Origin of hydrophobicity and enhanced water hydrogen bond strength near purely hydrophobic solutes

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Hydrophobicity plays an important role in numerous physicochemical processes from the process of dissolution in water to protein folding, but its origin at the fundamental level is still unclear. The classical view of hydrophobic hydration is that, in the presence of a hydrophobic solute, water forms transient microscopic “icebergs” arising from strengthened water hydrogen bonding, but there is no experimental evidence for enhanced hydrogen bonding and/or icebergs in such solutions. Here, we have used the redshifts and line shapes of the isotopically decoupled IR oxygen–deuterium (O–D) stretching mode of HDO water near small purely hydrophobic solutes (methane, ethane, krypton, and xenon) to study hydrophobicity at the most fundamental level. We present unequivocal and model-free experimental proof for the presence of strengthened water hydrogen bonds near four hydrophobic solutes, matching those in ice and clathrates. The water molecules involved in the enhanced hydrogen bonds display extensive structural ordering resembling that in clathrates. The number of ice-like hydrogen bonds is 10–15 per methane molecule. Ab initio molecular dynamics simulations have confirmed that water molecules in the vicinity of methane form stronger, more numerous, and more tetrahedrally oriented hydrogen bonds than those in bulk water and that their mobility is restricted. We show the absence of intercalating water molecules that cause the electrostatic screening (shielding) of hydrogen bonds in bulk water as the critical element for the enhanced hydrogen bonding around a hydrophobic solute. Our results confirm the classical view of hydrogen hydration.

Significance

Hydrophobicity governs a wide range of fundamental physicochemical processes, but its physical origin is unclear. The classical explanation of hydrophobicity is that tiny “icebergs” are formed near such solutes; however, no experimental proof has been advanced for their existence. Here, we used four small purely hydrophobic solutes (methane, ethane, krypton, and xenon) in water to study hydrophobicity at the most fundamental level. We present unequivocal experimental proof for strengthened water hydrogen bonds near purely hydrophobic solutes, matching those in ice and clathrates. The water molecules involved in the enhanced hydrogen bonds display extensive structural ordering resembling that in clathrates, thus indicating the fundamental interconnection between electrostatic screening (shielding) and the hydrophobic effect.

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decoupled IR oxygen-deuterium (O-D) stretching mode ($\nu_{OD}$) of four purely hydrophobic solutes in water together with ab initio molecular dynamics (MD) simulations of methane in water to study the origin of hydrophilicity at the most fundamental level.

**Results and Discussion**

**IR Spectroscopy of Water Molecules in the Neighborhood of Purely Hydrophobic Solutes.** The redshifts and line widths of the O-D stretching mode of the water molecules were measured when perturbed by small purely hydrophobic solutes: methane, ethane, krypton, and xenon. The very low solubility of these solutes in water was increased by using high pressure (14–56 bar) and low temperature (285–299 K). The schematic setup of the IR experiment is shown in SI Appendix, Fig. S1.

We focused on the decoupled O-D stretching mode ($\nu_{OD}$) centered at $\sim$2,500 cm$^{-1}$ (Fig. 1A and SI Appendix, Fig. S2) of HDO diluted in H$_2$O. Spectral line widths of the $\nu_{OH}$ and $\nu_{OD}$ of liquid water are very broad, mainly because of the large range of H-bond strengths and strong intra- and intermolecular couplings. To measure accurately the enhancement of H-bond strength of water near hydrophobic solutes, both couplings must be eliminated. Their removal is commonly achieved by diluting a small amount of D$_2$O into H$_2$O or vice versa (23, 27). In such solutions, the H-bonded structure remains virtually unchanged. Introducing small amounts of D$_2$O into H$_2$O gives, because of H/D exchange, a mixture of predominantly HDO and H$_2$O in equilibrium, in which the HDO molecules are surrounded by H$_2$O. A mixture of 1.4% (vol/vol) D$_2$O in H$_2$O gives $\sim$2.8% HDO, 97.2% H$_2$O, and only 0.02% D$_2$O. Because of the large differences between the frequencies of $\nu_{OH}$ and $\nu_{OD}$, the intra- and intermolecular couplings are completely removed. The spectrum of $\nu_{OD}$ of the water molecules perturbed by solute (Fig. 1B and C and SI Appendix, Fig. S4) was obtained by subtracting the spectra of pure H$_2$O and pure, unperturbed HDO from the spectrum of the same solution containing a small amount of hydrophobic solute. This double-subtraction procedure, introduced by Lindgren and coworkers (28), was modified to account for variable cell thickness and tested thoroughly using pure solvent (2.8% HDO in H$_2$O) and solutions of NaCl and methanol.

The effects of temperature and pressure on the difference spectra have been investigated (SI Appendix). The spectra of H$_2$O and HDO diluted in H$_2$O used for subtractions were obtained at the same temperatures and pressures as the spectra of solutions of alkanes and noble gases. The same high-pressure transmission cell was used to obtain all of these spectra. To preclude formation of solid clathrates, we used pressures and temperatures positioned on the right-hand side of the equilibrium line in the pressure–temperature (PT)-phase diagram (Fig. 1D and SI Appendix).

The resulting spectra of the water molecules that are perturbed by the solutes are shown in Fig. 1B and C and summarized in Table 1. All four solutes display a redshift of $\Delta \nu_{OD}$ (approximately $\sim$60 cm$^{-1}$, the same as those of HDO ice and HDO clathrates (Fig. 1C and Table 1). This observation shows that the strengths of the H bonds near purely hydrophobic solutes are enhanced to the level observed in ice and clathrates. Neither the temperature nor the pressure influence $\Delta \nu_{OD}$ significantly. The number of enhanced or ice-like H bonds per methane molecule is estimated to be between 10 and 15 (SI Appendix). The number of water molecules in hypothetically frozen structures is between five and eight per methane molecule. This amount is only a fraction of the number of waters in the first solvation layer of methane (i.e., 16) measured by neutron scattering (11). Our estimated number is in good agreement with the number obtained by Kauzmann (7). He estimated that, if icebergs around a methane molecule in solution are highly crystalline, they must contain less than half a dozen water molecules (7).

The line widths $\Delta \nu_{OD}$ of the water molecules perturbed by hydrophobic solutes are only slightly larger than those of clathrates.

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**Table 1.**

| Solvent Mixture | $\Delta \nu_{OD}$ (cm$^{-1}$) |
|----------------|-------------------|
| H$_2$O         | 60                |
| H$_2$O + NaCl  | 60                |
| H$_2$O + MeOH  | 60                |

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**Fig. 1.** Experimental data of aqueous solutions of methane, ethane, krypton, and xenon. (A) The purple line represents the IR spectrum of the solvent mixture (2.8% HDO in H$_2$O). The black line represents the spectrum of the pure unperturbed HDO molecules obtained by subtracting the spectrum of pure H$_2$O from the spectrum of the solvent mixture recorded under the same conditions. Inset shows the region of interest—the decoupled O-D stretching mode ($\nu_{OD}$). The black line represents the $\nu_{OD}$ of the pure unperturbed HDO molecules. The red line represents the $\nu_{OD}$ of the perturbed and unperturbed HDO molecules of the solution containing a small amount of methane. (B) Spectra of the $\nu_{OD}$ of the HDO molecules perturbed by methane, ethane, xenon, and krypton. (C) Comparison of the spectra of the $\nu_{OD}$ region: liquid HDO in H$_2$O (283 K and 1 bar; black), perturbed HDO molecules in the methane solution (red), methane HDO clathrate (blue), and HDO ice (green). The spectra are normalized to the same height. (D) Phase PT diagram of the equilibrium between solid methane clathrate and methane aqueous solution (circles 62) and line 63). The red circles show the positions of four measurements used to obtain spectra of methane solutions. The blue circle shows the position of solid methane clathrate used to obtain the spectrum in C.
Table 1. Spectral parameters of the νOD of liquid solvent, solutions of gases, NaCl solution, clathrates, and ice

| Sample                  | νOD (cm⁻¹) | ΔνOD (cm⁻¹) | Hw (Kelvin) | T (Kelvin) |
|-------------------------|------------|-------------|-------------|------------|
| Pure solvent*           | 293 ± 1    | 0 ± 2       | 164 ± 2     | 285 ± 56   |
| Pure solvent*           | 293 ± 56   | 0 ± 2       | 160 ± 2     | 293 ± 56   |
| Pure solvent*           | 285 ± 1    | 4 ± 2       | 158 ± 2     | 285 ± 56   |
| Pure solvent*           | 285 ± 56   | 4 ± 2       | 158 ± 2     | 285 ± 56   |
| CH₄ clathrate            | 291 ± 14   | 78 ± 8      | 86 ± 8      | 291 ± 14   |
| Xe clathrate            | 299 ± 16   | 60 ± 8      | 96 ± 8      | 299 ± 16   |
| Krypton solution*       | 288 ± 50   | 57 ± 8      | 87 ± 8      | 288 ± 50   |
| NaCl solution*          | 285 ± 53   | 34 ± 8      | 166 ± 8     | 285 ± 53   |
| C₂H₆Xe clathrate        | 275 ± 40   | 56 ± 2      | 60 ± 2      | 275 ± 40   |
| C₂H₆NaCl solution*      | 276 ± 7    | 60 ± 2      | 80 ± 2      | 276 ± 7    |
| Xe clathrate*           | 292 ± 18   | 60 ± 2      | 79 ± 2      | 292 ± 18   |
| Kr clathrate*           | 274 ± 56   | 56 ± 2      | 55 ± 2      | 274 ± 56   |
| Ice Ih                  | 271 ± 1    | 57 ± 2      | 37 ± 2      | 271 ± 1    |

Temperature T (Kelvin), pressure p (bar), frequency at maximum intensity νOD (centimeters⁻¹), frequency redshift ΔνOD (centimeters⁻¹), and half-width Hw (centimeters⁻¹) are shown. Hw is defined as the width of a peak at one-half height. Errors in measuring T and p are estimated at 0.5 K and 0.5 bars, respectively. All samples contain a solvent mixture of 2.8% HDO in H₂O.

Ab Initio MD Simulations of Purely Hydrophobic Solute in Water. The results so far obtained by Monte Carlo and MD simulations of purely hydrophobic molecules in water are inconsistent. Although some simulations confirm enhanced H bonding near apolar molecules and support the iceberg view (29–31), others do not (32–36).

Ab initio MD simulations currently provide the most accurate approach to addressing the structural and dynamic aspects of water–water H bonding in a condensed phase under ambient conditions, because in these methods, the interatomic forces are derived on the fly at the quantum mechanical level. Such simulations can explicitly capture electronic polarization effects and account for the proper response of water molecules to the local environment. We performed ab initio MD simulations of methan in D₂O at three temperatures (283, 293, and 300 K) using the dispersion-corrected density functional theory (DFT) approach as implemented in the program package VASP, version 5.3 (37) (Materials and Methods and SI Appendix). The most appropriate choice of exchange-correlation functional was found to be revPBE (38, 39) in combination with the Grimme D3 correction (40), which was shown in a series of test simulations of bulk water according to the ability to best reproduce experimental radial distribution functions, diffusion constants, line shape of νOD, and solvation enthalpy of liquid water at ambient conditions (SI Appendix). Time-dependent O-D stretching frequencies of individual water molecules were derived using the wavelet transform of the corresponding O-D distance time series (Materials and Methods).

Our analysis was focused on comparing the structural and energetic properties of the H-bond network of water molecules in the immediate vicinity of methane and relative to those of bulk water (Fig. 2). The νOD frequencies as a function of distance to methane (Fig. 2A) clearly show notable depressions in the region of the first hydration shell. The redshifts range from 14 to 7 cm⁻¹ with increasing temperature. Note, however, that the redshifts obtained by the ab initio MD simulations are not directly comparable with the experimental redshift (~60 cm⁻¹) (SI Appendix). Furthermore, we have analyzed the electrostatic interaction energies of water molecule pairs located in either the methane hydration layer or the bulk water. The electrostatic interaction potentials of water molecule pairs located in the methane hydration layer are consistently lower than those in the bulk water, indicating higher H-bond strength (Fig. 2B). The difference between hydration and bulk water pairs (Fig. 2B) shows clearly that the first four nearest water molecules surrounding a water molecule located in the first hydration shell around the methane interact more strongly with the tagged water molecule (0.1–0.15 kcal/mol) than molecules in bulk water, whereas the difference for the more distant neighbors (fifth onward) becomes negligible. This result shows that the structure of water is tetrahedral (four neighbors). The number of H bonds per water molecule and the tetrahedrality (Fig. 2C and D and SI Appendix), both as a function of distance to the methane, indicate that water H bonds in the hydration shell are more numerous and more...
tetrahedrally oriented than those in bulk water. Other structural and energy parameters that characterize H-bond strength are consistent with the results presented above (SI Appendix, Fig. S12). The correlation times, calculated by the ab initio MD simulations, show restricted orientational mobility of the water molecules near a methane solute (SI Appendix, Fig. S13), in accord with the NMR data from the work by Haselmeier et al. (8). The O-D vibrational redshift, the tetrahedrality, and the number of H bonds are larger at lower temperatures.

The simulations performed in this work clearly show that water molecules in the vicinity of methane have stronger, more numerous, and more tetrahedrally oriented H bonds than those in bulk water and that their mobility is restricted, which is consistent with the experimental results presented above.

**Origin of Strengthened Water H Bonds near Hydrophobic Solutes.** The results presented above show that the water H bonds in the neighborhood of purely hydrophobic solutes are as strong as those in ice or clathrates. These ice-like H bonds cause greater water ordering because of formation of icebergs as postulated by Kauzmann (7). Determining the physical origin of ice-like H bonds near hydrophobic solutes is, therefore, crucial for understanding hydrophobicity at the most fundamental level. Theoretical simulations show that water H bonds straddle small hydrophobic solutes in a way similar to that of H bonds in clathrates to maximize the number of H bonds (33). Such a constraint imposed on the H-bonding network can cause ordering of water molecules per se; however, it does not necessarily make H bonds stronger. The absence of a clear connection between steric constraint and H-bond strength led us to focus on electrostatic interactions, because water H bonds are predominantly electrostatic in nature (41). H bonds in water are known to be strongly cooperative (23, 42–44); their strength, therefore, depends on electrostatic interactions of an H-bonded pair with neighboring water molecules.

We, therefore, analyzed the effects of neighboring water molecules on the strength of the H bond between a pair of waters (Fig. 3A, yellow) during ab initio MD simulations. It has been shown that the instantaneous frequency, \( \nu_{OH} \), is proportional to the projection of an electric field on an H atom along the bond vector O-H (\( E_{O-H} \)) (23, 25, 45). The electric field on an H atom depends on the positions, orientation, and point atomic charges of water molecules in the vicinity. To verify this relationship, we calculated the electric field on D atoms using the Hirshfeld distances and the corresponding frequencies of the O-D stretching mode \( \nu_{OD} \) for short segments of the ab initio MD trajectory using the continuous wavelet transformation method (47). A remarkably simple linear relationship is seen to exist between the \( \nu_{OD} \) frequency (i.e., H-bond strength) and the projection of the electric field along the H-bond vector D’-O (\( E_{D’-O} \)) (Fig. 3A). We used here the values of \( E_{D’-O} \) instead of \( E_{OD} \), because the H bonds in liquid water are rarely collinear (average angle O-D < 167°). The linear relation between \( E_{D’-O} \) and \( \nu_{OD} \) is crucial to our analysis, because it enables the contribution of each neighboring water molecule to the H-bond strength to be evaluated. The strength of an H bond is enhanced if a larger force propels a positively charged atom D toward an acceptor O atom. The water molecules that are closest to the donor atom D make the largest contributions to \( E_{D’-O} \). The \( \nu_{OD} \) of an H-bonded water pair is affected predominantly by water molecules in the first solvation layer (cutoff < 5 Å) (Fig. 3B).

Three distinct classes of water molecule (Fig. 3B) were identified in the neighborhood of a water H-bond pair in liquid water. The first two classes comprise the water molecules that are H-bonded to the H-bonded pair; their contributions to the value of \( E_{D’-O} \) do not differ significantly from the contributions of the corresponding waters in ice. In ice, a pair of H-bonded water molecules is H-bonded to six water molecules located at the vertices of two fused tetrahedrons (Fig. 3C). In liquid water, the locations of H-bonded waters resemble fused tetrahedrons (Fig. 3D); however, some vertices are empty or overpopulated. The contributions of the first two classes of water molecules to the value of \( E_{D’-O} \) are determined primarily by the point atomic charges of atoms that are closest to the atom D of the H-bonded pair controlled by donor or acceptor characters of the corresponding H bonds. The first class is the H bond-enhancing water molecules, which increase the value of \( E_{D’-O} \) (Fig. 3B, red). The second class is the H bond-weakening waters, which decrease \( E_{D’-O} \) (Fig. 3B, blue). The average numbers of these two classes per H bond are 3.4 and 1.7; therefore, the net effect is to strengthen the H bond. In ice, the corresponding numbers are four and two.

The most interesting is the third class of intercalating water molecules. They are able to occupy transiently the space closest to the atom D of the H-bonded pair, which is empty in ice (Fig. 3B, green). To accommodate the intercalating water molecule, the angle O-D’O of the H-bonded pair must bend. Only ~6% of the H-bonded pairs in the bulk water have intercalating waters. (Fig. 3E) The environment of the two H-bonded waters in the first solvation layer of methane. Intercalating water molecules are replaced by methane—the resulting \( E_{D’-O} \) is larger than that of bulk water (red arrow).

![Fig. 3. Screening of a water H bond by an intercalating water molecule. (A) Frequency of \( \nu_{OD} \) as a function of projection of electric field \( E_{D’-O} \). Contributions of the H-bonded pair (yellow) to \( E_{D’-O} \) are ignored. (B) Distribution of water molecules as a function of distance to atom D and projection of electric field \( E_{D’-O} \). Red, blue, green, and yellow systematically correspond to classes I and II, intercalating waters, and the monitored H-bonded water pair, respectively. Class I and II waters are located on the vertices of two fused tetrahedrons. The environment of two H-bonded waters in ice. Waters centered on the vertices of tetrahedrons are marked by red or blue depending on their class. The circle shows the cutoff distance for intercalating waters. (D) The environment of the two H-bonded waters in bulk liquid water. Average electric field vectors and dipole moments for intercalating waters are shown as orange and green arrows, respectively. The resulting \( E_{D’-O} \) is shown as a red arrow. To accommodate the intercalating water molecule, the angle O-D’O of the H-bonded pair must bend. Only ~6% of the H-bonded pairs in the bulk water have intercalating waters. (E) The environment of the two H-bonded waters in the first solvation layer of methane. Intercalating water molecules are replaced by methane—the resulting \( E_{D’-O} \) is larger than that of bulk water (red arrow).](image-url)
primarily responsible for the lower strength of H bonds in liquid water than those in ice.

In the transfer of the H-bonded pair from the bulk to the first solvation layer of methane (Fig. 3E), the fraction of intercalating water molecules per H-bonded pair is reduced from 6 to 2%, because methane and intercalating water molecules tend to occupy the same space lateral to the direction of H bonds (Fig. 3D and E). Methane prefers this position, because water H bonds tend to straddle small hydrophobic solutes in a way similar to that in clathrates. By removing the intercalating water molecules, the value of $E_{DO}$ increases. A higher value of the projection of electric field $E_{DO}$ means that a larger force is pushing a positively charged D atom toward the acceptor O atom, which strengthens the H bond. This mechanism explains why water H bonds near methane are stronger than those in bulk water.

The explanation described above for the strengthening of water H bonds near hydrophobic solutes is in accord with the general concept that electrostatic interactions are stronger near hydrophobic groups. Berry and coworkers (51) have shown that water molecules in the neighborhood of a hydrophobic solute have a smaller dielectric susceptibility or in microscopic terms, less effective electrostatic screening (52). It has been shown that electrostatic screening is the main reason for the distinct backbone conformational preferences of amino acid residues in peptides and proteins (53), the nearest neighbor effect (54), and the formation of transient β-strands in unfolded proteins (55). It has also been shown, using amide to ester mutations, that H bonds in the protein interior are stronger than those exposed to solvent (56–58).

**Conclusion**

By applying methods of superior accuracy for measuring and calculating subtle effects of H-bonding in water, we have obtained evidence that supports, unequivocally, the iceberg view of hydrophobicity proposed by Frank and Evans (6) and Kauzmann (7). Our experimental results show that water H bonds near purely hydrophobic solutes are strengthened to the level observed in ice and clathrates. We have proposed a physical origin for hydrophobicity based on electrostatic screening that couples hydrophobic with electrostatic interactions. This coupling may be crucial in understanding protein folding and other complex phenomena.

**Materials and Methods**

**Materials.** Samples were prepared using milli-Q H$_2$O and D$_2$O (Euriso top, 99.90% D). Gases used were methane (Messer, 4.5), ethane (Messer, 3.5), krypton (Messer, 3.0), and xenon (Messer, 5.0).

**Instruments and Sample Preparation.** IR spectra were recorded on Bruker Vertex 80 and Tensor 27 Spectrometers and collected in the transmission mode with a nominal resolution of 4 cm$^{-1}$. Typically, 128 interferograms were averaged and apodized using the Gapp-Henzel function. A deuterated triglycerine sulphate (DTGS) detector was used throughout. The aperture of the IR beam was set to 326 μm and the sample thickness was 0.08 mm.

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