Stability Constants of Bio-Relevant, Redox-Active Metals with Amino Acids: The Challenges of Weakly Binding Ligands

Jaime M. Murphy,[a,b] Brian A. Powell,[a,c] and Julia L. Brumaghim[a,*]

[a] Department of Chemistry, Clemson University, Clemson, SC 29634-0973, USA

[b] Chemistry and Biochemistry Department, Harding University, Searcy, Arkansas 72149, USA

[c] Department of Environmental Engineering and Earth Sciences, Clemson University, Clemson, SC 29634, USA

*corresponding author, brumagh@clemson.edu

Abstract

Metal ion interactions with weakly coordinating ligands, such as amino acids, are dependent on several factors, including metal ion availability, metal ion propensity for hydrolysis, ligand availability, and thermodynamic stability, as measured by stability constants. Metal ions in biological systems are often controlled by highly specific chaperone, transport, and storage proteins. Disruption in the homeostasis of redox active metal ions, such as Cu(I), Cu(II), Fe(II), and Fe(III), has been linked to increased oxidative damage and disease. Weakly binding ligands such as amino acids may play an active role in mitigating this metal-mediated damage, but a comprehensive understanding of the availability and thermodynamic likelihood of coordination must be understood to accurately predict complex formation in a competitive environment. This review presents an overview of amino acid stability constants with Cu(I), Cu(II), Fe(II), and Fe(III), the most common redox-active metal ions in biological systems. Specific attention is given to sulfur- and selenium-containing amino acids, since their interactions with Cu(I) and Fe(II) is of particular biological interest. This review also describes methods available for stability constant determination, with particular attention to specific difficulties encountered when working with weakly binding ligands and each of these four metal ions. Finally, the potential biological implications of these results are discussed based on reported stability constants as well as amino acid, copper, and iron bioavailability.

Keywords: stability constants; amino acids; redox-active metals; copper; iron; sulfur; selenium

Abbreviations: Ala, alanine; Arg, arginine; Asn, asparagine; Asp, aspartic acid; Cys, cysteine, Gln, glutamine, Glu, glutamic acid; Gly, glycine, His, histidine; Ile, isoleucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Pro, proline; Ser, serine; Thr, threonine; Trp, tryptophan; Tyr, tyrosine; Val, valine, MeCys, methylcysteine; MeSeCys, methylselenocysteine; hCys, homocysteine; Pen, penicillamine; SeMet, selenomethionine,

Contents

1. Introduction

Metal uptake and transfer in biological systems is essential to enzyme function [1], oxygen and electron transfer [1], infection control [2], and redox balance [3]. Biological mechanisms for metal transfer and redox activity are often poorly understood due to the complexities of biological environments and a limited understanding of the quantities and localization of high-affinity and weakly binding ligands present in cells. For example, mis-regulation of copper and iron homeostasis is implicated in initiation and/or progression of Parkinson's and Alzheimer's diseases [4, 5], but the role of weakly chelating biomolecules in these diseases has not been addressed. It is often assumed that non-protein-bound metal ions are coordinated to low-molecular-weight oxygen- and nitrogen-containing ligands, but the nature of these ligands and how these interactions affect cellular processes is unknown.

Determination of *in vitro* stability constants is used to predict equilibria that may occur in more complex systems [6, 7] and to model speciation in biological fluids [8-11]. The goals of this review are to 1) examine weakly coordinating amino acid interaction with copper and iron under biological conditions, with an emphasis on sulfur and selenium amino acids, 2) examine the methods and method limitations for determination of stability constants describing complexation of redox-active metal ions with weakly binding ligands, and 3) emphasize specific needs for methods development and further research on these systems.

A comprehensive discussion of stability constants for weakly binding ligands present in significant quantities in the cell is lacking and is presented in this review. Specifically, this review focuses on the stability constants of amino acids with the biologically relevant, redox-active metals copper and iron. Copper and iron are of particular interest due to their availability in the cell, potential for chelation by wide variety of ligands, and known contribution to reactive oxygen species generation and oxidative damage. Ligand coordination to these metal ions can be

difficult to assess, due to their variable oxidation states and coordination geometries. Although the Smith and Martell database (NIST v.46) [12] contains a large set of externally evaluated stability constants, including some of the iron and copper complexes of interest to this review, this database is no longer being critically analyzed and curated.

This review places special emphasis on sulfur and selenium amino acids, since coordination of these ligands with copper and iron is of particular biological interest, and thiol and thioether coordination can stabilize the reduced forms of copper and iron [13-15]. A range of stability constant determination methods including potentiometric, spectrophotometric, and voltammetric analyses have been used to quantify formation of iron and copper complexes with amino acids under biologically relevant conditions. The review discusses and identifies the limitations of each method as it pertains to each metal and oxidation state and will evaluate the potential impact of amino acids on biologically relevant metal interactions by modeling of more complex systems.

2. Cellular Redox-Active Metal Ions and Amino Acids

Copper, iron, zinc, manganese, and cobalt are essential metal ions in biological systems that play crucial biochemical roles as cofactors in enzymes. Iron and copper are of particular interest due to their stability in multiple oxidation states which are often essential to biological processes [16, 17], but this activity makes assessing the validity of ligand coordination difficult even under tightly controlled systems such as *in vitro* analyses. The association and distribution of copper and iron, not only within the highly selective binding pockets of proteins, but also with more weakly binding ligands such as free amino acids has implications for the uptake, transfer, and redox states of these metal ions throughout the cell.

Complex formation is dependent on amino acid concentration, metal concentration, and the thermodynamic driving forces controlling complex formation. In human plasma, free amino acid concentrations can be divided into three categories: high abundance (200-500 μM), low abundance (10-200 μM), and trace abundance (less than 10 μM) [18-20]. Alanine, glutamine, glycine, leucine, lysine, proline, threonine, and valine fall into the high abundance category. With the exception of threonine, these amino acids have non-polar or positively charged side chains at pH 7, which limit their cation binding abilities to bidentate binding through the amine nitrogen and carboxylate oxygen groups. Arginine, aspartic acid, asparagine, cysteine, glutamic acid, histidine, isoleucine, serine, methionine, phenylalanine, tryptophan, and tyrosine fall into the low abundance category, and most of these have polar or negatively charged side chains at pH 7 that may allow tridentate coordination through the amine, carboxylate, and side chain groups. Trace-level amino acids include methylcysteine and the selenoamino acids selenomethionine, selenomethylcysteine, and selenocysteine. Methylcysteine concentrations in urine are reported to be 0.2-5 μM [21]; plasma or cellular concentrations are not reported. Selenoamino acid concentrations are also not reported, but total selenium concentration in human plasma averages 1.5-1.6 μM, with an estimated 90% incorporated into selenoprotein as selenocysteine or selenomethionine [22]. Although the abundance of selenoamino acids is extremely low, soft selenoether or selenolate groups may strongly interact with softer metals such Cu(I) and Fe(II) according to Pearson hard-soft acid-base theory.

Penicillamine is an amino acid not naturally found in cells, but it bears a close structural resemblance to cysteine. It is a highly effective copper chelator used to treat Wilson's disease [23, 24]. With a typical dosage of 750 mg/day, serum penicillamine levels can reach 100 μM in treated patients [25]. Although it binds copper, it may also influence iron homeostasis [26, 27].

In this work, stability constant determination is discussed separately with Cu(I) and Cu(II) and Fe(II) and Fe(III) due to the unique cellular roles and significantly different coordination characteristics of each ion. Each of these metal ions serves essential biological roles in electron transfer, oxygen transport, and catalysis [28]. Iron and copper are two of the most abundant transition metal ions in cells, and control of these potentially toxic ions is highly regulated by metallochaperones and storage proteins such as ferritin [29-31]. Total copper concentrations are in the range of 10-25 μM in human serum [32] and up to 100 μM in human brain tissue [33]. Labile (non-protein-bound) copper pools are also identified in cells, primarily as Cu(I) [34]. Cellular concentrations of labile copper are not quantified, but significant recent strides have been made in the development of methods to detect labile copper pools [35-37], and local labile copper concentrations may reach the very low micromolar range [38].

Total iron concentrations are 20 to 30 μM in human serum [39] but are approximately 300 times higher in human liver (6315 μM) [40]. Jhurry and coworkers quantified labile iron concentrations in the cytosol of human cells at 30 μM and in mitochondria at 210 μM [41], and typical labile Fe(II) pools are generally reported around 5-10 μM [42]. Mis-regulation of copper and iron homeostasis can lead to increased oxidative damage and protein misfolding or aggregation and is implicated in the development of neurodegenerative diseases such as Parkinson's and Alzheimer's diseases [4, 43-46]. In addition, redox cycling between Cu(I) and Cu(II) that is critical for the function of most copper enzymes is often controlled by amino acid coordination and protonation state [47-49]. In conjunction with reliable and complete stability constant determination and species identification, as well as the biological concentrations of the amino acids and metal ions, the extent of biological amino-acid-metal complex formation under equilibrium conditions can be predicted.

3. Amino Acids as Weakly Binding Ligands

Biological regulation of metal ions is dominated by strong chelation in highly specific binding pockets of proteins, often contributing to protein structural support and/or enzyme activation. It is more difficult to ascertain the role of metal-coordinating, small molecules, particularly at high metal concentrations resulting from loss of homeostasis [34, 50-54]. Amino acids have a wide range of stability constants (with log β values between ~4 and ~15 for Fe(II) and Cu(II), depending on stoichiometry and binding modes). In most cases, these metal-amino acid stability constants are lower than the stability constants of metalloproteins such as azurin [55], transferrin [56], and lactoferrin [57] that have stability constants in the range of 15-20. Small, coordinating molecules may function in a number of roles including: 1) cellular signaling agents, such as various hormones or cytokines, 2) molecules required for metabolism, such as sugars, 3) molecules needed for anabolism, such as amino acids or lipids, and 4) exogenous molecules, such as drugs, antioxidants, or toxins. Entire databases in bioinformatics and cheminformatics are committed to sorting, analyzing, and predicting chemical properties and biomolecular pathways for these types of metal-coordinating small molecules [58]. We focus on the coordination and stability of amino-acid-metal complexes, since amino acids coordinate strongly enough to Cu(I)/Cu(II) and Fe(II)/Fe(III) to infer that these complexes may form within a cell [6]. For the purposes of this review, binary systems of the metal ion and a single amino acid will be discussed, although the likelihood of mixed ternary complexes is high [59]. Dipeptides, tripeptides such as glutathione, and other oligopeptides are also potential metal-binding competitors [42, 60-67], but are also outside the scope of this review.

3.1 Potentially Bidentate Amino Acids

Because amino acids have varied potential metal-binding modes, discussion of amino acid coordination will be grouped according to their predicted denticity. Most amino acids only have the capability for bidentate coordination, through the α-carboxylate oxygen and α-amine nitrogen atoms, forming a five-membered chelate ring with the metal ion (Fig. 1). Since glycine is the simplest amino acid and primarily binds metals with bidentate coordination, this type of bidentate metal-amino-acid coordination is often referred as glycine-like binding [68].

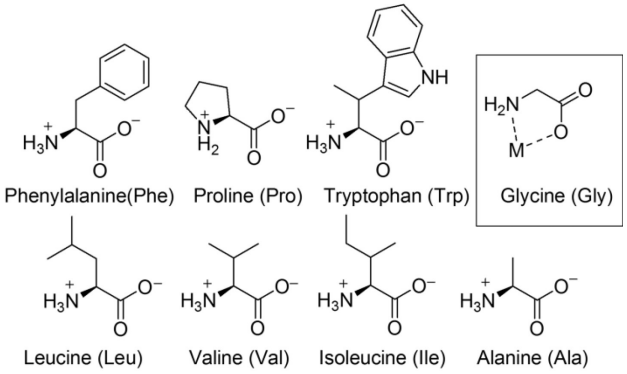

Fig. 1 Amino acids with non-coordinating aliphatic or aromatic side chains that have the capability to coordinate metal ions in a bidentate fashion. In box: complex showing bidentate binding to a metal ion (M) through the carboxylate oxygen and amine nitrogen, using glycine as an example.

3.2 Potentially Tridentate Amino Acids

Amino acids with polar or charged side chains may have the capability to bind in a tridentate fashion (Fig. 2), but often do not achieve full tridentate coordination. Alcohol, amine, and carboxylate groups all can potentially coordinate metals, but the influence of thermodynamic factors such as pK_a, steric strain, and entropy cost can lessen or prevent metal interactions. Predicting the likelihood of an amino acid binding in a tridentate fashion is not straightforward. For example, the polar side chains of arginine and lysine are positively charged at pH 7 (Fig. 2), with pK_a values above 10, properties that inhibit metal binding.

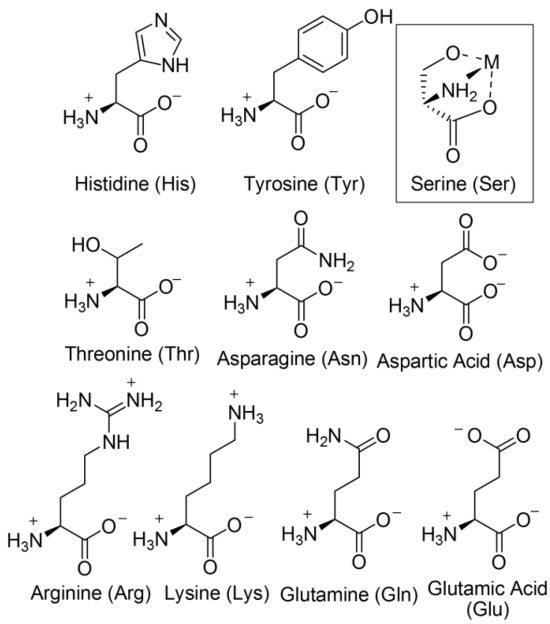

Fig. 2 Amino acids with polar or charged side chains that have the capability to bind metals in a tridentate fashion. In box: complex showing potential tridentate binding to a metal ion (M) through the α-carboxylate oxygen and α-amine nitrogen as well as a side chain atom, using binding to the oxygen atom of the deprotonated alcohol group in serine as an example.

Perhaps the best measure of the ability of an amino acid side chain to bind copper and iron is to consider the amino acid residues most often found in metalloprotein binding pockets. In a 2007 survey of the Protein Database, the three amino acids most commonly found in copper metalloprotein binding pockets were histidine, cysteine, and methionine, respectively [69]. Aspartic acid, glutamic acid, serine, threonine, glutamine, and asparagine also bind copper but much less commonly. For iron metalloproteins, histidine, glutamic acid, cysteine, aspartic acid, methionine, and tyrosine were the primary iron- binding amino acids, with serine and asparagine as minor contributors [69]. Based on these reports, it is reasonable to assume that these free amino acids also would potentially bind cellular copper and iron. Higher stability constants are expected for metal-amino acid complexes with tridentate binding compared to those with only bidentate coordination, since greater chelation confers higher thermodynamic stability.

3.3 Sulfur- and Selenium-Containing Amino Acids

In this review, special emphasis is given to iron and copper interactions with sulfur- and selenium-containing amino acids, including penicillamine, methylcysteine, cysteine, and selenomethylcysteine (Fig. 3). These amino acids not only show preferential binding to soft and borderline metal ions, such as Cu(I), Cu(II), and Fe(II), but they also influence redox activity of these metals [70]. Metal-sulfur and -selenium redox interactions can make it difficult to clearly interpret stability constant data for these systems, especially for thiol/selenol-containing amino acids with reduced metal ions [71-73]. Glutathione, a sulfur-containing peptide present in millimolar concentrations in cells, will not be discussed in-depth in this review although it likely has a significant impact on biological metal ion speciation [38, 74-77].

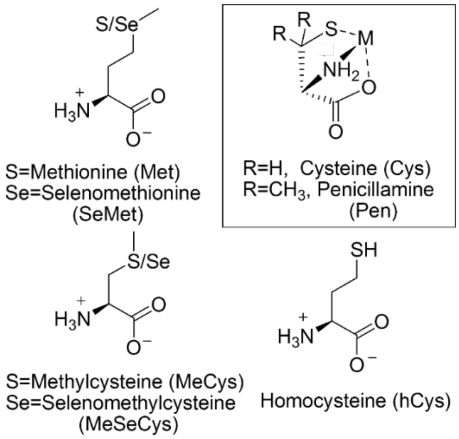

Fig. 3 Amino acids with sulfur- or selenium-containing side chains; all have the capability to bind metals in a tridentate fashion. In box: complex showing potential tridentate binding to a metal ion (M) through the α-carboxylate oxygen and α-amine nitrogen atoms as well as a side chain atom, using deprotonated sulfur in cysteine or penicillamine as an example.

Because of the S/Se atom in the side chain, these amino acids can potentially act as tridentate chelators to metal ions. Although selenoamino acids are less prevalent in the cell than their sulfur analogs, metal-selenocysteine binding is required for the activity of enzymes such as NiFeSe hydrogenases [78]. Selenoether-containing amino acids are not known to have primary metal-binding roles in metalloproteins, but selenomethionine can substitute indiscriminately for

methionine when Se levels are high [79] and has been well-studied for its ability to prevent metal-mediated oxidative damage [80].

4. Comparing Apples to Apples: Defining Parameters of Stability Constant Determination

The sheer volume of stability constant data for transition metal ions with amino acids is overwhelming and has been the subject of databases and extensive reviews [12, 68, 71, 81-83]. Previous reviewers [71, 84] noted that the wide range and seemingly inconsistent reports of these stability constants is attributable to the sensitivity of these systems to the specific conditions under which determinations are performed. Even when using the same analytical method, variables including the nature and concentration of supporting electrolyte, pH range, temperature, and solvent significantly affect the resulting stability constants. Whenever possible in this review, stability constants were chosen that represent the most consistent results, both with each other and with biological conditions. Thus, typical experimental conditions are 25-37 ˚C with 0.1-3.0 M supporting electrolyte. When limited data are available, the best or only reported metal-amino-acid stability constants are provided.

Clearly defining equilibrium constants is crucial to correctly interpreting stability constant data and identifying species formed across various analyses, especially for amino acids where charges can differ. In this review, amino acids are divided into three categories: 1) those likely to bind as bidentate ligands, composed of aliphatic or aromatic amino acids with nonpolar side chains (Fig. 1), 2) those that can potentially bind as tridentate ligands, composed of amino acids with polar or charged side chains (Fig. 2), and 3) sulfur- and selenium-containing amino acids (Fig. 3). Since the sulfur and selenium-containing amino acids have greater potential for redox activity compared to other amino acids, especially upon iron or copper coordination, it is

useful to treat these amino acids separately.

For all the amino acids, proton association constants can be expressed as stepwise protonation constants shown in equilibrium expressions 1 and 2.

$H^+ + L^- \rightleftharpoons HL$ protonation of $-NH_2$ group (1)

$H^+ + HL \rightleftharpoons H_2L^+$ protonation of $-COO^-$ group (2)

The equilibrium constant K_{HL} relates to the first protonation (equation 1) according to equation 3 and the equilibrium constant K_{H_2L} relates to the second protonation (equation 2) according to equation 4.

$$K_{HL} = \frac{[HL]}{[L^-][H^+]} \quad (3) \qquad K_{H_2L} = \frac{[H_2L^+]}{[HL][H^+]} \quad (4)$$

Equilibrium constants 3 and 4 apply for all amino acids that do not have side chains that can protonate or deprotonate, such as those shown in Fig. 1 and the thioethers shown in Fig. 3. The remaining amino acids have ionizable side chains that must be accounted for in additional equilibrium expressions.

For amino acids that are positively charged at pH 7, such as lysine, arginine, and histidine, the protonation equilibrium reactions 5, 6, and 7 apply.

$H^+ + L \rightleftharpoons HL$ protonation of $-NH_2$ group (5)

$H^+ + HL \rightleftharpoons H_2L^+$ protonation of side chain (6)

$H^+ + H_2L^+ \rightleftharpoons H_3L^{2+}$ protonation of $-COO^-$ group (7)

Thus, for protonation reactions of amino acids with ionizable side chains, equilibrium constants 3 and 4 apply, along with the additional equilibrium constant K_{H_3L} (8).

$$K_{H_3L} = \frac{[H_3L^{2+}]}{[H_2L^+][H^+]} \quad (8)$$

For amino acids that are negatively charged at pH 8, including glutamic acid, aspartic acid, cysteine, homocysteine, and penicillamine, the representative equilibria are 9, 10, and 11.

$H^+ + L^{2-} \rightleftharpoons HL^-$ protonation of $-NH_2$ group (9)

$H^+ + HL^- \rightleftharpoons H_2L$ protonation of side chain (10)

$H^+ + H_2L \rightleftharpoons H_3L^+$ protonation of $-COO^-$ group (11)

The related association constants are similar to those defined in equations 3, 4, and 8, although it is important to note that the charge on each species is different.

Association constants for metal-amino-acid coordination are defined in a similar manner. Because the charge of the metal ions (M) studied varies from +1 to +3 and the charges of the amino acids (L) also vary, charges on the species are typically not indicated in these general equilibrium expressions. When discussing specific species, charges will be shown whenever possible. Equilibrium equations for mono- and bis-coordinated complexes as well as their formation constant (β) expressions are represented by 12 and 13, respectively. The formation constant β is related to the thermodynamic stability of a complex, the association constant *K,* for each stepwise addition of a new ligand. At lower pH, the side chain of the amino acid may or may not be protonated, as shown in equations 14 and 12, respectively. At higher pH, some metal-amino acid systems coordinate a hydroxyl ligand, or deprotonate a coordinated water molecule, resulting in the ternary metal-ligand-hydroxide species [ML(OH)] (15).

$M + L \rightleftharpoons [ML]$ $\beta_{ML} = \frac{[ML]}{[L][M]}$ (12)

$M + 2\,L \rightleftharpoons [ML_2]$ $\beta_{ML2} = \frac{[ML_2]}{[L]^2[M]}$ (13)

$M + HL \rightleftharpoons MLH$ $\beta_{MLH} = \frac{[MLH]}{[H][ML]}$ (14)

$$[\mathrm{ML}] + \mathrm{OH} \rightleftharpoons [\mathrm{ML(OH)}] \qquad \beta_{MLOH} = \frac{[ML]}{[OH][ML]} \qquad (15)$$

The thermodynamic parameter for each stepwise formation constant, K can then be related to the standard free energy change ($\Delta G°$) at constant pressure (equation 16). The total enthalpy change $\Delta H°$ can be determined from the temperature dependence of K according to the van't Hoff equation (17). Although potentiometry is commonly used to determine metal-ligand formation constants, calorimetry is often used for enthalpy determinations, since potentiometric measurements may not be stable across the full temperature range needed to calculate a free energy change. Free energy and enthalpy thermodynamic parameters are not discussed in detail in this review.

$$\Delta G^{\circ} = -RT\ln K \qquad (16)$$

$$\frac{d(\ln K)}{dT} = \frac{\Delta H^{\circ}}{RT^2} \qquad (17)$$

To determine stability constants for metal-amino-acid complexes, any method can be used that can actively measure the formation and elimination of the species present, and books dedicated to methods development and analysis have been published [85-87]. General problems associated with determining metal-amino-acid stability constants for all metals are thoroughly reviewed [68, 82, 88-91], although these reviews are data-heavy and do not include a discussion of methods development. This review will focus specifically on the best methods for amino acid stability constant determination with Cu(I), Cu(II), Fe(II) and Fe(III), and the particular experimental limitations associated with these ions. Common methods are introduced here, and less common methods are discussed in each metal-specific section as relevant.

The three most common methods for stability constant determination are potentiometric, voltammetric, and spectrophotometric titrations. Potentiometric analyses are the most frequently

used method for amino acid-metal binding constant determinations. The precision and stability of this method makes it the ideal choice when conditions allow, and permits detection of minor species when coupled with the computational abilities of modeling software [68, 71, 73, 92]. While many of these species are inconsequential under biologically relevant titration conditions for simple systems, incorporation of these species into studies of more complex systems is imperative and can have significant effects, since their formation may influence formation of competitive species [8].

Potentiometric analyses are not always an option, and independent analyses are helpful, and in some cases necessary, in confirming complex speciation. Voltammetric or polarographic techniques permit measurements at a constant pH for pH-sensitive systems [93]. Often the resulting data are not as precise or as consistent as potentiometric methods, since typically only changes to the metal ion are measured. Spectrophotometric analyses work well with metal ions, ligands, and/or complexes that absorb in UV or visible wavelengths, but these methods may not be able to distinguish the binding modes of multidentate interactions. Development of methods such as electrophoresis, involving solvent-extraction of species, is a growing area. Table 1 provides an overview of the most common methods and their advantages and limitations.

For amino-acid-metal complex determinations, potentiometry is the most common method utilized, because the uptake and release of protons can be measured precisely [68, 82, 89-91, 94-96]. Electrode stability limits the analysis range, and data can be questionable at pH extremes (typically $pH < 2$ and $pH > 12$) [68, 91]. Although robust and precise, potentiometric analyses are limited to ligands or hydrolyzed metals with protons that associate and dissociate in the pH range investigated and can be limited by ligand and/or complex solubility across this pH range.

Table 1
Advantages and limitations of stability constant determination methods

Method	Advantages	Limitations
Potentiometric titration	High precision High accuracy	Ligands must protonate/deprotonate Species must be soluble across wide pH range Disproportionation issues with Cu(I) Curve fitting technique with no direct measurement of the metal or metal-ligand complex
Spectrophotometric titration	Can be run at narrow pH Direct probe of metal and metal-ligand complex	Either metal or ligand (or both) must be UV-vis active
Electrophoresis (paper or solution)	Simple detection Easy to identify species charges	Low precision Temperature and oxygen control more difficult Conditions differ from solution determination
Redox titration	Redox-active metals can be controlled	Species identification must be confirmed using independent methods
Solubility	Low solubility systems such as Fe(III)	Lengthy experiments due to slow equilibria between solid and solution
Electron paramagnetic resonance (EPR)	Indicates coordination modes	Often used in parallel with other methods for ease of interpretation Metal ion must be EPR active

Methods such as UV-visible, electron paramagnetic resonance (EPR), circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopies used in conjunction with potentiometric methods may provide greater insight into minor species, ligand coordination modes, and geometric orientation in solution, especially for less-predictable systems. Much of the burgeoning research in this area has focused on small peptide-metal complexes and has utilized a variety of spectroscopic methods in conjunction with potentiometry [60, 61]. The skill in pursuing research in these areas is matching the metal ion, ligand, and methods to maintain precision and accurately observe coordination events.

The Smith and Martell database (NIST v.46) contains a comprehensive list of all stability constant and associated thermodynamic data available for metal-amino-acid stability constant data reported up through 2001 [12]. Stability constants for most of the amino acids with a variety of metals are available for a wide range of temperatures and supporting electrolyte

concentrations. Many of the metal-amino-acid stability constants reported in the Smith and Martell database (NIST v.46) [12] and previous books [97-99] are reliable and accepted, therefore additional studies have not been performed. Various reviews present a more selective list of stability constants of metals with amino acids until 1997 [68, 71, 81-83]. While the database and reviews are comprehensive for the time periods indicated, they are cumbersome in the quantity of analyses given for some metals such as Cu(II) and reflect the lack of data for other metals such as Cu(I). Our analysis draws on these data and also comprehensively covers iron and copper data with amino acids up until early 2019.

5. The Gold Standard: Proof of Speciation

Unambiguous identification of the thermodynamically stable species present in solution is required to understand the solution equilibria of a metal-ligand complex [86, 87]. For potentiometric determinations, glass electrodes are used to track the change in potential as acid/base titrations are performed. Before the advent of modeling programs, best fit analyses were determined for the most likely species formed in the given system using graphical methods documented by Bjerrnum [100, 101] and Fronaeus [102]. More recently, programs such as SCOGS [103], HYSS [104], HYPERQUAD [105], MINIQUAD [106], SUPERQUAD [107], FITEQL [108], and other modeling systems [109-111] have made modeling and model-matching much easier to perform and have allowed for more precise data analysis. As a result of computational modeling methods and perhaps a more comprehensive understanding of solution equilibria for metal-amino acid complexes, the number of identified species in recent reports has expanded. While incorporation of additional species certainly improves model fit to the data, due to their low concentration and limited influence on metal complex formation, the existence of

such species are often difficult to confirm from titration data alone. For example, minor species such as amino acid complexes with protonated, unbound side chains may not play an active role in metal binding, but they may contribute to sample buffering [71, 112]. Thus, the gold standard for species determination should incorporate secondary methods to unambiguously identify these minor species. In this review, we describe the most consistent reported species, particularly emphasizing investigations that have demonstrated a high level of control of experimental conditions or used multiple methods of analysis to independently confirm the identified species.

6. Stability Constants of Non-Sulfur and -Selenium-Containing Amino Acids with Cu(II)

6.1 Cu(II) Complexes of Potentially Bidentate Amino Acids

Whether or not labile Cu(II) exists in the cell, Cu(II) plays a major role in organisms since activity and stability of Cu(II) metalloproteins depend on copper-amino acid interactions [1]. Compared to Cu(I), Fe(II), and Fe(III), Cu(II) is the most chemically well-behaved ion for analytical measurements. Most Cu(II) salts are soluble in aqueous solution and are not sensitive to air oxidation. It is not surprising, therefore, that hundreds of analyses to determine Cu(II)-amino-acid stability constants are reported [68, 71, 82, 88-90] using a wide variety of methods: polarography [113], spectrophotometry [114], circular dichroism [115], optical rotary dispersion [116], and electrophoresis [117, 118]. Although solubility of Cu(II)-amino acid complexes with hydrophobic side chains is limited in the basic pH range, this issue is not always discussed in published reports.

Table 2 shows a summary of the stability constants for Cu(II) for amino acids with non-coordinating side chains that are limited to bidentate coordination through the carboxylate oxygen and amine nitrogen of the amino acid (Fig. 1). Due to the plethora of available data for

Table 2
Stability constants for Cu(II) and Cu(I) with potentially bidentate amino acids

Ligand	[ML] ($\log \beta_{ML}$)[a]	[ML_2] ($\log \beta_{ML2}$)[b]	Temp. (°C)	Ionic Strength (M)	Method	Ref.
Stability Constants for Cu(II)						
Alanine	8.17(3)	14.94(5)	30	0.1 KNO_3	Potentiometry	[119]
Glycine	8.07(2)	14.86(3)	30	0.1 $NaClO_4$	Potentiometry	[120]
Isoleucine	8.50(6)	15.79(8)	25	0.1 $NaNO_3$	Potentiometry	[121]
Leucine	8.276(1)	15.174(1)	25	0.1 KNO_3	Potentiometry	[88]
Phenylalanine	7.93(1)	14.83(1)	25	0.1 KNO_3	Potentiometry	[88]
Proline	8.60(3)[c]	15.09(7)[c]	25	0.1 $NaNO_3$	Potentiometry	[122]
Tryptophan	8.02(1)	15.56(1)	25	0.1 KNO_3	Potentiometry	[88]
Valine	8.05(2)	14.91(2)	30	0.1 $NaClO_4$	Potentiometry	[120]
Stability Constants for Cu(I)						
Alanine	9.6[d]		25	0.3 K_2SO_4	Redox	[123]
Glycine	10.0[d]		25	0.3 K_2SO_4	Redox	[123]

[a] $\log \beta_{ML} = [ML]/[M][L]$ [b] $\log \beta_{ML2} = [ML_2]/[M][L]^2$ [c] Authors also reported the minor species [Cu(Pro)(OH)] ($\log \beta = 1.29(4)$) and $[Cu(Pro)(OH)_2]^-$ ($\log \beta = -8.58(3)$ [d] Error not reported

Cu(II)-amino-acid stability constants [12], the selected stability constants in Table 2 are those "recommended" in previous reviews due to their data quality and reproducibility [12, 68, 71-73, 81, 82], where possible. Beyond that, selected constants were 1) reported with errors, 2) determined within 25-37 ˚C, and 3) were conducted in a constant ionic strength medium (range 0.1-3 M) [124].

Analysis of Cu(II)-amino-acid stability constants with bidentate-coordinating amino acids (Fig. 1) is fairly straightforward and consistent. Coordination is typically through the α-carboxylate oxygen and α-amine for both [ML] and [ML_2] species and is supported by solid-state structures. The structure of [$Cu(Gly)_2$], a representative bidentate-[ML_2] species, is square planar with bidentate glycine ligands creating two five-membered, equatorial chelate rings around Cu(II) (Fig. 4A) [125]. The axial positions are vacant, with occasional coordination of water molecules or supporting electrolyte, such as in [$Cu(Gly)_2(H_2O)$] (Fig. 4B) [125-128].

No trend in stability constants relating to side chain hydrophobicity is observed for the aliphatic amino acids. Potential intermolecular interactions of aromatic side chains (e.g., phenylalanine) also do not impart added stability to the complexes, since all of the Cu(II)

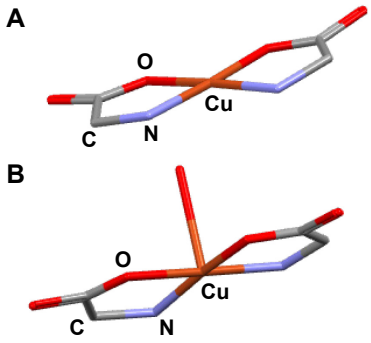

Fig. 4 A) Crystal structure diagram for $[Cu(Gly)_2]$ showing carboxylate and amine coordination with square planar geometry around the central Cu(II) ion [129]. B) Crystal structure diagram of $[Cu(Gly)_2(H_2O)]$ also showing carboxylate and amine coordination in the equatorial position, but with a water molecule coordinated in the axial position of the square pyramidal geometry [126, 128]. The Cu(II) ion is shown in orange, oxygen atoms are red, carbon atoms are grey, and nitrogen atoms are blue. Hydrogen atoms are omitted for clarity.

stability constants with bidentate amino acids are within one log unit of each other (Table 1). This stability constant uniformity indicates glycine-like [ML] and $[ML_2]$ complex formation for all of these amino acids with Cu(II).

Minor species have also been identified for these relatively simple systems. Berthon and coworkers [8] report $[Cu(HVal)]^{2+}$ and $[Cu(HVal)(Val)]^{+}$ as well as $[Cu(HGly)]^{2+}$, $[Cu(HGly)(Gly)]^{+}$, and $[Cu(HGly)_2]^{3+}$ species in their Val and Gly analyses, respectively. Because the side chains of Val and Gly cannot protonate, it can be assumed that these species arise from α-amine protonation and monodentate binding of the metal through the carboxylate oxygen. All of these species form below pH 3 and represent only a very small change in buffering of the system. While these species are chemically reasonable in terms of competition between a high proton concentration and Cu(II) for amino acid binding, they are formed at the accuracy limits of potentiometric measurements when formed at low pH and remain to be independently confirmed.

On the other end of the pH range, species with hydroxyl coordination, such as [ML(OH)] and $[ML_2(OH)]$, are reasonable and expected, especially since water is known to coordinate in the axial position in the solid state (Fig. 4B) [125, 130]. However, Cu(II)-amino acid complexes

typically precipitate in the alkaline range (pH > 9). Arena and coworkers note that signal drift can occur in potentiometric measurements at more basic pH when precipitation is seeding [131], and this signal drift can be misinterpreted as new species formation [132]. Thus, distinguishing between signal drift and minor hydroxyl species formation at basic pH is a core issue in determining accurate speciation for metal-amino-acid complexes.

Analyses across methods also are consistent for Cu(II) titrations with potentially bidentate amino acids, a promising sign for methods development, particularly for the determination of stability constants for ligands that may not have the ionizable protons needed for potentiometric analysis. Paper electrophoresis is an excellent method for separating species, although it is limited in precision and may not accurately represent "solution" equilibria. For potentiometric and paper electrophoresis results for the Cu(II)-alanine system, the paper electrophoresis stability constants reported by Jokl [133] are slightly higher: 8.5 and 15.2 for the $[Cu(Ala)]^+$ and $[Cu(Ala)_2]$ species (no errors are reported), respectively, compared to 8.17(3) and 14.94(5) using potentiometric methods [119]. Singh's $[Cu(Val)]^+$ and $[Cu(Val)_2]$ electrophoresis determinations [117] are consistent with or slightly lower than potentiometrically determined values (8.02 and 14.62, respectively, compared to 8.05(2) and 14.91(2), respectively) [120]. Separately, Tewari [134] reported paper electrophoresis stability constants for the Cu(II)-isoleucine system: 8.41(7) for $[Cu(Ile)]^+$ and 14.84(3) for $[Cu(Ile)_2]$, values consistent with or slightly lower than the potentiometric results of 8.50(6) and 15.79(8), respectively [121]. While this is not a comprehensive list of paper electrophoresis determinations for Cu(II) amino acid complexes, these representative data demonstrate method viability. Indications of the charge of species due to electrophoretic movement is an advantage of electrophoresis. If the detection limits are suitable, electrophoresis may be a method worth exploring for establishing the

existence of minor species.

Spectrophotometric analyses also are an option for spectrophotometrically active metals such as Cu(II) or ligands with aromatic groups that absorb or fluoresce in the UV or visible spectrum. However, concentrations required for species detection in the UV-visible range are dependent on the absorptivity of the species involved, which can be a limiting factor for systems with low absorptivity. Effects of metal-coordinating solvents or supporting electrolyte can also contribute to error in spectrophotometric methods. For example, a spectrophotometric analysis of the Cu(II)-isoleucine system by Bretton [135] with no supporting electrolyte results in considerably higher stability constants than those obtained by Ivicic [121] using potentiometric analyses with a supporting electrolyte of 0.1 M $NaNO_3$ (Table 2).

Other optical methods such as circular dichroism and optical rotary dispersion have also been used to determine stability constants with Cu(II), with results similar to those from potentiometric analyses, but it can be difficult to identify minor species using these methods [115, 116]. Perhaps the most compelling use of spectrophotometric methods to determine stability constants is in conjunction with potentiometric methods, since species identification can be supported by two independent methods. This combination is demonstrated by Davis [136] in determining stability constants for Cu(II)-valine-pyridoxal complexes.

Stability constants of Cu(II) with aliphatic amino acids are one of the most widely studied of all metal-amino-acid combinations. The relative stability of this metal ion with non-redox active ligands makes the resulting data easy to interpret, as long as the method is reliable in collecting quantifiable changes to the system, whether the release of protons or spectral changes. As a result, these systems provide the best arena for development of methods to examine metal coordination with weakly binding ligands.

6.2 Cu(II) Complexes of Potentially Tridentate Amino Acids

Amino acids with polar side chains, including serine, histidine, threonine, tyrosine, aspartic acid, glutamic acid, asparagine, and glutamine, may coordinate not only through the carboxylate oxygen and amine nitrogen atoms, but also through the polar side chain atoms. Although polar, the side chains of lysine and arginine are typically positively charged in aqueous solution with pK_a values of 10.54 and 12.48 [81], respectively, and therefore are not expected to coordinate positively charged Cu(II). Methionine and cysteine also have electronegative side chains with the potential for binding Cu(II), but these sulfur-containing amino acids have unique redox properties that present potential complications for stability constant determination and are discussed separately.

Due to the thermodynamic nature of stability constants, tridentate binding to Cu(II) should be reflected in considerably higher stability constants compared to bidentate binding. With Cu(II), stability constants for asparagine [137], glutamine [131], serine [138], threonine [88, 139-141], and tyrosine [142] (Table 3) are not significantly different from those for the bidentate amino acids (Table 2), suggesting only α-carboxylate and α-amine binding. Not surprisingly, the lowest stability constants for Cu(II) binding are observed for Lys and Arg (Table 3), likely indicating electrostatic repulsion between the positively charged amino acid and Cu(II). In contrast, Cu(II) stability constants of histidine ([ML] = 9.75, [ML_2] = 17.49) and aspartic acid ([ML] = 8.83, [ML_2] = 15.93) are considerably higher than the other potentially tridentate amino acids (Table 3), indicating side chain coordination.

Tridentate coordination to Cu(II) by aspartic acid [143] and histidine are supported by solid-state structures (Fig. 5) [144-147] and spectroscopic methods for glutamate [148, 149]. Stability constants for glutamic acid ([ML] = 8.30, [ML_2] = 15.03) are slightly elevated

Table 3
Stability constants for Cu(II) and Cu(I) with potentially tridentate amino acids

Ligand	[ML] ($\log \beta_{ML}$)[a]	[ML_2] ($\log \beta_{ML2}$)[b]	[ML(OH)] ($\log \beta_{MLOH}$)[c]	Other Species	Temp (°C)	Ionic Strength (M)	Method	Ref.
Stability Constants for Cu(II)								
Arginine	7.555(4)	14.007(5)			25	0.1 KNO_3	Potentiometry	[88]
Asparagine	7.788(3)	14.142(4)	4.17(2)	MLH 10.08(3) ML_2H 17.44(3)	37	0.15 $NaClO_4$	Potentiometry	[137]
Aspartic acid	8.83(3)	15.93(2)	24.0(1)	MLH 12.52(2) ML_2H 19.8(3) M_2L 10.34(6) M_2L_2 19.5(1)	25	0.1 KNO_3	Potentiometry	[150]
Glutamine	7.71(1)	14.12(1)			25	0.1 KNO_3	Potentiometry	[131]
Glutamic acid	8.30(4)	15.03(3)		MLH 12.52(2) ML_2H 19.6(3) M_2L 10.41(5) M_2L_2 18.6(2)	25	0.1 KNO_3	Potentiometry	[150]
Histidine[d]	9.75(1)	17.49(1)	2.2(2)	MLH 13.78(1) ML_2H 23.05(1) ML_2H_2 26.29(6) $ML_2(OH)$ 6.3(1)	37	0.15 NaCl	Potentiometry	[137]
Lysine	7.62(2)	13.94(2)		MLH 10.361(5) ML_2H 10.84(1)	25	0.1 KNO_3	Potentiometry	[88]
Serine	7.748(2)	14.083(5)	4.285(13)	MLH 10.030(16)	37	0.15 $NaClO_4$	Potentiometry	[137]
Threonine	7.98(4)	14.66(5)	4.81(3)	ML_2H_{-2} -6.0(1)	25	0.1 KNO_3	Potentiometry	[88, 139]
Tyrosine	7.90(2)	15.17(3)			25	0.1 KNO_3	Potentiometry	[142]
Stability Constants for Cu(I)								
Histidine	12.80[d]	25.20[d]			25	0.2 KNO_3	Redox	[151]

[a] $\log \beta_{ML} = [ML]/[M][L]$ [b] $\log \beta_{ML2} = [ML_2]/[M][L]^2$ [c] $\log \beta_{MLOH} = [ML(OH)]/[ML][OH]$ [d] The protonation state for His in the ML and ML_2 species assumes the histidine has one ionizable proton.

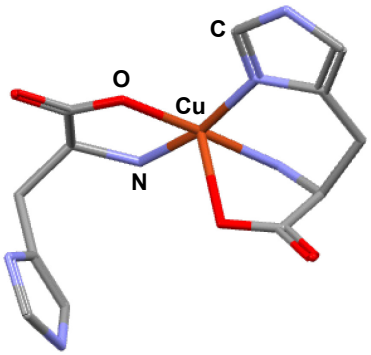

Fig. 5 Crystal structure diagram for the $Cu(His)_2$ complex, showing both tri- and bidentate binding of histidine to the Cu(II) center, as reported by Deschamps and coworkers [145]. The Cu(II) ion is shown in orange, oxygen atoms are red, carbon atoms are grey, and nitrogen atoms are blue. Hydrogen atoms are omitted for clarity.

compared to the other potentially tridentate amino acids, suggesting weaker side-chain coordination than for His or Asp. However, the only solid-state structure to support this tridentate binding mode is the glutamate complex with cadmium, $[Cd(Glu)(H_2O)]H_2O$ [152]. In general, most of the stability constants for the potentially tridentate ligands with Cu(II) do not indicate tridentate binding, and it is reasonable to assume that Cu(II) coordination by these amino acids is very similar to the bidentate amino acids with the exception of histidine and aspartate. Changes in side-chain protonation state, however, can complicate stability constant determination and make identifying minor species more difficult.

6.3 Challenges in Determining Cu(II)-Amino Acid Stability Constants

Determining the speciation of metal complexes with potentially tridentate amino acids is especially troublesome for modeling stability constants. These difficulties are primarily caused by reported potential minor species due to 1) inconsistency of identified species, 2) failure to independently characterize these species, and 3) absence of meaningful discussion about the relative importance or implications of the reported minor species. Thus, researchers may be adding minor species solely to optimize their model fitting to titration data, a particular issue given the unreliability of Cu(II)-amino acid titration data at pH > 9 due to precipitation. Collection of titration data is usually limited to the pH range over which all complexes remain in solution, but these pH limits are not always explained, and precipitation is rarely mentioned.

As an example, most studies describing binding constants for Cu(II)-serine complexes report only two species, [ML] and $[ML_2]$ [153]. More recent work reports two additional species, $[ML_2(OH)]$ and [MLH] [137]. The difficulties surrounding identification of additional, minor species are demonstrated by comparing the simulated titration data based on reported constants

for 1) a titration that incorporates just two primary species, $[Cu(Ser)_2]$ and $[Cu(Ser)]^+$ and 2) a titration that incorporates the additional minor species $[Cu(HSer)]^{2+}$ and [Cu(Ser)(OH)], for a total of four species (Fig. 6). Under typical titration conditions with a 1:2 Cu(II)-to-serine ratio, $[Cu(HSer)]^{2+}$ is present at less than 2% of total Cu and only below pH ~4, as modeled in Fig. 6B. Including this minor species results in no difference between the modeled two-species and four-species titration data at pH 4-8 for these concentrations (Fig. 6A). In contrast, adding the $[Cu(Ser)_2OH]^-$ species significantly affects titration buffering above pH 8. Including this additional species may improve the model fit to experimental data; however, many Cu(II)-amino-acid complexes precipitate above neutral pH as the concentration of the $[ML_2]$ species

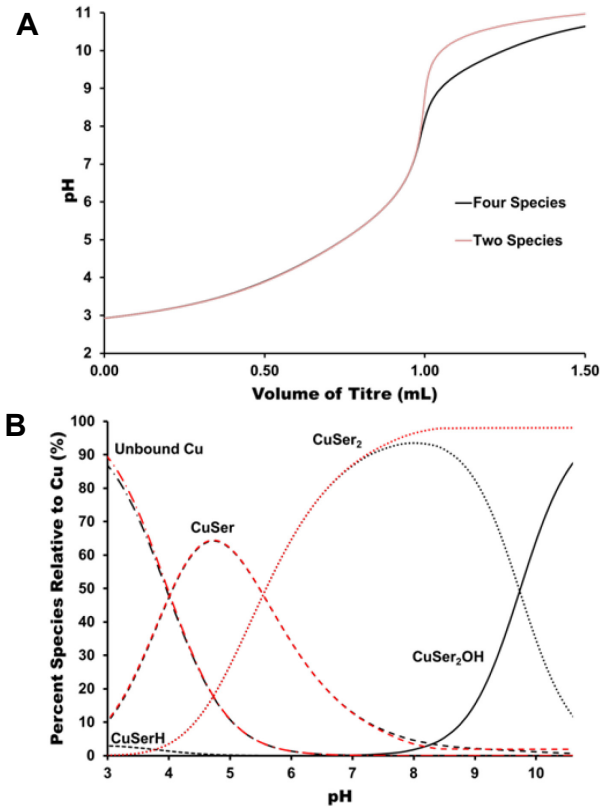

Fig. 6 A) Simulated titration with a strong base of Cu(II) and Ser with a 1:2 metal to ligand ratio. The "two-species" (red) line shows the modeled titration with only the $[Cu(Ser)]^+$ (log β = 7.92(1)) and $[Cu(Ser)_2]$ (log β_2 = 14.57(1)) species [153]. The "four-species" (black) line shows the modeled titration with four species, $[Cu(Ser)]^+$ (log β = 7.57), $[Cu(Ser)_2]$ (log β = 14.02), $[Cu(Ser)_2(OH)]^-$ (log β = 4.29), and $[Cu(Ser)H]^{2+}$ (log β = 10.03) [137]. B) A speciation diagram for the Cu(II) and Ser titration over the pH range 3-10.5 fit with two (red line) and four (black line) species.

increases (although this is often unreported), resulting in significant electrode drift and data inaccuracy. Under these conditions, the limited accuracy of experimental data may not support including minor species to increase modeling accuracy, and without independent characterization, these species may even be artefactual.

The Cu(II)-to-amino-acid ratio used in stability constant determinations also has a significant effect on complex speciation, as demonstrated with the four-species Cu(II)-Ser model titration (Fig. 6; at a 1:2 ratio) at metal-to-ligand ratios of 1:1 (Fig. 7A) and 1:10 (Fig. 7B). Concentrations of the two minor, potentially disputed, [MLH] and [$ML_2(OH)$] species are amplified by at least two-fold in the 1:10 simulation. These [MLH] and [$ML_2(OH)$] species are only present under very acidic or basic conditions, respectively, minimizing their impact in biological systems.

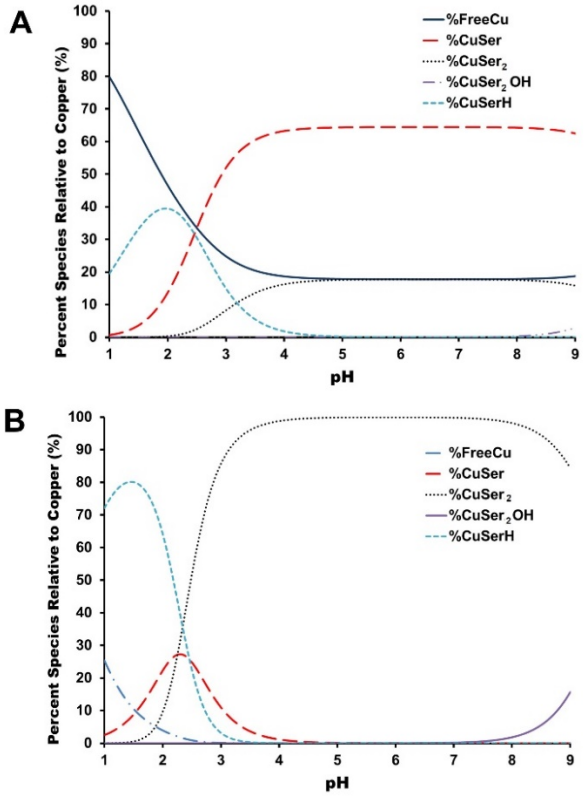

Fig. 7 A) Modeled speciation diagrams for the Cu(II)-Ser four-species system from Fig. 6 A) at a 1:1 Cu(II):Ser ratio and B) at a 1:10 Cu(II):Ser ratio.

To emphasize the inconsistency in identifying minor species, one can consider the example of Cu(II)-threonine titrations. From the wide range of data available, the primary [ML] and [ML_2] species are confirmed, and no [MLH] species is reported. Multiple studies identify the minor dihydroxide species, [$ML_2(OH)_2$] [88, 140, 146] but the presence and contribution of this hydroxide species is disputed given the conflicting data and lack of independent characterization.

Despite their uncertainties, the Cu(II)-Ser and Cu(II)-Thr systems are straightforward compared to Cu(II)-His titration modeling, where anywhere from four to thirteen species are identified (Table 2) [137, 154-156]. Understanding histidine-copper binding is of primary importance, since it is the most common amino acid in the binding pockets of copper metalloproteins and is the predominant non-protein-bound copper complex in blood plasma [69, 157, 158]. Reports of so many species, including dinuclear complexes, is indicative of inherent variability in His-Cu(II) coordination. The major species at pH 6-8 are [$Cu(His)_2H]^+$ and [$Cu(His)_2$]; however, minor species, such as [$Cu(His)]^+$, are present that could influence cellular speciation [82]. Kamyabi and coworkers [159] provided independent confirmation of [$Cu(His)]^+$, [Cu(His)H], [$Cu(His)_2$], [$Cu(His)_2H]^+$, and [Cu(His)(OH)] complexes using spectroscopic methods. The complexity and difficulties of determining Cu(II)-histidine speciation highlight core issues for stability constant determination. Even when the metal-ligand interaction is well-behaved and a variety of methods are available for analysis, confirmation of relevant species must be achieved for the data to be useful in large-scale modeling projects.

7. Stability Constants of Non-Sulfur and -Selenium-Containing Amino Acids with Cu(I)

Cu(I) is the least studied and the most poorly understood of the common copper and iron oxidation states in biological systems. In humans, cellular copper intake is tightly controlled

through the membrane transport protein hCTR1 [160-162], which has methionine-, cysteine-, and histidine-rich amino acid sequences in the Cu(I) binding site [13, 163]. Although hCTR1 and other copper transport proteins preferentially bind Cu(I) over Cu(II), Cu(I) stability constants are vastly under-examined due to the difficulties of working with this ion.

Cu(I) is highly unstable in aqueous systems and disproportionates to Cu(II) and Cu(0) in the presence of dioxygen. Cu(I) is also spectrophotometrically inactive, limiting spectrophotometric titrations to ligands that have absorbances in the UV or visible spectrum. In addition, the most commonly used Cu(I) salt, CuCl, is only sparingly soluble in aqueous systems, narrowly defining the parameters for which potentiometric methods can be utilized. Sharma and coworkers [164] used potentiometric methods to determine that Cu(I) is stabilized in aqueous systems with sufficient Cl^- support (1.0 M). Using potentiometric methods, they identified three species: CuCl, $[CuCl_2]^-$, and $[CuCl_3]^{2-}$, with step-wise stability constants of 2.68, 5.07, and 4.78, respectively. Given these difficulties with Cu(I) instability and solubility, reliable data for Cu(I) stability constants with amino acids lags far behind that of Cu(II) despite its biological importance.

Due to the significant limitations of potentiometric methods with Cu(I), stability constants have been primarily determined using redox methods. Since Cu(I) is unstable in aqueous solution, redox methods are preferred because metal oxidation state is controlled at the electrode surface. This method is dependent on predicting the potential at which half of the concentration is Cu(I) and half is Cu(II), and activity due to ionic strength is sometimes ignored in the calculations. Stability constants for Cu(I) with only three non-sulfur or -selenium amino acids are reported; Cu(I) stability constants with sulfur- and selenium-containing amino acids are discussed separately in the *Stability Constants of Sulfur- and Selenium-containing Amino Acids*

with Copper section.

Using redox analyses, stability constants of 9.6, 10.0, and 10.4 were found for [Cu^{I}(Ala)], [Cu^{I}(Gly)], and [Cu^{I}(His)], respectively [165]. Since alanine and glycine have non-coordinating side chains, and stability constants for all three complexes are very similar, these data suggest that all three amino acids are binding in bidentate fashion to Cu(I). It is surprising that His would show such weak Cu(I) binding, considering the role that histidine plays in stabilizing copper in metalloproteins [166]. The only other Cu(I)-His determination identifies formation of [Cu(HHis)] and $[Cu(HHis)_2]^-$ species with stability constants of 12.80 and 25.20, respectively (Table 3) [151], where "HHis" indicates protonation of the amine or imidazole nitrogen atom, implying only bidentate binding. These Cu(I)-His results seem contradictory, not only because the identified species are not the same, but because the Cu(I) species with a potentially tridentate-binding His ligand has a significantly lower stability constant than the Cu(I) species with only a bidentate-binding His ligand. Considering the importance of Cu(I) in biological systems, the fact that methods and stability constant data for Cu(I)-amino-acid complexes are not reliable enough to compare with similar Cu(II) data highlights the extreme difficulties inherent in studying this ion. To add these difficulties, even if reliable titration methods are identified, the propensity of Cu(I) to form multinuclear species [167-169] will provide an additional challenge for these measurements.

8. Stability Constants of Sulfur- and Selenium-Containing Amino Acids with Copper

Similar to histidine, sulfur-containing amino acids have been credited for the stability and redox activity of a wide variety of copper metalloproteins. Both methionine and cysteine are recognized for the structural and electronic stability that they contribute to blue copper proteins

[170, 171]. Thiols have such a high stability with copper that the drug penicillamine is administered as a copper chelator for treatment of Wilson's disease [23, 24]. Selenium compounds, such as selenocysteine, are crucial to the function of selenoproteins [172-174]. Selenocysteine coordinates nickel in NiFeSe hydrogenases [78], and selenomethionine is non-specifically incorporated into proteins in place of methionine [175, 176]. In addition, many sulfur and selenium species have been identified and extensively studied as antioxidants in *in vitro*, cellular, *in vivo*, and epidemiological studies [171, 177-181], in part due to their copper-binding properties. Selenium-containing supplements have been the subject of human studies for their potential as antioxidants, although results are limited and conflicting [80, 182-185].

All of the sulfur- and selenium-containing amino acids have the potential for tridentate binding through the carboxylate oxygen, the amine nitrogen, and the S/Se atom in the side chain. Table 4 summarizes the available data for stability constants of Cu(II) and Cu(I) with these amino acids; unsurprisingly, data for Cu(II) are much more complete than for Cu(I).

Table 4
Stability constants of Cu(II) and Cu(I) with sulfur- and selenium-containing amino acids

Cu(II) Stability Constants							
Ligand	**[ML] ($\log \beta_{ML}$)[a]**	**[ML_2] ($\log \beta_{ML2}$)[b]**	**Other Species**	**Temp (°C)**	**Ionic Strength (M)**	**Method**	**Ref.**
Homocysteine	11.92(1)	13.54(2)	7.57(1) (ML(OH))	25	0.1 KNO_3	Potentiometry	[186]
Methionine	7.85(2)	14.52(1)		25	0.1 KNO_3	Potentiometry	[88, 187, 188]
Methylcysteine	7.65[c]	14.13[c]		25	0.2 KCl	Potentiometry	[189]
Methylselenocysteine	8.2(1)	14.5(2)		25	0.1 $NaClO_4$	Potentiometry	[190]
Penicillamine	16.5[c]	21.7[c]		25	0.15 KNO_3	Potentiometry	[191]
Selenomethionine	7.77[c]	14.50[c]		25	0.1 $NaNO_3$	Potentiometry	[192]
Cu(I) Stability Constants							
Cysteine	10.164(6)	18.36(1)	20.34(2) (ML_3)	25	1.0 NaCl	Potentiometry	[11]
Methionine	9.1[c]			20	0.1 $NaClO_4$	Potentiometry	[163, 193]
Penicillamine	12.41(5)		18.72(1) (MLH) 22.29(2) (M_2LH) 34.44(1) (M_2L_2H)	25	1.0 NaCl	Potentiometry	[11]

[a] $\log \beta_{ML} = [ML]/[M][L]$ [b] $\log \beta_{ML2} = [ML_2]/[M][L]^2$ [c] Error not reported

8.1 Cu(II) Complexes of Sulfur- and Selenium-Containing Amino Acids

Thiol-containing cysteine, homocysteine, and penicillamine are redox-active in the presence of Cu(II), forming the respective disulfides and reducing Cu(II) to Cu(I) [194, 195]. This redox activity impacts the validity of stability constant determinations with these amino acids. Although Cu(II)-Cys stability constants have been errantly reported [12], previous reviewers [71] have explained the misidentification of species present in these analyses, and Pinto [186] suggested that these complexes are stable at ligand:metal ratios below one. The potential for redox reactions casts a shadow over the reliability of Cu(II)-thiol stability constant data and emphasizes the need for proof of speciation in these systems.

Penicillamine has the highest Cu(II) stability constants of the amino acids in Table 4, forming [Cu(Pen)] (log β = 16.5) and $[Cu(Pen)_2]^{2-}$ (log β_2 = 21.7) species [191]. The high affinity of penicillamine for Cu(II) is not surprising, since a primary use of penicillamine is as a copper chelator. Of the other sulfur and selenium amino acids examined, the thiol-containing homocysteine has a higher [ML] species stability constant (11.92(1)) [186] than the others (~7.8; Table 4), but the $[ML_2]$ species is slightly less stable at 13.54(2) than the $[ML_2]$ species of methionine, methylcysteine, and selenomethionine. Pinto [186] suggested that the amine and the soft thiolate of homocysteine binds borderline Cu(II) in the [ML] species, either in addition to the hard carboxylate oxygen or in place of it. This is reasonable, since EPR analysis of $[Cu(hCys)_2]^{2-}$ indicates tetrahedral geometry around Cu(II), with the thiolate sulfur replacing carboxylate oxygen binding. The [Cu(HhCys)] species has a significantly higher stability constant than the thio- or selenoethers, potentially indicating stability afforded by tridentate binding. When sterically hindered by a second ligand coordinating in the $[ML_2]$ species, the carboxylate oxygen coordination may be lost, and the two ligands likely coordinate in a bidentate

fashion through the amine nitrogen and thiolate sulfur atoms.

The presence of a thioether or selenoether group does not contribute to thermodynamic stability of Cu(II) complexes, since stability constants for Cu(II) with methionine, methylcysteine, and selenomethionine are similar to those of the bidentate-coordinating Cu(II)-amino-acid systems (Table 2). Solid-state structures also show no thioether or selenoether coordination for [ML_2] complexes of Cu(II), including [$Cu(Met)_2$] [196, 197], [$Cu(SeMet)_2$] [190], and [$Cu(MeCys)_2$] [198], although tridentate binding to Co(III) [199, 200] and softer metal ions such as rhenium [201] and ruthenium [202, 203] is observed.

8.2 Cu(I) Complexes of Sulfur- and Selenium-Containing Amino Acids

Stability constants for Cu(I) with sulfur and selenium amino acids are limited to two thiolates (Cys and Pen), only one thioether (Met), and no selenium-containing species (Table 4). This paucity of data makes evaluation difficult, as does the fact that the Cu(I)-Met results [193] have not been replicated in sixty years, and no other Cu(I) stability constants have been reported under these conditions. If these results are valid, only the [$Cu^{I}(Met)$] species has a higher stability constant (log $\beta = 9.1$) than the analogous Cu(II) species, $[Cu^{II}(Met)]^{+}$ (log $\beta = 7.85(1)$) [88]. The higher stability of the Cu(I) complex may suggest tridentate binding, or at least a different binding mode than the glycine-like coordination of the $[Cu^{II}(Met)]^{+}$ species. No X-ray diffraction structures are reported for $Cu^{I}(Met)$, but X-ray absorption spectroscopy studies supported by NMR results in aqueous solution indicate Cu(I) coordination through the thioether sulfur and the amine nitrogen atoms in a bidentate fashion [204].

Cu(I)-Cys is one of the most thoroughly investigated Cu(I) systems, with stability constant determination attempted using at least four different methods with vastly different

results. Using polarographic methods, only the [ML] species was identified with a stability constant of 19.19 [205]. By spectrophotometry, the stability constant for the same [ML] species was reported as 11.38 [206]. In a review, Berthon [71] highlighted the inconsistencies between these two reports, attributing the difference to interference by NH_3 or redox issues in the polarographic determinations. Walsh and Abner utilized fluorescent probes to determine stability constants of 23.0 for the [ML and 38.4 for the [ML_2] species, significantly different values compared to previously reported data [207]. More recently, Konigsberger [11] attempted to determine Cu(I)-Cys stability constants in a ternary system using potentiometric analyses with penicillamine. Although penicillamine addition may expand the solubility range of the system beyond pH 5.2, it adds multiple species into an already complicated model. Four different species were reported to form throughout the full pH range, including species with multiple cysteine protonation states and three dinuclear species. Polynuclear copper-thiolate complexes are well known, but suggesting the formation of multiple dinuclear species based on model fit alone is insufficient for this system. Adding to this complexity, kinetics analyses suggest a dinuclear, mixed-valent Cu(II/I)-Cys complex also may form as an intermediate between the [ML] and [ML_2] species [208].

Similar to Cys coordination, the Cu(I)-penicillamine system has also been extensively studied [11, 208-210] with no agreement on either the stability constants or species present (Table 5). Again, the tendency of Cu(I) to form dinuclear and polynuclear complexes significantly complicates species determination. Most notably, Persson and coworkers [209] determined stability constants of 39.18 and 101.5 for the $[Cu(HPen)_2]^-$ and $[Cu_5Pen_4]^{3-}$ species, respectively, high values that indicate penicillamine strongly stabilizes Cu(I).

To explore differences in speciation between Cu(I) and Cu(II) with penicillamine, a

Table 5
Speciation and stability constants for Cu(I) with penicillamine (Pen)

Species	log β	Ionic Strength	Temp(˚C)	Method	Ref.
[Cu(Pen)]	10.470(6)	1 M NaCl	25	Potentiometry	[11]
[Cu(HPen)]	18.46(1)				
$[Cu_2(HPen)]$	20.48(1)				
[Cu(Pen)]	12.25(2)	1 M NaCl	25	Potentiometry	[210]
[Cu(HPen)]	18.34(1)				
$[Cu(Pen)_2]$	15.44(3)				
$[Cu_4(Pen)_3]$	49.15(7)				
$[Cu(HPen)_2]$	39.18	0.5 M $NaClO_4$	25	Potentiometry	[209]
$[Cu_5(Pen)_4]$	101.5				

model of the speciation of copper (100 μM) with penicillamine (1000 mM) at 100 mV was calculated with Geochemist's Workbench [211] using the stability constants and species reported by Persson [209], including the protonation constants of penicillamine. The database parameters for this model that were added to Visual MINTEQ are listed in Table S1. Below pH 5, Cu(I) hydrolysis species predominate (Fig. 8A). As the pH increases and the thiolate of penicillamine deprotonates, formation of Cu(II)- and Cu(I)-Pen species increases, but the Cu(II) species are the more prevalent species, by a factor of 100. As the electrochemical potential decreases, Cu(I)-Pen species are stabilized (Fig. 8B).

Copper binding to selenium-containing amino acids is vastly understudied compared to their sulfur analogs. Data for the Cu(II)-SeMet system are reported, with stability constants of 7.77 and 14.50 for the ML and ML_2 species, respectively (Table 4). Cu(II)-MeSeCys stability constants have been recently determined [190] and are consistent with the other thio- and selenoether amino acids with stability constants of 8.2(1) and 14.5(2) for the [ML] and $[ML_2]$ species, respectively. Because these stability constants are similar to those of Cu(II)-Met systems and for the bidentate-binding amino acids (Table 2), Cu(II) likely binds selenomethionine and methylselenocysteine in a glycine-like manner. No stability constant data could be found for

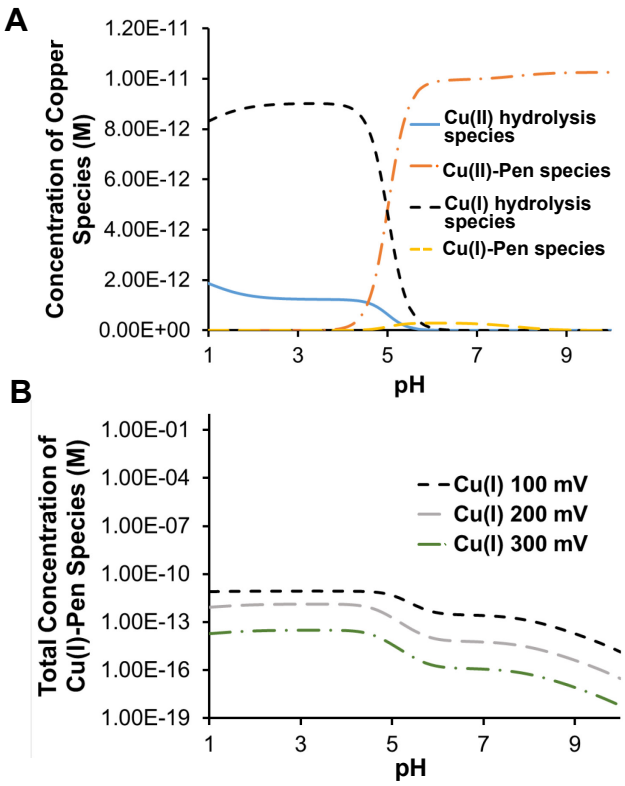

Fig. 8 A) Speciation comparison between Cu(II) and Cu(I) complexes of penicillamine at a 10:1 ligand-to-metal ratio and 100 mV, showing the favorable stability of Cu(II) over Cu(I) complexes. B) As the electrochemical potential decreases, Cu(I) complexes increase in stability.

other selenoamino acids such as selenocysteine. Selenocysteine is highly redox sensitive [212], greatly complicating these measurements, but determining its stability constants with copper would significantly contribute to the greater body of knowledge regarding biological selenium-metal interactions.

8.3 Challenges in Determining Copper Stability Constants with Sulfur- and Selenium-Containing Amino Acids

For the vast majority of Cu(II)-amino acid complexes, potentiometric analyses indicate formation of [ML] and [ML_2] species with stability constants of approximately 8 and 14, respectively (Tables 2, 3, and 4). Only histidine, glutamate, aspartate, and penicillamine stability

constants are high enough to suggest tridentate or partially tridentate Cu(II) coordination in the [ML] species. In general, stability of glycine-like amino acid binding to Cu(II) is greater than to Cu(I), although increased stability constants suggest that thiols, thioethers, and selenoethers coordinate Cu(I) through the sulfur or selenium, either in addition to or in place of the carboxylate oxygen. The difficulties in controlling the redox chemistry of Cu(I) with thiols and selenols has discouraged researchers from pursuing the determination of these stability constants with Cu(I). For both Cu(I) and Cu(II), formation of a variety of multinuclear species with sulfur and selenium amino acids also significantly hinders stability constant analysis and interpretation.

9. Stability Constants of Non-Sulfur and -Selenium-Containing Amino Acids with Fe(II)

Labile Fe(II) pools contribute to reactive oxygen species formation and cellular oxidative stress [213], and iron interactions with low-molecular-weight species such as amino acids may alter this behavior. Although not as robust as Cu(II) due to its tendency to form Fe(III) in the presence of oxygen, Fe(II) is fairly well-behaved in closed reaction vessels or under an inert atmosphere of nitrogen or argon. Hydrolysis constants of Fe(II) ($[Fe(OH)]^+$ $\log \beta = -9.5$ and $[Fe(OH)_2]$ $\log \beta = -20.5$ [214]) are low enough to be a factor only at high pH and/or high metal-to-ligand ratios. Since Fe(II) is spectrophotometrically inactive, similar to Cu(I), most stability constant measurements with this ion are performed using potentiometric methods.

Stability constants have been determined for most amino acids with Fe(II); however, a majority of these data are individual analyses, making accuracy evaluation difficult. For some amino acids, only one stability constant for either the [ML] or $[ML_2]$ species is reported, with little analysis or attempts to identify minor species. Since all the Fe(II)-amino-acid stability constants are determined using potentiometry, comparisons with other methods are not possible.

9.1 Fe(II) Complexes of Potentially Bidentate Amino Acids

Similar to Cu(II), Fe(II) stability constants with bidentate-coordinating amino acids all fall within one log unit of each other (3.39 to 4.13 for the [ML] species, and 7.1 to 8.3 for the [ML_2] species; Table 6). Glycine, with the relatively low log β values of 4.13 and 7.65 for the [ML] and [ML_2] species, respectively, forms the most stable Fe(II)-amino acid species [92]. Proof of speciation and details about coordination environment are scarce for these potentially bidentate Fe(II)-amino-acid complexes, since [$Fe(Pro)_2$(phenanthroline)] (Fig. 9) is the only reported Fe(II) structure with any single amino acid ligand [215]. In this complex, Fe(II) is coordinated in distorted octahedral geometry, with both bidentate Pro ligands coordinating through the amine nitrogen and carboxylate oxygen atoms. Given the similarity of [ML_2] stability constants for Fe(II) binding to all the potentially bidentate amino acids, it is reasonable to assume similar amine and carboxylate coordination for all the amino acids in Table 6.

Table 6
Stability constants of Fe(II) and Fe(III) with potentially bidentate amino acids

Stability Constants of Fe(II)							
Ligand	**[ML] (log β)[a]**	**[ML_2] (log β_2)[b]**	**[ML_3] (log β_3)[c]**	**Temp (°C)**	**Ionic Strength (M)**	**Method**	**Ref.**
Alanine	3.54[d]			20	1.0 KCl	Potentiometry	[217]
Glycine	4.13[d]	7.65[d]		25	0.1 KNO_3	Potentiometry	[92]
Leucine	3.42[d]			20	1.0 KCl	Potentiometry	[217]
Phenylalanine	3.74(1)	7.19(3)	10.7(2)	25	3.0 $NaClO_4$	Potentiometry	[216]
Proline		8.3[d]		20	0.01[e]	Potentiometry	[218]
Tryptophan	3.92[d]	7.39[d]	~9.5[d]	25	3.0 $NaClO_4$	Potentiometry	[216]
Valine	3.39[d]			20	1.0 KCl	Potentiometry	[217]
Stability Constants of Fe(III)							
Alanine	10.98[d]			30	1.0 KCl	Polarography	[219]
Glycine	10[d]			25	1.0 $NaClO_4$	Redox	[193]
Leucine	9.9[d]			20	1.0 $NaClO_4$	Redox	[193]
Phenylalanine	10.39(4)	19.1(1)	26.0(7)	25	3.0 $NaClO_4$	Potentiometry[f]	[216]
Proline	10.0(3)			20	1.0 $NaClO_4$	Potentiometry	
Tryptophan	9.0[d]			20	1.0 $NaClO_4$	Redox	[193]
Valine	9.6[d]			20	1.0 $NaClO_4$	Redox	[193]

[a] $\log \beta = [ML]/[M][L]$ [b] $\log \beta_2 = [ML_2]/[M][L]^2$ [c] $\log \beta_3 = [ML_3]/[M][L]^3$ [d] No error reported by author. [e] The identity of the electrolyte was not reported; titrations were run at approximately 0.01 M ligand. [f] Data also supported by calorimetry.

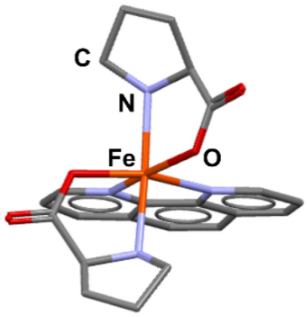

Fig. 9 Solid-state structure of [Fe(Pro)$_2$(phenanthroline)] [215], showing bidentate proline coordination through the carboxylate oxygen and the amine nitrogen atoms. The Fe(II) ion is shown in orange, oxygen atoms are red, carbon atoms are grey, and nitrogen atoms are blue. Hydrogen atoms are omitted for clarity.

A single study identifies the [FeL$_3$] species for phenylalanine and tryptophan with log β values of 10.7(2) and ~9.5, respectively [216]. Fe(II) binding to a third amino acid must out-compete formation of the [Fe(OH)]$^+$ and [Fe(OH)$_2$] species, the latter of which has limited solubility. If all three ligands of the [ML$_3$] species bind in a bidentate fashion, they are almost certainly arranged in octahedral geometry around the Fe(II) center. Both Phe and Trp have aromatic side chains that would result in considerable steric encumbrance to the complex. The predictable stepwise formation constants (log K_1 = 3.74, log K_2 = 3.45, and log K_3 = 3.5) suggests there is no enthalpic penalty due to increasing coordination, and the reported potentiometric results are supported by calorimetry measurements. In all cases, the stability constants for the [FeL]$^+$ and [FeL$_2$] species are weak and indicate that high ligand-to-metal concentrations are required for complex formation.

9.2 Fe(II) Complexes of Potentially Tridentate Amino Acids

Similar to the bidentate amino acids, stability constant data for potentially tridentate amino acid binding to Fe(II) are incomplete. Most of the stability constants for [ML] and [ML$_2$] complexes of these amino acids (Table 7) are similar to stability constants for Fe(II) with the bidentate amino acids (Table 6), consistent with glycine-like binding to Fe(II) without significant

Table 7
Stability constants of Fe(II) and Fe(III) with potentially tridentate amino acids

Ligand	[ML] (log β_{ML})[a]	[ML_2] (log β_{ML2})[b]	[ML_3] (log β_{ML3})[c]	Temp (°C)	Ionic Strength (M)	Method	Ref.
Stability Constants of Fe(II)							
Arginine	3.20[d]			20	0.01[e]	Potentiometry	[132]
Asparagine	4.37(3)	7.57(3)	10.26(5)	25	3.0 $NaClO_4$	Potentiometry	[220]
Aspartic acid	5.34[d]	8.57[d]		25	0.1[e]	Potentiometry	[221]
Glutamic acid	3.50[d]			20	1.0 KCl	Potentiometry	[193]
Histidine	5.88[d]	10.43[d]		25	3.0 $NaClO_4$	Potentiometry	[222]
Lysine	4.5[d]			20	0.01[e]	Potentiometry	[132]
Serine	4.299[d]	7.377[d]	10.299[d]	20	3.0 $NaClO_4$	Potentiometry	[223]
Threonine	3.69[d]	6.50[d]		40	0.2 KNO_3	Potentiometry	[224]
Tyrosine		7.1[d]		20	0.01[e]	Potentiometry	[218]
Stability Constants of Fe(III)							
Arginine	8.7(3)			20	1.0 $NaClO_4$	Potentiometry	[193]
Asparagine	8.6(1)			20	1.0 $NaClO_4$	Potentiometry	[193]
Aspartic acid	11.4(3)			20	1.0 $NaClO_4$	Potentiometry	[193]
Glutamic acid	13.39[d]			20	1.0 $NaClO_4$	Potentiometry	[225]
Histidine	4.7(4)			20	1.0 $NaClO_4$	Potentiometry	[193]
Serine	9.2(4)			20	1.0 $NaClO_4$	Potentiometry	[193]
Threonine	8.6(3)			20	1.0 $NaClO_4$	Potentiometry	[193]

[a] $\log \beta = [ML]/[M][L]$ [b] $\log \beta_2 = [ML_2]/[M][L]^2$ [c] $\log \beta_3 = [ML_3]/[M][L]^3$ [d] No error reported by author. [e] No supporting electrolyte was reported; titrations were run at approximately 0.01 M ligand.

stability contributed by the polar side chain. Many of the reported constants have not been replicated or independently confirmed by other methods.

Histidine and aspartic acid have somewhat higher stability constants (5.88 and 5.34 for the [ML] species and 10.43 and 8.57 for the [ML_2] species, respectively; Table 7) than the majority of the

other potentially tridentate amino acids, suggesting possible tridentate binding or Fe(II) stabilization through bridging ligands. [ML_3] stability constants are determined for asparagine [220] and serine [223], further supporting bidentate coordination of these amino acids, at a maximum. It is surprising that minor species have not been identified in the low pH range in these Fe(II)-amino acid systems, but the stability of iron hydrolysis species above pH 7 outcompetes weakly binding amino acid ligands. Fe(II) stability constants with the majority of the potentially bidentate and tridentate amino acids are fairly consistent: [ML] stability constants are approximately 3-4; [ML_2] stability constants are approximately 7; and the few [ML_3] stability

constants reported are approximately 10. Extrapolating from scarce structural and supporting speciation data, it is likely that most amino acids coordinate Fe(II) in a bidentate fashion through the carboxylate oxygen and amine nitrogen atoms. Aspartic acid and histidine have somewhat higher stability constants for the [ML] (5.88 and 5.32, respectively) and [ML_2] (10.43 and 8.57, respectively) species (Tables 6 and 7), suggesting that the His and Asp side chains participate significantly in coordination.

9.3 Challenges in Determining Fe(II)-Amino Acid Stability Constants

Although stability constants of divalent metals with amino acids have been the focus of a few comprehensive studies [132, 217, 218, 224], most Fe(II) amino acid stability constants are limited to these few studies with little speciation analysis. The majority of the published data were obtained using potentiometric titrations. Although Fe(II) salts are water soluble, Fe(II) oxidizes to Fe(III) in air, so oxygen-free conditions must be employed. This air sensitivity limits analysis techniques to methods that can be performed in a glove box or in closed cells. Fe(II) also forms hydrolysis compounds above pH 7. Although these complexes are not as stable as Fe(III) hydrolysis products, they do compete with amino acids for metal binding in the upper pH range. Fe(II) is also spectrochemically inactive, like Cu(I), and therefore not an option for spectrophotometric techniques with non-UV-active amino acids. As a whole, these issues have limited the data availability for Fe(II) with amino acids.

9.4 Comparison of Cu(II) and Fe(II) Stability Constants of Non-Sulfur and-Selenium-Containing Amino Acids

Cu(II) and Fe(II) have the same valency and are both considered borderline Lewis acids; both also have the potential to coordinate ligands in octahedral geometry. However, stability

constants of Cu(II) and Fe(II) with amino acids are significantly different, with Cu(II)-amino acid complexes significantly more stable than analogous Fe(II)-complexes. With ML constants of approximately 9 for $[CuL]^+$ species (Table 3) and between 3 and 4 for most of the $[FeL]^+$ complexes (Table 7), all of the non-sulfur- and non-selenium-containing amino acids show a higher affinity for the Cu(II) ion. A similar comparison can be made for $[CuL_2]$ and $[FeL_2]$ species with stability constants of ~14 and 7-8, respectively. With Cu(II), only His ([ML] log β = 9.75(1), $[ML_2]$ log β = 17.49(1)) Asp ([ML] log β = 8.83, $[ML_2]$ log β =15.93(2)), and Glu ([ML] log β = 8.30(4), $[ML_2]$ log β = 15.03(3)) have large enough stability constants to suggest the potential for tridentate coordination. With Fe(II), stability constants with His ([ML] log β = 5.88, $[ML_2]$ log β = 10.43) and Asp ([ML] log β = 5.34, $[ML_2]$ log β = 8.57) are somewhat elevated compared to bidentate-binding amino acids but are still considerably lower than stability constants with Cu(II).

Although solid-state structural data support tridentate His coordination in $[Cu(His)_2]$ (Fig. 5), no comparable Fe(II) structures exist to show tridentate amino acid coordination. His and Asp may be tridentate ligands binding Fe(II) through the amine, carboxylate, and side-chain N or O atoms or, alternatively, adopt bidentate coordination through the N or O atom of the side chain and either the amine nitrogen or carboxylate oxygen atom. Regardless of coordination mode, the stability of Fe(II) with non-sulfur- or selenium amino acids is significantly weaker than Cu(II) and therefore less biologically significant.

10. Stability Constants of Non-Sulfur and –Selenium-Containing Amino Acids with Fe(III)

Most Fe(III) in the cell is sequestered in ferritin storage as ferrihydrite [226], although Fe(III) also exists in the mitochondria [226]. Fe(III) does not generate hydroxyl radical as does

Fe(II), and it is poorly soluble and therefore not readily available in the aqueous environment of a cell. Poor Fe(III) solubility, due to the stability of the hydrolysis species, also contributes to a deficit of Fe(III) stability constants with amino acids, since it restricts the use of potentiometric titrations to a very narrow pH range. Accordingly, the Fe(III) stability constants reported in Table 7 were measured below pH 5 [193].

10.1 Fe(III) Complexes of Potentially Bidentate and Tridentate Amino Acids

For most amino acids, regardless of potential denticity, Fe(III) stability constants for only the [ML] species have been quantified (Table 7) with the exception of a single study by Williams [223]. Many of these constants were determined using redox measurements in one 1958 report by Perrin [193], and the lack of precision inherent to the redox method is reflected in the reported values.

Stability constants for the [ML] species of Fe(III) and a majority of the amino acids are consistently in the 8-10 range (Tables 6 and 7). Notable exceptions to this trend are glutamic acid, with a higher [ML] stability constant of 13.39, and histidine, with a lower [ML] stability constant of 4.7(4), respectively. The considerably higher Fe(III)-Glu stability constant was determined under different experimental conditions [225] compared to most of the other amino acids, but these experimental differences would not explain such a significant disparity. The considerably lower stability constant for the $[Fe^{III}(His)]^{+}$ seems to indicate that His coordination does not greatly stabilize Fe(III). In contrast, the only solid-state structure of Fe(III) bound to an amino acid incorporates histidine in a tridentate coordination mode: the oxo-bridged, dinuclear complex, $[Fe_2(His)_2(bipy)_2(\mu\text{-}O)]$ (bipy = 2,2'-bipyridyl; Fig. 10) [227]. Although this complex was not crystallized out of aqueous solution, it indicates that histidine is certainly capable of

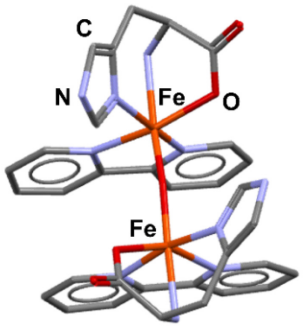

Fig. 10 Solid-state structure of $[Fe_2(His)_2(bipy)_2(\mu\text{-}O)]$, showing tridentate coordination of histidine through a nitrogen atom of the imidazole side chain as well as the carboxylate oxygen and amine nitrogen atoms [227]. The Fe(III) ion is shown in orange, oxygen atoms are red, carbon atoms are grey, and nitrogen atoms are blue. Hydrogens are omitted for clarity.

tridentate coordination to Fe(III).

10.2 Determining Fe(III) Stability Constants Using the Solubility Method

One method that has not been discussed thus far but has been used in environmental chemistry for determining stability constants of ions with sparingly soluble hydrolysis products is the solubility method. Complexes of neptunium(V) and plutonium(IV) are of environmental concern, and the solubility method has proven to be a technique to allow control of redox state yet span a wide range of pH and/or temperature [228-230]. With this technique, insoluble metal ions are slowly dissolved by complex formation with an aqueous-phase ligand. Concentration of the soluble complex can then be determined through methods such as inductively-coupled-plasma mass spectrometry or scintillation techniques with radioisotopes. By varying the ratio of ligand to metal, a continuous plot can be derived to track mass transfer from solid state to aqueous solution. Due to the extreme insolubility and stability of Fe(III) hydrolysis products, the solubility method is an optimal tool for stability constant determination in this system. In this method, competition for Fe(III) is measured through addition of increasing concentration of ligand to a suspension of $[Fe(OH)_3]$. The amount of complex is then determined by measurement

of pH and Fe(III) in solution. Limitations of this method include the difficulty in characterizing both the liquid and solid species formed as the pH changes [231, 232]. The solubility method is by no means the easiest or the fastest method for determining stability constants, but it may overcome the difficulties inherent in using most other methods for measuring Fe(III) stability constants due to the highly insoluble Fe(III) hydrolysis species.

A model Fe(III) solubility experiment was calculated using Geochemist's Workbench using low, moderate, and high amino acid stability constant values with Fe(III) as exemplified by Met, Glu, and Phe [193, 216, 225] (database parameters that were added to Visual MINTEQ can be seen in Table S1). In this model, amino acid concentrations are increased until the Fe(III)-amino-acid species out-compete the insoluble iron-hydrolysis species. In Fig. 11A, a complex forming with a stability constant of 9.1, the same as for $[Fe(Met)]^{2+}$ and comparable to most of the other [ML] species reported by Perrin [193, 217] has very little ability to dissolve the solid ferric hydrolysis species to form $[Fe(Met)]^{2+}$ in aqueous solution. Increasing the stability constant by four log units to 13.39, as reported for $[Fe(Glu)]^{+}$ [225] significantly increases the amount of Fe(III) dissolved in solution (Fig. 11B).

Utilizing the only multi-species data reported for Fe(III) complexes with non-sulfur or selenoamino acids, $[Fe(Phe)]^{2+}$, $[Fe(Phe)_2]^{+}$, and $[Fe(Phe)_3]$ species with stability constants of 10.39(4), 19.1(1), and 26.0(7), respectively [216], results in a significantly higher amount of dissolved Fe(III) (Fig. 11C) compared to that in the Fe(III)-Met and Fe(III)-Glu systems (Fig. 11A and 11B). It is entirely possible that multiple species form in all of the Fe(III)-amino acid systems, but identification of these species may be hindered by high-pH precipitation of iron hydrolysis species in the potentiometric and redox titrations. Solubility titrations could provide insight into formation of additional species in these systems, although this method is limited by

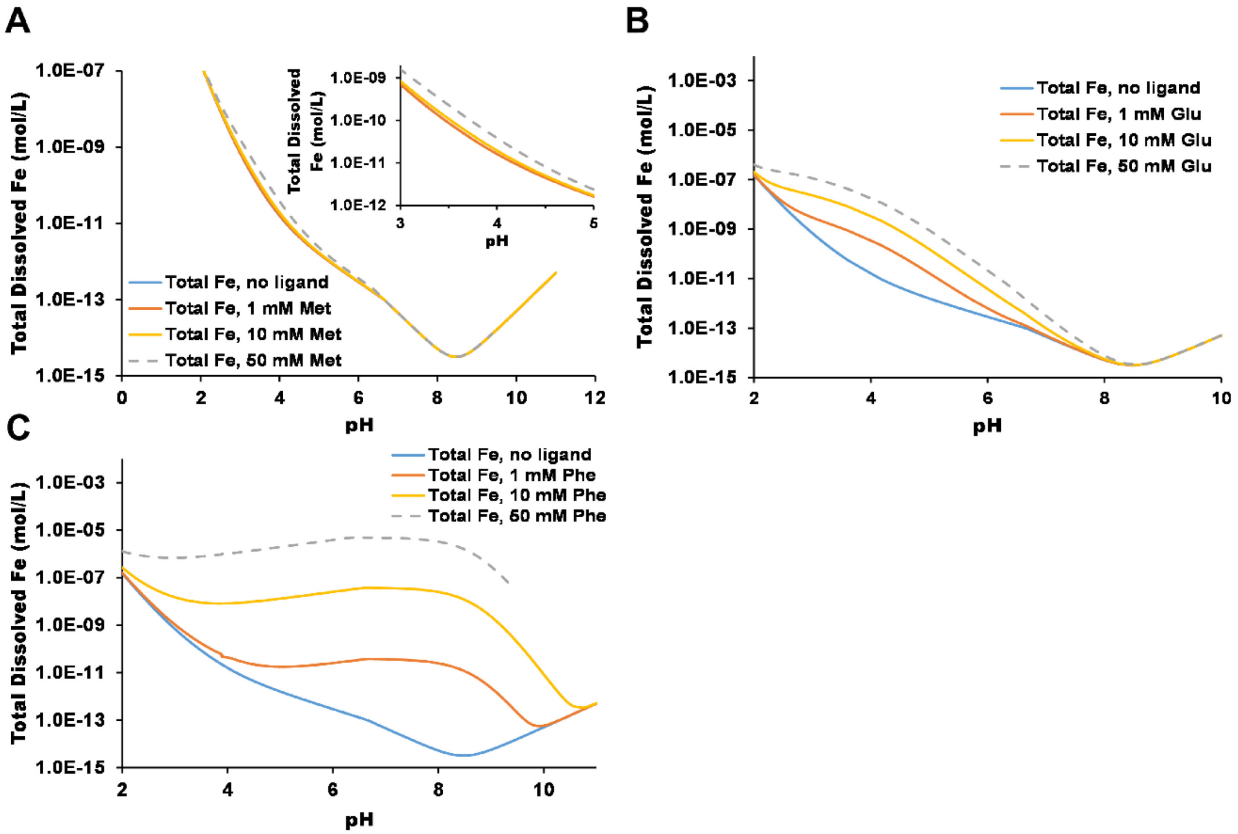

Fig. 11 Modeled solubility method data for Fe(III) complexes with A) methionine ([ML] log β = 9.1) [217], B) glutamate ([ML] log β = 13.39) [233], and C) phenylalanine ([ML] log β = 10.39, [ML_2] log β = 19.11, and [ML_3] log β = 26) [216]. Comparing graphs A and B shows the effect a change in log β by 4 log units has on ferrihydrite solubility. Comparing graphs B and C shows the significant effects of higher stability constants and multiple species on aqueous Fe(III) solubility.

the aqueous solubility of the resulting Fe(III)-amino acid complexes. It is reasonable to expect, however, that even low-solubility complexes would remain in solution at the extremely low total iron concentrations found in these modeled systems (pM to nM range). Thus, solubility titrations represent a viable but almost unexplored method for Fe(III) stability constant determination.

11. Stability Constants of Sulfur- and Selenium-Containing Amino Acids with Iron

Sulfur-containing metalloproteins such as rubredoxins, ferredoxins, and hemerythrin play a crucial role in electron transfer through iron-sulfur interactions [166]. Despite their biological importance, Fe(II) and Fe(III) stability constant determinations with sulfur- and

selenium-containing amino acids are so limited that it is difficult to assess the accuracy of the experimental results or to identify trends.

11.1 Fe(II) Complexes of Sulfur- and Selenium-Containing Amino Acids

In contrast to Cu(II), stability constants of thioether- and selenoether-containing amino acids with Fe(II) are low: 3.2 to 3.9 for the $[FeL]^+$ species (Table 8). These constants are consistent with those of Fe(II)-amino-acid species with potentially bidentate amino acids (Table 6), suggesting at most bidentate coordination or perhaps only amine or carboxylate binding. Thus, it is unlikely that the thio- or selenoether S or Se atom plays a significant role in Fe(II) coordination. Based on limited data, the selenoether-containing amino acids have slightly higher stability constants with Fe(II) than analogous thioether amino acids, although two data points (Met/SeMet and MeCys/MeSeCys) do not necessarily indicate a trend.

In contrast, Fe(II) stability constants with the thiol-containing amino acids cysteine and penicillamine are significantly higher (Table 8). Interestingly, Fe(II) cysteine and penicillamine

Table 8
Stability constants of Fe(II) and Fe(III) with sulfur- and selenium-containing amino acids

Ligand	[ML] (log β)[a]	[ML_2] (log β_2)[b]	Temp (°C)	Ionic Strength (M)	Method	Ref.
Fe(II) Stability Constants						
Cysteine	6.69(2)	11.90(3)	20	0.1 $NaClO_4$	Potentiometry	[234]
Methionine	3.24[c]		20	1.0 KCl	Potentiometry	[193]
Methylcysteine	3.49(4)		25	0.1 NaCl	Potentiometry	[190]
Methylselenocysteine	3.84(1)		25	0.1 NaCl	Potentiometry	[190]
Penicillamine	7.58(1)	13.74(2)	20	0.1 $NaClO_4$	Potentiometry	[234]
Selenomethionine	3.51(7)		25	0.1 NaCl	Potentiometry	[190]
Fe(III) Stability Constants						
Cysteine	10.85[c]	14.49[c]	20	0.15 KNO_3	Potentiometry	[235]
Methionine	9.1[c]		20	1.0 $NaClO_4$	Redox	[193]
Methylcysteine	8.37(5)	13.92(1)	25	0.1 M KNO_3	Electrophoresis	[236]
Penicillamine	11.27[c]	16.25[c]	20	0.15 KNO_3	Potentiometry	[235]

[a] $\log \beta = [ML]/[M][L]$ [b] $\log \beta_2 = [ML^2]/[M][L^2]$ [c] No error reported.

stability constants are similar to those of bidentate Cu(II)-amino-acid complexes (Table 2), potentially indicating coordination through the thiolate and amine groups rather than tridentate binding. Studies to confirm coordination modes for these amino acids have not been performed, and there are no reported Fe(II) stability constants for selenol-containing amino acids. The lack of independent characterization of the species identified in these Fe(II) stability constant studies provides only a very indirect understanding of these coordination complexes

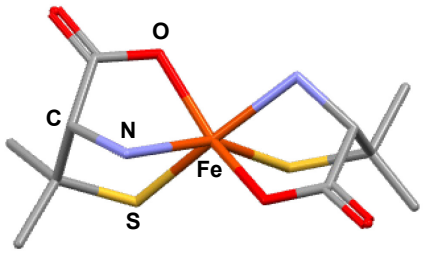

Fig. 12 Solid-state structure for Th[Fe(Pen)$_2$]. The Fe(II) ion is shown in orange, oxygen atoms are red, carbon atoms are grey, nitrogen atoms are blue, and the sulfur atoms are yellow. The counterion and hydrogen atoms are omitted for clarity.

11.2 Fe(III) Complexes of Sulfur- and Selenium-Containing Amino Acids

Only a handful of stability constant determinations with Fe(III) and sulfur amino acids have been reported, and none are reported for selenoamino acids (Table 8) . A variety of methods have been used for the few reported analyses, including potentiometric titrations [218, 234], paper electrophoresis [236], and redox methods [217]. Where comparisons can be made, the data conflict. For the Fe(III)-Met system, Tewari [118] used paper electrophoresis to identify two different species $[Fe(Met)]^{2+}$ and $[Fe(Met)_2]^{+}$ with stability constants of 7.95(7) and 12.65(6), respectively (Table 8). In contrast, a 1958 study by Perrin and coworkers [193] reported a stability constant of 9.1 for the $[Fe(Met)]^{2+}$ species using potentiometric methods. Due to the limited competition of methionine binding with formation of Fe(III) hydrolysis products (Fig. 11), it is not surprising that a $[ML_2]$ stability constant was not determined using this method. While paper electrophoresis is limited to low pH (1-4) to maintain solubility, this method

promotes separation of species through electrophoresis, directly establishing the number of species formed.

The thiol-containing amino acids cysteine and penicillamine have Fe(III) stability constants in the 10.8-11.3 range for the $[ML]^+$ species, significantly higher than those with thioether-containing amino acids. Thus, it is reasonable to assume that in aqueous solution, Cys and Pen ligands interact with Fe(III) through tridentate coordination of the thiolate sulfur, amine nitrogen, and carboxylate oxygen atoms. This binding mode is supported by the solid-state structure of $Th[Fe(Pen)_2]$, a $[Fe^{III}(Pen)_2]^-$ complex with a Th^+ counterion (Fig. 12) [237]. In this complex, both Pen ligands bind in tridentate fashion to Fe(III), with bond angles closer to trigonal bipyramidal than octahedral geometry.

11.3 Challenges in Determining Iron Stability Constants with Sulfur- and Selenium-Containing Amino Acids

Determination of iron stability constants with sulfur- and selenium-containing amino acids is plagued by issues common to Fe(II) and Fe(III) titrations with any amino acid. With Fe(II), experiments must be conducted in oxygen-controlled environments, UV-visible analyses are limited to spectrochemically active ligands, and potentiometric analyses are limited above pH 7. Fe(III) stability constant determinations with weakly binding ligands are even more limited due to the high stability of Fe(III) hydroxide species.

In addition to these problems, the thiol-containing cysteine and penicillamine are also redox-active with Fe(III) [238, 239]. Although Fe(III) stability constants are reported for these amino acids, conditions must be tightly controlled, and data misinterpretation is not uncommon. Sisley's kinetic analysis of the interaction of redox-active metals, including iron, with these

thiol-containing amino acids is detailed and specific about the limitations of these measurements [239]. Due to these significant limitations, few analyses are reported, and stability constant values can vary depending on experimental methods and conditions for these redox-active systems.

11.4 Comparison of Copper and Iron Stability Constant Determinations with Sulfur- and Selenium-Containing Amino Acids

The most complete stability constant data with Cu(II), Cu(I), Fe(II), and Fe(III) exists for the thiol-containing amino acids cysteine and penicillamine. Cu(II)-penicillamine complexes are extremely stable with log β values of 16.5 and 21.7 for the [ML] and [ML_2] species, respectively [191] (Table 4). The stepwise log *K* values for these constants, 16.5 for [ML] and 5.2 for [ML_2], suggest that penicillamine may coordinate Cu(II) as a tridentate ligand in the [ML] species. The significantly lower stability increase upon adding a second penicillamine ligand suggests that the second ligand may have only mono- or bidentate binding. Mixed tridentate and bidentate amino acid-Cu(II) complexes are structurally characterized [144-146], and rhenium-bound penicillamine adopts a structure where the two Pen ligands coordinate in tridentate and bidentate fashion simultaneously [240]. Fe(II)-Pen stability constants are significantly lower than the analogous Cu(II) species (7.58(1) and 13.74(2) for the [ML] and [ML_2] species, respectively [234]; Table 8) but significantly higher than stability constants of other Fe(II)-amino acid complexes (except Cys). These lower stability constants are most consistent with bidentate coordination.

A comparison of stability constants for Cu(I), Fe(II), and Fe(III) with cysteine is somewhat surprising (Tables 4 and 8). Cu(I) and Fe(III) have similar [ML] stability constants of

10.164(6) and 10.85, respectively, but similar Fe(II) values are lower at 6.69(2) [11, 234, 235]. This trend is slightly surprising since Cu(I) is significantly softer than Fe(III), with Fe(II) and Cu(II) falling in between. Comparing the [ML_2] stability constants, the Cu(I)-Cys species has a considerably higher stability of 18.36(1), as compared to 14.49 with Fe(III) and 11.90(3) with Fe(II). Perhaps this unexpected trend can be attributed to cysteine binding all of the metal ions in a tridentate fashion, but the relative Lewis acidity of the coordinating ligand atoms is also mixed, with hard carboxylate and amine groups and a softer thiolate group. It should be noted that Pen stability constants exhibit the same trends, with the Cu(II) species having the greatest stability compared to the other ions.

The thioether-containing methionine is the only other sulfur- or selenium-containing amino acid with analyses reported for Cu(II), Cu(I), Fe(II), and Fe(III). Cu(II)-Met stability constants of 7.82(2) and 14.52(1) for the [ML] and [ML_2] species, respectively, are well-supported by a variety of authors [88, 187, 188] and are consistent with results obtained for other amino acids with aliphatic side chains (Table 1). Methionine stability constants with Cu(I), Fe(II), and Fe(III) were reported in a single 1958 study by Perrin [193], making comparisons questionable. Methionine binding does not provide added stability compared to amino acids with non-coordinating side chains, suggesting that the thioether sulfur atom does not coordinate in the [ML] or [ML_2] species, a result supported by solid-state structures [196, 197]. Assuming Perrin's results are accurate, the same trend is observed for Met as for Cys and Pen. Methionine binding to Cu(I) and Fe(III) has higher stability for the [ML] species ($\log \beta = 9.1$ for both) than for the Fe(II) species ($\log \beta = 3.24$).

Although methionine and selenomethioneine are also bidentate Cu(I) chelators, the soft sulfur or selenium atom coordinates Cu(I) in addition to the amine nitrogen, with no bonding of

the carboxylate group. The best characterization has been obtained for the Cu(II)-Met and -SeMet complexes, where IR and X-ray diffraction data support the bidentate coordination of the amine and carboxylate groups. Differing amino acid coordination modes likely affect the measured stability constants, so evaluating trends across metals is not meaningful.

12. Iron and Copper Coordination to Weakly Binding Ligands: Biological Relevance, Methods Development, and Outlook

With their similar structures and diversity of side-chain functional groups, amino acids are an ideal system for developing more accurate methods to determine metal stability constants with weakly binding ligands. The four metal ions treated in this review, Cu(II), Cu(I), Fe(II), and Fe(III) also span the range between easy to examine (Cu(II)) and extremely difficult to study due to redox activity and insoluble hydrolysis products (Cu(I) and Fe(III)). Developing methods specifically designed to work around these issues, such as solubility titrations for Fe(III) stability constant measurements, would provide a substantial advance in this field and provide a foundation for stability constant determination for metal complexes with any weakly binding ligands. While most stability constants previously reported by Smith and Martell are still reliable today, spectroscopic methods such as circular dichroism and electron paramagnetic resonance used in conjunction with potentiometry have the potential to give insight into the speciation issues that remain [241]. In addition, accurate determination of metal-amino-acid stability constants can then be used to model complex biological systems and predict competition concentrations that may be relevant for understanding metal homeostasis and mis-regulation [8, 9, 11, 112, 242].

Taking into account the biological concentrations of metals and amino acids, we can use

established stability constants to predict the likelihood of complex formation in binary systems. A model was built using HYPERQUAD [105] with a constant pH of 7, corresponding to cellular pH. For this model, only [ML] species were considered, with static protonation constants of 9.2 and 11.2 for the ligand, corresponding to the approximate stability constants for the amine and carboxylate groups of amino acids with non-protonating side chains. Fig. 13A shows the percentage of complex formation as the amino acid ligand concentration varies from 1 μM to 500 μM, the typical range of blood amino acid concentrations, assuming 10 μM of available metal ion (as discussed in the *Cellular Redox-Active Metal Ions and Amino Acids* section). Fig. 13B shows complex formation for the amino acid range from 1-10 μM, where the metal (10 μM) is in excess of the ligand. Percent complex formation for these binary systems is predicted,

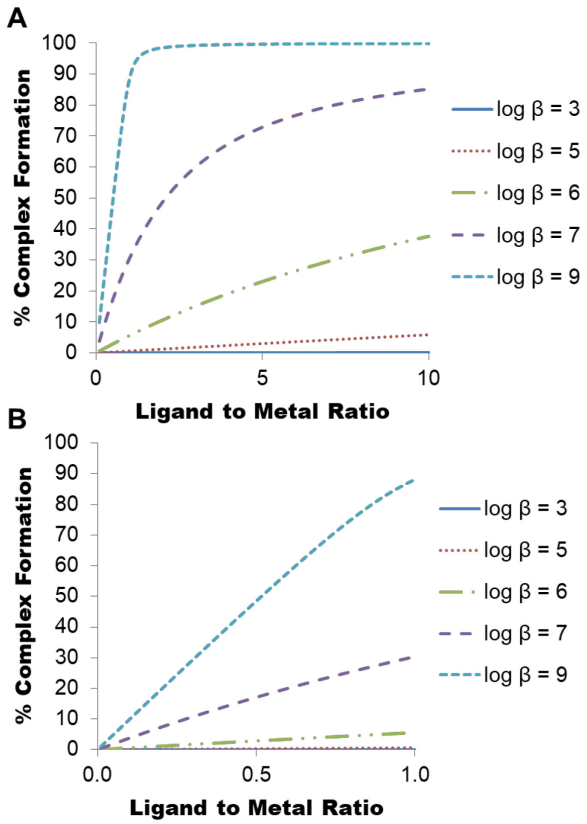

Fig. 13 Percent complex formation of the [ML] species for aqueous solutions containing 10 μM metal ion and 0-100 μM amino acid showing A) the full ligand-to-metal ratio range and B) the ligand-to-metal ratio range of 0 to 1. Formation constants for the amino acid (AA) of 9.2 for HAA and 11.2 for H_2AA were included to model a representative amino acid with amine and carboxylate protons and pH was held constant at 7.

depending on stability constants for the metal-amino-acid complexes.

Most of the Cu(II)-amino-acid complexes have [ML] stability constants of at least 7, indicating bidentate binding and resulting in approximately 40% to 70% of metal bound within a 1:1 to 1:10 ligand-to-metal ratio. For thiol-containing amino acids and histidine that have Cu(II) [ML] stability constants upward of 10, it is expected that 90-100% of the metal ion would be coordinated to the amino acid at a 1:1 or greater metal-to-amino-acid ratio. Ten-fold higher metal ion concentrations (100 μM) with the same amino acid concentration range result in decreased complex formation compared to 10 μM metal over the same stability constant range (Fig. S1 in *Supplementary Material*). All of the limited number of Cu(I)-amino-acid stability constants are higher than the Cu(II) stability constants with the same amino acid, even for amino acids such as alanine and glycine that are only bidentate chelators. Thus, in the reducing cellular environment, it is reasonable to expect that labile Cu(I) also would be bound by free amino acids, if potential ligands with higher stability constants were not available.

On the other end of the spectrum, the Fe(II)-amino-acid stability constants for the [ML] species are very low, approximately 3-4. With these low stability constants, even at a 10:1 ligand-to-metal ratio, less than 10% of Fe(II) would be bound (Fig. 13). With Fe(II), only cysteine and penicillamine with stability constants of 6.69 and 7.58, respectively, would form an appreciable amount of complex. Although Fe(III)-amino-acid complexes have high enough stability constants (in the 8-13 range) to expect amino acid coordination at the modeled concentrations, amino acids would not outcompete formation of Fe(III) hydrolysis products at reasonable biological pH ranges.

From these simple models, it is evident that amino acids with higher stability constants will dominate complexation with labile metal ions. For Cu(I) and Cu(II), histidine, aspartic acid,

cysteine, and penicillamine would out-compete other amino acid binding, as long as amino acid concentrations were relatively similar. For Fe(II) and Fe(III), cysteine and penicillamine coordination would dominate in the absence of Fe(III) hydrolysis, although penicillamine would not be present unless administered as a drug.

From this overview, it is evident that there is a need for more complete analyses of the redox active metals with sulfur- and selenium-containing amino acids. Although Cu(II) has been extensively studied, the other metal ions, Cu(I), Fe(II), and Fe(III) are just as biologically relevant, but data are poor. Before beginning an amino acid stability constant study, it is important to understand the advantages and disadvantages of not just the methods used, but of the metal and ligands to be studied. Cu(II) is a robust ion with high solubility in aqueous systems, and its stability constants with a wide variety of amino acids and other ligands have already been thoroughly examined. Thus, the Cu(II) system is ideal for new methods development, since the breadth of data available would provide dependable comparisons. Because Cu(II) is a redox-active metal, the redox activity of the ligand must be considered when selecting experimental parameters.

Cu(I) binds a variety of amino acids in metalloenzymes and also contributes to oxidative damage within the cell, if not controlled through cellular mechanisms and complexation. Determining Cu(I) complex stabilities with available small molecules is a wide-open field with significant biological implications. Cu(I) is extremely difficult to work with due to redox activity, oxygen sensitivity, limited solubility, tendency for disproportionation, and lack of spectrochemical activity. There is much need for methods development for stability constant determination with this ion. Measuring stability in high ionic strength media may provide the best path forward for potentiometric analysis. Other methods, such as zero-current potentiometry

and electrophoresis under atmosphere-controlled conditions, are worth developing and validating.

Fe(II)- and Fe(III)-amino-acid stability constant data is also lacking. Since potentiometric analysis is not ideal due to low solubility of iron hydrolysis products, other methods need to be explored. The solubility method has the potential to open up the Fe(III) determinations, especially with mass spectrometry techniques capable of detecting and quantifying individual species. Other methods such as EPR and NMR spectroscopies, as well as mass spectrometry have been utilized for the analysis of more stable and uniform complexes such as metalloproteins, but methods development for smaller ligands such as amino acids has been limited due to the variety of species that may simultaneously form in solution.

Determining stability constants for copper and iron binding to sulfur and selenium amino acids is also critical for understanding biological systems. Sulfur amino acids are required for maintaining cellular redox balance, and simple modeling studies indicate that these amino acids may bind biological iron and copper. Selenoamino acids and related species are implicated in cancer prevention and as antioxidants to prevent metal-mediated oxidative damage, yet selenium interaction with biometals is thoroughly underexplored and requires more dedicated study.

“The difficulty lies not so much in developing new ideas as in escaping from old ones” (John Maynard Keynes). One of the primary difficulties with this field is that the easiest systems have been thoroughly studied, but the more problematic ones only have single analyses or no data at all. Revisiting some of the analyses that were performed more than 50 years ago as well as using and/or developing new methods to confirm these results and continue the study of weakly binding ligands is worth exploring. Understanding and predicting the interactions between metal ions and small molecules can have far reaching effects into the efficacy of drug

development and oxidative-damage prevention in biological systems.

13. Acknowledgments and Funding

This work was supported by the National Science Foundation grants CHE 1213912 and 1807709, and J.M.M. was supported by Clemson University with a Doctoral Dissertation Completion Grant.

14. References

[1] C. Andreini, I. Bertini, G. Cavallaro, G.L. Holliday, J.M. Thornton, J. Biol. Inorg. Chem. 13 (2008) 1205.

[2] M.I. Hood, E.P. Skaar, Nat. Rev. Microbiol. 10 (2012) 525.

[3] K. Keyer, J.A. Imlay, Proc. Natl. Acad. Sci. U.S.A. 93 (1996) 13635.

[4] S. Bolognin, L. Messori, P. Zatta, NeuroMol. Med. 11 (2009) 223.

[5] R. Gillard, R. Mason, N.C. Payne, G.B. Robertson, J. Chem. Soc. A Inorg. Phys. Theor. 12 (1969) 1864.

[6] P.S. Hallman, D.D. Perrin, A.E. Watt, Biochem J 121 (1971) 549.

[7] R.J. Motekaitis, A.E. Martell, Mar. Chem. 21 (1987) 123.

[8] G. Berthon, M.J. Blais, M. Piktas, K. Houngbossa, J. Inorg. Biochem. 20 (1984) 113.

[9] W.R. Harris, R.D. Sammons, R.C. Grabiak, J Inorg Biochem 116 (2012) 140.

[10] M.C. White, Plant Physiology 67 (1981) 301.

[11] L.-C. Konigsberger, E. Konigsberger, G. Hefter, P.M. May, Dalton Trans. 44 (2015) 20413.

[12] A.E. Martell, R.K. Smith, Critical Stability Constants, Standard Reference Database 46, Version 6.30, National Institute of Standards, Gaithersburg, MD, 2001.

[13] J.T. Rubino, P. Riggs-Gelasco, K.J. Franz, J. Biol. Inorg. Chem. 15 (2010) 1033.

[14] M. Okada, T. Miura, J. Inorg. Biochem. 159 (2016) 45.

[15] W.-Y. Sun, N. Ueyama, A. Nakamura, Biopolymers 46 (1998) 1.

[16] R.M. Roat-Malone, Bioinorganic Chemistry, John Wiley & Sons, Inc., Hoboken, New Jersey, 2002, 151-230.

[17] R.L. Jurado, Clin. Infec. Dis. 25 (1997) 888.
[18] J. Metcoff, J. Am. Coll. Nutr. 5 (1986) 107.
[19] K. Fukuda, Y. Nishi, T. Usui, J. Pediatr. Gastroenterol. Nutr. 3 (1984) 432.
[20] L.D. Stegink, L.J. Filer, M.C. Brummel, G.L. Baker, W.L. Krause, E.F. Bell, E.E. Ziegler, Am. J. Clin. Nutr. 53 (1991) 670.
[21] F.M. Rubino, M. Pitton, D. Di Fabio, G. Meroni, E. Santaniello, E. Caneva, M. Pappini, A. Colombi, Biomed. Chromatogr. 25 (2011) 330.
[22] R.F. Burk, B.K. Norsworthy, K.E. Hill, A.K. Motley, D.W. Byrne, Cancer Epidemiol., Biomarkers Prev. 15 (2006) 804.
[23] B. Sarkar, Chem. Rev. 99 (1999) 2535.
[24] G.J. Brewer, F.K. Askari, J. Hepatol. 42 (2005) S13.
[25] A.O. Muijsers, R.J. van de Stadt, A.M.A. Henrichs, H.J.W. Ament, J.K. van der Korst, Arthritis Rheumatol. 27 (1984) 1362.
[26] V. Medici, V. Di Leo, F. Lamboglia, C.L. Bowlus, S.C. Tseng, R. D'Inca, P. Irato, P. Burra, D. Martines, G.C. Sturniolo, Scand. J. Gastroenterol. 42 (2007) 1495.
[27] W.H. Lyle, D.F. Pearcy, M. Hui, Proc. R. Soc. Med. 70 (1977) 48.
[28] S.J. Lippard, J.M. Berg, Principles of Bioinorganic Chemistry, University Science Books, Mill Valley, 1994.
[29] R. Meneghini, Free Radic Biol Med 23 (1997) 783.
[30] T.I. Mzhel'skaya, Bull Exp Biol Med 130 (2000) 719.
[31] E.L. Que, D.W. Domaille, C.J. Chang, Chem Rev 108 (2008) 1517.
[32] J. Stockel, J. Safar, A.C. Wallace, F.E. Cohen, S.B. Prusiner, Biochemistry 37 (1998) 7185.
[33] E. Gaggelli, H. Kozlowski, D. Valensin, G. Valensin, Chem. Rev. 106 (2006) 1995.
[34] L. Yang, R. McRae, M.M. Henary, R. Patel, B. Lai, S. Vogt, C.J. Fahrni, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 11179.
[35] H.Y. Au-Yeung, C.Y. Chan, K.Y. Tong, Z.H. Yu, J. Inorg. Biochem. 177 (2017) 300.
[36] J.A. Cotruvo, Jr., A.T. Aron, K.M. Ramos-Torres, C.J. Chang, Chem. Soc. Rev. 44 (2015) 4400.
[37] P. Verwilst, K. Sunwoo, J.S. Kim, Chem. Commun. 51 (2015) 5556.

[38] L. Yang, R. McRae, L.L. Henary, R. Patel, B. Lai, S. Vogt, C.J. Fahrni 102 (2005) 11179.
[39] L.J. Que, R.Y.N. Ho, Chem. Rev. 96 (1996) 2607.
[40] G.E. Griesmann, A.C. Hartmann, F.F. Farris, Int. J. Environ. Health Res. 19 (2009) 231.
[41] N.D. Jhurry, M. Chakrabarti, S.P. McCormick, G.P. Holmes-Hampton, P.A. Lindahl, Biochemistry 51 (2012) 5276.
[42] R.C. Hider, X. Kong, Dalton Trans. 42 (2013) 3220.
[43] S.A. Bellingham, B. Guo, A.F. Hill, Biol. Cell. 107 (2015) 389.
[44] M. Mital, N.E. Wezynfeld, T. Fraczyk, M.Z. Wiloch, U.E. Wawrzyniak, A. Bonna, C. Tumpach, K.J. Barnham, C.L. Haigh, W. Bal, S.C. Drew, Angew. Chem. Int. Ed. Engl. 54 (2015) 10460.
[45] M. Kawahara, D. Mizuno, Biomed. Res. Trace Elem. 26 (2015) 10.
[46] A. Robert, Y. Liu, M. Nguyen, B. Meunier, Acc. Chem. Res. 48 (2015) 1332.
[47] L.C. Jeuken, R. Camba, F.A. Armstrong, G.W. Canters, J. Biol. Inorg. Chem. 7 (2002) 94.
[48] L.J. Jeuken, M. Ubbink, J.H. Bitter, P. van Vliet, W. Meyer-Klaucke, G.W. Canters, J. Mol. Biol. 299 (2000) 737.
[49] E.A. Ambundo, L.A. Ochrymowycz, D.B. Rorabacher, Inorg. Chem. 40 (2001) 5133.
[50] A. Ala, A.P. Walker, K. Ashkan, J.S. Dooley, M.L. Schilsky, Lancet 369 (2007) 397.
[51] N. Leone, D. Courbon, P. Ducimetiere, M. Zureik, Epidemiology 17 (2006) 308.
[52] K. Ando, K. Ogawa, S. Misaki, K. Kikugawa, Free Radic. Res. 36 (2002) 1079.
[53] T.D. Rae, P.J. Schmidt, R.A. Pufahl, V.C. Culotta, T.V. O'Halloran, Science 284 (1999) 805.
[54] E. Prus, E. Fibach, Br. J. Haematol. 142 (2008) 301.
[55] M.L. North, D.E. Wilcox, J. Am. Chem. Soc. 141 (2019) 14329.
[56] P. Aisen, A. Leibman, J. Zweier, J. Biol. Chem. 253 (1978) 1930.
[57] P. Aisen, A. Leibman, Biochim. Biophys. Acta 257 (1972) 314.
[58] D.S. Wishart, PLoS Comput. Biol. 8 (2012) e1002805.
[59] P. Patel, P.K. Bhattacharya, J. Inorg. Biochem. 54 (1994) 187.
[60] I. Sovago, C. Kallay, K. Varnagy, Coord. Chem. Rev. 256 (2012) 2225.
[61] I. Sovago, K. Osz, Dalton Trans. 32 (2006) 3841.

[62] D. Sanna, C.G. Agoston, G. Micera, I. Sovago, Polyhedron 20 (2001) 3079.

[63] M. Remelli, C. Conato, A. Agarossi, F. Pulidori, P. Mlynarz, H. Kozlowski, Polyhedron 19 (2000) 2409.

[64] W.J. Puspita, A. Odani, O. Yamauchi, J. Inorg. Biochem. 73 (1999) 203.

[65] K. Varnagy, B. Boka, I. Sovago, D. Sanna, P. Marras, G. Micera, Inorg. Chim. Acta 275-276 (1998) 440.

[66] N. Lihi, M. Lukacs, M. Raics, G. Szunyog, K. Varnagy, C. Kallay, Inorg. Chim. Acta 472 (2018) 165.

[67] J. Aurelie, J. Latour, S. Olivier, Dalton Trans. 43 (2014) 3922.

[68] T. Kiss, I. Sovago, A. Gergely, Pure Appl. Chem. 63 (1991) 597.

[69] I. Dokmanic, M. Sikic, S. Tomic, Acta Crystallogr., Sect. D: Biol. Crystallogr. 64 (2008) 257.

[70] A. Rigo, A. Corazza, M.L. di Paolo, M. Rossetto, R. Ugolini, M. Scarpa, J. Inorg. Biochem. 98 (2004) 1495.

[71] G. Berthon, Pure Appl. Chem. 67 (1995) 1117.

[72] A. Gergely, I. Sovago, Bioinorg. Chem. 9 (1978) 47.

[73] I. Sovago, B. Harman, A. Gergely, Inorg. Chim. Acta 46 (1980) 91.

[74] A. Krezel, W. Bal, Acta Biohim. Pol. 46 (1999) 567.

[75] M.T. Morgan, L.A.H. Nguyen, H.L. Hancock, C.J. Fahrni, J. Biol. Chem. 292 (2017) 21558.

[76] J. Liu, H. Liu, Y. Li, H. Wang, J. Biol. Phys. 40 (2014) 313.

[77] M.Y. Hamed, J. Silver, Inorg. Chim. Acta 80 (1983) 115.

[78] M.C. Marques, C. Tapia, O. Gutierrez-Sanz, A.R. Ramos, K.L. Keller, J.D. Wall, A.L. De Lacey, P.M. Matias, I.A.C. Pereira, Nat. Chem. Biol. 13 (2017) 544.

[79] K.T. Suzuki, J. Health Sci. 51 (2005) 107.

[80] E.E. Battin, M.T. Zimmerman, R.R. Ramoutar, C.E. Quarles, J.L. Brumaghim, Metallomics 3 (2011) 503.

[81] O. Yamauchi, A. Odani, Pure Appl. Chem. 68 (1996) 469.

[82] L.D. Pettit, Pure Appl. Chem. 56 (1984) 247.

[83] I. Sovago, T. Kiss, A. Gergely, Pure Appl. Chem. 65 (1993) 1029.

[84] M.T. Beck, Pure Appl. Chem. 49 (1977) 127.

[85] M.T. Beck, Chemistry of Complex Equilibria, Akademiai Kiado, Budapest, 1969.

[86] M.T. Beck, I. Nagypal, Chemistry of Complex Equilibria, Ellis Horwood Ltd., Chichester, 1989.

[87] F. Rossotti, H. Rossotti, The Determination of Stability Constants, McGraw-Hill, New York, 1961.

[88] G. Brookes, L.D. Pettit, J. Chem. Soc., Dalton Trans. 19 (1977) 1918.

[89] G. Anderegg, Pure Appl. Chem. 54 (1982) 2693.

[90] G.H. Nancollas, M.B. Thomson, Pure Appl. Chem. 54 (1982) 2676.

[91] A. Braibanti, G. Ostacoli, P. Paoletti, L.D. Pettit, S. Sammartano, Pure Appl. Chem. 59 (1987) 1721.

[92] A. Gergely, Acta Chim. Acad. Sci. Hung. 59 (1969) 309.

[93] A.L.J. Rao, M. Singh, S. Sanjiv, Rev. Anal. Chem. 8 (1986) 283.

[94] J. Stary, J.O. Liljenzin, Pure Appl. Chem. 54 (1982) 2557.

[95] D.G. Tuck, Pure Appl. Chem. 55 (1983) 1477.

[96] P. Paoletti, Pure Appl. Chem. 56 (1987) 491.

[97] R.K. Smith, A.E. Martell, Critical Stability Constants, 4, Plenum Press, New York, 1976.

[98] A.E. Martell, R.M. Smith, Critical Stability Constants, 5, Plenum Press, New York, 1982.

[99] A.E. Martell, Critical Stability Constants, 6, Plenum Press, New York, 1989.

[100] Bjernum, Metal Ammine Formation in Aqueous Solutions, Haas & Sons, Copenhagen, 1941.

[101] M. Calvin, K.W. Wilson, J. Am. Chem. Soc. 67 (1945) 2003.

[102] S. Fronaeus, Acta Chem. Scand. 4 (1950) 72.

[103] I.G. Sayce, Talanta 15 (1968) 1397.

[104] L. Alderighi, I. Gans., D. Peters, A. Sabatini, A. Vacca, Coord. Chem. Rev. 184 (1999) 311.

[105] P. Gans, A. Sabatini, A. Vacca, Talanta 43 (1996) 1739.

[106] A. Sabatini, A. Vacca, P. Gans, Talanta 21 (1974) 53.

[107] P. Gans, A. Sabatini, A. Vacca, J. Chem. Soc., Dalton Trans. 6 (1985) 1195.

[108] A.L. Herbelin, J.C. Westall, FITEQL, A computer program for determination of chemical equilibrium constants from experimental data, 3.1, Oregon State University, Corvallis, OR, 1994.

[109] M. Meloun, M. Javurek, J. Havel, Talanta 33 (1986) 513.
[110] Z. Leggett, Computation Methods for the Determination of Formation Constants, Plenum Press, New York, 1985, 291.
[111] D.J. Leggett, W.A.E. McBryde, Anal. Chem. 47 (1975) 1065.
[112] L.C. Tran-Ho, P.M. May, G.T. Hefter, J. Inorg. Biochem. 68 (1997) 225.
[113] S.K. Shah, C.M. Gupta, Talanta 27 (1980) 823.
[114] R.C. Mercier, M. Bonnet, M. Paris, Bull. Soc. Chim. France 10 (1965) 2926.
[115] A. Bonniol, P. Vieles, J. Chim. Phys. 65 (1968) 414.
[116] M.M. Ramel, M.R. Paris, Bull. Soc. Chim. France 4 (1967) 1359.
[117] S. Singh, H.L. Yadava, P.C. Yadava, K.L. Yadava, Bull. Soc. Chim. France 11 (1984) 349.
[118] B.B. Tewari, Rev. Inorg. Chem. 23 (2003) 349.
[119] G. Arena, R. Cali, V. Cucinotta, S. Musumeci, E. Rizzarelli, S. Sammartano, J. Chem. Soc. Dalton Trans. 1983 (1983) 1271.
[120] B.R. Arbad, D.N. Shelke, D.V. Jahagirdar, Inorg. Chim. Acta 46 (1980) L17.
[121] N. Ivicic, V. Simeon, J. Inorg. Nucl. Chem. 43 (1981) 2581.
[122] A.A. Shoukry, J. Solution Chem. 40 (2011) 1796.
[123] B.R. James, R. Williams, J. Chem. Soc. (1961) 2007.
[124] P. Debye, E. Huckel, Phys. Z. 24 (1923) 185.
[125] K. Tomita, Bull. Soc. Chim. Jpn. 34 (1961) 297.
[126] S. Konar, K. Gagnon, A. Clearfield, C. Thompson, J. Hartle, C. Ericson, C. Nelson, J. Coord. Chem. 63 (2010) 3335.
[127] H. Davies, J. Park, R.D. Gillard, Inorg. Chim. Acta 356 (2003) 69.
[128] B.M. Casari, A.H. Mahmoudkhani, V. Langer, Acta Crystallogr. E60 (2004) m1949.
[129] K. Tomita, Bull. Sco. Chim. Jpn 34 (1961) 297.
[130] A. Wojciechowska, A. Gagor, J. Jezierska, M. Duczmal, RSC Adv. 4 (2014) 63150.
[131] G. Arena, C. Conato, A. Contino, F. Pulidori, R. Purrello, M. Remelli, G. Tabbi, Ann. Chim. 88 (1998) 1.
[132] A. Albert, Biochem. J. 50 (1952) 690.
[133] V. Jokl, J. Chromatogr. 14 (1964) 71.
[134] B.B. Tewari, J. Mex. Chem. Soc. 52 (2008) 219.

[135] P. Bretton, J. Chim. Phys. 55 (1958) 61.
[136] L. Davis, F. Roddy, D.E. Metzler, J. Am. Chem. Soc. 83 (1961) 127.
[137] V. Brumas, N. Alliey, G. Berthon, J. Inorg. Biochem. 52 (1993) 287.
[138] V. Brumas, B. Brumas, G. Berthon, J Inorg Biochem 57 (1995) 191.
[139] H.C. Freeman, R.P. Martin, J. Biol. Chem. 244 (1969) 4823.
[140] L.D. Pettit, J. Swash, J. Chem. Soc. ,Dalton Trans. 23 (1976) 2416.
[141] H.-K. Lin, Z.-Z. Gu, X.-M. Chen, Y.-T. Chen, Thermochim. Acta 123 (1988) 201.
[142] M.L. Barr, E. Baumgartner, K. Kustin, J. Coord. Chem. 2 (1973) 263.
[143] L. Antolini, L.P. Battaglia, C.A. Bonamartini, G. Marcotrgiano, L. Menabue, G.C. Pellacani, M. Saladini, M. Sola, Inorg. Chem. 26 (1986) 2901.
[144] T. Ono, H. Shimanouchi, Y. Sasada, T. Sakurai, O. Yamauchi, A. Nakahara, Bull. Soc. Chim. Jpn. 52 (1979) 2229.
[145] P. Deschamps, P.P. Kulkarni, B. Sarkar, Inorg. Chem. 43 (2004) 3338.
[146] H.C. Freeman, J.M. Guss, R.P. Healy, C.E. Martin, C.E. Nockolds, Chem. Commun. (London) (1969) 225.
[147] L.H. Abdel-Rahman, L.P. Battaglia, P. Sgarabotto, M.R. Mahmoud, J. Chem. Cryst. 24 (1994) 567.
[148] K.M. Wellman, T.G. Mecca, W. Mungall, C.R. Hare, J. Am. Chem. Soc. 90 (1968) 805.
[149] B.V.M. Rao, G. Venkatanarayana, P. Lingaiah, Indian J. Chem. 27A (1988) 261.
[150] P. Daniele, P. Amico, G. Ostacoli, Ann. di Chim. 74 (1984) 105.
[151] A. Zuberbuhler, Helv. Chim. Acta. 53 (1970) 669.
[152] H. Soylu, D. Ulku, J.C. Morrow, Z. Kristallogr., Kristallgeom., Kristallphys., Kristllchem. 140 (1974) 281.
[153] T.P. I, G. Nancollas, Inorg. Chem. 11 (1972) 2414.
[154] V.G. Shtyrlin, Y.I. Zyavkina, E.M. Gilyazetdinov, M.S. Bukharov, A.A. Krutikov, R.R. Garipov, A.S. Mukhtarov, A.V. Zakharov, Dalton Trans 41 (2012) 1216.
[155] G.G. Gorboletova, A.A. Metlin, Russ. J. Phys. Chem. A 89 (2015) 218.
[156] M.A. Doran, S. Chaberek, A.E. Martell, J. Am. Chem. Soc. 86 (1964) 2129.
[157] B. Sarkar, T.P. Kruck, Can. J. Chem. 45 (1967) 2046.
[158] P.Z. Neumann, A. Sass-Kortsak, J. Clin. Invest. 46 (1967) 646.
[159] A. Abbaspour, M.A. Kamyabi, Anal. Chim. Acta 512 (2004) 257.

[160] L. Yang, Z. Huang, F. Li, J. Pept. Sci. 18 (2012) 449.
[161] I. Voskoboinik, J. Camakaris, J. Bioenerg. Biomembr. 34 (2002) 363.
[162] S. Lutsenko, R.G. Efremov, R. Tsivkovskii, J.M. Walker, J. Bioenerg. Biomembr. 34 (2002) 351.
[163] J.T. Rubino, M.P. Chenkin, M. Keller, P. Riggs-Gelasco, K.J. Franz, Metallomics 3 (2011) 61.
[164] V.K. Sharma, F.J. Millero, J. Solution Chem. 19 (1990) 375.
[165] B.R. James, R. Williams, J. Chem. Soc. (1961) 2007.
[166] J. Liu, S. Chakraborty, P. Hosseinzadeh, Y. Yu, S. Tian, I. Petrik, A. Bhagi, Y. Lu, Chem. Rev. 114 (2014) 4366.
[167] P.C. Ford, E. Cariati, J. Bourassa, Chem. Rev. 99 (1999) 3625.
[168] I.J. Pickering, G.N. George, C.T. Dameron, B. Kurtz, D.R. Winge, I.G. Dance, J. Am. Chem. Soc. 15 (1993) 9498.
[169] D.N. Heaton, G.N. George, G. Garrison, D.R. Winge, Biochemistry 40 (2001) 743.
[170] U. Ryde, M. Olsson, K. Pierloot, Theoretical and Computational Chemistry 9 (2001) 1.
[171] H. Tapiero, D.M. Townsend, K.D. Tew, Biomed. Pharmacother. 57 (2003) 386.
[172] R.S. Glass, W.P. Singh, W. Jung, Z. Veres, T.D. Scholz, T.C. Stadtman, Biochemistry 32 (1993) 12555.
[173] Y. Ohta, K.T. Suzuki, Toxicol. Appl. Pharmacol. 226 (2008) 169.
[174] G.M. Lacourciere, T.C. Stadtman, Biofactors 14 (2001) 69.
[175] D. Behne, A. Kyriakopoulos, Annu. Rev. Nutr. 21 (2001) 453.
[176] T.C. Stadtman, FASEB J. 1 (1987) 375.
[177] E.E. Battin, J.L. Brumaghim, J. Inorg. Biochem. 102 (2008) 3036.
[178] G. Atmaca, Yonsei Medical Journal 45 (2004) 776.
[179] C. Ip, H.E. Ganther, Carcinogenesis 13 (1992) 1167.
[180] A. Pezzini, E. Del Zotto, A. Padovani, Curr. Med. Chem. 14 (2007) 249.
[181] M.T. Zimmerman, C.A. Bayse, R. Ramoutar, J.L. Brumaghim, J. Inorg. Biochem. 145 (2015) 30.
[182] L.C. Clark, B. Dalkin, A. Krongrad, G.F. Combs, Jr., B.W. Turnbull, E.H. Slate, R. Witherington, J.H. Herlong, E. Janosko, D. Carpenter, C. Borosso, S. Falk, J. Rounder, Br. J. Urol. 81 (1998) 730.

[183] G.F. Combs, Jr., Br. J. Cancer 91 (2004) 195.

[184] S.M. Lippman, E.A. Klein, P.J. Goodman, M.S. Lucia, I.M. Thompson, L.G. Ford, H.L. Parnes, L.M. Minasian, J.M. Gaziano, J.A. Hartline, J.K. Parsons, J.D. Bearden, 3rd, E.D. Crawford, G.E. Goodman, J. Claudio, E. Winquist, E.D. Cook, D.D. Karp, P. Walther, M.M. Lieber, A.R. Kristal, A.K. Darke, K.B. Arnold, P.A. Ganz, R.M. Santella, D. Albanes, P.R. Taylor, J.L. Probstfield, T.J. Jagpal, J.J. Crowley, F.L. Meyskens, Jr., L.H. Baker, C.A. Coltman, Jr., JAMA 301 (2009) 39.

[185] M.E. Reid, A.J. Duffield-Lillico, E. Slate, N. Natarajan, B. Turnbull, E. Jacobs, G.F. Combs, Jr., D.S. Alberts, L.C. Clark, J.R. Marshall, Nutr. Cancer 60 (2008) 155.

[186] L.D. Pinto, P. Puppin, V.M. Behring, O. Alves, N. Rey, J. Felcman, Inorg. Chim. Acta 386 (2012) 60.

[187] G. Lenz, A. Martell, Biochemistry 3 (1964) 745.

[188] M. Israeli, L.D. Pettit, J. Inorg. Nucl. Chem. 37 (1975) 999.

[189] I. Sovago, G. Petocz, J. Chem. Soc. Dalton Trans. 7 (1987) 1717.

[190] J.M. Murphy, A.E. Gaertner, T. Williams, C.D. McMillen, B.A. Powell, J.L. Brumaghim, J. Inorg. Biochem. 195 (2019) 20.

[191] E.J. Kuchinkas, Y. Rosen, Arch. Biochem. Biophys. 97 (1962) 370.

[192] H. Zainal, W. Wolf, Transition Met. Chem. (London) 20 (1995) 225.

[193] D.D. Perrin, J. Chem. Soc. (1958) 3125.

[194] C.H. Anderson, R.A. Holwerda, J. Inorg. Biochem. 23 (1985) 29.

[195] D.D. Perrin, I.G. Sayce, J. Chem. Soc. A Inorg. Phys. Theor. 1 (1968) 53.

[196] C. Ou, D. Powers, J. Thich, T. Felthouse, D. Hendrickson, J. Potenza, H. Schugar, Inorg. Chem. 17 (1978) 34.

[197] M. Veidis, G. Palenik, J. Chem. Soc. D 21 (1969) 1277.

[198] E. Dubler, N. Cathomas, G.B. Jameson, Inorg. Chim. Acta 123 (1986) 99.

[199] T.W. Hambley, Acta. Crystallogr. Sect. B 44 (1988) 601.

[200] P. De Meester, D.J. Hodgson, J. Chem. Soc., Dalton Trans. 7 (1976) 618.

[201] C. Tessier, F.D. Rochon, A.L. Beauchamp, Inorg Chem 43 (2004) 7463.

[202] W.S. Sheldrick, R. Exner, J. Organomet. Chem. 386 (1990) 375.

[203] T.A. Balakaeva, A.V. Churakov, M.G. Ezernitskaya, L.G. Kuz'mina, B.V. Lokshin, I.A. Efimenko, Koord. Khim. 25 (1999) 579.

[204] H.C. Wang, M. Riahi, J. Pothen, C.A. Bayse, P. Riggs-Gelasco, J.L. Brumaghim, Inorg. Chem. 50 (2011) 10893.
[205] W. Stricks, I.M. Kolthoff, J. Am. Chem. Soc. 73 (1951) 1723.
[206] G.A. Bagiyan, I.K. Koroleva, N.V. Soroka, Zh. Neorg. Khim. 23 (1978) 2422.
[207] M.J. Walsh, B.A. Ahner, J Inorg Biochem 128 (2013) 112.
[208] S.P. Mezyk, D.A. Armstrong, Can. J. Chem. 67 (1989) 736.
[209] R. Oesterberg, R. Ligaarden, D. Persson, J. Inorg. Biochem. 10 (1979) 341.
[210] G. Hefter, P.M. May, P. Sipos, Chem. Commun. (1993) 1704.
[211] C. Bethke, Geochemical and Biogeochemical Reaction Modeling, Cambridge Press, Cambridge, 2008.
[212] T.C. Stadtman, Annu. Rev. Biochem. 65 (1996) 83.
[213] S.J. Dixon, B.R. Stockwell, Nat. Chem. Biol. 10 (2014) 9.
[214] C.F. Wells, M.A. Salam, Nature 205 (1965) 690.
[215] C.P. Magill, C. Floriani, A. Chiesi-Villa, C. Rizzoli, Inorg. Chem. 33 (1994) 1928.
[216] D.R. Williams, P.A. Yeo, J. Chem. Soc., Dalton Trans. 18 (1972) 1988.
[217] D.D. Perrin, J. Chem. Soc. (1959) 290.
[218] A. Albert, Biochem. J. 47 (1950) 531.
[219] R.C. Kapoor, K.C. Mathur, J. Polarogr. 13 (1967) 86.
[220] A. Baxter, D. Williams, J. Chem. Soc., Dalton Trans. 11 (1974) 1117.
[221] R. Gowda, M. Venkatappa, J. Electrochem. Soc. India 30 (1981) 336.
[222] D.R. Williams, J. Chem. Soc. A Inorg. Phys. Theor. 9 (1970) 1550.
[223] D.R. Williams, J. Chem. Soc. Dalton Trans. (1973) 1064.
[224] E.V. Raju, H.B. Mathur, J. Inorg. Nucl. Chem. 30 (1968) 2181.
[225] P. Djurdjevic, R. Jelic, Trans. Met. Chem. 22 (1997) 284.
[226] M.C. Linder, Nutrients 5 (2013) 4022.
[227] M. Roy, T. Bhowmick, R. Santhanagopal, S. Ramakumar, A.R. Chakravarty, Dalton Trans. 24 (2009) 4671.
[228] L. Rao, T.G. Srinivasan, A.Y. Garnov, P. Zanonato, P.D. Bernardo, A. Bismondo, Geochim. Cosmochim. Acta 68 (2004) 4821.
[229] D. Rai, Radiochim. Acta 35 (1984) 97.

[230] D. Rai, H.J. Bolton, D.A. Moore, N.J. Hess, G.R. Choppin, Radiochim. Acta 89 (2001) 67.

[231] V. Neck, Geochim. Cosmochim. Acta 70 (2006) 4551.

[232] L. Rao, T.G. Srinivasan, A.Y. Garnov, P. Zanonato, P.D. Bernardo, A. Bismondo, Geochim. Cosmochim. Acta 70 (2006) 4821.

[233] H.L. Girdhar, S. Parveen, M.K. Puri, Indian J. Chem. 14A (1976) 1021.

[234] D.A. Doornbos, J.S. Faber, Pharmaceutisch Weekblad 99 (1964) 289.

[235] T.D. Zucconi, G.E. Janaeur, S. Donahe, C. Lewkowicz, J. Pharm. Sci. 68 (1979) 426.

[236] B.B. Tewari, J Chromatogr A 962 (2002) 233.

[237] A. Muller, M. Straube, E. Krickemeyer, H. Bogge, Die Naturwissenschaften 79 (1992) 323.

[238] L. Michaelis, E.S. Guzman Barron, J. Biol. Chem. 83 (1929) 191.

[239] M.J. Sisley, Inorg. Chem. 34 (1995) 6015.

[240] P. Leibnitz, G. Reck, H. Pietzsch, H. Spies, Forschungszentrum Rossendorf FZR-311 (2001) 1.

[241] K. Nagaj, K. Stokowa-Soltys, E. Kurowska, T. Fraczyk, M. Jezowska-Bojczuk, W. Bal, Inorg. Chem. 52 (2013) 13927.

[242] C.M. Weekley, H.H. Harris, Chem. Soc. Rev. 42 (2013) 8870.