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Hydrophobic interactions and small-angle neutron scattering in aqueous solutions

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Abstract - First hydrophobic hydration and hydrophobic interactions are defined and some of their characteristic features are described. Then the connection between macroscopic properties of solutions and molecule-molecule potential and distribution functions is discussed. Finally it will be shown on the example of the aqueous solution of tetramethylurea how the small-angle neutron scattering can be applied to studying the different aspects of hydrophobic interactions, such as their temperature and concentration dependence; solvent-separated vs. contact-pair solute-solute interaction; solute induced perturbation of the solvent structure.

INTRODUCTION

Hydrophobic effects seem to play an important role in rationalizing of a great variety of phenomena. These include low solubility of nonpolar substances, such as hydrocarbon liquids, in water, formation of micelles and biological membranes, stability and thermal denaturation of globular proteins, binding of a substrate to an enzyme etc. Although hydrophobic effects have been studied very extensively and have been the subject of an extensive literature over the past twenty years (see e.g ref. 1-8) numerous molecular details of hydrophobic effects remain to be elucidated along with a need for a better understanding of the role of hydrophobic effects in complex biochemical and biological processes. In this paper I should like to review very briefly the definition and a few features of hydrophobic effects, their connection to molecule-molecule potential and distribution functions and then the possibilities offered by the small angle neutron scattering (SANS) technique to study hydrophobic interactions.

HYDROPHOBIC EFFECTS

Hydrophobic effects are used to describe two different but interrelated phenomena: hydrophobic hydration and hydrophobic interactions.

In the first case we consider a process of transferring of a simple solute (e.g. methane) from a nonpolar liquid, such as cyclohexane, into water. If both solutions are infinitely diluted then the work required to transfer one solute molecule from a fixed position in the solvent into a fixed position in water is the standard free energy of transfer (ΔG^0_{tr}) (ref. 1). $\Delta G^0_{tr}<0$ indicates that the solute molecule prefers the aqueous environment to nonaqueous solvent, and vice versa for $\Delta G^0_{tr}>0$, thus ΔG^0_{tr} can be interpreted as the relative " phobia" of the solute molecule for the two solvents. The transfer of nonpolar solutes from organic

solvents (or from the gas phase) to water is accompanied by a very large decrease in entropy which has been rationalized by postulating that the nonpolar solute reorganizes the solvent molecules in its neighbourhood.

This process leads to a more ordered state, which can be described by various terms, such as "icebergs", "water becomes more solid-like", "flickering-clusters", "clathration shells", "structure making" etc. This effect is called hydrophobic hydration. Note that ΔG^0_{tr} gives the difference in the solute-solvent interactions between the two solvents.

The term hydrophobic interaction (HI) is used to describe the interaction between two (or more) nonpolar molecules in a solvent, particularly in water (in nonaqueous systems the use of the term "solvophobic interaction" would be more appropriate).

The hydrophobic interaction between pairs of simple solute molecules in a solvent can be characterized by the Gibbs free energy change during the hydrophobic interaction process (ref. 1):
Two solute molecules found at fixed positions but at infinite separation ($r_{12}=\infty$) in the solvent are brought close together, say to a distance $r_{12}=\sigma$, where σ is the molecular diameter of the solute molecules. The Gibbs free energy change during this process (T and P are kept constant) can be given as

$$\Delta G(\infty\rightarrow\sigma)=\Delta G(\sigma)=G(T,P,solvent,r_{12}=\sigma)-G(T,P,solvent,r_{12}=\infty)\,. \tag{1}$$

In other words, hydrophobic interaction is the tendency of two nonpolar solute molecules to come together in aqueous solutions.

$\Delta G(r)$ is the potential of mean force, from which the average force operating between two solute molecules in the solvent (F_{ss}) can be obtained by

$$F_{ss}(r)=-\frac{\partial\Delta G(r)}{\partial r}\,. \tag{2}$$

In general there is more than one minimum in $\Delta G(r)$, whereas the potential function which describes the interaction of two solute molecules in vacuum $(U_{ss}(r))$ has only one minimum. For example, $\Delta G(r)$ obtained for a 5 mol% aqueous solution of methanol by Monte Carlo calculation displays two minima and the second minimum corresponding to the solvent-separated association of two methanol molecules is deeper than the one related to the direct association (contact pair) of methyl groups (ref. 9).
It is convenient to divide $\Delta G(r)$ into two parts

$$\Delta G(r)=U_{ss}(r)+\delta G^{HI}(r) \tag{3}$$

where $U_{ss}(r)$ is due to the direct solute-solute interaction and $\delta G^{HI}(r)$ arises from the presence of the solvent. $U_{ss}(r)$ is independent of the type of the solvent, T and P, therefore the contribution of the solvent to the force between the two solute molecules is represented by $\delta G^{HI}(r)$.

The potential of mean force is related to the solute-solute pair correlation function $(g_{ss}(r))$ through

$$g_{ss}(r)=\exp(-\Delta G(r)/kT)\,. \tag{4}$$

For spherically symmetric molecules the function $g_{ss}(r)$ gives the relative probability of finding a second solute molecule located at the distance r from another solute molecule simultaneously located at the origin, i.e. the quantity $n_s g_{ss}(r)4\pi r^2 dr$ gives the average number of solute molecules in a spherical shell of width dr at a distance r from the centre of a given solute molecule (n_s is the number density of the solute).

SOLUTE-SOLUTE INTERACTIONS IN DILUTE SOLUTIONS

By analogy with the virial expansion of the pressure of real gases the osmotic pressure of a solution (Π) can be expanded in terms of the number densities of the solute molecules

$$\Pi/kT = n_s + Bn_s^2 + Cn_s^3 + \ldots \tag{5}$$

where B and C are the second and third osmotic virial coefficients, respectively, which measure the deviations from the ideal behaviour due to solute-solute interactions. For spherical molecules the relationships between B, g_{ss}^0 (solute-solute pair correlation function at infinite dilution) and $\Delta G(r)$ can be given as

$$B=-\frac{1}{2}\int_0^\infty [g_{ss}^0(r)-1]\,4\pi r^2 dr=-\frac{1}{2}\int_0^\infty \{\exp[-\Delta G(r)/kT]-1\}4\pi r^2 dr. \tag{6}$$

B can be determined from the results of osmotic pressure, freezing point depression and vapour pressure measurements (ref. 10). For spherical solutes with a hard-core diameter σ one can split the integral (Eq.(6)) into two terms:

$$B=-\frac{1}{2}\int_0^\sigma (-4\pi r^2)\,dr-\frac{1}{2}\int_\sigma^\infty [g_{ss}^0(r)-1]\,4\pi r^2 dr=4v_s-B_{attr} \tag{7}$$

where v_s is the volume of the solute molecule; the first term represents the contribution to B from the repulsive part of solute-solute interactions, whereas B_{attr} measures the essentially attractive region. B becomes more negative with increasing temperature (e.g. ref. 10), which indicates that the pairwise association between solute molecules increases, i.e. the hydrophobic interaction becomes more attractive at higher temperatures (ref. 5).

After this introduction into the hydrophobic effects I should like to list some of the questions and problems in this field, part of which - as will be shown below- can successfully studied by the SANS technique: how does a nonpolar solute change the structure of the solvent?; how far does the solute-induced solvent structure extend?; temperature and concentration dependence of HI; solvent-separated solute-solute interaction vs. contact pair interaction; nonadditivity of HI; which role does the unique structure of liquid water play in HI?; separation of direct solute-solute interactions from hydrophobic interactions (the last two problems could be studied by comparing solutions of the same nonpolar solute in water and nonaqueous solvents).

SMALL ANGLE NEUTRON SCATTERING (SANS)

SANS investigations on solutions of small molecules are not easy to perform as the scattering cross section of each single particle decreases with the square of its volume. Another difficulty arises from the fact that one is mainly interested in the low concentration range, where the most interesting structural changes take place and the results can be interpreted by taking into account only the interactions between pairs of solute molecules. On the other hand, the perturbation of solvent properties caused by the solute in its vicinity can be expected to be studied easier in the case of small molecules, since the contribution to the scattering pattern coming from the small particle plays a less dominant role. A further advantage which is offered by the SANS technique in solving the problems of the scattering on small molecules is the possibility of using isotope substitution in the solvent as well as in the solute (generally known as the method of contrast variation).

In the following it will be shown on the example of the aqueous solution of tetramethylurea (TMU) (ref. 11,12) how the SANS technique can be applied to studying the various problems of hydrophobic interactions.

The scattered intensity ($i(q)$) in SANS is given by (ref. 13)

$$i(q)=n_s(B_x-\rho_0 v_s)^2\langle|F(q)|^2\rangle S(q) \tag{8}$$

where n_s is the number density of the solute molecules, B_x is the sum of coherent scattering lengths taken over a particle, ρ_0 is a similar sum taken over a unit volume of the solvent, v_s is the volume of the particle, $\langle|F(q)|^2\rangle$ is the single particle form factor squared and averaged over the particle orientations; $S(q)$ is the interparticle structure factor which reflects the correlation of the mutual arrangement of particles caused by interparticle interactions in the solution and $q=4\pi\sin(\theta/2)/\lambda$ (θ: scattering angle, λ: wavelength). $S(q)$ depends on concentration and temperature; in infinitely dilute solution, where there is no solute-solute interaction, $S(q)=1$.
Equation (8) can be rewritten in a Guinier form

$$i(q)=i(0)\exp(-1/3q^2R_g^2) \tag{9}$$

with

$$i(0)=n_s(B_x-\rho_0 v_s)^2 S(0) \tag{10}$$

$$R_g^2=-3d[\ln(\langle|F(q)|^2\rangle)+\ln(S(q))]/dq^2 \tag{11}$$

where $i(0)$ is the forward scattering cross section and R_g is the apparent value of radius of gyration.
The interparticle structure factor $S(q)$ is related to the solute-solute pair correlation function by the expression (ref. 14)

$$S(q)=1+\phi/v_s\int[g_{ss}(r)-1]\sin(qr)/(qr)\,dV \tag{12}$$

where ϕ is the volume fraction of the solute.
Expanding sin(qr)/(qr) in a power series and keeping the first two terms one obtains

$$S(q)\approx 1-2\phi(B/v_s)\,[1-q^2D_{corr}^2/3]+O(q^4) \tag{13}$$

where

$$D_{corr}^2=\frac{1}{2}\int[g_{ss}(r)-1]\,r^2dV/\int[g_{ss}(r)-1]\,dV \tag{14}$$

is the measure of the mean-square distance at which the positions of two particles in the solution are correlated.

By combining eqs. (10), (11) and (13) one obtains an expression for the determination of D_{corr}

$$D_{corr}^2=i_0(0)\,dR_g^2/di(0) \tag{15}$$

where $i_0(0)$ is the value of $i(0)$ at infinite dilution.

Figure 1 shows the $i(0)/n_s$ values for solutions of TMU in heavy water as a function of concentration at different temperatures (ref. 11). It can be seen that the data at different temperatures can adequately be described by straight lines which reflect the concentration dependence of $S(0)$. Then from Eq.(13) it follows that

$$S(0)\approx 1-2\phi(B/v_s)\,. \tag{16}$$

The values for the dimensionless second virial coefficient (B/v_s) 0.93, 0.32 and ≈0 obtained at 8, 21 and 41^{0}C respectively, are rather small compared with 4 for the case of hard sphere (Eq.(7)). This indicates the presence of significant attractive solute-solute interactions in the solution, which become progressively more pronounced with increasing temperature in accord with view that hydrophobic interaction becomes more attractive at higher temperatures (ref.5).

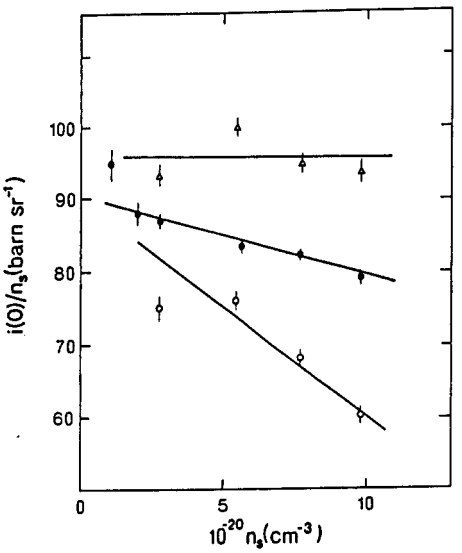

Fig. 1. Forward scattering cross -section per single molecule of TMU in heavy water ($i(0)/n_s$) vs. number density (n_s) at different temperatures: Δ 41, ● 21 and o 8°C (ref. 11,12).

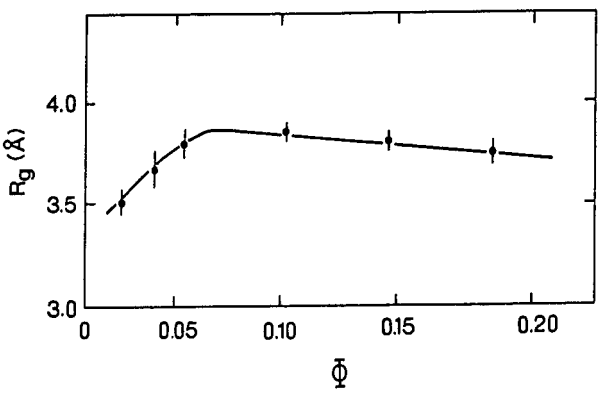

Fig. 2. Radius of gyration of TMU (R_g) vs. volume fraction of the solute (ϕ) at 21°C.

The observed R_g values for 21°C are plotted as a function of concentration (ϕ) in Fig.2. The maximum at $\phi \approx 0.07$ can be taken as a strong indication of a structural change taking place in the solution in this concentration range. One plausible explanation may be that with increasing concentration more and more contact solute pairs are formed at the expense of water-separated pairs. The elaboration of a model which would explain the linear concentration dependence of $i(0)/n_s$ and the maximum in R_g at the same time must await until more precise experimental data for the low-concentration range become available.

From the experimental data for the high-concentration range (ϕ>0.07) one obtains by using Eq.(15) a value of 4.3 Å for D_{corr}. By comparing this value of the mean-correlation distance between the solute molecules with the characteristic sizes of the TMU molecules - the equivalent radius is 3.6 Å, and the lengths of the semiaxes of the equivalent ellipsoid are 4.96, 3.8 and 2.4 Å (ref. 15) - it seems safe to conclude that contact hydrophobic association plays a significant role in this concentration range (ϕ=0.07-0.18).

The method of contrast variation (using isotope substitution) is capable of giving information on the changes brought about by the solute in the solvent structure in its neighbourhood. The results of the investigation of aqueous TMU solutions (for details see ref. 11) showed that for v_s (the volume occupied by the solute molecule in the solution) it is appropriate to use the partial molar volume of the solute (obtained from the concentration dependence of the density of the solution) in the equations used in the evaluation of SANS data (see e.g. Eqs.(8) and (10)). The analysis of the data of the contrast variation experiments can also give information on the preferred orientation of water molecules in the neighbourhood of a solute particle which can then be compared with the findings of molecular dynamics and Monte Carlo simulations.

SANS investigations on dilute aqueous and nonaqueous solutions of the same nonpolar solute would lead to the separation of the direct solute-solute interaction ($U_{ss}(r)$) from the indirect, solvent-induced HI (Eq.(3)) and thereby to information on the role which the unique structure of liquid water may play in the phenomenon of hydrophobic interaction.

CONCLUSIONS

It has been shown that the SANS technique offers some new possibilities to study the hydrophobic interactions in aqueous solutions of rather small solute molecules. The results can shed a great deal of light on the factors which influence these interactions in dilute solutions.

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