Bull. Inst. Chem. Res., Kyoto Univ., Vol. 61, No. 4, 1983

Solvent Effects on a Protein Molecule: A Theoretical Inspection

Ken NISHIKAWA

Received June 7, 1983

A confusion exists in the literature in the treatment of solvent effects upon a protein molecule. The solvent effect arising from surrounding water is formally expressed as an extra free energy term in addition to the conformational energy of a protein computed in vacuum. Some people have employed the transfer free energy of a solute from nonaqueous to aqueous solvents for the calculation of the extra free energy. On the other hand, the corresponding parameter used in the hydration-shell model developed by Gibson and Scheraga has a different form from the above transfer free energy. The present inspection clarifies that the treatment of the hydration-shell model is valid and the parameter used in the model has a physical meaning of the transfer free energy of a solute into water from gas phase, instead of from nonaqueous solvent. In relation to this subject, we also discuss other confusions caused by coexistence of two different definitions for *hydrophobicity*, one defined by Kauzmann and the other by Ben-Naim. A serious error involved in the Ben-Naim's theory is pointed out.

KEY WORDS: Hydration free energy/ Hydrophobicity/ Protein conformation/

INTRODUCTION

The solvent effect was first incorporated into the conformational energy calculation of a protein by Gibson-Scheraga.[1] The method was later developed as hydration-shell model by Hopfinger[2,3] and Hodes *et al.*[4,5] In the hydration-shell model, the solvent effect is treated as an additional free energy term, ΔF, onto the conformational energy computed in vacuum. The latter includes all the intramolecular noncovalent interactions among constituting atoms of a protein. The total energy, including solvent effects, is just the sum of the two terms, *i.e.*,

$$F_{total}=E_{conf}+\Delta F \qquad (1)$$

According to Gibson-Scheraga, the additional term, ΔF, originates from the free energy change when one solvent (water) molecule is removed from the first water layer around a solute molecule considered. They stated that the parameter values used for ΔF in their computation were deduced from the study of Némethy-Scheraga[6], which dealt with aqueous-hydrocarbon solution by statistical thermodynamics. It is not obvious, however, how the parameter values for ΔF have been derived because no corresponding quantity explicitly appears in the paper[6] cited. The "definition" quoted above is not clear enough to understand the exact physical meaning of ΔF. The situation remains unchanged on consulting other papers of the successors.

* 西川 建 : Laboratory of Physical Chemistry of Enzyme, Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611.

On the other hand, the solvent effect has also been treated in simpler ways by other people (Levitt,[7] and Robson-Osguthorpe[8]). While they introduced several simplified assumptions in the presentation of a protein molecule as well as interaction energies, the basic expression of the conformation energy in solution is formally the same as that of eq. 1. However, they used for the calculation of sidechain-solvent interactions which corresponds to the term ΔF here, the standard free energy for transfer of amino acid residues (more generally, solute A) from water to nonaqueous solution, denoted in this study as $\Delta\mu^0_{tr}(W\rightarrow A)$.

A question then arises whether or not ΔF used in the hydration-shell model has the same meaning of the transfer free energy in the latter usage. In order to answer to this question, an attempt was made to deduce the explicit form for ΔF from the original paper of Némethy-Scheraga[6]. The result (shown in Appendix A) turns out that it differs from the transfer free energy itself having a form proportional to such a quantity as, $\Delta\mu^0_{tr}(W\rightarrow A)-U(A)$, where $U(A)$ is the potential energy of solute molecule A in its pure liquid state.

With this discrepancy at hands, we will examine which one of these quantities is principally valid as an additional free energy term expressing solvent effects. Through the present inspection the physical meaning of the ΔF term in the hydration-shell model may become clear. Since the subject of solvent effects upon protein conformation is closely related to the concept of hydrophobic bonds, we will also investigate an unique concept of "hydrophobic interaction" proposed by Ben-Naim[9] and discuss other confusions seen in the understanding of the hydrophobic effects, in relation to the above problem.

THEORETICAL ANALYSIS

Folding process of a protein as a model system

Instead of going into the microscopic details of solvent interactions with a protein, we consider the change in energy accompanied with conformational change of a globular protein as a model, and see the energy change in two different ways; one takes solvent as well as protein molecules explicitly into account, and the other takes only a protein molecule into the explicit consideration, leaving the solvent as a whole of its continuous background. Since both lines of the aspects should give the same result, we could decide from this analysis what kind of form should be required for the additional term ΔF in eq. 1.

We begin with the first line of the above considerations. Since our main concern in this study is the solvent effect upon a protein molecule, the distinctions among protein-solvent, solvent-solvent and intramolecular interactions within a protein are crucial but not so in detailed distinctions among intramolecular noncovalent interactions, such as van der Waals forces, hydrogen bonds, *etc.* Thus, a very simple model is introduced in the following. All the protein atoms and atomic groups (*e.g.*, -CH_2-, CH_3-, *etc.* as units) are assumed to have the same size and interact with each other with an averaged interaction energy of the same amount for each one of intramolecular interactions. Similarly, a water molecule is treated as a single entity having also the same size as that of protein atoms (and groups), and water molecules interact with protein

atoms as well as with each other. The types of interaction energies (per one noncovalent interaction) are denoted as,

ε_{AA}: average atom-atom interaction energy
ε_{AW}: average atom-water interaction energy
ε_{WW}: average water-water interaction energy

In the following discussion, we ignore the chain entropy of a protein for the sake of simplicity. While the chain entropy is one of the major factors governing the protein stability in general, it is not necessary at present when considering the solvent effect in connection with the conformational energy, the usual computations of which also ignore it.

We consider next the native (N) and denatured (D) states of a protein at some fixed value of an external variable (*e.g.,* temperature). The change of the external variables causes a transition between the two states. In the present model these states (at a fixed temperature) are characterized with the number of the interactions considered in respective states: We denote them

$I_{AA}(N)$ and $I_{AA}(D)$: number of atom-atom interactions
$I_{AW}(N)$ and $I_{AW}(D)$: number of atom-water interactions
$I_{WW}(N)$ and $I_{WW}(D)$: number of water-water interactions

for the N and D states, respectively. Then, ignoring the chain entropy, the energy difference of the total system in protein folding is given as,

$$\begin{aligned}\Delta E_{D\to N} &= E_{tot,N} - E_{tot,D} \\ &= (I_{AA}(N)\varepsilon_{AA} + I_{AW}(N)\varepsilon_{AW} + I_{WW}(N)\varepsilon_{WW}) \\ &\quad - (I_{AA}(D)\varepsilon_{AA} + I_{AW}(D)\varepsilon_{AW} + I_{WW}(D)\varepsilon_{WW}) \end{aligned} \tag{2a}$$

Here holds a restriction condition that the total number of interaction sites of all atoms in protein are conserved irrespective of conformational change. A similar condition holds for interaction sites of all the water molecules. Namely,

$$2I_{AA}(N) + I_{AW}(N) = 2I_{AA}(D) + I_{AW}(D)$$

and

$$2I_{WW}(N) + I_{AW}(N) = 2I_{WW}(D) + I_{AW}(D)$$

By using these conditions, eq. 2a reduces to eq. 2b below.

$$\Delta E_{D\to N} = (I_{AA}(N) - I_{AA}(D))\cdot\Delta\varepsilon \tag{2b}$$

with

$$\Delta\varepsilon = \varepsilon_{AA} + \varepsilon_{WW} - 2\varepsilon_{AW} \tag{3a}$$

The energy difference $\Delta\varepsilon$ corresponds to the following reaction of bond redistribution.

$$2(A-W) \longrightarrow (A-A) + (W-W) \tag{4a}$$

Thus, eq. 2b simply implies that the energy change in the folding process is equal to the number of increased atom-atom interactions times the energy difference for creation of a single atom-atom interaction. The result is the same in its form as the energy

difference for separation and mixing of binary solutions treated with a simple lattice theory of solution (*e.g.*, the Bragg-Williams approximation). This is natural because our treatment with this level of approximation can be regarded as a sort of the lattice model, comparable to these used in general theory of polymer solutions.[10]

The essential feature above does not change at all even a more complex model used at the beginning. For instance, if we assume the interaction site for an atom-atom pair not being the same as for an atom-water interaction but m times larger than the latter, the result is unchanged except that $\Delta\varepsilon$ is replaced to

$$\Delta\varepsilon=\varepsilon_{AA}+m\cdot\varepsilon_{WW}-2m\cdot\varepsilon_{AW} \tag{3b}$$

corresponding to a process

$$2m(A-\mathrm{W}) \longrightarrow (A-A)+m(W-W) \tag{4b}$$

As imagined from this reaction scheme, we are able to accommodate easily the hydrophobic character of nonpolar atoms into the expression of $\Delta\varepsilon$, only with a slight modification of definitions of enrgy terms (ε_{AA}, ε_{WW} and ε_{WA}) to free energies. Therefore, we can regard that the essential feature of energy change in the folding process is given by eq. 2b, apart from the gross change in chain entropy.

Now, the same result as above should be reached by starting along the second line of the considerations. It takes only a protein molecule explicitly into account and regards the solvent effect as some excess interactions arising from the interface between the protein molecule and the solvent. This is a typical way to incorporate the solvent effect into the conformational energy calculation as mentioned at the beginning. According to this treatment the total energy for a given protein conformation is written as

$$E_{tot}=E_{conf}+E_X \tag{5}$$

where E_X is the total excess energy expressing the solvent effect. In order to compare this with the previous consideration, we rewrite it with the same notation, *i.e.*,

$$E_{conf}=I_{AA}\cdot\varepsilon_{AA} \tag{6a}$$

$$E_X=I_{AW}\cdot\varepsilon_X \tag{6b}$$

here ε_X is the excess energy per one interaction site of protein atoms located at the interface. Applying this form to the folding process as before, we get

$$\begin{aligned}\Delta E_{D\to N}&=E_{tot,N}-E_{tot,D}\\&=(I_{AA}(N)\varepsilon_{AA}+I_{AW}(N)\varepsilon_X)-(I_{AA}(D)\varepsilon_{AA}+I_{AW}(D)\varepsilon_X)\end{aligned} \tag{7a}$$

By using again the conservation condition for the total interaction sites of the protein atoms, it reduces to

$$\Delta E_{D\to N}=(I_{AA}(N)-I_{AA}(D))\cdot(\varepsilon_{AA}-2\varepsilon_X) \tag{7b}$$

From the comparison with eq. 2b, the proper form of ε_X is obtained as

$$\varepsilon_X=1/2\cdot(\varepsilon_{AA}-\Delta\varepsilon) \tag{8}$$

together with either form of $\Delta\varepsilon$ used (eqs. 3a and 3b).

The expression of ε_X obtained in eq. 8 is to be compared with the additional term ΔF in eq. 1. To do this, we have to check the content of the free energy of transfer, $\Delta\mu^0_{tr}$, in terms of the interaction energies used in the above discussion. If the free energy of transfer corresponds to $\Delta\varepsilon$, then the result of eq. 8 suggests that the correct form of ΔF would be one given in the hydration-shell model.

Transfer free energy of solute from water to nonaqueous solvent

According to Kauzmann[11], the free energy of transfer is defined as the difference of the standard chemical potential of a solute in two phases of water and of some nonaqueous solvent. The latter phase is either defined as pure liquid of the solute or as some organic solvent, both of which are mimic for nonaqueous environment in protein interior. Therefore, we do not distinguish them in this study. Although only nonpolar solutes are usually considered in relation to hydrophobic bonds, the following discussion holds for general transfer process of a solute irrespective of nonpolar or polar characters. Then, the definition above is written as

$$\Delta\mu^0_{tr}(W\rightarrow A)=\mu^0_A-\mu^0_W \tag{9a}$$

where μ^0_W and μ^0_A are the standard chemical potential of solute A in water and in pure liquid state of A, respectively.

Fom now on we mainly follow Ben-Naim[9] who analysed the subject with a statistical mechanical theory. Eq. 9a is rewritten by Ben-Naim as follows,

$$\Delta\mu^0_{tr}(W\rightarrow A)=\Delta\mu^0_{tr}(G\rightarrow A)-\Delta\mu^0_{tr}(G\rightarrow W) \tag{9b}$$

$$=W(A|A)-W(A|W) \tag{9c}$$

The first equality (eq. 9b) comes from the fact that the transfer process of solute molecule A from water to pure liquid A is identical to two successive transfer processes combined; first the solute is transferred from water to ideal gas phase and then from gas phase to pure liquid state (or to nonpolar medium) under the equilibrium condition. The second equality (eq. 9c) means that the difference in eq. 9b is the same as that between coupling works of solute A against water and the pure liquid of A, respectively. According to Ben-Naim the "coupling work" is a work required to incorporate a single solute molecule (A) into a given medium (W or A). This work consists of two parts, *i.e.*, the work to create a hole in the medium to accommodate a solute molecule and the work to insert the solute into the hole.

In relation to our present model the pure liquid state of A is considered as liquid lattice where each one of molecules interacts with z nearest neighbors by average interaction energy of ε_{AA}. Similarly, when a solute is in water it interacts with zm water molecules by an amount of ε_{AW}, where m has the same meaning as before. In this context, the coupling work of $W(A|W)$ is interpreted as that several (*i.e.*, $zm/2$) water-water bonds are broken because of a hole creation and then new several (*i.e.*, zm) solute-water bonds are produced upon insertion of a solute molecule in it. It is written as

$$W(A|W)=z/2\cdot(2m\varepsilon_{AW}-m\varepsilon_{WW}) \tag{10a}$$

The same interpretation applies to $W(A|A)$, *i.e.*,

$$W(A|A)=z\varepsilon_{AA}-z/2\cdot\varepsilon_{AA}=z/2\cdot\varepsilon_{AA} \tag{10b}$$

Substituting them into eq. 9c we get

$$\Delta\mu^0{}_{tr}(W\rightarrow A)=z/2\cdot(\varepsilon_{AA}+m\varepsilon_{WW}-2m\varepsilon_{AW})=z/2\cdot\Delta\varepsilon \tag{11}$$

While eq. 11 is here derived from just a qualitative consideration on the "coupling work", entirely the same result can be obtained by estimating the standard chemical potentials of $\mu^0{}_A$ and $\mu^0{}_W$ (eq. 9a) with a statistical mechanical treatment based on the general lattice theory of binary solution.[12]

Equation 11 shows that the transfer free energy $\Delta\mu^0{}_{tr}(W\rightarrow A)$ carries essentially the same content as that of the association process of two solute molecules expressed by eq. 4b. Only a difference is in their magnitudes; the transfer energy is defined for the whole molecule of a single solute while the latter is partial, defined for one solute-solute pair interaction (or two interaction sites of the solute). From the identity in their physical meaning we can say that the driving force of the protein folding expressed by eq. 2b is the transfer of protein atoms from aqueous environment to protein interior. This result is consistent with the current argument on protein stability.[13] Ben-Naim derived a different result and disapproved the above statement in his book (Appendix 5 in Ref. 9). However, the same conclusion as above would be attained from his treatment if a serious mistake involved in his theory were corrected, as shown in Appendix B.

Note that the transfer free energy is not identified here as the hydrophobic force as already pointed out. It is a wider concept than the hydrophobic force, applying not only to nonpolar but also polar groups. This distinction may be important in explaining the experimental data: For example, a large positive enthalpy (as well as entropy) change commonly observed in thermal denaturation of a protein cannot be explained when the hydrophobic force (*i.e.,* contribution of *nonpolar* groups) alone is regarded as a dominant factor for the protein stabilization.[14] This subject will be further discussed elsewhere.

Returning to our present concern we can say from eqs. 8 and 11 that the transfer free energy $\Delta\mu^0{}_{tr}(W\rightarrow A)$ itself cannot be used as the excess energy of the solvent effect. On the other hand, usage in the hydration-shell model (see Appendix A) seems to be consistent with the excess energy of eq. 8. In order to make this point clearer, we consider next about what physical meaning is contained in the excess energy term.

Physical meaning of the excess energy term

First, we shall briefly refer to the theoretical treatment of Ben-Naim.[9] His definition of "hydrophobic interaction" is quite different from the usual one. He considers an association process of two nonpolar solute molecules in water (similar to the scheme of eq. 4). He defines the free energy change for such a process that two solute molecules initially infinitely separated with each other come to close contact with a fixed distance. (His definition is rigorous to consider a fixed disposition of solutes for both initial and final states in order to avoid the external freedom of solutes as well as the mixing entropy). He divides the total free enregy change of this process into two; one is a solute-solute *direct* interaction term independent of the surrounding solvent

and the other is an *indirect* term corresponding to the net effects of solvents. Namely,

$$\Delta G = U_{AA} + \delta G^{HI} \tag{12}$$

The first term at the right-hand side is the potential energy between two solutes A in vacuum and the second term, δG^{HI}, is regarded as the pure "hydrophobic interaction" arisen from solvent effects.

Although our present model is very simple the above process considered by Ben-Naim is essentially the same as the reaction scheme given by eq. 4. Thus, we can put the following relations.

$$\Delta G \equiv \Delta\varepsilon \tag{13a}$$

$$U_{AA} \equiv \varepsilon_{AA} \tag{13b}$$

All these quantities are defined per one solute-solute pair interaction (or per two interaction sites of solute molecules). Then

$$\begin{aligned} \delta G^{HI} &= \Delta G - U_{AA} \\ &= \Delta\varepsilon - \varepsilon_{AA} = -2\varepsilon_X \end{aligned} \tag{14}$$

The last relation above comes from eq. 8. Thus we can assign the same physical meaning of δG^{HI} to the excess energy ε_X, except for the differences in the sign and the magnitude (ε_X is defined for one interaction site instead of two). That is, $(-\varepsilon_X)$ implies the net "hydrophobic interaction" in the association process of nonpolar solute molecules in water, which is realized when the direct interaction between solutes in vacuum is "switched off."[9]

Ben-Naim developed a method to estimate the extent of δG^{HI} and its derivatives (*i.e.*, δS^{HI}, δH^{HI}, *etc.*) in experimental and semi-theoretical ways. He introduced there an unique concept of "fused molecule": For instance, two methane molecules in contact to each other to make a "dimer" is approximately replaced by a single molecule of ethane. With this assumption, δG^{HI} for the association process of two methane molecules is expressed by using the standard chemical potentials of methane and ethane, values of which are experimentally obtainable. This assumption is written as

$$\delta G^{HI}(r) = \Delta\mu^0_{Et}(G \rightarrow W) - 2\Delta\mu^0_{Met}(G \rightarrow W) \tag{15}$$

In this way he obtained numerical values of δG^{HI} (and its derivatives) for various model compounds. We could use them for the excess energy of the solvent effects. A resulting value of eq. 15 is, however, only valid under the retention that the final configuration of the "dimerized" methane has an abnormally close carbon-carbon contact with bond distance of ethane (*i.e.*, $r \fallingdotseq 1.5$A).

From a close inspection on his treatment, however, we can proceed furthermore. It is recognized from eq. 15 that both terms at the right-hand side are expressed with the same quantity, *i.e.*, standard free energy of transfer from gas phase to water, although one is for "dimer" and the other for monomer. Because of their identity in nature we can add them up to get a sum, *i.e,*.

$$\delta G^{HI} = \Delta\mu^0{}_{di} - 2\Delta\mu^0{}_{mono}$$
$$= -2\alpha\cdot\Delta\mu^0{}_{mono}(G \rightarrow W) \qquad (16)$$

Here α is some proportional constant; it is reasonable to define α as the ratio between the accessible surface area decreased due to the dimer formation and the total accessible surface of a monomer. A proper value could be estimated from a simple geometriacal consideration. We also obtain here another expression for ε_X from comparison between eqs. 14 and 16, *i.e.,*

$$\varepsilon_X = \alpha\cdot\Delta\mu^0{}_{mono}(G \rightarrow W) \qquad (17)$$

These relations of eqs. 16 and 17 seem to me much simpler to express δG^{HI} (and ε_X) with measurable quantities. We do not need the approximation of "fused molecule" anymore and also free from the retention attached to it.

To ensure the validity of eq. 16, I will show in the following that essentially the same result is also obtained from a different consideration. Recalling that the transfer process from water to nonaqueous environment has the same content with respect to energy as the association process of solutes under consideration, we return to eq. 9. There the standard free energy change for transfer of solute A from gas phase to pure liquid state is the same as the coupling work of A against the liquid composed of A[9]. Therefore, we get the following relations.

$$\Delta\mu^0{}_{tr}(G \rightarrow A) = W(A|A)$$
$$= z/2\cdot\varepsilon_{AA} = U(A) \qquad (18)$$

The second equality above comes from eq. 10b. The last equality shows that the quantity $(z/2\cdot\varepsilon_{AA})$ is just the average potential energy of solute A in the medium composed of the same kind of molecules A. This statement is clear from the very definition of $\Delta\mu^0{}_{tr}(G \rightarrow A)$, indicating the transfer of one solute from gas phase to the medium without the change of external freedom of the solute.[9] Then we can rewrite eq. 9b as

$$\Delta\mu^0{}_{tr}(W \rightarrow A) = U(A) - \Delta\mu^0{}_{tr}(G \rightarrow W) \qquad (19)$$

This relation is just parallel to eq. 12 given by Ben-Naim. Only a difference is again in the definition of whether it applies to the whole molecule of a solute or only to the interacting sites. We clearly see not only ΔG proportional to the total transfer energy of $\Delta\mu^0{}_{tr}(W \rightarrow A)$, but also the net hydrophobic part of δG^{HI} proportional to the transfer energy from gas to water $-\Delta\mu^0{}_{tr}(G \rightarrow W)$. The latter relation is identical to the expression of eq.16.

Equation 19 is the final formulation which we have sought for in the present study. It tells us that the quantity of $[\Delta\mu^0{}_{tr}(W \rightarrow A) - U(A)]$, which is used as the additional free energy term in the hydration-shell model, is identical to the free energy change in the transfer of a solute (or an atomic group of a protein) from gas phase into water (eq. 19), instead of nonaqueous solvent to water. This quantity $\Delta\mu^0{}_{tr}(G \rightarrow W)$ is usually called as "standard free energy of hydration (solvation)" of a given solute or also called as "solubility" in reference to its gas phase instead of pure

liquid phase ("solubility" in the latter sense corresponds to the total transfer energy $\Delta\mu^0_{tr}(W \rightarrow A)$).

Our conclusion is as follows: The solvent effect should be incorporated into the conformational energy calculation by subtracting the free energy of solvation of those protein atoms that are buried in the protein interior, or conversely by adding the free energy of solvation of those atoms that are exposed to the solvent. This conclusion is quite natural when we note that conformational energy in vacuum implies conformational energy in "gas phase" In this sense, the excess energy indicates the energy difference of a protein between in vacuum and in solution, and therefore goes to zero as the solvent is gone away. This would not apply if the total transfer energy $\Delta\mu^0_{tr}(W \rightarrow A)$ were used in place of ΔF in eq. 1.

DISCUSSION

There exist some confusions even at the very basic level of understanding about solvent effect, including hydrophobic ones, upon solutes particularly of macromolueclar ones. Therefore, a primitive model of the protein folding employed in this study, which may correspond to the zero-th ordered approximation in the solution theory[12] is still effective as has shown to resolve some ambiguities of this basic level. The following points have been made clear from the present inspection.

(i) Use of the transfer free energy based on solubility data (*e.g.,* Nozaki and Tanford[15]) in addition to the conformational energy of a protein in vacuum, is wrong in its physical meaning.

(ii) The free energy of hydration defined in the hydration-shell model has a general meaning of the standard free energy for transfer of a solute from gas phase into water.

The point (ii) is consistent in the physical meaning with the study of Amidon *et al,*[16] who referred the above transfer energy of nonpolar solutes to the "intrinsic hydrophobicity" in contrast to the usual hydrophobic concept defined for the total transfer process from water to nonaqueous solvent (but see below for their main argument). The same physical meaning is also carried by a concept of the net "hydrophobic interaction" defined by Ben-Naim,[9] as already pointed out. From both points of (i) and (ii), it is clear enough that no direct comparison is allowed between the free energy values used in the hydration-shell model and those derived by Nozaki-Tanford[15] or related values[17], although it is sometimes done (*e.g.,* Banaszak *et al*[18]).

(iii) From a basic point of view, the folding process of a globular protein can be regarded as the transfer process of constituent atoms (or groups) from aqueous to nonaqueous environments. An antagonism raised by Ben-Naim is based on an erroneous theoretical treatment (see Appendix B).

The statement (iii) is, however, only valid in a rough sense. It is well known that the standard free energy of transfer experimentally obtained from the solubility of low molecular-weight compounds, which is in principle valid in the limit of the infinite dilution cannot give a good result for a solution of high concentration[19]. This situation applies to protein molecules in which constituent atoms and groups are regarded as being "locally" concentrated.[20] The reason for this discrepancy is recognized as that

structural changes of the solvent (water) would not be restricted within the first layer but extend beyond layers around solute particles as the solute concentration is getting high.

This problem is often stated as: the association (*e.g.*, "dimerization") process of hydrophobic groups is not simply a "partial reversal" of the solution process in water.[20] If the opposite to the above discussion is also true, we can expect that the concept of "partial reversal" holds well for a small solute in the relatively low concentration. In such a simpler situation,[21] we can also expect that the "first-ordered" approximation holds well. An example may be the one assumed in the Némethy-Scheraga theory and later employed in the hydration-shell model in which structural changes of water are assumed to occur only in the first-hydration layer of a solute. Therefore, speaking in short, it is likely that the partial-reversal concept may hold well in such a case where the "first-ordered" approximation is applicable with a good precision.

In this context, a comment may be in order for the opposite preposition of "non partial-reversal process" stressed in the literature. For instance, using various kinds of mixed water-organic solvents, Oakenfull and Fenwick[22] made a direct experimental comparison between free energy changes of the pairwise hydrophobic interaction and of the transfer of a nonpolar solute from a mixed solvent to a nonpolar medium. No clear relationships between them were obtained, including even a negative correlation in the case of *t*-butanol-water mixed solvent. They have concluded from this experiment that "hydrophobic interaction (*i.e.*, a pairwise interaction between hydrophobic groups) is not simply a partial reversal of solution of the nonpolar molecules in water". The results, however, may not be extended to the general level including a simple case mentioned above because the mixed solvent system is fairly complex containing at least three different components of nonpolar solute, organic solvent and water (See the review by Franks[19] for various anomalities of mixed aqueous solvents). Ben-Naim has also generalized the same preposition from a similar reason based on experimental data of mixed aqueous solvents although the discrepancy observed between the solution process and the pairwise hydrophobic interaction seems to be much smaller than that observed by Oakenful and Fenwick (Sec. 3. 4 in Ref. 9).

Another reason raised by Ben-Naim comes from the opposite behavior of the volume change accompanied in the two processes. The volume change for the hydrophobic interaction between two methane molecules is given by Ben-Naim (Sec. 5. 5 in Ref. 9) as

$$\delta V^{HI}=\partial\delta G^{HI}/\partial P\fallingdotseq \bar{V}_{Et}{}^{0}-2\bar{V}_{Met}{}^{0} \tag{20}$$

where $\bar{V}_{Et}{}^{0}$ and $\bar{V}_{Met}{}^{0}$ are the partial molar volumes of ethane and methane, respectively. A "dimer" of methane is approximated by a "fuzed" molecule of ethane, as already explained. However, the definition above does not give a true measure of the volume change for the "hydrophobic interaction", because a simple sum of the partial molar volumes in eq. 20 includes the "fused" volume as well. The volume decrease due to this "fusion" is considerably large (Think about the closest contact distance of two methanes ($\sim$4 Å) in comparison with the C-C distance of 1.5 Å in ethane). Thus, it is natural to result always large negative values, which are opposite

in sign from the volume change expected from the *reversed* solution process of nonpolar solute in water.[11] Ben-Naim has claimed that this is an "evidence" for the fact that the hydrophobic interaction is a non partial-reversal process of the transfer from nonaqueous to aqueous environments. Curiously enough, he made no attempt to correct the above artifact.

Besides more detailed levels of arguments, another question has been raised toward the propriety of the very concept of hydrophobic effects (or interactions) themselves.[16,24~26] The clearest appeal among them may be the one raised by Cramer.[26] He argues that, if the dominant factor in hydrophobic phenomena is really the structural change of water around a solute, then the free energy change for the total transfer process (*i.e.,* water → organic solvent) should be parallel to the "intrinsic hydrophobicity" (*i.e.,* for a transfer: water → vapor), because the other term (*i.e.,* for a transfer: organic solvent → vapor) is independent from the contribution of water (see eqs. 9b and 19). He demonstrated no simple parallelism observed between them for various nonpolar and inert solutes, and also the magnitude of the *intrinsic* hydrophobicity of -CH_2- group, for instance, being small compared with total free energy change. A similar conclusion of the small (or sometimes even unfavourable) contribution of the *intrinsic* hydrophobicity was reached by Amidon *et al*[16] and Wolfenden-Lewis.[25]

As already refuted by Némethy *et al*,[27] they dealt mainly with the free energy instead of more crucial quantities for the hydrophobicity, i.e., entropy and heat capacity. In addition, we must remember that the original work of Frank and Evans,[28] based on which the hydrophobic concept has later been established, concerned with the vaporization process of various kinds of solutes from various solvents including water. The vaporization is the very process from which the above question to the hydrophobic concept was raised. The difference of Frank and Evans from the antagonists, however, lies at the point that the unique role of water as a solvent (*i.e.,* extra large entropy and heat capacity changes accompanied with vaporization of nonpolar solutes) was elucidated from comparison between water and other *normal* solvents. Therefore, if the transfer process for a solute from water to nonpolar solvent is divided into the interaction energy in vacuum and the solvation term (see eq. 19) the magnitude (increment of the free energy change[29]) of the former may appear dominant or larger than the latter as they showed (particularly, Amidon *et al*[16]). Even so, this fact cannot deny the unique and large role of water in comparison with the *normal* solvent. Note that a "small" contribution of the intrinsic solvent effect (the second term above) is measured from its 0 value, *i.e.,* against a reference frame of vacuum. It is not a measure of "hydrophobicity" but a measure of the free energy of solvation (or the hydration free energy[30]) as mentioned before.

In this respect, the terminology of "intrinsic hydrophobicity" (Amidon *et al*) as well as the net "hydrophobic interaction" (Ben-Naim) both of which are measured from the reference state of vacuum,[31] may not be adequate, leading readily to confusions as above. For another example, Amidon et al referred aliphatic and aromatic hydrocarbons to intrinsically hydrophobic and intrinsically hydrophilic, respectively, because of their respective positive and negative values in the hydration free energy. This classification is odd in comparison with the usual one (*e.g.,* Franks

and Reid[32]).

The appropriate measure of the hydrophobicity, on the other hand, should be the one based on the direct comparison of solubilities of a substance in water and in a *normal* solvent, *i.e.,* the original definition of Kauzmann.[11] So, we have returned to the starting point.

A special comment on the Ben-Naim's theory

We admire that the theoretical treatment of Ben-Naim[9] has an advantage of dealing with the hydrophobic interaction in terms of the statistical mechanics. It provides us microscopic insights into the phenomena of transfer processes as well as hydrophobic interactions, in contrast to the usual thermodynamic treatments. Moreover, his theory has been developed on the general statistical mechanics without introducing any particular model for a system considered in contrast to other authors' treatments (*e.g.,* Némethy-Scheraga[6]). This is the reason that we have followed him and largely employed his notations in the present study.

A glance at the sophisticated theory he employed seems to be sound and rigorous. However, it involves sometimes careless mistakes (*e.g.,* see the above diccussion on the volume change, δV^{HI}), and also a severe error at the fundamental level as mentioned below. Ben-Naim has claimed so far several contradictory generalizations against the conventional hydrophobic concepts: For instances, i) the association (dimerization) process of two nonpolar solutes in water is emphasized as not a "partical reversal" of the solution process (this may be partially correct, but not so in the basic level as already discussed), ii) "structural changes in the solvent, induced by the hydrophobic interaction process, cannot affect the *strength* of the hydrophobic interaction",[33] iii) the folding process of a protein, even at the very basic level, cannot be regarded as the transfer process of constituting atoms from a solvent-exposed state to protein interior. All these concepts have originated or been deduced either from careless mistakes or from the basic fault described in Appendix B.

ACKNOWLEDGMENTS

The author expresses his sincere thanks to Professor R. Huber in Max-Planck-Institut für Biochemie in Martinsried (FRG) for kind support and providing the research facilities. The present work was performed under the scholarships of the Alexander von Humboldt Foundation in the Federal Republic of Germany during a period of Oct. 1981-Mar. 1983.

APPENDIX A

Derivation of an explicit form of the *the hydration free energy* from the Némethy-Scheraga theory

In treating aqueous hydrocarbon solutions by statistical mechanics, the basic assumption made by Némethy and Scheraga[6] is that the energy levels and the distribution of water molecules in "the water layer next to the hydrocarbon" are shifted from those in the bulk water due to different interactions between the water and hydrocarbon molecules. Based on this assumption, the standard free energy of solution,

corresponding to the transfer of one mole of solute (hydrocarbon) from its pure liquid state to water is given as a sum of two parts, one for free energy change of the solute and the other for free energy change of the water:

$$\Delta F^0=(F_A{}^S-F_A{}^0)+Y^C(F_W{}^C-F_W{}^0) \tag{A1}$$

Here, $F_A{}^0$ and $F_W{}^0$ are the standard molar free energies of the solute and the water in their pure liquid states, respectively. $F_A{}^S$ is the molar free energy of the solute surrounded by water, and $F_W{}^C$ is the molar free energy of water contained in the first layer around the solute molecules, pertaining to the Y^C moles of water per mole of solute.

The first part at the right-hand side of eq. A1 is expressed with molecular parameters in the paper (ignoring here the term for configurational entropy change of a solute molecule, ΔS_{conf}) as follows:

$$(F_A{}^S-F_A{}^0)=Y^C\Delta E_l-E_{RR} \tag{A2}$$

where $Y^C\Delta E_l$ is the energy gain per mole of hydrocarbons due to solute-water interactions, while $-E_{RR}$ is the energy loss due to breaking the solute-solute interactions on removal from the pure liquid state.

The second part of eq. A1, on the other hand, was not given in their paper in terms of the corresponding molecular parameters. Following their discussions closely, however, we may write the desired expression as

$$Y^C(F_W{}^C-F_W{}^0)=Y^C[\chi_4{}^C\Delta E_l+(1-\chi_4{}^C)\Delta E_r] \tag{A3}$$

Here, $\chi_4{}^C$ is the mole fraction of the tetra-hydrogen-bonded species of water in the first layer of solutes. This species is assumed in the model to gain the energy by an amount of ΔE_l per water-solute interaction pair. The fraction of $(1-\chi_4{}^C)$ indicates the mole fraction of all the rest of water species in the first hydration layer. Each one of these species is assumed to loose the energy commonly by ΔE_r per mole of the water.

Substituting eqs. A2 and A3 into eq. A1, we get

$$\Delta F^0=Y^C\Delta f-E_{RR} \tag{A4}$$

with

$$\Delta f=(1+\chi_4{}^C)\Delta E_l+(1-\chi_4{}^C)\Delta E_r \tag{A5}$$

It is clear from eq. A4 that Δf obtained above implies the free energy change of solvation per water molecule hydrated to the solute. Thus, the quantity $(-\Delta f)$ corresponds to the parameter used in the hydration-shell model,[1] where it is denoted as F^0. Numerical values of Δf for aliphatic and aromatic hydrocarbons which are obtainable from the parameter values given by Némethy-Scheraga[6] are in accord with those listed by Gibson-Scheraga[1] (the latter authors seem to have used the temperature of 20°C for $\chi_4{}^C$, which is a function of T).

In relation to the notation in the text, ΔF^0 corresponds to $-\Delta\mu^0{}_{tr}$ $(W\rightarrow A)$, and E_{RR} is the potential energy of one solute molecule (A) in its pure liquid state, *i.e.*, $U(A)$. Then rewriting eq. A4,

$$-\Delta f=1/Y^C\cdot[\Delta\mu^0{}_{tr}(W\rightarrow A)-U(A)] \tag{A6}$$

The quantity $-\Delta f$ deduced here is the free enregy of removing one water molecule from the first-hydration shell of the solute, in accordance with the statement of Gibson and Scheraga[1].

APPENDIX B

A fundamental error involved in the Ben-Naim's theory

1. On the definition of the "binding energy"

According to Ben-Naim (Appendix 1 in Ref. 9), the standard free energy of a solute (S) transferred from gas phase to some fixed position ($\boldsymbol{R}_S$) in the solvent (W) is expressed in terms of other microscopic quantities (with $\beta=1/kT$) as

$$\Delta\mu^0_{tr}(G\rightarrow W)=W(S|W)=-kT\ln\langle\exp[-\beta B_S(\boldsymbol{R}_S)]\rangle \tag{B1}$$

where $W(S|W)$ is the "coupling work" of the solute against the solvent as explained in the text, and $B_S(\boldsymbol{R}_S)$ is called as "binding energy" of the solute to the rest of all particles in the solution (in the following, a process of a single solute molecule transferred in the pure solvent is considered for simplicity). The symbol $\langle\ \rangle$ implies the statistical average over all configurations of the solvent molecules, *i.e.*,

$$\langle\exp[-\beta B_S(\boldsymbol{R}_S)]\rangle=\frac{\int\cdots\int d\boldsymbol{R}^W\cdot\exp[-\beta U(\boldsymbol{R}^W)-\beta B_S(\boldsymbol{R}_S)]}{\int\cdots\int d\boldsymbol{R}^W\cdot\exp[-\beta U(\boldsymbol{R}^W)]} \tag{B2}$$

where $U(\boldsymbol{R}^W)$ is the total potential energy of the solvent at a certain configuration specified with $\boldsymbol{R}^W$. Note that the integrals only concern to the solvent molecules.

Equation B2 comes from the definition of B_S as expressing the difference of the total potential energy of the system between before and after the addition of a solute S, *i.e.*,

$$B_S(\boldsymbol{R}_S)=U(S+W)-U(W) \tag{B3}$$

This difference is in turn identical (Sec. 5.6 in Ref. 9) to

$$B_S=\sum_{i=1}^{N}U_{s,i}(\boldsymbol{R}_S,\boldsymbol{R}^W) \tag{B4}$$

Namely, the sum of pairwise interaction energy $U_{s,i}$, between the solute S and all N solvent molecules. These relations are the definition of the "binding energy" made by Ben-Naim.

Eqs. B3 and B4 are defined for a certain configuration of the solvent molecules, $\boldsymbol{R}^W$. We realize, however, that these relations do not always apply to all possible configurations of the pure solvent. In other words, they only apply to those solvent configurations which are realized after a solute molecule added in the solvent. In order to accommodate a solute, the range of the possible locations of the solvent molecules is obviously restricted within a smaller range in comparison to that of the pure solvent. Thus eq. B2 (with retaining eqs. B3 and B4) is no longer valid: The range of the integral in the numerator should be changed as

$$\frac{\int\cdots\int_{V-v(s)}\cdots\int d\boldsymbol{R}^W\cdot\exp[-\beta U(\boldsymbol{R}^W)-\beta B_S(\boldsymbol{R}_S)]}{\int\cdots\int_{V}\cdots\int d\boldsymbol{R}^W\cdot\exp[-\beta U(\boldsymbol{R}^W)]} \tag{B5}$$

indicating that the integrals in the numerator should be carried out in a range excluding a small volume $v(s)$ occupied by the solute molecule S.

Actually almost the same expression as eq. B5 was used by Ben-Naim in a different place to explain the application of the scaled-particle theory to the hydrophobic phenomena (Chap. 2 and Appendix 4 in Ref. 9). There eq. B1 is casted in a different form:

$$\begin{aligned}\Delta\mu^0{}_{tr}(G\rightarrow W)&=W(S|W)\\&=W(\mathrm{rep}|W)+W(\mathrm{att}|W)\end{aligned}\tag{B6}$$

and

$$W(\mathrm{rep}|W)\fallingdotseq W(\mathrm{cav}|W)=-kT\ln P_0(\mathrm{cav})\tag{B7}$$

and

$$P_0(\mathrm{cav})=\frac{\int\ldots_{V-v(\mathrm{cav})}\ldots\int d\boldsymbol{R}^W\cdot\exp[-\beta U(\boldsymbol{R}^W)]}{\int\ldots_V\ldots\int d\boldsymbol{R}^W\cdot\exp[-\beta U(\boldsymbol{R}^W)]}\tag{B8}$$

The coupling work is first divided into the repulsive and attractive parts of the interaction energy of the solute particle (eq. B6). Then the repulsive part is approximated by a work to create cavity with a suitable volume for the solute (eq. B7). Eq. B8 together with eq. B7 conveys a reasonable meaning that the work to create a cavity in the solvent to accommodate a particle originates from the restriction imposed on the configurational space of the solvent molecules. Therefore, it is clear that eq. B5 which differs from eq. B8 only by the term of B_S is the formal expression for the entire coupling work including the work for the attractive part appeared in eq. B6. This is natural because after insertion of a solute into the solvent the interaction energy B_S acts essentially as an attractive one (namely, the "binding energy" in its literal sense; this naming is however only suitable under the situation of *post*-insertion).

It is now clear from these inspections that eqs. B1-B4 completely lack the contribution of positive energy arising from the cavity creation in the solvent, accounting only for the "binding" (attractive) energy between a oslute and the solvent. This is a serious fault because these (rather than eqs. B6–B8) are the equations which Ben-Naim has so far used as the basic relations to deduce various other formulations from. Therefore, whenever eqs. B1–B4 are explicitly applied in his theories, they inevitably involve serious errors producing of contradictory concepts. Some examples of utmost importance in the general discussion on solution process are shown in the following.

2. "Application" to the protein folding process

In a simplified-model study on the folding process of a protein (Appendix 5 in Ref. 9), Ben-Naim introduced the binding energy for sidechain groups located within the protein interior (S) as

$$\begin{aligned}\Delta\mu^0{}_{tr}(G\rightarrow S)&=-kT\ln\langle\exp(-\beta B_S)\rangle\\&\fallingdotseq B_S=\sum_{j=1}^{m}U_{ij}\end{aligned}\tag{B9}$$

That is, the binding energy of the i-th sidechain to the rest parts of the protein in the folded state is equated to the sum of pairwise interaction energies between the i-th and other sidechain j, in accord with the definition of eq. B4.

However, in order to equate it to the transfer energy in eq. B9, we have to add the work to create a cavity. The same process as above is already considered in eq. 10b in the text. In the present case, the final term in eq. B9 should be multiplied by a factor (1/2) because of subtracting the potential energy of the i-th sidechain ($=1/2\cdot\sum_{j=1}^{m} U_{ij}$), which is identical (but opposite in sign) to the hole creation work.

The correction above leads to the final equation below in place of eq. A106 in Ref. 9 for the total free energy change accompanied with folding process of a protein (composed of m sidechains).

$$\Delta\mu^0 = m\Delta\mu^0_{tr}(W \rightarrow S) + kT\ln(q_0/q) \qquad \text{(B10)}$$

This corrected form is in accord with the result obtained in the text. Namely, the transfer of sidechains from water to protein interior drives the folding process, which is counterbalanced with change of the chain entropy indicated by the second term in the right-hand side of eq. B10 (See Ben-Naim[9] for the details).

3. Change of *solvent structure* in the hydrophobic interaction

Ben-Naim is now famous with the advocacy stating that structural changes in the solvent induced by the hydrophobic interaction cannot contribute to the standard free energy of the process although they may affect the standard enthalpy, entropy and other partial derivatives of the free energy. In the original paper,[33] he claimed that the same statement generally holds for a wide range of processes in solution composed of any solvent; *e.g.*, no contribution of structural changes in the solvent for the Henry's law constant, and for equilibrium constants of association or of conformational change of biopolymers.

A ground on which all the conclusions above have been derived is the fact that the standard free energy of solution for a solute (*i.e.*, $\Delta\mu^0_{tr}$ defined in eq. B1) contains only an average over the distribution function of the pure solvent, whereas other thermodynamic derivatives (ΔH, ΔS etc.) contain configurational integrals including the solute as well as solvent molecules. However, as already pointed out, eq. B1 itself (together with eqs. B2–B4) is wrong, completely devoid of the contribution of cavity creation. It is easy to imagine that the energy loss arisen from cavity creation is closely related to the cause of entropy loss (*i.e.*, "structural change" in the usual meaning) of water molecules in the immediate vicinity of hydrophobic solutes. In fact, Ben-Naim himself has suggested an intimate relationship between the cavity creation and the surface tension of the solvent (Sec. 2.5), the latter of which is in turn relevant to the hydrophobic effects as is well known.[11,34] After the criticism made by Marcelja *et al*[35] from a different point of view from the present one, Ben-Naim has excused that what he is concerned with is not the "structure of the solvent around the solute" but the "structural changes in the solvent" (probably in the bulk solvent) (Sec. 5.9).——Curiously enough his original paper[33] is no more cited in the reference list of his book lately written[9]——. We should say that such a definition of "structure of water" as above is no longer

relevant to the "hydrophobic interaction" because water molecules of predominant importance in hydrophobic interactions are undoubtedly those ones "around the solute" instead of the bulk water.

Ben-Naim further made an attempt to confirm his conclusion in comparison with a simpler model system of adsorbing process, whcih has the advantage to be exactly solved with the general statistical mechanics. In this case study (Sec. 5.10 in Ref. 9) he demonstrates that the exact theory leads to the analogous (same) result as well, *i.e.*, indicating the independence of the free energy change of the adsorption from the "structural change of the solvent" (defined there in analogy to the solution process; see his book for the details). However, this coincidence is not surprising. Because the model he has chosen there is an adsorption process of gaseous particles onto lattice sites (a model of actual carriers such as polymers), only a work involved in the process is "binding". No work for cavity creation is involved there in nature (another example like this may be any process taking place in a gas phase). Therefore, the exact theory has verified that his theory is applicable for such a process as adsorption, but not for general processes in solution.

REFERENCES AND NOTES

(1) K. D. Gibson and H. A. Scheraga, *Proc. Natl. Acad. Sci. USA*, **58**, 420 (1967).
(2) A. J. Hopfinger, *Macromolecules*, **4**, 731 (1971).
(3) K. H. Forsythe and A. J. Hopfinger, *Macromolecules*, **6**, 423 (1973).
(4) Z. I. Hodes, G. Nemethy and H. A. Scheraga, *Biopolymers*, **18**, 1565 (1979).
(5) Z. I. Hodes, G. Nemethy and H. A. Scheraga, *Biopolymers*, **18**, 1611 (1979).
(6) G. Némethy and H. A. Scheraga, *J. Chem. Phys.*, **36**, 3401 (1962).
(7) M. Levitt, *J. Mol. Biol.*, **104**, 59 (1976).
(8) B. Robson and D. J. Osguthorpe, *J. Mol. Biol.*, **132**, 19 (1979).
(9) A. Ben-Naim, "Hydrophobic Interactions", Plenum, N. Y. (1980).
(10) P. J. Flory, "Principles of Polymer Chemistry", Cornell Univ. Press, Ithaca, USA (1953).
(11) W. Kauzmann, *Adv. Protein Chem.*, **14**, 1 (1959).
(12) N. Saito, "Polymer Physics" (in Japanese) Shokabo, Tokyo (1968).
(13) C. Tanford, *J. Am. Chem. Soc.*, **84**, 4240 (1962).
(14) H. A. Scheraga, G. Némethy and I. Z. Steinberg, *J. Biol. Chem.*, **237**, 2506 (1962).
(15) Y. Nozaki and C. Tanford, *J. Biol. Chem.*, **246**, 2211 (1971).
(16) G. L. Amidon, R. S. Pearlman and S. T. Anik, *J. theor. Biol.*, **77**, 161 (1979).
(17) C. Chothia, *Nature*, **248**, 338 (1974), *ibid* **254**, 304 (1975).
(18) L. J. Banaszak, J. J. Birktoft and C. D. Barry, in "Protein-Protein Interactions" (C. Freidman and L. W. Nichol, ed.) John Wiley, N. Y. (1981).
(19) F. Franks, "Water: A comprehensive treatise", Vol. 4, pp. 1–93, Plenum, N. Y. (1975).
(20) G. Némethy and H. A. Scheraga, *J. Phys. Chem.*, **66**, 1773 (1962).
(21) This is a hypothetical situation because "dimerization" of solute molecules in diluted solution would not be realized due to large effects of mixing entropy.
(22) D. Oakenfull and D. E. Fenwick, *J. Chem. Soc. Faraday Trans., I* **75**, 636 (1979).
(24) J. H. Hildebrand, *J. Phys. Chem.*, **72**, 1841 (1968).
(25) R. Wolfenden and C. A. Lewis, Jr., *J. theor. Biol.*, **59**, 231 (1976).
(26) R. D. Cramer, *J. Am. Chem. Soc.*, **99**, 5408 (1977).
(27) G. Némethy, W. J. Peer and H. A. Scheraga, *Ann. Rev. Biophys. Bioeng.*, **10**, 459 (1981).
(28) H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).
(29) Experimental values obtained from, *e.g.*, the Henry's law constant depend on the choice of the standard states in both vapor and solution phases. Therefore, only the relative value is usually meaningful.

(30) Small magnitudes of the hydration free energy for hydrophobic compounds arise from compensating effects between large changes of entropy and enthalpy.
(31) If the reference value is shifted from zero to some appropriate negative value, this is logically identical to change the scale from "intrinsic hydrophobicity" to the usual hydrophobicity defined for the total transfer process.
(32) F. Franks and D. S. Reid, "Water: A comprehensive treatise", Vol. 2, pp. 323–380, Plenum, N. Y. (1973).
(33) A. Ben-Naim, *Biopolymers,* **14**, 1337 (1975).
(34) C. Tanford, *Proc. Natl. Acad. Sci. USA,* **76**, 4175 (1979).
(35) S. Marcelja, D. J. Mitchell, B. W. Ninham and M. J. Sculley, *J. Chem. Soc. Faraday Trans., II* **73**, 630 (1977).