ELSEVIER

Biophysical Chemistry 70 (1998) 57–63

Biophysical
Chemistry

Thermodynamics of the hydration of non-polar substances

Ricardo L. Mancera [a,b,*], Bengt Kronberg [c], Miguel Costas [d], Rebecca Silveston [c]

[a] Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge CB2 1EW, UK
[b] Drug Design Group, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ, UK
[c] Institute for Surface Chemistry, P.O. Box 5607, S-114 86 Stockholm, Sweden
[d] Laboratorio de Termofísica, Departamento de Física y Química Teórica, Facultad de Química, Universidad Nacional Autónoma de México, México D.F. 04510, Mexico
[e] Westvaco, Charlston Research Center, 5600 Virginia Ave., SC 29406, USA

Received 12 February 1997; accepted 21 August 1997

Abstract

We re-examine the numerical value and physical significance of T_S, the temperature where the entropy of transfer $\Delta_L^W S$ from the pure hydrocarbon liquid into water is zero. It is shown that the numerical value of T_S depends on the convention adopted for calculating $\Delta_L^W G$ from solubility data at 25°C and on the $\Delta_L^W C_P$ fitting function. It is concluded that the interpretation of T_S as the temperature where hydration ceases cannot be sustained. As previously reported [R.L. Baldwin, N. Muller, Proc. Natl. Acad. Sci. USA, 89 (1992) 7110], hydration must vanish at a temperature $T' > T_S$, where its experimental manifestation, i.e., $\Delta_L^W C_P$, is zero. We discuss the concept of water relaxation around a non-polar solute molecule and its relation to the hydration process. © 1998 Elsevier Science B.V.

Keywords: Hydrophobic effect; Aqueous solutions; Protein folding

1. Introduction

The dissolution of non-polar substances into water has attracted the attention of numerous workers. It is generally agreed that understanding the low solubility of non-polar substances in water is essential to comprehend protein stabilisation and denaturation, as well as the hydrophobic effect resulting in surfactant micellisation and adsorption. The transfer of a hydrocarbon from its pure liquid into an aqueous phase is found to generate a positive free energy that reaches a maximum around 140°C, where the entropy of transfer is zero. This entropy of transfer is found to be negative and decreasing in magnitude with temperature and asymptotically approaching zero, or some other value, depending on the choice of standard state, as will be shown below. The enthalpy, on the other hand, is negative at low temperatures and positive at higher temperatures. The crossover, zero enthalpy, is normally found at temperatures between 15 and 25°C, corresponding to the observed minimum in the solubility of these species in water.

These phenomena have, through the years, been interpreted in several different ways [1]. Some years ago, Privalov and Gill [2,3] presented an interpretation scheme in which the temperature T_S, where the entropy of transfer from the pure hydrocarbon liquid

---

[*] Corresponding author.

0301-4622/98/$19.00
PII S0301-4622(97)00110-5

into water is zero, plays an important role. This interpretation has been used by several authors [4–7] primarily to extract information on the dissolution properties of non-polar solutes in water and, in particular, to isolate the so called hydration free energy, enthalpy, entropy and heat capacity responsible for the hydrophobic effect. On the other hand, difficulties with this interpretation scheme in terms of T_S have been discussed in the literature from various viewpoints [8–12]. Here we comment on both the numerical value of T_S for hydrocarbons and its physical significance, and discuss in detail the concept of hydration. It should be pointed out that, although the hypothesis that the hydration of non-polar substances goes to zero at T_S is believed to be incorrect by several workers in the field, it is equally true that an equally numerous group of researchers uses it in the analysis of their results. In this context, this paper also has as one of its goals to explain in detail why this hypothesis is not correct.

2. The numerical value of T_S

The entropy of transfer of a solute from its pure liquid into water $\Delta_L^W S$ is given by

$$\Delta_L^W S(T) = \Delta_L^W S(T_O) + \int_{T_O}^{T} (\Delta_L^W C_P/T)\mathrm{d}T \qquad (1)$$

where $\Delta_L^W C_P$ is the heat capacity of transfer from the pure hydrocarbon liquid into water and $\Delta_L^W S(T_O)$ is the entropy of transfer at T_O (25°C). $\Delta_L^W S(T_O)$ is obtained as $(\Delta_L^W H(T_O) - \Delta_L^W G(T_O))/T_O$, where both the enthalpy ($\Delta_L^W H$) and Gibbs free energy ($\Delta_L^W G$) of transfer are experimentally accessible independently of each other. While $\Delta_L^W H(T_O)$ is directly obtainable experimentally, $\Delta_L^W G(T_O)$ needs a convention (choice of standard state) in order to be calculated from, for example, the solubility or vapour pressure data. Hence, the numerical value of $\Delta_L^W S(T_O)$ in Eq. (1) depends on the convention used to obtain $\Delta_L^W G(T_O)$. The temperature T_S is defined as the temperature where $\Delta_L^W S$ is equal to zero [2,3]. It can be shown that for a given non-polar substance, the numerical value of T_S depends on two factors: (i) the particular functional form for $\Delta_L^W C_P$ chosen to fit the experimental data and (ii) the value of $\Delta_L^W S(T_O)$, which in turn depends on the convention adopted for the calculation of $\Delta_L^W G(T_O)$. In order to illustrate the sensitivity of T_S to these two factors, we have chosen two $\Delta_L^W C_P$ fitting functions and also the often used constant heat capacity (independent of temperature) assumption:

$$\Delta_L^W C_P(T) = \Delta_L^W C_P(T_O)\exp \times[-a(T-T_O)] \qquad (2)$$

$$\Delta_L^W C_P(T) = \Delta_L^W C_P(T_O)(T/T_O)\exp[-b(T-T_O)] \qquad (3)$$

$$\Delta_L^W C_P(T) = \Delta_L^W C_P(T_O) \qquad (4)$$

and the following different conventions for obtaining $\Delta_L^W G(T_O)$ from solubility data:

$$\Delta_L^W G(T_O) = -RT_O \ln x \qquad (5)$$

$$\Delta_L^W G(T_O) = -RT_O \ln\phi = -RT_O[\ln x + \ln(V_S/V_W)] \qquad (6)$$

$$\Delta_L^W G(T_O) = -RT_O\,[\ln\phi + (1-(V_S/V_W))] \qquad (7)$$

In Eqs. (5) and (6) the solubility of the non-polar substance in water is expressed as mole fraction (x) and volume fraction (ϕ), respectively. In Eqs. (6) and (7), which are only valid at very low concentrations, V_S and V_W are the molar volumes of the solute and water, respectively. It has been shown [13] that when dealing with free energies and entropies, the use of mole fractions is appropriate only when dealing with equal-sized molecules. For mixtures of molecules of different sizes, it is necessary to use expressions such as Eqs. (6) and (7) which take into account such size differences. In Eq. (7), the Flory–Huggins expression for the free energy has been used, and for hydrocarbons in water the resulting $\Delta_L^W G(T_O)$ has been shown to be independent of the solute–water size difference [14,15]. Some efforts have also been made to include the effect of curvature [16] and shape [17] of the solute when calculating free energies of solution. The ideal gas equations, combined with experimental molar volumes, have also been used to account for entropy changes induced by volume changes by equating this term to its equivalent ideal gas work, recovering the Flory–Huggins expressions under additive volume conditions [18]. The use of Flory–Huggins theory, developed originally for polymer solutions, has nevertheless been criticised by some authors [19,20]. From the theoretical point of view, it has been argued that the theory is not suitable for the description of partition experiments of non-polar solutes from their pure liquids to water, and that the use of the molarity scale is the correct way to calculate the solvation free energy change [19]. Recent experimental evidence seems to have a better correlation with the solvent-accessible area of the solute without the inclusion of a molecular volume term [20]. Nevertheless, a recent theoretical and computer simulation analysis of Flory–Huggins theory confirmed its applicability to account for molecular size effects, although it seems that they are overestimated by about 25% [21].

Table 1 shows T_S values for benzene and toluene obtained using Eqs. (2)–(4) and Eqs. (5)–(7). In particular, Privalov and Gill [2] used Eqs. (2) and (5) to obtain $T_S = 139°\mathrm{C}$ and 145°C for benzene and toluene, respectively. Table 1 clearly indicates that the value of T_S depends on both the $\Delta_L^W C_P$ fitting function and the convention adopted for calculating $\Delta_L^W G(T_O)$, the dependence on the latter being more pronounced. Note that, for a given choice of $\Delta_L^W C_P$ and $\Delta_L^W G(T_O)$ equations, T_S remains essentially constant in going from benzene to toluene. Using Eqs. (5) and (2) with the parameter a kept constant, Privalov and Gill [2] showed that T_S is approximately constant for many non-polar substances (140°C). In fact, it is possible to show that if all imaginable contributions to the entropy of transfer $\Delta_L^W S$ are proportional to solute size, T_S must be the same for all non-polar solutes. Then, the fact that T_S is taken as a universal constant for many solutes [2,3] appears to be true; however, as shown in Table 1, its actual numerical value depends on the manner it is evaluated. Even if accurate heat capacity data for many solutes were available in a wide temperature interval, the value of T_S would still depend on the convention adopted for the calculation of $\Delta_L^W G(T_O)$.

Table 1
Temperature T_S for benzene and toluene using different ΔC_P fitting functions and conventions to obtain $\Delta_L^W G(T)$[a]

$\Delta_L^W C_P$	$\Delta_L^W G\ (T_O)$	Benzene		Toluene	
		$\Delta_L^W S(T_O)$ J K^{-1} mol^{-1}	T_S (°C)	$\Delta_L^W S(T_O)$ J K^{-1} mol^{-1}	T_S (°C)
Eq. (2)	Eq. (5)	−58	139	−71	145
Eq. (2)	Eq. (6)	−45	103	−56	110
Eq. (2)	Eq. (7)	−78	208	−97	232
Eq. (3)	Eq. (5)	−58	125	−71	119
Eq. (3)	Eq. (6)	−45	96	−56	93
Eq. (3)	Eq. (7)	−78	185	−97	177
Eq. (4)	Eq. (5)	−58	107	−71	103
Eq. (4)	Eq. (6)	−45	87	−56	85
Eq. (4)	Eq. (7)	−78	141	−97	137

[a] Parameters in Eqs. (2) and (3) were fitted to the experimental $\Delta_L^W C_P(T)$ [22]: $a = 0.0041$ for benzene and toluene [2], $b = 0.00677$ for benzene and toluene. In Eq. (4), $\Delta_L^W C_P(T_O) = 238$ J K^{-1} mol^{-1} for benzene and 305 J K^{-1} mol^{-1} for toluene [22] with $T_O = 298$ K. Solubilities are (mole fraction) 4.01×10^{-4} for benzene [23] and 1.01×10^{-4} for toluene [24]. Molar volumes (cm^3 mol^{-1}) at 25°C are 89.4, 106.9 and 18.0 for benzene, toluene and water, respectively. $\Delta_L^W H(T_O)$ values used to calculate $\Delta_L^W S(T_O)$ are 2.08 kJ mol^{-1} and 1.73 kJ mol^{-1} [2] for benzene and toluene, respectively.

3. The physical meaning of T_S and the concept of hydration

Because the entropy is a temperature derivative of the Gibbs free energy, $\mathrm{d}\Delta_L^W G/\mathrm{d}T = -\Delta_L^W S$, the existence of T_S implies that the free energy of transfer has a maximum at this temperature and hence its importance. Although the term hydration has been used many times in the literature, its meaning has seldom been explained. Privalov and Gill [2] defined hydration clearly and we will use their concept here. Hydration is defined as the transfer process to water from a hypothetical compact state, whose properties are those of a compressed hydrocarbon gas at T_S, i.e., a fluid without molecular interactions. It was then proposed [2,3] that at T_S water does not solvate the non-polar solute, i.e., T_S is the temperature at which hydration ceases. This interpretation has been used [4–7] not only to study aqueous solutions of simple non-polar solutes, but also to analyse more complex problems such as protein stabilisation and denaturation. Our purpose here is to re-examine the above conceptualisation of hydration and the accompanying physical meaning of T_S. To do so, it is necessary to break down the non-polar liquid to water transfer into its possible components at the molecular level. A reasonable division was proposed earlier [15], consisting of the following steps: (1) Removal of a solute molecule from the pure liquid, breaking solute–solute contacts. (2) Closing of the cavity therein. (3) Creating a cavity in water to accommodate the solute molecule. (4) Creation of solute–water contacts. (5) Relaxation of water molecules around the solute.

In order to evaluate these components, it is necessary to construct one or several molecular models. Recently, this five-step scheme, together with a first-principles molecular model of the relaxation component (step 5), was used to analyse the experimentally obtained free energies of transfer for a series of alkylbenzenes into water and to discuss the origin of hydrophobicity [15]. It is useful then to consider the enthalpic and entropic contributions from each of the above steps. Steps 1 and 2 together have a *positive enthalpy* change arising from the interaction between solute molecules in their pure liquid phase; these two steps are nearly temperature-independent. There is also an associated small positive entropy change due to the removal of a solute molecule from its pure liquid phase and the closure of the cavity left behind.

Rather than proceeding to describe individually steps 3 to 5, we would now like to propose an accompanying description of the process of insertion of the solute based on clearly defined physical stages. This arises from the need to define unambiguously the molecular meaning of hydration. The molecular processes involved in steps 3 to 5 are not independent, since all of them involve the destruction and formation of hydrogen bonds. The contribution to the relaxation energetics from the formation of a cavity was also recognised by Lee [25,26]. To be able to better understand the thermodynamic contributions associated with steps 3 to 5, we define the following two stages, which are actually ensemble averages over different configurations of the water molecules and the solutes: (a) Water molecules of the neat liquid are expanded to infinity at *fixed* relative orientations, and then they are contracted back to *minimum* energy in their fixed orientations in the presence of solute molecules. (b) Water molecules in the neighbourhood of the solute molecules are allowed to have rotational and translational freedom, in a process we denote as *relaxation*.

The enthalpic and entropic contributions associated with these stages can be rationalised as follows. Stage (a) has a *positive enthalpy* arising from the excess of broken hydrogen bonds in the liquid expansion over the reformed ones during the contraction, since only *translational* freedom is allowed in this process and the presence of the solute prevents the formation of several hydrogen bonds and/or permits only the formation of bent hydrogen bonds. There is also, of course, a negative, but comparatively small, enthalpic contribution coming from the interactions between the solute and the water molecules. After this process, water is said to be in an *unrelaxed* state in the mixture. The whole expansion–contraction process has a positive *combinatorial entropy* change which can be described, for example, by the Flory–Huggins theory, and is essentially temperature independent [15]. It can now be seen that stage (a) comprises both steps 3 and 4 above; since it involves the net breaking of hydrogen bonds (or from a different perspective, the replacement of strong water–water interactions with weaker solute–water ones), it is accompanied by a *positive free energy* change which is nearly temperature independent. Stage (b) involves a temperature-dependent structural relaxation of the water molecules in the vicinity of the solute, brought about by the need of water molecules to minimise their energy. This relaxation is accompanied by a *negative enthalpy* change since the translational and rotational freedom of water molecules involves changes in their distances and orientations to the solute and other water molecules, thus allowing for the formation of new hydrogen bonds (or less strained ones). The resultant reduced local free volume and orientational freedom due to the presence of the solute produces as well a *negative entropy* change. The whole process of relaxation, i.e., stage (b) or step 5 above, is a spontaneous one, so it involves a *negative free energy* change which, as mentioned before, is temperature dependent.

Within the framework of our description of the process of inserting a solute in water, hydration would be constituted by stages a and b (or steps 3, 4 and 5). Hence the total entropy of transfer $\Delta_L^W S$ can be written as

$$\begin{aligned}\Delta_L^W S &= \Delta S(1+2) + \Delta S(3+4+5)\\ &= \Delta S(3+4) + \Delta S(5) = \Delta S(\mathrm{a}) + \Delta S(\mathrm{b})\\ &= \Delta S(\mathrm{hydration}) \qquad (8)\end{aligned}$$

according to the various transfer components and stages previously defined. Note that setting $\Delta S(1+2) = 0$ is analogous to the entropy of transfer from the pure liquid solute to the compact state being zero in Privalov and Gill's interpretation scheme of the thermodynamic transfer quantities [2]. Eq. (8) indicates that, within the five-component framework described before, the temperature T_S has no physical significance but rather it is only the temperature at which $\Delta S(\mathrm{b}) = -\Delta S(\mathrm{a})$. T_S is then the temperature where the combinatorial (positive) contribution to the entropy of transfer and the (negative) contribution arising from the relaxation of water molecules balance each other. Thus at T_S hydration cannot cease; in fact, hydration does not vanish when $T > T_S$, as it has already been pointed out earlier [11]. Fig. 1 summarises schematically the above discussed situation. Since $\Delta_L^W S = \Delta S(\mathrm{hydration})$ depends on the choice of convention or, more precisely, on the $\Delta_L^W G$ equation (Eqs. (5)–(7)) used, $\Delta S(3+4) = \Delta S(\mathrm{a})$ also depends on this choice. Fig. 1 shows two possible reference states producing, as shown numerically in Table 1, two different $\Delta S(\mathrm{a})$ values and hence two different T_S values. In both cases, and in fact for any choice of convention to calculate $\Delta S(\mathrm{a})$, hydration continues for $T > T_S$. It should be noted that the assigned physical meaning to T_S as the temperature where hydration ceases, produces an inconsistency in the two-step description of dissolution presented by Privalov and Gill [2]. This can be expressed in the following manner: since one of the properties of the hypothetical compact state is that the entropy of transfer of the solute from its pure liquid state to the compact state is zero, the total entropy of transfer $\Delta_L^W S = \Delta S(\mathrm{hydration})$; on the other hand, since hydration is believed to vanish at T_S, then $\Delta_L^W S$ should be zero at $T > T_S$. What is then the origin of the positive $\Delta_L^W S$ at $T > T_S$ which is calculated from Eq. (1)? (see Figs. 11, 12, 14 and 16 in Ref. [2] and Fig. 5 in Ref. [3]) The answer to this question is given by Eq. (8), which indicates that hydration contains the positive, temperature-independent combinatorial entropy $\Delta S(\mathrm{a})$. $\Delta_L^W S$ becomes positive for $T > T_S$ because starting at T_S the positive $\Delta S(\mathrm{a})$ overcomes the negative $\Delta S(\mathrm{b})$. This is in agreement with the conclusions of Lee [25] and de Young and Dill [14], who propose that the difference in molecular size, which must be reflected in the combinatorial entropy, has a major contribution to the hydrophobicity of non-polar solutes in water.

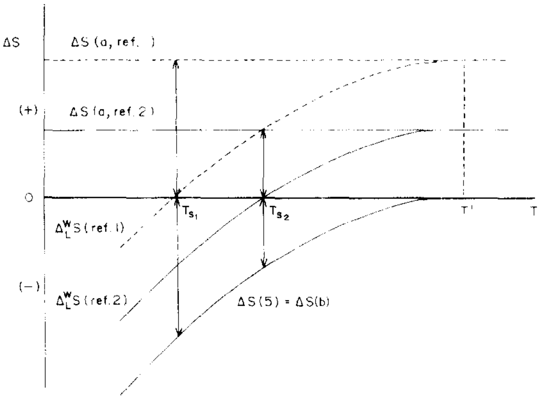

Fig. 1. Schematic representation of the entropy of transfer against temperature. $\Delta_L^W S$ (Ref. [1]) and $\Delta_L^W S$ (Ref. [2]) are the total entropies of transfer obtained using two different conventions for the calculation of $\Delta_L^W S\ (T_0)$ (see Eq. (1) and text). $\Delta S(5)$ is the contribution to the total entropy of transfer due to the relaxation of water molecules around the solute (step 5 or stage b). $\Delta S(\mathrm{a}, [1])$ and $\Delta S(\mathrm{a}, [2])$ are the combinatorial contributions to the entropy of transfer due to components 3 and 4 (stage a). Following Privalov and Gill [2], $\Delta S(\mathrm{hydration}) = \Delta S(3+4) + \Delta S(5)$. T_{S1} and T_{S2} are the temperatures, corresponding to each reference, where both contributions to the total entropy of transfer $\Delta S(5)$ and $\Delta S(3+4)$ cancel each other. T' is the temperature where $\mathrm{d}\Delta_L^W S(T)/\mathrm{d}T = \Delta_L^W C_p(T)/T = 0$ (negligible) and $\Delta S(5)$ effectively vanishes.

Based on the five-component scheme for the transfer thermodynamic quantities given above, it is possible to offer an alternative point of view or rationalisation of the temperature dependence of $\Delta_L^W S$. The experimental finding that $\Delta_L^W C_P$ is much greater than the heat capacity of the pure liquid solute [1–3] has almost unanimously been interpreted [1–3,11,27] (an exception is that given in Ref. [28]) as a reliable indication of the relaxation of water molecules around the solute, i.e., that $\Delta_L^W C_P$ is mainly caused by water solvating the non-polar solute. Then, although in principle all five components have a contribution to $\Delta_L^W C_P$, it is reasonable to assume that those corresponding to steps 1 to 4 are very small (an order of magnitude smaller) compared to that from step 5. Consequently, the experimental $\Delta_L^W C_P$ and its temperature dependence are due only to the relaxation of water. This $\Delta_L^W C_P$ does not vanish at T_S, and actually experiments [22,29,30] and model calculations [27,31] with several solutes indicate that $\Delta_L^W C_P$ values at T_S are between 0.3 and 0.5 times as large as those at room temperature [11]. In other words, at and above T_S water is still capable of relaxing itself around the solute. Since $\mathrm{d}\Delta_L^W S(T)/\mathrm{d}T = \Delta_L^W C_P/T$, the relaxation of water molecules around the solute ceases when $\Delta S(\mathrm{b})$ in Eq. (8) levels off. Hydration then vanishes at a temperature higher than T_S, where its experimental manifestation, i.e., $\Delta_L^W C_P$, is zero; this conclusion was first reached by Baldwin and Muller [10]. Since $\Delta_L^W C_P$ data appears to be well fitted by exponentially decreasing functions with temperature (such as those given by Eqs. (2) and (3)), there is no finite value of this temperature, only a temperature T' (see Fig. 1) where the magnitude of $\Delta_L^W C_P$ is effectively negligible. It thus appears that the physically significant temperature is T' rather than T_S. In terms of the entropy of transfer, the more meaningful contribution to $\Delta_L^W S$ is that arising from the relaxation of water molecules around the solute, $\Delta S(5)$ or $\Delta S(\mathrm{b})$. According to Eq. (8), $\Delta S(5)$ can be obtained by simply subtracting from $\Delta_L^W S$ the combinatorial contribution $\Delta S(\mathrm{a})$. Such a subtraction, performed over the experimentally obtained free energies of transfer of alkylbenzenes, has been done in the past [15] using the Flory–Huggins theory and leading to a satisfying and consistent picture of the dissolution of non-polar solutes in water. The removal of the combinatorial contribution from the thermodynamic transfer functions does not cancel their dependence with a reference state or convention used, but at least it does not mix opposite-sign contributions which can lead, as seen above, to serious conceptual problems and inconsistencies. In this context, the concept of hydration in Refs. [2] and [3], although appealing because of its apparent simplicity, obscures some of the essential features of the dissolution of non-polar solutes in water which, we propose, are better understood if: (i) the transfer process is divided into the five components described above and a proper account of the thermodynamic contributions to each step is made, and (ii) the combinatorial contributions are explicitly taken into account. On the other hand, it is important to call attention to the fact that the present results do not modify one of the main conclusions of Privalov and Gill [2,3], namely that the hydration of non-polar molecules increases or promotes their solubility in water, a conclusion that was first stated by Shinoda and Fujihara [32] and Shinoda [33] on pure thermodynamic grounds.

In studying protein unfolding, the general trend is to split the unfolding process into polar and non-polar contributions [2,4,7–10]. The latter is often modelled by the liquid to water transfer of small non-polar molecules [2,8,9] assuming a temperature independent $\Delta_L^W C_P$, which clearly also affects the numerical value of T_S (see Table 1). It is also common to use a temperature T_S^* [34] where the specific entropies of folding of a set of proteins are the same. T_S^* is then closely related to T_S and it is obtained by plotting ΔS vs. ΔC_P at a given temperature [4,8,35] and assuming a temperature independent ΔC_P and Eq. (5) as the convention to calculate ΔG and hence ΔS. In view of the results presented here, these two assumptions determine both the numerical value of T_S^* and its physical meaning. Hence, we believe that the above criticism of the numerical value of T_S (and T_S^*), the physical interpretation of T_S and the proposal of $\Delta_L^W C_P = 0$ as opposed to $\Delta_L^W S = 0$ as the reference point where hydration disappears, might have an effect in the current understanding and modelling of protein unfolding. In this respect, a recent theoretical treatment of the thermodynamics of protein folding [36] has abandoned the use of T_S^*, although the reasons for doing so have not been stated or clarified.

4. Conclusions

We have shown that the numerical value of T_S, the temperature where the entropy of transfer from the pure hydrocarbon liquid into water is zero, depends on the convention adopted for calculating G from solubility data and on the $\Delta_L^W C_P$ fitting function. It is concluded that the interpretation of T_S as the temperature where hydration ceases cannot be sustained, since proper account of the combinatorial entropy of transfer has to be made. As reported earlier [10], hydration must vanish at a temperature $T' > T_S$, where its experimental manifestation, i.e. $\Delta_L^W C_P$, is zero. A molecular description of the process of hydration has been defined to allow for the rationalisation of the temperature dependence of its thermodynamic contributions.

Acknowledgements

BK, MC and RS gratefully acknowledge the support of the Swedish Institute and the Consejo Nacional de Ciencia y Tecnología (Grant E-3904) of Mexico. RS thanks The Foundation for Surface Chemistry Research for financial support. RLM thanks Prof. A.D. Buckingham for his advice and useful comments.

References

[1] C. Tanford, The Hydrophobic Effect: Formation of Micelles and Biological Membranes, Wiley, New York, 1973.
[2] P.L. Privalov, S.J. Gill, Adv. Prot. Chem. 39 (1988) 191.
[3] P.L. Privalov, S.J. Gill, Pure Appl. Chem. 61 (1989) 1097.
[4] K.P. Murphy, P.L. Privalov, S.J. Gill, Science 247 (1990) 559.
[5] A.J. Doig, D.H. Williams, Biochemistry 31 (1992) 9371.
[6] A.J. Doig, D.H. Williams, J. Mol. Biol. 222 (1991) 389.
[7] K.P. Murphy, S.J. Gill, J. Mol. Biol. 222 (1991) 699.
[8] R.L. Baldwin, Proc. Natl. Acad. Sci. USA 83 (1986) 8069.
[9] B. Lee, Proc. Natl. Acad. Sci. USA 88 (1991) 5154.
[10] R.L. Baldwin, N. Muller, Proc. Natl. Acad. Sci. USA 89 (1992) 7110.
[11] N. Muller, Acc. Chem. Res. 23 (1990) 23.
[12] K.A. Dill, Biochemistry 29 (1990) 7133.
[13] D. Patterson, Y.B. Tewari, H.P. Schreiber, J.E. Guillet, Macromolecules 4 (1971) 356.
[14] L.R. de Young, K.A. Dill, J. Phys. Chem. 94 (1990) 801.
[15] M. Costas, B. Kronberg, R. Silveston, J. Chem. Soc., Faraday Trans. 90 (1994) 1513.
[16] K.A. Sharp, A. Nicholls, R.F. Fine, B. Honig, Science 252 (1991) 106.
[17] J.M. Sánchez-Ruiz, J. Phys. Chem. 99 (1995) 12076.
[18] K.A. Sharp, A. Nicholls, R. Friedman, B. Honig, Biochemistry 30 (1991) 9686.
[19] A. Holtzer, Biopolymers 34 (1995) 595.
[20] D.J. Giesen, C.J. Cramer, D.J. Truhlar, J. Phys. Chem. 98 (1994) 4141.
[21] S.K. Kumar, I. Szleifer, K. Sharp, P.J. Rossky, R. Friedman, B. Honig, J. Phys. Chem. 99 (1995) 8382.
[22] G.L. Makhatadze, P.L. Privalov, J. Chem. Thermodyn. 20 (1988) 405.
[23] F. Franks, M. Gent, H.H. Johnson, J. Chem. Soc., (1963) 2716.
[24] C. McAuliffe, J. Phys. Chem. 70 (1966) 1267.
[25] B. Lee, Biopolymers 31 (1991) 993.
[26] B. Lee, Biopolymers 24 (1985) 813.
[27] S.J. Gill, S.F. Dec, G. Olofsson, I. Wadsö, J. Phys. Chem. 89 (1985) 3758.
[28] A. Ben-Naim, in: F. Franks (Ed.), Water, A Comprehensive Treatise, Vol. 2, Chap. 11, Plenum, New York, 1973.
[29] M.S. Ramadan, D.F. Evans, R. Lumry, J. Phys. Chem. 87 (1983) 4538.
[30] R. Crovetto, R. Fernández-Prini, M.L. Japas, J. Chem. Phys. 76 (1982) 1077.
[31] N. Muller, J. Solut. Chem. 17 (1988) 661.
[32] K. Shinoda, M. Fujihara, Bull. Chem. Soc., Japan 41 (1968) 2612.
[33] K. Shinoda, J. Phys. Chem. 81 (1977) 1300.
[34] R. Ragone, P. Stiuso, G. Colonna, Proteins 24 (1996) 388.
[35] J.M. Sturtevant, Proc. Natl. Acad. Sci. USA 74 (1977) 2236.
[36] G.I. Makhatadze, P.L. Privalov, Adv. Protein Chem. 47 (1995) 307.