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Enzymatic strategies for selenium incorporation into biological molecules

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Abstract

The trace element selenium (Se) is essential to the physiology of most organisms on the planet. The most well documented of Se's biological forms are selenoproteins, where selenocysteine often serves as the catalytic center for crucial redox processes. Se is also found in several other classes of biological molecules, including nucleic acids, sugars, and modified amino acids, although its role in the function of these metabolites is less understood. Despite its prevalence, only a small number of Se-specific biosynthetic pathways have been discovered. Around half of these were first characterized in the past three years, suggesting that the selenometabolome may be more diverse than previously appreciated. Here, we review the recent advances in our understanding of this intriguing biochemical space, and discuss prospects for future discovery efforts.

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Introduction

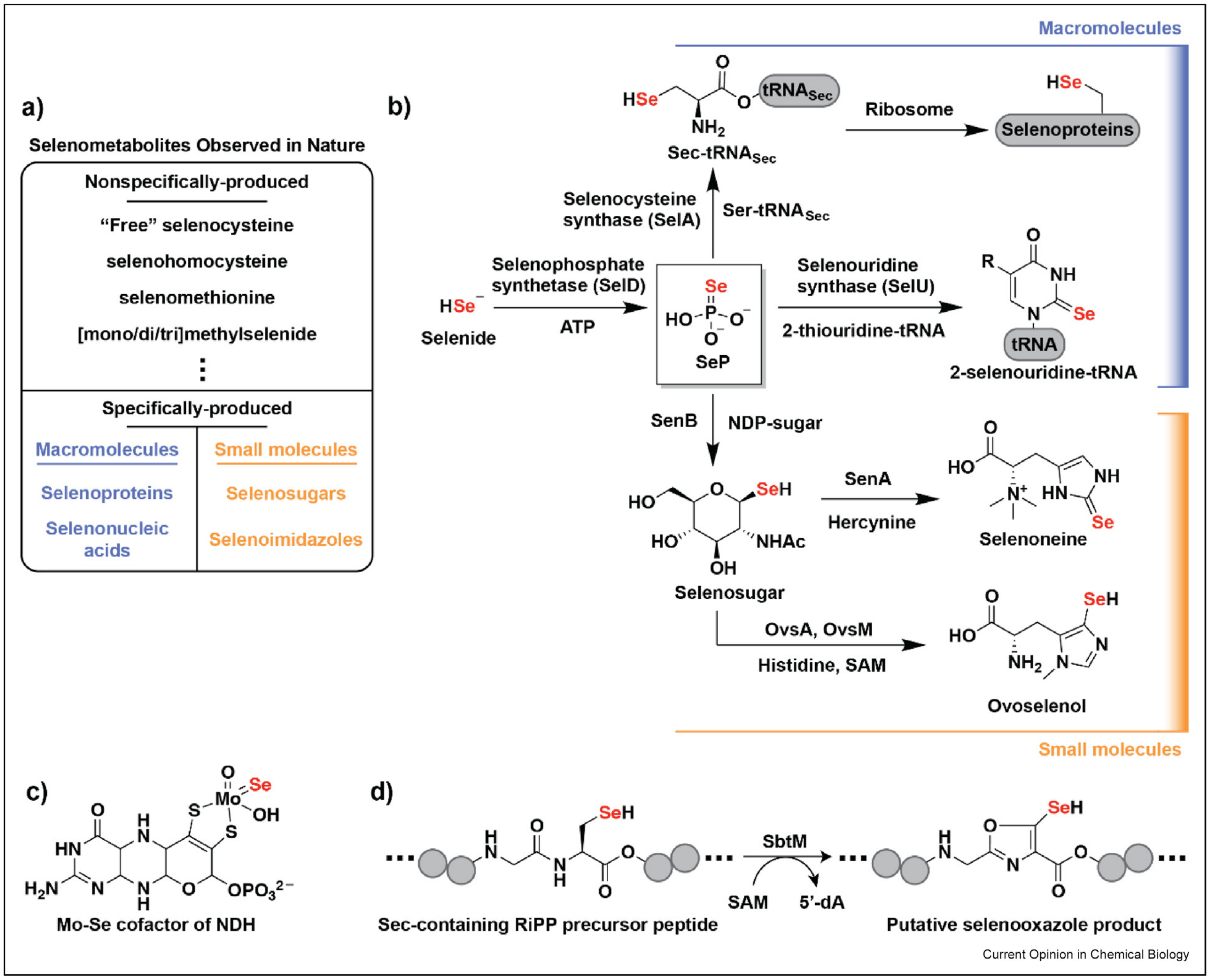
Selenium (Se) is one of the most intriguing elements in biology as it is essential across all kingdoms of life yet only present in a small number of biomolecules [1]. This apparent dichotomy between taxonomic ubiquity and molecular scarcity of biological Se is largely due to its inherent chemical reactivity, endowing selenocompounds with both beneficial and toxic properties [2]. The chemical properties of Se, relative to sulfur (S), are consistent with differences in going from smaller to larger, more polarizable, elements in the same group. Alkyl selenols are significantly more acidic, by 3–4 pK_a units yet more nucleophilic and better leaving groups than the corresponding thiols [1]. At neutral pH, they are in the nucleophilic selenolate form, whereas thiols are protonated. Moreover, they have lower one- and two-electron reduction potentials and are therefore better reductants. Nature, therefore, appears to relegate this powerful element to a very specialized set of metabolites.

Beginning with the discovery of selenocysteine (Sec) incorporation into proteins in the 1970s, only four classes of selenometabolites have since been associated with specific biosynthetic pathways: selenoproteins, selenonucleic acids, selenosugars, and selenoimidazoles, with reports of pathways for the latter two only emerging in recent years [3–6]. The known list of selenium–carbon (Se–C) bond-forming enzymes, central to these pathways, is equally short. However, recent activity in the field suggests a renewed interest in organoselenium biochemistry. In this review, we highlight Nature's enzymatic strategies for selenium incorporation into biological molecules, focusing particularly on insights gained within the last three years. We also point to several open questions, including both longstanding ones and those that have recently surfaced.

Natural selenium incorporation pathways

Natural selenometabolites can be classified in several ways (Figure 1a). One useful distinction is between those produced specifically (i.e. by Se-specific biosynthetic pathways) and those produced nonspecifically (i.e. by adventitious promiscuity of sulfur-utilizing enzymes). Given the chemical similarities between Se and sulfur (S), enzymes often poorly discriminate between the two [7–9]. Hence, while many Se-containing compounds have been detected in biological samples following exposure to exogenous Se, we prefer not to designate them as bona fide selenometabolites until specific biosynthetic, regulatory, or otherwise functional mechanisms are presented, which distinguish them from their sulfur isologues. Selenometabolites can be further subdivided into either macromolecules (i.e. proteins and nucleic acids) or small molecules as these distinctions may parallel their functional roles in primary or secondary metabolism, respectively.

Figure 1



Classification of selenometabolites. **(a)** Selenometabolites observed in Nature can be categorized as nonspecifically produced versus specifically produced, as well as macromolecule versus small molecule. **(b)** Overview of characterized selenometabolite biosynthetic pathways. **(c,d)** Additional, putative classes of selenometabolites including **(c)** the Mo–Se cofactor and **(d)** Sec-containing RiPPs. Abbreviations: Sec = selenocysteine, Ser = serine, NDP = nucleoside diphosphate, SAM = S-adenosylmethionine, NDH = nicotinate dehydrogenase.

An important feature of all known Se-specific biosynthetic pathways is the common intermediate metabolite, selenophosphate (SeP) (Figure 1b) [10]. Generation of SeP from ATP and selenide by SeP synthetase, SelD, represents the first dedicated step toward producing both selenomacromolecules and small selenometabolites [11]. SelA, SelU, and SenB use SeP as a substrate to generate the Se–C bonds found in selenoproteins, selenonucleic acids, and selenosugars, respectively [3–5]. SenA and OvsA utilize a selenosugar substrate (the product of SenB) to catalyze 2′- or 5′-selenylation of a histidine-derived imidazole moiety on route to selenoneine and ovoselenol, respectively [6]. Pathways for producing selenonucleic acids, selenosugars, and selenoimidazoles have thus far been strictly limited to prokaryotes, whereas Sec incorporation into proteins also occurs in protozoa and animals [10,12,13].

Two additional, putative classes of selenometabolites exist, though further investigation is needed to confirm the details of their biosynthetic pathways. The first is the molybdenum–selenium (Mo–Se) cofactor found in a small number of bacterial and archaeal enzymes such as nicotinate dehydrogenase (Figure 1c) [14,15]. The terminal Se within this pyranopterin cofactor is expected to originate from SeP due to its genetic dependence on *selD* [16,17]. Two genes, *yqeB* and *yqeC*, are often colocalized with *selD* in these systems and are expected to be involved in cofactor biogenesis, but their functions are unknown [18]. The second putative class of selenometabolites is a recently explored family of bacterial ribosomally synthesized post-translationally modified peptides (RiPPs) bearing a Sec residue (Figure 1d) [19,20]. While these are technically a subset of the selenoprotein class, they are unique in that they are thought to involve a radical S-adenosylmethionine (RaS) enzyme, SbtM, that modifies the Sec side chain. In vitro experiments involving the precursor selenopeptide and associated RaS enzyme imply a four-electron oxidation of the substrate, proposed to furnish a selenooxazole. However, the identity of the final product of this pathway remains to be determined.

SelD: selenophosphate synthetase

While the list of characterized Se biotransformations is relatively short, they are carried out by a surprisingly diverse set of enzyme classes. The first of these is SelD, which is functionally annotated as a kinase with homology to several other ATP-dependent enzyme families, including thiamine monophosphate kinase ThiL [21]. SelD, however, is unique in that in addition to phosphorylating selenide, it also catalyzes the additional hydrolysis of adenosine diphosphate (ADP) to yield SeP, adenosine monophosphate, and orthophosphate (P_i), further driving the reaction toward products; hence, its formal designation as a selenide—water dikinase (Figure 2a) [22]. Biochemical and structural studies have unraveled several mechanistic details of the reaction, including a catalytic Cys (or Sec) residue that reacts with the selenide equivalent, likely the thioselenide (or perselenide) form [23,24], to generate a chromophore that is putatively designated as a covalent adduct at this residue. Results from isotope-labeling experiments suggest formation of an ATP-derived phosphoryl-enzyme intermediate, poised to undergo nucleophilic attack by the enzyme—selenide equivalent, followed by release of SeP by selenosulfide (or diselenide) reduction [25,26]. However, this phosphoryl-enzyme intermediate has never been observed, and alternate mechanisms for Se—P bond formation have been proposed [27,28]. The precise mechanism of selenide delivery to SelD also remains unclear. While the enzyme accepts free HSe^- in vitro, the toxicity of this species suggests it is unlikely to be the predominant substrate in vivo. An attractive candidate for delivery is Sec lyase (SCL), which functions to salvage selenide from Sec during selenoprotein degradation. Interestingly, SCL forms a direct protein—protein interaction with SelD, and several studies have demonstrated the catalytic competence of selenide-bound SCL in the SelD reaction [29,30]. In addition to the identities of these reaction intermediates, the order of catalytic events (substrate binding, Se—P bond formation, ADP hydrolysis, and product release) also remains an open question.

SelA and SelU: Ser-tRNA$_{Sec}$ selenium transferase and 2-selenouridine synthase

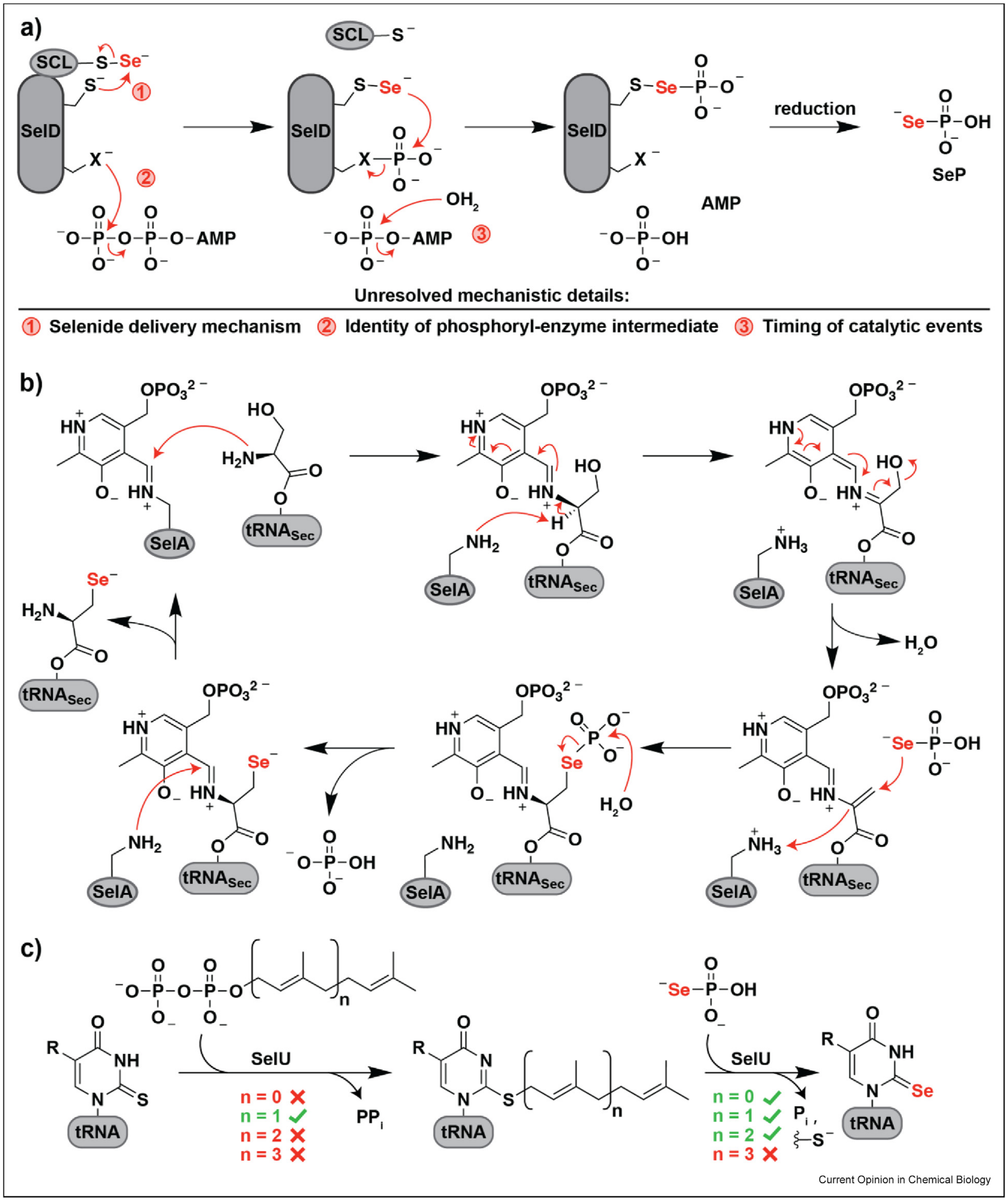
All known Se-specific biosynthetic pathways utilize SeP to generate selenoproteins, selenonucleic acids, selenosugars, or selenoimidazoles, each of which features a distinct class of Se—C bond-forming enzyme. The macromolecular selenometabolites (proteins and nucleic acids), studied extensively for the past three decades, are endowed with their requisite Se—C bonds through the activity of the pyridoxal 5′-phosphate (PLP)-dependent enzyme SelA and the rhodanese-like enzyme SelU, respectively. SelA follows an initial reaction sequence typical of many PLP-dependent enzymes, beginning with binding of substrate, which consists of serine tethered to a special Sec-decoding tRNA (Ser-tRNA$_{Sec}$). This is followed by external aldimine formation to free up an active-site Lys residue that acts as a Bronsted base to remove the α-proton (Figure 2b) [31]. The tethered Ser then undergoes β-elimination to a dehydroalanyl intermediate, followed by nucleophilic attack by SeP to furnish Sec-tRNA$_{Sec}$. This special aminoacyl tRNA is then shuttled to the ribosome to incorporate Sec into proteins [32,33].

The rhodanese-like enzyme SelU also operates on a tRNA-based substrate, albeit with a completely different mechanism, catalyzing Se—C bond formation to generate a selenonucleotide at the wobble position of tRNA$_{Lys}$, tRNA$_{Glu}$, or tRNA$_{Gln}$ in many species of bacteria (Figure 2c) [1,4]. The reaction of SelU involves SeP and a tRNA-bound 2-thiouridine residue (with additional 5′-modifications), on which the sulfur is swapped with Se to yield the hypermodified 2-selenouridine product [34]. Recent studies have revealed this to be a two-step transformation, both SelU-catalyzed, whereby the thiourea moiety is first prenylated, followed by nucleophilic attack by SeP [35—37]. Interestingly, the prenylation activity is specific for geranyl pyrophosphate, whereas the selenylation step can proceed with little discrimination for prenyl chain length. The sequence determinants of substrate binding, Se—C bond formation, and phosphate hydrolysis, as well as the detailed mechanism underlying the transformation, remain to be elucidated. Structural characterization will be an important future step toward understanding the inner workings of this intriguing enzyme.

SenB, SenA, and OvsA: selenosugar synthase, selenoneine synthase, and ovoselenol synthase

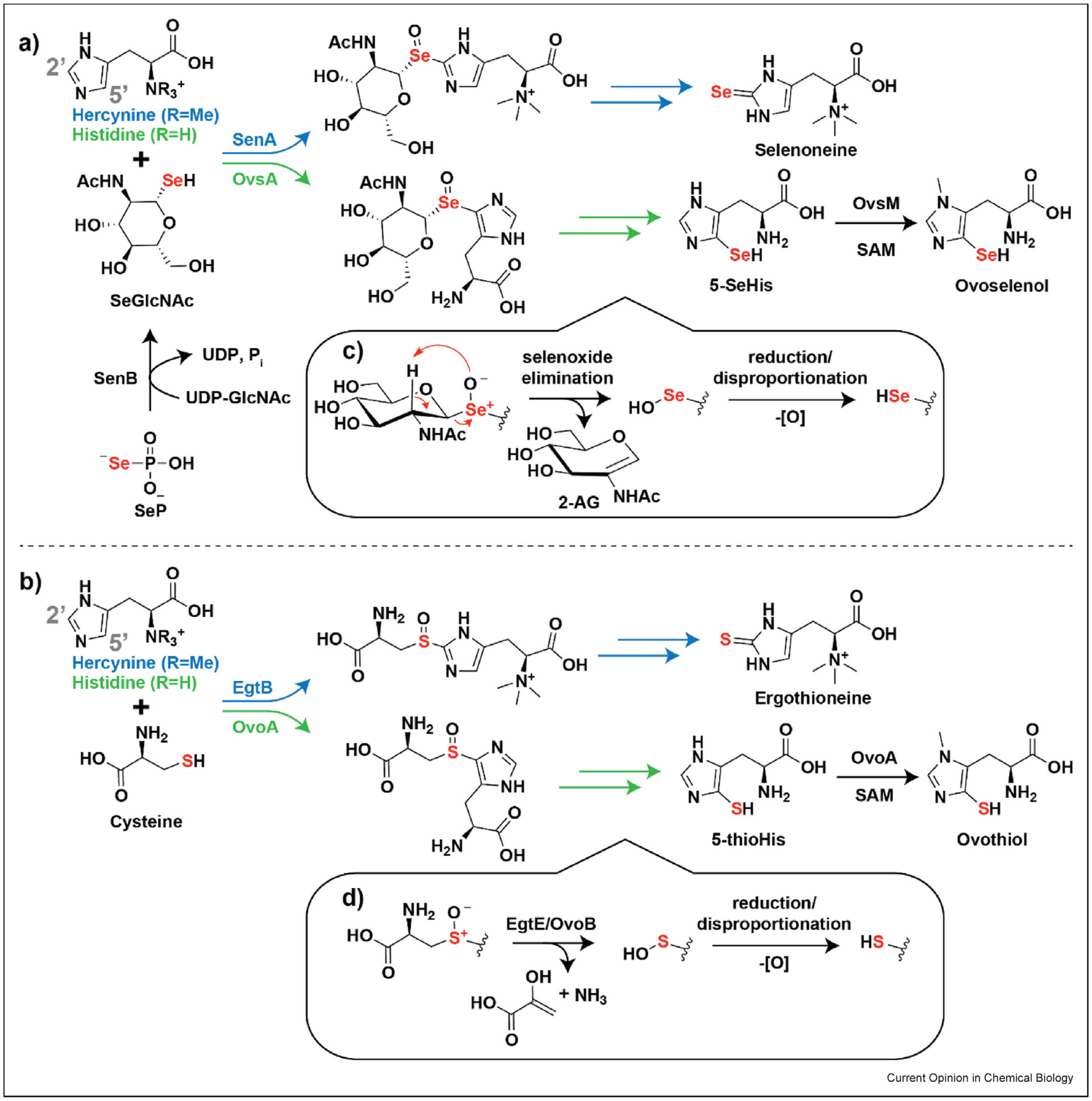
The most recent developments in the field of Se biochemistry pertain to the small molecule selenometabolites, namely selenosugars and selenoimidazoles. Pathways for these molecules (Figure 3a) were only recently discovered through dedicated genome-mining efforts, beginning in 2022 when a search for *selD*-colocalized biosynthetic genes uncovered two new Se—C bond-forming enzymes harbored by diverse bacterial species [5]. The first of these was the previously unannotated protein SenB, now known to be a selenosugar synthase. SenB carries out a Se-glycosylation reaction using a SeP and UDP-N-acetylglucosamine to furnish

Figure 2



Proposed catalytic mechanisms for **(a)** selenophosphate generation by SelD, **(b)** selenocysteine formation by SelA, and **(c)** selenouridine biosynthesis by SelU. Abbreviation: AMP = adenosine monophosphate.

Figure 3



(a) Biosynthesis of selenosugars and selenoimidazoles by the *sen* and *ovs* pathways in comparison to **(b)** biosynthesis of thioimidazoles by the *egt* and *ovo* pathways. **(c)** The *sen* and *ovs* pathways achieve Se–C bond cleavage through spontaneous selenoxide elimination, whereas **(d)** the *egt* and *ovo* pathways require additional enzymes for S–C bond cleavage.

N-acetyl-1-seleno-β-d-glucosamine (SeGlcNAc). The second enzyme discovered in this study was SenA, which catalyzes Se—C bond formation between the 2′-imidazole carbon of N-α-trimethyl-l-histidine (hercynine) and SeGlcNAc to generate the natural product selenoneine. An even more recent development revealed a similar reaction performed by OvsA, a divergent homolog of SenA, except with selenylation occurring at the 5′-position of His [6]. The product of this reaction, 5-selenohistidine (5-SeHis), undergoes a N-π methylation by the methyltransferase OvsM to complete the biosynthetic pathway of a novel selenometabolite, ovoselenol.

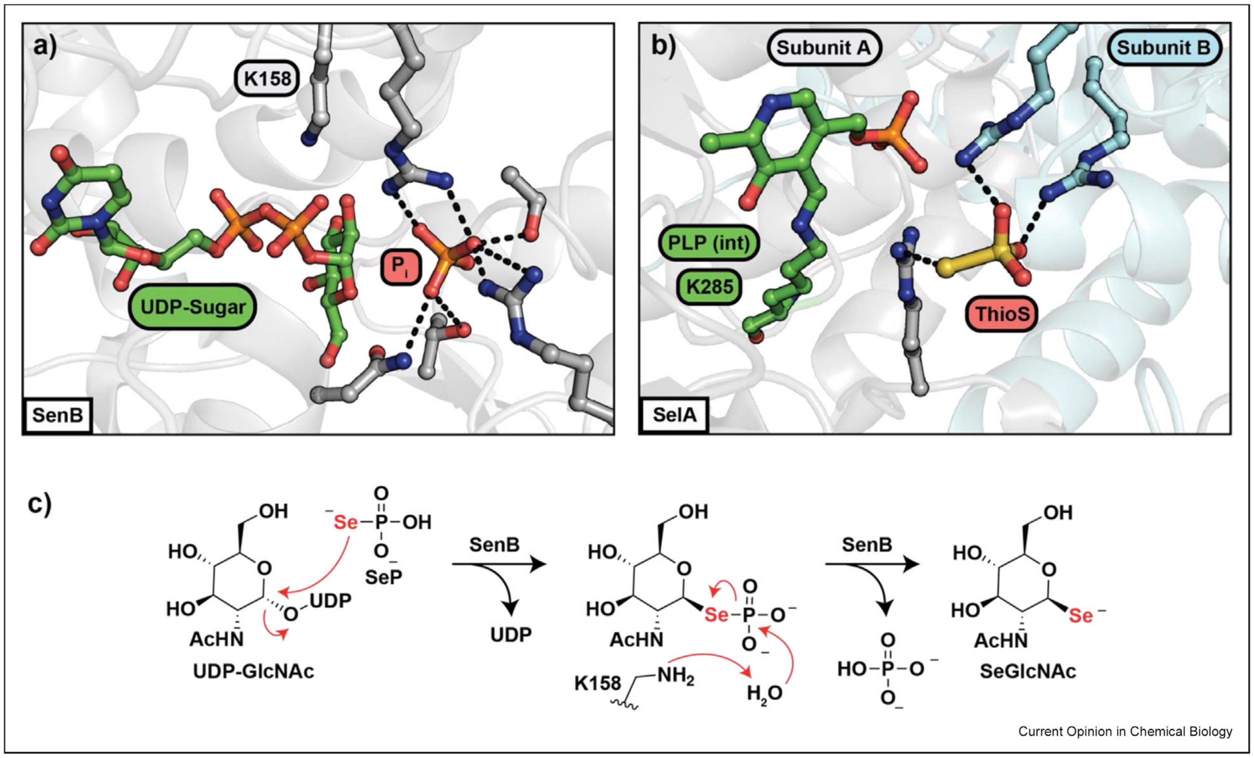
SenA and OvsA belong to a burgeoning class of metalloenzymes termed nonheme iron sulfoxide/selenoxide synthases (NHISS) that includes two other enzymes, EgtB and OvoA, involved in the biosynthesis of ergothioneine and ovothiol, the sulfur isologues of selenoneine and ovoselenol, respectively (Figure 3b) [38,39]. These enzymes all catalyze an interesting oxidative C—S/Se bond formation, linking their two substrates together with a sulfoxide or selenoxide bridge. In the cases of EgtB and OvoA, which utilize cysteine as their thiol substrate, additional enzymes (EgtE and OvoB, respectively) are required for the final C—S bond-cleavage step on route to the final product (Figure 3d) [38,40]. However, unlike sulfoxides that are chemically stable, the selenoxide products of SenA and OvsA undergo a spontaneous internal elimination (Figure 3c), obviating the need for an additional enzymatic step and underscoring Nature's resourceful use of Se's inherent reactivity [41,42].

Although only recently discovered, SenB, SenA, and OvsA have all been successfully crystallized, and the resulting ligand-bound X-ray structures reveal insights into their catalytic functions [43—45]. SenB, for example, is one of only three known SeP-utilizing enzymes, of which only SelA had been previously structurally characterized. It was thus not known whether these enzymes adopt a similar mode of SeP binding, which if true could imply a conserved motif that would facilitate in silico discovery of new SeP-utilizing enzymes. However, the structure of SenB with a bound SeP analog (P_i) revealed a completely different set of binding interactions compared to SelA, which forms a binding pocket for its SeP analog (thiosulfate) at the interface between two SelA protomers (Figure 4a,b) [44,31]. This implies evolutionarily convergent strategies for SeP binding by SenB and SelA. It remains to be determined whether SelU shares commonalities with either or exhibits yet another SeP binding mode.

Structural analyses of SenB have also unexpectedly revealed an overall topology and conformational dynamics similar to those of canonical glycosyltransferases, despite sharing only marginal homology [43]. Additionally, the active-site geometry and mutagenesis experiments support a two-step mechanism, whereby nucleophilic attack of SeP at the anomeric carbon forms the Se—C bond with inversion of stereochemistry, followed by base-activation of a bound water molecule by a conserved Lys to facilitate Se—P bond hydrolysis (Figure 4c) [44].

Substrate-bound crystal structures of SenA and OvsA have also helped decipher aspects of NHISS enzyme catalysis, including the sequence determinants of hercynine/His preference, as well as binding of the

Figure 4



Current Opinion in Chemical Biology

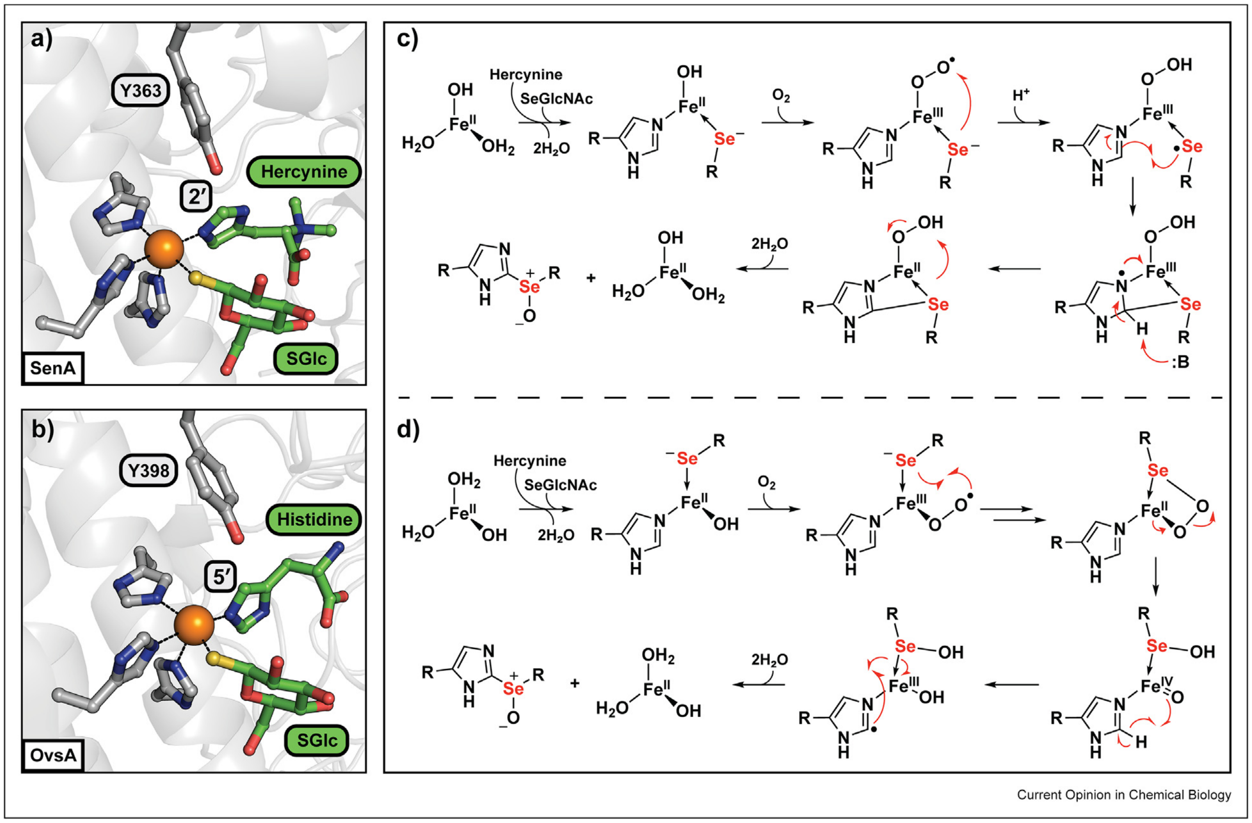
Comparison of **(a)** SenB (PDB: 8JJN, with bound SeP analog P_i) and **(b)** SelA (PDB: 3W1J, with bound SeP analog thiosulfate [ThioS]) active sites, revealing distinct SeP binding modes. **(c)** Proposed mechanism of selenosugar formation by SenB.

selenol/thiol donor in comparison to EgtB and OvoA [45,6]. These structures also reveal the basis for regioselectivity of Se–C bond formation, which is achieved through reorientation of the substrate imidazole ring in relation to the catalytic iron, which is coordinated by a three-His facial triad, and an essential tyrosine in the active site (Figure 5a,b). These features are conserved among all characterized NHISS enzymes, and in each case, the relevant imidazole carbon is pointed directly toward the catalytic tyrosine to effect 2′- or 5′-selenylation. The precise mechanism of NHISS-dependent C–S/Se bond formation, including the role of the catalytic tyrosine, is currently unknown. However, drawing from previous experimental and computation studies of EgtB and OvoA, two of many plausible mechanisms are shown (Figure 5c,d) [46–51]. In the first, ligation of selenol to the ferrous form and reaction with O_2 results in a ferric superoxide that may be converted to the hydroperoxo species via electron transfer from the Se-ligand (Figure 5c). The resulting radical could add into the His π-system to establish the Se–C bond and a N-centered radical. Rearomatization via reduction of the Fe center and dissociation of the Se ligand facilitated by cleavage of the weak O–O bond would complete turnover and generate the selenoxide that can undergo internal elimination, as described earlier. Alternatively, a mechanism involving ferryl-iron can be envisioned (Figure 5d). In this case, the ferric superoxide, formed analogously to the first pathway, could react with the Se ligand to form a ferrous peroxide species that could give rise to the highly oxidizing Fe^{IV}-oxo species, which could abstract the C2′–H atom. Rebound of the Se-ligand would complete turnover and deliver the selenoxide. Other mechanisms can be envisioned as well, and detailed kinetic and spectroscopic studies are necessary to differentiate between the possibilities.

Prospects for discovering new selenometabolite pathways

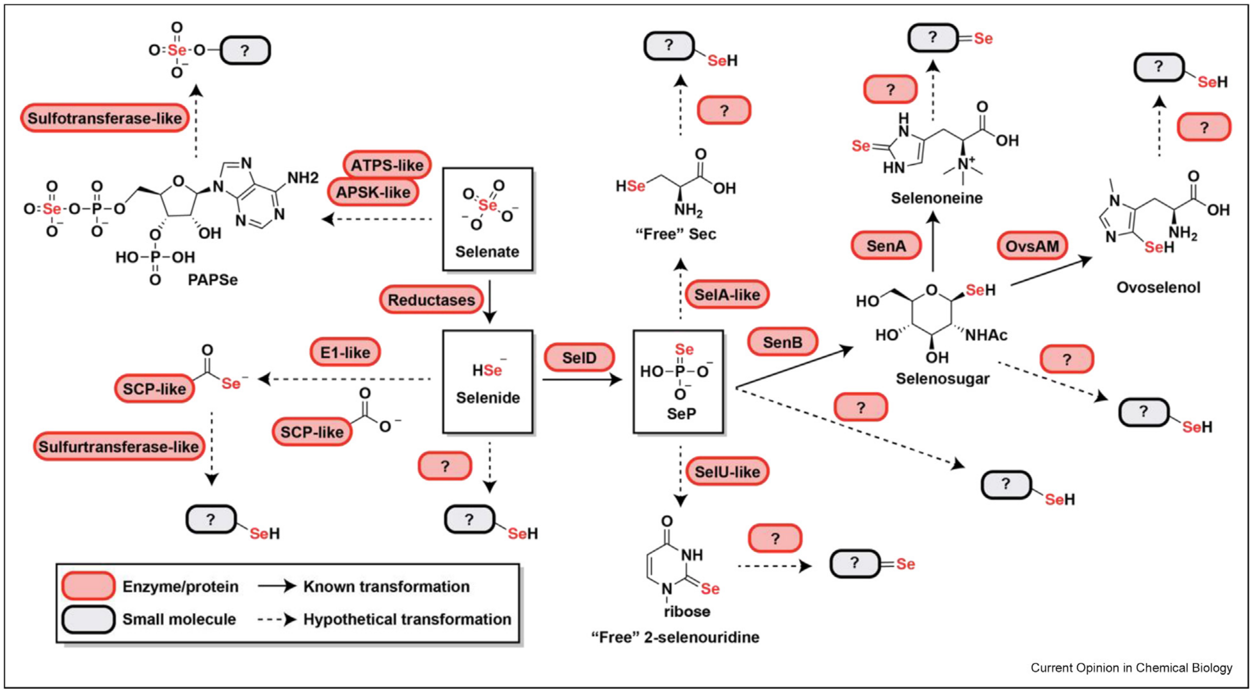
While much recent progress has been made toward identifying new regions of selenometobolism, there still remain relatively few specific biosynthetic pathways. However, we believe it is unlikely that the field has uncovered the entirety of Nature's biological Se repertoire, and we suspect many new and diverse pathways await discovery (Figure 6). For example, there is still no

Figure 5



Regioselectivity of Se–C bond formation by **(a)** SenA (PDB: 8K5I, with bound substrate analog thioglucose [SGlc]) and **(b)** OvsA (PDB: 8U41, with SGlc modeled in based on its orientation in SenA) is dictated by the orientation of the substrate imidazole with respect to an essential tyrosine. **(c,d)** Two possible mechanisms of selenoimidazole formation by NHISS enzymes. The function of the essential tyrosine is unknown. See text for further description.

Figure 6



Hypothetical selenometabolite pathways that may exist in nature. Pathways branching from selenide or selenate (i.e. SelD-independent) have never been observed, but are hypothesized in analogy to sulfur metabolism. Abbreviations: APSK = adenosine phosphosulfate kinase, ATPS = ATP sulfurylase, PAPSe = phosphoadenosine phosphoselenate, SCP = sulfur-carrier protein.

known mechanism for specific production of the free amino acid Sec, though it is conceivable that a SelA-like enzyme could perform this function by operating on a small-molecule (not tRNA-bound) substrate. Such a pathway could provide Sec as a building block for complex natural product pathways involving nonribosomal peptide synthetases, cyclodipeptide synthetases, ATP-grasp enzymes, PLP-dependent enzymes, or other amino-acid-accepting biocatalysts. Likewise, a hypothetical SelU-like enzyme could furnish free 2-selenouridine as an intermediate to selenonucleoside natural products. Furthermore, there may be additional SeP-utilizing enzymes that install novel Se—C bonds, as well as alternate biosynthetic fates of selenosugars and selenoimidazoles.

Another intriguing possibility would be Se-specific pathways that do not involve a SeP intermediate (i.e. SelD-independent), involving enzymes that instead accept more primitive substrates such as selenide. These could plausibly mirror sulfurtransferase pathways, which use as a substrate the terminal thiocarboxylate of a sulfur-carrier protein (SCP) generated by E1-like ligases from a sulfide equivalent [52,53]. A recent study has demonstrated the feasibility of this concept through chemical selenylation of SCPs, which were then found to be catalytically competent in enzymatic selenium-transfer reactions [54]. Other selenide-utilizing enzymes can also be envisioned (i.e. rhodanese-like), which, for instance, could offer an attractive alternative to the oxygen-dependent NHISS enzymes for selenoimidazole production in anaerobic organisms, as demonstrated previously in the anaerobic ergothioneine biosynthetic pathway [55].

In addition to selenide, SelD-independent pathways could also initiate directly from more oxidized forms of selenium, such as selenate or selenite. For example, akin to the ubiquitous sulfation pathway mediated by the sulfate donor phosphoadenosine phosphosulfate, there may exist an analogous pathway for producing phosphoadenosine phosphoselenate on route to selenated metabolites [56].

New strategies are clearly needed for identifying these putative pathways, although which specific approaches will be successful remains to be seen. Arguably the most likely reason for the current shortage is the fact that traditional nature product discovery protocols do not include Se supplementation in microbial culture media and are thus effectively blind to selenometabolites. Thus, perhaps the most obvious next step will be to subject diverse microorganisms to exogenous Se, followed by traditional techniques for microbial natural product discovery. Of course, further pathway characterization will be crucial to filter out compounds resulting from nonspecific Se-incorporation. On the other hand, genome-guided approaches may provide a more direct route toward identifying Se-specific pathways, although creative hypotheses and bioinformatic hooks are required to fish out these elusive genes. Computational approaches may also prove useful, for example to identify direct interactions between SelD and new SeP-utilizing enzymes through predictive modeling of protein–protein interfaces. While many unsolved problems remain, the selenometabolome is now ripe for discovery.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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