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A Simple Two-Structure Model for Liquid Water

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Abstract: Using no adjustable parameters but only known data on ice and on water dimers in the gas phase, it has been possible for the first time to construct a model involving isomerization between clusters which reproduces (±2%) the anomalous heat capacity of liquid water from 0 to 100 °C. The clusters are a polycyclic, cubic-shaped octamer which can dissociate into two cyclic tetramers. These clusters are held together by H bonds and though very labile appear to be the principal species present in liquid water. Polycyclic decamers and monocyclic pentamers may also exist and play a similar role. It is shown that a random H bond breaking model cannot account for the heat capacity of water.

Liquids can be loosely divided into two classes. The first are described as "regular" or unstructured liquids whose molecules are held together by relatively weak nondirectional, van der Waals forces and fill space fairly well. The second are the structured liquids whose molecules are held together by much stronger and oriented forces, usually hydrogen-bonded or polar. Regular liquids follow well the simple empirical rules such as Trouton's rule, the Guy Guildberg rule, and simple two-parameter equations of state such as the Berthelot equation. They generally have close packing with about 10–11 nearest neighbors. Structured liquids show marked departures from the simple empirical rules and can have many fewer nearest neighbors, in the case of water as few as 4.5 on the average. They do not fill space well and have voids.

Among the structured liquids water is perhaps the most unique with a maximum in its density at 4 °C and an anomalously high heat capacity. No simple van der Waals liquid can show such behavior.

These unusual properties have been generally thought to stem from structure imposed by the existence of the hydrogen bond in the liquid state. Surely no liquid has been more studied than water, and yet no adequate model for its thermal and volumetric properties has been presented. Recent efforts have focused on exploring the feasibility of long-lived clusters of water molecules to account for the observed heat capacity of liquid water, a value well in excess of that derivable from its gas properties. In a previous paper, it was shown that the excess heat capacity could be explained in terms of energy changes accompanying chemical isomerization in water,[1] but the identity of the isomers of water was not pursued. Many researchers have tried to treat the anomalous heat capacity as arising from the breaking of H bonds.[2–6] The most successful[2,5] have started with the data on C_p and simply assumed the values of ΔH and ΔS for the process needed to reproduce the values of C_p from 0 to 100 °C. Others, starting with spectroscopic data[3] in an effort to deduce C_p, have not been successful. Angell[6] showed that simple bond breaking without large values of ΔS for the process could not come close to fitting C_p. We will show later that random breaking of H bonds by itself does not have a sufficiently large entropy increase accompanying it to contribute significantly to C_p. We will also show that only an equilibrium between discrete species or clusters can have a sufficiently large entropy to contribute to C_p. In the present paper, we shall identify these species and estimate their contributions to C_p for liquid water.

Water Clusters

Energetic considerations restrict appreciably the possible candidates for an isomerization process. Starting with ice whose structure and enthalpy are well known, we can assign the bulk of its energy of sublimation, 11.7 kcal/mol, to hydrogen bonds.[7] In ice each water molecule shares four hydrogen bonds with its nearest neighbors, resulting in a net of 2 mol of hydrogen bonds/mol of water. Assigning all of the binding in ice to H bonds would put an upper limit of 5.85 kcal/mol as the energy of the H bond at 0 °C. From the value of the second virial coefficient of water over the temperature range 100–200 °C,[7] we find a value for the dissociation enthalpy of water dimers to monomer of 5.7 kcal/mol at 100 °C, and this puts a lower limit on the energy of the H bond of 4.9 kcal/mol. The difference of 0.8 kcal corresponds to the difference between enthalpy and energy at 150 °C. The lower limit is discussed later. Note also that the O···H–O distance in ice is appreciably smaller than that in the gas-phase dimer. For the clusters we employ here we will use a value of 5.5 for the H bond energy.

The enthalpy of melting of ice is 1.4 kcal/mol. Since the density change on melting is only 10%, it seems reasonable to ascribe this in major part to hydrogen bond breaking which would correspond to about 0.25 or $^1/_4$ of a hydrogen bond per water molecule in ice.

If we simply broke at random $^1/_4$ of the H bonds in ice which would leave 1.75 H bonds per H_2O, we should hardly expect that it would result in a fluidlike as opposed to a solidlike substance. For fluid properties, discrete units, $(H_2O)_n$, which can move independently of each other are required. The smallest structural units which had been suggested earlier,[1] linear polymeric chains or rings, suffer from the fact that they have at most one H bond per water molecule, which is too few to account for the energy of vaporization of water (9.9 kcal/mol or about 1.7 H bonds per water molecule at 300 K).[7] The next simplest candidate would be a polycyclic structure with a larger number of H bonds per water molecule. Sandwich-type structures consisting of totally joined cyclic rings fall into this category.

The simplest ring structure composed of relatively unstrained H bonds is a cyclic tetramer. If we fuse two such cyclic tetramers together, we arrive at a cubelike structure composed of eight water molecules with their oxygen atoms at the corners of the cube. Some tetramers and octamers are shown in Figures 1 and 2. The H–O–H angle in the water molecule is 104° while the expected tetrahedral angle for icelike structures is 109.5°. The 90° angle between oxygen atoms in the tetramer and cubic octamer would require a 7–10° distortion of the hydrogen bond, which involves a relatively small cost in energy per bond.[7]

Each water in the cubic octamer shares three H bonds corresponding to a net of 1.5 H bonds per water molecule. This is much closer to approximating the energy of liquid water of about 1.7 H bonds per water molecule. We could gain an additional H bond per octamer by joining three of them in a symmetrical ring. There is, however, no way of fusing two octamers together by more than one bond without having highly strained H bonds. We shall show

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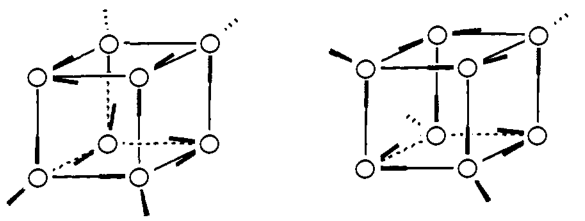
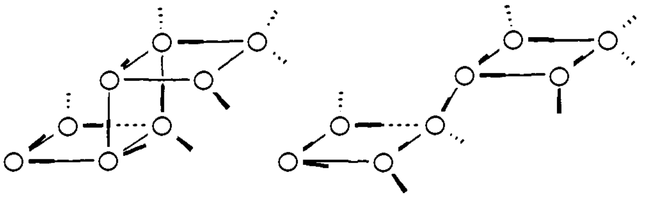


a) Low Symmetry Cube
1.5 H-Bonds/H_2O

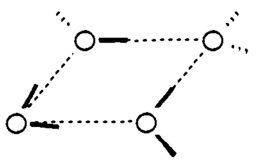
b) High Symmetry Cube
1.5 H-Bonds/H_2O



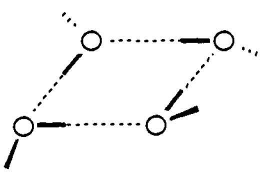
c) Tricyclic Octamer
1.25 H-Bonds/H_2O

d) Bicyclic Octamer
1.125 H-Bonds/H_2O

Figure 1. Some octamers of water. H atoms are represented by solid or dashed bars extending from O atoms.



a) Polar Tetramer



b) Non-Polar Tetramer

Figure 2. Some cyclic tetramers of water (see legend of Figure 1).

that the extra energy requirement of the octamer amounting to about $^{1}/_{4}$ H bond per water molecule (or about 1.4 kcal/mol of water) can be provided by van der Waals interactions. This is not possible for linear polymers or cyclic rings so that the octamer is our first candidate for a discrete entity in liquid water.

Kistenmacher et al.[8] have examined theoretically a number of low-energy octamers and found them to have hydrogen bonds with energies between 4.5 and 5.2 kcal/mol. Stillinger and David,[9,10] using a polarization model potential function, explored an octamer with the structure of bicyclooctane, having nine H bonds and found it to have even stronger H bonds of about 8.1 kcal/mol of H bond. Brink and Glasser[11] examined all of these models using a number of different potential functions and concluded that the cubic structure shown in Figure 1b was the most stable of all the octamers. They did not explore the structure shown in Figure 1a in which the dipoles of the water molecules occupying corner positions of the cubes are favorably aligned; this is probably a more stable structure than Figure 1b in which water dipoles in two of the faces are unfavorably aligned. We will return to this question of octamer structure later.

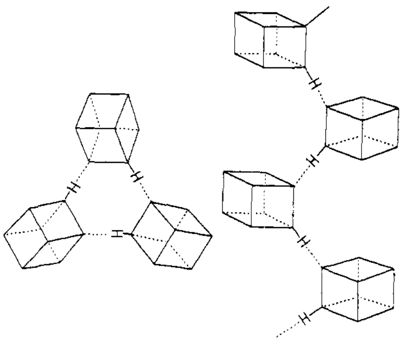


Figure 3. Some H-bonded oligomers of H-bonded octamers (see legend of Figure 1).

The simplest equilibrium which we will explore in the present paper is that between the cubic octamer (Figure 1) and the cyclic tetramer (Figure 2):

$$(H_2O)_8 \rightleftharpoons 2(H_2O)_4 \qquad (1)$$

The octamers shown in Figure 1a,b have been assigned oxygen–oxygen distances of 2.83 Å consistent with a recent calculation on small water clusters.[12] When the van der Waals radius of 1.51 Å is assigned to the oxygen atoms at the corners of the cube, the cluster assumes the approximate shape of a cube with four rounded corners and four corners with small protuberances due to the hydrogen–oxygen bonds which extend about 0.7 Å beyond the oxygen atoms. If we neglect these H atoms, the octamer cube has an edge of approximately 5.85 Å = [2.83 + 2(1.51)]. If these octamers were stacked in a simple cubic array to form a liquid, they would occupy a volume of 120.5 cm^3/mol of octamer or about 15.1 cm^3/mol of H_2O. Making allowances for the four H atoms would increase this volume to about 133 cm^3/mol of cluster or 16.6 cm^3/mol of H_2O. (This is based on the increment of 6.3 cm^3/mol for successively adding H_2 to benzene to form cyclohexadiene, cyclohexene, and cyclohexane.)

A similar calculation of the approximately half-cube slab for the tetramer using an oxygen–oxygen distance of 2.89 Å[12] as shown in Figure 2 yields a volume of 63.5 cm^3/mol of tetramer or about 15.9 cm^3/mol of H_2O. Accounting for the four H's on the tetramer as for the octamer increases the volume of the tetramer to 71.5 cm^3/mol or 17.9 cm^3/mol of H_2O. Here the H atom increment is based on the 4 cm^3 difference in molar volumes between cyclobutane (77.8 cm^3/mol) and cyclobutene (73.7 cm^3/mol). Both clusters have molar volumes that are too small to account for the observed molar volume of liquid water. This immediately suggests that if liquid water is an assembly of these units, there must be significant H-bonding *between* clusters to account for the larger molar volume of liquid water.

Such H-bonding would prevent the efficient space-filling suggested by the cubic and half-cube shapes of these simple clusters without adding too much to the cohesion of liquid water. A linear polymer made up of octamers would have at most one H bond per cluster amounting to about 5.7/8 or 0.7 kcal/mol of H_2O. The proposed structure of the octamer lends itself to forming cyclic trimers with unstrained H bonds. This is illustrated in Figure 3. A looser dimer consisting of two octamers with one H bond between them would have an additional cohesive energy of about 0.35 kcal/mol of H_2O.

The tetramers could similarly hydrogen bond to form looser dimers (isooctamers) with either one or two hydrogen bonds as shown in Figure 1. These dimers, containing eight molecules of water, would have cohesions intermediate between the tetramer and octamer with 0.7 kcal/mol of H_2O (one H bond *per iso-*

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octamer) or 1.4 kcal/mol of H_2O (two H bonds *per isooctamer*). A liquid composed of our simplest building blocks, the tetramers and octamers, could similarly be composed of random assemblies of H-bonded clusters. Note that while two tetramers could be joined by two relatively unstrained H bonds, this would not be true of two octamers or of an octamer and a tetramer.

In considering the energetics of space-filling for the simple clusters, we must estimate the van der Waals energy of attraction of a liquid consisting of either pure octamer or pure tetramer. An analogy can be made between our water clusters and the CH_2 unit in cyclic hydrocarbons. We would expect the van der Waals energies to be very close. As an example we find that the heats of vaporization of cyclopentane ($(\overline{(CH_2)_5})$) and tetrahydrofuran ($\overline{CH_2(CH_2)_3O}$) are 6.5 and 6.8 kcal/mol, respectively, despite the dipole present in the furan. In similar fashion cyclohexane and 1,4-dioxane have ΔH_{vap} of 7.1 and 7.5 kcal, respectively, while $\overline{CH_2(CH_2)_4O}$ has ΔH_{vap} of 7.3 kcal. We also find that the molar volumes of the cyclic hydrocarbons correspond to about 18 cm^3/mol per CH_2 unit, very close to the value we estimate for the water clusters.

On this basis we will use cyclobutane ΔH_{vap} as the basis for assigning a van der Waals energy to the tetramer. It comes to about 1.3-kcal cohesion energy per CH_2 unit. For cyclooctane it is about 1.2 kcal per CH_2 unit. Similar numbers can be arrived at from the energies of vaporization of liquid O_2, estimating the number of O···O contacts and assuming similar van der Waals cohesion for H_2O contacts and O···O contacts.

One further comment on the disposition of the H bonds in these oligomers is of interest. In Figure 2b we show a symmetrical *counterclockwise array of H bonds* in a cyclic tetramer. These water molecules have the favored conformation of the H bond in the dimer of water.[12] They have negligible dipole–dipole interactions, and the symmetrical arrangement of the H bonds results in zero net dipole moment for the assembly. Hence, we have termed these clusters "nonpolar". The polar tetramer shown in Figure 2a has a head-to-tail dipole attraction of two of the water molecules across the ring. With the 2.89-Å distance between O atoms estimated for the tetramer, this yields a 1.4-kcal stabilization energy for this polar conformation or about 0.35 kcal per H_2O. It is estimated to have a net dipole moment in excess of 4.0 D. The octamer shown in Figure 1a may be visualized as being constructed from two of these polar tetramers with their net dipoles adjacent and antiparallel. In such a fusion of the tetramers, the water molecules with two H atoms pointing out of the ring will be rotated down by about 45° so that one of these H atoms bonds to the O atom below. In the nonpolar octamer (Figure 1a) the head-to-tail conformation of dipoles that existed in the polar tetramer (Figure 2b) has lost about 30% of its attractive energy by this rotation or about 0.11 kcal/mol of H_2O. Despite this loss, the preferred conformations will still be the polar tetramer and the low-symmetry cubic octamer it can form on dimerization.

Liquid Water

In the present paper we simplify the structural considerations by neglecting the associations between clusters to form larger units. Instead, we treat the equilibrium between octamers and tetramers as that obtaining in a regular, unstructured liquid. We assume an ideal mixture, i.e., that these forms of water are miscible in all proportions, and that they have a negligible heat of mixing. The enthalpy of the mixture can then be written as

$$H_{mix} = n_o H_o + n_t H_t$$

where n_o is the number of moles of water molecules bound up as octamer, n_t is the number of moles of water molecules bound up as tetramer, H_o is the enthalpy per mole of water bound up in the octamer, and H_t is the enthalpy per mole of water bound up in the tetramer. For 1 mol of water, $n_o + n_t = 1$, and

$$H_{mix} = H_o + n_t \Delta H \tag{2}$$

where $\Delta H = H_t - H_o$, the difference in enthalpy between a mole of water bound as octamer and that of a mole of water bound as tetramer.

The heat capacity of the water mixture is obtained by taking the derivative of the enthalpy with respect to temperature. At constant pressure

$$C_{mix} = (dH/dT)_p = C_{p,o} + n_t \Delta C_p + \Delta H\,(dn_t/dT) \tag{3}$$

where $\Delta C_p = C_{p,t} - C_{p,o}$, the difference between the heat capacity of 1 mol of water tied up as tetramer ($C_{p,t}$) and 1 mol tied up as octamer ($C_{p,o}$).

The number of moles of water as tetramer as a function of temperature is found using the equilibrium constant expression for eq 1 in terms of mole fractions, x_i, of each species:

$$K_1 = x_t^2/x_o$$

When the mole fraction is expressed in terms of number of moles of tetramer and octamer, the equilibrium constant becomes

$$K_1 = n_t^2/(n_{total} n_o) \tag{4}$$

When eq 4 is rewritten in terms of the number of moles of water molecules bound as the oligomer, $n_o = n_o/8$ and $n_t = n_t/4$, and for 1 mol of water ($n_o + n_t = 1$), the equilibrium constant for eq 1 becomes

$$K_1 = 4n_t^2/(1 - n_t^2) \tag{5}$$

whereupon $n_t^2 = K_1/(4 + K_1)$, an expression very similar to that derived earlier for a more general equilibrium.[1]

Equation 3 can be simplified further by using an alternate expression for dn_t/dT. Taking the natural logarithm of eq 5 and differentiating with respect to temperature gives

$$\frac{d \ln K_1}{dT} = \frac{2}{n_t(1 - n_t^2)} \frac{dn_t}{dT} \tag{6}$$

or

$$\frac{dn_t}{dT} = \frac{n_t(1 - n_t^2)}{2} \frac{d \ln K_1}{dT}$$

From thermodynamics, $d \ln K/dT = \Delta H/RT^2$, where ΔH is the change in enthalpy for the equilibrium in eq 1. Note that the enthalpy change per mole of water equals ΔH previously defined in eq 2:

$$\tfrac{1}{8}\Delta H_{reaction} = \tfrac{1}{8}(2H_t - H_o) = \tfrac{1}{8}(2(4H_t) - 8H_o)$$

$$\Delta H = H_t - H_o \tag{7}$$

Substitution of eq 7 into eq 3 gives

$$C_{mix} = C_{p,o} + n_t \Delta C_p + [n_t(1 - n_t^2)R/2](\Delta H/RT)^2 \tag{8a}$$

Equation 8a becomes our working equation, where the heat capacity of liquid water composed of a mixture of octamers and tetramers results from three terms: (1) the heat capacity of one structure (we have chosen the octamer), (2) a term arising from the intrinsic difference between the heat capacities of the two forms, and (3) a term referred to as the contribution due to isomerization, C_{pi}.

The third term on the right-hand side of eq 8a represents the contribution to the heat capacity of the isomerization equilibrium, C_{pi}. We can use eq 5 to express C_{pi} in terms of only the thermochemical quantities ΔH and K_1.

$$C_{pi} = \frac{R}{2}\left[\frac{(K_1/4)^{1/2}}{(1 + K_1/4)^{3/2}}\right]\left[\frac{\Delta H}{RT}\right]^2 \tag{8b}$$

At fixed values of $\Delta H/RT$, C_{pi} has a very broad maximum at $K_1 \approx 2$. Values of $K_1 = 1$ or 4 have a value of C_{pi} only 10% less than its value at this maximum. At the maximum $n_t = 0.58$ while it is 0.71 at $K_1 = 4$, and 0.45 at $K_1 = 1$. Hence, n_t is twice as sensitive to K_1 than is C_{pi}. In contrast we see that C_{pi} is most sensitive to the value of ΔH. Note that n_t goes monotonically with K_1 from 0 at $K_1 = 0$ to 1 at $K_1 \to \infty$.

The task now is to determine realistic values for the heat capacity of 1 mol of water bound as octamer, the same for the

Table I. Number of Degrees of Freedom for Octamer and Tetramer

	total	intramolecular	librational	torsional	int (intracluster)
octamer	72	24	3	3	42
tetramer	36	12	3	3	18

Table II

	O•••O distance (Å)	no. of shared H bonds per H_2O	no. of H bonds per H_2O
ice	2.77	4	2
octamer	2.83	3	1.5
tetramer	2.89	2	1

tetramer, the equilibrium constant (from which the number of moles of water bound as tetramer, n_t, can be determined), and the ΔH ($=H_t - H_o$) per mole of water for the isomerization equilibrium between octamers and tetramers (eq 1). Calculation of the equilibrium constant, K_1 will require both ΔH_1 and ΔS_1.

In calculating reasonable values of heat capacity and entropy for the octamer and tetramer in the liquid state, the intracluster bends and stretches of the individual water molecules themselves along with the six modes of motion (three hindered translational or librational and three hindered rotational or torsional modes) of the cluster are considered. In addition, the internal motion in the cluster associated with the hindered movement of one water molecule against another must be considered. Table I shows the assignment of the $3N$ (where N is the number of atoms) degrees of freedom for each cluster.

Due to the high frequency of the O–H stretch ($\nu = 3600$ cm^{-1}) and bending motions ($\nu = 1700$ cm^{-1}), it is safe to neglect any *intra*molecular contribution of the water molecules themselves to the heat capacity and entropy of the water cluster. Further, the hindered translational (librational) and torsional modes of the clusters are expected to contribute classically. The remaining internal motion of one molecule against another within the cluster has been assumed to be "icelike"; we have calculated their contributions on the basis of the heat capacity and entropy of ice. The following sections describe in more detail how these internal contributions of each cluster to thermodynamic quantities were determined.

Heat Capacity

The heat capacity of each water cluster in the liquid state is the sum

$$C_p = C(\text{libl}) + C(\text{torsl}) + C(\text{int}) + C(\text{lattice exp}) \qquad (9)$$

The classical assignment of the three librational and three torsional modes gives $6R$ or 12 entropy units to the heat capacity of each cluster in the liquid. The contributions of the internal modes of freedom (42 for the octamer and 18 for the tetramer) are the icelike internal modes of the cluster. This contribution is found by first extrapolating the molar heat capacity of ice, which varies essentially linearly over the temperature range from 100 to 273 K, to temperatures up to 100 °C:[7]

$$C_p(\text{ice}) = 9.10 + 0.034(T - 273)$$

Since C_p(ice) includes a contribution arising from lattice expansion, we must estimate this contribution from the thermodynamic relation

$$C_p - C_v = VT\alpha^2/\beta \qquad (10)$$

where α is the coefficient of volume expansion and β is the compressibility of ice. From data on ice[7] between −143 and −10 °C, it is possible to evaluate α and β as functions of temperature:

$$10^6\alpha_{\text{ice}}\ (\text{K}^{-1}) = 164 + 0.85(T - 273)$$

and

$$10^6\beta_{\text{ice}}\ (\text{bar}^{-1}) = 12.8 + 0.015(T - 273)$$

Note that α is much more sensitive to temperature than β. The contribution to the heat capacity at constant pressure from the lattice expansion is calculated and subtracted to find heat capacity at constant volume for the following treatment (see Table III).

Table III. Average Frequencies in Water Clusters from a Fit of Heat Capacity

temp (K)	C_p(ice) (eu)	C_v(ice) (eu)	C_v(ice)/6 (eu)	ν_{ice} (cm^{-1})	ν_o (cm^{-1})	ν_t (cm^{-1})
253	8.42	8.21	1.37	373	316	242
263	8.76	8.52	1.42	369	313	240
273	9.10	8.83	1.47	363	309	236
298	9.95	9.58	1.60	340	288	221
323	10.80	10.32	1.72	298	253	194
348	11.65	11.03	1.84	231	196	150
373	12.50	11.72	1.95	*a*		

[a] The value is so close to the classical limit R that identification with a classical oscillator loses meaning.

Table IV. Heat Capacity of Water Clusters (cal/(mol K))

	o/t			
temp (K)	int	lattice	total per mole of cluster	total per mole of H_2O
253	64.13/30.62	9.73/5.23	85.86/47.85	10.73/11.96
263	65.74/31.04	10.11/5.44	87.85/48.48	10.98/12.12
273	67.32/31.47	10.50/5.64	89.82/49.11	11.23/12.28
298	71.24/32.52	11.46/6.16	94.70/50.68	11.84/12.67
323	75.08/33.55	12.42/6.68	99.50/52.23	12.44/13.06
348	78.85/34.58	13.38/7.20	104.23/53.78	13.03/13.44
373	82.56/35.63	14.34/7.71	108.90/55.34	13.61/13.84

The remaining heat capacity of ice (at constant volume) is attributed to the six modes of oscillation in the solid corresponding to hindered libration and rotation in the solid. Taking an average contribution per mode (by dividing C_v by 6), an average frequency (ν_{ice}) corresponding to each oscillation is found. The next task is to adjust realistically the frequencies in order to account for longer O•••O distances in the clusters than in ice and to account for the fact that there are fewer hydrogen bonds per water molecule in each cluster than in ice. Our model calls for the parameters listed in Table III.

The vibrational frequency within each cluster is lowered from the average value in ice for two reasons. First, the fractional increase in bond length in the cluster relative to ice gives

$$\nu'_o = 2.77/2.83\nu_{\text{ice}} = 0.98\nu_{\text{ice}} \qquad (11a)$$

$$\nu'_t = 0.92\nu_{\text{ice}}$$

Then, because the frequency of a harmonic oscillator varies as the square root of restoring force in a bond, the frequency is inversely proportional to the square root of the number of hydrogen bonds per molecule. To account for the change in the number of H bonds per molecule in the cluster compared to that in ice, the frequencies of the octamer and tetramer are lowered:

$$\nu_o = (1.5/2)^{1/2}\nu'_o \qquad \nu_t = (1/2)^{1/2}\nu'_t \qquad (11b)$$

The adjusted frequencies determined at several temperatures between −20 and +100 °C are given in Table III. On the basis of these adjusted frequencies, the contribution to the heat capacity from each "average" oscillation can be calculated.[16] The octamer in the liquid state has contributions from 42 such average internal oscillations (see Table I), while the tetramer in the liquid state has 18. The internal heat capacity of each cluster is listed in Table IV.

The infrared and Raman spectra of ice show broad liberational (hindered translations) bands centered about 229 cm^{-1} and a broad torsional bond around 800 cm^{-1}.[7] Our choice of an average frequency for both of these district modes to simulate C_v for ice is a gross simplification which avoids a very complex analysis of these bonds and their anharmonicities. It is responsible for the

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Table V. Average Frequencies of Water Clusters from a Fit of Entropy

temp (K)	S(ice) (eu)	S(ice)/6 (eu)	ν_{av} (cm^{-1})	ν_o[a] (cm^{-1})	νt[a] (cm^{-1})
253	8.43	1.40	243	206	158
263	8.77	1.46	245	208	160
273	9.10	1.52	248	211	162
298	9.93	1.66	254	216	165
323	10.77	1.80	259	220	168
348	11.62	1.94	262	222	170
373	12.47	2.08	264	224	172

[a] It is interesting to note that this table shows the oscillator frequencies for entropy almost constant over the temperature range, while those for heat capacity (Table III) are definitely temperature dependent; these frequencies are selected in order to obtain the best fit to the entropy and heat capacity values of ice which have been extrapolated to temperatures of interest in this study.

different frequencies used for the $S°$ and $C_p°$ of ice (Tables III and V). We will discuss this later.

The total heat capacity of each cluster in the liquid at constant pressure contains a contribution from the PV term (the contribution of the lattice expansion, see eq 10). Because the molar volume of the octamer (V_o = 133 cm^3/mol) is nearly twice that of the tetramer (V_t = 71.5 cm^3/mol), the expansion correction must be accounted for. Using values of $\alpha = 1.1 \times 10^{-3}$ K^{-1} and $\beta = 1 \times 10^{-4}$ atm^{-1} typical of a normal liquid and neglecting any temperature dependence of V, α, and β, the lattice expansion is calculated to be on the order of 10 eu for the octamer and 5 eu for the tetramer at 300 K; values are listed in Table IV.

The total heat capacity for each cluster, the sum of the classical librational and torsional contributions, the internal contributions from the movement of one water molecule against another in the cluster, and a lattice expansion term to convert to C_p (eq 9) are given in Table VII. Note that the difference in heat capacity between 1 mol of water bound as tetramer and 1 mol bound as octamer, ΔC_p, is very small.

Entropy

The entropy of each cluster in the liquid phase is calculated by first calculating the entropy of the cluster in the vapor phase and then subtracting the entropy lost on condensation to a normal liquid:

$$S_{liq} = S_{vap} - \Delta S(\text{vaporization}) \qquad (12)$$

The entropy of the vapor is due to translation and rotation of the clusters, along with the internal entropy due to the motions of the water molecules against each other in the cluster:

$$S_{vap} = S_{transl} + S_{rotl} + S_{int}$$

Intramolecular contributions of H_2O to the entropy are neglected in this treatment.

The translational and rotational contribution to the entropy of the clusters as an ideal gas in the vapor phase are[16]

$$S°_{transl} = 37.0 + \tfrac{3}{2}R \ln (M/40) + \tfrac{5}{2}R \ln (T/298) + R \ln n \qquad (13)$$

where M is the molecular mass (amu) and n is the number of optical isomers (taken as 1 in each case). For rotation

$$S°_{rotl} = 11.5 + R \ln (I^{3/2}/\sigma) + \tfrac{3}{2}R \ln (T/298) \qquad (14)$$

where I^3 is the product of the moments of inertia about the x, y, and z axes and σ is the external symmetry number of the cluster. For the octamer, we take $\sigma = 1$ for the cube in Figure 1a with three identical principal moments of inertia each equal to 509 amu·A^2; for the polar tetramer in Figure 2a, we take $\sigma = 2$ with two identical principal moments of inertia each equal to 134 amu·A^2 and the third moment equal to 269 amu·A^2. The translational and rotational contributions to entropy of each cluster are listed in Table VI.

The internal contributions to entropy of each cluster due to the movement of one water molecule against another are found in a fashion similar to those contributions calculated for the heat capacity. First, the entropy of ice is extrapolated to higher temperatures using

$$S_T(\text{ice}) = S_{273\,K}(\text{ice}) + C_p(\text{ice}) \ln (T_2/273)$$

where $S_{273\,K}$(ice) is the calorimetric entropy taken as 9.10 eu (4) and C_p(ice) is the average heat capacity of "ice" over the temperature range considered (see Table III). The entropy of ice at temperature T is divided by 6 to determine the average contribution per degree of freedom, and this entropy contribution is ascribed to an average frequency of vibration. The vibrational frequency is adjusted as for heat capacity to account for the longer O···O distance and the difference in the number of hydrogen bonds per water molecule (eq 11). The adjusted frequencies based on a fit of entropy are essentially independent of temperature and are listed in Table V.

The contributions to the internal entropy of the respective clusters calculated from the adjusted frequencies in Table V are listed in Table VI.

The difference in entropy between the vapor phase and the liquid ($-\Delta S$(vaporization)) is based on the premise that a liquid composed of octamers, as well as one composed of tetramers, roughly follows Trouton's rule, and thus at its boiling point, the liquid has an entropy of 21 eu less than the corresponding vapor

Table VI

temp (K)	transl		rotl		int		total		ΔS(con)		S/mol		S_t at 1 M	ΔS_{t-o}/ mol H_2O
	o	t	o	t	o	t	o	t	o	t	o	t		
253	40.02	37.94	29.71	25.01	74.00	42.63	143.73	105.58	−29.55	−22.14	114.18	83.44	88.96	7.97
263	40.22	38.14	29.82	25.12	76.47	43.72	146.51	106.98	−29.01	−21.78	117.50	85.20	90.72	7.99
273	40.40	38.32	29.94	25.24	78.93	44.80	149.27	108.36	−28.48	−21.42	120.79	86.94	92.46	8.02
277	40.48	38.40	29.98	25.28	79.92	45.23	150.38	108.90	−28.27	−21.28	122.11	87.62	93.14	8.02
298	40.84	38.76	30.20	25.50	85.04	47.73	156.08	111.99	−27.17	−20.55	128.91	91.44	96.96	8.13
323	41.24	39.16	30.44	25.74	91.09	49.99	162.77	114.89	−25.88	−19.70	136.89	95.18	100.70	8.06
348	41.62	39.54	30.66	25.97	97.12	52.51	169.40	118.02	−24.62	−18.90	144.78	99.12	104.64	8.06
373	41.96	39.88	30.87	26.17	103.13	54.99	175.96	121.04	−23.38	−18.10	152.58	102.94	108.46	8.04

Table VII. Equilibrium Constants and Mole Fractions[a]

temp	ΔH	$\Delta H/RT$	ΔS	K	n_t	$C_{p,i}$	$C_{p,o}$	ΔC_p	C_p(water)
253	2.41	4.763	7.97	0.459	0.321	6.531	10.619	1.026	17.479
263	2.43	4.620	7.99	0.535	0.344	6.467	10.862	0.928	17.648
273	2.44	4.469	8.02	0.632	0.369	6.370	11.105	0.831	17.782
277	2.45	4.422	8.02	0.662	0.377	6.323	11.201	0.792	17.748
298	2.48	4.161	8.13	0.908	0.430	6.070	11.869	0.592	18.094
323	2.51	3.885	8.06	1.156	0.473	5.545	12.292	0.360	17.902
348	2.53	3.635	8.06	1.484	0.520	5.014	12.871	0.136	17.845
373	2.55	3.418	8.04	1.825	0.560	4.491	13.625	−0.077	18.116

[a] Units are Kelvin for temp and entropy units for C_p and S.

at the boiling point. A small temperature correction should be added to account for the fact that the temperatures of interest here (−20 to +100 °C) are not the boiling points of pure octamer or pure tetramer (T_{bp}):

$$-\Delta S(\text{vaporization})_T = -21\text{ eu} + \int ((C_{\text{liq}} - C_{\text{vap}})/T)\,\mathrm{d}T \tag{15}$$

where the integration limits are from T_{bp} to T.

To apply the temperature correction, the boiling points of the clusters must be known. The boiling point of the liquid composed of octamers can be estimated as the boiling point of cyclooctane, a liquid which follows Trouton's rule; the boiling point of a liquid of tetramers is taken to be that of cyclobutane. The enthalpy of vaporization at the boiling point, which reflects the energy of the van der Waals forces, is also listed. Values at 300 K are in parentheses.

	ΔH_{vap}(bp) (ΔH_{vap}(300K)) (kcal/mol)		T_{bp} (K)
cyclooctane	8.8 (10.2)	octamer of water	422
cyclobutane	6.0 (5.85)	tetramer of water	285

The difference in C_p between the liquid and vapor forms of the cluster is

$$C_{p,\text{vap}}(\text{transl+rotl+int+}PV) = \tfrac{3}{2}R + \tfrac{3}{2}R + C(\text{int}) + R$$

$$C_{p,\text{liq}}(\text{libl+torsl+int+lattice exp}) = 3R + 3R + C(\text{int}) + VT\alpha^2/\beta \tag{16}$$

$$\Delta C_{p,\text{liq-vap}} = 2R + VT\alpha^2/\beta$$

Upon substitution of eqs 16 into 15

$$-\Delta S(\text{vaporization}) = -21 + 2R\ln(T/T_{\text{bp}}) + (V\alpha^2/\beta)(T - T_{\text{bp}}) \tag{17}$$

The entropies of the liquid clusters, calculated from eqs 13, 14, and 17, and the internal entropies, are listed in Table VI.

One additional contribution to entropy appears in Table VI. This involves establishing the standard state where tetramer as solute is taken at 1.0 M concentration:[13]

$$S^{\circ}_{\text{liq,t}}(1\text{M}) = S(\text{pure, 15.8 mol of t/L}) - R\ln 1/M$$

$$\Delta S = -R\ln 1/M$$

The correction to the standard state of $1/M$ for the tetramer (from the tetramer molarity of 15.8 M) is 5.52 eu/mol of tetramer.

Finally, the entropy change on mixing tetramer and octamer should be mentioned: $\Delta S_{\text{mix}} = -n_{\text{t}}\ln x_{\text{t}} - n_{\text{o}}\ln x_{\text{o}}$. While the value of the entropy change on mixing depends on the relative concentrations of the two clusters, the contribution is small and is not considered significant in this treatment.

Enthalpy Change on Isomerization

The enthalpy change per mole of water on isomerization comes in large part from breaking half of a hydrogen bond per water molecule (Table II) in going from one octamer to two tetramer molecules. There are, in addition, small differences in the van der Waals attractions and thermal energy which may be accounted for in the following cycle:

$$\begin{tikzcd}
VT & \text{octamer(liq)} \arrow[r, "\Delta H_T"] \arrow[dd, "\Delta H_1"] & \text{2 tetramer(liq)} & T \\
& & \text{2 tetramer(liq)} \arrow[u, "\Delta H_6"] & T_{\text{bp}}(\text{t}) = 285\text{ K} \\
T_{\text{bp}}(\text{o}) = 422\text{ K} & \text{octamer(liq)} \arrow[d, "\Delta H_2"] & \text{2 tetramer(vap)} \arrow[u, "\Delta H_5"] & T_{\text{bp}}(\text{t}) = 285\text{ K} \\
T_{\text{bp}}(\text{o}) = 422\text{ K} & \text{octamer(vap)} \arrow[r, "\Delta H_3"] & \text{2 tetramer(vap)} \arrow[u, "\Delta H_4"] & T = 422\text{ K}
\end{tikzcd}$$

The change in enthalpy, ΔH_T at temperature T, for the equilibrium between octamer and tetramer (eq 1) is the sum of each term in the cycle. On a molar basis for the water molecule, the following contributions to enthalpy change are used:

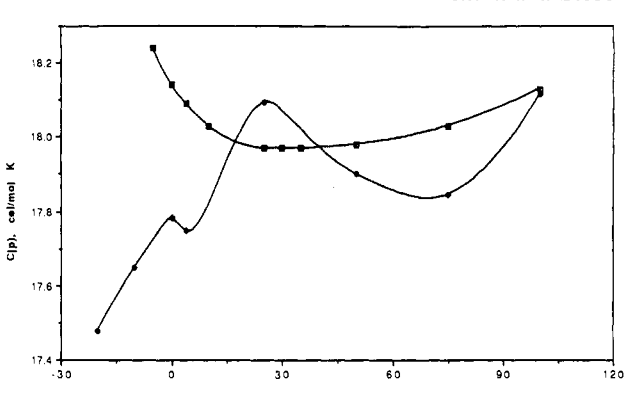

Figure 4. Heat capacity of water: ⊡, experimental values; ♦, calculated values (Table VII).

$\Delta H_1 = C_{p\text{o,liq}}(422\text{K}-T)$ where C_p is the average heat capacity per mole of water of the octamer over the temperature interval, and 422 K is the estimated boiling point of octamer (the boiling point of cyclooctane).

ΔH_2 = (8.8 kcal)/8 where 8.8 kcal, the heat of vaporization of cyclooctane at its boiling point, is used as an estimate of the van der Waals forces in the octamer; it is divided by 8 to put this value on a per mole water basis.

$\Delta H_3 = {}^1/_2\,(4.90 + 0.84/8)$ kcal where 4.95 kcal is the energy of a hydrogen bond, estimated at 100 °C and $0.84/8 = RT/8$ at T = 422 K from the gas dimer dissociation energy at 100 °C.

$\Delta H_4 = C_{p\text{vap,t}}(285\text{–}422\text{K})$ where 285 K is the estimated boiling point of tetramer (the boiling point of cyclobutane) and C_p is $R + {}^1/_4$ (C_v for the tetramer as vapor).

$\Delta H_5 = -(6.00/4)$ kcal where 6.00 kcal is the heat of vaporization of cyclobutane, the model for the water tetramer; it is divided by 4 to obtain a value per mole of water.

$\Delta H_6 = C_{p\text{liq,t}}(T\text{–}285\text{K})$ where $C_{p\text{liq,t}}$ is the average heat capacity per mole of water of the liquid tetramer over the temperature range. The enthalpy change at each temperature for the isomerization in eq 1 is calculated from the above contributions, and the total is listed in Table VII.

Calculation of the Heat Capacity of Water

The quantities necessary for a calculation of the heat capacity of water modeled on an equilibrium between two water clusters, the octamer and tetramer, are now available. Substitution into eq 8 of the calculated quantities at each temperature gives values of the heat capacity listed in Table VII. These calculated values of heat capacity at constant pressure are within 0.02–0.4 eu of the experimental values at each temperature, better than 2% agreement (Figure 4).

The average deviation over the range 0–100 °C is 0.7%. This at first somewhat remarkable agreement must be considered a bit fortuitous because of the sensitivity of the model to ΔH_1. We see in eq 8b that a 1% change in the value of ΔH_1 which would be about 0.03 kcal/mol would lead to a 2% change in C_{pi} or about 0.08 cal/(mol K), neglecting the accompanying change in K_1 due to a change in ΔH_1. Despite this caveat, the closeness of the fit must be considered very strong support for the cluster model of water. There have been many attempts over the last 30 years to model $C_p(H_2O)$, and none have done better than deviations of 15–25% over the range 0–100 °C.

Of special interest in Figure 4 is the very shallow minimum in the experimental heat capacity of water at about 35 °C. This is also shown by the estimated curve except that the minimum has been displaced to about 70 °C. It is quite likely that a refined treatment which would include the effect of further H-bonding between clusters at lower temperatures would also shift this minimum to lower temperatures where such equilibria would be more important. It is also possible that these equilibria would eliminate much of the decrease in C_p estimated below 0 °C for supercooled water.

Discussion

In ref 1 it was demonstrated that no simple one-fluid model of liquid water could reproduce the heat capacity of water to within 50%. It was further shown that even the simplest two-fluid model could account for the heat capacity of water over the range 0–100 °C quantitatively. It was also pointed out that only a narrow range of paired values of ΔH_1 and ΔS_1 for isomerization could do this. The present work presents two plausible structures which, with no arbitrary parameters, but only properties derived from the data on ice and the gas-phase H_2O dimer turn out to have values of ΔH_1 and ΔS_1 very close to the values indicated in the earlier work. This is not meant to imply that liquid water is solely a mixture of octamers and tetramers in equilibrium. These are rather the simplest structures which probably do exist as part of the structure of water.

Other structures, which are also plausible, are cyclic pentamers $(H_2O)_5$ along with their sandwich dimers, the decameric $(H_2O)_{10}$. These are in a way more attractive than the tetramer and octamer explored here since they consist of H bonds which are less bent than those in the octamer. The pentamer would have no bonds strained by more than 2° while the decamer would have only five bent by as much as 7°. While a cyclic hexamer and its dodecamer sandwich may seem equally attractive, it turns out that they have more strained H bonds than the pentamer/decamer or the tetramer/octamer pairs.

The same thermodynamic equations (eqs 4–8) apply to these dimerizations so that the formalism is the same for these alternative systems. It also turns out that the volumes per mole of H_2O of the pentamer/decamer pair are very close to those estimated for the tetramer/octamer pair. Without repeating the detailed calculation we would expect that C_{pi} would be very similar for the decamer/pentamer pair equilibrium. We can also have two decamers interacting to form two octamers plus a tetramer:

$$2n_{10} \rightleftharpoons 2n_8 + n_4$$

Such a process could take place via a series of steps, in each one of which one H bond would form while another broke so that the net change could be very rapid with minimal activation energy.

What can we say about $\Delta H_f°$ of the water formed from the octamer/tetramer or the decamer/pentamer system? We have not attempted to fit their properties to those of liquid water except semiquantitatively. The pure octamer liquid has an energy of vaporization to one mole of water monomer given by contributions from H-bonding (~8.4 kcal/mol), dipole interaction (0.4 kcal), van der Waals interactions (1.2 kcal), and RT (0.6 kcal/mol at 300 K) which total about 10.6 kcal/mol in excellent agreement with the measured value of 10.5 kcal/mol for the vaporization of liquid water. However, at 300 K water is 37% tetramer (Table VII), and this has a ΔH_i of 2.5 kcal. Thus, the mixture will be lower in enthalpy by an additional 0.9 kcal or will have a molar heat of vaporization of about 9.7 kcal/mol. It is not likely that enhanced van der Waals interactions could account for more than about 0.3 kcal of this total. Another 0.3 kcal could be accounted for in the uncertainty of the actual strength of the H bonds in the clusters while non-nearest-neighbor interactions and most likely random H bonds between clusters could make up the remaining differences.

It will not be a simple task to further refine the estimates of cluster energy described here. The differences in energy are too small, of the order of tenths of a kilocalorie, to yield simply to ab initio or empirical calculations. Many of the prior calculations have in fact ascribed much stronger bonding to the octamers than we have proposed here.

In ref 1 it was proposed that the isomeric form A (here octamer), more stable at lower temperatures, would have about a 20% lower molar volume than form B (here tetramer). The octamer and tetramer discussed here have similar molar volumes per water molecule. If allowances are made for the extraring H atoms, the octamer has about 10% smaller volume. To account for the volumetric behavior of water, we are forced to involve further equilibria in the form of H-bonded tetramers or octamers at lower temperatures. If we assume that there is a loss of about 20 eu in forming a hydrogen bond between two octamers or two tetramers, then such structures would become numerous below about 300 K, particularly the cyclic trimers of octamers (Figure 3) or tetramers (Figure 1c) since there is a much lower entropy loss per unit in ring formation than in simple dimerization. We do not attempt to deal with these equilibria here, but it may be noted that because they involve very small energies per water molecule they will have only very small contributions to C_{pi} (eq 8). Thus, one additional H bond between two octamers will have a C_{pi} contribution at 300 K of only 0.1 eu. It is for this reason that we neglected their contributions in the calculation of C_p.

More pertinent to our considerations is to consider the alternate proposals which have been made to account for the anomalous C_p simply in terms of random H bond breaking.[2–5] If we look upon liquid water as simply a disordered ice structure in which $^1/_8$ of the H bonds have been broken, we must inquire into the entropy increase which would make possible the breaking of these H bonds. Since the H bond energy is about 5.7 kcal, at 300 K we would need an entropy increase of about 5 eu to compensate for 1.44 kcal of H bonds in ice to be broken. However, the breaking of a single H bond in ice would be accompanied by almost trivially small entropy increases, in the neighborhood of a few tenths of an entropy unit per bond broken. Angell[6] commented on this problem. Large entropy increases occur only when discrete units are formed capable of independent orientational and translational freedom. The breaking of a single or even two adjacent H bonds in ice will not achieve this.

Dynamic Properties

Is there any direct evidence for the structures we have discussed here? The answer is unfortunately no. The half-life of a single H bond can be estimated from transition state theory[8] to be about 2×10^{-10} s at 300 K using an A factor of $10^{13.3}$ s^{-1} and an activation energy of 5.3 kcal/mol.

If we break a single H bond in the tetramer, we form an open chain species with appreciably larger entropy. (Dielectric relaxation times are about 10^{-11} s at 20 °C with $\Delta H^‡$ of about 4.0 kcal and $\Delta S^‡$ of about 5–6 eu.[7] These are in the range of values we might expect for bond breaking in the tetramer or for the rotation of the polar tetramer.) This is not the case for the octamer. One has to break at least two H bonds before any significant entropy increase occurs. Such processes if isolated might have half-lives of occurrence of about 2×10^{-6} s. However, it is more probable that H bond breaking occurs not in isolation but more likely as a cooperative process involving two oligomers which form new bonds as old bonds are broken. The result of such concerted or consecutive processes will be to give a very labile appearance to the water structure on a time scale of nanoseconds.

The temperature dependence of the viscosity of water gives some testimony on this score. Whereas for most liquids the viscosity is representable by a simple exponential Arrhenius form with an activation energy equal to about $^1/_4$ of the energy of vaporization, the viscosity of water changes very rapidly between 0 and 30 °C with an activation energy which changes rapidly from 5.5 kcal/mol at 0 °C to about 3.9 kcal/mol at 30 °C.[7] The viscosity of water is abnormally high for its molecular weight, being 1.79 cP at 0 °C and 0.80 cP at 30 °C. For comparison, cyclooctane has a value of 2.35 cP at 13 °C and cyclohexane has a value of 1.02 cP at 17 °C; *n*-octane has a viscosity of 0.54 cP at 20 °C and 0.71 cP at 0 °C.

Solvation in Water

When polar substances such as HF or NH_3 are dissolved in water, they do so exothermically with the energies appropriate to H bond formation, and it is likely that they simply fit into whatever oligomeric structures are present in water. However, when nonpolar solutes such as argon, hydrocarbons, carbon tetrachloride, or carbon dioxide dissolve in water, there are also appreciable heat releases[4,5] and large negative entropies of solvation. Even more significant is the fact that there is a large positive ΔC_p(solvn) accompanying the solution process. The exothermic solution of argon in water has been ascribed to the formation of a solvation shell (clathrate) of H-bonded molecules around the nonpolar solute, termed in the last 15 years as the "hydrophobic" effect. What has not been appreciated is that this by itself would result in a *negative* value for ΔC_p(solvn). To explain the observed positive value, we must assume that these clathrate cages are in dynamic equilibrium with less ordered cages so that the solute must be considered to exist in at least two different solvent environments, with the positive ΔC_p(solvn) arising from the "melting" of the ordered clathrates to a less ordered solvent cage. This would correspond to a heat capacity of isomerization.

A number of researchers have proposed that it is only the immediate solvent shell around a dissolved solute molecule that is needed to account for the anomalously large and positive values of ΔC_p(solvn). This is supported by data showing that ΔH(solvn) and ΔS(solvn) for a large number of non-H-bonding solutes correlate very well with the van der Waals surface estimated for these solutes.[19] However, none of these efforts have done better than about a 10–20% fit of ΔC_p(solvn) over a temperature range of 0–100 °C. Even this is done with the assumptions of rather arbitrary values of the H bond. Further, none of the efforts have employed discrete structures for liquid water in an effort to relate the ΔH(solvn) to specific structural changes.

One consequence of these very large positive values of ΔC_p(solvn) is that at higher temperatures near 90–100 °C the solution of argon or carbon tetrachloride in water is endothermic rather than exothermic and water appears to be much more of a "normal" solvent.[5]

Very similar arguments must apply to the solution of ions in water. About half or more of the solvation energy of ions comes from the first solvent shell,[14] while outside this highly ordered shell there are likely to be oligomeric structures. For ammonium ion and hydronium ion, recent investigations[15] have demonstrated the appearance of pentameric structures which form part of dodecahedral assemblies.

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Photoacoustic Calorimetry Study of Human Carboxyhemoglobin

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Abstract: Time-resolved photoacoustic calorimetry is used to study the dynamics of the enthalpy and volume changes associated with the formation of triply ligated carboxyhemoglobin from carboxyhemoglobin. The enthalpy for the formation is ΔH = 18.0 ± 2.9 kcal/mol, and the volume change is ΔV = 23.5 ± 0.5 mL/mol. All observed kinetic processes occur in less than 100 ns.

Introduction

Hemoglobin has been the most extensively studied allosteric protein, yet a dynamical molecular description for the mechanism of the allosteric effect remains to be fully elaborated.[1] Although there is a good description, in terms of structure and energetics, of the initial and final states for ligand binding, little is known regarding intermediate states that play a dominant role in cooperative ligand binding. This is attributed in part to low populations of intermediate states under equilibrium conditions. However, ligand dissociation induced by photophysical techniques has been successful in producing significant populations of intermediate species, which have been kinetically and vibrationally characterized.[2-7] Although kinetic techniques allow spectroscopic access to transient species, the associated energetics, which are fundamental to the development of an understanding of the allosteric effect, are exceedingly difficult to discern.

With the recent development of time-resolved photoacoustic calorimetry, it is now feasible to measure the dynamics of enthalpy and volume changes that accompany photoinduced ligand dissociation on the nanosecond–microsecond time scale.[8] This method has been applied to ligand dissociation in both sperm-whale and horse carboxymyoglobin where enthalpy changes associated with protein tertiary structural rearrangements are observed in the nanosecond time regime.[9-11] In this paper we report our first photoacoustic calorimetry study of human carboxyhemoglobin where the enthalpy and volume changes for ligand dissociation to produce triply ligated R-state carboxyhemoglobin are measured.

Experimental Section

Protein Purification. The A_0 component of human hemoglobin, removed of organic phosphates, was prepared by the method of Williams and Tsay[12] and stored as a frozen pellet in liquid nitrogen. Prior to the experiment it was dialyzed against 0.05 M phosphate buffer, pH 7.0, and diluted to a final concentration of 28 μM in hemoglobin. Carboxyhemoglobin was formed by bubbling CO through the solution.

Apparatus. The photoacoustic calorimeter has been described previously.[9] Briefly, a PRA nitrogen-pumped dye laser operating at 1 Hz is used to pass a beam with a wavelength of 500 nm, pulse energy of 15 μJ, and pulse width of 500 ps though the sample which is held under anaerobic conditions in a 1 cm × 1 cm thermostated cuvette. The acoustic waves are measured by a lead zirconate–lead titanate piezoelectric crystal operating at 0.5 MHz and are digitized by a 100-MHz Gould 4072

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