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HYDROPHOBIC EFFECT: SOLUBILITY OF NON-POLAR SUBSTANCES IN WATER, PROTEIN DENATURATION AND MICELLE FORMATION

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Abstract

The ‘hydrophobic effect’ of the dissolution process of non-polar substances in water has been analysed under the light of a statistical thermodynamic molecular model. The model, based on the distinction between *non-reacting* and *reacting* systems explains the changes of the thermodynamic functions with temperature in aqueous systems. In the dissolution of non-polar substances in water, it follows from the model that the enthalpy change can be expressed as a linear function of the temperature ($\Delta H_{\mathrm{app}}=\Delta H^{o}+n_{\mathrm{w}}C_{\mathrm{p,w}}T$). Experimental solubility and calorimetric data of a large number of non-polar substances nicely agree with the expected function. The specific contribution of n_{w} solvent molecules depends on the size of solute and is related to destructuring ($n_{\mathrm{w}}>0$) of water molecules around the solute. Then the study of ‘hydrophobic effect’ has been extended to the protein denaturation and micelle formation. Denaturation enthalpy either obtained by van’t Hoff equation or by calorimetric determinations again depends linearly upon denaturation temperature, with denaturation enthalpy, ΔH_{den}, increasing with T. A portion of reaction enthalpy is absorbed by a number n_{w} of water molecules ($n_{\mathrm{w}}>0$) relaxed in space around the denatured moieties. In micellization, an opposite process takes place with negative number of restructured water molecules ($n_{\mathrm{w}}<0$) because the hydrophobic moieties of the molecules joined by hydrophobic affinity occupy a smaller cavity.

Keywords: hydrophobic effect, protein denaturation, thermodynamics

Introduction

The study of the so-called ‘hydrophobic effect’ has in the past been linked to the analysis of the thermodynamic parameters of the dissolution process of non-polar substances in water [1–11]. The values of Gibbs energy, enthalpy, entropy and heat capacity of transfer of a substance from its liquid state or from its gaseous state to water have been examined to explain phenomena such as the formation of micelles and biological membranes, the folding of proteins, etc. The fundamental point has been the definition of some reference state in order to be able to calculate the changes of the thermodynamic functions of transfer. We are now proposing a new point of view based on a molecular statistical thermodynamic model which seems to be well suited to interpret such processes and their connections with hydrophobic effect.

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Typically, the solubility of non-polar substances in water reaches a minimum at some temperature near room temperature. The plot of the logarithm of the solubility constant or Henry constant which represents free energy

$$2.302\log K_{\mathrm{H}} = -\Delta G^{\varnothing}/RT \tag{1}$$

vs. the reciprocal temperature $1/T$ shows in fact a mimimum (Fig. 1).

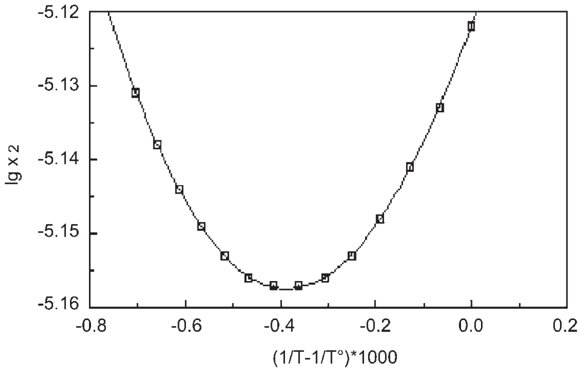

Fig. 1 Solubility of helium as the function of reciprocal temperature ($T°$=273.15 K)

The shape of the curve implies that according to van't Hoff law

$$\partial(-\Delta G^{\varnothing}/RT)/\partial(1/T) = -\Delta H^{\varnothing}/R \tag{2}$$

the process presents a negative enthalpy at low temperature ($1/T > (1/T)_{\min}$) where the slope is positive and a positive enthalpy at high temperature ($1/T < (1/T)_{\min}$). The point of minimum solubility is also the point at which the transfer is adiabatic ($-\Delta H^{\phi}/R=0$).

On the other hand, if one plots the function ($T\ln K$) *vs.* T, by recalling that $(-\Delta G^{\phi}/R+\Delta H^{\phi}/R)/T=\Delta S^{\phi}/R$, we get from the Gibbs-Helmholtz equation

$$\partial(-\Delta G^{\varnothing}/R)/\partial T = \Delta S^{\varnothing}/R \tag{3}$$

the entropy of transfer. The curve $(T\ln K)=f(T)$ shows a minimum for many substances near 120–140°C. This means that around these temperatures, the entropy change for the transfer of a pure substance from liquid to water is null, null being the tangent calculated by Eq. (3). Privalov and Gill [3] supposed that at this temperature the hydration of the non-polar solute by water ceases. This interpretation has not only been used to study the solubilization of non-polar solutes in water but has been extended to analyse complex problems such as protein folding and unfolding. More recently, Kronberg *et al.* [1, 2] supposed that the transfer of non-polar liquids to water can be broken down into several molecular steps, namely (1) removal of a non-polar molecule from pure liquid breaking molecule-molecule contacts, (2) closing of the cavity formed therein, (3) creation of a cavity in water to accomodate the non-polar solute, (4) creation of solute-water contacts, (5) relaxation of water around the solute molecule.

We have analysed these solubilization processes under the light of a thermodynamic statistical model [12–20] and reached conclusions which are consistent with some of the proposals of Kronberg *et al.* [1, 2]. According to the model, a *reacting* thermodynamic system can be seen as a set of discrete enthalpy levels over which the different species are variably distributed depending on the concentrations of reactants and on the temperature. The pressure is assumed constant if reactions in solution are taken into account. By assuming that a set of successive reactions is taking place, say $M+iA=MA_i$ each enthalpy level H_i is associated to one species MA_i. The ground level enthalpy H_0 is associated to free M. Each enthalpy level H_i is the mean value of a set of enthalpy sublevels $H_{i,j}$ each of which has an enthalpy only slightly different from that of the adjacent sublevels, thus giving rise to a continuous sequence of enthalpy sublevels grouped around the species mean enthalpy level $H_i=<H_{i,j}>$. The distribution of species in *reacting* ensembles is described in probability space [12, 16] by a grand canonical partition function which we denote in general as Z_M where M indicate the receptor. The pure compounds or solutions of a single species are *non-reacting* systems. They are characterized by a continuous distribution of enthalpy levels analogous to that observed in each subset $H_{i,j}$ of a reacting system. Even each set of sublevels, therefore, taken per se represents a non-reacting system. The distribution of molecules is described in probability space by a canonical partition function which we denote in general by ζ_A where A indicates the solute. Complex systems formed by a combination of *reacting* and *non-reacting* components are described by a convoluted partition function or probability product which we denote in general by $\Xi=Z_M\cdot\zeta_A$.

Solubility as a chemical equilibrium

In order to explain the hydrophobic effect, we refer again to the solubilization process of a typical hydrophobic simple molecule such as a noble gas. The solubility of a gas [18] such as He in water, W (cfr. Fig. 1) can be treated as an equilibrium between He and W

$$\mathrm{He}+x\mathrm{W}=\mathrm{HeW}_{x-n_w}+n_w\mathrm{W} \tag{4}$$

where xW is the portion of the bulk water involved in the solubilization process, and HeW_{x-nw} is the gas molecule trapped in a cavity surrounded by a cage formed by $(x-n_w)$ water molecules. The n_w water molecules are destructured (relaxed) around the cavity hosting the gas molecule. The constant of this equilibrium is

$$K_s=[\mathrm{HeW}_{x-n_w}][\mathrm{W}]^{n_w}/[\mathrm{He}][\mathrm{W}]^x \tag{5}$$

At constant gas pressure, the concentration $[\mathrm{HeW}_{x-n_w}]$ is a measure of solubility if we assume that free [He] is constant because it is in equilibrium with gas at constant pressure. The solubility is usually identified with the molar fraction x_2 at unit pressure, where index 2 indicates solute. At unit pressure, the molar fraction x_2 is reciprocal of Henry constant K_H. Equation (5) can be written as a solubility product,

$$P_s = [W]^{n_w} / K_H = x_2 [W]^{n_w} \tag{6}$$

where P_s includes the conversion factor deriving from the inhomogeneity of the concentration scales of gas and water. Here $(1/K_H) = x_2$ is, in probability space, a convoluted partition function or solubility distribution function ($=\Xi$) resulting from the product of a grand canonical partition function P_s ($=Z_M$) and a canonical partition function $[W]^{-n_w}$ ($=\zeta_A$).

By taking the logarithms of partition functions, one moves from probability space to affinity thermodynamic space [12, 16] whereby relative changes of probability are measured by changes of thermodynamic functions. The logarithm of Eq. (6), therefore, represents thermodynamic changes

$$\ln x_2 = \ln P_s - n_w \ln[W] \tag{7}$$

The solubility distribution function can be expressed in the affinity thermodynamic space ($\ln x_2 = -\Delta G^{ø}_{app}/RT$) by a Taylor expansion as the function of $x = (1/T)$. If the polynomial fitting the experimental data $\ln x_2 = f(1/T)$ is written as

$$y = a + bx + cx^2 + dx^3 + ex^4 \tag{8}$$

then the thermodynamic quantities ΔH_{app} and $\Delta C_{p,app}$, which are the first and second derivatives respectively of the distribution function, can be calculated from the coefficients of the polynomial (8).

By deriving the distribution function with respect to the reciprocal temperature, we get at a chosen reference temperature $T = \theta$

$$\{\partial \ln x_2 / \partial (1/T)\}_\theta = \{\partial \ln P_s / \partial (1/T)\}_\theta - n_w \{\partial \ln[W] / \partial (1/T)\}_\theta \tag{9}$$

which can be identified for a sum of enthalpy contributions

$$-\{\Delta H_{app}\}_\theta = -\{\Delta H^{ø}\}_\theta + \{\Delta H_w\}_\theta \tag{10}$$

The l.h.m. $\{\Delta H_{app}\}_\theta$ can be obtained from Eq. (8) as tangent at temperature $T = \theta$ of the plot of the function $\ln x_2 = f(1/T)$. The true enthalpy of the process $\{\Delta H^{\phi}\}_\theta$ can be obtained by assuming the hypothesis that at a first approximation the solubility product P_s satisfies van't Hoff equation. By recalling the concept of *thermal equivalent dilution, TED*, the enthalpy $\{\Delta H_w\}_\theta$ can be obtained from the dependence of ln[W] upon T which can be expressed [19] as

$$-n_w \partial \ln[W] / \partial \ln T = n_w C_{p,w} / R \tag{11}$$

where $C_{p,w}$ is the molar heat capacity of water. By transformation into the derivative with respect to $1/T$, Eq. (11) yields

$$n_w T \partial \ln[W] / \partial (1/T) = \{-\Delta H_w / R\}_T = n_w C_{p,w} T / R \tag{12}$$

Table 1 Enthalpy ΔH^{ϕ} and number n_w of water molecules for solubility of inert gases in water

Compound	Method	n_w	ΔH^{ϕ}	α-plz*	Ref.	Compound	Method	n_w	ΔH^{ϕ}	α-plz	Ref
He	solub.	1.57	–35.69	0.204	[24]	N_2F_4	solub.	5.35	–140.70		[24]
Ne	solub.	1.87	–42.25	0.393	[24]	N_2O	solub.	1.99	–66.04		[24]
Ar	solub.	2.48	–68.64	1.630	[24]	NO	solub.	2.50	–68.18		[24]
Kr	solub.	2.84	–78.92	2.460	[24]	H_2S	solub.	2.31	–69.20		[24]
Xe	solub	3.24	–92.62	4.000	[24]	SF_6	solub.	6.91	–175.38		[24]
Rn	solub.	3.89	–108.82	5.860	[24]	H_2Se	solub.	1.08	–40.04		[24]
H_2	solub.	1.86	–45.88	0.802	[24]	AsH_3	solub.	2.11	–64.45		[24]
N_2	solub.	2.96	–76.90	1.740	[24]	Air	solub.	2.39	–64.84		[24]
O_2	solub.	2.70	–72.65	1.570	[24]	CH_4	calor.	3.20	–85.10		[25]
CO	solub.	2.57	–68.87	1.930	[24]	C_2H_6	calor.	4.22	–114.02		[25]
CO_2	solub.	2.31	–71.75		[24]	C_3H_8	calor.	5.16	–138.77		[25]
CH_4	solub.	2.76	–75.81	2.700	[24]	C_4H_{10}	calor.	5.63	–152.34		[25]
C_2H_6	solub.	3.98	–109.19	4.330	[24]	C_2H_6	calor.	3.64	–101.32		[27]
C_2H_4	solub.	2.13	–62.75	3.700	[24]	C_3H_8	calor.	4.22	–118.08		[27]
C_2H_2	solub.	2.37	–68.16	3.190	[24]	CH_4	calor.	2.77	–75.65		[26]
C_3H_8	solub.	4.91	–132.82		[24]	CH_4	solub.	3.24	–83.50		[28]
C_4H_{10}	solub.	4.93	–136.78		[24]	C_2H_6	solub.	3.66	–99.98		[28]

Table 1 Continued

Compound	Method	n_w	ΔH^{ϕ}	α-plz*	Ref.	Compound	Method	n_w	ΔH^{ϕ}	α-plz	Ref
$(CH_3)_2C=CH_2$	solub.	3.64	–106.53		[24]	C_4H_{10}	solub.	5.10	–137.77		[28]
$1.3C_4H_6$	solub.	7.69	–210.51		[24]	O_2	calor.	2.72	–73.25		[25]
neoC_5H_{12}	solub.	6.83	–181.44		[24]	He	calor.	1.79	–40.86		[25]
FCH_3	solub.	2.00	–63.14		[24]	Ne	calor.	1.94	–47.02		[25]
$ClCH_3$	solub.	2.58	–81.00		[24]	Ar	calor.	2.64	–71.48		[25]
$BrCH_3$	solub.	2.47	–80.98		[24]	Kr	calor.	2.92	–80.90		[25]
CF_4	solub.	5.04	–128.24	2.53	[24]	Xe	calor.	3.30	–93.30		[25]
$CHClF_2$	solub.	4.77	–135.63		[24]	CF_4	solub.	5.73	–142.20		[29]
C_2F_4	solub.	4.07	–108.87	1.70	[24]	C_4F_8	solub.	11.14	–272.53		[29]
C_3F_6	solub.	0.75	–37.13		[24]	C_2F_6	solub.	10.12	–245.47		[29]
COS	solub.	3.42	–101.40		[24]	CF_4	solub.	7.01	–172.79		[30]
NF_3	solub.	3.86	–102.17		[24]						

*Volume polirizability

By combining Eqs (10) and (12), if the constant P_s follows the van't Hoff equation with constant true enthalpy, ΔH^o and constant heat capacity of water, $C_{p,w}$, we get

$$\{-\Delta H_{app}/R\}_\theta = -\Delta H^o/R - n_w C_{p,w}\theta/R \tag{13}$$

This equation shows how the apparent enthalpy, ΔH_{app} is a linear function of the variable $T=\theta$ with slope $n_w C_{p,w}$. According to Eq. (13), the contribution of the solvent molecules to the apparent enthalpy should depend linearly upon the temperature and the slope of the line should give the number of water molecules involved. This behaviour is shown in general by the experimental data of the gases examined and makes possible the determination of the number of water molecules, n_w involved in the reaction. Therefore, the enthalpy change observed in the solubilization of noble gases in water is dependent not only on the reaction heat, ΔH^ϕ, but it is also dependent on the heat, ΔH_w, absorbed (or released) by n_w water molecules taking part in the reaction. The number of water molecules, n_w is dependent upon the size of the solute molecule and the heat ΔH_w is linearly dependent upon the temperature. The same behaviour as that of helium is also presented by the protonation constants of carboxylic acid at different temperatures.

The model has been successfully applied to treat the changes with temperature of *i*) the protonation constant log$K$ of several carboxylic acids [20], *ii*) the solubility constant logK_H or Henry constant in water of noble gases [18], *iii*) the solubility constant logK_s in water of inert gases and liquids [21], *iv*) the unfolding enthalpy ΔH_{den} of proteins [22], *v*) the micellization enthalpy [23]. For any process of these series, the enthalpy at different temperatures can be represented by the Eq. (13), thus giving two basic pieces of information, namely ΔH^ϕ and n_w.

Table 2 Enthalpy ΔH^ϕ and number n_w of water molecules for solubility of liquids in water

Compound	n_w	ΔH^ϕ	Method	Ref.
Benzene	2.93	–63.93	calor.	[31]
Benzene	2.99	–64.94	calor.	[32]
D_6-benzene	2.75	–59.73	calor.	[32]
Toluene	3.50	–76.90	calor.	[32]
Ethylbenzene	4.19	–92.08	calor.	[32]
Propylbenzene	5.16	–113.65	calor.	[32]
Cyclohexane	4.74	–106.42	calor.	[32]
Pentane	5.31	–121.26	calor.	[32]
Hexane	5.84	–131.19	calor.	[32]

The values of ΔH^ϕ and n_w for many solubilization processes concerning gases in water are reported in Table 1. The enthalpy has been obtained either from solubility determinations from the slopes of the diagram $\ln x_2=f(1/T)$ via van't Hoff equation or from calorimetric determinations of the solution enthalpy. It is worth noting that whenever both experimental routes have been followed, congruent results are obtained. Beyond the set of values reported in Table 1, very few compounds (seven out of 64) show a different behaviour with negative values of n_w. We assume that the solubilization in water for these non-inert compounds (namely, C_3H_6, C_3H_4, cC_3H_6, iCH_4H_{10}, NH_3, Cl_2, ClO_2) takes place through different hydration routes. Values concerning the solubility of liquid substances are reported in Table 2.

Size of solute and structure of solvent

The number of water molecules n_w depends on the size of the solute molecule, as already observed for noble gases. In fact, the smaller the molecules such as Ne or H_2, the smaller the value of n_w (n_w=1.7 and 1.9 for Ne and H_2 respectively). The number n_w in noble gases nicely correlates with volume polarizability α_2 and hard sphere parameter σ_2 (Fig. 2) thus confirming that it is related to some physical property depending on the the size of the molecules. The parameters α_2 and σ_2 have been used by Pierotti [33] to explain the solubility of gases in water on the basis of scaled particle theory which, with the idea of cavity formation offers a way to understand the role of water molecules. In fact, a possible mechanism of the solubilization process of inert molecules in water places water molecules in relation with the 'relaxed' water molecules proposed by Costas *et al.* [1] (Fig. 3).These water molecules, according to Abou-Aiad *et al.* [34], are related to the surface of the solute but are much fewer than those required for complete occupation of the surface.

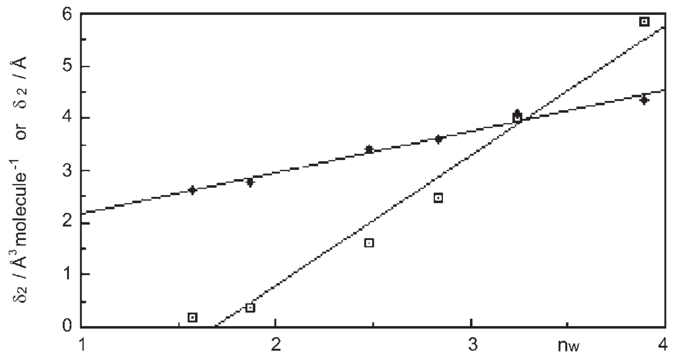

Fig. 2 Relationship of volume polarizability and hard sphere diameter with n_w for noble gases. Data from Pierotti [33]

Values of n_w higher than those found in noble gases, have been observed in hydrocarbons, with values ranging from n_w=2.8 for methane to n_w=5.6, 5.8, and 5.2 for longer molecules such as butane, hexane, propylbenzene, respectively. Other polar or inductive factors and related solute-solvent interactions, however, are also effective, as shown by the even higher values observed for perfluorocarbon compounds such as c-C_4F_8 and C_2F_6 for which n_w=11.1 and 10.1, respectively. This is in agreement with the 'hydrophobic' tendency of perfluorocarbon compounds which is greater than that of alkanes [29, 35]. A peculiar behaviour is that observed in gaseous and liquid hydrocarbons when the numbers n_w are compared with the length of the chain expressed by the number n_C of carbon atoms in the chain (Fig. 4). The gaseous and liquid hydrocarbons lay on different parallel lines. The equal slope of the regression lines indicates that in both groups there is a constant increment $\Delta n_w \approx 0.8$ for each $-CH_2$ added to the chain. The linearity of the dependence suggests that the interaction of each portion of molecule with the solvent is not cooperative and proportional to the surface area of the non polar solute [36]. The parallel displacement of the lines for liquid and gaseous hydrocarbons indicates that at equal length of the chain, the liquid compound moves ≈1.3 water molecules less than the corresponding gaseous compound. This fact could be explained by considering that the liquid molecules which exert stronger reciprocal cohesion forces than towards the molecules of the solvent are actually associated to form dimers or trimers and yield destructured surrounding zones per molecule smaller than the corresponding gaseous solutes. The existence of dimers in

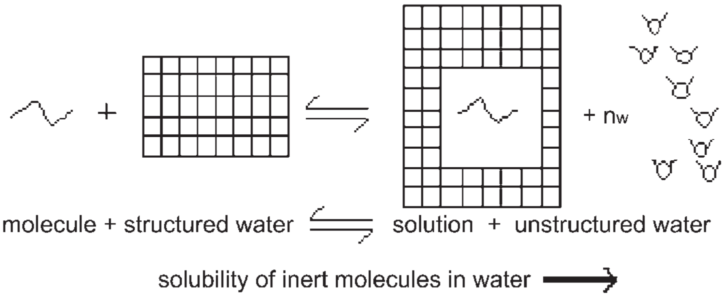

Fig. 3 The solubilization process of inert molecules in water is determined by the formation of a zone of relaxed destructured water molecules around the solute

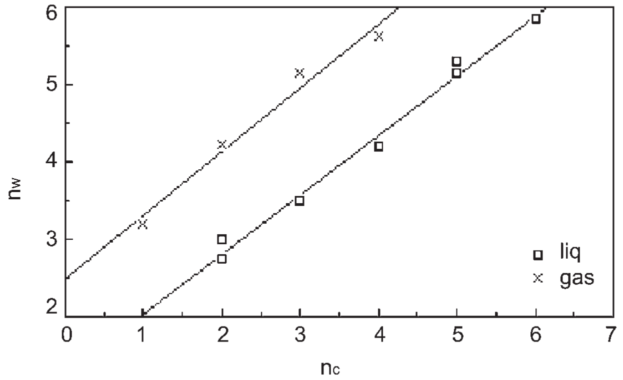

Fig. 4 Relation between number of water molecules (n_w) and number of carbon atoms in the chain (n_C) in gaseous hydrocarbons and liquid hydrocarbons. The benzene ring is assumed equivalent in length to $-CH_2-CH_2-$

aqueous solution of benzene was demonstrated by Tucker *et al.* [37, 38] by vapor pressure determinations and by Hallén *et al.* [39] by calorimetric determinations.

Solubilization entropy

The minimum of the solubility curve corresponds also to the point at which the transfer is adiabatic ($-\Delta H_{\mathrm{app}}/R=0$) and at this point

$$(-\Delta G^{ø}_{\mathrm{app}}/RT)_{\mathrm{min}}=\Delta S^{ø}/R \qquad (14)$$

Values of the entropy change have been calculated from values of $\log x_2$ at the minimum of each curve. Values of the entropy change ΔS^{ϕ} for a set of gases are reported in Table 3. When plotted *vs.* n_w, the solubilization entropy yields data not correlated to n_w. Most of the entropy values for gases are centred around $<\Delta S^{ø}_{\mathrm{sol}}>=19.5\pm5$ J K^{-1} mol^{-1}. By referring to T=298 K, the entropy term $T<\Delta S^{ø}_{\mathrm{sol}}>$ is worth 5.81±1.5 kJ mol^{-1} at most and in any case rather smaller than the enthalpy change per water molecule. When plotted against $1/T_m$ (Fig. 5), where T_m is the temperature of the minimum the solubilization entropy from data in Table 3 yields a very good correlation, with equation $\Delta S^{ø}_{\mathrm{sol}}=-70.0+19.117(1/T_m)$. This result indicates that the solubilization step of gases is endothermic with ΔH_{sol}=+19.1 kJ mol^{-1} for any compound, obtained from

$$\Delta S^{ø}_{\mathrm{sol}}=+\Delta S_{\mathrm{sol,a}}+\Delta S_{\mathrm{sol,b}}(T)=-70+\Delta H_{\mathrm{sol}}/T_m \qquad (15)$$

It is coupled with negative entropy change ($\Delta S_{\mathrm{sol,a}}=-70$ J K^{-1} mol^{-1}) which is a measure of change of degrees of freedom of the molecule from gaseous state to trapped solution state.

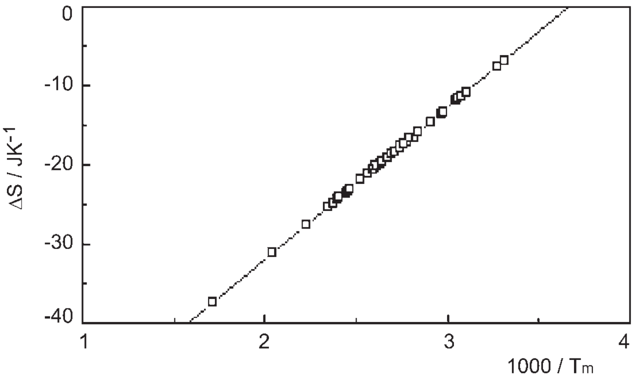

Fig. 5 Entropy change from the minimum of the solubility curve as the function of $1/T_m$

Table 3 Solubilization entropy at the minimum of the curve

Coumpound	T_m	$\lg K_m$	ΔS/J $K^{-1}mol^{-1}$	n_w	Ref.
CH_4	356.74	–0.858	–16.426	3.24	[28]
C_2H_6	538.55	–1.947	–37.275	3.66	[28]
C_4H_{10}	450.13	–1.439	–27.549	5.10	[28]
$(CH_3)_2C{=}CH_2$	390.16	–1.098	–21.021	3.64	[24]
$BrCH_3$	418.39	–1.271	–24.333	2.47	[24]
$CHClF_2$	380.02	–1.030	–19.719	4.77	[24]
C_2F_4	352.88	–0.827	–15.833	4.07	[24]
C_3F_6	157.29	2.697		0.75	[24]
CF_4	338.07	–0.703	–13.459	5.07	[24]
COS	344.92	–0.762	–14.588	3.42	[24]
NF_3	354.86	–0.843	–16.139	3.86	[24]
N_2F_4	364.36	–0.916	–17.536	5.35	[24]
N_2O	365.46	–0.925	–17.709	1.99	[24]
NO	361.88	–0.898	–17.192	2.50	[24]
SF_6	336.73	–0.691	–13.229	6.91	[24]
H_2Se	406.43	–1.201	–22.993	1.08	[24]
H_2	327.49	–0.607	–11.621	1.86	[24]
N_2	344.71	–0.760	–14.550	2.96	[24]
O_2	357.90	–0.867	–16.598	2.70	[24]
CO	356.03	–0.852	–16.311	2.57	[24]
CO_2	416.20	–1.258	–24.084	2.31	[24]
C_3H_8	359.81	–0.882	–16.886	4.91	[24]
C_2H_4	410.62	–1.226	–23.471	2.13	[24]
C_2H_2	384.28	–1.059	–20.274	2.37	[24]
$1.3C_4H_6$	384.58	–1.061	–20.312	3.64	[24]
neoC_5H_{12}	352.41	–0.823	–15.756	6.83	[24]
FCH_3	427.58	–1.322	–25.309	2.00	[24]
$ClCH_3$	422.85	–1.296	–24.811	2.58	[24]
H_2S	408.70	–1.214	–23.242	2.31	[24]
AsH_3	386.20	–1.072	–20.523	2.11	[24]
Air	360.33	–0.886	–16.962	2.39	[24]
He	305.40	–0.387	–7.409	1.57	[24]
Ne	323.10	–0.566	–10.836	1.87	[24]
Ar	369.20	–0.952	–18.226	2.48	[24]
Kr	374.10	–0.988	–18.915	2.84	[24]
Xe	396.10	–1.136	–21.749	3.24	[24]
Rn	371.50	–0.969	–18.552	3.89	[24]
C_4F_8	328.50	–0.617	–11.810	11.10	[29]
C_2F_6	305.63	–0.389	7.450	10.10	[29]

Extrapolated enthalpy and hydrophobic effect energy

The extrapolated or true enthalpy ΔH^{ϕ} for the solubilization processes gives the energy released when the molecule reacts with structured water before that part of that energy be absorbed by destructured water molecules. The extrapolated enthalpy ΔH^{ϕ} also depends on the number of water molecules. When values for gases are plotted against n_w, a linear function is obtained (Fig. 6). The coefficients of the function

$$\Delta H^{\varnothing} = \Delta H_0 + \Delta h_w n_w \tag{16}$$

are for gases $\Delta H_0 = -12.65$ and $\Delta h_w = -23.66$ kJ mol^{-1} n_w^{-1}, indicating that the binding energy for each water molecule Δh_w is more or less that attributed to hydrogen bonds.

The extrapolated enthalpy ΔH^{ϕ} is comprehensive of the energy released by the combination of the molecule with water and of the energy absorbed or released to form the cavity. From the slope Δh_w of the line we get an evaluation of the interaction

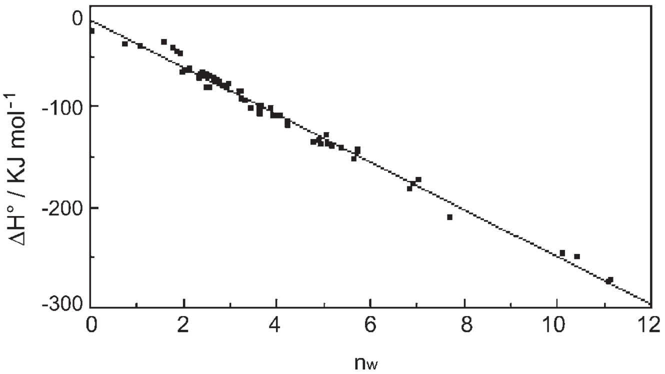

Fig. 6 The extrapolated enthalpy *vs.* number of water molecules n_w for molecular gases

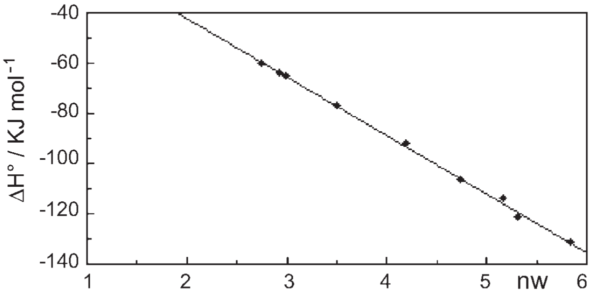

Fig. 7 Extrapolated enthalpy *vs.* number of water molecules n_w for solubility of liquids

enthalpy per water molecule in the set of processes examined. The value of ΔH_0 gives the balance between the interaction of gas with the structure of solvent and the energy required to form the cavity. This balance is negative for gases.

The same plot of Eq. (16) for liquids yields ΔH_0=+4.5 and Δh_w= –23.28 kJ mol^{-1} n_w^{-1} (Fig. 7). This indicates that the interaction energy per relaxed water molecule in liquids is almost equal to that for gases whereas the binding and cavity formation energy for liquids is endothermic. The enthalpy is about 80% of free energy for hydrogen bonds (ca 29 kJ mol^{-1}), what is reasonable but is about fifteen times the energy calculated for Lennard-Jones pair potential by Pierotti [33] according to scaled particle theory.

Denaturation and van't Hoff equation

We can now extend the analysis to systems where protein denaturation or micelle formation takes place, with hydrophobic interactions playing a fundamental role. These systems have in common with gases and liquids dissolving in water that the enthalpy of the process depends linearly upon the absolute temperature.

The criteria derived from the equivalence of chemical and thermal dilution [19] of the free solvent, W, and applied to the solubility of gases in water, can be applied to the interpretation of the denaturation of proteins as well. By assuming a two-state process, the equilibrium between native, N, and hydrated denatured, D_{hyd}, states of a protein can be expressed by the denaturation constant

$$K_{den}=[D_{hyd}]/[N] \tag{17}$$

Then by introducing the hydration equilibrium, Eq, (17) yields

$$K_{den}=K_0[W]^{-n_w} \tag{18}$$

K_{den} itself can be considered as a grand canonical partition or saturation function; $[W]^{n_w}$ is the canonical partition function ζ_w for the solvent and K_0 is the convoluted partition function.

By taking the logarithms and differentiating with respect to $1/T$, we get

$$\partial \ln K_{den}/\partial(1/T)=\partial \ln K_0/\partial(1/T)-n_w \partial \ln[W]/\partial(1/T) \tag{19}$$

By applying the van't Hoff equation to Eq. (19), the denaturation enthalpy, ΔH_{den}, is obtained

$$-\Delta H_{den}(1/R)=-\Delta H^{ø}(1/R)-n_w \partial \ln[W]/\partial(1/T) \tag{20}$$

The last term of this equation can be transformed by applying the principle of *thermal equivalent dilution*

$$\partial \ln[W]/\partial \ln T=-C_{p,w}/R \tag{21}$$

By the introduction of the Eq. (21) into (20) and by multiplication by $-R$, the denaturation enthalpy of the equation can be expressed as

$$\Delta H_{den}=\Delta H^{ø}+n_w T C_{p,w} \tag{22}$$

which corresponds to Eq. (13). By plotting the denaturation enthalpy *vs*. T, a straight line is obtained (Fig. 8). In the cases examined so far, the denaturation processes are two-state processes. The relationships can be extended to cope with multi-state processes. Several sets of data concerning denaturation enthalpy are reported in Table 4.

Table 4 Dehydration numbers, n_w in protein denaturation

Protein	pH	$\Delta C_{p,app}$/J mol^{-1} K^{-1}	n_w	ΔH^Θ/kJ mol^{-1}	Ref.
HEW		6701.00	88.90	–1767.788	[22]
WildT4		9199.00	122.00	–2463.413	[22]
T157 (T4)		9903.00	131.40	–2701.723	[22]
R96H (T4)		10539.00	139.40	–2896.170	[22]
βLG	7.00	5036.00	66.81	–1521.890	[42]
	6.50	6005.00	79.661	–838.090	[42]
	2.50	6715.00	88.09	–2068.260	[42]
	2.00	6516.00	86.44	–2016.120	[42]
	1.50	6623.00	87.87	–2044.240	[42]
	1.00	6333.00	84.02	–1947.160	[42]
βLG (ur.)	2.50	8911.92	118.23	–2655.830	[43]
	2.50	9037.44	119.90	–2693.240	[43]
	2.71	8493.52	112.68	–2531.160	[43]
	3.00	8995.60	119.34	–2680.780	[43]
	2.57	9748.72	129.33	–2905.210	[43]
	2.78	8116.96	107.68	–2418.940	[43]
	2.55	7782.24	103.24	–2319.190	[43]
	3.20	8786.40	116.57	–2618.440	[43]
Ribonucl.		10878.40	144.32	–3241.550	[43]
Ribonucl.		9623.20	127.67	–2867.530	[43]
Chimotr.		14644.00	194.27	–4363.760	[43]
Myogl.		5857.60	77.71	–1745.390	[43]
βLG (ur.)		8789.50	116.57	–2702.060	[43]
βLG (GuHCl)		8786.40	116.57	–2618.440	[43]
Ribonucl.	1.13	10331.00	137.10	–2795.028	[41]
	2.10	13302.00	176.50	–3668.442	[41]
	2.50	14183.00	196.50	–4167.174	[41]
	2.77	15567.00	206.50	–4403.301	[41]
	3.15	21309.00	282.70	–6244.693	[41]

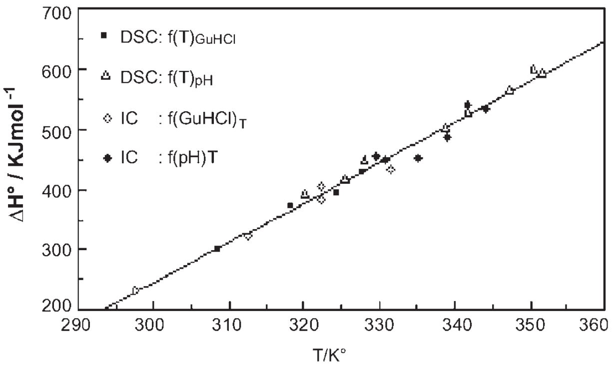

Fig. 8 Denaturation enthalpy of HEW lysozyme *vs. T.* IC: isothermal calorimetry, DSC: differential scanning calorimetry, (Data from [22])

Number of water molecules and size of proteins

The number of water molecules n_w depends on the size of the solute molecule, as already observed for noble gases and other non-polar molecules even if the number of water molecules is less than that required to saturate the total free surface of the solute molecule, according to Abou-Aiad *et al.* [34]. In fact, a possible mechanism of denaturation is analogous to that proposed for the solubilization process of inert molecules in water. When unfolded, the chains of the protein occupy a cavity in the solvent. Other polar or inductive factors and related solute-solvent interactions, however, are also effective, in agreement with 'hydrophobic' character of the substituents.

Values of n_w obtained in protein denaturation are much higher than those found in noble gases and simple small molecules. The number of water molecules for different types of lysozyme changes from n_w=88.9 for HEW with molecular mass 14.100 Da to n_w=122.0 for T4wild type with molecular mass 18.700 Da. Again, besides the size of the molecule, other hydrophobic factors contribute to increase the number of water molecules as shown by mutants of T4wild type. In fact, for mutants of wild lysozyme T4Ala (Thr157Ala) and T4His (Arg96His) the introduction of the group alanine for threonine and histidine for arginine produces higher hydrophobicity and consequently values n_w=131.4 and n_w=139.8, respectively, to be compared with n_w=122.0 mentioned above for T4wild type. In general, the size of the molecule is the main factor determining the number of water molecules, but the protonation state of the protein can produce changes as shown by the data for ribonuclease. All these data are consistent with a picture of the process whereby the inserted molecules forms a cavity in the structured water and some water molecules relax around the uncoiled chain. When, however, aqueous micelle solutions are considered (Table 5) the slope of the enthalpy *vs.* temperature is negative thus indicating a negative number of water molecules. This is the case of aqueous micellar solutions of surfactants, reported in Table 5. Again, the amount of water molecules is low as for the solubilization of small molecules in water and, again, it is proportional to the size of the molecule. A negative number n_w of water molecules implies that water molecules are included into the structured water and this can be accomplished, accompanied by the effect of the counterion, by way of the micellization mechanism depicted schematically in Fig. 9. The hydrophobic surfactant molecules before micellization are in the solution surrounded by a cavity like a sheath via a mechanism analogous to that depicted for the solubilization of gases and liquids. When two such hydrophobic moieties with their sheaths get close to each other, the single separated cavities coalesce and give rise to a resulting cavity which is smaller than the sum of the two original sheaths. The excess empty volume is then occupied by a convenient number of water molecules that transform from relaxed to structured (n_w<0).

Table 5 Dehydration numbers n_w for micellization

Surfactant	Type	$\Delta C_{p,app}$/ J mol^{-1} K^{-1}	n_w	ΔH^{Φ}/ kJ mol^{-1}	Ref.
$C_{14}H_{29}OSO_3Na$	anionic	–33.5	–4.45	98.639	[44]
	anionic	–603	–8.00	178.178	[45]
$CH_3(CHSO_3)C_{12}H_{25}Na$	anionic	–343	–4.55	103.004	[44]
$CH_3C_2H_4(CHOSO_3)C_{10}H_{21}Na$	anionic	–351	–4.66	107.149	[44]
$C_{12}H_{25}OSO_3Na$	anionic	–527	–6.99	155.536	[46]
	anionic	–457	–6.06	142.079	[47]
	anionic	–476	–6.32	140.917	[53]
	anionic	–315	–4.18	94.269	[44]
	anionic	–283	–6.58	85.757	[48]
	anionic	–496	–3.82	148.113	[45]
C_{12}NPyr I	cationic	–390	–5.18	103.600	[49]
$C_{12}NPyrOCH_3$ Cl	cationic	–446	–5.92	134.778	[49]
$C_{12}OPyrNCH_3$ Br	cationic	–594	–7.89	170.104	[49]
$C_{14}NPyrOCH_3$ Br	cationic	–581	–7.71	162.691	[49]
C_9NACl	cationic	–299	–3.97	91.615	[23]
$C_{10}NACl$	cationic	–385	–5.10	116.369	[23]
$C_{10}N(CH_3)_3Br$	cationic	–311	–4.13	93.106	[50]
$C_{12}N(CH_3)_3Br$	cationic	–1437	–19.10	421.491	[51]
	cationic	–1270	–16.05	355.016	[51]
$C_{14}N(CH_3)_3Br$	cationic	–607	–8.05	177.444	[51]
$C_{16}N(CH_3)_3Br$	cationic	–573	–7.60	161.150	[50]
	cationic	–352	–4.67	93.470	[52]

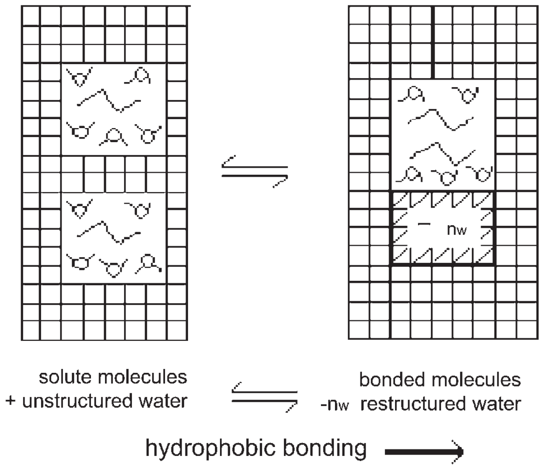

Fig. 9 Formation of a hydrophobic bond is accompanied by restructuring of water molecules to fill the excess of the cavity

Hydrophobic energy

The extrapolated enthalpy ΔH^{ϕ} which is calculated from the plot of ΔH_{app} *vs.* T is on its turn dependent on the number of water molecules n_{w}. When the extrapolated enthalpy, ΔH^{ϕ}, is plotted *vs.* n_{w}, a linear function is obtained. The coefficient Δh_{w} of the function

$$\Delta H^{ø}_{\mathrm{class}} = \Delta H_0 + \Delta h_{\mathrm{w}} n_{\mathrm{w}} \tag{23}$$

obtained for each class of compounds yields the energy Δh_{w} per water molecule released or entrapped. The extrapolated enthalpy, ΔH^{ϕ}, for the solubilization and denaturation processes gives the energy released when the molecule reacts with structured water before that part of that energy be absorbed by the water molecules destructured.

This extrapolated enthalpy is inclusive of the energy released by the combination of the molecule with water and of the energy absorbed to form the cavity. From the slope Δh_{w} of the lines, we get the energy released per water molecule in the various processes examined. Δh_{w} gives an evaluation of the interaction enthalpy between one water molecule and a piece of solute. The value obtained for proteins is $\Delta h_{\mathrm{w}} = -21.90$ kJ mol^{-1} n_{w}^{-1}, which is somewhat less than the energy attributed to a hydrogen bond ($\Delta H^{\phi} = -30$ kJ mol^{-1}). The zero point enthalpy is $\Delta H_0 = 14.53$ kJ mol^{-1}, thus indicating an endothermic step of cavity formation in protein denaturation. The value of Δh_{w} for proteins is very close to that $\Delta h_{\mathrm{w}} = -23.3$ kJ mol^{-1} n_{w}^{-1} found for non-polar gases. A value which practically coincides is found (Fig. 11) from the plot of micellization enthalpy *vs.* n_{w} ($\Delta h_{\mathrm{w}} = -21.99$ kJ mol^{-1} n_{w}^{-1}, $\Delta H_0 = 0.51$ kJ mol^{-1}). The set of values taken as a whole indicates that the average energy per water molecule is very similar. Even the micellization process, therefore, which correctly comes out to

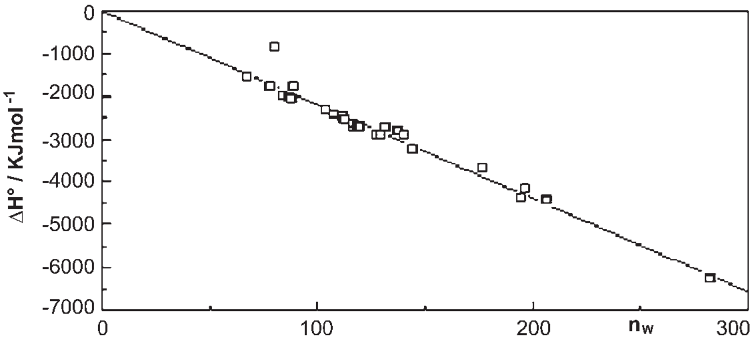

Fig. 10 Extrapolated enthalpy as the function of water molecules n_w (ΔH^{Φ}=14.531–21.90 n_w kJ mol^{-1})

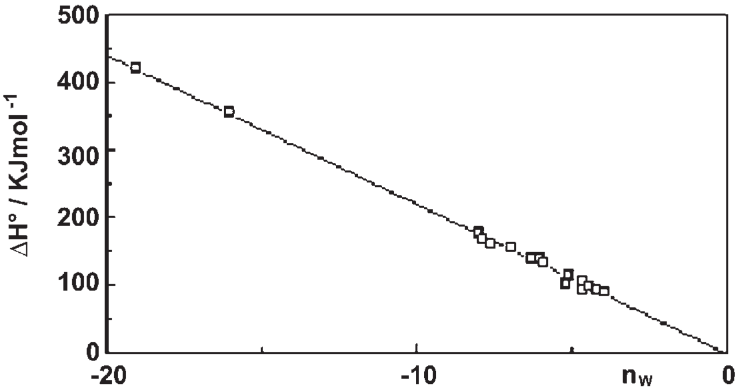

Fig. 11 Extrapolated enthalpy from micellization of cationic and anionic surfactants as the function of n_w water molecules (ΔH_0=0.501–21.99 n_w kJ mol^{-1})

be endothermic, falls within the expected range. The zero point enthalpy for micellization, ΔH_0=0.51 kJ mol^{-1}, is almost null and in fact there is no cavity formation, but rather a cohesion force ($\Delta H_{coh}\approx -14$ kJ mol^{-1}) between solutes.

Conclusions

The solubilization process of non-polar gases in water can be interpreted on the basis of the statistical thermodynamic model. The whole system can be described as the convolution of *reacting* and *non-reacting* subsystems. Gas and part of water are *reacting* species whereas the bulk of water is the *non-reacting* component. The properties of the whole system can be described by a convoluted partition function resulting from the product of canonical and grand canonical partition functions, respectively. The changes of the solubility parameter representing the convoluted partition function with changing temperature can be explained by assuming that the solubilization reaction takes place by forming a cavity in the solvent to allocate the solute. Part of the water molecules removed relax around the solute and absorb part of the enthalpy change of the reaction in a manner that linearly depends upon the temperature. This explains why the experimental solubilization heat is linearly related to the absolute temperature T. This mechanism, involving n_w water molecules depending on the size of the dissolved molecule, is a general scheme for the series of substances examined and implies a value of enthalpy change per water molecule which is constant throughout the series. The step of solubilization process concerning these water molecules can be considered as being equivalent to that occurring in the disruption of a hydrophobic bond.

The dependence of denaturation enthalpy on the temperature can be explained by considering a similar mechanism whereby, at denaturation, an interaction with the solvent takes place analogous to that proposed for the solubilization process of non-polar substances in water. Starting from the expression for the partition function, the same results should be obtained, either by calculating the enthalpy from equilibrium data at different temperatures either by calorimetry. The process of micellization occurs in an opposite way to denaturation, by trapping some water molecules. The latter occupy the excess of cavity void after the coupling of those molecular moieties which were separate before micelle formation. This step is an example of the hydrophobic effect. According to the mechanism proposed here, this type of interaction occurs by trapping water molecules, which release heat. Trapping is a process which implies a loss of configuration entropy. This finding is in contrast with the previously proposed mechanisms of hydrophobic bonding whereby water molecules are released at the very moment of hydrophobic bonding leading to increasing configurational entropy. An energy balance of micellization is, apart from the contribution of counterion, coincident with the energy of hydrophobic interaction. The enthalpy change of hydrophobic bond is endothermic but the amount of heat absorbed is counterbalanced by heat released by restructured water molecules. The latter energy is that which stabilizes the hydrophobic interaction. The denaturation of proteins is, in contrast, a process accompanied by breaking of hydrophobic attractions. The analogy of the two opposite processes of micellization and denaturation is confirmed by the quantitative evaluation of the hydration enthalpy per water molecule which is practically equal in both processes.

* * *

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