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Differences between pair and bulk hydrophobic interactions

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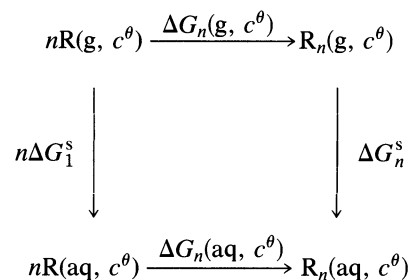
ABSTRACT It is now well known that the pair interaction between two hydrocarbon molecules in water has distinctly different properties from the bulk hydrophobic interaction familiar to the biochemist, which is modeled by the transfer of a hydrocarbon from aqueous solutions to pure liquid hydrocarbon. We consider experimental data for pair interactions, which have been fitted by a simple empirical potential function, and point out some of their properties. (i) Surface free energy and cosphere overlap models, of the type considered until now, cannot reproduce correctly both the pair and bulk hydrophobic interactions. (ii) Pair interactions though still attractive are strikingly weaker in aqueous solution than in the gas phase, in contrast to the usual view of hydrophobic interactions. (iii) For pair interactions in water, the solvent-separated configuration is less important than the contact configuration if the hydrocarbon has more than two carbon atoms.

Hydrophobic “bonding” involves the association of nonpolar groups in an aqueous environment. Kozak *et al.* (1) have drawn the distinction between bulk hydrophobic interactions—that is, “interactions involving large clusters of nonpolar groups as may, for instance, be found in the interior of a protein molecule”—and interactions between small numbers of nonpolar groups as may, for instance, be found at the surface of a protein molecule. These qualitatively different interactions are generally lumped together in the analysis of biochemical systems. For macromolecules, models of bulk hydrophobic interactions are appropriate for the buried molecular interior, while models for pair hydrophobic interactions are probably more appropriate at the molecular surface where there are many neighboring water molecules present. The distinction between these two kinds of interactions is important because they can be quite different in magnitude. As described below, the relative effect of water, in general, is to increase the bulk interaction and decrease the pair interaction for saturated hydrocarbons, although both are decreased for benzene. The macroscopic features of both bulk and pair hydrophobic interactions have been extensively studied and much is known about them (2–23). At the molecular scale a knowledge of aqueous hydrophobic interactions implies a knowledge of the potentials of average force between the hydrophobic species, and many questions about these potentials remain unanswered. For a complete understanding of these interactions in biochemical processes, we need a detailed knowledge of the forces involved. This paper presents some conclusions about the effects of water on bulk and pair interactions of hydrophobic molecules and suggests some tests of molecular scale models of these interactions.

Pair Versus Bulk Interactions

The relationships between pair interactions, bulk interactions, hydrophobic solvation, and the transfer of hydropho-

bic groups between water and hydrophobic medium is illustrated by the following thermodynamic cycle.



Here R is a hydrophobic molecule and c^θ is the chosen standard state concentration ($c^\theta = 1 \text{ mol/dm}^3$). For $n = 2$, $\Delta G_2(g) = -RT \ln K_2(g)$ is a measure of the association of a pair of gas-phase molecules, while the corresponding $\Delta G_2(aq)$ and equilibrium constant, $K_2(aq)$, is a measure of the association of a pair of hydrophobic molecules in water.

For the bulk interaction, the four-sided cycle collapses to a triangular one because the thermodynamic properties of $R_n(g)$ become equal to those of $R_n(aq)$ in the limit of large n ; a large enough n -mer is a liquid drop of R. The bulk interaction familiar to the biochemist is characterized by $(1/n)\Delta G_n(aq, c^\theta)$, which is the Gibbs energy change for transfer of a hydrocarbon from aqueous solution to pure liquid hydrocarbon when n is large. As proposed originally by Kauzmann (2), this process can be used to model the transfer of a hydrocarbon side chain, exposed to aqueous solution in the unfolded protein, to the interior of the protein as folding occurs. [Note that there is a different bulk interaction in the gas phase, characterized by $(1/n)\Delta G_n(g, c^\theta)$.] This free energy of transfer from an aqueous phase to a hydrocarbon phase is a measure of bulk hydrophobic interactions. The corresponding reaction for $n = 2$ is a measure of the pairwise hydrophobic interaction, and both of these reactions can be compared with the corresponding reactions in the gas phase [at the same concentration to avoid standard-state problems (24, 25)]. This comparison allows the effect of the aqueous environment on pair and bulk interactions to be measured. A more fundamental comparison is of the osmotic second virial coefficients, $B_2(aq)$ (26), with the corresponding gas-phase virial coefficients, $B_2(g)$, since the virial coefficients are integrals of the direct or vacuum potential U [for $B_2(g)$] and of the potential of mean force U^* [for $B_2(aq)$]. Thus,

$$B_2(g) = -\frac{1}{2} \int \{\exp(U/kT) - 1\} 4\pi r^2 dr, \quad [1]$$

and $B_2(aq)$ is the same integral with U^* substituted for U .

Returning to the diagram ΔG_n^s is the free energy of solvation from the gas phase into water of an n -mer. The thermodynamic cycle gives the relationship between hydrophobic interactions and the free energies of solvation of the n -mer and the monomer. A knowledge of any three of the above processes allows one to calculate the fourth process.

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A variety of methods for estimating bulk hydrophobic interactions exist, most of which involve the partitioning of nonpolar compounds between water and either the gas phase or nonpolar solvents or the partitioning of hydrophobic groups between the surface and interior of proteins (2–14, 27–30). It is well known that the bulk hydrophobic interaction for saturated hydrocarbons is much more attractive in water than in the gas phase (3), and this is easily demonstrated from the thermodynamic cycle described above. If the ΔG_1^s is positive, as it is for saturated hydrocarbons,[§] then for large n , $\Delta G_n(\text{aq})/n$ must be more negative than $\Delta G_n(\text{g})/n$, since $\Delta G_n^s/n$, being primarily a surface effect, must approach zero as n becomes large. (The free energy of immersing a large aggregate in water, ΔG_n^s , is proportional to $n^{2/3}$ for a reasonably shaped aggregate and the limit $n \rightarrow \infty$ $\Delta G_n^s/n = 0$.) This analysis of bulk interactions for hydrophobic solutes leads to Stryer's (32) statement that "water accentuates the interaction of nonpolar molecules."

Estimates of pairwise interactions of two nonpolar solutes are obtained from osmotic second virial coefficients in aqueous solutions, $B_{AB}(\text{aq})$, which are a measure of the tendency of two molecules to associate above and beyond the association that would be present in a random solution of A and B in water (1, 15–22). For strongly associating species $B_{AA}(\text{aq})$ becomes equal to minus the ordinary thermodynamic association constant $[-K_2(\text{aq})]$.[¶] Franks has reviewed early evidence for the differences between pair and bulk interactions (23). Recently, Watanabe and Andersen (33) have shown that hydrophobic solvation does not necessarily imply the existence of hydrophobic association. In their simulation of a model for krypton dissolved in water, they found that the krypton molecules exhibited typical hydrophobic solvation (ΔG_1^s positive) but had net repulsive interactions between each other in water [positive $B_{AB}(\text{aq})$] and, thus, no tendency to associate in an aqueous solution. Thus, neither hydrophobic hydration nor attractive bulk interactions have to be associated with attractive pairwise hydrophobic interactions. Clark *et al.* (20) pointed out that the experimental $B_{AB}(\text{aq})$ data then available, as well as their model for the hydrophobic interaction between two aqueous alcohols, indicated that pairwise association in an aqueous solution was less than that in a vacuum $\Delta G_2(\text{g}) < \Delta G_2(\text{aq})$. The experimental data were limited and their model used just one spherically symmetric attractive interaction to model all of the interactions between two alcohol molecules (methyl–methyl, methyl–hydroxyl, and hydroxyl–hydroxyl group interactions).

Recent experimental evidence and virial coefficient models (26) show even more conclusively that the tendency of two hydrocarbon molecules containing more than a couple of carbon atoms to associate in an aqueous solution is less than their tendency to associate in a vacuum. This is in contrast to bulk hydrophobic interactions. The consequence of this is that, in water, the hydrophobic groups on the surface of a protein or other macromolecule will be less likely to interact attractively with a neighboring hydrophobic group than in a vacuum while the clustering of large numbers of hydrophobic groups (such as in the interior of a protein) will be more likely. It also means that simulations that do not take into account the differences between bulk and pair hydrophobic interactions will not be accurate.

The new experimental data, which allow the estimation of pairwise hydrophobic interactions, are shown in Table 1, which gives $B_{AB}(\text{aq})$ for the interactions of cyclohexanol with itself and with cyclohexane, as well as the gas-phase second

Table 1. Comparison of gas phase [$B_{AB}(\text{g})$] and osmotic [$B_{AB}(\text{aq})$] second virial coefficients for cyclohexane, cyclohexanol, and benzene

A	B	$B_{AB}(\text{aq})$, $\text{cm}^3 \cdot \text{mol}^{-1}$	$B_{AB}(\text{g})$, $\text{cm}^3 \cdot \text{mol}^{-1}$
C ₆ H ₁₁ OH	C ₆ H ₁₁ OH	–209*	
C ₆ H ₁₁ OH	C ₆ H ₁₂	–368 [†]	
C ₆ H ₁₂	C ₆ H ₁₂	–527, [‡] –487 [§]	–1734 [¶]
		–410, –402**	
		–596 ^{††}	
C ₆ H ₆	C ₆ H ₆	–388 [†]	–1480 [¶]

*From ref. 34.

[†]From ref. 35.

[‡]Estimated by a linear extrapolation of the data in this table versus number of OH groups.

[§]Estimated from $B_{AB}(\text{aq})$ for C₆H₁₁OH – C₆H₁₂ corrected for the CH₂–OH interactions using the group additivity principle (21, 22).

[¶]From ref. 36.

^{||}Estimated from $B_{AB}(\text{aq})$ for C₆H₁₁OH – C₆H₁₁OH corrected for the CH₂–OH and OH–OH interactions using the group additivity principle (21, 22).

**Estimated from the group additivity principle (21, 22).

^{††}Estimated from a site-interaction model that fits $B_{AB}(\text{aq})$ for eight alcohols (26).

virial coefficient [$B_{AB}(\text{g})$] of cyclohexane with itself. From the aqueous solution data we can estimate the $B_{AB}(\text{aq})$ for cyclohexane with itself in a variety of ways (26) (see Table 1). These estimates involve (i) the extrapolation of $B_{AB}(\text{aq})$ as a function of the number of hydroxyl groups on the molecules, (ii) the correction of $B_{AB}(\text{aq})$ for the effect of the OH group interactions, using a group additivity principle (21, 22), and (iii) the direct group additivity prediction of $B_{AB}(\text{aq})$ (21, 22). All of these estimates are in reasonable agreement and show clearly that the osmotic second virial coefficient in water, $B_{AB}(\text{aq})$, is less than one-third of the gas-phase osmotic second virial coefficient, $B_{AB}(\text{g})$, and, thus, that the tendency of two cyclohexane molecules to associate in aqueous solution is about one-third of their tendency to associate in the gas phase.

Rosky and Friedman (37) pointed out that for benzene $B_{AB}(\text{aq})$ is also much less than $B_{AB}(\text{g})$. Although in principle the hydrophobic interactions between aromatic rings could be quite different than the interactions between saturated hydrocarbons, Table 1 shows that the experimental $B_{AB}(\text{aq})$ for benzene of Tucker *et al.* (35) is similar to that of cyclohexane (–388 versus $-500 \pm 100 \text{ cm}^3 \cdot \text{mol}^{-1}$) and less than one-third of the gas-phase value, $B_{AB}(\text{g}) = -1480 \text{ cm}^3 \cdot \text{mol}^{-1}$ (36). Analysis of the previous thermodynamic cycle leads to the conclusion that for benzene the tendency for bulk hydrophobic association is weaker in aqueous solution than in the gas phase since ΔG_1^s is negative when the same concentration standard states are used in both the gas and liquid phases. As Ben Naim and Marcus have shown, use of these standard states is essential if one wants to compare intrinsic solvation energies in different solvents (24, 25).

The site-interaction model for the osmotic second virial coefficients, $B_{AB}(\text{aq})$, of a series of aqueous alcohols (26) uses interaction sites on the carbon and the oxygen atoms together with square-well potentials of average force to model the $B_{AB}(\text{aq})$. It was found that the carbon–carbon interaction was not pairwise additive but that it could be modeled by a narrow, contact square well and a nonpairwise additive interaction such as would be found if the interaction were mainly due to the overlap of solvation cospheres. This model fits the experimental data for eight aqueous alcohol systems, including *myo*-inositol with itself, cyclohexanol with *myo*-inositol, cyclohexanol with itself, and cyclohexanol with cyclohexane. Using the same model to predict the cyclohexane–cyclohexane interaction gives $B_{AB}(\text{aq}) = -596 \text{ cm}^3$.

[§]Recent evidence indicates ΔG_1^s is negative for C_nH_{2n+2} for $n > 18$ (31).

[¶]With K_2 in units of $\text{dm}^3 \cdot \text{mol}^{-1}$, $\Delta G^\theta = -RT \ln K_2$ for the 1 mol·dm⁻³ standard state. Note that even for cyclohexane the association is weak.

mol^{-1} , as given in Table 1 (26). There is good agreement between the estimate from the model and the estimates from experimental data given above, confirming the conclusion that the pairwise interaction in water, although still attractive, is less than that in the gas phase by a factor of ≈ 3 (-596 versus $-1734 \text{ cm}^3 \cdot \text{mol}^{-1}$). Similar estimates of $B_{AB}(\text{aq})$ for methane, ethane, propane, 2-methylpropane, and 2,2-dimethylpropane are all negative but less negative than the corresponding gas-phase virial coefficient, $B_{AB}(\text{g})$, although the difference is small for methane (26).

The difference between $B_{AB}(\text{g})$ and $B_{AB}(\text{aq})$ is small for two methane molecules and increases for the larger hydrocarbons like cyclohexane (26, 36). The potentials that fit the osmotic virial coefficients (26) indicate that the difference is due mainly to the nonadditivity of the interaction site potentials. Reducing attractive interactions by use of a nonadditivity algorithm is equivalent to adding a repulsive component to this potential of mean force. Since this nonadditivity is due to solvent forces, detailed analyses of these forces using modern theories as well as molecular simulations are needed.

Surface Free Energy Models

There is no *a priori* reason to expect pairwise interactions to be stronger in water simply because bulk interactions are stronger. This expectation is usually based on surface free energy (5–9) or cosphere overlap models (21, 27–30) in which the surface free energy term is calculated from experimental free energies of hydration or transfer and is added to the direct (vacuum) interaction between the solutes. Such an effect always gives a negative (attractive) contribution to the free energy. The failure of this model can be easily demonstrated by noting that as two hydrophobic solutes approach each other in water, the accessible surface must always be less than the accessible surface of the isolated particles. Thus, the surface free energy term lowers the potential of interaction between the particles and represents an attractive interaction at all distances between the particles. In contrast to this, theoretical calculations (38–43) and simulations (44–46) agree that between solvent-separated and contact distances there is a region where the effect of the solvent is to raise the interaction potential. This repulsive tendency is a packing effect and is present in essentially all dense fluids and even in solutions of hard-sphere solutes in a hard-sphere solvent. Thus, the effect of the solvent is to change the broad attractive dispersion interaction into a narrow attractive region at contact, another attractive region at solvent-separated distances, and a repulsive region in between. A simple surface free energy or cosphere overlap model (5–9, 27–30) cannot reproduce these effects. Surface free energies have been shown to work very well for predicting the free energies of solvation and transfer of a variety of molecules (5–9, 27–30). Since these models do not reproduce the expected contact and solvent-separated minima, which are present in essentially all liquid models, it seems clear that these models will not yield accurate estimates of the potential of average force for the approach of two nonpolar groups surrounded by water on the surface of a macromolecule. The aggregation of the surface groups will be overestimated because these models predict stronger pairwise association in water. These are very serious flaws for models of macromolecules in water.

Contact Versus Solvent-Separated Interactions

In the recent literature there has also been some controversy as to the importance of solvent-separated interactions in the tendency of hydrophobic species to associate (23). Stryer states that “substrates are bound to enzymes at active site clefts, from which water is largely excluded when the sub-

strate is bound” (32), indicating that the interaction is mainly a contact interaction and not a solvent-separated interaction. On the other hand, most theoretical calculations and simulations of the interactions of models for methane interacting with methane in an aqueous solution indicate that interactions at solvent-separated distances are more important in the association process than interactions at contact (38–43). The same is true for interactions between larger spherical molecules (40–42). However, one recent theoretical calculation seems to indicate that contact interactions are more important (43). Also, the interaction site model for aqueous alcohols could not fit the experimental data when appreciable amounts of solvent-separated interactions were included (26). The potential of mean force for this model is mainly determined by the interactions of the relatively large alcohols in the data set, so that the experimental evidence (at least for the larger hydrocarbon groups) indicates that the association process is mainly through contact interactions, in agreement with the evidence from biochemical interactions (32).

Theories and simulations indicate that even when solvent-separated interactions for two spherical molecules are more likely than contact interactions, the contact interactions involve a deeper (although narrower) well. This behavior gives a plausible reason why contact interactions appear to be more likely for larger hydrophobic molecules (26). For configurations with multiple C–C site interactions, $\exp(-\beta U^*)$ is a rapidly increasing function of the number of contacts (especially when $\beta U^* < -1$), so that the difference in energy between contact and solvent-separated configurations becomes much more important than the effect of the configuration volume element at the larger separations.

Simulations

The above discussion shows that the hydrophobic interaction is a very complicated interaction with quantitative differences that depend on the existence of neighboring sites (nonpairwise additive interactions). It seems unlikely that these effects can be modeled by treating the role of the water with a surface free energy term. This is unfortunate because the explicit introduction of water molecules into simulations greatly increases the needed computer resources and, in fact, we do not even know whether the use of any of the presently known water models would be able to mimic the properties of both bulk and pairwise hydrophobic interactions. Normally the water parameters in the potential models are adjusted to give the correct properties for bulk water, the hydrocarbon parameters are adjusted to give the properties for bulk hydrocarbons or hydrocarbon crystals, and the water–hydrocarbon parameters are adjusted to give the correct free energy of solvation. Most models for water–water interactions that are used for simulations of biochemical phenomena leave out the nonadditive or cooperative nature of these interactions; that is, when a water molecule is already hydrogen bonded to one molecule, its hydrogen bonds to another water are stronger. It is at least possible that this nonadditivity plays an important role in determining pairwise hydrophobic interactions. Thus, it is an open question as to whether models with parameters adjusted in this way are able to reproduce correctly the details of the pairwise hydrophobic interaction and, in fact, until such tests are made we cannot be certain that present models are adequate to mimic hydrophobic phenomena.

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