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# Entropy/enthalpy compensation: hydrophobic effect, micelles and protein complexes

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Molecular interpretations are here presented of the hydrophobic effect, which is the cause of the low solubility of apolar substances in water. The solubilization process of substances such as the noble gases consists in the formation of a cavity in the solvent with expulsion of $n_w$ water molecules. The process is associated to an entropy/enthalpy ($S/H$) compensation linearly dependent upon the temperature. The observed enthalpy $\Delta H_{app}$, either determined calorimetrically or by van't Hoff equation, shows $\Delta C_{p,app} \neq 0$ and positive. We set $\Delta C_{p,app} = n_w C_{p,w}$ where $C_{p,w}$ is the isobaric heat capacity of water. The number $n_w$ ($n_w > 0$) of relaxed water molecules is proportional to the size of the solute molecule and hence of the cavity. The term $n_w C_{p,w} T$ is actually an entropy term, which compensates for part of the reaction enthalpy ($\Delta H_0 < 0$). The entropy change at 298 K linearly depends on $n_w$, thus showing that cavity formation is associated to a negative entropy change ($\Delta s_{cav} = -23.2$ J K$^{-1}$ mol$^{-1}$ $n_w^{-1}$). Beyond a temperature $T_{min}$ typical of each compound, the reaction becomes endothermic. The highly negative entropy change $\Delta S_{min}$ (at $T_{min}$ we have $\Delta H_{app} = 0$) is related to the loss of kinetic energy by the solute molecule when trapped in the cage. Another example of $S/H$ compensation occurs in the formation of micelles. The resultant cage volume after formation of the micelle is smaller than the sum of the cavities previously hosting the single separated apolar moieties. Therefore, some floating water molecules need to be reintroduced into the structure of the solvent ($n_w < 0$) to fill the void. The contraction of the cavity is associated to a positive entropy change ($\Delta s_{fill} = 22.4$ J K$^{-1}$ mol$^{-1}$ $|n_w|^{-1}$). Protein *folding* and protein–substrate association behave in a way similar to micellisation ($n_w < 0$). The present interpretation of the complexation reactions of proteins, and also of micellisation, leads to a new formulation of the so-called 'hydrophobic bond': the positive entropy change for cavity contraction is the main driving force of hydrophobic bonding. In the denaturation process as opposite to *folding*, the denaturation enthalpy $\Delta H_{den}$ at different temperatures $T_{den}$ depends on positive numbers ($n_w > 0$) of water molecules. The presence of polar groups and/or charges in the solute molecule, on the other hand, exerts on the water molecules the same action as that produced by micellisation ($n_w < 0$).

## Introduction

Apolar substances show a very low solubility in water, a property that is defined as 'hydrophobicity' (*i.e.* aversion to water) or 'hydrohobic effect'. This phenomenon has been widely and deeply studied and its interpretations are strictly related to the possible structure assigned to water. The study starts from the analysis of the thermodynamic parameters of the dissolution process of non-polar substances in water.[1–24] Gibbs energy, enthalpy, entropy and heat capacity of transfer of a slightly soluble substance from its liquid or gaseous state to aqueous solution or from another solvent to water are considered. These thermodynamic data are the starting steps to the understanding and explanation of other phenomena such as the formation of micelles and biological membranes and the *folding* of proteins. Compensation of entropy and enthalpy within the total Gibbs energy change of these processes has been found to be almost ubiquitous. The solubility of apolar substances does not imply in itself the formation of a hydrophobic bond between apolar moieties, due to the action of a driving force, although an interaction with the structure of the solvent certainly takes place. Therefore, one speaks rather of hydrophobic hydration to mean the processes accompanying the interaction between a solute molecule and the solvent water.

Guillot and Giussani[13] evaluated the temperature dependence of hydrophobic hydration by molecular dynamics calculations of the solubility of rare gases or methane in water. They found that the evaluation of the pair distribution function between solute and solvent enables establishing the formation of clathrate type cages around the solute. Lee[14] suggests the hypothesis that the transfer of a hydrophobic molecule from non-aqueous phase to water involves two different but related compensation behaviours. The temperature dependence of the thermodynamics of this process exhibits a compensating behaviour in the sense that both enthalpy and entropy changes vary monotonically with the temperature. Free energy changes, on the other hand, show different trends in different temperature ranges. It is typical, in fact, of all these dissolution processes that the solubility free energy of every non-polar substance in water reaches a minimum at temperature near room temperature (Fig. 1) and then increases. Moreover, hydrophobic hydration is accompanied by solvent reorganisation, which is a compensating process, and, as such, it should be irrelevant as far as the free energy of hydrophobic bond is concerned. Lee concludes that the true cause of the hydrophobicity must be sought elsewhere other than in solvent reorganisation.

Starting from the observations concerning the parabolic shape of the solubility curve of apolar substances, we have developed a statistical thermodynamic molecular model[16–22] which is suited to explain the changes of thermodynamic functions with the temperature in aqueous systems. It is our purpose here to show that, starting from the grounds of this model, we can propose a molecular interpretation of the phenomenon of hydrophobicity. The process is associated with

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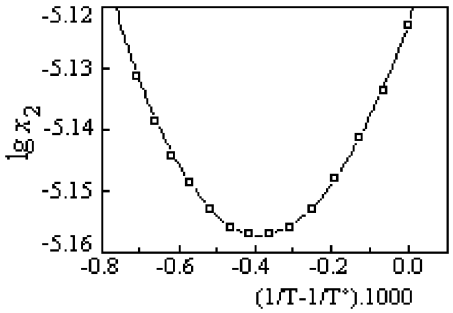


**Fig. 1** Solubility of helium as the function of reciprocal temperature ($T^{\circ} = 273.15$ K).

entropy/enthalpy ($S/H$) compensation. The entropy/enthalpy ($S/H$) compensation principle can then be extended to the thermodynamics of micelle formation, protein denaturation and protein complexation. On this basis, we suggest a possible molecular mechanism for these processes, whereby a compensation takes place between the thermal energy of floating water molecules and heat produced in the reaction.

This statistical model[17–21] is based on the distinction between *reacting* and *non-reacting* thermodynamic systems. The *reacting* system can be seen as a set of separated 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 considered. We assume that a set of successive reactions is taking place, say $M + iA = MA_i$. Each enthalpy level $H_i$ is associated with one species $MA_i$. The ground level $H_0$ is associated with free M. Each enthalpy level $H_i$ is the mean value of a set of enthalpy sublevels only slightly different from one another, thus giving rise to a continuous sequence of enthalpy sublevels $H_{i,j}$ grouped around the species mean enthalpy level $H_i = \langle H_{i,j} \rangle$. The pure compounds or solutions of a single species are *non-reacting* systems. They are characterised by a continuous distribution of enthalpy levels analogous to that observed in each subset $H_{i,j}$ of a *reacting* system. Thus also each set of sublevels, taken *per se* represents a *non-reacting* system. A corollary of this model is the TED (*thermal equivalent dilution*) principle[21]

$$-\partial \ln [A]/\partial \ln T = C_p/R \qquad (1)$$

whereby an increase in temperature is equivalent in *non-reacting* ensembles to diluting the solute species A, the dilution being calculated as the reciprocal of concentration [A].

## Solubility of apolar substances and $S/H$ compensation

In order to evidence $S/H$ compensation processes, we refer to the solubilisation process of a typical hydrophobic simple molecule such as a noble gas. The plot (see Fig. 1) of the logarithm of the solubility constant which represents standard free energy

$$2.302 \log K_H = -\Delta G^{\ominus}/RT \qquad (2)$$

against the reciprocal temperature $1/T$ shows in fact a minimum at $(1/T_{min})$. This implies that according to the van't Hoff law

$$\partial(-\Delta G^{\ominus}/RT)/\partial(1/T) = -\Delta H^{\ominus}/R \qquad (3)$$

the process presents a positive slope (*i.e.* negative enthalpy) at low temperatures ($1/T > (1/T_{min})$) and a negative slope (*i.e.* positive enthalpy) at high temperatures ($1/T < (1/T_{min})$).

The solubility of a gas[16] such as He in water W can be treated as an equilibrium between He and W

$$He + n_w W_l = He(W_m)_{n_w} + n_w W \qquad (4)$$

where $n_w W_l$ is the portion of the bulk water involved in the solubilisation process, and $He(W_m)_{n_w}$ is the gas molecule trapped within a cage formed by $n_w$ water clusters ($W_m$) (with $m = l - 1$, where $m = 4, 6$). This is in accordance with the existence of three forms of water structures, namely form I, $(W_l)_I$, form II, $(W_m)_{II}$, and form III, $(W)_{III}$ (Fig. 2). Forms I and II are clusters of water molecules in accordance with the suggestion of Schmid.[23] The cluster $(W_m)_{II}$ forms a cage of water around the solute; these water molecules are held together by strong hydrogen bonds and form a structure of high density.[25,26] The cage could correspond to the clathrate suggested by Guillot and Giussani[13] as well as by Franks[27] and by Matubayasi *et al.*[28] The clusters $(W_l)_I$, forming the bulk of the solvent, are held together by weaker hydrogen bonds and form a structure of low density. Finally, the third form $(W)_{III}$ consists of floating isolated water molecules that are destructured (relaxed) around the solute within the cage hosting the solute molecule. The same equilibria exist in the structure of pure water[29] and explain the trend of the isobaric heat capacity of water itself at different temperatures. The constant of this equilibrium is, with $(W_l)_I$ in excess,

$$K_S = [He(W_m)_{n_w}][W]^{n_w}/[He] \qquad (5)$$

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

$$P_S = [W]^{n_w}/K_H = x_2[W]^{n_w} \qquad (6)$$

where $P_S$ includes the conversion factor deriving from the non-homogeneity of the concentration scales of gas and water. By taking the logarithms, one moves from probability space to affinity thermodynamic space[19] whereby relative changes of probability are measured by changes of thermodynamic functions. The logarithm of eqn. (6), therefore, represents thermodynamic changes

$$\ln x_2 = \ln P_S - n_w \ln [W] \qquad (7)$$

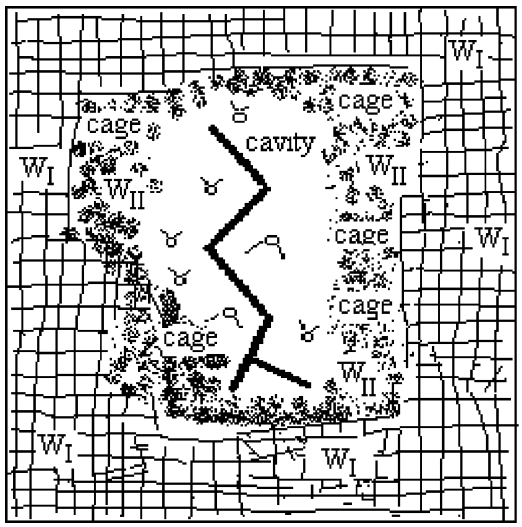


**Fig. 2** Solubility in water of an apolar substance. The cage wall consists of a network of water molecules $W_{II}$ bound by strong hydrogen bonds (high density). The cavity inside the cage is occupied by the solute and by $n_w$ relaxed water ($W_{III}$) molecules ($n_w > 0$). The structured sector is the bulk solvent $W_I$ (low density).

The solubility distribution function can be expressed in the affinity thermodynamic space ($\ln x_2 = -\Delta G^{\ominus}_{app}/RT$) by a Taylor expansion as the function of $(1/T)$.

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

$$\{\partial \ln x_2/\partial(1/T)\}_\theta = \{\partial \ln P_S/\partial(1/T)\}_\theta - n_w \{\partial \ln [W]/\partial(1/T)\}_\theta \quad (8)$$

which can be identified for a combinations of enthalpies

$$-\{\Delta H_{app}\}_\theta = -\{\Delta H_0\}_\theta + \{\Delta H_w\}_\theta \quad (9)$$

The left hand side (l.h.s.) of eqn. (9), $\{\Delta H_{app}\}_\theta$, can be obtained as tangent at temperature $T = \theta$ of the plot of the function $\ln x_2 = f(1/T)$. The true enthalpy $\{\Delta H_0\}_\theta$ refers to the separation of levels and is in principle constant. On the other hand, 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 $\ln T$ which can be expressed[21] as

$$-n_w \partial \ln [W]/\partial \ln T = n_w C_{p,w}/R \quad (10)$$

where $C_{p,w}$ is the molar heat capacity of water. The heat capacity of water can be considered constant in the interval 0–100 °C because the observed values vary less than 0.3%. By transformation into the derivative with respect to $1/T$, eqn. (10) yields

$$n_w \partial \ln [W]/\partial(1/T) = \{-\Delta H_w/R\}_T = n_w C_{p,w} T/R \quad (11)$$

By combining eqns. (9) and (11), one obtains, if the constant $P_S$ has constant true enthalpy $\Delta H_0$ at constant heat capacity of water $C_{p,w}$

$$\{\Delta H_{app}/R\}_\theta = \Delta H_0/R + n_w C_{p,w} T/R \quad (12)$$

The observed apparent enthalpy, determined either calorimetrically or by the van't Hoff equation, when plotted against $T$, produces a straight line. This behaviour was observed by processing the experimental solubility data of about forty substances (Table 1) at different temperatures between 0 and 75 °C. Sometimes the determinations go beyond 75 °C and in a few cases are up to 100 °C.[30–36] The behaviour conforms to the finding that in every solubilisation process in water of apolar substance it has been observed that the heat capacity change is positive[12,37–43] ($\Delta C_{p,app} > 0$).

Eqn. (12) is an example of $S/H$ compensation because the r.h.s. second term is actually a $T\Delta S/R$ term although not included in the $T\Delta S^{\ominus}$ entropy term of the reaction. The compensation consists in the exploitation of the energy released by the reaction of gas with water to provide kinetic energy to some water molecules relaxed from the bulk of the solvent. At upper temperatures, beyond a temperature $T_{min}$ typical of each compound, the reaction becomes endothermic and the total heat supplied is compensated for by the increased kinetic energy of $n_w$ water molecules. The $n_w$ water molecules move within the cage surrounding the solute. The compensation yields a cancellation of part of the reaction enthalpy ($\Delta H_0 < 0$). Therefore, the observed enthalpy $\Delta H_{app}$ is apparent. The true enthalpy $\Delta H_0$ of the reaction is obtained by linear extrapolation of $\Delta H_{app}$ to $T = 0$ by eqn. (12). The temperature at which the enthalpy is completely compensated corresponds to the minimum of the curve $\ln x_2 = f(1/T)$.

On the ground of these findings, we propose a molecular mechanism for the solubilisation process of apolar substances (Fig. 2). The structure of liquid water is in general well described by a dual structure, in equilibrium or fluctuation from one another.[1,11,23–26] One structure (high density) consists of a network of hydrogen bonded clusters $(W_m)_{II}$ (with $m =$ 4, 6) of molecules, whereas the second structure (low density)

**Table 1** Enthalpy $\Delta H_0$ and number $n_w$ of water molecules for solubility of apolar gases in water[a]

| Compound | Method | $n_w$ | $\Delta H_0$/kJ mol$^{-1}$ | Ref. | Compound | Method | $n_w$ | $\Delta H_0$/kJ mol$^{-1}$ | Ref. |
|---|---|---|---|---|---|---|---|---|---|
| He | Solub. | 1.38 | −31.9 | 30 | $N_2F_4$ | Solub. | 5.33 | −140.2 | 30 |
| Ne | Solub. | 1.98 | −48.3 | 30 | $N_2O$ | Solub. | 1.71 | −59.8 | 30 |
| Ar | Solub. | 2.35 | −65.2 | 30 | NO | Solub. | 0.60 | −46.7 | 30 |
| Kr | Solub. | 2.76 | −77.6 | 30 | $H_2S$ | Solub. | 2.32 | −69.4 | 30 |
| Xe | Solub | 2.61 | −77.2 | 30 | $SF_6$ | Solub. | 6.99 | −177.2 | 30 |
| Rn | Solub. | 3.88 | −108.6 | 30 | $H_2Se$ | Solub. | 1.01 | −38.5 | 30 |
| $H_2$ | Solub. | 1.87 | −46.2 | 30 | $AsH_3$ | Solub. | 2.11 | −64.5 | 30 |
| $N_2$ | Solub. | 2.96 | −76.9 | 30 | Air | Solub. | 3.10 | −73.4 | 30 |
| $O_2$ | Solub. | 2.68 | −68.2 | 30 | $CH_4$ | Calor. | 3.20 | −85.1 | 31 |
| CO | Solub. | 2.57 | −69.0 | 30 | $C_2H_6$ | Calor. | 4.22 | −114.0 | 31 |
| $CO_2$ | Solub. | 2.31 | −71.7 | 30 | $C_3H_8$ | Calor. | 5.16 | −138.8 | 31 |
| $CH_4$ | Solub. | 2.75 | −75.6 | 30 | $nC_4H_{10}$ | Calor. | 5.63 | −152.3 | 31 |
| $C_2H_6$ | Solub. | 3.98 | −109.2 | 30 | $C_2H_6$ | Calor. | 3.64 | −101.3 | 33 |
| $C_2H_4$ | Solub. | 2.27 | −66.3 | 30 | $C_3H_8$ | Calor. | 4.22 | −118.1 | 33 |
| $C_2H_2$ | Solub. | 2.36 | −67.8 | 30 | $CH_4$ | Calor. | 2.77 | −75.7 | 32 |
| $C_3H_8$ | Solub. | 4.89 | −132.4 | 30 | $CH_4$ | Solub. | 3.24 | −83.5 | 34 |
| $nC_4H_{10}$ | Solub. | 4.95 | −137.1 | 30 | $C_2H_6$ | Solub. | 3.66 | −100.0 | 34 |
| $(CH_3)_2C{=}CH_2$ | Solub. | 3.63 | −106.2 | 30 | $nC_4H_{10}$ | Solub. | 5.10 | −137.8 | 34 |
| $1.3C_4H_6$ | Solub. | 7.70 | −210.8 | 30 | $O_2$ | Calor. | 2.72 | −73.3 | 31 |
| $(CH_3)_4C$ | Solub. | 6.83 | −181.4 | 30 | He | Calor. | 1.79 | −40.9 | 31 |
| $FCH_3$ | Solub. | 2.00 | −63.2 | 30 | Ne | Calor. | 1.94 | −47.0 | 31 |
| $ClCH_3$ | Solub. | 2.58 | −81.2 | 30 | Ar | Calor. | 2.64 | −71.5 | 31 |
| $BrCH_3$ | Solub. | 2.43 | −80.2 | 30 | Kr | Calor. | 2.92 | −80.9 | 31 |
| $CF_4$ | Solub. | 5.07 | −129.1 | 30 | Xe | Calor. | 3.30 | −93.3 | 31 |
| $CHClF_2$ | Solub. | 4.86 | −137.9 | 30 | $CF_4$ | Solub. | 5.73 | −142.2 | 35 |
| $C_2F_4$ | Solub. | 3.99 | −107.0 | 30 | $C_4F_8$ | Solub. | 11.14 | −272.5 | 35 |
| $C_3F_6$ | Solub. | 0.76 | −37.3 | 30 | $C_2F_6$ | Solub. | 10.12 | −245.5 | 35 |
| COS | Solub. | 3.34 | −99.7 | 30 | $CF_4$ | Solub. | 7.01 | −172.8 | 36 |
| $NF_3$ | Solub. | 3.83 | −101.6 | 30 | | | | | |

[a] $\Delta H_0 = \Delta H_0^{(w=0)} + \Delta h_w n_w = -11.97 - 23.919\ n_w$ kJ mol$^{-1}$ ($R^2 = 0.9815$). Cumulative with protein denaturation of Table 5: $\Delta H_0 = \Delta H_0^{(w=0)} + n_w \Delta h_0 = -17{,}758 - 21.626\ n_w$ kJ mol$^{-1}$ ($R^2 = 0.9927$). Accuracy (within single set): $\sigma(\Delta H_0) = \pm 0.3$ kJ mol$^{-1}$, $\sigma(n_w) = \pm 0.04$. Precision (from discrepancies between different methods): $\delta(\Delta H_0) = \pm 7$ kJ mol$^{-1}$, $\delta(n_w) = \pm 0.4$.

**Table 2** Enthalpy $\Delta H_0$ and number $n_w$ of water molecules for solubility of liquids in water

| Compound | $n_w$ | $\Delta H_0$/kJ mol$^{-1}$ | Method | Ref. |
|---|---|---|---|---|
| Benzene | 2.93 | −63.9 | Calor. | 44 |
| Benzene | 2.99 | −64.9 | Calor. | 45 |
| $D_6$benzene | 2.75 | −59.7 | Calor. | 45 |
| Toluene | 3.50 | −76.9 | Calor. | 45 |
| Ethylbenzene | 4.19 | −92.1 | Calor. | 45 |
| Propylbenzene | 5.16 | −113.7 | Calor. | 45 |
| Cyclohexane | 4.74 | −106.4 | Calor. | 45 |
| Pentane | 5.31 | −121.3 | Calor. | 45 |
| Hexane | 5.84 | −131.2 | Calor. | 45 |

consists of blocks of $(W_l)_I$ molecules (with $l = m + 1$). We assume that the passage from $(W_l)_I$ to the second structure $(W_m)_{II}$ is accompanied by dissociation or relaxation of $n_w$ water molecules, $(W_m)_{III}$ and by formation of a cavity within a cage whose walls are formed by the high-density structure $(W_m)_{II}$. The number of water molecules involved in the cage, therefore, is not equal to $n_w$ but is proportional to it.

The relation of $n_w$ with the structure of the solute is consistent with the existence of a linear dependence of $n_w$ upon the number of carbon atoms, $n$C, in the chain.[38,39] Several authors, in fact, have shown that the heat capacity changes could be calculated as the sum of group contributions.[42,43] An analogous dependence, with very similar slopes, is shown by other series of molecules of increasing size. For instance, the parallel trends of $n_w$ in gases and liquids of equal size can be compared. The values[44,45] of $n_w$ for the liquid substances (Table 2) are systematically lower by about 2 than the value of the corresponding gaseous substances of equal length.[38,39] This behaviour has been attributed[39] to partial association of the liquids producing a phenomenon equal to that observed in micelle formation with reduction in $n_w$, as will be shown below.

The relationship between $n_w$ and size of solute molecule is further confirmed by considering the changes of volume involved. According to Kharakoz,[46,47] each water molecule occupies a volume of 18.5 cm$^3$ mol$^{-1}$. This corresponds to a sphere of radius $r = 3.111$ Å per molecule. The expulsion of water molecules ($n_w > 0$) from the structure of the solvent produces a cavity of volume

$$V_{cav} = n_w \times 18.5 \text{ cm}^3 \text{ mol}^{-1} \quad (13)$$

The volume of cavity thus calculated is larger than the partial molar volume calculated by Kharakoz for the solute and a shrinkage, therefore, has taken place (Table 3). This smaller volume could be due to the fact that the water molecules are expelled from the denser water structure, $(W_m)_{II}$ having a partial molar volume smaller than 18.5 cm$^3$ mol$^{-1}$. This contraction is confirmed by the data reported by Franks[27] concerning the excess partial volumes $V_2^E$ of solutes in water as function of solute concentration. A negative $V_2^E$ implies net volume shrinkage when the solute is added to water. In other words, the solute in aqueous solution does not occupy as much volume as it does in its pure liquid state. The concentration dependence of $V_2^E$ shows that for apolar compounds the partial volume decreases with increasing concentration. Moreover, the diminution is higher as the size of the hydrophobic moiety increases, *i.e.* the larger $n_w$ becomes.

**Table 3** Volume of cavity and volume of solute[46,47]

| | $n_w$ | $V_{cav}$/cm$^3$ mol$^{-1}$ | $V_2$/cm$^3$ mol$^{-1}$ | $\Delta V$/cm$^3$ mol$^{-1}$ |
|---|---|---|---|---|
| Methane | 2.75 | 50.88 | 26.5 | −24.38 |
| Ethane | 3.98 | 75.63 | 53.0 | −22.63 |
| Propane | 4.89 | 90.47 | 68.9 | −21.57 |

The compensation here observed between enthalpy and $n_w C_{p,w} T$ is an actual cancellation and leads to reduction of the experimental enthalpy. In general, however, one speaks of $S/H$ compensation between entropy and enthalpy measured separately. The compensation, therefore, is only potential and produces a reduction of free energy. This type of compensation also takes place in the solubility process of apolar substances in water. The compensation between the extrapolated enthalpy and the entropy effect in the solubility of apolar substances yields low (positive) values of free energy. The potential compensation is between a high exothermic effect and an even higher negative configurational entropy effect.

## Entropy change for cavity formation

In 1990 Murphy, Privalov and Gill[48] showed that, by plotting the entropy change *versus* the heat capacity change at 25 °C for series of homologous compounds undergoing the same physico-chemical process in water straight lines are obtained with practically the same slope. The linear dependence on $\Delta C_p$ implies a linear dependence on $n_w$. By plotting the entropy values at 25 °C reported by Abraham and Matteoli[49] *versus* $n_w$ we obtain a straight line

$$\begin{aligned} \Delta S_{app} &= \Delta S_0^{(w=0)} + \Delta s_{cav} n_w \text{ JK}^{-1} \text{ mol}^{-1} \\ &= -65.0 - 23.18\, n_w \text{ JK}^{-1} \text{ mol}^{-1} \end{aligned} \quad (14)$$

whose slope indicates the change of entropy per water molecule and hence the entropy change of formation of a cavity having a volume corresponding to one water molecule. The cavity formation entropy change per water molecule removed is calculated as $\Delta s_{cav} = -23.2$ J K$^{-1}$ mol$^{-1}$ $n_w^{-1}$ whereas the intercept $\Delta S_0^{(w=0)} = -65.0$ J K$^{-1}$ mol$^{-1}$ represents the average loss of configurations of solute when trapped in the cage. In fact, according to Murphy *et al.*,[48] the data of gas dissolution give in eqn. (14) a slope $\Delta s_{cav} = -21.1$ J K$^{-1}$ mol$^{-1}$ $n_w^{-1}$ and an intercept $\Delta S_0^{(w=0)} = -78.5 \pm 23$ J K$^{-1}$ mol$^{-1}$. The latter value is very close to $\langle \Delta S_{min} \rangle = -81 \pm 22$ J K$^{-1}$ mol$^{-1}$ found from the minima of the solubility curves that will be shown (see below) to be related to the loss of kinetic energy by the gas molecule. This interpretetion of the extrapolated entropy is indirectly confirmed by the fact that the dissolution of liquid hydrocarbons which gives in eqn. (14) a rather similar slope $\Delta s_{cav} = -18.1$ J K$^{-1}$ mol$^{-1}$ $n_w^{-1}$ yields an intercept $\Delta S_0^{(w=0)} = -0.5 \pm 3$ J K$^{-1}$ mol$^{-1}$ with almost null loss of kinetic energy. On the other hand, the dissolution of solid cyclic dipeptides yields a similar slope $\Delta s_{cav} = -17.3$ J K$^{-1}$ mol$^{-1}$ $n_w^{-1}$ and a positive intercept $\Delta S_0^{(w=0)} = 16 \pm 1$ J K$^{-1}$ mol$^{-1}$ which is in accordance with the dilution of the molecules transferring from solid to solution and generating positive configurational entropy. The same holds for the unfolding of proteins.

## Free energy, enthalpy and entropy of gas/water equilibria

The solubility distribution function can be expressed in the affinity thermodynamic space ($\ln x_2 = -\Delta G_{app}^{\ominus}/RT$) by a Taylor expansion as the function of $(1/T)$. The tangent of the curve at any point is the enthalpy change $\{-\Delta H_{app}/R\}$. At the minimum, the tangent is null and so is the enthalpy ($\{-\Delta H_{app}/R\}_{min} = 0$). At this point, from

$$\{-\Delta G_{app}/RT\} = \{-\Delta H_{app}/RT\} + \{\Delta S/R\} \quad (15)$$

one obtains

$$\{-\Delta G_{app}/RT\}_{min} = + \{\Delta S/R\}_{min} \quad (16)$$

which corresponds to the minimum of the curve $\ln x_2 = f(1/T)$. At this point, the reaction is apparently adiabatic. The values of $\Delta S_{min}$ have been calculated in Table 4 for all the apolar substances.

**Table 4** Free energy $\Delta G_{min}$ and entropy $\Delta S_{min}$ at the minimum of the solubility curve (data from ref. 30)[a]

| Compound | $\Delta H_0$/kJ mol$^{-1}$ | $n_w$ | $T_{min}$/K | log $k_{min}$ | $\Delta G_{min}$/kJ mol$^{-1}$ | $\Delta S_{min}$/J K$^{-1}$ mol$^{-1}$ |
|---|---|---|---|---|---|---|
| He | −31.9 | 1.38 | 305.4 | −5.15 | 30.2 | −98.7 |
| Ne | −48.3 | 1.98 | 323.2 | −5.1148 | 31.7 | −97.9 |
| Ar | −65.2 | 2.35 | 367.9 | −4.8154 | 33.9 | −92.2 |
| Kr | −77.6 | 2.76 | 372.6 | −4.6437 | 33.1 | −88.9 |
| Xe | −77.2 | 2.61 | 391.2 | −4.5286 | 33.9 | −86.7 |
| Rd | −108.6 | 3.88 | 371.1 | −4.1698 | 29.6 | −79.8 |
| $H_2$ | −46.2 | 1.87 | 326.9 | −4.8807 | 30.6 | −93.5 |
| $N_2$ | −76.9 | 2.96 | 344.7 | −5.0597 | 33.4 | −96.9 |
| $O_2$ | −68.2 | 2.68 | 337.0 | −5.4538 | 37.2 | −104.1 |
| CO | −69.0 | 2.57 | 355.5 | −4.9291 | 33.6 | −94.4 |
| $CO_2$ | −71.8 | 2.31 | 413.2 | −3.7438 | 29.6 | −71.7 |
| $C_2H_6$ | −109.2 | 3.98 | 364.2 | −4.8087 | 33.5 | −92.1 |
| $C_2H_4$ | −66.3 | 2.27 | 387.1 | −4.4029 | 32.6 | −84.3 |
| $C_3H_8$ | −132.4 | 4.89 | 359.3 | −4.9228 | 33.9 | −94.3 |
| $(CH_3)_4C$ | −181.4 | 6.83 | 352.2 | −5.3623 | 36.2 | −102.7 |
| $CH_3F$ | −63.2 | 2.00 | 419.6 | −3.4816 | 28.0 | −66.7 |
| $CH_3Cl$ | −81.2 | 2.58 | 418.5 | −3.3704 | 27.0 | −64.5 |
| $H_2S$ | −69.4 | 2.32 | 397.2 | −3.1449 | 23.9 | −60.2 |
| $AsH_3$ | −64.5 | 2.11 | 425.1 | −4.2613 | 34.7 | −81.6 |
| AIR | −73.4 | 3.1 | 314.9 | −3.5945 | 21.7 | −68.8 |
| $CH_4$ | −75.6 | 2.75 | 364.8 | −4.8351 | 33.8 | −92.6 |
| $C_2H_2$ | −67.8 | 2.36 | 381.6 | −3.4313 | 25.1 | −65.7 |
| $nC_4H_{10}$ | −137.1 | 4.95 | 367.9 | −5.118 | 36.1 | −98.0 |

[a] Estimated errors are $\sigma(\Delta G_{min})$ ±2 kJ mol$^{-1}$ and $\sigma(\Delta S_{min})$ ±6 J K$^{-1}$ mol$^{-1}$.

The analysis of the values of entropy $\Delta S_{min}$ shows interesting peculiarities. If the values of $\Delta S_{min}$ for noble gases and alkanes are plotted (Fig. 3) against the atomic or molecular radius, a V-shaped distribution is obtained very similar, with opposite sign, to that observed by Graziano[50] for free energies. The entropy raising branch is due to noble gases and the decreasing branch to alkanes. This means that the entropy for noble gases increases (*i.e.* it becomes less negative) with increasing size whereas the entropy decreases with size for alkanes. It is important, however, to know that for all these substances taken together the values of $n_w$ against the radius show a monotonic trend (Fig. 4). This means that $n_w$ is proportional to the radius of the volume of water displaced by the solute, *i.e.* the radius is proportional to a cavity volume of constant density in every compound. The entropic kinetic energy of the solute particles, however, depends on both volume and density of solute and hence on its own mass. This density effect is particularly enhanced for noble gases, for which the atomic weight change from 4 for He to 222 for Rn. The relationship of entropy must be searched for rather with the de Broglie thermal wavelength $\Lambda$ of a single species[6]

$$\Lambda = \{h^2/(2\pi mkT)\}^{1/2} = h(2\pi mkT)^{-1/2} \qquad (17)$$

where $h$ is the Planck constant, $k$ the Boltzman constant, and $m$ molecular mass. $\Lambda$, that is dimensionally a length, can be considered as a mean oscillation diameter of the solute particle. $\Lambda$ raised to the third power and multiplied by the Avogadro number $N_L$ gives the molar oscillation volume

$$V_m = \Lambda^3 = \{h^2/(2\pi mkT)^{3/2}\} \qquad (18)$$

The plot of $\Delta S_{min}$ against $1/V_m$ shows a homogeneous behaviour for noble gases and alkanes (Fig. 5). Residual deviations from the general trend are maintained by the alkanes probably because their structure, shape, and surface are not spherically symmetrical. In contrast, the entropy values $\Delta S_{min}$ do not show any peculiar relationship either if they are plotted as the function of $(1/T_{min})$ or of $T_{min}$ or of $n_w$. Most of the values, however, are around a mean value $\langle \Delta S_{min} \rangle = -81 \pm 22$ J K$^{-1}$ mol$^{-1}$. This highly negative mean entropy should be associated to the loss of oscillation by the solute trapped in the cage of water molecules as already said above in the comments to eqn. (14).

The extrapolated enthalpy $\Delta H_0$ that is calculated from the plot of $\Delta H_{app}$ against $T$ by eqn. (12) is also dependent upon the number of water molecules $n_w$. In fact, when the extrapolated enthalpy, $\Delta H_0$, is plotted against $n_w$, a linear function is obtained (Fig. 6). The coefficient $\Delta h_w$ of the function

$$\Delta H_0 = \Delta H_0^{(w=0)} + \Delta h_w n_w = -17.76 - 21.63\, n_w \text{ kJ mol}^{-1} \qquad (19)$$

obtained ($R^2 = 0.9927$) for the class of apolar compounds together with the values of $\Delta H_0$ obtained for the denaturation of proteins (*cf.* Table 5 below) yields the energy $\Delta h_w = -21.6$ kJ mol$^{-1}$ $n_w^{-1}$ per water molecule relaxed. This

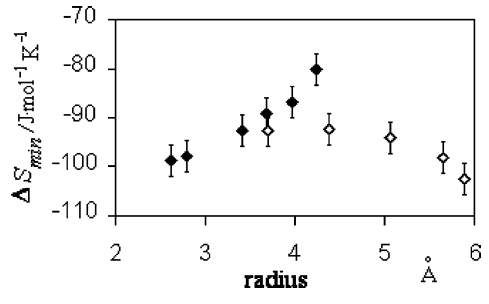


**Fig. 3** Entropy values $\Delta S_{min}$ plotted against molecular radius for noble gases (black) and alkanes (empty) show different trends for noble gases and alkanes, respectively.

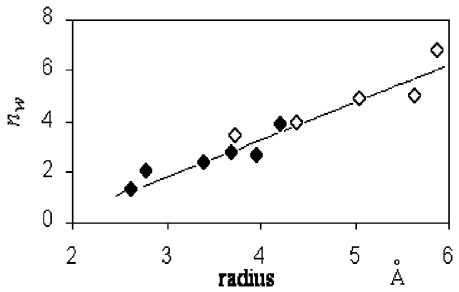


**Fig. 4** $n_w$ plotted against molecular radius for noble gases (black) and alkanes (empty) shows a unique monotonic behaviour.

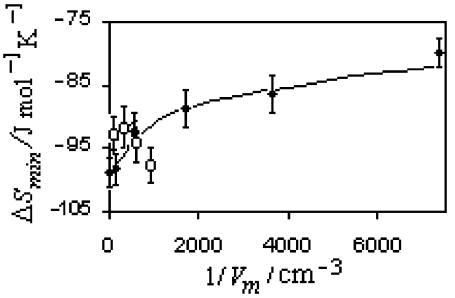


**Fig. 5** Entropy $\Delta S_{\min}$ as the function of $1/V_{\mathrm{m}}$ for noble gases (black) and alkanes (empty) shows a uniform trend. The deviations of alkanes can be attributed to the failing of spherical symmetry, both as structure and surface.

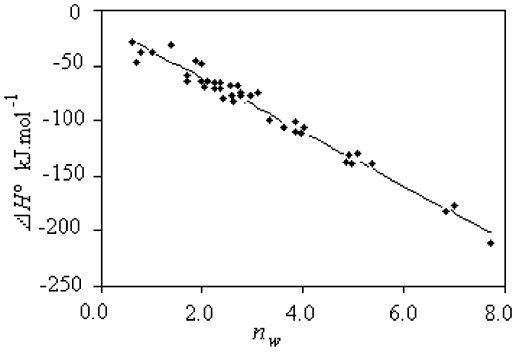


**Fig. 6** Energetics of hydrophobic hydration: enthalpy is $h_{\mathrm{w}} = -21.63$ kJ mol$^{-1}$ $n_{\mathrm{w}}^{-1}$ per relaxed water mole (*cf.* eqn. (19)).

means that the solubilisation process is highly exothermic. The extrapolated enthalpy, $\Delta H_0$, for the solubilisation and denaturation processes gives the energy released when the molecule reacts with structured water before the destructured water molecules absorb part of that energy. The enthalpy change is opposed by a highly negative entropy effect. By assuming that the entropy corresponds to the mean value $\langle \Delta S_{\min} \rangle = -81 \pm 22$ J K$^{-1}$ mol$^{-1}$ we have at 298 K a very roughly estimated value of free energy $\Delta G_{\mathrm{w}} = -0.02$ kJ mol$^{-1}$ per water molecule removed. This again is an example of $S/H$ potential compensation between potential enthalpy and configurational entropy.

## Micelle formation

Another example of $S/H$ direct (not potential) compensation occurs in the formation of micelles. It can be assumed that at the very moment of micelle formation some water molecules previously floating become fixed in the fluctuating structure of solvent. They lose kinetic energy,[51] and the kinetic energy is transmitted as heat out of the system (exothermal). In fact, when aqueous solutions of micelles are considered, the slope of the plot of enthalpy against temperature is negative thus indicating a negative number $n_{\mathrm{w}}$ of floating water molecules. This is the case of aqueous micellar solutions of surfactants,[52–62] reported in Table 6. The amount of $n_{\mathrm{w}}$ water molecules involved is as low as for the solubilisation of small molecules in water and, again, it is proportional to the size of the monomeric molecule. A negative number $n_{\mathrm{w}}$ of water molecules implies that some previously floating water molecules are included in the structured water. This can be accomplished, accompanied by the effect of the counterion, by way of the micellization mechanism depicted schematically in Fig. 7. Each hydrophobic surfactant molecule before micellization is in the solution surrounded by a sheath of water $(W_m)_{II}$, forming a cage *via* a mechanism analogous to that proposed for the solubilisation of gases and liquids. When two such hydrophobic moieties with their sheaths get close to each other, the single

**Table 5** Dehydration numbers, $n_{\mathrm{w}}$ in protein denaturation[a]

| Protein | pH | $\Delta C_{\mathrm{p,app}}$/J K$^{-1}$ mol$^{-1}$ | $n_{\mathrm{w}}$ | $\Delta H_0$/kJ mol$^{-1}$ | Ref. |
|---|---|---|---|---|---|
| HEW | | 6701 | 88.9 | −1768 | 77 |
| WildT4 | | 9199 | 122.0 | −2463 | 77 |
| T157 (T4) | | 9903 | 131.4 | −2702 | 77 |
| R96H (T4) | | 10539 | 139.4 | −2896 | 77 |
| βLG | 7.0 | 5036 | 66.8 | −1522 | 80 |
| | 6.5 | 6005 | 79.7 | −838 | 80 |
| | 2.5 | 6715 | 88.1 | −2068 | 80 |
| | 2.0 | 6516 | 86.4 | −2016 | 80 |
| | 1.5 | 6623 | 87.9 | −2044 | 80 |
| | 1.0 | 6333 | 84.0 | −1947 | 80 |
| βLG (ur.) | 2.50 | 8912 | 118.2 | −2656 | 81 |
| | 2.50 | 9037 | 119.9 | −2693 | 81 |
| | 2.71 | 8494 | 112.7 | −2531 | 81 |
| | 3.00 | 8996 | 119.3 | −2681 | 81 |
| | 2.57 | 9749 | 129.3 | −2905 | 81 |
| | 2.78 | 8117 | 107.7 | −2419 | 81 |
| | 2.55 | 7782 | 103.2 | −2319 | 81 |
| | 3.20 | 8786 | 116.6 | −2618 | 81 |
| Ribonucl. | | 10878 | 144.3 | −3241 | 81 |
| Ribonucl. | | 9623 | 127.7 | −2867 | 81 |
| Chimotrip. | | 14644 | 194.3 | −4363 | 81 |
| Myogl. | | 5858 | 77.7 | −1745 | 81 |
| βLG (ur.) | | 8790 | 116.6 | −2702 | 81 |
| βLG(GuHCl) | | 8786 | 116.6 | −2618 | 81 |
| Ribonucl. | 1.13 | 10331 | 137.1 | −2795 | 82 |
| | 2.10 | 13302 | 176.5 | −3668 | 82 |
| | 2.50 | 14183 | 196.5 | −4167 | 82 |
| | 2.77 | 15567 | 206.5 | −4403 | 82 |
| | 3.15 | 21309 | 282.7 | −6245 | 82 |

[a] $\Delta H_0 = \Delta H_0^{(w=0)} + n_{\mathrm{w}} \Delta h_0 = +24.488 - n_{\mathrm{w}}\, 21.918$ kJ mol$^{-1}$ ($R^2 = 0.9604$). Cumulative with $\Delta H_0$ of apolar subst. of Table 1: $\Delta H_0 = \Delta H_0^{(w=0)} + n_{\mathrm{w}}$ $\Delta h_0 = -17.758 - n_{\mathrm{w}}\, 21.626$ kJ mol$^{-1}$ ($R^2 = 0.9927$).

**Table 6** Hydration numbers $n_w$ for micellization

| Surfactant | Type | $\Delta C_{p,app}$/J K$^{-1}$ mol$^{-1}$ | $n_w$ | $\Delta H_0$/kJ mol$^{-1}$ | Ref. |
|---|---|---|---|---|---|
| $C_{14}H_{29}OSO_3Na$ | Anionic | −33.5 | −4.5 | 98.6 | 52 |
| | Anionic | −603 | −8.0 | 178.2 | 53 |
| $CH_3(CHSO_3)C_{12}H_{25}Na$ | Anionic | −343 | −4.6 | 103.0 | 52 |
| $C_3H_7(CHOSO_3)C_{10}H_{21}Na$ | Anionic | −351 | −4.7 | 107.1 | 52 |
| $C_{12}H_{25}OSO_3Na$ | Anionic | −527 | −7.0 | 155.5 | 54 |
| | Anionic | −457 | −6.0 | 142.1 | 55 |
| | Anionic | −476 | −6.3 | 140.9 | 56 |
| | Anionic | −315 | −4.2 | 94.3 | 52 |
| | Anionic | −283 | −6.6 | 85.8 | 57 |
| | Anionic | −496 | −3.8 | 148.1 | 59 |
| $C_{12}$NpyrI | Cationic | −390 | −5.2 | 103.6 | 58 |
| $C_{12}NPyrOCH_3Cl$ | Cationic | −446 | −5.9 | 134.8 | 58 |
| $C_{12}OPyrNCH_3Br$ | Cationic | −594 | −7.9 | 170.1 | 58 |
| $C_{14}NPyrOCH_3Br$ | Cationic | −581 | −7.7 | 162.7 | 58 |
| $C_9$NaCl | Cationic | −299 | −4.0 | 91.6 | 59 |
| $C_{10}$NaCl | Cationic | −385 | −5.1 | 116.4 | 59 |
| $C_{10}N(CH_3)_3Br$ | Cationic | −311 | −4.1 | 93.1 | 60 |
| $C_{12}N(CH_3)_3Br$ | Cationic | −1437 | −19.1 | 421.5 | 61 |
| | Cationic | −1270 | −16.0 | 355.0 | 61 |
| $C_{14}N(CH_3)_3Br$ | Cationic | −607 | −8.0 | 177.4 | 61 |
| $C_{16}N(CH_3)_3Br$ | Cationic | −573 | −7.6 | 161.2 | 60 |
| | Cationic | −352 | −4.7 | 93.5 | 62 |

separated cages coalesce and give rise to a resulting cage with a cavity smaller than the sum of the two original ones. The excess empty volume is then occupied by a convenient number of water molecules ($n_w < 0$) that transform from a relaxed $(W)_{III}$ to a structured $(W_l)_I$ state. The contraction of the cavity is accompanied by a positive change of entropy ($\Delta S_{fill}$ = 22.8 J K$^{-1}$ mol$^{-1}$ $|n_w|^{-1}$). This entropy change for filling the cavity can be calculated from the data of Cox *et al.*[63] for the protonation equilibria of alkylammonium bases. By plotting $\Delta S^\circ$ against $\Delta C_p/C_{p,w} = n_w$, we obtain

$$\Delta S^\circ = +4.67 - 22.78\ n_w \text{ J K}^{-1} \text{ mol}^{-1} \quad (20)$$

which, by considering that $n_w < 0$, can be rewritten in general form as

$$\Delta S^\circ = \Delta S_0^{(w=0)} + \Delta s_{fill}\ |n_w| \text{ J K}^{-1} \text{ mol}^{-1} \quad (21)$$

At the same time, $S/H$ compensation takes place between the heat expended by water molecules to enter the structure and the kinetic energy lost by the same water molecules.

The negative value of $n_w$ is confirmed by analysing $\Delta C_p$ for micelle formation in the plot of $C_p$ *versus* molality for surfactants. The change of $C_p$ at c.m.c. at 298 K for decyltrimethylammonium bromide[64] is negative and corresponds to negative $\Delta n_w$.

$$\Delta C_p/75.38 = \Delta n_w = -4.9 \quad (22)$$

The value of $\Delta n_w$ depends on the size of the chain, as shown by nonyltrimethylammonium bromide[65] for which we calculate $\Delta n_w = -3.5$.

Even the change in partial volume observed at c.m.c. is in accordance with the proposed mechanism. In fact, in sodium decanoate[66] at c.m.c. at different temperatures a positive change of volume is observed, which agrees with the proposed restructuring of $n_w$ ($n_w < 0$) water molecules as structure $(W_l)_I$. The connection between restructuring of water ($n_w < 0$) and increase of partial volume is confirmed by observing that in decyltrimethylammonium bromide[66] by changing the temperature from 324.6 to 374.6 K the variation of $C_p$ becomes positive ($n_w > 0$) but the change of partial volume[67] becomes negative.

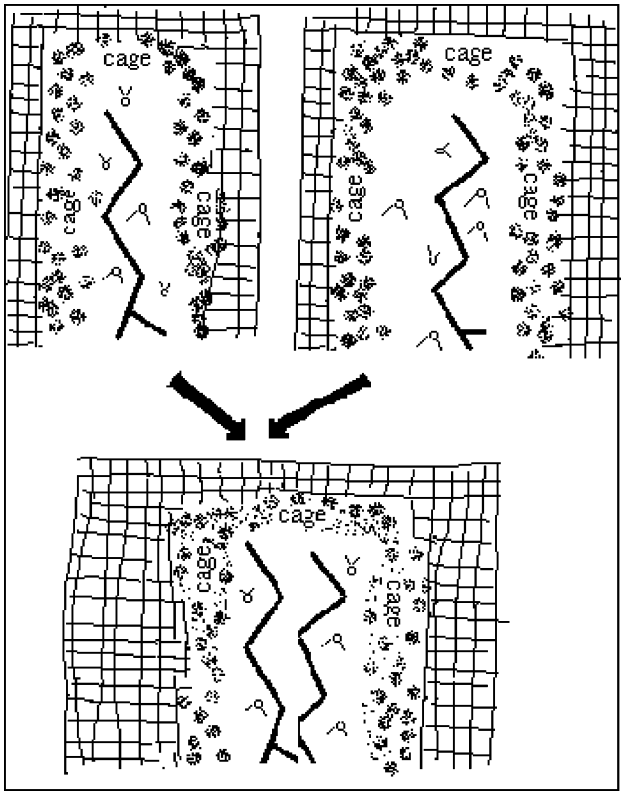


**Fig. 7** Micelle formation. The resulting section of the wall and the volume inside the cage are smaller than the sum of the component parts. The number of relaxed water molecules also decreases ($n_w < 0$) because some of them readopt structure $W_I$ (low density) to occupy the voids.

## Protein complexes

A mechanism analogous to that of micelle formation can be invoked to explain the mechanism of folding in proteins or of association between protein and substrate or inhibitor. The folding of proteins consists of the association between chains, which are surrounded by both cages and relaxed water molecules. When unfolded, each chain of the protein, surrounded by relaxed water molecules, occupies a cage in the solvent.

 

After the association of two chains, the volume of the resulting cage is smaller than the sum of the previous separated cages. The same holds for the section of the wall of the cage. Consequently, some water molecules ($n_w < 0$) need to be restructured in the bulk of the solvent. Other polar or inductive factors and related solute–solvent interactions, however, are also effective, in agreement with the 'hydrophobic' character of the substituents. This type of bonding, occurs even in some protein–substrate associations. The size and number of chains involved in bonding, however, are larger in protein association than in micelle formation. In fact, Frisch *et al.*,[68,69] studied the thermodynamics of the interaction between the ribonuclease barnase and its natural inhibitor barnstar by several different combinations of mutations in the interface. From the values of $\Delta C_p$ obtained by them, values of $n_w$ from −19 to −23 can be calculated. Knapp *et al.*[70] confirms this behavior for other compounds. These authors have studied the affinity of binding of TCF4 of different lengths with β-catenin armadillo. They found that $\Delta C_p = -6.276$ kJ K$^{-1}$ mol$^{-1}$ for the combinations of β-catenin armadillo with constructs TCF4(1-53) and TCF4(1-56) what corresponds to an average $n_w = -83.2$. We have calculated the values for the two combinations considered separately and found for TCF4(1-53) $n_w = -82.4$ and for TCF4(1-56) $n_w = -90.1$, which is consistent with the different lengths of the complexing agents. Even DNA interactions conform to this mechanism. The binding enthalpy of chartreusin to DNA is linearly changing with temperature[71] with slope $\Delta C_p = -1.636$ kJ K$^{-1}$ mol$^{-1}$, from which we calculate $n_w = -21.7$. Analogously,[72] the uncharged multivalent intercalator actinomycin D shows $n_w = -20.8$. The observed $n_w$'s are clearly higher than those for other intercalators such as ethidium bromide for which we calculate $n_w = -7.7$, in accordance with the smaller size of this molecule. Not very different from this is $n_w = -8.9$ for the charged intercalator daunorubicin. Ha *et al.*[73] and others[74–76] have determined the stability constants at different temperatures of other site-specific protein–DNA complexes and have again found negative values of $\Delta C_p$ whereby we calculate negative values of $n_w$. Values of $\Delta C_p$, $n_w$, and $\Delta H_0$ for all these complexes are reported in Table 7.

The values of the extrapolated enthalpy $\Delta H_0$ for every surfactant of Table 6 and every complex of Table 7 against $|n_w|$ give a straight line ($R^2 = 0.9985$)

$$\Delta H_0 = \Delta H_0^{(w=0)} + |n_w|\Delta h_w = +7.07 + |n_w|21.40 \text{ kJ mol}^{-1} \quad (23)$$

thus indicating that the extrapolated enthalpy $\Delta H_0$ is proportional to $n_w$ and that the value $\Delta h_w = 21.40$ kJ mol$^{-1}$ per water molecule is practically equal, with a change of sign, to the heat released ($\Delta h_w = -21.6$ kJ mol$^{-1}$ per water molecule) in the opposite solubilisation or denaturation process.

In the plot of the complexation constant for protein complexes $\log K$ against $1/T$ we observe a maximum $\log K_{max} = \{-\Delta G/RT\}_{max}$ at which $\{-\Delta H_{app}/RT\} = 0$. In analogy with eqn. (15) we can calculate $\{-\Delta G/T\}_{max} = \{\Delta S\}_{max}$. We find that the values of the entropy change are highly positive. For example, we calculate for the complex of β-catenine with TCF4(1-53)[70] $\{\Delta S\}_{max} = 162$ J K$^{-1}$ mol$^{-1}$, for the complex of *lac repr* with DNA[73] $\{\Delta S\}_{max} = +213$ J K$^{-1}$ mol$^{-1}$ and for the complex *ecoRI* with DNA $\{\Delta S\}_{max} = +200$ J K$^{-1}$ mol$^{-1}$. These high entropy values can be attributed to the dilution effect (increase of configurational entropy) accompanying the binding of the ligand to the receptor to form a unique molecular unit. We recall here the point that a formation constant for a simple complex AB is expressed in {concentration}$^{-1}$ units and hence in dilution units and consequently $\exp(S/R)$ probability units.

## Hydrophobic bonding

The complexation reactions of proteins, as well as micellisation, can be considered as examples of the so-called 'hydrophobic bonding'. The present interpretation of the complexation mechanism leads to a new formulation of it. This bond is generally believed to take place between two aliphatic or aromatic apolar moieties in water with expulsion of water molecules from the contact interface. These disordered water molecules should render the bonding entropy-driven in the sense that the dominant effect in the free energy change is the highly positive contribution from the disordered water molecules. According to our model, the hydrophobic bonding between apolar moieties is again entropy-driven but the positive configurational entropy contribution arises from the contraction of the cavity and from the dilution of the solute due to the bonding. Moreover, the free energy is the result of $S/H$ compensation, whereby most of the energy expended ($\Delta H > 0$) by water molecules to enter the structure is supplied by the kinetic energy lost ($T\Delta S_w = Tn_wC_{p,w} < 0$) by the same restructured water molecules.

**Table 7** Hydration numbers for protein complexation[a]

| Compound | Ligand | $\Delta C_p$/J K$^{-1}$ mol$^{-1}$ | $n_w$ | $\Delta H_0$/kJ mol$^{-1}$ | Ref. |
|---|---|---|---|---|---|
| β-caten[b] | TCF4-53 | −6211 | −82.4 | 1734 | 70 |
| β-caten | TCF4-56 | −6795 | −90.1 | 1909 | 70 |
| b*[c] | bn[c] | −1519 | −20.1 | 371 | 67 |
| b* | bn | −1552 | −20.6 | 382 | 67 |
| b* | bn(73Q) | −1678 | −22.3 | 459 | 67 |
| b* | bn(73W) | −1728 | −22.9 | 480 | 67 |
| b* | bn(73A) | −1636 | −21.7 | 459 | 67 |
| b* | bn(73F) | −1439 | −19.1 | 392 | 67 |
| DNA | chartr[d] | −1616 | −21.4 | 443 | 71 |
| GAATTC[e] | Eco-RI[f] | −6276 | −83.3 | 1880 | 73 |
| O$^{symg}$ | lac repr[g] | −5439 | −72.2 | 1596 | 73 |
| O+[h] | lacreI | −3766 | −49.9 | 1107 | 74 |
| Omnt[h] | Mnt rep | −14226 | −188.7 | 4065 | 75 |
| lPr pro[i] | E$\sigma$Pol[i] | −10042 | −133.2 | 3165 | 76 |

[a] $\Delta H_0 = \Delta H_0^{(w=0)} + (-n_w)\,\Delta h_0 = -39.52 + (-n_w)\,22.402$ kJ mol$^{-1}$ ($R^2 = 0.9953$) cumulative with $\Delta H_0$ of micelles of Table 6: $\Delta H_0 = \Delta H_0^{(w=0)} + (-n_w)\,\Delta h_w = +7.07 + (-n_w)\,21.40$ kJ mol$^{-1}$ ($R^2 = 0.9985$). [b] β-catenin = member of armadillo family, TCF4-53 = member of high mobility group protein. [c] Barnase (bn) = extracellular ribonuclease, barnstar (b*) = intracellular inhibitor. [d] Chartreusin = antitumor antibiotic that contains the chromophore chartarin. [e] Site sequence on DNA is –GAATTC–. [f] *Eco*RI endonuclease. [g] *lac* = *E. coli* lactose receptor, O$^{sym}$ = Plasmid pAS8-1 with symmetric operator O$^{sym}$. [h] O+, Mnt rep = P22 Mnt repressor with its operator site $O_{Mnt}$, *lacre*I = wild type *lac* operator. [i] E$\sigma$Pol$^b$ = E$\sigma^{70}$ RNAPolymerase, DNA site is $\lambda P_r$promoter.

On the ground of eqn. (12), (14) and (23) we can calculate the free energy change for a hydrophobic bond

$$\begin{aligned}\Delta G_{\text{hyphob}} =& \Delta H_{\text{app}} - T\Delta S_{\text{app}} \\ =& (\Delta H_0^{(w=0)} - n_{\text{w}}\Delta h_{\text{w}} + n_{\text{w}} C_{\text{p,w}} T) \\ & - T(\Delta S_0^{(w=0)} - n_{\text{w}}\Delta s_{\text{fill}})\end{aligned} \quad (24)$$

By introducing the numerical values of the coefficients, we obtain (remember that $n_{\text{w}} < 0$)

$$\Delta H_{\text{app}} = 4720 - 21400\ n_{\text{w}} + 75.36\ n_{\text{w}} T\ [\text{J mol}^{-1}] \quad (25)$$

and

$$T\Delta S_{\text{app}} = T\ (5.38\ -22.4\ n_{\text{w}})\ [\text{J mol}^{-1}] \quad (26)$$

The free energy $\Delta G_{\text{hyphob}}$ refers to one monomeric unit of the micelle and not to the whole micelle that is composed of several monomers. The free energy can be calculated at 298 K, for $n_{\text{w}} = -2$, as $\Delta G_{\text{hyphob}} = -12.3$ kJ (mol monomer)$^{-1}$ and for $n_{\text{w}} = -4$ as $\Delta G_{\text{hyphob}} = -27.8$ kJ (mol monomer)$^{-1}$. This means that the strength of the hydrophobic bond is of the same order of magnitude as that of a hydrogen bond and that the hydrophobic bond becomes stronger as the length of the chain increases. Another peculiarity of this bond is that from the eqn. (24) we can calculate the temperature at which the free energy becomes null (cold denaturation) which are $T_{\text{cold}} = 236$ K for $n_{\text{w}} = -2$ and $T_{\text{cold}} = 227$ K for $n_{\text{w}} = -4$, respectively. If we assume that the hydrophobic bond is in competition with a hydrogen bond with $\Delta G_{\text{HB}} = -20$ kJ (mol monomer)$^{-1}$, we obtain $T_{\text{cold}} = 336$ K for $n_{\text{w}} = -2$ and $T_{\text{cold}} = 278$ K for $n_{\text{w}} = -4$, respectively. These results seem to suggest that at ambient temperature the micelle formed by a short anphiphilic molecule is unstable and that formed by a long one is stable.

## Denaturation

The denaturation process of proteins shows denaturation enthalpies $\Delta H_{\text{app}}$, which, against the denaturation temperature, produce linear plots with a positive slope. This means that $\Delta C_{\text{p,app}}$ depends on a positive number $n_{\text{w}}$ of water molecules. In fact, by integration[22] of the apparent isobaric heat capacity $\Delta C_{\text{p,app}}$ of the protein during thermal denaturation we obtain the denaturation heat $\Delta Q_{\text{den}}$ as

$$\Delta Q_{\text{den}} = \Delta H + n_{\text{w}} T_{\text{den}} C_{\text{p,w}} \quad (27)$$

with $T_{\text{den}} = (1/2)(T_1 + T_2)$. This shows that the denaturation heat is the balance of two contributions, one of which is linearly dependent on the temperature. The denaturation enthalpy has been found in any case to be linearly dependent on the temperature.[77,78] This is equivalent to saying that $\Delta C_{\text{p,app}}$ is large and positive,[79] which is the characteristic signature of hydrophobic interactions.

A set of values of $n_{\text{w}}$ calculated for the denaturation of some proteins are reported in Table 5. Values of $n_{\text{w}}$ obtained in protein denaturation are much higher than those found in noble gases and simple small molecules and depend on the size of the protein, thus confirming the existence of a relationship between solute size and $n_{\text{w}}$. For instance, the number of water molecules for different types of lysozyme changes from $n_{\text{w}} = 88.9$ for HEW, with molecular weight 14 100 Da, to $n_{\text{w}} = 122.0$ for T4 wild type, with molecular weight 18 700 Da.

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 alanine group for threonine and histidine for arginine produces higher hydrophobicity. Consequently values $n_{\text{w}} = 131.4$ and 139.8, respectively, are found, comparable with $n_{\text{w}} = 122.0$ mentioned above for T4wild type. In general, the size of the molecule is the main factor determining the number of water molecules, but even the protonation state of the protein can produce changes in the cage by examining the effect of charge, as shown below in the following chapter. All these data are consistent with a picture of the process whereby the inserted unfolded chain is floating within a cage of structured dense water, with $n_{\text{w}}$ water molecules relaxed inside the cage (Fig. 8). The volume of the cage and the total cross section of its wall are larger than those surrounding the folded state, where the chains were facing each other. The number of floating water molecules $n_{\text{w}}$ is positive ($n_{\text{w}} > 0$) upon unfolding.

## Polar compounds and substituents

The expression of eqn. (12) with $n_{\text{w}} C_{\text{p,w}} \neq 0$ is equivalent to stating that $\Delta C_{\text{p}} \neq 0$. In fact, Cabani *et al.*[38,83] reported a long list of hydrophobic compounds for which $\Delta C_{\text{p}} > 0$. The values of $\Delta C_{\text{p}}$ can be transformed into values of $n_{\text{w}}$ by division by $C_{\text{p,w}}$. Cabani *et al.*[38,83] searched for a composition rule of $\Delta C_{\text{p}}$. He then proposed a formula

$$\Delta C_{\text{p}} = A_{\text{Z}} + \textstyle\sum_j n_j B_{\text{Z}}(j) \quad (28)$$

where $A_{\text{Z}}$ is a general extrapolation constant. He attributed to each $j$ group or moiety of a molecule a typical constant contribution $B_{\text{Z}}(j)$, and $n_j$ is the number of times the $j$th group appears in the molecule. Translated into the language of floating water molecules, this corresponds to the assigning of a $\Delta n_{\text{w}}(j) = B_{\text{Z}(j)}/C_{\text{p,w}}$ value to each substituent. The relationship (28) works well for monofunctional compounds. For polyfunctional compounds a correction term $+C_{\text{Z}}$ ($Y_1$, $Y_2 \ldots Y_m$) is introduced in eqn. (28), with ($Y_1$, $Y_2, \ldots Y_m$) indicating the substituents. With hydrophilic substituent, even the correction terms fail to reproduce the experimental data. For monofunctional compounds, however, it is possible to calculate the value of $\Delta C_{\text{p}}$. Cabani divided the $B_{\text{Z}}$ coefficients into two categories, one for non-polar groups with positive coefficients and another for polar groups or components with

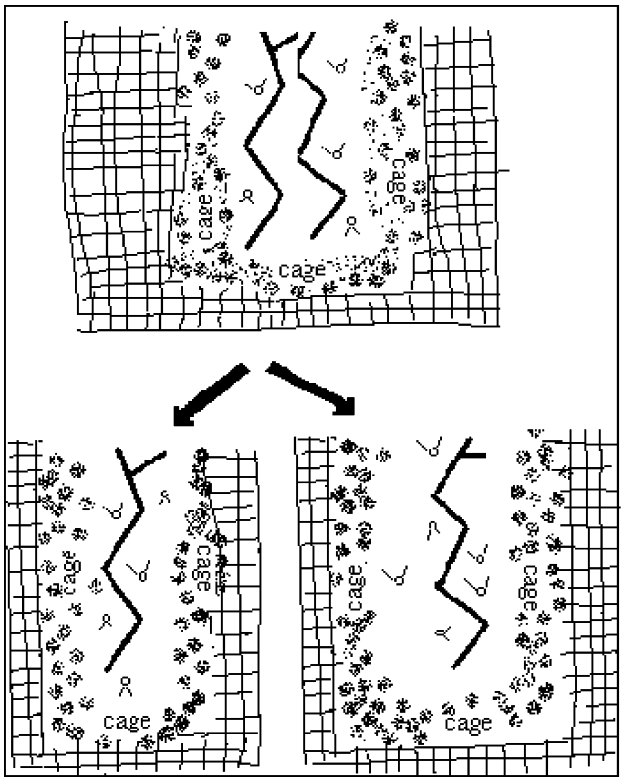


**Fig. 8** Effect of unfolding on cage volume and relaxed water molecules ($n_{\text{w}} > 0$). The effect is analogous to that occurring in the dissolution of apolar substances.

**Table 8** Group contributions $A_Z$ (J K$^{-1}$ mol$^{-1}$) and $B_Z$ ($=\Delta\Delta C_p$) (J K$^{-1}$ mol$^{-1}$) transformed into $\Delta n_w$[a]

| Group | $B_Z$ | $\Delta n_w$ | Group | $B_Z$ | $\Delta n_w$ |
|---|---|---|---|---|---|
| $CH_3$ | 66.4 | 0.881 | $NH_2$ | −75.5 | −1.002 |
| $CH_2$ | 64.5 | 0.856 | NH | −7.7 | −0.102 |
| CH | 67.8 | 0.899 | N | −39.0 | −0.517 |
| C | 44.7 | 0.593 | $N_{ar}$ | −12.0 | −0.159 |
| C=C | 100.8 | 1.337 | O | −62.6 | −0.830 |
| C=–C | 44.3 | 0.588 | OH | −44.4 | −0.589 |
| $CH_2$ | 47.3 | 0.628 | SO | −152.0 | −2.017 |
| $CH_{ar}$ | 26.0 | 0.345 | F | −30.3 | −0.402 |
| $C_{ar}$ | 0.0 | 0.000 | Cl | 2.0 | 0.027 |
| $C_{cond}$ | 0.1 | 0.001 | Br | 18.4 | 0.244 |
| CHO | −91.0 | −1.207 | I | 235.2 | 3.120 |
| CO | −79.5 | −1.055 | NHCONH | −81.0 | −1.075 |
| COO | −37.0 | −0.491 | $NH_2CON$ | −99.9 | −1.325 |
| COOH | −84.7 | −1.124 | NCON | −88.4 | −1.173 |
| $CONH_2$ | −84.6 | −1.122 | OH(Φ) | −32.3 | −0.429 |
| CONH | −87.4 | −1.159 | | $A_Z$ | $\Delta n_w$ |
| CON | −112.4 | −1.491 | — | 115.9 | 1,538 |

[a] Values from Ref. 45.

negative coefficients. The coefficients can be transformed into values of $\Delta n_w$ (Table 8). The positive coefficients for non-polar groups, corresponding to values of $\Delta n_w > 0$, are indicative of the same effect (*i.e.*, formation of cavity and cage with floating water molecules) on the surrounding solvent as the compounds of Table 1. The negative coefficients of polar groups, however, transformed into values of $\Delta n_w < 0$, indicate that the presence of polar groups in the molecule exerts on the water molecules floating in the cage the same transformation as that produced by micellisation. In the latter case, the contraction of the cage when two hydrophobic chains become close to each other generates the need to occupy the void by some restructured water molecules. In the former case, the electrostatic field causes contraction of the cage section and freezing of the floating water molecules in structured solvent (Fig. 9). It is worth noting that the passage from fluoro-substitution to chloro-, bromo-, or iodo-substitution produces a change from $\Delta n_w = -0.4$ for F, to $\Delta n_w = +0.03$ for Cl, to $\Delta n_w = +0.24$ for Br and $\Delta n_w = +3.12$ for I. This fact underlines the effect of solute polarity on the structure of the solvent. The strong-electronegative fluorine promotes the condensation of water molecules whereas the weak-electronegative iodine facilitates the relaxation. Substitution of a polar moiety for part of a hydrocarbon molecule of the same size yields shrinkage of the volume of the cage. This shrinkage can be particularly noticeable as for amide and peptide groups, which is about 10 cm$^3$ mol$^{-1}$ (Cabani[83]). It is not possible to calculate the value of the extrapolated enthalpy $\Delta H_0$ from these data

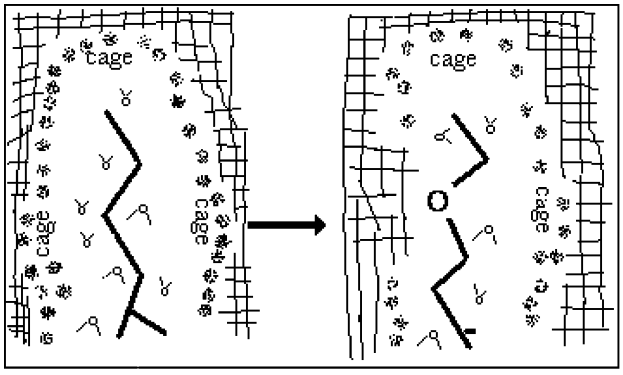


**Fig. 9** Effect of polar group on cage section and relaxed water molecules ($n_w < 0$).

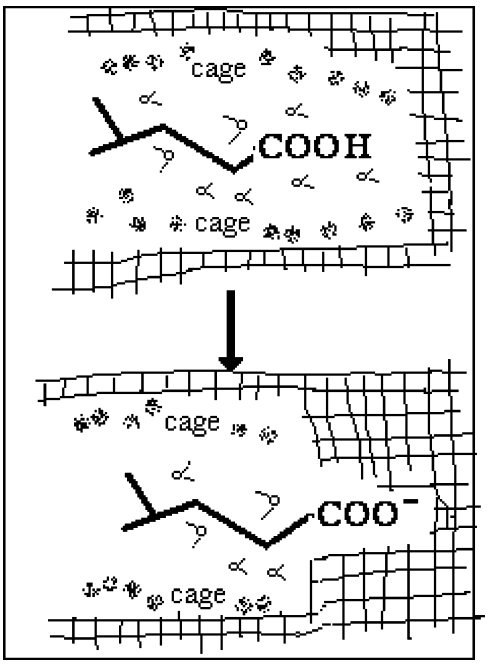


**Fig. 10** Effect of charge on cage section and relaxed water molecules ($n_w < 0$).

because the values of $\Delta H_{app}$ are not known. The extrapolated enthalpy, however, can be calculated from the data reported by Guinto and Di Cera[84] for the binding constant of $Na^+$ to thrombin. Binding of $Na^+$ to thrombin is characterized by a modest dependence upon ionic strength and a negative heat capacity change of $-4.60 \pm 0.4$ kJ K$^{-1}$ mol$^{-1}$. This corresponds to $n_w = -61.0$ water molecules. The authors, in fact, propose that this change be linked to burial of a large cluster of water molecules in the $Na^+$ binding pocket upon $Na^+$ binding.

This mechanism of water blockage by charge is confirmed by the analysis of the protonation constants of mono-carboxylic acids by changing the temperature.[16] The reaction $R–COO^- + H_3O^+ \rightarrow RCOOH + H_2O$, which, in contrast to that of thrombin, consists of a removal of charge and the diminution of electrostatic field, presents $n_w = 2.1$ in many acids. Protonation of the carboxylato anion neutralises the polarity and lets 2.1 water molecules float freely around the solute. In contrast, the deprotonation of RCOOH with the consequent introduction of charge produces an effect analogous to that of the polar group with $n_w = -2.1$ (Fig. 10).

## Conclusions

The analysis of the solubilisation of apolar substances in water has offered an example of molecular mechanism with entropy/enthalpy compensation. The heat released at the very moment of solubilisation is absorbed by a certain number of water molecules that become free to float. The relaxed water molecules leave a cavity which is occupied by the solute with contraction. The formation of the cavity implies a negative entropy change. The same type of mechanism can be transferred to interpret the denaturation process of proteins. Moreover, a process just opposite to this, whereby the floating water molecules are trapped in the structure of water by the loss of kinetic energy with the release of heat, can be suggested to interpret the formation of micelles. When a micelle is formed, the two chains that come close to each other produce the disappearance of part of the cavity. The contraction of the cavity implies a positive change of entropy. The void corresponding to the diminution of the cavity is occupied by some restructuring water molecules. This same mechanism takes place in the association of proteins with substrate or inhibitor. Micelle formation and association of proteins with

substrate or inhibitor are examples of entropy-driven hydrophobic bonding.

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## References

1 M. Costa, B. Kronberg and R. Silveston, *J. Chem. Soc., Faraday Trans.*, 1994, **90**, 1513.
2 R. L. Mancera, B. Kronberg, M. Costa and R. Silveston, *Biophys. Chem.*, 1998, **70**, 57.
3 P. L. Privalov and S. J. Gill, *Adv. Protein Chem.*, 1998, **39**, 191.
4 P. L. Privalov and S. J. Gill, *Pure Appl. Chem.*, 1989, **61**, 1097.
5 C. Tanford, *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Wiley, New York, 1973.
6 A. Ben-Naim, *Solvation Thermodynamics*, Plenum, New York, 1987.
7 L. K. Daggett, *J. Chem. Phys.*, 1996, **100**, 5616.
8 B. Lee and G. Graziano, *J. Am. Chem. Soc.*, 1996, **118**, 5163.
9 Y. P. Pertsemlidis, A. Saxena, A. Soper, T. Head-Gordon and R. Glaser, *Proc. Natl. Acad. Sci. USA*, 1996, **93**, 10769.
10 A. Ben-Naim, *J. Chem. Phys.*, 1978, **82**, 792.
11 N. Muller, *J. Solution Chem.*, 1988, **17**, 661.
12 S. Cabani, *Thermodynamics of Aqueous Dilute Solutions of Non-charged Molecules*, in *Advances in Solution Chemistry*, ed. I. Bertini, L. Lunazzi and A. Dei, Plenum, Amsterdam, 1981.
13 B. Guillot and Y. Giussani, *J. Chem. Phys.*, 1993, **99**, 8075.
14 B. Lee, *Biophys. Chem.*, 1994, **51**, 271.
15 B. Madan and B. Lee, *Biophys. Chem.*, 1994, **51**, 279.
16 A. Braibanti, E. Fisicaro, F. Dallavalle, J. D. Lamb, J. L. Oscarson and F. X. Ughi, *J. Phys. Chem.*, 1993, **97**, 8071.
17 A. Braibanti, E. Fisicaro and C. Compari, *Thermochim. Acta*, 1998, **320**, 253.
18 A. Braibanti, E. Fisicaro and C. Compari, *Thermochim. Acta*, 1998, **320**, 265.
19 A. Braibanti, E. Fisicaro and C. Compari, *Thermochim. Acta*, 1998, **320**, 101.
20 A. Braibanti, E. Fisicaro and C. Compari, *Thermochim. Acta*, 1998, **320**, 277.
21 A. Braibanti, E. Fisicaro and C. Compari, *J. Phys. Chem. B*, 1998, **102**, 8537.
22 A. Braibanti, E. Fisicaro and C. Compari, *Phys. Chem. Chem. Phys.*, 2000, **2**, 4870.
23 R. Schmid, *Monatsh. Chem.*, 2001, **132**, 1295.
24 K. Soda, *Adv. Biophys.*, 1993, **29**, 1.
25 G. E. Walrafen, *J. Chem. Phys.*, 1968, **42**, 244.
26 G. E. Walrafen, M. R. Fisher, M. S. Hokmabady and W.-H. Yang, *J. Chem. Phys.*, 1986, **85**, 6970.
27 F. Franks, *Water*, The Royal Society of Chemistry, London, 1983, p. 45.
28 N. Matubayasi, L. H. Reed and R. M. Levy, *J. Phys. Chem.*, 1994, **98**, 10640.
29 A. Braibanti, E. Fisicaro, A. Ghiozzi and C. Compari, *Thermochim. Acta*, 1996, **51**, 286.
30 E. Wilhelm, R. Battino and R. J. Wilcock, *Chem. Rev.*, 1974, **77**, 219.
31 G. Olofsson, A. A. Oshodi, E. Qvarnström and I. Wadsö, *J. Chem. Thermodyn.*, 1984, **16**, 1041.
32 H. Naghibi, F. F. Dec and S. J. Gill, *J. Phys. Chem.*, 1986, **90**, 4621.
33 H. Naghibi, F. F. Dec and S. J. Gill, *J. Phys. Chem.*, 1987, **91**, 245.
34 A. Ben-Naim, J. Wilf and H. Yaacobi, *J. Phys. Chem.*, 1973, **77**, 95.
35 A. Muccitelli, *J. Solution Chem.*, 1979, **3**, 225.
36 P. Scharlin and R. Battino, *J. Solution Chem.*, 1992, **21**, 675.
37 A. Cooper, C. M. Johnson, J. H. Lakey and M. Nöllmann, *Biophys. Chem.*, 2001, **93**, 215.
38 S. Cabani, G. Conti, A. Martinelli and E. Matteoli, *J. Chem. Soc., Faraday Trans. 1*, 1973, **69**, 2112.
39 A. Braibanti, E. Fisicaro and C. Compari, *J. Therm. Anal. Calorim.*, 2000, **61**, 461.
40 B. Madan and K. Sharp, *J. Phys. Chem. B*, 1996, **100**, 7713.
41 K. Sharp and B. Madan, *J. Phys. Chem. B*, 1997, **101**, 4343.
42 G. Graziano and G. Barone, *J. Am. Chem. Soc.*, 1996, **118**, 1831.
43 S. Cabani, P. Gianni, V. Mollica and L. Lepori, *J. Solution Chem.*, 1981, **10**, 563.
44 S. J. Gill, S. F. Nichols and I. Wadsö, *J. Chem. Thermodynamics*, 1975, **7**, 175.
45 S. J. Gill, S. F. Nichols and I. Wadsö, *J. Chem. Thermodynamics*, 1976, **8**, 445.
46 D. P. Kharakoz, *J. Solution Chem.*, 1992, **21**, 569.
47 D. P. Kharakoz, *Biophys. Chem.*, 1989, **34**, 115.
48 K. P. Murphy, P. L. Privalov and S. J. Gill, *Science*, 1990, **247**, 559.
49 M. H. Abraham and E. Matteoli, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 1985.
50 G. Graziano, *J. Phys. Chem. B*, 2001, **105**, 2079.
51 M. E. Paulaitis, *Curr. Opin. Colloid Interface Sci.*, 1997, **2**, 315.
52 B. D. Flockhart, *J. Colloid Sci.*, 1961, **16**, 484.
53 Y. Moroi, N. Nishikido, H. Uehara and R. Matuura, *J. Colloid Interface Sci.*, 1975, **50**, 254.
54 G. C. Kresheck and W. Hargraves, *J. Colloid Interface Sci.*, 1974, **48**, 481.
55 V. K. Swarma, R. Bhat and J. C. Ahluwalia, *J. Colloid Interface Sci.*, 1987, **115**, 396.
56 E. M. Woolley and T. E. Burchfield, *J. Phys. Chem.*, 1984, **88**, 2155.
57 E. D. Goddard and G. C. Benson, *Can. J. Chem.*, 1957, **35**, 986.
58 P. Stenius, S. Backlund and P. Ekwall, in *Thermochimica Transport Properties of Organic Salts*, ed. P. Franzosini and M. Sanesi, Pergamon Press,Oxford, 1980.
59 E. Fisicaro, M. Barbieri, E. Pelizzetti and P. Savarino, *J. Chem. Soc., Faraday Trans.*, 1991, **87**, 2983.
60 M. T. Bashford and E. M. Woolley, *J. Phys. Chem.*, 1985, **89**, 3173.
61 B. W. Barry and G. F. J. Russel, *J. Colloid Interface Sci.*, 1972, **40**, 174.
62 S. Bergström and G. Olofsson, *Thermochim. Acta*, 1986, **109**, 155.
63 M. C. Cox, D. H. Everett, D. A. Landsman and R. J. Munn, *J. Chem. Soc. B*, 1968, 1373.
64 R. De Lisi, C. Ostiguy, G. Perron and J. E. Desnoyers, *J. Colloid Interface Sci.*, 1979, **71**, 147.
65 R. De Lisi, G. Perron and J. E. Desnoyers, *Can. J. Chem.*, 1980, **58**, 959.
66 D. G. Archer, *J. Chem. Thermodyn.*, 1987, **19**, 407.
67 D. G. Archer, V. Majer, A. Inglese and R. H. Wood, *J. Colloid Interface Sci.*, 1988, **124**, 591.
68 C. Frisch, G. Schreiber, C. M. Johnson and A. R. Fersht, *J. Mol. Biol.*, 1997, **267**, 696.
69 A. Cooper, in *Microcalorimetry of protein–protein interactions*, ed. J. E. Ladbury and B. Z. Chowdhry, *Biocalorimetry: The Application of Calorimetry in the Biological Sciences*, Wiley, New York, 1998, pp. 103–111.
70 S. Knapp, M. Zamai, D. Volpi, V. Nardese, N. Avanzi, J. Breton, S. Plyte, M. Fiocco, M. Marconi, A. Isacchi and V. R. Caiolfa, *J. Mol. Biol.*, 2001, **306**, 1179.
71 F. Barceló, D. Capó and J. Portugal, *Nucleic Acids Res.*, 2002, **30**, 4567.
72 J. Ren, T. C. Jenkins and J. B. Chaires, *Biochemistry*, 2000, **39**, 8439.
73 J.-H. Ha, R. S. Spolar and M. T. Record Jr., *J. Mol. Biol.*, 1989, **209**, 801.
74 P. A. Whitson, J. S. Olson and K. S. Metthews, *Biochemistry*, 1986, **25**, 3852.
75 A. K. Vershon, S. M. Liao, W. R. McClure and R. T. Sauer, *J. Mol. Biol.*, 1987, **195**, 311.
76 J. H. Roe, R. R. Burgess and M. T. Record Jr., *J. Mol. Biol.*, 1985, **184**, 441.
77 A. Braibanti and E. Fisicaro, *Thermochim. Acta*, 1994, **241**, 131.
78 P. L. Privalov, *Pure Appl. Chem.*, 1976, **47**, 293.
79 A. Cooper, *Biophys. Chem.*, 2000, **85**, 25.
80 N. K. D. Kella and G. E. Kinsella, *Biochem. J.*, 1998, **255**, 113.
81 N. C. Pace and C. Tanford, *Biochemistry*, 1968, **7**, 198.
82 J. F. Brandts, *J. Am. Chem Soc.*, 1964, **86**, 4291.
83 S. Cabani, *Port. Electrochim. Acta*, 1985, **3**, 5.
84 E. R. Guinto and E. Di Cera, *Biochemistry*, 1996, **35**, 8800.

 