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Hydration and convergence temperatures: on the use and interpretation of correlation plots *

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Abstract

An understanding of the energetics of hydration of protein functional groups is essential to understanding the stability and folding of proteins. Much can be learned about hydration energetics by the study of the transfer of model compounds into water. An important feature, common to model compound dissolution and to protein unfolding, is the presence of convergence temperatures at which $\Delta S°$ (or $\Delta H°$) for each compound in a series takes on a common value. Here we review the relationship between convergence and group additivity. Analysis of the aqueous dissolution of gaseous alcohols and alkanes shows a large negative entropy change for the alcohols relative to the alkanes. While this has been taken as leading to entropic stabilization of hydrogen bonding in proteins, it is shown that this negative $\Delta S°$ arises from changes in internal degrees of freedom and should not be applied to the analysis of protein energetics.

Key words: Hydration; Entropy; Hydrogen bonding; Protein stability; Convergence temperature

1. Introduction

The ability to predict the energetics of folding and binding reactions of proteins based on knowledge of their structure is a long-standing goal of biophysical chemistry. Recently, several investigators have demonstrated that the energetics of folding/unfolding transitions in globular proteins can be reproduced with a high degree of accuracy based on correlations of accessible surface area (ASA) changes with thermodynamic parameters [1–6]. The idea that the energetics of protein stability are correlated to buried surface has been explored for nearly twenty years [7–9] and is related to earlier observations of the correlation of the energetics of the aqueous dissolution of small molecules with surface areas [10]. These empirical methods hold considerable promise, not only for understanding protein folding energetics, but also for understanding protein–ligand interactions [11,12].

The development and the interpretation of these empirical analyses stress the importance of changes in heat capacity, ΔC_p, as indicative of changes in the interaction of protein surfaces with water [13–20]. Given the central role of ΔC_p in hydration phenomena, correlations of other

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thermodynamic parameters with ΔC_p have been suggested as a means of dissecting the energetics of transfer processes into their constituent contributions [16,19].

For the transfer into water of a homologous series of compounds, there can exist a temperature at which the $\Delta S°$ of transfer is the same for each member of the series. Thus, for compounds with varying amounts of apolar surface the entropies are independent of the apolar groups at this temperature. This temperature is commonly referred to as a convergence temperature and also is observed for $\Delta H°$ of transfer. The entropy convergence temperature is designated as T_S^* and the residual $\Delta S°$ at this temperature is designated ΔS^*. For ΔH, we have the convergence temperature T_H^* at which the $\Delta H°$ is equal to ΔH^*. The thermal denaturation of many globular proteins is also characterized by convergence of $\Delta H°$ and $\Delta S°$ when these parameters are normalized to the number of amino acid residues in the protein [17,21]. Correlations of $\Delta H°$ and $\Delta S°$ with ΔC_p are directly related to convergence phenomena and hydration [19].

The interpretation of ΔC_p correlations, or convergence phenomena, in proteins has been the subject of considerable discussion recently [1,4–6,16,17,19,22–25]. In this paper the interpretation of convergence in terms of group additivity will be reviewed and applied to the hydration of alcohols and alkanes. Comparison of these two systems illustrates the utility of convergence phenomena in assigning energetic contributions to a process, but also illustrates that care must be taken in applying model compound transfer results to the analysis of protein stability. In particular, it is shown that the apparent $\Delta S°$ of hydration of polar groups in alcohols is largely due to changes in internal degrees of freedom (molecular rotation) and this contribution should not be applied to the analysis of protein data.

2. Theory

We have previously published the theory detailing how group additivity leads to convergence phenomena and ΔC_p correlations [1,26] and an independent, but very similar, treatment has been given by Lee [22]. The concept of group additivity has found wide application in solution thermodynamics [26–29]. Group additivity is the assumption that a thermodynamic quantity for a given compound can be given as the sum of the contributions of the individual groups that make up that compound. In equation form:

$$\Delta X = \sum N_i \Delta x_i \tag{1}$$

where ΔX is the change in any thermodynamic property, Δx_i is the contribution to ΔX of group i, and N_i is the number of such groups in the compound.

As an example, consider the aqueous dissolution of gaseous normal alcohols. According to Eq.(1), the enthalpy change, $\Delta H°$, is given as

$$\Delta H° = N_{\text{-OH}} \Delta h_{\text{-OH}} + N_C \Delta h_C \tag{2}$$

where Δh indicates the group contribution to $\Delta H°$, and the subscripts indicate the hydroxyl (–OH) and methyl(ene) (C) groups. Similarly, $\Delta S°$ and ΔC_p can be written as

$$\Delta S° = N_{\text{-OH}} \Delta s_{\text{-OH}} + N_C \Delta s_C \tag{3}$$

and

$$\Delta C_p = N_{\text{-OH}} \Delta c_{\text{-OH}} + N_C \Delta c_C, \tag{4}$$

For the normal alcohols $N_{\text{-OH}}$ is equal to one and only N_C varies for the series.

Convergence of $\Delta H°$ for the alcohols would mean that at some temperature, T_H^*, all the compounds would have the same $\Delta H°$ of dissolution, ΔH^*. In other words, at T_H^* $\Delta H°$ is independent of the specific compound within the series but depends only on the nature of the series itself. According to Eq. (2), this would require that Δh_C be zero at T_H^* and ΔH^* is then equal to $\Delta h_{\text{-OH}}$. Similarly, according to Eq. (3), convergence of $\Delta S°$ values at T_S^* requires Δs_C to be zero at this temperature.

As has been discussed [19], the convergence phenomena can be recast in terms of correlations with ΔC_p. Convergence is then seen as a linear plot of $\Delta H°$ or $\Delta S°$ versus ΔC_p. These plots have been dubbed MPG (Murphy, Privalov, Gill) plots by Lee [22]. This approach has the advantage of eliminating the need for long temperature extrap-

olations. Using T_H^* as a reference temperature, the temperature dependence of ΔH° for a given compound is given as

$$\Delta H^\circ = \Delta H^* + \Delta C_p(T - T_H^*) \quad (5)$$

If the series of compounds shows convergence, then ΔH^* is the same for each compound and one need only know ΔC_p and T_H^* to describe the $\Delta H^\circ(T)$ function for any given compound in the series. Rather than considering Eq. (5) as describing $\Delta H^\circ(T)$ for a single compound however, one can also consider it as $\Delta H^\circ(\Delta C_p)$ for a series of compounds at some defined temperature T. A plot of ΔH° (298 K) versus ΔC_p for this series will then yield a straight line with slope $(298 - T_H^*)$ and intercept ΔH^*.

The interpretation of T_H^* and ΔH^* in terms of group additivity is obtained by solving Eq. (4) for N_C and substituting the result into Eq. (2) to yield

$$\Delta H^\circ = N_{-OH}\left[\Delta h_{-OH} - \Delta c_{-OH}\left(\frac{\Delta h_C}{\Delta c_C}\right)\right] + \Delta C_p\left(\frac{\Delta h_C}{\Delta c_C}\right). \quad (6)$$

Comparison of Eqs. (6) and (7), with $N_{-OH} = 1$, indicates that

$$(T - T_H^*) = \left(\frac{\Delta h_C}{\Delta c_C}\right) \quad (7)$$

and

$$\Delta H^* = \Delta h_{-OH} - \Delta c_{-OH}\left(\frac{\Delta h_C}{\Delta c_C}\right). \quad (8)$$

According to Eq. (7), when T equals T_H^* then Δh_C equals zero. Eq. (8) states that ΔH^* is the hydroxyl contribution to ΔH° at the temperature T_H^*.

The reasoning presented here can be generalized to any series of compounds in which one contributing group is constant (the –OH in the case of the alcohols) and another contributing group varies (the C in the alcohols). For the alcohol system one can then conclude that the apolar groups have a Δh of zero at the convergence temperature and that the observed ΔH^* is due to the transfer into water of the polar –OH group. Thus, ΔH^* contains contributions from van der Waals interactions and specific –OH/water interactions (i.e. hydrogen bonds), as well as any contributions from restructuring of water around the –OH group. The sum of all these contributions is generally considered as hydration.

Similar reasoning applies to the entropy convergence, but somewhat more care must be applied in the interpretation. The group additivity analysis suggests that the convergence entropy change, ΔS^* can be attributed to whatever entropic contributors are common to each compound, while the convergence temperature, T_S^*, is the temperature at which the variable contributor(s) have zero Δs. In the case of the alcohols above, ΔS^* will include not only the ΔS° of hydration of the polar groups (i.e. restructuring of water around the –OH group), but also translational and vibrational contributions, and contributions from internal and overall molecular rotations that are constant for the process and can depend on the choice of standard state. This is an important point and will be examined in detail below.

The slope of the MPG plot is related to the convergence temperature as

$$\ln(T/T_S^*) = \left(\frac{\Delta s_C}{\Delta c_C}\right), \quad (9)$$

indicating that Δs_C is zero at T_S^*. The intercept is the convergence ΔS°,

$$\Delta S^* = \Delta s_{-OH} - \Delta c_{-OH}\left(\frac{\Delta s_C}{\Delta c_C}\right), \quad (10)$$

which is the contribution resulting for the –OH group at T_S^*.

It is also interesting to note that group additivity leads to correlation of ΔH° and ΔS°, often referred to as enthalpy–entropy compensation [30,31]. The temperature dependence of ΔS°, using T_S^* as the reference temperature is

$$\Delta S^\circ = \Delta S^* + \Delta C_p \ln(T/T_S^*). \quad (11)$$

Solving for ΔC_p and substituting into Eq. (6) yields

$$\Delta H^\circ = \left(\Delta H^* - \Delta S^* \frac{T - T_H^*}{\ln(T/T_S^*)} \right) + \Delta S^\circ \frac{T - T_H^*}{\ln(T/T_S^*)}. \quad (12)$$

The slope of a plot of ΔH° versus ΔS°, usually called the compensation temperature T_c [30,31], is given by the ratio of the slopes of the ΔH° and ΔS° MPG plots and depends on temperature. For the alcohols then:

$$T_c = \frac{T - T_H^*}{\ln(T/T_S^*)} = \Delta h_C / \Delta s_C \quad (13)$$

The intercept, α, is:

$$\alpha = \Delta H^* - \Delta S^* \frac{T - T_H^*}{\ln(T/T_S^*)}, \quad (14)$$

which is also temperature dependent. Processes involving homologous series of compounds for which group additivity applies will therefore show enthalpy–entropy compensation with a compensation temperature determined by the group contributions of the variable functional group.

For processes involving series of hydrophobic compounds, Eq. (13) can be used to determine T_H^*, even in the absence of ΔC_p data. The value of T_S^* has been shown to be universal for processes involving the transfer of hydrophobic compounds into water, independent of the originating phase (gas, liquid, solid or protein interior) [16,19] and is 385 K. Thus knowledge of T_c allows determination of T_H^* for such processes.

Before proceeding with the application of this formalism to dissolution data, mention of the analysis of the protein results seems appropriate. A more complete discussion of the various interpretations of the protein data is given elsewhere [32]. As has been discussed, the ΔH° and ΔS° of denaturation of globular proteins both show convergence when the data are normalized to the number of amino acid residues in the protein [17,19,21]. We have interpreted these phenomena in a manner exactly analogous to the model compound dissolution data [1,2,19] arguing that at the convergence temperatures, the exposure of apolar surface makes no contribution to the thermodynamic term. This argument requires that the amount of apolar surface area per residue exposed upon unfolding varies within the set of proteins being examined, while the amount of polar surface area per residue exposed upon unfolding stays constant. This appears to be the case [2,33]. As regards the entropy convergence, our interpretation is consistent with the original analysis of Baldwin [16].

Other explanations of convergence in proteins have been put forth however. Lee [22] has argued that, while the group additivity approach outlined above is valid for understanding model compound data, the protein data are best understood differently. He argues that at the convergence temperatures the polar and apolar contributions are equivalent per unit surface area. This is expected when the proteins vary in the *percentage* of buried polar surface. Recently however, we have demonstrated that this analysis is correctly applied when the data are normalized to the total buried surface, rather than to the number of residues [2,33]. Consequently, Lee's analysis and ours are quite consistent with each other [2,33].

Yang et al. [23] have suggested that the enthalpy convergence occurs because of the enthalpically destabilizing hydration of the hydrogen bonding groups in proteins. They argue that the normalized values of ΔH° decrease with increasing ΔC_p because the more hydrophobic proteins (i.e. those with larger ΔC_p) bury more hydrogen bonding groups per residue or bury them more deeply. Thus, upon unfolding, there is an increasingly negative polar hydration term with increasing ΔC_p. This explanation is consistent with the analysis given be Privalov and Makhatadze [5]. While plausible, this analysis fails to explain the convergence behavior observed in the dissolution of cyclic dipeptides. These dipeptides have the same number of hydrogen bonding groups in each compound and thus the polar hydration should be constant within the series [26]. Nevertheless, it is observed that ΔH° decreases with increasing ΔC_p for the dissolution of these compounds, contrary to the expectation from the arguments of Yang et al.

It should also be mentioned that several investigators [34,35] have offered the following as explanation of the enthalpy convergence. The globular proteins all have similar normalized ΔS values near 112°C and also have similar melting temperatures, T_m, on the absolute temperature scale. Consequently, they are required to have similar normalized ΔH values at high temperature. While this line of reasoning is certainly true, it has the effect of changing the question from Why do we observe convergence of ΔH? to Why do we see similar values of T_m? However, the same physical mechanism must underlie both questions and this analysis sheds no light on this issue as has been noted previously [5,32].

3. Applications and discussion

Table 1 lists the energetics of dissolution from gas to water for the normal alcohols and alkanes at 298.15 K. Dissolution $\Delta H°$ and ΔC_p values were determined calorimetrically and are taken from Hallén et al. [36] for the alcohols and from Dec and Gill [37] for the alkanes. Alcohol $\Delta H°$ and ΔC_p data were corrected to the gas phase using the vaporization data [38]. $\Delta C_{p,v}$ were calculated as $(\Delta H_v(298) - \Delta H_{v,b})/(298 - T_b)$ where the subscript b indicates the boiling temperature.

Table 1
Energetics of aqueous dissolution of alcohols and alkanes from the gas phase. Data are from Hallén et al. [36], Dec and Gill [37], and Cabani et al. [27] as described in the text. Units are kcal mol^{-1} for $\Delta H°$ and $\Delta G°$ and cal K^{-1} mol^{-1} for $\Delta S°$ and ΔC_p

Compound	$\Delta G°$	$\Delta H°$	$\Delta S°$	ΔC_p
methanol	−5.11	−10.8	−19.0	31.6
ethanol	−5.01	−12.6	−25.4	52.2
propanol	−4.82	−13.8	−30.0	70.0
butanol	−4.72	−14.7	−33.6	86.4
pentanol	−4.47	−15.5	−37.1	104
hexanol	−4.36	−16.3	−39.9	120
heptanol	−4.24	−17.1	−43.2	137
octanol	−4.09	−17.8	−45.9	153
methane	2.00	−3.15	−17.3	52.0
ethane	1.83	−4.67	−21.8	67.9
propane	1.96	−5.56	−25.2	79.4
butane	2.08	−6.20	−27.8	93.2

The calculated value for propanol appeared anomalously low, and an interpolated value was used instead. Values of ΔC_p for heptanol and octanol were extrapolated using a second order polynomial, as values $\Delta H_{v,b}$ were unavailable. No additional corrections were made to $\Delta H°$ or ΔC_p so that the results can be directly compared to those of Cabani et al. (1981). Values of $\Delta G°$ are those tabulated by Cabani et al. [27] using a molar standard state for both the gas and solution phases. This choice of standard state excludes the translational entropy contribution from the transfer energetics, provided that the momentum partition function is the same in both states, but does not exclude changes in internal degrees of freedom [39]. Values of $\Delta S°$ are calculated as $\Delta S° = (\Delta H° - \Delta G°)/298.15$.

3.1. Enthalpy correlations

The convergence of $\Delta H°$ values for the two series of compounds is illustrated in the enthalpy MPG plots (Fig. 1). The slopes of the two lines are similar, being -74.6 ± 8.6 for the alkanes and -55.8 ± 2.9 for the alcohols. The reported errors are the standard errors of the linear fit.

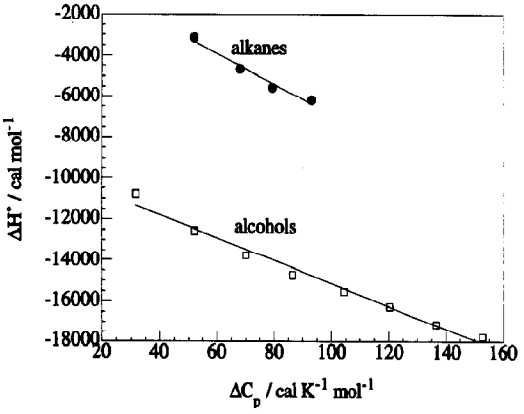

Fig. 1. Correlation $\Delta H°$ with ΔC_p for the aqueous dissolution of gaseous alcohols (open squares) and alkanes (filled circles). The slope of the line $(298 - T_H^*)$ yields the convergence temperature, T_H^*, at which $\Delta H°$ depends only on the series but is independent of the specific compound. The intercept gives the convergence enthalpy change, ΔH^* which is characteristic of the series. The solid lines are the linear least squares fit with slopes of −74.6 (alkanes) and −55.8 (alcohols), and intercepts 600 (alkanes) and −9560 (alcohols).

These slopes correspond to T_H^* values of 373 ± 9 and 354 ± 3 K respectively. The difference in slopes (or convergence temperatures) reflects the slight curvature in the data which is indicative of deviations from simple group additivity. The initial slope in the alcohol data is steeper and more similar to the alkane slope.

Analysis of the data in Fig. 1, in terms of the group additivity scheme outlined above suggests that the dissolution of the aliphatic groups is accompanied by a significant negative $\Delta H°$. This negative $\Delta H°$ decreases in magnitude with increasing temperature and passes through zero at some high temperature near 373 K (under the assumption of a constant ΔC_p). The intercept value is nearly zero (0.6 ± 0.6 kcal mol^{-1}) for the alkanes as expected from the group additivity formalism discussed above. The intercept for the alcohols (-9.7 ± 0.3 kcal mol^{-1}) is the contribution of the hydroxyl, -OH, at the convergence temperature. This value compares well to that calculated from the group values of Cabani et al. [27] of -9.49 kcal mol^{-1}. This value is also very close to $-\Delta H°_v$ of water (-10.52 kcal mol^{-1} [40] p. 109).

3.2. Entropy correlations

The MPG entropy plots of the alkanes and alcohols are shown in Fig. 2. The slopes are nearly parallel as expected [19]. The values of -0.258 ± 0.018 for the alkanes and -0.216 ± 0.009 for the alcohols correspond to T_S^* values of 386 ± 7 and 370 ± 5 K. The difference between the two slopes is again the result of the curvature in the alcohol data. The average slope for the aqueous dissolution of apolar compounds is -0.256 corresponding to a convergence temperature of 385 K [14,19] (solid lines in the figure). At this temperature, the values of ΔS^* for the alkanes and alcohols are -4.3 ± 0.2 and -10.1 ± 0.7 cal K^{-1} mol^{-1}.

The interpretation of Fig. 2 in terms of group additivity is that the apolar hydration is accompanied by a large negative $\Delta S°$ which decreases in magnitude with increasing temperature up to approximately 385 K (again assuming a constant ΔC_p). The intercept value for the alkanes is small, and represents entropic contributions common to the dissolution process, primarily rotational and vibrational entropy changes. The large, negative ΔS^* for the alcohols is associated with the effect of the hydroxyl on the transfer from gas to water.

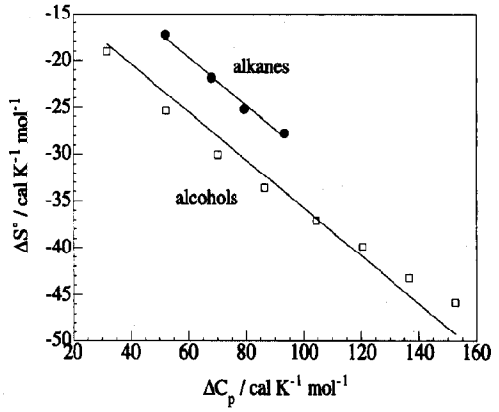

Fig. 2. Correlation $\Delta S°$ with ΔC_p for the aqueous dissolution of gaseous alcohols (open squares) and alkanes (filled circles). The slope ($\ln(T/T_S^*)$) yields the convergence temperature, T_S^*, at which $\Delta S°$ depends only on the series but is independent of the specific compound. The intercept gives the convergence entropy change, ΔS^*, which includes changes in internal degrees of freedom. The solid lines are the linear least squares fit assuming $T_S^* = 385$ K, yielding intercepts of -4.3 (alkanes) and -10.1 (alcohols).

What is the source of this negative ΔS^* for the hydroxyl group? One interpretation is to consider it as an entropy of hydration, that is, as an entropy associated with the restructuring of solvent around the solute -OH group. This is the interpretation suggested by Privalov and Makhatadze [5] who obtain a value of -10.9 cal K^{-1} mol^{-1} for the hydroxyl of serine at 398 K. The 398 K value is the most appropriate for comparison to the ΔS^* at 385 K because Privalov and Makhatadze include a temperature dependence in ΔC_p [5]. Based upon this large negative $\Delta S°$ for polar groups, in conjunction with their analysis of hydration enthalpies, they conclude that hydrogen bonding in proteins is entropically driven [5].

In the context of the group additivity scheme as outlined above, the ΔS^* term represents all contributions to $\Delta S°$ which are independent of the apolar surface. These will include hydration of the hydroxyl, as well as changes in translational, rotational, and vibrational degrees of freedom. The choice of the molar standard state

eliminates translational contributions to $\Delta S°$ of transfer [39]. One can thus envision the process as the transfer of an alcohol molecule from a fixed point in the gas phase to a fixed point in solution. Changes in internal degrees of freedom (rotation and vibration) still contribute to $\Delta S°$, however, in addition to hydration [39].

While ΔS^* for the gas to water transfer contains contributions from hydration and changes in internal degrees of freedom, the change in entropy upon vaporization, $\Delta S°_v$, has no hydration component by definition. Table 2 lists the boiling points, T_b, enthalpies of vaporization, $\Delta H°_v$, and entropies of vaporization, $\Delta S°_v$, for several normal alcohols and alkanes at 298 K. $\Delta S°_v$ was calculated as:

$$\Delta S°_v = \frac{\Delta H°_v}{T} + R \ln\left(\frac{p_{sat}}{p°}\right), \quad (15)$$

where $\Delta H°_v$ is the vaporization enthalpy at $T =$ 298 K, R is the gas constant, p_{sat} is the saturated vapor pressure of the liquid, and $p°$ is the reference vapor pressure of 1 atm. Values of p_{sat} were determined by extrapolation or interpolation of vapor pressure data [41] according to the Clausius–Clapeyron equation. These values are also given in the table.

Table 2
Vaporization thermodynamics for normal alkanes and alcohols. Vaporization enthalpies, $\Delta H°_v$ are from [38]. Saturated vapor pressures at 298 K are interpolated from data in [41]. $\Delta S°_v$ is calculated as described in the text

Compound	T_b (K)	p_{sat} (atm)	$\Delta H°_v$ (kcal mol^{-1})	$\Delta S°_v$ (cal K^{-1} mol^{-1})
ethane	184.55	45.9	2.33	15.4
propane	231.05	9.78	3.88	17.6
butane	272.65	2.34	5.17	19.0
pentane	309.25	0.611	6.39	20.5
hexane	342.15	0.173	7.58	22.0
heptane	371.55	0.0544	8.76	23.6
octane	398.85	0.0175	9.92	25.2
methanol	338.15	0.150	9.04	26.6
ethanol	351.65	0.0715	10.1	28.8
propanol	370.55	0.0262	11.4	30.8
butanol	390.35	0.0101	12.5	32.9
pentanol	410.45	0.00337	13.6	34.4
hexanol	431.15	0.00147	14.7	36.4
heptanol	449.15	0.000416	16.0	38.1
octanol	467.55	0.000178	17.0	39.7

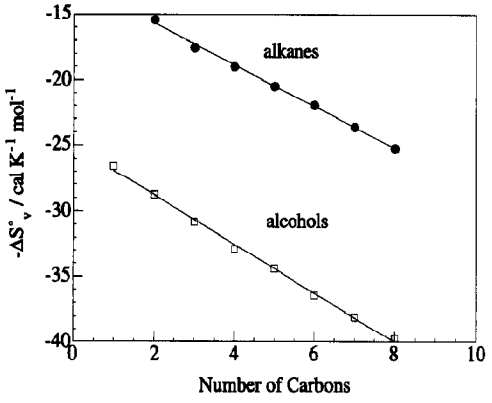

Fig. 3. $-\Delta S°_v$ as a function of the number of carbon atoms in the chain for the normal alcohols (open squares) and alkanes (filled circles). The lines are the linear least squares fits with slopes of -1.6 (alkanes) and -1.9 (alcohols), and intercepts of -12.5 (alkanes) and -25.1 (alcohols).

Fig. 3 shows $-\Delta S°_v$ versus the number of carbon atoms in the chain for both the alkanes and alcohols. The negative of the vaporization is plotted for comparison to the dissolution process. Both sets of data have very similar slopes (-1.59 ± 0.04 and -1.87 ± 0.04 cal K^{-1} mol^{-1} per heavy atom for the alkanes and alcohols respectively) but different intercepts. For the alkanes the intercept is -12.5 ± 0.2 cal K^{-1} mol^{-1}, while for the alcohols it is -25.1 ± 0.2 cal K^{-1} mol^{-1}. The difference between the intercepts is -12.6 ± 0.3 cal K^{-1} mol^{-1}, close to the ΔS^* value for the alcohol dissolution.

The vaporization entropy, as defined by Eq. (15), is for the transfer of a molecule from the neat liquid to vapor at 1 atm and 298 K. It therefore includes entropic effects associated with changes in translational, rotational and vibrational degrees of freedom. The translational contribution can be approximated as $R \ln(V_g/V_l)$ where V_g and V_l are the molar volumes of the gas and liquid phases receptively. Strictly, the free volumes (corrected for excluded volume) should be used [42], but the approximate values are suitable to illustrate the general effects. The molar volumes of the neat liquids were calculated from the densities [41]. No correction for the temperature dependence of the densities was made. The gas volumes were calculated assuming ideality.

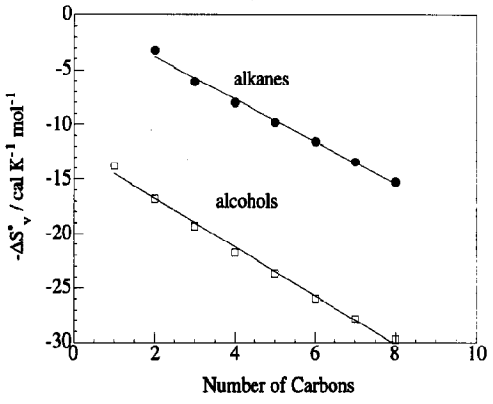

Fig. 4. Negative vaporization entropies corrected for volume differences, $-\Delta S^*_v$, versus the number of carbon atoms in the chain for the alcohols (open squares) and alkanes (filled circles). The solid lines are the linear least squares fits with slopes of -1.9 (alkanes) and -2.2 (alcohols), and intercepts of 0.1 (alkanes) and -12.3 (alcohols).

Fig. 4 shows a plot of $-\Delta S^\circ_v$ corrected for the translational contribution, $-\Delta S^*_v$, for the alkanes and alcohols, plotted versus the number of carbons. We again observe very similar slopes, -1.94 ± 0.07 for the alkanes and -2.24 ± 0.7 for the alcohols. The primary difference from Fig. 3 is the intercepts. For the alkanes the intercept is now essentially zero (0.1 ± 0.4 cal K^{-1} mol^{-1}). This is expected since the only contribution to ΔS^*_v of a point mass is the translational ΔS. In contrast, the alcohols show an intercept of -12.3 ± 0.4 cal K^{-1} mol^{-1}.

The significantly higher ΔS^*_v for associated liquids, such as alcohols, relative to 'regular' liquids such as the alkanes, has been discussed for a long time. Barclay and Butler [43] stated in 1938:

"It is well known that the entropy of vaporisation [*sic*] of associated liquids, such as water, alcohols, etc., is greater than that of normal liquids. In these cases the entropy of the liquid compared with the vapour is abnormally small, which is easily accounted for on the consideration that *the molecular rotations are incompletely developed in the liquid owing to hydrogen links between molecule and molecule*" (my emphasis).

Smaller deviations of vaporization entropies in 'regular' liquids have also been attributed to restricted molecular rotations in the liquid [44].

In addition to restricted molecular rotations in the liquid, there are also hydrogen bonds present in the neat alcohols which, upon vaporization to an ideal gas, are lost. The fundamental vibrational frequencies of hydrogen bonds range from 250–50 cm^{-1} [45] which corresponds to entropies around 3 cal K^{-1} mol^{-1} at 298 K. Thus, although hydrogen bonds contribute to ΔS°_v of the alcohols, they tend to counteract the entropy loss associated with decreased molecular rotations.

The observation that the ΔS^* for the alcohols is very similar to the –OH contribution as given by the intercept in Fig. 4 suggests that ΔS^* for the alcohols primarily results from the restriction of molecular rotations in solution and not from solvent restructuring, i.e. hydration. If the restructuring of water around a solute is primarily a function of the solute size, then it is perhaps not surprising that the hydration ΔS° for the hydroxyl group should be negligible at T_S^*, as is observed for apolar groups.

It should be emphasized that the above analysis suggests that the hydration ΔS° of polar groups is negligible at T_S^*, which is equal to 385 K under the assumption of constant ΔC_p. A plot of ΔC_p for the dissolution of gaseous alcohols versus the number of carbons (not shown) gives a straight line with equal slope and intercept (17.2 ± 1.3 cal K^{-1} (mol OH)$^{-1}$ for the intercept and 17.1 ± 0.3 cal K^{-1} (mol C)$^{-1}$ for the slope), suggesting that the hydroxyl and methylene groups make similar contributions. At 298 K then, the hydration contribution to the entropy would be essentially the same for both groups (-4 cal K^{-1} mol^{-1}). This is predicted by recent theoretical studies on hydration entropies of polar and nonpolar molecules [46].

The positive ΔC_p for the hydroxyl dissolution might seem to be at odds with recent empirical analyses suggesting that the polar contribution to ΔC_p of protein unfolding is negative [1,4,26,47,48]. The –OH group contribution to ΔC_p for liquid to water transfer is only 8 ± 2 cal K^{-1} mol^{-1}, however, and it seems likely that the formation of strong hydrogen bonds in the protein interior is the explanation for the negative ΔC_p contribution as has been suggested previously [48].

The above example illustrates that care must

be taken in applying the analysis of model compound data to larger systems such as proteins. Similar differences in dissolution $\Delta S°$ values are observed between the amines and alkanes as between the alcohols and the alkanes [5]. It seems reasonable that these differences can also be attributed to differences in rotational entropies in the liquid phase.

If the difference in dissolution $\Delta S°$ for alcohols (or amides) and alkanes instead is assumed to correspond to the hydration of the hydrogen bonding groups, inappropriate values for the hydrogen bonding contribution to $\Delta S°$ of unfolding of proteins will be obtained. In the empirical schemes being utilized [2,3,5], the configurational entropy change is taken as the total entropy of unfolding less the hydrophobic and hydrogen bonding contributions. Consequently, excessively large values for the configurational entropy will also be obtained. Theoretical and empirical estimates of configurational $\Delta S°$ values [11,49–52] are in good agreement and result in $\Delta S°$ per residue averaging between 4 and 5 cal K^{-1} mol^{-1} [53]. In contrast, consideration of a large, polar hydration contribution results in estimates of 19 cal K^{-1} (mol res)$^{-1}$ [5], far in excess of the theoretical value [12,32].

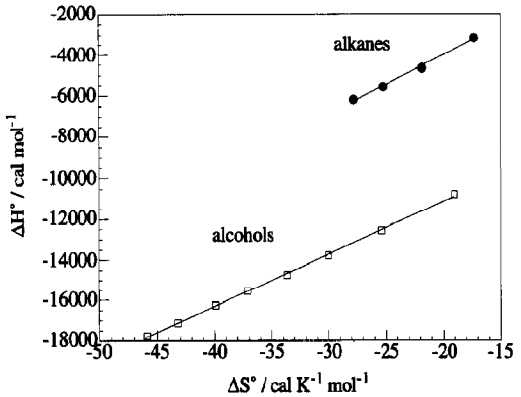

Fig. 5. Enthalpy entropy compensation plot for the aqueous dissolution of gaseous alcohols (open squares) and alkanes (filled circles). The solid lines are the linear least squares fits with solpes of 260 (alcohols) and 290 (alkanes), and intercepts of 1790 (alkanes) and −5970 (alcohols).

3.3. Enthalpy–entropy compensation

As discussed above, systems which show group additivity will also show enthalpy–entropy compensation. This is illustrated for the alcohols and alkanes in Fig. 5. As expected, the plot of $\Delta H°$ versus $\Delta S°$ results in a straight line. The slopes, yielding the compensation temperatures T_c, are similar, being 290 ± 15 K for the alkanes and 260 ± 3 K for the alcohols. Values of α are 1800 $\pm$ 400 cal mol^{-1} for the alkanes and -6000 ± 300 cal mol^{-1} for the alcohols.

T_H^*, calculated from Eq. (13) using $T_S^* = 385$ K, is 372 K for the alkanes and 390 K for the alcohols. The alkane value is the same as that given from the slope of the MPG enthalpy plot, while the alcohol value is somewhat higher. This reflects the somewhat lower apparent value for T_S^* of the alcohols.

4. Conclusions

The study of the forces involved in stabilizing proteins is complicated due to the complexity of the interactions which occur in the protein interior. The simplicity of model compound systems, on the other hand, allows for a more detailed understanding of the energetics of the component interactions. Theories of the stability of proteins should be consistent with what is known about model compound systems, but care must be taken in appropriately assigning the energetic contributions in these systems.

The $\Delta S°$ of dissolution of polar compounds provides a clear example of the potential for misinterpreting the model compound data. Whereas comparison of the vaporization and dissolution energetics indicates that the large negative $\Delta S°$ (as compared to the alkanes) results from changes in internal degrees of freedom, most likely rotation, some investigators have applied this contribution entirely to hydration [3,5].

As this entropic term contributes to the 'hydration' $\Delta G°$ of polar compounds, use of uncorrected model compound $\Delta G°$ values, such as those of Ooi et al. [54], is also likely to be misleading. Such numbers are also used in deriving empirical

potentials for molecular mechanics. The rotational entropy contribution should also be excluded in these cases.

In our own studies, we have assumed that the $\Delta S°$ of hydration of polar groups is zero at T_S^* as it is for apolar groups [1,2,11,33]. The analysis of the alcohol and alkane data supports this assumption, at least as a first approximation. The average value of ΔS^* per residue, 4.3 cal K^{-1} mol^{-1}, can thus be taken as the configurational entropy change as originally suggested by Privalov [21].

The current, empirical approaches to correlating the structures and energetics of proteins have been highly successful [2–5,33]. The continued refinement of the empirical parameters and the acquisition of additional model compound and protein data, promise to advance our understanding of the many forces, including hydration, responsible for the stability of proteins.

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Discussion to the paper by K.P. Murphy

Comments

By B.K. Lee

In your discussion of the entropy correlations (the middle of the second paragraph after your quotation of Barclay and Butler), it is stated that "ΔS^* for the alcohols primarily results from the restriction of molecular rotations in solution and not from solvent restructuring, i.e., hydration". I suppose that there are many ways to define the "solvent restructuring", but certainly one major component of the "solvent restructuring" associated with introduction of a hydroxyl group in water can be restriction of rotational motion of the solvent water. Why does not the fact that the ΔS^* values are similar for the hydration and vaporization processes simply mean that both represent the restriction of rotational (and translational) motion of the solvent molecules, water in the case of hydration and other alcohol molecules in the case of vaporization? Is the orientational restriction of the water molecules around the hydroxyl group not a part of the hydration process?

The fact that the existence of the convergence temperatures implies also the enthalpy–entropy compensation, since both arise from a linear free energy relation, was pointed out to me earlier by Dr. B.W. Sigurskjold (personal communication).

By K. Sharp, B. Honig, A.-S. Yang

Murphy presents a detailed analysis of enthalpy/entropy vs. heat capacity (MPG) plots, using these to analyze the process of hydroxyl group hydration in alcohols. It is important to point out, as Murphy does, that the intercept of these plots (where $\Delta C_p = 0$), is not the contribution of the constant portion of a homologous solute series at 25°C, i.e. for the example of alcohols, dH^* is not equal to ΔH_{oh}. The intercept of the plot is the contribution of the constant group at the convergence temperature, and must be converted back to 25°C using Eqs. (8) or (10) as appropriate.

A crucial issue for interpreting plots of thermodynamic properties with homologous solute series is whether linearity necessarily requires group additivity. Additivity presumably means that the contribution of the common group (i.e. –OH for the alcohols) to ΔH or ΔS of a process is the same in all the compounds. The deduction, from the very similar intercept entropies of alcohol hydration (Fig. 2) and alcohol vaporization (Fig. 4), that the –OH entropy comes mostly from the solute's loss of molecular motions relies on an additivity assumption: If putting an OH group into say $n = 8$ (octanol, where there is no water, takes the same entropy (≈ 12 cal/mol/K) as putting it into water, then water ordering does not contribute. However as the chain is shortened the pure alcohol becomes a more polar solvent, and the chance of one OH group interacting with others, and being differentially solvated increases. It is unlikely that solvation contribution is constant with n. The compound corresponding to the intercept in Fig. 4 (a zero carbon alcohol) is effectively water, and the intercept entropy is almost exactly that of water vaporization, 12.1 cal/mol/K. Thus the entropy at intercept in Fig. 4 actually represents the introduction of an OH group into water, not into anhydrous environment. The plot may be linear without additivity if the change in OH solvation is linear with the number of carbons: This is quite plausible, since virtually every other thermodynamic property varies linearly with n. Group additivity is a good assumption for a solute transferred from one dilute phase to another (e.g., gas to water), but when one of the phases is the pure solute it is likely to break down.

B.K. Lee (1991) and Yang et al. (1992) were

interested in explaining not so much the linearity of dH versus dC_p plots for proteins, but the very negative slope (close to the nonpolar gas to water transfer values). The two possible causes are (a) There is a *favourable* enthalpic interaction which *decreases* with increasing dC_p (i.e. increasing apolar area burial). (b) There is an *unfavourable* ehthalpy which *increases* with increasing dC_p. Lee proposed (a) arising from decreased polar area burial. We proposed (b), arising from increasing desolvation of polar groups. The latter is consistent with the cyclic dipeptides also having a very negative slope of dH versus dCp plots. Even though the number of polar groups per cyclic dipeptide molecule is constant, the energy of taking these groups from water into the crystal would change as the overall crystal environment is made more apolar by increasing side chain hydrophobicity. The contribution of polar groups to solvation in water is likely to be constant because the solute is delute and additivity will hold here. However, in the crystal phase, like the pure alcohol phase, the environment of the polar portion of the molecule is significantly changed since there are many hydrophobic 'side chains' close by. A serious difficulty in interpreting MPG or other plots of homologous solute thermodynamics is that with the additivity assumption by definition anything that changes (the slope value) is interpreted entirely as a property of the variable portion of the molecule, and the contribution from the constant part is what's left. While this might be true in dilute solution, in condensed phase (crystal, pure liquid, protein interior) it need not hold. Linearity is not sufficient evidence of additivity, since linearity can arise when there are multiple but compensating changes.

As Murphy points out, explaining the linearity of protein MPG plots in terms of similar T_m's only changes the question. A better explanation should be sought in the free energy, since this is what actually governs the behaviour of proteins: Most globular proteins work within a narrow range of ΔGfold (ΔGfold/residue is effectively zero) presumably because of biological and functional requirements (which are sensitive to free energy), and Yang et al. (1992) have argued that this will result in linear plots.

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[2] A. Yang, K. Sharp, B. Honig, J. Mol. Biol. 227 (1992) 889.

By A. Rashin

(1) In our paper in this Issue we found that there is a differences of about 30% in entropy per Å^2 of the nonpolar accessible surface area (slope of $T\Delta S$/ASA plot) for linear alkanes and alcohols. A reasonable explanation of this difference involves a restriction of configurational space of alcohols in water compared to the gas phase. However, we find that at room temperature entropies per Å^2 are of the same order of magnitude for polar and nonpolar groups. Compared to alkanes they seem to be somewhat higher for hydroxyl and amino-groups and somewhat lower for carbonyl groups.

In our model calculations for spherical particles (where no rotational entropy of the solute can be invoked) we obtain the same trend [2,3]. Thus, it is likely that this hydration entropy is caused by restrictions on positions of waters of the solvent around polar groups which is basically a definition of the cause of hydration entropy. The magnitude of these entropies is not negligible which agrees with your note on similar magnitudes of hydration entropies of hydroxyl and methylene groups.

Then why would not a burial of these polar groups upon a hydrogen bond formation stabilize hydrogen bonds to the same extent as the burial of apolar surfaces? While Privalov's values may be somewhat too high, I do not see why they should be negligible according to your own data at least at room temperature.

(2) Your estimates of vibrational entropies are based on in vacuo frequencies and correspond to a rather strong binding. In solution this binding is much weaker as electrostatics is partially screened by the solvent [3] and, thus, frequencies are much lower.

(3) While vaporization of alcohols do not involve *hydration* entropy, it involves the entropy of solvation in a polar fluid. This can be expected to be similar to that of hydration. If so, your attempt to use the similarity between vaporization and 'hydration' entropies as a contradiction proving your point may be invalid.

[1] A.A. Rashin, L. Young and I. Topol, this Issue.
[2] A.A. Rashin and M.A. Bukatin, J. Phys. Chem. 98 (1994) 386.
[3] A.A. Rashin and M.A. Bukatin, in this Issue.

By P.L. Privalov

The configurational entropy which we calculated by excluding hydration entropy from the entropy of protein unfolding actually corresponds to protein unfolding in a vacuum. This value appeared to be larger than the theoretically calculated values for the polypeptide chain in vacuum. That is why we were interested in your suggestion that configurational entropy of protein unfolding might be smaller than what we obtained because of restriction of torsional motions of polar groups in water. Unfortunately this does not help. Configurational entropy estimated as you suggest could be lower if water indeed reduces torsional motion of polar groups. However, one cannot compare its value with the theoretical values which were calculated assuming that all groups in the unfolded polypeptide chain are free. Therefore, it is unclear what you mean by saying that "Theoretical and empirical estimates of configurational ΔS values are in good agreement".

My other concern is the enthalpy of hydration. How could we explain the large, negative and temperature-dependent enthalpy of hydration of polar groups if not by the rearrangement and reinforcement of the hydrogen bonding network in water surrounding polar group. But could that happen without significant entropy decrease of water? The split of hydration entropy in two components, one associated with water and other with polar groups, can hardly be done reliably based on the single example of alcohol vaporization. Because of this, it is unclear whether it is worth doing at all. The restriction of torsional motion of polar groups by water is also the effect of hydration. On the other hand, it is much easier to deal with the polypeptide chain in a vacuum than in a quasi-viscous fluid in considering theoretically its configurational entropy.

By G. Makhatadze

The observations presented in your paper are very interesting. In your analysis you compare the entropies of dissolution in water and the entropies of vaporization for alkanes and alcohols. The similarity between the entropies of dissolution and vaporization per hydroxyl group is obtained. The conclusion made out of this observation is that the negative entropy of hydration of hydroxyl group "*... arises from changes in internal degrees of freedom and should not be applied to the analysis of protein energetics.*" This conclusion would be correct if the same behavior could be observed for series of other compounds. Unfortunately it does not seem to be so. Fig. 1 shows a plot of the entropy of vaporization (corrected on the translational contribution) of *n*-alkanes, *n*-alcohols, *n*-alkylamines, esters (formates) and carboxylic acids plotted versus the number of heavy atoms. Data for *n*-alkanes and *n*-alcohols was taken from Table 2 of your paper and the data for *n*-alkylamines, esters and carboxylic acids was for taken from A. Ben-Naim and Y. Marcus (J. Chem. Phys. 81 (1984) 2016–2027). In contrast to what one can expect from your analysis, the behavior of *n*-alkylamines, esters and carboxylic acids is very similar to that of *n*-alkanes. One can argue that *n*-alkylamines and esters cannot form hydrogen bonds in their pure liquid state and that is why they behave in a similar way to the *n*-alkanes. However, carboxylic acids can form hydrogen bond in the pure liquid state and yet, upon extrapolating they intersect the *y* axis very close to zero. This means that there is some kind of anomaly in the behavior of alcohols. One probable explanations could be incorrect experimental values for alcohols. It is very difficult to obtain anhydrous alcohols (see S. Cohen, Y. Marcus, Y. Migron, S. Dickstein and A. Shafran, J. Chem. Soc. Faraday, Trans. 89 (1993) 3271–3275). Significant traces of water in "pure" alcohol can affect the thermodynamics of vaporization, which in this case will include the effects of dehydration as well. The sigh and magnitude of observed anomalous effects in entropy of vaporization of alcohols support this explanation.

Responses by K.P. Murphy to Comments

To B.K. Lee

I agree with Dr. Lee that orientational restrictions of the water molecules around the hydroxyl

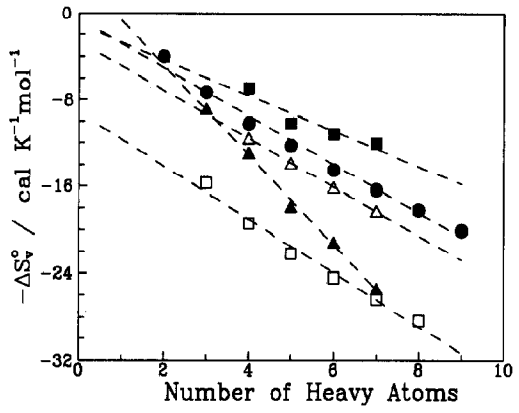

Fig. 1. Negative vaporization entropies corrected for volume differences, $-\Delta S_v^\circ$, versus the number of heavy atoms in the chain for the *n*-alcohols (□), *n*-alkanes (●), *n*-amines (△), esters (formates) (■) and carboxylic acids (▲).

group should be considered a part of the hydration process. If the hydroxyl contribution to ΔS^* (and $\Delta S_{vap}^{\cdot}$) did arise from solvent restructuring, one might expect it to decrease with increasing chain length of the solvent because fewer solvent molecules could 'pack' around the hydroxyl. However, as illustrated in Fig. 4, the difference in $-\Delta S_{vap}^{\cdot}$ between the alcohols and alkanes is constant, or perhaps even increasing, with increasing chain length. Additionally, the term "hydration" is generally taken as referring to "special" properties of water as a solvent (see, for example, [1]), which does not seem to be the case for the hydroxyl ΔS°.

[1] K.A. Dill, Science 250 (1990) 297.

To Sharp, Honig and Yang

Sharp et al. raise several important points in their commentary. They are quite correct in pointing out that linearity in thermodynamic properties for a homologous series of compounds does not require group additivity. What I have tried to emphasize here is that linearity, and convergence, is required *if* we have group additivity. While more complex explanations are possible, and perhaps likely, they are not necessary in order to explain the data.

In discussing Fig. 4 in the text, I have suggested that the intercept for the alcohols can be interpreted as arising predominantly from decreased molecular rotational degrees of freedom in the liquid state. Sharp et al. suggest an alternative interpretation, namely that the intercept essentially represents the entropy of vaporization of water and that the slope arises from the change in solvation of the –OH with increasing chain length. They suggest that the linear effect of chain length on solvation is quite plausible since "...every other thermodynamic property varies linearly with *n*". I would contend that the other thermodynamic properties vary linearly with *n* because group additivity holds. Moreover, the slope for the alkanes is nearly identical to that of the alcohols, although no –OH solvation is available to be effected by chain length. Another way of viewing the highly similar slopes is that the addition of an –OH to a linear alkane results in the same loss of entropy regardless of the chain length. The similarity of the slopes for the two series seems inconsistent with their interpretation.

The validity of group additivity in analyzing the crystalline model compound data is also a valid question. Our initial analysis [1] suggested that group additivity provides an adequate empirical description of the dissolution energies. While the sidechains may effect the peptide interactions in the crystal, this is not apparent in the known crystal structures of these compounds [2]. The dissolution data of Barone et al. [3] add further weight to our use of group additivity in this case, as the group values obtained for the dissolution of the N-alkyl amino acid amide series are very similar to those derived from our data on cyclic dipeptides, even though the compounds are significantly different.

[1] K.P. Murphy and S.J. Gill, Thermochim, Acta 172 (1990) 11.
[2] K.P. Murphy, Ph. D. (University of Colorado, 1990).
[3] G. Barone, G. Della Gatta, P. Del Vecchio, C. Giancola and G. Graziano, Biophys. Chem. 51 (1994) 89.

To A. Rashin

(1) I essentially agree with you here, at least as regards the gas to water transfer. An unanswered point in this regard is the empirical observation that ΔC_p for the transfer of polar groups from

solids, liquids, and proteins appears to be negative [1–3]. If the hydration entropy for both polar and apolar surface become negligible at T_s^*, and if the empirical contribution is associated with hydration, then the hydration entropy of burying these hydrogen-bond forming groups would be unfavorable. This issue requires further study.

(2) The primary point regarding the vibrational entropy term is that it is not large enough and of the wrong sign to explain the observed ΔS values.

(3) This point is similar to that raised by B.K. Lee and by Sharp et al. and I would refer you to my replies there.

(4) I look forward to seeing your comments on Privalov's work and his replies.

[1] K.P. Murphy and S.J. Gill, Thermochim. Acta 172 (1990) 11.
[2] P.L. Privalov and G.I. Makhatadze, J. Mol. Biol. 224 (1992) 715.
[3] R.S. Spolar, J.R. Livingstone and M.T. Record Jr., Biochemistry 31 (1992) 3947.

To P.L. Privalov

I agree that what we would all like to calculate as the configurational entropy refers to the entropy change of unfolding of the polypeptide chain *in vacuo*. I am not suggesting that we can calculate restrictions of torsional motion in protein groups, but that the loss of entropy due to restricted molecular rotations in solution must be considered in trying to extract hydration entropies from data on the gas to water dissolution of model compounds. While water may affect the torsional motion of polar groups in proteins, this is a separate issue. The results I presented suggest that the polar contribution to the hydration entropy is negligible at T_s^*. This leads to the empirical estimates of the configurational entropy which are in good agreement with the theoretically derived values.

As noted in my article, a negligible hydration entropy at T_s^* does not require a negligible hydration entropy at all temperatures. Indeed it is the situation observed for the apolar groups. If the hydration entropy arises from intrinsic properties of water, it seems reasonable that it would become zero at some temperature for all groups, polar and apolar. It is important to note that I am not suggesting that the hydration entropy can be split into two components. Rather, I am suggesting that the gas to water dissolution entropy contains contributions from hydration and from changes in internal degrees of freedom. However, your point that the single example of the alcohols is limited is perfectly valid. It would be desirable to have additional examples in addition to rotational spectroscopic data of such molecules in both the gaseous and aqueous phases. Nevertheless, the available data do seem to suggest that the difference between ΔS^* of dissolution of the alkanes and the alcohols is not due to hydration of the polar hydroxyl.

Finally I am uncertain to what your reference to a quasi viscous liquid is referring, and thus I cannot address this point.

To G. Makhatadze

You are correct that my conclusions would predict that the carboxylic acid vaporization entropies should, like the alcohols, be of greater magnitude than the alkanes. If you include the carboxylic acid data of Ben-Naim and Marcus [1] on my Fig. 4 you obtain the Fig. 2 below. The difference between this plot and yours is that the data are plotted versus the number of carbons in

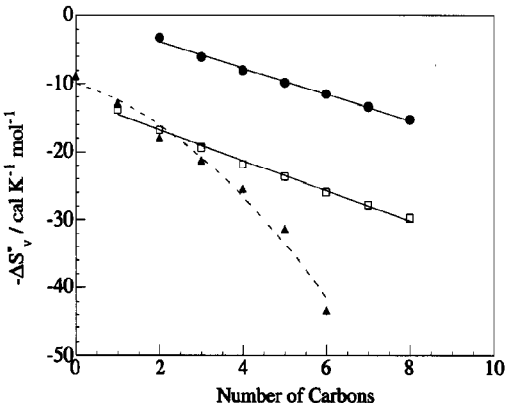

Fig. 2. Negative vaporization entropies corrected for volume differences versus the number of carbons in the alkyl chain for the alcohols (open squares), alkanes (filled circles), and carboxylic acids (filled triangles). Carboxylic acid data are from Ben-Naim and Marcus [1]. Other data are taken from Fig. 4 of the main text.

the alkyl chain, as described in the text, rather than the total number of heavy atoms as in your figure. In this way the intercept is expected to yield the contribution of the functional group. While there is curvature in the acid data, they clearly resemble the alcohols rather than the alkanes as predicted. The curvature might be the result of the assumption of the saturated vapor as an ideal gas, since the carboxylic acids readily associate in the vapor phase [2].

I have not included the amines or the esters on this plot. I agree that the esters are not expected to form hydrogen bonds. The amines can form a –NH...N hydrogen bond, but this is weaker bond than that formed by the alcohols [3]. The two *n*-alkylamines listed by Ben-Naim and Marcus [1] fall intermediate between the alkanes and the alcohols. However, the formate esters do so as well for which I have no explanation.

Finally in regards to the reliability of the experimental data, I would expect that the alcohol data are more reliable than the carboxylic acid data in general. Few calorimetric data on the heats of vaporization of carboxylic acids are available due to their "chemical aggressiveness" [2]. Additionally, their ready association in the vapor phase complicates the interpretation of the data [2]. In contrast, there are abundant calorimetric data on the alcohols. The problem of trace water is not likely to have a large effect on the results. While the solubility of water in alcohols can complicate water to alcohol transfer experiments, anhydrous alcohols can be prepared for vaporization studies. For example, anhydrous methanol can be obtained with $\leqslant 0.1\%$ water. This is equivalent to less than one molecule of water per 500 molecules of methanol which should not make a detectable contribution to the vaporization energetics.

[1] A. Ben-Naim and Y. Marcus, J. Chem. Phys. 81 (1984) 2016.

[2] V. Majer and V. Svoboda, Enthalpies of vaporization of organic compounds (Blackwell Scientific Publications, Oxford, 1985).

[3] G.C. Pimentel and A.L. McClellan, The hydrogen bond (W.H. Freeman, San Francisco, 1960).