Received: 9 January 2024 | Revised: 18 April 2024 | Accepted: 21 April 2024

DOI: 10.1002/pro.5013

THE PROTEIN SOCIETY WILEY

RESEARCH NOTE

Quantitative entropy–enthalpy compensation in intraprotein interactions from model compound data

Thomas W. Redvanly | **Gary J. Pielak**

Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

Correspondence
Gary J. Pielak, Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.
Email: gary_pielak@unc.edu

Funding information
National Science Foundation, Grant/Award Number: CHE-2203505

Review Editor: Nir Ben-Tal

Abstract

Many small globular proteins exist in only two states—the physiologically relevant folded state and an inactive unfolded state. The active state is stabilized by numerous weak attractive contacts, including hydrogen bonds, other polar interactions, and the hydrophobic effect. Knowledge of these interactions is key to understanding the fundamental equilibrium thermodynamics of protein folding and stability. We focus on one such interaction, that between amide and aromatic groups. We provide a statistically convincing case for quantitative, linear entropy–enthalpy compensation in forming aromatic–amide interactions using published model compound transfer-free energy data.

KEYWORDS

compensation, enthalpy, entropy, entropy–enthalpy compensation, equilibrium thermodynamics, protein stability

The amino acid sequence of a globular protein determines its structure, stability, and function (Anfinsen, 1973). Proteins are stabilized by hydrogen bonds, other polar interactions and contacts between nonpolar atoms. Although weak, the large number of contacts is enough to offset the conformational entropy required to transform the disordered, unfolded state into the structured and stable folded state. Although it is now possible to predict structure from amino acid sequence (Jumper et al., 2021) and to design, de novo, sequences consistent with a given fold (Watson et al., 2023), fundamental features are not fully understood (Chen et al., 2023), including the energetics of the interactions that allow folding.

To fold, entities on proteins must lose at least some hydrating water to form the stabilizing interactions that facilitate folding. For years, investigators postulated the existence of a quantifiable relationship between the entropy and enthalpy of forming such interactions (Lumry & Rajender, 1970). For polar interactions, the idea is that the stronger the water–solute interaction, the more heat is required to break interactions with water and the larger the concomitant increase in the entropy of the water that is released on forming the intraprotein interaction. For nonpolar interactions, the change in entropy drives solvation but the enthalpy of H_2O—H_2O hydrogen bonds is also important (Chandler, 2005). The simplest form of such a relationship is linear,

$$\Delta H^{o\prime} = T_c \Delta S^{o\prime} + \Delta H_0{}^{o\prime}, \quad (1)$$

where $\Delta H^{o\prime}$ is enthalpy change, $\Delta S^{o\prime}$ is the entropy change, T_c is the slope, and $\Delta H_0^{o\prime}$ is the intercept ($\Delta H_0^{o\prime}$ is also $\Delta G_0^{o\prime}$ because $\Delta S_0^{o\prime}$ is zero at the intercept) (Leffler, 1955).

This linear effect, if it exists, is extrathermodynamic. That is, the laws of thermodynamics do not require a relationship between enthalpy and entropy except as stated by the Gibbs equation,

$$\Delta G^{o\prime} = \Delta H^{o\prime} - T\Delta S^{o\prime}, \quad (2)$$

where G is Gibbs free energy and T is the absolute temperature.

Identifying quantitative linear entropy–enthalpy compensation involves examining a series of related reactants. Here, we consider interactions between amides and aromatic functional groups, using data from Zytkiewicz et al. (2023). Their efforts focus on amides in aqueous naphthalene solutions to obtain, quoting from their publication, "the chemical potential derivative $(\partial\mu_2/\partial m_3)_{T,P,m_2}$ ..., a model-independent fundamental thermodynamic coefficient which can be interpreted as a transfer free energy and which quantifies the free energy of the preferential interaction of the two solutes, relative to their interactions with water" (Zytkiewicz et al., 2023), where μ is the chemical potential, P is the pressure, m is the molal concentration of the amide (subscript 2) or naphthalene (subscript 3). That is, $(\partial\mu_2/\partial m_3)_{T,P,m_2}$ quantifies the strength of amide–aromatic interactions. Such data are useful for applying Thomas Record's additivity-based solute partitioning model (Record et al., 2013) to understanding the interactions that stabilize globular proteins and how cosolutes affect stability (Capp et al., 2009).

The unique feature of the data reported by Zytkiewicz et al. (2023) is the temperature dependence of $(\partial\mu_2/\partial m_3)$, which allowed parsing of the free energy into its enthalpic and entropic components. The enthalpy was quantified via van't Hoff analysis. The entropic component was obtained from the Gibbs equation using a defined temperature, 298 K. However, as described next, the road to deriving a quantitative relationship between the entropy and enthalpy (Lumry & Rajender, 1970) is paved with false starts and controversy (Beasley et al., 2002; Krug et al., 1976a, 1976b, 1976c; Sharp, 2001).

For a series of reactions, the slope of a plot of each enthalpy change on the y-axis and each entropy change on the x-axis has units of temperature and is called the compensation temperature, T_c, because at T_c the free energy change is the same for all the reactions. Lumry and Rajender (1970) state that compensation temperatures for proteins "lie in a relatively narrow range, from about 250 to 315 K," but as shown by Krug et al. (1976a, 1976b, 1976c) and reinforced by others (Beasley et al., 2002; Sharp, 2001), this range mostly reflects the average temperature, T_{av} (more strictly the harmonic mean temperature) (Krug et al., 1976a, 1976b, 1976c) used to construct the van't Hoff plots that yield the enthalpy change of each reaction.

The van't Hoff enthalpy is determined from dependence of each equilibrium constant on inverse temperature, usually over the same narrow temperature range for each reaction. For the narrow range used here (283–318 K), the mean temperature (T_{av}, 299 K), the harmonic mean temperature (299 K), and temperature used to construct the plots (298 K) are essentially identical.

The entropy change is determined from the van't Hoff enthalpy via Equation (2), which means that the enthalpy and entropy are determined from the same data. Under these circumstances, there is a large chance that an observed linear relationship between enthalpy and entropy arises from the correlation of the uncertainties in the enthalpies and entropies rather than a real extrathermodynamic effect (Krug et al., 1976a, 1976b, 1976c). To be confident that T_c represents an extrathermodynamic effect, T_c and its uncertainty must not overlap T_{av}.

Two null hypotheses are useful for assessing the veracity of data purporting to show linear entropy–enthalpy compensation (Beasley et al., 2002; Krug et al., 1976b, 1976c). If these hypotheses cannot be rejected at the 95% confidence interval, we assume that the apparent relationship is consistent with uncorrelated data, and we conclude that there is no extrathermodynamic effect. The first hypothesis is that the slope of a plot of the standard-state van't Hoff enthalpy change ($\Delta H^{o\prime}$) on y versus the standard-state entropy change ($\Delta S^{o\prime}$) on x (Equation 1) is the same as the mean temperature used to derive the enthalpies (Krug et al., 1976c). Accepting this hypothesis is consistent with the correlation arising from the correlated uncertainties in the enthalpy and entropy data. These ideas were tested two decades ago on protein denaturation data; no evidence for quantitative compensation was found (Beasley et al., 2002).

Turning to the second hypothesis, Krug et al. (1976c) note that the uncertainty in the standard-state free energy change at T_c ($\Delta G^{o\prime}_{T_c}$) is uncorrelated with the uncertainty in $\Delta H^{o\prime}$. The slope of a plot of $\Delta H^{o\prime}$ on y versus $\Delta G^{o\prime}_{T_{av}}$ on x, which they call γ, contains a term for T_c,

$$\gamma = \frac{1}{1-(T_{av}/T_c)}. \tag{3}$$

Equation (3) can be obtained by combining Equations (1) and (2) (The derivation is shown in the Supporting Information.). The limiting case where T_{av} equals T_c merits comment. In this instance, γ equals infinity, which means that a $\Delta H^{o\prime} - \Delta G^{o\prime}_{T_{av}}$ plot is not useful for detecting linear entropy–enthalpy compensation (Krug et al., 1976c).

As discussed by Krug et al. (1976c), a slope of one suggests that the data are consistent with Equation (2), and there is no need to invoke an extrathermodynamic effect. Therefore, the second null hypothesis is that γ equals one. Rejecting both hypotheses suggests the existence of an extrathermodynamic effect.

Zytkiewicz et al. (2023) refer to $(\partial\mu_2/\partial m_3)_{T,P,m_2}$ as $\mu_{2,3}$ and the associated enthalpy and entropy changes for

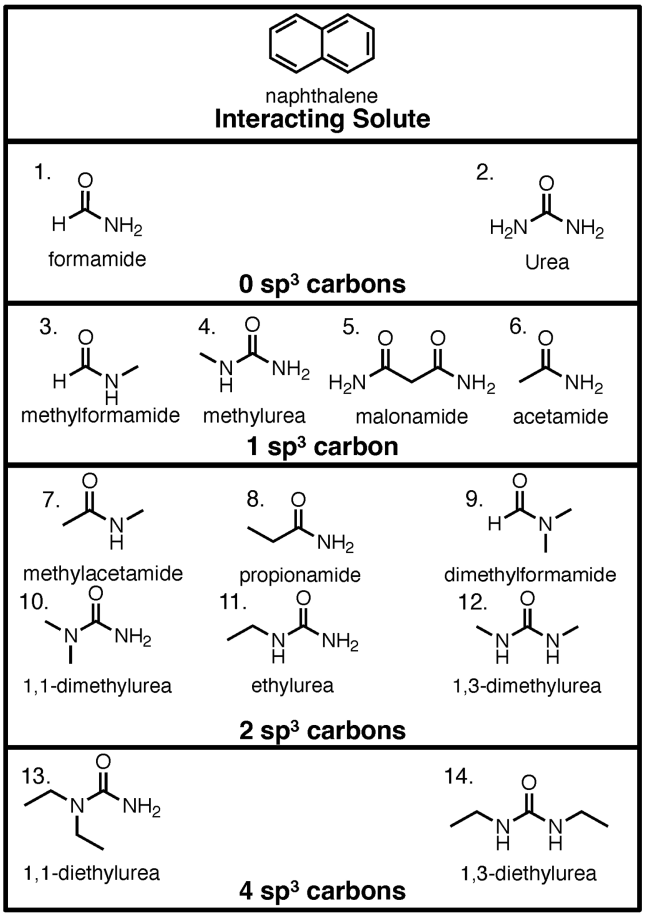

FIGURE 1 Structures of naphthalene and amides with number of sp^3 carbons.

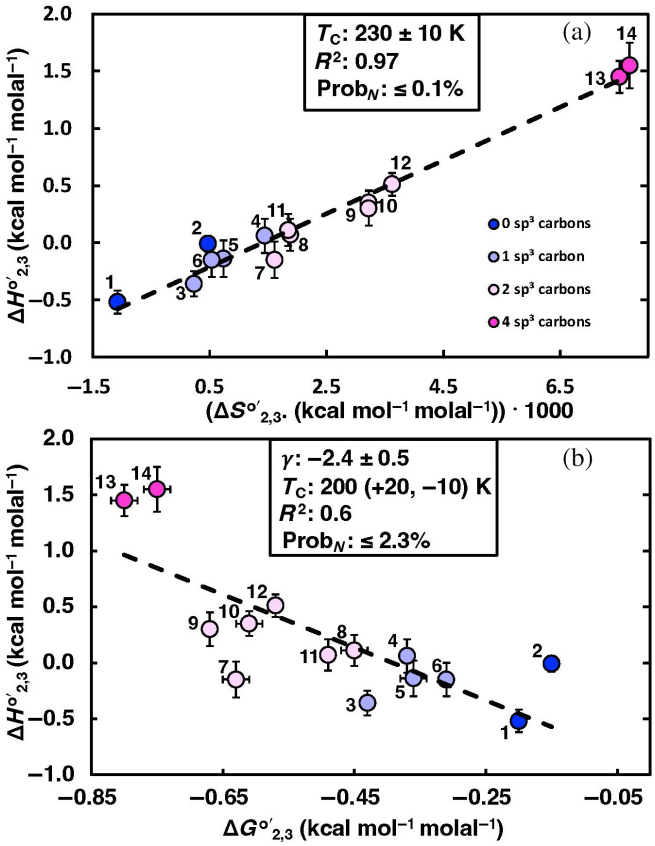

FIGURE 2 (a) Transfer enthalpy of naphthalene, $\Delta H^{o\prime}_{2,3}$, versus transfer entropy of naphthalene, $\Delta S^{o\prime}_{2,3}$. (b) $\Delta H^{o\prime}_{2,3}$ versus transfer free energy of naphthalene, $\Delta G^{o\prime}_{2,3}$, at 298 K. The number of sp^3 carbons in the amides is indicated by the color scheme and the identity of the amides in Figure 1 is indicated by numbers.

the interaction as $\Delta h_{2,3}$ and $\Delta s_{2,3}$ but we use the more familiar notations, $\Delta G^{o\prime}_{2,3}$, $\Delta H^{o\prime}_{2,3}$, and $\Delta S^{o\prime}_{2,3}$, respectively. They also assume, justifiably, that there is no heat capacity difference between the products and reactants over the temperatures studied, such that $\Delta H^{o\prime}_{2,3}$ and $\Delta S^{o\prime}_{2,3}$ are temperature independent. We used the values and uncertainties from tab. 1 of Zytkiewicz et al. (2023). The structures of the solutes are shown in Figure 1. Our analysis is shown in Figure 2.

We evaluated the data for the first hypothesis (Figure 2a) using linear least-squares analysis. T_c is 230 ± 10 K, 53 K lower than the lowest temperature used to acquire the data, suggesting that T_c does not arise from correlation of uncertainties. The enthalpy change increases as the entropy change increases. This trend correlates with an increasing number of sp^3 carbons (Figure 1). Additional evidence comes from statistical analysis. Given the Pearson correlation coefficient (R^2) of 0.97, the probability that the correlation from these 14 points arises from uncorrelated data is ≤0.1% (Taylor, 1982). Thus, the first hypothesis is rejected.

We tested the second hypothesis by plotting $\Delta H^{o\prime}_{2,3}$ against $\Delta G^{o\prime}_{2,3}$ at 298 K (Figure 2b). The enthalpy change decreases as the free energy change increases, and the points again group by the number of sp^3 carbons (Figure 1). We used linear least-squares analysis to calculate γ and its uncertainty. We then used Equation (3) to determine T_c and estimated its uncertainty by using Equation (3) and the uncertainty in γ. T_c is 200 (+20, −10) K, which just overlaps with the value from the enthalpy–entropy plot (Figure 2b). Given the R^2 of 0.6, the probability that the correlation from these 14 points arises from unrelated data is ≤2.3%. Thus, the second hypothesis is also rejected. We conclude that there exists an extrathermodynamic effect described by a linear relationship between the entropy and enthalpy for interactions between amides and aromatics.

Entropy–enthalpy compensation probably exists for other interactions, and one might anticipate that studies like that of Zytkiewicz et al. (2023) could be repeated for other noncovalent intraprotein interactions. However,

this dataset is perhaps unique for two reasons. First, it depends on the low solubility of naphthalene. Other side chain models are too soluble for these kinds of studies, which means that osmometry would need to be used, leading to the second limitation, as stated by the authors (Zytkiewicz et al., 2023): simple osmometry can only assess a few temperatures making it less suited to measuring van't Hoff enthalpies.

The hypothesis that intraprotein interactions should exhibit quantitative entropy–enthalpy has been around for over 50 years (Lumry & Rajender, 1970), but there was little statistically significant analysis. Analysis of model compound data from Zytkiewicz et al. (2023) provides quantitative support for the idea of compensation in intraprotein interactions. The observation of authentic entropy–enthalpy compensation increases our fundamental knowledge of protein hydration and can be further exploited to manipulate protein thermodynamics by introducing non-natural amino acids (Kwon, 2023).

AUTHOR CONTRIBUTIONS

Thomas W. Redvanly: Conceptualization; investigation; writing – original draft; methodology; visualization; writing – review and editing; formal analysis; data curation. **Gary J. Pielak:** Conceptualization; writing – original draft; funding acquisition; methodology; validation; writing – review and editing; formal analysis; supervision; resources.

ACKNOWLEDGMENTS

Our laboratory is supported by the National Science Foundation (CHE-2203505). We thank Elizabeth Pielak for comments on the manuscript. T.W.R. and G.J.P. designed the research and wrote the paper.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Thomas W. Redvanly https://orcid.org/0009-0008-1785-1975

Gary J. Pielak https://orcid.org/0000-0001-6307-542X

REFERENCES

Anfinsen CB. Principles that govern the folding of protein chains. Science. 1973;181:223–30.

Beasley JR, Doyle DF, Chen L, Cohen DS, Fine BR, Pielak GJ. Searching for quantitative entropy–enthalpy compensation among protein variants. Proteins. 2002;49:398–402.

Capp MW, Pegram LM, Saecker RM, Kratz M, Riccardi D, Wendorff T, et al. Interactions of the osmolyte glycine betaine with molecular surfaces in water: thermodynamics, structural interpretation, and prediction of m-values. Biochemistry. 2009; 48:10372–9.

Chandler D. Interfaces and the driving force of hydrophobic assembly. Nature. 2005;437:640–7.

Chen S-J, Hassan M, Jernigan RL, Jia K, Kihara D, Kloczkowski A, et al. Opinion: protein folds vs. protein folding: differing questions, different challenges. Proc Natl Acad Sci USA. 2023;120:e2214423119.

Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al. Highly accurate protein structure prediction with AlphaFold. Nature. 2021;596:583–9.

Krug RR, Hunter WG, Grieger RA. Statistical interpretation of enthalpy-entropy compensation. Nature. 1976a;261:566–7.

Krug RR, Hunter WG, Grieger RA. Enthalpy–entropy compensation. 1. Some fundamental statistical problems associated with the analysis of van't Hoff and Arrhenius data. J Phys Chem. 1976b;80:2335–41.

Krug RR, Hunter WG, Grieger RA. Enthalpy–entropy compensation 2. Separation of the chemical from the statistical effect. J Phys Chem. 1976c;80:2341–51.

Kwon D. How scientists are hacking the genetic code to give proteins new powers. Nature. 2023;618:874–6.

Leffler JE. The enthalpy-entropy relationship and its implications for organic chemistry. J Org Chem. 1955;20:1202–31.

Lumry R, Rajender S. Enthalpy–entropy compensation and phenomena in water solutions of proteins and small molecules: a ubiquitous property of water. Biopolymers. 1970;9:1125–7.

Record MT, Guinn E, Pegram L, Capp M. Introductory lecture: interpreting and predicting Hofmeister salt ion and solute effects on biopolymer and model processes using the solute partitioning model. Faraday Discuss. 2013;160:9–44.

Sharp K. Entropy—enthalpy compensation: fact or artifact? Protein Sci. 2001;10:661–7.

Taylor JR. An introduction to error analysis. Mill Valley, CA: University Science Books; 1982.

Watson JL, Juergens D, Bennett NR, Trippe BL, Yim J, Eisenach HE, et al. De novo design of protein structure and function with RFdiffusion. Nature. 2023;620:1089–100.

Zytkiewicz E, Shkel IA, Cheng X, Rupanya A, McClure K, Karim R, et al. Quantifying amide–aromatic interactions at molecular and atomic levels: experimentally determined enthalpic and entropic contributions to interactions of amide sp^2O, N, C and sp^3C unified atoms with naphthalene sp^2C atoms in water. Biochemistry. 2023;62:2841–53.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Redvanly TW, Pielak GJ. Quantitative entropy–enthalpy compensation in intraprotein interactions from model compound data. Protein Science. 2024;33(6):e5013. https://doi.org/10.1002/pro.5013