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A study on the enthalpy–entropy compensation in protein unfolding

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Abstract

A large number of thermodynamic data including the free energy, enthalpy, entropy, and heat capacity changes were collected for the denaturation of various proteins. Regression indicated that remarkable enthalpy–entropy compensation occurred in protein unfolding, which meant that the change in enthalpy was almost compensated by a corresponding change in entropy resulting in a smaller net free energy change. This behavior was proposed to result from the water molecule reorganization, which contributed significantly to the enthalpy and entropy changes but little to the free energy change in protein unfolding. It turned out that the enthalpy–entropy compensation could provide novel insights into the problem of enthalpy and entropy convergence in protein unfolding. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Enthalpy–entropy compensation; Enthalpy and entropy convergence; Solvent reorganization; Protein unfolding

1. Introduction

As one of the centric topics for understanding life at the molecular level, protein unfolding has attracted great attention in physics, biology and chemistry for decades [1–5]. Despite the considerable progress that has been made on this issue, a number of problems still remain poorly understood, such as the forces that stabilize the native proteins [6,7] and the famous Privalov's puzzle of enthalpy and entropy covergence [8].

Physicochemical studies provide fundamental knowledge on protein unfolding [9]. To date, vast amount of thermodynamic data have been accumulated for the denaturation of various proteins. Systematic studies on these data have recently

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attracted considerable interests and turned out much valuable information [10–14]. Herein, the intriguing phenomenon of enthalpy–entropy compensation (EEC) in protein unfolding was reported. This phenomenon, which meant that in many chemical processes the change in enthalpy was partially or almost compensated by a corresponding change in entropy resulting in a smaller net free energy change, has been widely observed. However, the physical origin of the behavior remains unclear.

2. Enthalpy–entropy compensation in protein unfolding

2.1. Experimental observations

Protein unfolding appears as a two-state transition between a native, N, and an unfolded, U, state [15,16]. The native state corresponds to the compact global protein molecule, with most nonpolar side chains located in the interior of the conformation. The unfolded state approaches a random flight coil with a significant degree of solvent exposure of its side chains and backbone [17]. $\Delta G_{un}(T)$, $\Delta H_{un}(T)$, $\Delta S_{un}(T)$, and $\Delta C_{un}(T)$ are the free energy, enthalpy, entropy and heat capacity changes for the N ⇋ U transition at temperature T. They have been extensively measured especially by the high-sensitivity Differential Scanning Calorimetry (DSC) [18–20].

Since detailed DSC measurement showed that $\Delta C_{un}(T)$ is slightly temperature-dependent [21,22], it is reasonable to assume ΔC_{un} to be invariant within a moderate temperature range. Therefore, if the thermodynamic data at a certain temperature T_0 are available, the thermodynamic data at another temperature T are also known according to the following equations [9]:

$$\Delta H_{un}(T) = \Delta H_{un}(T_0) + \Delta C_{un}(T - T_0) \quad (1)$$

$$\Delta S_{un}(T) = \Delta S_{un}(T_0) + \Delta C_{un} \ln \frac{T}{T_0} \quad (2)$$

and

$$\begin{aligned}\Delta G_{un}(T) &= \Delta H_{un}(T) - T\Delta S_{un}(T) \\ &= \Delta H_{un}(T_0) - T\Delta S_{un}(T_0) \\ &\quad + \Delta C_{un}\left[(T - T_0) - T \ln \frac{T}{T_0}\right]\end{aligned} \quad (3)$$

In the present study, 3224 sets of experimental thermodynamic data including $\Delta G_{un}(T)$, $\Delta H_{un}(T)$, $\Delta S_{un}(T)$, and ΔC_{un}, were collected for the denaturation of various proteins under different kinds of conditions. For a meaningful investigation [23], all the thermodynamic data were converted to ΔG_{un} (298 K), ΔH_{un} (298 K), and ΔS_{un} (298 K) according to Eqs. (1)–(3), respectively. Linear correlations between ΔH_{un} (298 K) and T (298 K) ΔS_{un} (298 K), as well as ΔH_{un} (298 K) and ΔG_{un} (298 K) were performed on the whole data (see Fig. 1). The correlation results fit the following equations:

$$\begin{aligned}&T\,(298\text{K})\Delta S_{un}\ (298\ \text{K}) \\ &\quad = 0.909\,\Delta H_{un}\ (298\ \text{K}) - 12.34 \\ &\quad (r = 0.991,\ \text{S.D.} = 15.67,\ n = 15.60, \\ &\quad n = 3224,\ P < 0.0001)\end{aligned} \quad (4)$$

and

$$\begin{aligned}\Delta G_{un}(298\ \text{K}) &= 0.091\ \Delta H_{un}\ (298\ \text{K}) + 12.34 \\ &\quad (r = 0.600,\ \text{S.D.} = 15.60, \\ &\quad n = 3224,\ P < 0.0001)\end{aligned} \quad (5)$$

From the above results, it is obvious that protein unfolding exhibits excellent EEC, an intriguing phenomenon widely observed in chemistry and biophysics [24,25]. The correlation coefficient (0.991) for such a huge sample ($n = 3224$) is remarkable. The slope (0.909) is close to unity, which means that any variation in ΔH will be offset by a corresponding variation in ΔS, resulting in a small variation in ΔG. However, the correlation between ΔH and ΔG is very poor with a correlation coefficient of 0.600, indicating no mutual dependence between the two variables. The quite small slope (0.091) also indicates the enthalpy–entropy compensation.

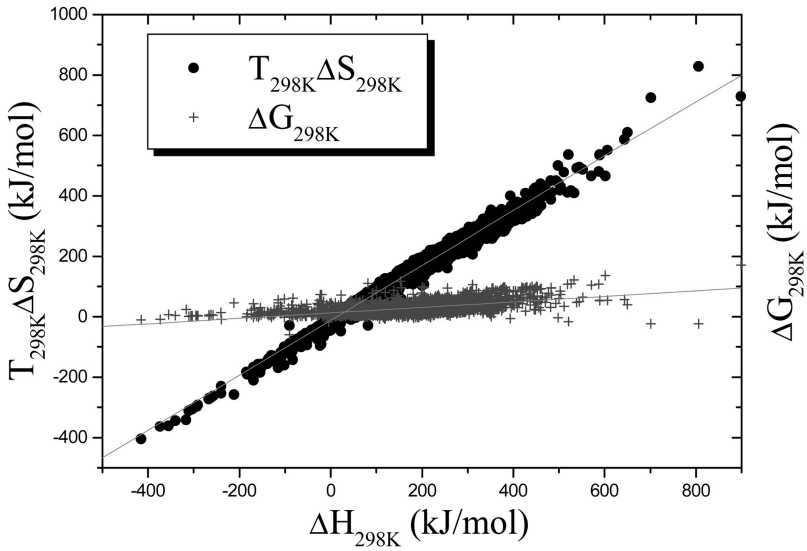

Fig. 1. Enthalpy–entropy compensation in protein unfolding. The data were collected from the various literature (see text).

2.2. Theoretical explanation

EEC remains an unsettled puzzle. Despite wide documentation [24,25], it is still often regarded as a statistical artifact from the van't Hoff plot [26–30]. However, the thermodynamic data used in the present analysis were mostly obtained via the direct calorimetric measurements; the size of the sample was also very large. Therefore, the compensation observed here seemed a fact resulting from certain physical reasons.

Although there has been many studies on the relationship between different thermodynamic quantities in protein unfolding [10–14], little has been reported on the remarkable EEC effect. This seems an omission in understanding the behaviors of proteins. In 1970, Lumry and Rajender [24] firstly noticed the compensation of the changes in ΔH and ΔS of the unfolding of ribonuclease when adding ethanol to the aqueous solution. The initial report of the EEC between the mutants of a protein appeared in 1980s [31,32], and Shortle et al. [33] discovered a large compensation effect in the denaturation of the mutants of *Staphylococcal* nuclease. In 1995, Makhatadze et al. reported the solvent isotope effect in protein stability [34]. The changes in the hydration of proteins in D_2O compared to H_2O were found to produce EEC and thus little effect on the protein stability. Very recently, Johnson et al. [35] reported another observation of EEC in the denaturation of mutants of barnase with disulfide crosslinks. However, the physical origin of the EEC effect remains unclear [36,37].

To date, there still lacks a general model capable of connecting the experimental EEC with a theoretical explanation at the molecular level [38,39]. Although some theories have been proposed, they often rely heavily in the specific details of the systems under consideration and were not generally applicable [40–50]. Nevertheless, a recent viewpoint that EEC is the outcome of the solvent reorganization seems interesting and reasonable [24,51–59].

Herein, a theoretical model based on the concept of solvent reorganization was strictly developed for the EEC in protein unfolding. Considering that there is a certain amount of protein in a dilute aqueous solution, in which there thus will be five distinguishable species at equilibrium, i.e. W/w, N/w, W/n, U/w, and W/u [56–62]. The capital letters stand for the water molecules (W), the protein molecules in the native state (N) and in the unfolded state (U). The lower-case letters immediately following describe the environmental constraint sensed by the species. For example,

W/w represents a water molecule in bulk aqueous solution and hence surrounded solely by water, while W/n represents the water molecule in the hydration shell of a folded protein molecule and hence contacting that molecule.

The enthalpy of the solution at temperature T and pressure P can be written as

$$H_{\text{initial}} = h_{\text{N/w}} \cdot n_{\text{N/w}} + h_{\text{U/w}} \cdot n_{\text{U/w}} + h_{\text{W/n}} \cdot n_{\text{W/n}} + h_{\text{W/u}} \cdot n_{\text{W/u}} + h_{\text{W/w}} \cdot n_{\text{W/w}} \quad (6)$$

where h represents the partial molar enthalpy, and n the quantities of the species.

Considering that the protein unfolding undergoes a sequence of quasistatic processes, thus

$$\Delta H = \int \mathrm{d}H \quad (7)$$

In each quasistatic process, the enthalpy change ($\mathrm{d}H$) is caused by the changes in the partial molar enthalpies and the amounts of the solution species, i.e. $\mathrm{d}h_i$ and $\mathrm{d}n_i$ (herein i represents N/w, U/w, W/n, W/u, and W/w, respectively). Therefore,

$$\mathrm{d}H = n_{\text{N/w}} \cdot \mathrm{d}h_{\text{N/w}} + h_{\text{N/w}} \cdot \mathrm{d}n_{\text{N/w}} + n_{\text{U/w}} \cdot \mathrm{d}h_{\text{U/w}} + h_{\text{U/w}} \cdot \mathrm{d}n_{\text{U/w}} + n_{\text{W/n}} \cdot \mathrm{d}h_{\text{W/n}} + h_{\text{W/n}} \cdot \mathrm{d}n_{\text{W/n}} + n_{\text{W/u}} \cdot \mathrm{d}h_{\text{W/u}} + h_{\text{W/u}} \cdot \mathrm{d}n_{\text{W/u}} + n_{\text{W/w}} \cdot \mathrm{d}h_{\text{W/w}} + h_{\text{W/w}} \cdot \mathrm{d}n_{\text{W/w}} \quad (8)$$

According to Gibbs–Duhem's equation [63,64], the changes in the partial molar enthalpy obey the following equation,

$$n_{\text{N/w}} \cdot \mathrm{d}h_{\text{N/w}} + n_{\text{U/w}} \cdot \mathrm{d}h_{\text{U/w}} + n_{\text{W/n}} \cdot \mathrm{d}h_{\text{W/n}} + n_{\text{W/u}} \cdot \mathrm{d}h_{\text{W/u}} + n_{\text{W/w}} \cdot \mathrm{d}h_{\text{W/w}} = 0 \quad (9)$$

Therefore,

$$\mathrm{d}H = h_{\text{N/w}} \cdot \mathrm{d}n_{\text{N/w}} + h_{\text{U/w}} \cdot \mathrm{d}n_{\text{U/w}} + h_{\text{W/n}} \cdot \mathrm{d}n_{\text{W/n}} + h_{\text{W/u}} \cdot \mathrm{d}n_{\text{W/u}} + h_{\text{W/w}} \cdot \mathrm{d}n_{\text{W/w}} \quad (10)$$

Since the total amount of the protein molecules remains invariant, i.e.

$$\mathrm{d}n_{\text{N/w}} + \mathrm{d}n_{\text{U/w}} = 0 \quad (11)$$

Eq. (10) can be further simplified as

$$\mathrm{d}H = (h_{\text{N/w}} - h_{\text{U/w}}) \cdot \mathrm{d}n_{\text{N/w}} + h_{\text{W/n}} \cdot \mathrm{d}n_{\text{W/n}} + h_{\text{W/u}} \cdot \mathrm{d}n_{\text{W/u}} + h_{\text{W/w}} \cdot \mathrm{d}n_{\text{W/w}} \quad (12)$$

In consequence

$$\Delta H = \int \mathrm{d}H = \int (h_{\text{N/w}} - h_{\text{U/w}}) \cdot \mathrm{d}n_{\text{N/w}} + \int h_{\text{W/n}} \cdot \mathrm{d}n_{\text{W/n}} + \int h_{\text{W/u}} \cdot \mathrm{d}n_{\text{W/u}} + \int h_{\text{W/w}} \cdot \mathrm{d}n_{\text{W/w}} \quad (13)$$

Similarly, the entropy, free energy and heat capacity changes in protein unfolding are:

$$\Delta S_{\text{un}} = \int (s_{\text{N/w}} - s_{\text{U/w}}) \cdot \mathrm{d}n_{\text{N/w}} + \int s_{\text{W/n}} \cdot \mathrm{d}n_{\text{W/n}} + \int s_{\text{W/u}} \cdot \mathrm{d}n_{\text{W/u}} + \int s_{\text{W/w}} \cdot \mathrm{d}n_{\text{W/w}} \quad (14)$$

$$\Delta G_{\text{un}} = \int (\mu_{\text{N/w}} - \mu_{\text{U/w}}) \cdot \mathrm{d}n_{\text{N/w}} + \int \mu_{\text{W/n}} \cdot \mathrm{d}n_{\text{W/n}} + \int \mu_{\text{W/u}} \cdot \mathrm{d}n_{\text{W/u}} + \int \mu_{\text{W/w}} \cdot \mathrm{d}n_{\text{W/w}} \quad (15)$$

and

$$\Delta C_{un} = \int (c_{N/w} - c_{U/w}) \cdot dn_{N/w} + \int c_{W/n} \cdot dn_{W/n} + \int c_{W/u} \cdot dn_{W/u} + \int c_{W/w} \cdot dn_{W/w} \quad (16)$$

where s, μ and c represent the partial molar entropy, chemical potential and partial heat capacity, respectively.

Interestingly, since the solution remains in a thermodynamic equilibrium during every quasistatic process, the following chemical potentials are always equal [49,50],

$$\mu_{\text{W/n}} = \mu_{\text{W/u}} = \mu_{\text{W/w}} \quad (17)$$

Meanwhile, the total amount of the water molecules is invariant, i.e.

$$\mathrm{d}n_{\text{W/n}} + \mathrm{d}n_{\text{W/u}} + \mathrm{d}n_{\text{W/w}} = 0 \quad (18)$$

Therefore, the last three terms in ΔG actually disappear, i.e.

$$\Delta G = \int(\mu_{N/w} - \mu_{U/w}) \cdot dn_{N/w} \quad (19)$$

This means that the corresponding three terms in ΔH_{un} and ΔS_{un} offset each other, i.e.

$$\int(h_{W/n} \cdot dn_{W/n} + h_{W/u} \cdot dn_{W/u} + h_{W/w} \cdot dn_{W/w}) = \int(s_{W/n} \cdot dn_{W/n} + s_{W/u} \cdot dn_{W/u} + s_{W/w} \cdot dn_{W/w}) \cdot T \quad (20)$$

Eqs. (19) and (20), which are obtained strictly from the fundamental thermodynamics, probably provide the physical origin of the EEC in protein unfolding. Since the compensation terms are related to the reorganization of the water molecules, the water molecule reorganization does not contribute to the overall free energy change of protein unfolding.

However, the water molecule reorganization would significantly contribute to the overall ΔH_{un} and ΔS_{un} in protein unfolding, because the species of the same compounds at thermodynamic equilibrium but with different environmental restrictions generally differ in their partial molar enthalpy and entropy (e.g. $h_{W/n} \neq h_{W/u} \neq h_{W/w}$, and $s_{W/n} \neq s_{W/u} \neq s_{W/w}$ in general) [44,45]. In aqueous solution, these differences could even be very large due to the strong solvent–solvent hydrogen-bonding interactions [65]. Recently, the thermodynamics of the solvent reorganization during the hydration of some organic compound were investigated [66,67]. The entropy changes of the solvent reorganization at 25°C were estimated to be 23.3 J/K·mol on average for all the compounds, while the variation of these entropy changes could be as large as 40.0 J/K·mol from one compound to the other. These values were instructive when compared to the configurational entropy change of protein unfolding, which was estimated to centralize in a rather small range from 15 to 20 J/K per mole of amino acid residue at 25°C. Obviously, the magnitude and range of the entropy change from the configuration change in protein unfolding, which is defined as the entropy in the absence of solvent, is much smaller than that of the accompanying solvent reorganization.

In brief, the enthalpy and entropy changes in protein unfolding could be written as

$$\Delta H_{un} = \Delta H_P + \Delta H_w \quad (21)$$

and

$$\Delta S_{un} = \Delta S_P + \Delta S_w \quad (22)$$

in which P indicates that the parameters are related to the structural changes of the protein molecules itself (i.e. the first term in Eqs. (13) and (14)), while w means that the parameters are related to the accompanying water molecule reorganization (i.e. the last three terms in Eqs. (13) and (14)) during protein unfolding. Since the enthalpy and entropy related to water molecule reorganization compensate each other, i.e.

$$\Delta H_w = T\Delta S_w \quad (23)$$

the free energy change of protein unfolding is

$$\Delta G_{un} = \Delta G_P = \Delta H_P - T\Delta S_P \quad (24)$$

As indicated above, the variation in ΔH_W (or ΔS_W) for the unfolding of different protein is much larger than in ΔH_P (or ΔS_P), i.e.

$$|\delta\Delta H_P| \ll |\delta\Delta H_w| \quad (25)$$

Hence,

$$|\delta\Delta G_{un}| = |\delta\Delta G_P| \ll |\delta\Delta H_{un}| \quad (26)$$

This clearly accounted for the observed enthalpy–entropy compensation.

2.3. Discussion

It is worth pointing out that the present model of EEC is strictly deduced from the fundamental thermodynamics without any relation to the theories of the hydrophobic interactions. This argument is important, since the theories on the hydrophobic interaction [68] are currently in an unprecedented degree of confusion [69–71].

Traditionally, hydrophobicity was considered as the result of the enhanced structure of the water molecules in the near vicinity of the non-polar solute, which would bring about a usually large entropy loss during the hydration [72]. Sometimes, this explanation was even overemphasized, resulting in the postulation of the iceberg- or clathrate-like structures of the hydrophobic hydration shell [73,74]. According to this model, the destructive overlap of the hydrophobic hydration shell, which was entropically favorable due to the release of the structure hydration water, constituted a driving force for the aggregation of non-polar solutes in aqueous solution [75–78]. This driving force was usually named hydrophobic interaction [79–81].

However, the above microscopic picture of the hydrophobic effect was greatly challenged recently. Neither the neutron scattering measurements nor the computer simulations indicated any evidence that the structure of the hydration water close to a non-polar group was more ordered than that of water in the bulk [82–86]. It was argued that the loss of entropy upon hydrophobic hydration [87] could be well explained alternatively without assuming the formation of a more rigid structure [88,89], for the reduction of rotational freedom and the compression of water molecules in the hydration shell could produce the entropy loss as well [90].

3. Enthalpy and entropy convergence in protein unfolding

3.1. Introductory remarks

As an independent rule, EEC could offer fresh insights into the understanding of protein unfolding. Firstly, EEC can serve as an empirical rule to test the reliability of experimental thermodynamic data. If the data when added to the plot in Fig. 1 deviate significantly from the compensation line, the data are questionable. Secondly, the present model of EEC provides new information to the unsettled problem of enthalpy and entropy convergence in protein unfolding.

The phenomenon of the enthalpy and entropy convergence was discovered by Privalov [91–94], who insightfully suggested that ΔH_{un} and ΔS_{un} converged at some characteristic temperature around 100°C when normalized with respect to the number of the amino acid residues in the protein [91–94]. This intriguing behavior has been carefully reexamined in many following studies, but no firm conclusions have been reached yet. Although sometimes the convergence temperatures for the enthalpy and entropy changes (T_H and T_S, respectively) were proposed to be identical [8,72], it was also suggested that T_H was centered around 100°C whereas T_S was located near 110°C [95,96].

In 1986, Baldwin provided the first explanation for the behavior [97,98], who regarded the thermodynamics of the hydration of liquid hydrocarbons in water as a reasonable model for the temperature dependence of the hydrophobic interaction in protein unfolding. It was found that at the temperature around 113°C an aqueous solution of a non-polar substance appeared to be a regular solution, in which the entropy of transfer reached zero. Since this temperature was strikingly similar to the entropy convergence temperature of protein unfolding, the hydrophobic interaction seemed to play a key role in entropy convergence.

Lee [99] provided an elegant explanation for the entropy and enthalpy convergence based on the linear relationships between the thermodynamic quantities (i.e. ΔG_{un}, ΔH_{un}, ΔS_{un} and ΔC_{un}) and a temperature-independent molecular property. For protein unfolding, this property was suggested to measure the polar/non-polar mix of the internal interaction within the protein interior. The coincidence of T_S and T_H was regarded as the consequence of the fact that the free energy changes upon exposure of the polar and non-polar groups happened to be closely similar on a per area basis at T_S.

Murphy and Gill [100,101] also regarded the linear relationships of ΔH_{un}, and ΔS_{un} with ΔC_{un} as the result of group additivity. T_H and T_S were proposed as the temperatures at which the contributions of the apolar group exposure to the overall enthalpy and entropy changes approached zero. The analysis of thermodynamic data of the

dissolution of solid cyclic dipeptide into water was original, although whether the solid or the liquid [102,103] model compounds should be chosen for protein unfolding was still under debate. Interestingly, in a later paper, Murphy [104] chose the aqueous dissolution of gaseous alcohols and alkanes to study the protein unfolding.

Later, Baldwin et al. [105] modified their previous conclusions. The coincidence of T_H and T_S was thought to arise straightforwardly from the fact that the temperature midpoints (T_m) of the unfolding of different proteins were close to one another. This interesting idea was similar with that of Doig and Williams [106], who considered the enthalpy convergence of the protein unfolding as the consequence of the fact that proteins have evolved to have similar stability around room temperatures. Meanwhile, Fu and Freire [107] measured the temperature dependence of the protein unfolding thermodynamics at different concentrations of methanol in an aqueous solution. Since ΔH_{un} and ΔS_{un} in different methanol concentrations were observed to converge, it was concluded that the convergence temperature corresponded to the temperature at which the hydrophobic contributions to the thermodynamic quantity was zero.

Ragone and Colonna performed a series of theoretical studies on the enthalpy and entropy convergence in protein unfolding. T_H and T_S were carefully reexamined [108], and it was concluded that T_H typical of protein unfolding actually reflected the total enthalpic effects arising from non-polar and peptide backbone hydration [109]. Unlike previous studies choosing compact gases [110] or organic solids [100,101] as model compounds, the liquid amide dissolution thermodynamics was investigated [111]. This method was instructive, and it was inferred that the adaptation of thermophilic proteins to best function at very high temperatures did not require the introduction of any structural peculiarity [112]. Based on the results, a new approach to dissect the unfolding free energy of global proteins, in terms of solid-like and liquid-like contributions was also proposed [113]. However, this method remained to be challenged. Some resent studies still regarded the organic solids [114] or the pure hydrocarbon liquids [115] as reasonable model compounds in the study of the thermodynamics of protein unfolding.

Notably, a very recent theoretical study [116–118] at the level of statistical thermodynamics evaluated the temperature dependence of hydrophobic solvation of small molecules by an information theory model. The entropies of hydration for the solutes were found to converge around 460 K, and this behavior constituted a good explanation for the entropy convergence in the protein unfolding. This result was remarkable and exciting, although the enthalpy convergence still waits for such a theoretical investigation.

To summarize, although quite different approaches were used, one conclusion was reached unanimously, i.e. the occurrence of the enthalpy and entropy convergence in protein unfolding was closely related to the hydration effect during the water exposure of the buried surface. Debates remain on: (1) whether gaseous, liquid, or solid model compounds should be chosen for protein unfolding; (2) whether the similarity between T_H and T_S is a true behavior or a simple coincidence; (3) whether T_H (or T_S) corresponds to the temperature at which the contribution from the hydrophobic hydration disappears, or the temperature at which the contributions from the polar and non-polar hydration balance with one another.

3.2. Explanation in the present study

As seen, the species of the same compounds at thermodynamic equilibrium but with different environmental restrictions generally differ in their partial molar enthalpy and entropy (e.g. $h_{W/n} \neq h_{W/u} \neq h_{W/w}$, and $s_{W/n} \neq s_{W/u} \neq s_{W/w}$ in general) [44,45]. However, it is still possible that at certain temperature, these partial molar enthalpies or entropies can be equal, i.e. $h_{W/n} = h_{W/u} = h_{W/w}$, and $s_{W/n} = s_{W/u} = s_{W/w}$. This is not surprising, since fundamental thermodynamics tells that in a second phase transition, not only is true that $\mu_{W/n} = \mu_{W/u} = \mu_{W/w}$, but also it is right that $h_{W/n} = h_{W/u} = h_{W/w}$, and $s_{W/n} = s_{W/u} = s_{W/w}$. Supposing that this temperature is T^*. At T^*, similar to the deduction in Section 2.3,

we have not only $\Delta G_W(T^*) = 0$, but also $\Delta H_W(T^*) = 0$ and $\Delta S_W(T^*) = 0$. Therefore, at T^*, $\Delta G_{un}(T^*) = \Delta G_P(T^*)$, $\Delta H_{un}(T^*) = \Delta H_P(T^*)$, and $\Delta S_{un}(T^*) = \Delta S_P(T^*)$.

On the other hand, ΔS_P is the entropy change contributed from the structural changes of the protein molecule itself in protein unfolding. This quantity was indicated by many studies to be correlated with the number of the amino acid residues (N_r) in the protein [119–121], i.e.

$$\frac{\Delta S_P}{N_r} \cong \text{constant} \tag{27}$$

It was also suggested that $\Delta S_P(T)$ was very slightly temperature-dependent [122,123].

From the above deduction, the entropy changes in the unfolding of different proteins at T^* should converge to a constant when normalized with respect to the number of the amino acid residues in the protein, i.e.

$$\frac{\Delta S_{un}(T^*)}{N_r} = \frac{\Delta S_P(T^*)}{N_r} \cong \text{constant} \tag{28}$$

Similarly, since the enthalpy change for the melting of an average residue was approximately 6.0 kJ $(\text{mol}\cdot\text{res})^{-1}$ for most the native proteins and temperature-independent [124], $\Delta H_P/N_r \cong$ constant. Therefore, the enthalpy changes should also converge at T^* for protein unfolding. In brief, the temperature T^* equals both the enthalpy and entropy convergence temperatures in the protein unfolding, i.e.

$$T^* = T_H = T_S \tag{29}$$

At the other temperatures, because of the existence of non-zero ΔH_W and ΔS_W, which do not depend on the number of the amino acid residues of the protein, the enthalpy and entropy cannot converge. On the contrary, the enthalpy and entropy compensation will occur with the emergence of ΔH_W and ΔS_W, because $\Delta G_W = 0$. This argument is well supported by the experimental observations. Herein, the correlation between the enthalpy and entropy changes per mole of amino acid residue upon protein unfolding at both 298 and 383 K [125] is shown in Fig. 2. Clearly, at 298 K, there is no enthalpy or entropy convergence, but protein unfolding exhibits excellent EEC with a correlation coefficient of 0.979. However, at 383 K, the enthalpy and entropy convergence takes place, but very poor EEC occurs in protein unfolding. Therefore, the enthalpy–entropy compensation and the enthalpy and entropy convergence are two antithetic phenomena in protein unfolding; one's occurrence will inhibit the occurrence of the other. Interestingly, they arise from the same physical origin.

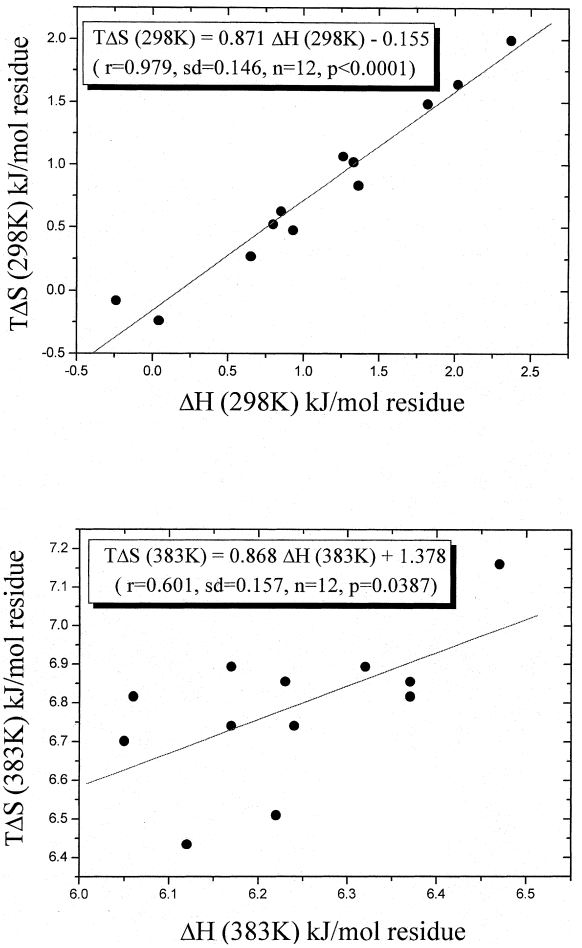

Fig. 2. Enthalpy–entropy compensation in the protein unfolding at 298 K (a) and 383 K (b). The data were taken from the literature [2,3].

3.3. Discussion

The present model indicated that T_H and T_S were the same. This result is different from the former postulations either that they were coincidentally analogous [126] or that they were actually different [95,96]. However, as known, T_H and T_S were determined based on the linear extrapolation with the assumption that ΔC_{un} was temperature-independent. This method can yield approximate results but not precise ones, for the assumption is not strictly correct. Recently, the temperature-dependence of ΔC_{un} was carefully measured. It was found while at 25°C the heat capacity of the unfolded state was approximately 1.45 times larger on average than that of the native state, at 100°C it was only 1.15 times larger [127–129]. Therefore, an insignificant difference of 10° between T_H and T_S is probably unreliable. A qualitative conclusion that T_H approaches or equals T_S sounds better. Although some studies have formulated several possible reasons for such a coincidence [99,105,106], the present study concludes that T_H may be strictly the same as T_S.

The present model dissects the thermodynamics of protein unfolding in terms of protein structure change and water molecule reorganization contributions. The convergence temperature is that at which the contribution from water molecule reorganization approaches zero. With the assumption that ΔC_P is always zero [122–124], and that ΔS_W and ΔH_W approach to zero at 110°C, the dissection of the thermodynamic quantities at 25°C for the unfolding of 12 well-documented proteins [8] leads to the results shown in Fig. 3. The variation of the specific enthalpy and entropy changes contributed from the protein structural changes is small. In comparison, the variation of the specific enthalpy and entropy changes contributed from the water molecule reorganization is much larger. This again explains the EEC in protein unfolding.

The above conclusion helps explain why a typical protein has evolved to have a remarkable small stability at room temperature [130], which is important to the lives on earth. Too strong stability will make the protein difficult to degrade and function, and easy to be trapped in a wrongly or even lethally folded structural configuration [106]. The present study, which has evaluated the stability of different proteins under various conditions at 298 K, also indicated this phenomenon (see Fig. 1, in which ΔG_{un} was distributed in a much smaller range than ΔH_{un}). Although this phenomenon has been employed to explain the enthalpy and entropy convergence in the protein unfolding [105,106], it is shown here that this experimental observation actually results from the reason that $\Delta H_P/N_r$ and $\Delta S_P/N_r$ are approximately the same for most proteins [122–124]. Since ΔG_W is always zero, the stability of most proteins, which is thus only determined by ΔG_P, is spanned in a narrow range. Water molecule reorganization actually does not play a role in the stabilization of native proteins, in accordance with the gradually emerged views that the non-bonded van der Waals and electrostatic forces constitute the major driving forces in protein folding [131,132]. Furthermore, this also challenges the idea that an increased hydrophobicity of the protein core made the thermophilic protein stable at high temperature [112,133,134].

Admittedly, although the present model can explain the enthalpy and entropy convergence in protein unfolding, it can not fully explain why the convergence temperature will be a specific one, e.g. 110°C. Computer-aided simulations at the level of statistical thermodynamics [116–118] may be helpful on this problem, and this study is presently being conducted in our laboratory. Interestingly, a recent work [135] using the computer simulations gave an explanation for the temperature at which the transfer entropy of non-polar solutes is zero: below this temperature, shell water molecules have more hydrogen bonds than bulk water molecules; above this temperature, the reverse is true. Although the work only considered the gas → water transfer, the results indicate the validity of our suggestion that T_H and T_S should correspond to the temperature at which the enthalpy and entropy changes contributed from the water molecular reorganization are zero.

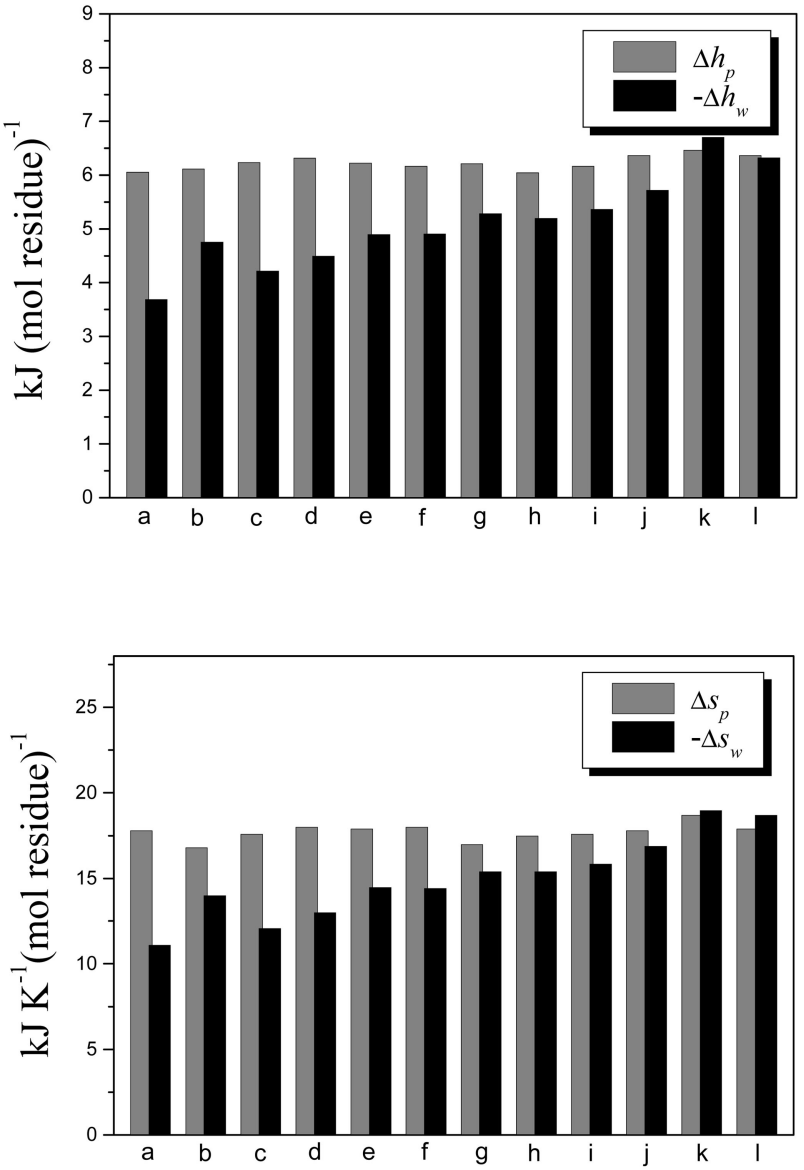

Fig. 3. The specific enthalpy and entropy changes in 12 types of proteins: ribonuclease A (a); parvalbumin (b); egg-white lysozyme (c); fragment K4 of plasminogen (d); β-trypsin (e); α-chymotrypsin (f); papain (g); *Staphylococcus* nuclease (h); carbonic anhydrase (i); cytochrome *c* (j); pepsinogen (k); myoglobin (l).

4. Conclusion

Remarkable enthalpy–entropy compensation was proposed based on a large number of thermodynamic data for protein unfolding. According to a strict thermodynamics deduction, it was found that the water molecular reorganization involved in protein unfolding constituted the physical origin of the behavior. It turned out that the present theory of enthalpy–entropy compensation could also provide novel insights into the problem of

enthalpy and entropy convergence in protein unfolding.

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