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Enthalpy convergence temperatures: proteins and model compounds ☆,[1]

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Abstract

It is suggested that the classical model of non-polar hydration in proteins does not take into account a large negative enthalpy due to the solvation of polar surface, mostly represented by the peptide group. Analysis of the dissolution thermodynamics of several organic compounds, containing functional groups typical of proteins, clarifies that the penalty associated with the burial of polar surface in proteins shifts the temperature at which the water transfer enthalpy of non-polar moieties goes to zero, from room temperature to approx. 376 K. This seems to be the clue for reconciling opposing views on the role played by non-polar hydration in proteins.

Keywords: Enthalpy; Hydration; Model; Protein

1. Introduction

Nowadays the most debated aspect of protein thermodynamics is perhaps the role played by hydrophobic solvation in protein unfolding. A strong controversy exists about the reason why the water transfer enthalpy of non-polar surface in protein seems to vanish near $T_h^* \approx 376$ K [1], i.e. very far from room temperature,

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where the solution enthalpy of non-polar molecules is usually zero. This has raised the issue of whether dispersion forces are in fact the dominant factor in hydrophobic interactions involved in the stabilisation of protein structure [2,3]. What is not clear is why van der Waals interactions should be involved in the solution thermodynamics of small molecules to a lesser extent than that invoked for protein unfolding [4]. Recently, Yang et al. [1] have argued that the combined effects of hydrogen bond formation and close packing predict a large positive unfolding enthalpy near room temperature. Thus, the small enthalpy changes that accompany protein unfolding at 25°C can be explained only if the burial of polar groups provides for a sufficiently large, negative contribution to effectively cancel those effects. Theoretical calculations indicate that the penalty associated with burying polar surface should contribute approximately -7 to -9 kJ mol^{-1} residue^{-1} in order to account for the experimental enthalpies [1]. Here we try to show how the solution thermodynamics of some organic compounds satisfactorily accounts for this view, also evidencing that the classical approach to the modelling of protein unfolding by water solution thermodynamics calls for some adjustment.

2. Experimental

Solution enthalpies ($\Delta_{sol}H^{\ominus}$) at 298 K and heat capacity changes ($\Delta C_p^{\ominus}$) of several organic substances available in the literature were used to calculate the temperature (T_h) at which $\Delta_{sol}H^{\ominus}$ goes to zero, with the assumption that $\Delta C_p^{\ominus}$ is constant with temperature. Data were from Arnett et al. [5] for alcohols, Konicek and Wadsö [6] for acids, amines and amides, except for *N*-methyl-acetamide and *N*-butyl-acetamide, which were taken from Sköld et al. [7]. The T_h of individual compounds was computed according to the equation

$$T_h = 298\ \text{K} - \Delta_{sol}H^{\ominus}(298\ \text{K})/\Delta C_p^{\ominus} \quad (1)$$

The error on T_h (ΔT_h) was evaluated assuming $\Delta T_h = (\delta T_h/\delta \Delta_{sol}H^{\ominus})\Delta\Delta_{sol}H^{\ominus} + (\delta T_h/\delta \Delta C_p^{\ominus})\Delta\Delta C_p^{\ominus}$, where $\Delta\Delta_{sol}H^{\ominus}$ and $\Delta\Delta C_p^{\ominus}$ are the uncertainties affecting $\Delta_{sol}H^{\ominus}$ and $\Delta C_p^{\ominus}$, respectively.

The protein composition was expressed in terms of n_C (number of alkyl carbons) per residue assuming that n_C is half the number of non-polar hydrogens, computed according to Murphy and Gill [8]. The proteins used were those reported by Privalov and Gill [2], for which the amino acid composition is available in the literature.

3. Results

It has already been noticed [9] that relating the solution enthalpy ($\Delta_{sol}H^{\ominus}$) of hydrophobic substances in water to the heat capacity change ($\Delta C_p^{\ominus}$) through

$$\Delta_{sol}H^{\ominus} = \Delta C_p^{\ominus}(T - T_h) \quad (2)$$

does not imply that T_h (the temperature at which $\Delta_{sol}H^{\ominus}$ is zero) always lies in the

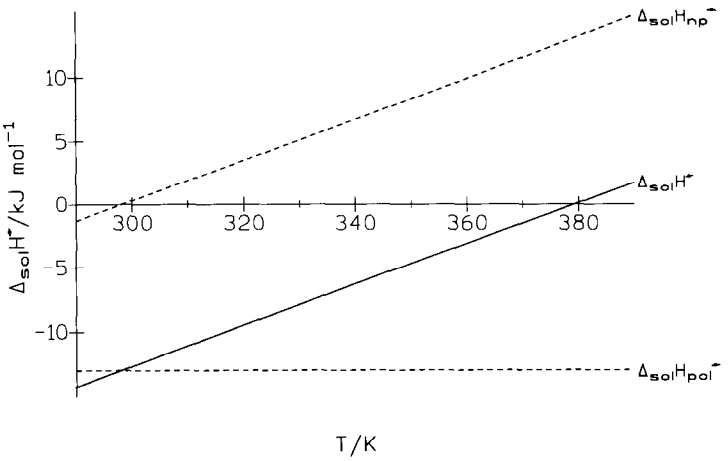

Fig. 1. Effect of a polar contribution on $\Delta_{sol}H^{\ominus}_{np}$ of a hypothetical liquid hydrocarbon. The line (dashed) was drawn by Eq. (2) using $\Delta C_p^{\ominus} = 160$ J mol^{-1} (corresponding to about 3 alkyl carbons) and $T_h = 298$ K, which is typical of liquid hydrocarbons. The total $\Delta_{sol}H^{\ominus}$ was evaluated by adding a constant polar contribution ($\Delta_{sol}H^{\ominus}_{pol} = -13\,000$ J mol^{-1}) to $\Delta_{sol}H^{\ominus}_{np}$.

region 295 ± 8 K, as found for a number of hydrocarbons [10]. For many other substances, the values of T_h must be substantially higher than for hydrocarbons. For instance, it was argued that for alcohols the value of T_h must be 30–50 K above 298 K and a similar statement would hold for many other organic compounds dissolved in water [9]. That knowledge of T_h values is essential, if we are to draw significant information from the solution enthalpy, was stated sixteen years ago [9]. This fact cannot be overlooked if we wish to mimic protein unfolding by the solution behaviour of organic molecules.

As an example, Fig. 1 shows how the solution enthalpy temperature dependence of a hypothetical hydrocarbon could be affected by the negative contribution due to a polar group. In particular, it is evident that the x-axis intercept (the zero-enthalpy temperature) shifts to a higher value. That this is really the case is shown in Table 1, where T_h values for a number of organic substances are calculated assuming that $\Delta C_p^{\ominus}$ is independent of temperature. Of course, the nature of the polar group bound to the hydrocarbon moiety determines the extent to which T_h moves away from 298 K. It is worth noting that a similar behaviour is observed for the transfer enthalpy of gaseous compounds into water. Here T_h shifts to higher temperatures (intriguingly close to T_h^* of protein unfolding [1,8,11–13]) because the total hydration enthalpy results from the sum of the solution enthalpy, which is positive above room temperature, and the condensation enthalpy, which is strongly negative. This fact, which appears as an entirely coincidental occurrence, has perhaps suggested that protein unfolding resembles gas dissolution more than liquid dissolution [1].

It is also evident from Table 1 that, within a given class of compounds, individual T_h values approach room temperature as the non-polar part of the molecule

Table 1
Thermal properties of the dissolution of organic compounds into water

Compound	$-\Delta_{sol}H^{\ominus}$(298 K) in J mol^{-1}	$\Delta C_p^{\ominus}$ in J mol^{-1} K^{-1}	T_h in K
Me-COOH	1176 ± 4	42 ± 2	326.0 ± 1.4
Et-COOH	1544 ± 4	102 ± 2	313.1 ± 0.3
Pr-COOH	1460 ± 4	159 ± 1	307.2 ± 0.1
i-Pr-COOH	1418 ± 4	161 ± 1	306.8 ± 0.1
Bu-COOH	370 ± 8	235 ± 6	299.6 ± 0.1
Et-OH	10180 ± 50	164 ± 11	360.1 ± 4.5
Pr-OH	10121 ± 29	236 ± 14	340.9 ± 2.7
i-Pr-OH	13071 ± 42	232 ± 14	354.3 ± 3.6
Bu-OH	9410 ± 42	300 ± 16	329.4 ± 1.8
i-Bu-OH	9314 ± 38	284 ± 18	330.8 ± 2.2
Pe-OH	7816 ± 46	349 ± 22	320.4 ± 1.5
Pr-NH_2	24620 ± 20	167 ± 4	445.4 ± 3.7
i-Pr-NH_2	27450 ± 80	178 ± 16	452.2 ± 14.3
Bu-NH_2	23330 ± 10	234 ± 3	397.7 ± 1.3
i-Bu-NH_2	23370 ± 20	222 ± 8	403.3 ± 2.9
Pe-NH_2	22040 ± 20	297 ± 4	372.2 ± 1.1
Hex-NH_2	20660 ± 20	351 ± 4	356.9 ± 0.1
Me-NHCO-Me	13090 ± 20	107 ± 3	420.3 ± 3.6
Me-NHCO-Et	14870 ± 20	155 ± 3	393.9 ± 2.0
Me-NHCO-Pr	16020 ± 20	227 ± 4	368.6 ± 1.3
Me-NHCO-i-Pr	15790 ± 20	222 ± 5	369.1 ± 1.7
Me-NHCO-Bu	15030 ± 20	286 ± 4	350.6 ± 0.8
Et-NHCO-Me	15480 ± 20	163 ± 4	393.0 ± 2.5
Pr-NHCO-Me	15760 ± 20	230 ± 4	366.5 ± 1.3
i-Pr-NHCO-Me	17240 ± 20	230 ± 4	373.0 ± 1.4
Bu-NHCO-Me	14720 ± 30	280 ± 1	350.6 ± 3.0

(represented, for example, by the number of alkyl carbons) becomes larger. In particular, it seems interesting to analyse the dependence of T_h on the reciprocal number of alkyl carbons (n_C) in order to obtain the extrapolated value of T_h when n_C goes to infinity. This is shown in Fig. 2. Of course, each class of compounds shows a particular dependence of T_h on n_C, which reflects differences in the polar contribution to $\Delta_{sol}H^{\ominus}$. In this regard it is worth noting that T_h values for acids are not too far from room temperature, probably because the carboxyl contributes only slightly to $\Delta_{sol}H^{\ominus}$. In other words, the dissolution into water of carboxylic acids shows a thermal behaviour very similar to that of hydrocarbons. We will not discuss this point further, because it is beyond the scope of this work. Another point is that extrapolated values of T_h (y-axis intercept) are close to room temperature for alcohols and acids, while amides show a slightly larger value. Amines are characterised by an intercept well below the freezing point of water. This fact deserves some comment, because we would expect values not too far from room temperature when the non-polar part is very large, as is the case of acids, alcohols and, to a minor extent, amides. Amines seem to escape this rule. A possible explanation is that the enthalpic contributions of alkyl chain and functional group

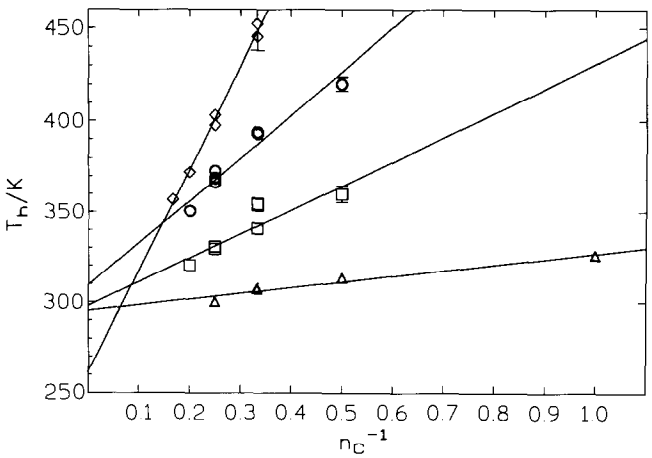

Fig. 2. Dependence of T_h of organic liquids on the reciprocal number of alkyl carbons. Data from Table 1 were fitted to the equation $T_h = T_{np} + k(n_C)^{-1}$. The parameters of the linear regression analysis were △, acids: $T_{np} = 295.2 \pm 2.4$ K, $k = 31.8 \pm 4.3 n_C$ K, $r = 0.973$; □, alcohols: $T_{np} = 297.9 \pm 9.3$ K, $k = 133.2 \pm 28.7 n_C$ K, $r = 0.919$; ○, amides: $T_{np} = 309.3 \pm 6.1$ K, $k = 234.4 \pm 20.3 n_C$ K, $r = 0.975$; ◇, amines: $T_{np} = 261.5 \pm 6.0$ K, $k = 559.9 \pm 22.7 n_C$ K, $r = 0.997$. The equation found for amides was used to predict T_h^* of proteins (see Table 2).

Table 2
Protein composition and predicted values of T_h^* [a]

Protein	Non-polar hydrogens	Alkyl carbons per residue	T_h^* in K
Ribonuclease A	664	2.68	396.8
Parvalbumin	619	2.87	391.1
Lysozyme	690	2.68	396.9
β-Trypsin	1238	2.71	396.0
α-Chymotrypsin	1364	2.79	393.3
Papain	1202	2.84	392.0
Nuclease, *straphylococcus*	922	3.10	385.0
Carbonic anhydrase	1511	2.90	390.3
Cytochrome *c*	632	3.04	386.4
Pepsinogen	2089	2.83	392.3
Myoglobin	974	3.19	382.9
Plasminogen, fragment K4	446	2.60	399.6

[a] Predicted values of T_h^* were evaluated using the linear regression parameters found for amides (see legend to Fig. 2).

are not strictly additive because of correlation between them. This aspect was largely discussed by Ben-Naim, who introduced the concept of conditional hydration to explain how the hydrophobic behaviour of non-polar molecules is affected

by the vicinity of a functional group [14]. In particular, this should not be forgotten whenever we make the choice of modelling hydration thermodynamics in protein unfolding by means of very simple model compounds [14].

What can be done with proteins? If we assume that the polar surface buried upon folding is mostly represented by the peptide moiety, enthalpic effects associated with the transfer of this group into water should be adequately mimicked by amides. This choice provides for a quite simple explanation of the high value of T_h^* typical of protein unfolding, as anticipated in a recent communication [15]. The dependence of T_h on n_C found for amides actually represents the balance between opposing enthalpic contributions due to the transfer of alkyl chains (positive) and amide group (negative) from the pure liquid phase into water. Thus, we can model heat effects linked to the immersion of a fully buried residue into water. In order to evaluate the non-polar enthalpic contribution in proteins we must only express the composition of an average amino acid residue in terms of n_C equivalents. Such calculation has been performed for several globular proteins, assuming that n_C is properly represented by half the number of non-polar hydrogens, as shown in Table 2. It can be appreciated that predicted values of T_h^* fall within 380–400 K, i.e. within the experimental uncertainty affecting the amide model, which seems quite good in the light of the approximations used. It is also very likely that such a result holds for every globular protein, because proteins are not very different from each other in terms of amino acid composition.

4. Discussion

The water solution enthalpy of organic molecules containing polar groups is usually strongly negative at 298 K [5–7]. This fact has been disappointingly forgotten in the small-molecule-based modelling of protein unfolding [16]. Because the solution enthalpy of hydrocarbons is nearly zero at room temperature, it appears that a negative contribution should be ascribed to the presence of polar functional groups in the molecule. This observation is of utmost importance for justifying the large enthalpy gap between protein unfolding and non-polar hydration of liquid hydrocarbons. Accordingly, we have shown that the classical model of non-polar hydration in proteins [16], which has been largely used to date, does not take into account that T_h^* of protein unfolding is far from T_h of hydrocarbon dissolution into water because of a large negative contribution due to the solvation of the buried peptide moiety. The explanation proposed in this work seems to provide experimental support to theoretical calculations leading to the same conclusions [1]. This result should not displease researchers upholding the use of liquid compounds for studying the interaction of non-polar groups with water. At the same time, people invoking the necessity of a new view or definition of hydrophobic behaviour, for which we believe the operational definition of Dill [17,18] to be satisfactory, could find a reasonable answer to the supposed anomalous behaviour of proteins.

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