Structural Thermodynamics of Hydration

Tigran V. Chalikian*

Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Toronto, 19 Russell Street, Toronto, Ontario M5S 2S2, Canada

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A simple two-state structural model of solute hydration has been developed. In this model, both water in the bulk state and water of solute hydration are assumed to consist of two structural species: a high density/high enthalpy species, structurally similar to ice III, and a low density/low enthalpy species, structurally similar to ice I. It is assumed that structural and thermodynamic distinctions between bulk and hydration water originate solely from the differential fractional composition, whereas the two structural species and thermodynamic parameters associated with each species are identical for bulk and hydration water. This model has been used in conjunction with volumetric data reported in the literature to analyze the hydration properties of charged, polar, and nonpolar groups at 25 °C. The equilibrium between the two structural species of water of hydration of charged and polar groups is shifted toward the high density/high enthalpy species. In contrast, the equilibrium between the two species of water solvating nonpolar groups is shifted toward the low density/low enthalpy species. Solvent reorganization was found to be thermodynamically unfavorable for any atomic group independent of its chemical nature. However, the enthalpy, entropy, and heat capacity of solvent reorganization are strongly dependent on the chemical nature of solvent exposed groups. In the aggregate, our results provide foundation for more reliable interpretations of thermodynamic data in terms of hydration. In addition, these results underscore the importance and potential usefulness of combining volumetric and calorimetric data for a more complete thermodynamic description of microscopic events, in particular, solute hydration.

1. Introduction

In an aqueous environment, peptides, proteins, and nucleic acids are extensively hydrated.[1−9] Solvent molecules adjacent to these solutes interact with their external atomic groups thereby becoming structurally, dynamically, and thermodynamically distinct from bulk water.[8,10−14] Because solute-induced modification of solvent molecules is thermodynamically "costly", it should contribute to the structural/conformational energetics of the solute molecule. In fact, structural/conformational preferences of proteins and nucleic acids at specific solution conditions are, to a large extent, dictated by the energetics of solute−solvent interactions (commonly referred to as hydration).[7,8,15−18]

Molecular recognition also is affected by hydration. Molecular recognition events, including protein folding and binding, are generally accompanied by alterations in the number and/or chemical nature of solvent-exposed atomic groups. This, in turn, leads to a change in solute hydration: some water molecules, previously in the hydration phase, may be released to the bulk phase, whereas other water molecules, previously in the bulk phase, may be taken up by the solute hydration shell. Because water of hydration is thermodynamically distinct from bulk water, any redistribution of water molecules between the bulk and the hydration phases should have a significant impact on the net energetics of a molecular event.

The importance of hydration for conformational stability of proteins, nucleic acids, and their complexes and for the specificity and affinity of folding and binding events has been recognized long ago.[19−26] Generally speaking, an understanding of solute hydration at the microscopic and macroscopic levels is an absolutely necessary initial step toward identifying, resolving, and characterizing individual thermodynamic determinants of the conformational stability and functional activity of biologically relevant molecules. To this end, much effort has gone into the search for theoretical and experimental approaches to studying the properties of bulk and hydration water in solutions of biological compounds (e.g., see refs 27−36). There have been numerous attempts to quantify the structural properties of liquid water in terms of hydrogen bonded and non-hydrogen bonded species differing in energies, distances, and angles between adjacent molecules. It is worth noting that, in many instances, both the structural and the thermodynamic properties of liquid water have been satisfactorily described by extremely simple two-state models.[27,29,37−43] In these models, the entire spectrum of water structures is presented as an effective mixture of two species characterized by differential values of free energy, ΔG, enthalpy, ΔH, entropy, ΔS, heat capacity, ΔC_P, volume, ΔV, expansibility, ΔE, isothermal, ΔK_T, and adiabatic, ΔK_S, compressibility, etc.

In this work, a simple yet intrinsically consistent thermodynamic model of solute hydration has been developed based on a two-state structural presentation of liquid water. In this model, liquid water is presented as an effective mixture of the high density/high enthalpy and low density/low enthalpy structural species. In contrast to previously described two-state models, in our model, water of solute hydration and bulk water are

* To whom correspondence should be addressed. Tel: (416) 946-3715. Fax: (416) 978-8511. E-mail: chalikan@phm.utoronto.ca.

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assumed to consist of the same structural species. We further assume that all structural and thermodynamic changes in the hydration shell of a solute can be accounted for by a shift in equilibrium between the two structural species caused by solute−solvent interactions. We use a combination of volume and compressibility data as a thermodynamic signature of solute-induced solvent reorganization. Significantly, volumetric data in conjunction with the model developed allow one to determine the complete energetics (ΔG, ΔH, ΔS, ΔC_P) of solvent reorganization.

This model is of practical importance since it can be used to evaluate the amount and structural composition of water solvating atomic groups of different chemical nature based on experimental thermodynamics data. Furthermore, the model may find potential applications in determining the hydration contribution to the energetics of biomolecular recognition, including protein folding, helix-to-helix and helix-to-coil transitions of nucleic acids, biopolymer−ligand interactions, protein−protein and DNA-protein recognition, etc.

2. Bulk Water. In a two-state representation of liquid water, only two structural species are considered. Some of the proposed two-state models include hydrogen bonded and non-hydrogen bonded species, whereas other models include species with bent hydrogen bonds.[27] Jhon et al. in the 1960s[44] and more recently Robinson and collaborators[23,45−48] have proposed that liquid water effectively consists of dynamically interconverting microdomains of two structural types of hydrogen-bonded molecular species. One structural species, which predominantly exists at low temperatures and pressures, is arranged into the regular tetrahedrical structure of four water molecules surrounding a central molecule, similar to that in ordinary ice I. The second structural species, which predominantly exists at high temperatures and pressures, forms a more densely packed irregular tetrahedrical structure similar to that of ice II and ice III.

Because the high density structural species predominates at high temperatures, it should have a higher enthalpy than the low density species. From this perspective, ice III, perhaps, is a more relevant approximation for the densely packed high temperature/high pressure species than ice II. Strictly speaking, ice II cannot exist in equilibrium with liquid water.[27] In addition, ice III is characterized by a higher enthalpy than ice I (ΔH is 94 cal mol^{-1} at 2 kbar and −22 °C), whereas the enthalpy of ice II is smaller than that of ice I ($\Delta H = -180$ cal mol^{-1} at 2 kbar and −35 °C).[27] One cannot exclude, however, the possibility that, when extrapolated to atmospheric pressure and room temperature, the relative enthalpies of ice I, ice II, and ice III change in magnitude and even in sign. In each of the three ice polymorphs, the four hydrogen-bonded nearest neighbors are separated from the central molecule by a distance of roughly 2.8 Å. In ice I with its regular tetrahedrical structure, the distance between the non-hydrogen-bound outer shell neighbor water molecules is about 4.5 Å. In the more densely packed polymorphs ice II and ice III (where the tetrahedrical structure is irregular), this distance is near 3.4 Å.[44−48]

Using this simple two-state representation of liquid water, Robinson and collaborators have succeeded in accounting for many anomalous properties of liquid water.[45−48] It should be noted, however, that a certain amount of broken hydrogen bonds must be present in both subpopulations of water molecules. The presence of molecules with broken hydrogen bonds is required for fluidity of liquid water. In addition, a comparison of the heat of fusion of ice I (1.44 kcal mol^{-1}) with the heat of its sublimation (12.20 kcal mol^{-1}) indicates that, in liquid water, there are ∼10% broken hydrogen bonds.[27]

Equilibrium between the low density/low enthalpy, ice I-like structural species and the high density/high enthalpy, ice III-like structural species of water molecules can be presented as follows

$$(H_2O)_n = n/m\ (H_2O)_m \tag{1}$$

where n and m are the cooperativity numbers for the two structural species of water molecules; $(H_2O)_n$ denotes the n-meric cluster of water molecules of the first type (high density/high enthalpy); and $(H_2O)_m$ denotes the m-meric cluster of water molecules of the second type (low density/low enthalpy). In general, the values of n and m may or may not be equal.

The equilibrium constant K for reaction (1) is given by the relationship

$$K = C_2^{n/m}/C_1 = (X_2/m)^{n/m}/(X_1/n) = [(f_2/m)^{n/m}/(f_1/n)]\ [H_2O]^{n/m-1} \tag{2}$$

C_1 and C_2 are the molar concentrations of the first species' n-mers and the second species' m-mers, respectively; X_1 and X_2 are, respectively, the molar concentrations (per monomer) of the first and second species; f_1 and f_2 are the mole fractions of the first and second species, respectively; and $[H_2O]$ is a constant representing the total amount of moles of water in the system. Because $f_1 + f_2 = 1$, one finally obtains

$$K = \{(1 - f_1)/m]^{n/m}/(f_1/n)\}\ [H_2O]^{n/m-1} \tag{3}$$

The partial molar volume, $V_0°$, of water can be calculated as a weighed sum of the contributions from the two species

$$V_0° = f_1V_1 + f_2V_2 = f_1V_1 + (1 - f_1)V_2 = V_2 + f_1\Delta V \tag{4}$$

where $\Delta V = V_1 - V_2$; V_1 and V_2 are the partial molar volumes of the first and second structural species, respectively.

The partial molar isothermal compressibility, $K_{T0}°$, of water is equal in magnitude and opposite in sign to the first pressure derivative of the partial molar volume, $V_0°$, and can be calculated by differentiating eq 4

$$K_{T0}° = -(\partial V_0°/\partial P)_T = K_{T2} + f_1\Delta K_T - \Delta V(\partial f_1/\partial P)_T \tag{5}$$

where P is the pressure; T is the absolute temperature; $K_{T2} = -(\partial V_2/\partial P)_T$ is the partial molar isothermal compressibility of the second (low density/low enthalpy) structural species of water; and $\Delta K_T = K_{T1} - K_{T2}$ is the differential partial molar isothermal compressibility of the two species. The relationship for $(\partial f_1/\partial P)_T$ can be derived as follows

$$(\partial f_1/\partial P)_T = (\partial f_1/\partial \ln K)_T\ (\partial \ln K/\partial P)_T \tag{6}$$

Note that $(\partial \ln K/\partial P)_T = -n\Delta V/RT$, whereas the expression for $(\partial f_1/\partial \ln K)_T$ can be obtained by differentiating eq 3

$$(\partial f_1/\partial \ln K)_T = mf_1(1 - f_1)/[n(1 - f_1) + mf_1] \tag{7}$$

Finally, one obtains the following relationship for $(\partial f_1/\partial P)_T$

$$(\partial f_1/\partial P)_T = -(\Delta V/RT)nmf_1(1 - f_1)/[n(1 - f_1) + mf_1] \tag{8}$$

Substituting eq 8 into eq 5, one derives the following expression

for the partial molar isothermal compressibility, $K_{T0}°$, of water

$$K_{T0}° = K_{T2} + f_1\Delta K_T + (\Delta V^2/RT)nmf_1(1 - f_1)/[n(1 - f_1) + mf_1] \quad (9)$$

An analogous relationship can be obtained for the partial molar adiabatic compressibility, $K_{S0}°$

$$K_{S0}° \approx -(\partial V_0°/\partial P)_S = K_{S2} + f_1\Delta K_S + (\Delta V^2/RT)nmf_1(1 - f_1)/[n(1 - f_1) + mf_1] \quad (10)$$

where S is the entropy.

In eqs 9 and 10, the first two terms represent the structural or instantaneous contribution to the compressibility of water. The third term, $(\Delta V^2/RT)nmf_1(1 - f_1)/[n(1 - f_1) + mf_1]$, is the relaxation contribution to compressibility which results from the pressure-induced redistribution of water molecules between the two structural species (an increase in pressure will bring about a shift in the equilibrium toward the species with smaller volume and vice versa). Strictly speaking, the relaxation term in eq 10 is frequency-dependent:[49−51]

$$K_{S0}° = K_{S2} + f_1\Delta K_S + (\Delta V^2/RT)\, nmf_1(1 - f_1)/[n(1 - f_1) + mf_1]\,[1/(1 + \omega^2\tau^2)] \quad (11)$$

In this equation, τ is the relaxation time for reaction (1), and ω is the angular frequency of positive and negative changes in pressure applied to the system for measuring its compressibility. The adiabatic compressibility of water and aqueous solutions is usually determined by combining measurements of ultrasonic velocity and density.[51,52] For such measurements, ω represents the angular frequency of ultrasonic waves. Inspection of eq 11 reveals that the frequency-dependent relaxation term subsides to zero at very high frequencies [$1/(1 + \omega^2\tau^2)$ becomes equal to zero when ω approaches ∞]. Thus, at very high frequencies, the compressibility of water is determined only by the structural contribution ($K_{S2} + f_1\Delta K_S$). On the other hand, at low frequencies (when ω is close to 0), the value of $1/(1 + \omega^2\tau^2)$ becomes equal to unity and the relaxation contribution to compressibility reaches its maximum value. Relaxation time of structural rearrangements of water molecules, τ, is on the order of picoseconds ($\sim 10^{-12}$ sec),[27,37,50] whereas the typical range of ultrasonic frequencies, ω, used in biophysical/biochemical investigations is only on the order of 10^6 to 10^7 Hz.[51,52] Therefore, for practical calculations, the term $1/(1 + \omega^2\tau^2)$ in eq 11 can be effectively taken equal to unity.

Using the same approach, one can derive expressions for the partial molar heat capacity at constant pressure, $C_{P0}°$, and expansibility, $E_0°$, of water. In these derivations, the partial molar enthalpy, $H°$, of water is given as a linear combination of the contributions from the two species

$$H_0° = f_1H_1 + (1 - f_1)H_2 = H_2 + f_1\Delta H \quad (12)$$

where H_1 and H_2 are, respectively, the partial molar enthalpies of the first (high density/high enthalpy) and second (low density/low enthalpy) structural species; and $\Delta H = H_1 - H_2$. Partial molar heat capacity at constant pressure, $C_{P0}°$, represents the first temperature derivative of partial molar enthalpy, $H_0°$, and, hence, can be found by differentiating eq 12

$$C_{P0}° = (\partial H_0°/\partial T)_P = C_{P2} + f_1\Delta C_P + \Delta H(\partial f_1/\partial T)_P \quad (13)$$

where $C_{P2} = (\partial H_2/\partial T)_P$ is the partial molar heat capacity of the second structural species; and $\Delta C_P = C_{P1} - C_{P2}$ is the differential partial molar heat capacity of the two water species.

The third term in eq 13, $\Delta H(\partial f_1/\partial T)_P$, represents the relaxation contribution to the heat capacity of water resulting from temperature-induced redistribution of water molecules between the two structural species (an increase in temperature will bring about a shift in the equilibrium toward the species with a higher enthalpy and vice versa). The relationship for $(\partial f_1/\partial T)_P$ can be obtained as follows

$$(\partial f_1/\partial T)_P = (\partial f_1/\partial \ln K)_P\,(\partial \ln K/\partial T)_P \quad (14)$$

Combining eqs 7 and 14 and taking into account that $(\partial \ln K/\partial T)_P = \Delta H/RT^2$, one can derive the following

$$(\partial f_1/\partial T)_P = (\Delta H/RT^2)nmf_1(1 - f_1)/[n(1 - f_1) + mf_1] \quad (15)$$

Substituting eq 15 into eq 14, one finally obtains

$$C_{P0}° = C_{P2} + f_1\Delta C_P + (\Delta H^2/RT^2)\, nmf_1(1 - f_1)/[n(1 - f_1) + mf_1] \quad (16)$$

Partial molar expansibility, $E_0°$, equals the first temperature derivative of partial molar volume, $V_0°$, and can be found by differentiating eq 4

$$E_0° = (\partial V_0°/\partial T)_P = E_2 + f_1\Delta E + \Delta V(\partial f_1/\partial T)_P \quad (17)$$

where $E_2 = (\partial V_2/\partial T)_P$ is the partial molar expansibility of the second (low density/low enthalpy) structural species of water; and $\Delta E = E_1 - E_2$ is the differential partial molar expansibility of the two structural species. Substituting eq 15 into eq 17, one obtains the following relationship

$$E_0° = E_2 + f_1\Delta E + (\Delta V\Delta H/RT^2)\, nmf_1(1 - f_1)/[n(1 - f_1) + mf_1] \quad (18)$$

The partial molar entropy, $S°$, of a system containing Z moles of water is given by the following expression[53]

$$S_0° = S_2 + f_1\Delta S - R[f_1 \ln f_1 + (1 - f_1)\ln(1 - f_1) + \ln Z!] \quad (19)$$

where S_1 and S_2 are, respectively, the partial molar entropies of the first and second structural species; $\Delta S = S_1 - S_2$. Combining eqs 12 and 19, one calculates the partial molar free energy (chemical potential) of liquid water

$$G_0° = H_0° - TS_0° \quad (20)$$

On the basis of the foregoing discussion, the thermodynamics of liquid water can be calculated within the framework of a two-state model if the cooperativity numbers (n, m), mole fractions of the two structural species (f_1, f_2), and the thermodynamic parameters (H, S, C_P, V, E, K_T, K_S) for each of the two species are known. In principle, these values can be determined from the two-state fitting of thermodynamic functions of water based on eqs 1−20.

3. Water of Hydration. Solute dissolution can be viewed as consisting of three steps with thermodynamic penalties/gains associated with each step.[54] The first step is to create in the solvent a cavity, large enough to accommodate the solute molecule. The second step is to place the solute molecule into the cavity. And the third step is to "switch on" solute−solvent interactions that may structurally reorganize the solute and

solvent molecules. Solute-induced reorganization of solvent represents an elusive yet major determinant of the energetics of solute dissolution. The energetic contribution of solvent reorganization is difficult to separate and characterize because waters of hydration (structurally and thermodynamically modified water molecules in the vicinity of solute) are chemically identical to bulk water. It is a formidable task to reliably discriminate between hydration and bulk water and quantitatively characterize the extent of structural reorganization of solvent molecules.

As shown below, the simple two-state analysis of liquid water described above can be extended to characterizing the thermodynamics of solute-induced solvent reorganization. We assume that the two structural species present in bulk water are the same species that present in the hydration shell of a solute. However, the distribution between the two structural species in the hydration shell of a solute may differ from that in the bulk state. This is a plausible assumption since, as shown below, a shift in structural equilibrium is sufficient to account for the entire range of the observed partial molar volumes and adiabatic compressibilities of water molecules solvating all types of atomic groups. Hence, at least from the point of view of volumetric observables, there is no need to introduce new structural species to account for the hydration-induced changes in solvent thermodynamics. With this assumption, the thermodynamic properties of water of hydration (denoted by subscript h) can be expressed as follows

$$V_h^\circ = V_2 + f_{1h}\Delta V \tag{21}$$

$$H_h^\circ = H_2 + f_{1h}\Delta H \tag{22}$$

$$S_h^\circ = S_2 + f_{1h}\Delta S - R[f_{1h}\ln f_{1h} + (1 - f_{1h})\ln(1 - f_{1h}) + \ln Z!] + \Psi_h \tag{23}$$

$$K_{Th}^\circ = K_{T2} + f_{1h}\Delta K_T + (\Delta V^2/RT)\, nmf_{1h}(1 - f_{1h})/[n(1 - f_{1h}) + mf_{1h}] \tag{24}$$

$$K_{Sh}^\circ = K_{S2} + f_{1h}\Delta K_S + (\Delta V^2/RT)\, nmf_{1h}(1 - f_{1h})/[n(1 - f_{1h}) + mf_{1h}] \tag{25}$$

$$C_{Ph}^\circ = C_{P2} + f_{1h}\Delta C_P + (\Delta H^2/RT^2)\, nmf_{1h}(1 - f_{1h})/[n(1 - f_{1h}) + mf_{1h}] \tag{26}$$

$$E_h^\circ = E_2 + f_{1h}\Delta E + (\Delta V\Delta H/RT^2)\, nmf_{1h}(1 - f_{1h})/[n(1 - f_{1h}) + mf_{1h}] \tag{27}$$

In these equations, the differential thermodynamics of hydration and bulk water is determined by the differential fractional composition (f_{1h} versus f_1). In eq 23, the term Ψ_h reflects a possible decrease in translational and orientational degrees of freedom of water molecules in the hydration shell of a solute.

If the hydration shell of a solute consists of n_h water molecules, then the thermodynamic effects of solute-induced solvent reorganization can be expressed by the following general relationship

$$\Delta Y_h = n_h(Y_h - Y_0) \tag{28}$$

where Y is some thermodynamic parameter; and n_h is the hydration number.

From eq 28, one obtains the following relationships for the thermodynamic functions of solvent reorganization in the solute hydration shell

$$\Delta V_h = n_h\Delta V(f_{1h} - f_1) \tag{29}$$

$$\Delta H_h = n_h\Delta H(f_{1h} - f_1) \tag{30}$$

$$\Delta S_h = n_h\{\Delta S(f_{1h} - f_1) - R[f_{1h}\ln f_{1h} + (1 - f_{1h})\ln(1 - f_{1h}) - f_1\ln f_1 - (1 - f_1)\ln(1 - f_1)] + \Psi_h\} \tag{31}$$

$$\Delta K_{Th} = n_h\Delta K_T(f_{1h} - f_1) + n_h(\Delta V^2/RT)\{nmf_{1h}(1 - f_{1h})/[n(1 - f_{1h}) + mf_{1h}] - nmf_1(1 - f_1)/[n(1 - f_1) + mf_1]\} \tag{32}$$

$$\Delta K_{Sh} = n_h\Delta K_S(f_{1h} - f_1) + n_h(\Delta V^2/RT)\{nmf_{1h}(1 - f_{1h})/[n(1 - f_{1h}) + mf_{1h}] - nmf_1(1 - f_1)/[n(1 - f_1) + mf_1]\} \tag{33}$$

$$\Delta C_{Ph} = n_h\Delta C_P(f_{1h} - f_1) + n_h(\Delta H^2/RT^2)\{nmf_{1h}(1 - f_{1h})/[n(1 - f_{1h}) + mf_{1h}] - nmf_1(1 - f_1)/[n(1 - f_1) + mf_1]\} \tag{34}$$

$$\Delta E_h = n_h\Delta E(f_{1h} - f_1) + n_h(\Delta V\Delta H/RT^2)\{nmf_{1h}(1 - f_{1h})/[n(1 - f_{1h}) + mf_{1h}] - nmf_1(1 - f_1)/[n(1 - f_1) + mf_1]\} \tag{35}$$

A crucial task in thermodynamic description of solute hydration is to determine the values of hydration number, n_h, and fractional composition, f_{1h}. The hydration number, n_h, reflects the "quantity" of solute hydration, whereas the fractional composition, f_{1h}, reflects the "quality" of hydration, which is the extent of solute-induced structural changes in the solvent. The hydration number, n_h, of a solute is often estimated (quite arbitrarily) as the number of water molecules contacting or forming direct hydrogen bonds with a solute. Alternatively, one may use eqs 29−35 to develop a more rigorous algorithm of determining the values of n_h and f_{1h}. Provided that the differential thermodynamic parameters (ΔV, ΔK_T, ΔK_S, ΔE, ΔH, ΔS, ΔC_P) and the fractional composition, f_1, of bulk water are known, the values of n_h and f_{1h} can be determined if any pair of the hydration functions (ΔV_h, ΔK_{Th}, ΔK_{Sh}, ΔE_h, ΔH_h, ΔS_h, ΔC_{Ph}) is measured. The values of n_h and f_{1h} for a given solute or an atomic group can be calculated mathematically by solving the system of a corresponding pair of equations.

Compared to calorimetric observables (free energy, enthalpy, entropy, heat capacity), volumetric observables (volume, compressibility, expansibility) are more convenient for use in practical hydration-related calculations because they can be more easily resolved into the contribution from solvent reorganization and other contributions.[52,54−59] Many characteristics of solute dissolution that are not directly related to solvent reorganization (such as potential energy of solute−solvent and solute−solute interactions, entropic factors) strongly influence calorimetric parameters. Nevertheless, these characteristics are volumetrically silent which simplifies interpretation of volumetric observables in terms of solute-induced solvent reorganization. In general, the fact that volumetric and calorimetric parameters often provide complementary descriptions of the same phenomena emphasizes the need of combining volumetric and calorimetric measurements in thermodynamic studies. Such a combination often yields a more complete and multifaceted characterization of a thermodynamic system or a process.

Among volumetric characteristics, the partial molar volume, V°, and adiabatic compressibility, K_S°, of biological compounds

have been studied most widely. This makes volume and compressibility good candidates for hydration analysis [based on eqs 29 and 33]. In general, $V°$ and $K_S°$ can be resolved in terms of intrinsic and hydration contributions as follows[52,54−59]

$$V° = V_C + \Delta V_h = V_M + V_T + \Delta V_h + \beta_{TO}RT \quad (36)$$

$$K_S° = K_M + \Delta K_{Sh} \quad (37)$$

where β_{TO} is the coefficent of isothermal compressibility of solvent; $V_C = V_M + V_T$ is the volume of the cavity enclosing the solute molecule; V_M is the intrinsic volume of the solute; V_T is the thermal volume resulting from the thermally induced mutual vibrations of solute and solvent molecules; ΔV_h, which is, sometimes, called the "interaction volume", represents the volume change accompanying solute-induced solvent reorganization; K_M is the intrinsic compressibility of the solute; and ΔK_{Sh} is the change in adiabatic compressibility accompanying solvent reorganization. For small molecules and nucleic acids, the intrinsic compressibility, K_M, in eq 37 is effectively zero.[52,58] Consequently, for these solutes, the partial molar adiabatic compressibility, $K_S°$, is determined by the compressibility effect of solvent reorganization ΔK_{Sh}. By contrast, for globular proteins, the value of K_M is large and positive and cannot be neglected.[52,58,59] Consequently, one needs to take into account the intrinsic compressibility, K_M, of a globular protein when calculating its hydration contribution to compressibility, ΔK_{Sh}, from eq 37.

Provided that the values of ΔV_h and ΔK_{Sh} of a solute have been determined experimentally, the hydration number, n_h, and fractional composition, f_{1h}, can be calculated by solving the system of eqs 29 and 33. Clearly, a precondition for such an analysis is that the values of ΔV, ΔK_S, and f_1 for bulk water are known. In the section that follows, we use the data on ΔV_h and ΔK_{Sh} for charged, polar, and nonpolar groups reported in the literature in conjunction with eqs 29 and 33 to determine the hydration numbers, n_h, and fractional compositions, f_{1h}, for each group type.

4. Hydration of Atomic Groups of Different Chemical Nature. The values of V_2, K_{S2}, ΔV, ΔK_S, and f_1 in eqs 4 and 10 can be determined as a function of temperature and pressure by fitting thermodynamic characteristics of liquid water using the two-state approximation. There are a number of such determinations reported in the literature.[27,39,43−45] Survey of the literature reveals that the values of V_2, K_{S2}, ΔV, ΔK_S, and f_1 may differ significantly from one author to another, even though most of them satisfactorily describe the thermodynamic (acoustic, densimetric, and calorimetric) properties of liquid water. At this stage, it is out of the scope of the present paper to perform another accurate determination of the values of V_2, K_{S2}, ΔV, ΔK_S, and f_1 as a function of temperature and/or pressure. Instead, the analysis presented below is limited to a single temperature of 25 °C and atmospheric pressure and aimed at demonstrating the potential applicability of the proposed approach. At this temperature, volumetric data on biological compounds are the most abundant. In addition, the thermodynamics of the majority of recognition events studied to date has been determined at 25 °C. However, ultimately, the proposed approach should be extended to a wider range of temperatures and pressures. In fact, such data obtained as a function of temperature and pressure may further refine many of the adjustable parameters and could represent a critical test for the proposed hydration model.

Our approximate choice of the values of V_2, ΔV, K_{S2}, ΔK_S, and f_1 in eqs 4 and 10 is as follows: $V_2 = 19.1$ cm^{3}mol^{-1}; $\Delta V = -3.7$ cm^{3}mol^{-1}; $K_{S2} = 3.0 \times 10^{-4}$ cm^{3}mol^{-1}bar^{-1}; $\Delta K_S = -1.0 \times 10^{-4}$ cm^{3}mol^{-1}bar^{-1}; and $f_1 = 0.27$. These values are within the range of similar values obtained by other researchers.[27,39,43−45] The values of V_2 and K_{S2} have been chosen close to the partial molar volume and adiabatic compressibility of ice I, whereas ΔV, ΔK_S, and f_1 have been approximately adjusted using eqs 4 and 10 to obtain the values of $V_0°$ and $K_{S0}°$ of liquid water at 25 °C. The cooperativity numbers, m and n, in reaction (1) have been both set to be equal to five. This choice is reasonable in view of the tetrahedrical arrangements of water molecules in ice I and ice III. We have used eqs 4 and 10 to calculate the partial molar volume, $V_0°$, and adiabatic compressibility, $K_{S0}°$, of liquid water as a function of mole fraction of the high-density species, f_1, at 25 °C. Figure 1 presents results of these calculations. As is seen from Figure 1, the relaxation contribution to the compressibility of bulk water (at $f_1 = 0.27$) is about 65%, in good agreement with earlier estimates.[37,60]

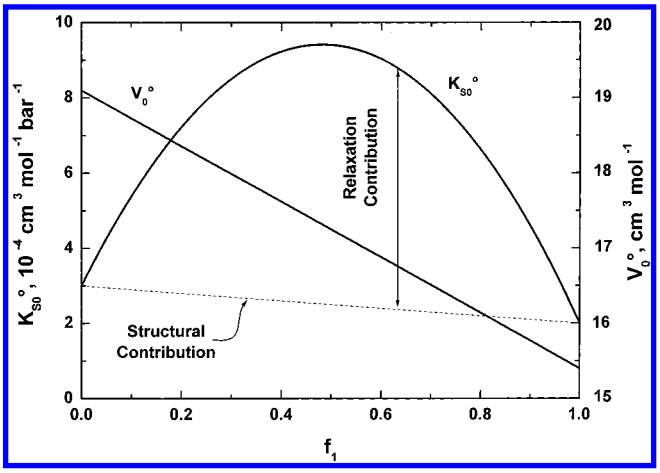

Figure 1. Partial molar volume, $V_0°$, and adiabatic compressibility, $K_{S0}°$, of liquid water as a function of its fractional composition, f_1, as calculated from eqs 4 and 10.

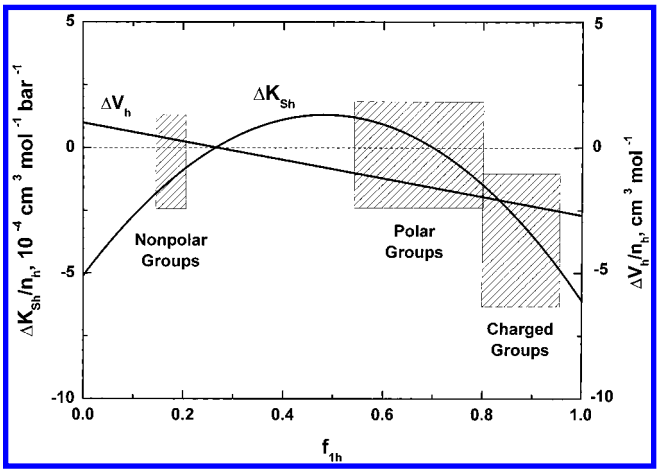

Figure 2. Solvent reorganization-induced changes in volume, $\Delta V_h/n_h$, and adiabatic compressibility, $\Delta K_{Sh}/n_h$, normalized per mole of hydration water as a function of its fractional composition, f_{1h}, as calculated from eqs 29 and 33. The shaded areas correspond to charged ($f_{1h} \approx 0.80$ to 0.95), polar ($f_{1h} \approx 0.55$ to 0.80), and nonpolar ($f_{1h} \approx 0.15$ to 0.20) groups (see in text).

Figure 2 shows how the solvent reorganization-induced changes in volume, $\Delta V_h/n_h$, and adiabatic compressibility, $\Delta K_{Sh}/n_h$, normalized per mole of water of hydration depend on the fractional composition, f_{1h}. These dependences have been calculated using eqs 29 and 33 for $n_h = 1$ and $f_1 = 0.27$. In fact, $\Delta V_h/n_h$ and $\Delta K_{Sh}/n_h$ respectively represent the differential partial molar volume and adiabatic compressibility of water of

TABLE 1: Volume, ΔV_h, and Adiabatic Compressibility, ΔK_{Sh}, Effects of Solvent Reorganization, Hydration Numbers, n_h, and Fractional Composition, f_{1h}, of the Hydration Shell for Electrolytes and the Amino Acid Glycine at 25 °C

solutes	ΔV_h cm^3mol^{-1}	ΔK_{Sh} 10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$	n_h	f_{1h}
LiCl	−20.7[a]	−36.6[b]	8.5	0.93
NaCl	−25.8[a]	−50.5[b]	10.2	0.96
KCl	−23.9[a]	−44.1[b]	9.6	0.94
RbCl	−23.2[a]	−41.4[b]	9.5	0.93
CsCl	−23.3[a]	−37.7[b]	9.8	0.91
NaBr	−25.3[a]	−43.0[c]	10.5	0.92
KBr	−23.4[a]	−36.0[c]	10.0	0.90
RbBr	−22.7[a]	−33.2[c]	9.9	0.89
CsBr	−22.8[a]	−29.7[c]	10.3	0.87
NaI	−24.7[a]	−31.7[c]	11.2	0.87
KI	−22.8[a]	−24.7[c]	10.8	0.84
RbI	−22.1[a]	−21.9[c]	10.7	0.83
CsI	−22.2[a]	−18.4[c]	11.2	0.80
glycine	−24.1[d]	−26.6[d]	11.4	0.84

[a] Data are from ref 60. [b] Data are from ref 62. [c] Data are from ref 61. [d] Data are from ref 67.

TABLE 2: Volume, ΔV_h, and Adiabatic Compressibility, ΔK_{Sh}, Effects of Solvent Reorganization, Hydration Numbers, n_h, and Fractional Composition, f_{1h}, of the Hydration Shell for Nucleic Acid Duplexes Groups at 25 °C

solutes	ΔV_h [a] $cm^3\,mol^{-1}$	ΔK_{Sh} [a] 10^{-4} $cm^3\,mol^{-1}\,bar^{-1}$	n_h	f_{1h}
poly(dAT)poly(dAT)	−51.8	−57.5	24.5	0.84
poly(dA)poly(dT)	−51.5	−57.5	24.3	0.84
poly(dGC)poly(dGC)	−71.5	−73.0	34.5	0.83
poly(dIC)poly(dIC)	−95.8	−101.8	45.7	0.84
poly(rA)poly(rU)	−55.7	−52.2	27.4	0.82
poly(rA)poly(dT)	−57.9	−55.2	28.4	0.82
poly(dA)poly(rU)	−50.5	−41.4	25.6	0.80
Salmon Testes	−37.5	−36.3	18.3	0.82
Human Placenta	−40.9	−39.1	20.0	0.82
Herring Testes	−35.9	−32.6	17.8	0.82
E. Coli	−39.9	−40.4	19.3	0.83
M. Lysodeikticus	−55.5	−54.4	27.0	0.83

[a] Data are from refs 64−66.

hydration and bulk water [see eq 28]. Inspection of Figure 2 reveals that, depending on f_{1h}, solutes with the same number of water molecules in their hydration shell, n_h, may exhibit vastly different (in magnitude *and* sign) values of ΔV_h and ΔK_{Sh}. This observation underscores limitations of qualitative characterizations of solutes as "more hydrated" or "less hydrated". Such characterizations may be misleading and often lacking any rigorous physical meaning.

Below, we examine the hydration properties of charged, polar, and nonpolar atomic groups based on their volume and compressibility contributions.

4.1 Charged Groups. Charged solutes typically exhibit highly negative values of ΔV_h and ΔK_{Sh}. Some examples of solutes, the hydration shells of which are dominated by electrostatic solute−solvent interactions, are electrolytes, zwitterionic amino acids, and nucleic acids. Table 1 shows the values of ΔV_h (second column) and ΔK_{Sh} (third column) at 25 °C for some alkali halides and glycine. Table 2 presents similar data for double stranded polynucleotides. For alkali halides, the data on ΔV_h have been reported by Hirata and Arakawa,[61] whereas the ΔK_{Sh} data have been reported by Millero et al.[62] and Mathieson and Conway.[63] The values of ΔV_h and ΔK_{Sh} for nucleic acid duplexes have been reported by Chalikian et al.[64,65] and Chalikian and Breslauer,[66] while ΔV_h and ΔK_{Sh} for glycine are from ref 67. In Table 2, ΔV_h and ΔK_{Sh} of the polynucleotide duplexes are expressed as per mole of nucleotide. The interaction volume, ΔV_h, for the polynucleotides have been calculated using eq 36 from the data on partial molar volumes, $V°$, intrinsic volumes, V_M, and solvent-accessible surface areas, S_A, reported for each duplex by Chalikian et al.[65] In these calculations, the thermal volume, V_T, is assumed to be proportional to the solvent-accessible surface area, S_A: $V_T = \delta S_A$. The proportionality coefficient, δ, of 0.56 Å represents the thickness of the thermal volume around the solute molecule.[57,68−70]

For each solute in Tables 1 and 2, the values of ΔV_h and ΔK_{Sh} have been used in conjunction with eqs 29 and 33 to calculate the hydration number, n_h, and the fractional composition, f_{1h}. Tables 1 and 2 list the calculated values of n_h (fourth column) and f_{1h} (fifth column). Inspection of Tables 1 and 2 reveals a number of important observations. First, judging by the value of f_{1h} (between 0.80 and 0.96 as compared to f_1 of 0.27 for bulk water), the equilibrium between the two water species in the hydration shell of charged groups is strongly shifted toward the more densely packed structural species.

Second, the hydration number, n_h, for alkali halides is relatively constant (8.5 to 11.2) even though the size of the cation and anion changes significantly, going from Li^+ to Cs^+ and from Cl^- to I^-. The individual hydration properties of these electrolytes are mostly determined by the fractional composition, f_{1h}, which varies between 0.80 and 0.96. In fact, the value of f_{1h} reflects the electrostriction in the hydration shell of alkali halides: the stronger the electrostriction the closer the value of f_{1h} to unity. As is seen from Table 1, in agreement with conventional wisdom, electrostriction of alkali halides decreases with increasing the size of the cation or anion. For example, f_{1h} is equal to 0.96 and 0.80 for NaCl and CsI, respectively.

Third, for zwitterionic amino acid glycine, f_{1h} is equal to 0.84. Analogous to electrolytes, the equilibrium between the two structural species of water molecules in the hydration shell of glycine is shifted toward the high density/high enthalpy species. The hydration number, n_h, of 11.4 is in reasonable agreement with our earlier estimate of 15 ± 1.[67]

Fourth, for the nucleic acid duplexes, the values of f_{1h} are within the narrow range of 0.80 to 0.84. The individual hydration properties of double stranded nucleic acids differing in structure, sequence, and composition are reflected in the "quantity of hydration" (n_h) rather than in the "quality of hydration" (f_{1h}). The values of n_h range from 17.8 for herring testes DNA to 45.7 for poly(dIC)poly(dIC) [Note that there are only about 20 water molecules within the first coordination layer of a nucleic acid duplex[64]]. These numbers, which are in excellent agreement with our earlier estimates,[65,66] suggest the presence of a multilayer hydration shell around the more extensively solvated duplexes [e.g., poly(dIC)poly(dIC) or poly(dGC)poly(dGC)]. This conclusion is consistent with the results of theoretical calculations and X-ray characterizations of DNA hydration.[71−73]

Inspection of Figures 1 and 2 reveals that water molecules solvating charged groups (f_{1h} is between 0.80 and 0.96) are strongly contracted with the average partial molar volume, V_h, between 15.5 and 16.0 cm^3mol^{-1} (85 to 90% of that of bulk water). In addition, owing to a strong reduction in the relaxation contribution, the partial molar adiabatic compressibility, K_{Sh}, of water of electrostatic hydration is reduced to 3.2×10^{-4} to 6.6×10^{-4} $cm^3mol^{-1}bar^{-1}$ (see Figure 1) that correspond to 40 to 80% of the partial molar adiabatic compressibility, K_{S0}, of bulk water.

4.2 Polar Groups. The "interaction volume", ΔV_h, of a polar group is negative and relatively insensitive to its position with respect to other polar groups in a solute molecule (at 25 °C,

TABLE 3: The Volume, ΔV_h, and Adiabatic Compressibility, ΔK_{Sh}, Effects of Solvent Reorganization, Hydration Numbers, n_h, and Fractional Composition, f_{1h}, of the Hydration Shell for Polar Atomic Groups at 25 °C

groups	ΔV_h [a] cm^3mol^{-1}	ΔK_{Sh} [a] 10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$	n_h	f_{1h}
-OH (in serine)	−7.0[a]	−6.0[b]	3.5	0.81
-OH (in threonine)	−5.5[a]	−5.5[b]	2.7	0.83
-OH (in tyrosine)	−6.5[a]	−1.3[b]	3.9	0.72
$-CONH_2$ (in asparagine)	−13.0[a]	−10.0[b]	6.7	0.80
$-CONH_2$ (in glutamine)	−12.0[a]	−2.7[b]	7.1	0.72
-OH (in pentoses)	−4.8[c]	−2.2[c]	2.7	0.76
-OH (in hexoses)	−5.0[c]	−2.2[c]	2.8	0.75
-OH (in 6-deoxyhexoses)	−4.7[c]	−2.5[c]	2.6	0.77
-OH (in 2-deoxypentoses)	−4.1[c]	−0.1[c]	2.6	0.70
-OH (in 2-deoxyhexoses)	−4.4[c]	−0.1[c]	2.8	0.70
-CONH- (in oligoglycines)	−10.5[d]	0.5[d]	6.8	0.69
-OH (in aliphatic alcohols)	−4.6[e]	3.8b	4.0	0.58
an average polar group in globular proteins	−26.0[f]	−15.5[f]	14.0	0.77

[a] Data are from ref 69. [b] Data are from ref 74. [c] Data are from ref 75. [d] Data are from ref 5. [e] Data are from ref 57. [f] Data are from ref 76.

$\Delta V_h \approx -5.5 \pm 1.5\ cm^3mol^{-1}$).[57,69] By contrast, the compressibility contribution of a polar group strongly depends on its proximity to other polar or charged groups of a solute.[58,74] For example, at 25 °C, the compressibility contribution, ΔK_{Sh}, of a "single" polar group, which is separated from other polar groups of a solute by more than five covalent bonds, is positive and may be as large as $5 \times 10^{-4}\ cm^3mol^{-1}bar^{-1}$. The compressibility contribution, ΔK_{Sh}, of a polar group in close proximity to other polar or charged groups (separated by less than three covalent bonds) is negative ranging from -6×10^{-4} to $-2 \times 10^{-4}\ cm^3mol^{-1}bar^{-1}$. Intermediate polar groups are characterized by compressibility contributions close to zero. As shown below, the two-state model of solute hydration described above provides a simple explanation for this long-standing intriguing observation.

Table 3 shows the volume, ΔV_h, and adiabatic compressibility, ΔK_{Sh}, effects of solvent reorganization in the hydration shell of polar groups in amino acids,[69,74] simple sugars,[75] aliphatic alcohols,[57,74] and globular proteins.[76] Equations 29 and 33 have been used to calculate the hydration number, n_h, and fractional composition, f_{1h}, for each polar group listed in Table 3. Inspection of Table 3 reveals that the hydration number, n_h, of a polar group in a small solute varies from 2.6 to 4.0, whereas f_{1h} ranges from 0.58 to 0.83.

Inspection of Figures 1 and 2 reveals that, when the value of f_{1h} is below 0.70 (but above 0.27), the partial molar adiabatic compressibility, K_{Sh}, of water of hydration is enhanced relative to bulk water due to a greater relaxation contribution. This observation enables one to account for the differential compressibility behavior of single and closely located polar groups. Single polar groups exhibit the values of f_{1h} between 0.58 and 0.70 and, consequently, are characterized by positive values of ΔK_{Sh}. By contrast, closely located polar groups influence their solvating water molecules more strongly thereby shifting f_{1h} to higher values (between 0.70 and 0.83). For these values of f_{1h}, the partial molar adiabatic compressibility, K_{Sh}, of water of hydration is smaller (due to a smaller relaxation contribution) than that of bulk water (see Figures 1 and 2). Hence, closely located polar groups exhibit negative values of ΔK_{Sh}. These results suggest that the differential hydration properties of single and closely located polar groups are determined by the strength of solute−solvent interactions, as reflected in f_{1h}, rather than by the amount of affected water molecules, as reflected in n_h.

TABLE 4: Volume, ΔV_h, and Adiabatic Compressibility, ΔK_{Sh}, Effects of Solvent Reorganization, Hydration Numbers, n_h, and Fractional Composition, f_{1h}, of the Hydration Shell for Nonpolar Atomic Groups at 25 °C

groups	ΔV_h [a] $cm^3\ mol^{-1}$	ΔK_{Sh} [a] 10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$	n_h	f_{1h}
$-C_6H_5$ (benzene ring in Gly-Gly-Phe)	0.7	−2.6[a]	2.6	0.20
$-CH_2-$ (in α,ω-aminocarboxylic acids)	0.4	−1.6[b]	1.0	0.16

[a] Data are from ref 79. [b] Data are from ref 67.

Compared to polar groups in small molecules, an average polar group in a globular protein exhibits significantly more negative values of ΔV_h ($-26.0\ cm^3mol^{-1}$) and ΔK_{Sh} ($-15.5 \times 10^{-4}\ cm^3mol^{-1}bar^{-1}$). Our analysis reveals that, on average, a polar group in a globular protein has the hydration number, n_h, of 14 (see Table 3). In other words, a polar group in a globular protein (with n_h of 14) affects 4 to 5 times more water molecules than a polar group in a small solute (with n_h of 2.6 to 4). These results are suggestive of cooperative water networks formed in the vicinity of polar protein groups. The average value of f_{1h} for a polar protein group is 0.77, which is within the range typical for closely located polar groups.

Armed with the values of f_{1h} between 0.58 and 0.83, the volume and compressibility of water molecules solvating polar groups can be estimated from Figure 1. Water molecules solvating polar groups are characterized by the partial molar volume, V_h, of 16.0 to 16.9 cm^3mol^{-1} (90 to 95% of that of bulk water). The partial molar adiabatic compressibility, K_{Sh}, of water molecules solvating polar groups may be larger or smaller than that of bulk water: K_{Sh} is between 6.5×10^{-4} and $9.1 \times 10^{-4}\ cm^3mol^{-1}bar^{-1}$ (80 to 110% of that of bulk water).

4.3 Nonpolar Groups. Aliphatic and aromatic groups, which are collectively referred to as nonpolar or hydrophobic groups, do not directly interact with solvating water molecules. Instead, water molecules in the hydration shell of nonpolar groups are forced to form their complement solvent−solvent hydrogen bonds within a more limited space thereby exhibiting altered structural/thermodynamic properties. It is thought that the volume effect of solvent reorganization, ΔV_h, around nonpolar solutes is close to zero.[57,77] By contrast, the compressibility effect of solvent reorganization, ΔK_{Sh}, is not zero and steeply changes from highly negative values below 35 °C to positive values at higher temperatures.[58,67,74,78] At 25 °C, the values of ΔK_{Sh} for an aliphatic $-CH_2-$ and aromatic $-C_6H_5$ groups are equal to -1.6×10^{-4} and $-2.6 \times 10^{-4}\ cm^3mol^{-1}bar^{-1}$, respectively[67,79] (see Table 4).

Equations 29 and 33 have been used to characterize the hydration properties of aliphatic and aromatic groups. In this analysis, the use of ΔV_h equal to zero or slightly negative values does not yield any meaningful numbers for f_{1h} and n_h. In

contrast, the use of small *positive* values of ΔV_h greatly improves the quality of calculations yielding reasonable numbers. Hence, we have used in our analysis the values of ΔV_h equal to 0.4 and 0.7 cm^3mol^{-1} for the $-CH_2$- and $-C_6H_5$ groups, respectively. These small positive values are within experimental error for most volumetric measurements on solutions of biological compounds. In fact, small positive values have been predicted for ΔV_h for a number of nonpolar solutes in aqueous solutions based on concepts of scaled particle theory.[57,77] Inspection of Table 4 reveals that, for an aromatic $-C_6H_5$ ring, f_{1h} equals 0.20 and n_h equals 2.6, whereas, for an aliphatic methylene $-CH_2$- group, f_{1h} is 0.16 and n_h is 1. Note that, in contrast to polar and charged groups, nonpolar groups exhibit the f_{1h} values smaller than that of bulk water. This observation indicates that, at 25 °C, the distribution between the two structural species in water molecules solvating nonpolar groups is shifted toward the low density/low enthalpy species. As seen from Figures 1 and 2, the negative sign of ΔK_{Sh} reflects a diminution in the relaxation contribution to the partial molar adiabatic compressibility, K_{Sh}, of water molecules solvating nonpolar groups (f_{1h} is between 0.15 and 0.20) relative to waters in the bulk state.

Our calculated hydration numbers, n_h, for aliphatic and aromatic groups are three to four times smaller than the number of water molecules in direct contact with these groups. In fact, this discrepancy is not entirely unexpected in view of the current understanding of aqueous solvation of small hydrophobic species.[80−82] It has been proposed that the hydration bond network of water molecules in the vicinity of small hydrophobic solutes is not distorted significantly. Hydrogen bonds between adjacent water molecules may remain intact even when these molecules are in direct contact with the solute. Hydrogen bonds simply may go around the solute. However, there is an entropic cost to the net free energy of hydrophobic hydration: the presence of small hydrophobic species limits the configurational space available for hydrogen bonding.

5. Solutes. In general, biological compounds may contain all three types of chemical groups (charged, polar, and nonpolar). Hence, the hydration shell of a biological solute is heterogeneous with respect to local "thickness" (local hydration number, n_{hi}) and fractional composition (local fractional composition, f_{1hi}). The volume, ΔV_h, and compressibility, ΔK_{Sh}, effects of solvent reorganization around solute molecule should contain contributions from all solvent-exposed atomic groups

$$\Delta V_h = \sum_{i=1}^{N} \Delta V_{hi} \tag{38}$$

$$\Delta K_{Sh} = \sum_{i=1}^{N} \Delta K_{Shi} \tag{39}$$

where N is the number of solvent-exposed atomic groups; ΔV_{hi} and ΔK_{Shi} are, respectively, the local values of ΔV_h and ΔK_{Sh} in the vicinity of the i-th atomic group; ΔV_{hi} and ΔK_{Shi} are given by the following relationships

$$\Delta V_{hi} = n_{hi}\Delta V(f_{1hi} - f_1) \tag{40}$$

$$K_{Shi} = n_{hi}\Delta K_S(f_{1hi} - f_1) + n_{hi}(\Delta V^2/RT)\{nmf_{1hi}(1 - f_{1hi})/[n(1 - f_{1hi}) + mf_{1hi}] - nmf_1(1 - f_1)/[n(1 - f_1) + mf_1]\} \tag{41}$$

Note that the local value of n_{hi} for a solvent-exposed group in eqs 40 and 41 may or may not correlate with its solvent-accessible surface area. This consideration is important since solvent-accessible surface area is often invoked as a quantitative measure of the amount of solute hydration.

TABLE 5: Volume, ΔV_h, and Adiabatic Compressibility, ΔK_{Sh}, Effects of Solvent Reorganization, Hydration Numbers, n_h, and Fractional Composition, f_{1h}, of the Hydration Shell for Monosaccharides at 25 °C

solutes	ΔV_h [a] $cm^3\ mol^{-1}$	ΔK_{Sh} [a] 10^{-4} $cm^3\ mol^{-1}\ bar^{-1}$	n_h	f_{1h}
2-Deoxyribose	−16.2	−5.7	9.3	0.74
Ribose	−22.8	−13.1	12.3	0.77
Arabinose	−25.6	−21.1	13.0	0.80
Xylose	−22.8	−13.7	12.2	0.77
Lyxose	−24.2	−14.3	13.0	0.77
2-Deoxyglucose	−20.7	−4.6	12.3	0.72
2-Deoxygalactose	−22.8	−8.3	13.0	0.74
6-Deoxyglucose	−22.6	−15.4	11.9	0.79
6-Deoxygalactose	−24.5	−21.4	12.4	0.81
Glucose	−29.9	−19.3	15.8	0.78
Mannose	−29.7	−15.2	16.3	0.76
Galactose	−31.0	−21.5	16.2	0.79
Allose	−30.8	−21.5	16.1	0.79
Tallose	−29.2	−12.7	16.4	0.75

[a] Data are from ref 75.

When analyzing solute hydration, one needs to separate the hydration contributions of constituent atomic groups based on eqs 38−41. Such a separation is model dependent, not straightforward, and not always possible. Alternatively, the "effective" hydration properties of a solute can be determined by using the experimentally measured values of ΔV_h and ΔK_{Sh} directly without breaking them down into group contributions. However, the danger is that the determined effective values of n_h and f_{1h} may not have any realistic meaning. Specifically, the effective value of n_h may not be equal to $\sum_{i=1}^{N} n_{hi}$, whereas the effective value of f_{1h} may not be equal to $N^{-1} \sum_{i=1}^{N} f_{1hi}$. Consequently, such an analysis may be highly misleading, especially when the hydration properties of chemically diverse molecules are compared. Nevertheless, such an analysis may sometimes be justified yielding useful data. One example is when the hydration shell of a solute is dominated by one type of solute−solvent interactions (e.g., the hydration shell of polyanionic nucleic acids is overwhelmingly dictated by electrostatic solute−solvent interactions). Another example is when one compares chemically similar molecules (e.g., homologous proteins). In this case, a qualitative distinction in terms of "stronger" or "weaker" hydration between solutes, all belonging to the same class, can be obtained.

As an example, we analyze below the hydration properties of monosaccharides and globular proteins (It should be noted that other macromolecules such as polysaccharides also can be treated in a similar way). Monosaccharides consist of only aliphatic and hydroxyl groups all of which are solvent accessible. On the other hand, globular proteins exhibit remarkably constant solvent accessible surface areas of charged (14 ± 4%), polar (33 ± 7%), and nonpolar (53 ± 5%) groups.[76] Therefore, one may plausibly assume that, on average, the hydration shells of monosaccharides and native globular proteins are homogeneous within the class. With this assumption, the effective hydration properties of these solutes can be justifiably analyzed using eqs 29 and 33. Tables 5 and 6 list the calculated values of n_h (fourth column) and f_{1h} (fifth column) for some monosaccharides and globular proteins, respectively. Table 6 also shows the numbers of water molecules within the first coordination layer for each protein (fourth column, in the parenthesis). These numbers have been estimated by dividing the solvent accessible surface area, S_A, of each protein by 9 $Å^2$, the effective cross-section of a water molecule. The values of ΔV_h and ΔK_{Sh} for the mono-

TABLE 6: Volume, ΔV_h, and Adiabatic Compressibility, ΔK_{Sh}, Effects of Solvent Reorganization, Hydration Numbers, n_h, and Fractional Composition, f_{1h}, of the Hydration Shell for Globular Proteins at 25 °C

solutes	ΔV_h [a] $cm^3\ mol^{-1}$	ΔK_{Sh} [a] 10^{-4} $cm^3\ mol^{-1}$ bar^{-1}	n_h	f_{1h}
Hemoglobin	−7525	−6086	3822 (2834)	0.80
Ovalbumin	−5821	−4588	2972 (1882)	0.80
Pepsin	−5738	−4029	2994 (1524)	0.79
α-Chymotrypsinogen A	−5365	−3579	2825 (1202)	0.78
α-Chymotrypsin	−5249	−3561	2756 (1184)	0.78
Trypsin	−4995	−3565	2599 (1056)	0.79
Trypsinogen	−4497	−3013	2367 (1075)	0.78
Myoglobin	−3103	−1664	1692 (857)	0.77
α-Lactalbumin	−3333	−1901	1801 (802)	0.77
Lysozyme	−3417	−2170	1814 (752)	0.78
Ribonuclease A	−3436	−2108	1835 (754)	0.78
Cytochrome *c*	−2139	−1519	1114 (679)	0.79

[a] Data are from ref 76. [b] The number of water molecules within the first hydration shell of each protein is shown in parentheses.

saccharides are from ref 75. For each protein, the value of ΔV_h has been calculated from eq 36 using the data on the partial molar volume, $V°$, intrinsic volume, V_M, and solvent-accessible surface area, S_A.[76] The thermal volume, V_T, is assumed to be proportional to S_A: $V_T = \delta S_A$. The thickness, δ, of the thermal volume around a globular protein is equal to 1 Å.[76] The values of ΔK_{Sh} of the proteins have been calculated using eq 37. The intrinsic compressibility, K_M, of a protein is proportional to its intrinsic volume, V_M: $K_M = \beta_M V_M$. The intrinsic coefficient of adiabatic compressibility, β_M, for a globular protein is 25×10^{-6} bar^{-1}.[59,76,83]

5.1 Monosaccharides. Inspection of Table 5 reveals that the values of f_{1h} for the monosaccharides are between 0.72 and 0.81, a range typical for polar groups. This observation is consistent with the hydration properties of monosaccharides being dominated by polar groups and serves to justify our analysis in terms of "effective" hydration. The hydration numbers, n_h, for the monosaccharides range between 9.3 and 16.4 and generally correlate with the number of heavy atoms (carbons or oxygens) in the molecule. An important observation is that the differential hydration properties of isomeric saccharides are determined by f_{1h} rather than n_h. For example, for 2-deoxyglucose and 6-deoxyglucose which exhibit significantly different partial molar adiabatic compressibilities (-4.6×10^{-4} $cm^3 mol^{-1} bar^{-1}$ versus -15.4×10^{-4} $cm^3 mol^{-1} bar^{-1}$), f_{1h} is equal to 0.72 and 0.79, respectively, whereas n_h is, practically, the same (12.3 and 11.9, respectively).

5.2 Proteins. Inspection of Table 6 reveals that the hydration shell of native globular proteins is very homogeneous with respect to the average value of f_{1h} (f_{1h} is between 0.77 and 0.80). This range is typical for polar groups. This observation is consistent with the hydration properties of globular properties being dominated by polar groups. The relative "size" of the hydration shell of a globular protein may vary significantly: the ratio of n_h to the number of water molecules within the first coordination layer ranges from 1.35 (hemoglobin) to 2.46 (trypsin). However, for all the proteins studied, the effective number of solvating waters is significantly larger than the number of water molecules within the first hydration layer. This observation suggests that solvent networks involving waters from more than one coordination sphere are formed at the protein surface.

On the basis of Figure 1, the average partial molar volume, V_h, of water of protein hydration is about 16.2 $cm^3 mol^{-1}$ (90% of that of bulk water), whereas the average partial molar adiabatic compressibility, K_{Sh}, is about 6.8×10^{-4} $cm^3 mol^{-1} bar^{-1}$ (~80% of that of bulk water). Our estimate of the partial molar volume of water of protein hydration is in good agreement with recent experimental data of Svergun et al.[84] These authors have investigated the globular proteins lysozyme, E. coli thioredoxin reductase, and protein R1 of *E. coli* ribonucleotide reductase by both X-ray and neutron scattering in H_2O and D_2O solutions. Their data reveal that the first hydration shell of a globular protein has an average density 10% larger than that of the bulk solvent.

6. Implications for the Energetics of Solute Hydration. Solvent reorganization in the vicinity of a solute molecule is not an independent process: it is thermodynamically coupled with other events accompanying solute dissolution (cavity formation, direct solute−solvent interactions, solvent-induced solute modification, disruption of solute−solute interactions, entropic factors, etc.).[11,85] Therefore, it is very difficult to reliably separate the energetics of solute reorganization from the rest of the energetics of solute dissolution using purely calorimetric approaches. An alternative way for calculating the energetic functions of solvent reorganization (ΔG_h, ΔH_h, ΔS_h, and ΔC_{Ph}) is based on the use of eqs 30, 31 and 34. For these calculations, one needs to know the differential values of ΔH, ΔS, and ΔC_P for the two structural species of water molecules, which can be determined by fitting thermodynamic functions of liquid water. Accurate determination of these quantities is out of the scope of this paper. Instead, we will focus on qualitative discussion of energetic trends of solvent reorganization in the vicinity of charged, polar, and nonpolar groups.

The approximate values of ΔH, ΔS, and ΔC_P can be estimated based on the assumption, used in developing our model, that the two structural species of liquid water can be approximated by the ice I and ice III polymorphs. The partial molar heat capacity, $C_P°$, of ice I is 9 cal $mol^{-1}K^{-1}$, whereas that of ice III is 13 cal $mol^{-1}K^{-1}$ [from the temperature dependence of the enthalpy of ice I-to-ice III transition[27]]. Hence, ΔC_P should be roughly equal to 4 cal $mol^{-1}K^{-1}$. The differential enthalpy, ΔH, of the two structural species can be approximately evaluated from eq 17. With ΔC_P of 4 cal $mol^{-1}K^{-1}$ and f_1 of 0.27, ΔH should be roughly equal to ~1.2 kcal mol^{-1} to obtain from eq 17 the partial molar heat capacity, $C_{P0}°$, of bulk water of ~18 cal $mol^{-1}K^{-1}$. Our estimate of 1.2 kcal mol^{-1} is within the range of ΔH values calculated by other investigators within the framework of two-state models of liquid water (0.5 to 3.1 kcal mol^{-1}).[27] The value of ΔS can be calculated from $\Delta G = -RT \ln[f1/(1 - f1)] = \Delta H - T\Delta S$. Because ΔG is ~600 cal mol^{-1} (based on $f_1 = 0.27$), ΔS is roughly 2.0 cal $mol^{-1}K^{-1}$.

Armed with these approximate values of ΔH, ΔS, and ΔC_P, we now use eqs 30, 31 and 34 to calculate the normalized values of $\Delta H_h/n_h$, $\Delta S_h/n_h$, and $\Delta C_{Ph}/n_h$ as a function of the fractional composition, f_{1h}, of water of hydration. Figures 3−5 present results of these calculations. In Figure 4, $\Delta S_h/n_h$ has been calculated without taking into account a possible contribution of Ψ_h, a decrease in translational and orientational degrees of freedom of water molecules within the solute hydration shell. Therefore, the real values of $\Delta S_h/n_h$ should be more negative than the calculated ones.

Inspection of Figures 3−5 reveals a number of important observations. First, relative to bulk water, water molecules solvating nonpolar groups (f_{1h} is between 0.15 and 0.20) exhibit a lower enthalpy (Figure 3), entropy (Figure 4), and heat capacity (Figure 5). The values of $\Delta H_h/n_h$, $\Delta S_h/n_h$, and $\Delta C_{Ph}/n_h$ are on the order of −200 cal mol^{-1}, −0.5 cal $mol^{-1}K^{-1}$, and −3 cal $mol^{-1}K^{-1}$, respectively. The negative sign of

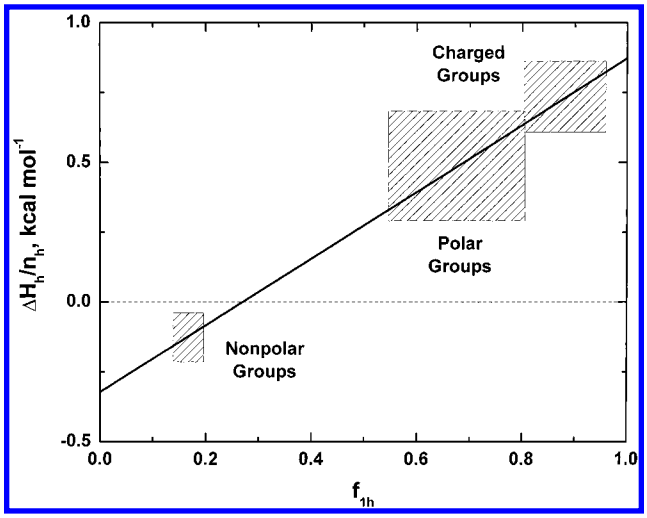

Figure 3. Solvent reorganization-induced changes in enthalpy, $\Delta H_h/n_h$, normalized per mole of hydration water as a function of its fractional composition, f_{1h}, as calculated from eq 30. The shaded areas correspond to charged ($f_{1h} \approx 0.80$ to 0.95), polar ($f_{1h} \approx 0.55$ to 0.80), and nonpolar ($f_{1h} \approx 0.15$ to 0.20) groups (see in text).

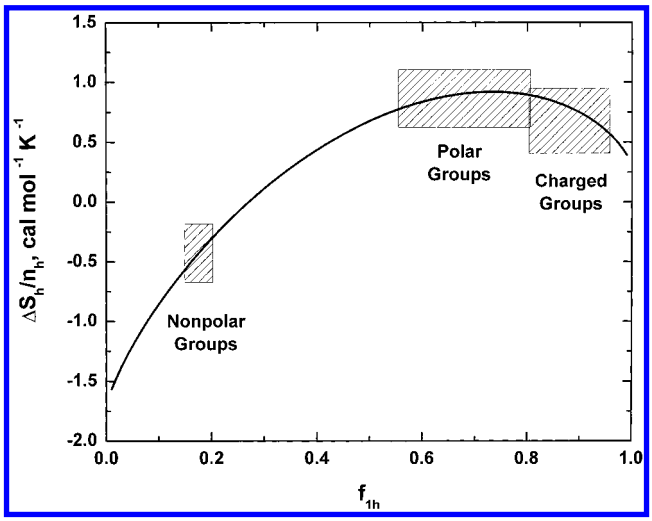

Figure 4. Solvent reorganization-induced changes in entropy, $\Delta S_h/n_h$, normalized per mole of hydration water as a function of its fractional composition, f_{1h}, as calculated from eq 31. The shaded areas correspond to charged ($f_{1h} \approx 0.80$ to 0.95), polar ($f_{1h} \approx 0.55$ to 0.80), and nonpolar ($f_{1h} \approx 0.15$ to 0.20) groups (see in text).

$\Delta H_h/n_h$ reflects the shift in equilibrium between the two structural species of water molecules solvating nonpolar groups toward the low density/low enthalpy state. The negative sign of $\Delta H_h/n_h$ and $\Delta S_h/n_h$ coincides with the sign of the enthalpy and entropy of hydrophobic hydration at room temperature derived from calorimetric and solute transfer thermodynamics data.[18] By contrast, the negative sign of $\Delta C_{Ph}/n_h$ (reflecting a decrease in the relaxation contribution to $C_{Ph}°$) is opposite to the positive sign of experimentally determined heat capacity of hydrophobic hydration.[18]

It should be noted that care must be exercised when our data are compared with the solute transfer thermodynamics data reported, for example, by Makhatadze and Privalov [ref 18 and other similar studies]. In general, direct comparison between the two sets of data is incorrect. The energetics of solute hydration, as defined in ref 18, is given by the change in Gibbs free energy, enthalpy, entropy, and heat capacity accompanying the transfer of a solute molecule from a fixed position in an ideal gas phase into a fixed position in water. Consequently,

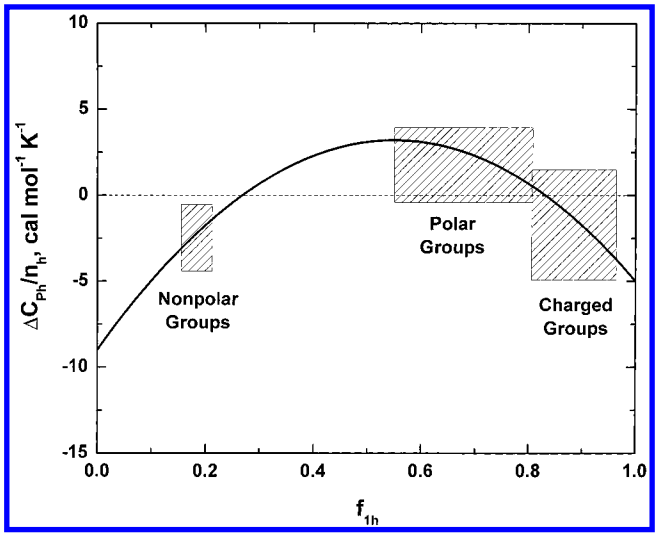

Figure 5. Solvent reorganization-induced changes in heat capacity, $\Delta C_{Ph}/n_h$, normalized per mole of hydration water as a function of its fractional composition, f_{1h}, as calculated from eq 34. The shaded areas correspond to charged ($f_{1h} \approx 0.80$ to 0.95), polar ($f_{1h} \approx 0.55$ to 0.80), and nonpolar ($f_{1h} \approx 0.15$ to 0.20) groups (see in text).

the free energy, enthalpy, entropy, and heat capacity of solute hydration determined in ref 18 include not only the contribution from solvent reorganization but also contributions from cavity formation, solute−solvent interactions, and solute reorganization if any. By contrast, our estimates of ΔH_h, ΔS_h, and ΔC_{Ph} have been calculated specifically for solvent reorganization. With this distinction in mind, the observed disparity between $\Delta C_{Ph}/n_h$ from this work and ΔC_P of hydrophobic hydration from ref 18 may originate from the positive heat capacity of cavity formation and solute−solvent interactions which prevail over the negative heat capacity of solvent reorganization.

Second, water molecules solvating polar groups (f_{1h} is between 0.55 and 0.8) exhibit higher enthalpy, entropy, and heat capacity relative to bulk water. Specifically, $\Delta H_h/n_h$, $\Delta S_h/n_h$, and $\Delta C_{Ph}/n_h$ are on the order of 500 cal mol^{-1}, 1 cal mol^{-1}K^{-1}, and 2 cal mol^{-1}K^{-1}, respectively. The positive sign of $\Delta H_h/n_h$ reflects the shift in the structural equilibrium of water molecules solvating polar groups toward the high density/high enthalpy species. The positive sign of $\Delta C_{Ph}/n_h$ reflects an increase in the relaxation contribution to the partial molar heat capacity, $C_{Ph}°$, of water solvating polar groups.

It is worth noting that the values of enthalpy, entropy, and heat capacity reported for polar hydration are all negative.[18] The negative sign of enthalpy of polar hydration from ref 18, perhaps, can be accounted for by a very large negative enthalpy of direct solute−solvent interactions (hydrogen bonding plus van der Waals interactions) that prevails over the positive contributions from cavity formation and solvent reorganization. The negative sign of entropy of polar hydration from ref 18 may be related to the unaccounted negative contribution of Ψ_h, a decrease in translational and orienational entropy of water molecules in the hydration shell of polar groups. In addition, a significant decrease in entropy may result from the reduction in rotation of polar groups caused by hydrogen bonding with solvating water molecules.[86] Negative heat capacity of polar hydration from ref 18 may reflect a decrease in the absolute value of enthalpy of solute−solvent interactions with increasing temperature.

Third, water molecules solvating charged groups (f_{1h} is between 0.8 and 0.95) exhibit a larger enthalpy and entropy but smaller heat capacity relative to water in the bulk state.

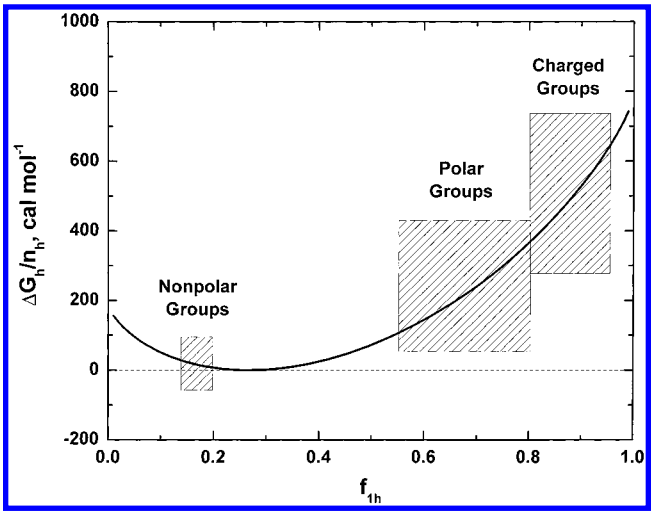

Figure 6. Solvent reorganization-induced changes in free energy, $\Delta G_h/n_h = \Delta H_h/n_h - T\Delta S_h/n_h$, normalized per mole of hydration water as a function of its fractional composition, f_{1h}. The shaded areas correspond to charged ($f_{1h} \approx 0.80$ to 0.95), polar ($f_{1h} \approx 0.55$ to 0.80), and nonpolar ($f_{1h} \approx 0.15$ to 0.20) groups (see in text).

Specifically, $\Delta H_h/n_h$, $\Delta S_h/n_h$, and $\Delta C_{Ph}/n_h$ are on the order of 700 cal mol^{-1}, 0.7 cal mol^{-1}K^{-1}, and -0.5 cal mol^{-1}K^{-1}, respectively. The positive sign of $\Delta H_h/n_h$ reflects the shift in structural equilibrium of water molecules solvating charged groups toward the high density/high enthalpy species, while the negative sign of $\Delta C_{Ph}/n_h$ reflects a decrease in the relaxation contribution to $C_{Ph}°$. The positive sign of $\Delta H_h/n_h$ and $\Delta S_h/n_h$ is opposite to the negative sign of the enthalpy and entropy of hydration of charged groups determined from calorimetric and solute transfer thermodynamics data.[18] Analogous to polar groups, the negative sign of enthalpy of hydration of charged groups may originate from a very large negative enthalpy of solute−solvent interactions (charge-dipole interactions plus van der Waals interactions), whereas the negative sign of hydration entropy is probably due to the unaccounted contribution of Ψ_h.

Finally, combining the data presented in Figures 3 and 4, one may calculate the free energy of solvent reorganization, $\Delta G_h/n_h = \Delta H_h/n_h - T\Delta S_h/n_h$. Figure 6 plots $\Delta G_h/n_h$ as a function of f_{1h}. Inspection of Figure 6 reveals that $\Delta G_h/n_h$ is positive for nonpolar (50 to 100 cal mol^{-1}), polar (100 to 400 cal mol^{-1}), *and* charged (400 to 700 cal mol^{-1}) groups. Thus, solvent reorganization is thermodynamically unfavorable for any atomic group, with charged groups exhibiting the most unfavorable free energy followed by polar and nonpolar groups. *In other words, the contribution of solvent reorganization to the net energetics of solute dissolution is always unfavorable (ΔG_h is positive) independent of the chemical nature of the solute molecule.* Even though this conclusion may sound somewhat counterintuitive (especially, for polar and charged groups), it is in agreement with conventional wisdom. *If ΔG_h were not positive for any solute or atomic group, then the solute-specific solvent reorganization would occur even in the absence of the solute.*

One implication of this observation is that intramolecular hydrogen bonding interactions between polar groups within a protein or DNA molecule should be energetically more favorable than intermolecular hydrogen bonding interactions between polar groups and adjacent water molecules. In intramolecular hydrogen bonding, polar groups satisfy their hydrogen bonding propensities without unfavorable contribution from concomitant solvent reorganization. Thus, the burial of a polar group should have a positive impact on the conformational stability of proteins and nucleic acids. This conclusion is consistent with and provides rationalization for the two recent experimental reports of the highly favorable contribution of polar groups forming hydrogen bonds within the interior of a globular protein to its conformational stability.[87,88] In fact, Pace, based on his site-directed mutagenesis studies, has concluded that the burial of an amide group contributes more to protein stability than the burial of an aliphatic group of an equivalent size.[87] To account for this observation, Pace has suggested that "the hydrogen bonding and van der Waals interactions of peptide groups in the tightly packed interior of folded protein are more favorable than similar interactions with water in the unfolded protein".[87] This suggestion is consistent with unfavorable free energy of solvent reorganization in the vicinity of solvent-exposed polar groups.

7. Concluding Remarks

A simple two-state structural model of solute hydration has been developed. In this model, both water in the bulk state and water of solute hydration are presented as consisting of two structural species: a high density/high enthalpy species, structurally similar to ice III, and a low density/low enthalpy species, structurally similar to ice I. It is assumed that structural and thermodynamic distinctions between bulk and hydration water originate solely from the differential fractional composition, whereas the two structural species and thermodynamic parameters associated with each species are identical in bulk and hydration water. On the basis of this model, analytical expressions for changes in volume, compressibility, expansibility, free energy, enthalpy, entropy, and heat capacity accompanying solvent reorganization in the solute hydration shell have been derived. These expressions provide the link between the solute-induced changes in solvent structure, energetics, and volumetric characteristics.

The derived analytical expressions have been used in conjunction with volumetric data reported in the literature to analyze the hydration properties of charged, polar, and nonpolar groups at 25 °C. The equilibrium between the two structural species of water of hydration of charged groups is strongly shifted toward the high density/high enthalpy species. The partial molar volume and adiabatic compressibility of water of hydration of charged groups are significantly smaller than those of bulk water. For water of hydration of polar groups, the equilibrium between the two structural species is still shifted toward the high density/high enthalpy species but not as strongly as for charged groups. The partial molar volume of water of hydration of polar groups is smaller than that of bulk water. The partial molar adiabatic compressibility of water of polar hydration can be larger or smaller than that of bulk water depending on how strongly the equilibrium between the two species is shifted toward the high density/high enthalpy species. In contrast to charged and polar groups, the equilibrium between the two species of water solvating nonpolar groups is shifted toward the low density/low enthalpy species. The partial molar volume of water of nonpolar hydration is slightly higher than that of bulk water, whereas the partial molar adiabatic compressibility is lower relative to bulk water.

Solvent reorganization was found to be thermodynamically unfavorable for any atomic group, with charged groups exhibiting the most unfavorable free energy followed by polar and nonpolar groups. In other words, the contribution of solvent reorganization to the net energetics of solute dissolution is always unfavorable independent of the chemical nature of the solute molecule. However, the magnitude and the sign of

enthalpy, entropy, and heat capacity of solvent reorganization strongly depend on the chemical nature of solvent exposed groups.

In the aggregate, our results provide a foundation for the development of more refined thermodynamic models of solute hydration and a more rigorous interpretation of macroscopic data on solution thermodynamics in terms of microscopic events. In addition, these results underscore the potential importance and usefulness of combining volumetric and calorimetric data for a more complete thermodynamic description of microscopic events, in particular, solute hydration.

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