One Water Molecule Stiffens a Protein

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Molecular dynamics (MD) simulations have been performed to characterize the initial steps in the hydration of gas-phase BPTI $(M+6H)^{6+}$ (bovine pancreatic trypsin inhibitor with six protons). Experimentally, the first water molecule that binds to the BPTI $(M+6H)^{6+}$ ion in the gas phase is special, with a particularly large $-\Delta S°$.[1] The simulations suggest that the large $-\Delta S°$ results from tetrahedral coordination of a water molecule within a cavity in the protein. Occupation of this type of hydration site stiffens the low-frequency modes of the protein, and leads to a substantial decrease in the vibrational entropy. The decrease in the vibrational entropy appears to be characteristic of tetrahedral coordination and is not observed for 2- or 3-fold coordination.

Water is known to have a profound effect on protein structure, function, and stability.[2−6] The contribution of protein−solvent interactions to protein folding has been studied extensively.[7−9] While it has not been possible to determine accurately the thermodynamics of incorporating a single water molecule at the protein−solvent interface in solution,[10,11] advances in mass spectrometry now permit measurement of $\Delta H°$ and $\Delta S°$ for the hydration of dehydrated protein ions in the gas phase.[1,12] For BPTI $(M+6H)^{6+}$, $-\Delta H°$, and $-\Delta S°$ for the first water molecule adsorbed were found to be particularly large.[13] The $-\Delta S°$ is larger than can be accounted for by immobilizing a water molecule on the protein;[14] thus, the initial hydration step must cause a decrease in the entropy of the protein. Here we report the results of MD simulations performed to characterize the special hydration site on BPTI $(M+6H)^{6+}$.

Simulations were performed with the MACSIMUS[15] molecular modeling software using CHARMM-like potentials[16] with the 21.3 parameter set and the united atom representation. The CHARMM-adapted TIP3P model[17] was used for the water molecules. BPTI has six basic arginine and four basic lysine residues. Arginine is more basic than lysine,[18] thus we assume that the six protons in the $(M+6H)^{6+}$ ion reside on the arginine residues (although we also did simulations with some of the lysine residues protonated). All of the other residues were neutral, and a dielectric constant of 1.0 was used. For the hydration reaction

$$\mathrm{BPTI(M+6H)^{6+} + H_2O \rightleftarrows BPTI(M+6H)^{6+}{\cdot}H_2O} \quad (1)$$

we assume that $\Delta H°$ can be approximated by the difference between the potential energies of BPTI $(M+6H)^{6+}$ + H_2O and BPTI $(M+6H)^{6+}{\cdot}H_2O$. We used potential energies for energy-minimized structures obtained after the simulations reached equilibrium. To test how well the TIP3P model reproduced the geometries and energetics of hydration a series of singly charged ions with arginine and lysine side chains, and with varying degrees of solvation, were used as model systems. The structures and hydration energies obtained using the TIP3P potential are close to the results of ab initio calculations using a 6-31G* basis set.

The loss of three translational and three rotational degrees of freedom in an association reaction is offset by the formation of six new vibrations. Elementary statistical mechanics provides a good approximation for calculating the entropy change for an association reaction that involves noncovalent binding.[19] The translational and rotational entropies of BPTI $(M+6H)^{6+}$ are almost identical to those of BPTI $(M+6H)^{6+}{\cdot}H_2O$ (because the masses and structures are so similar).[20] The vibrational entropy of a water molecule is small, and thus the change in the water's vibrational entropy when it binds to the protein is negligible.[21] Thus, the entropy change for hydration of a single conformation is[22]

$$\Delta S° \approx -S°_{H_2O} + (S_{BPTI{\cdot}H_2O} - S_{BPTI})_{vib} \quad (2)$$

where $S°_{H_2O}$ is the total entropy of H_2O (186 J mol^{-1} K^{-1} at 1 atm and 273.2 K) and $(S_{BPTI{\cdot}H_2O} - S_{BPTI})_{vib}$ is the change in vibrational entropy that results when the water adsorbs. The vibrational entropies were calculated from the normal-mode frequencies using the standard quantum form.[22] The frequencies were determined from the Hessian of the potential for energy-minimized structures obtained after the simulations reached equilibrium.

A series of 1 ns simulations were performed to examine possible hydration sites on the BPTI $(M+6H)^{6+}$ ion. The starting point was the 5PTI crystal structure[23] with all crystal waters removed and a single water added. The protonated arginine groups are expected to provide energetically favorable hydration sites because of their high charge density. In addition, BPTI has several structural water molecules: W122 and a hydrogen-bonded trimer W111−W113; which are conserved in all three crystal structures reported for BPTI[23−25] and observed by NMR in solution.[26] W122 is tetrahedrally coordinated in a pocket to Thr11, Cys14, and Cys38 and appears to be in a particularly favorable hydration site. Thus, we considered the protonated arginines and the W122 site as possible hydration sites.

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(13) The measured $\Delta H°$ and $\Delta S°$ are for a standard state of 1 atm.
(14) The measured $\Delta S°$ is −259 J K^{-1} mol^{-1}. The $\Delta S°$ for hydration has contributions due to the loss of translational and rotational entropy of the water and the increase in vibrational entropy of the product. An entropy change of −105 to −145 J K^{-1} mol^{-1} is expected for localizing a water on the protein (see ref 1 for a more detailed discussion). According to the simulation performed here, an entropy change of −118 to −148 J K^{-1} mol^{-1} occurs when a water is localized on a charged arginine near the surface of the protein (see Table 1).
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Table 1. Enthalpy and Entropy Changes for Hydration at Different Sites on BPTI[a]

	$\Delta H°$ (kJ mol^{-1})	$(S_{BPTI·H_2O} - S_{BPTI})_{vib}$ (J K^{-1} mol^{-1})	$\Delta S°$ (J K^{-1} mol^{-1})
Experiment			
first water adsorbed	−89 ± 4		−259 ± 21
second to sixth waters	−55 to −66		−136 to −176
Charged Sites			
ARG1, ARG17, ARG20, ARG39, ARG42, ARG53	−50 to −84	+38 to +68	−118 to −148
Tetrahedral Coordinate			
THR11HG1, ASN441HD, ARG20O, PHE33O pocket	−54	−46	−232
W122 pocket (neutral BPTI)	−88	−26	−212
3-Coordinate			
GLY12O, GLY12H, LYS38O	−35	+16	−170
PRO8O, LYS41O, ASN431HD	−66	+52	−129

[a] Entropies were calculated using a temperature of 273.2 K.

In the crystal structure the arginines are on the surface of the protein. In the absence of a solvent the protonated arginines are self-solvated by hydrogen bonds mainly to backbone carbonyl groups. The water also hydrogen bonds to the protonated arginines. The calculated hydration enthalpy depends on the amount of self-solvation. The calculated $\Delta H°$ for charged arginines with one to two oxygens in the self-solvation shell (ARG39 and ARG17) is around −84 kJ mol^{-1}, with three oxygens (ARG20, ARG42, and ARG53) it is −63 to −71 kJ mol^{-1}, and with four oxygens (ARG1) it is around −50 kJ mol^{-1}. For comparison, the measured enthalpy change for binding the first water molecule to BPTI $(M+6H)^{6+}$ is −89 ± 4 kJ mol^{-1}. The computed $(S_{BPTI·H_2O} - S_{BPTI})_{vib}$ for binding a water at a charged arginine is 38−68 J mol^{-1} K^{-1}, and this is not correlated with self-solvation. $(S_{BPTI·H_2O} - S_{BPTI})_{vib}$ is expected to be positive because six new vibrational modes are created when the water adsorbs. The calculated $\Delta S°$ from eq 2 is −118 to −148 J mol^{-1} K^{-1}. This is substantially less negative than the measured value of −259 ± 21 J mol^{-1} K^{-1} for the first water adsorbed on BPTI $(M+6H)^{6+}$, but close to the entropy change for the second to sixth water molecules adsorbed (see Table 1).

When the water is placed at the W122 cavity, it moves out and usually hydrates ARG39, the nearest arginine. This migration is reproducible and independent of the initial velocities in the MD simulation. ARG39 is an attractive hydration site because it only has two oxygens in its self-solvation shell. In the crystal structure the arginine side chains tend to point away from the surface of the protein, and they retain this geometry in the MD simulations. To increase the self-solvation of ARG39 and stabilize the water in the W122 pocket, the arginine side chains were folded over and bent toward the protein at the beginning of the simulation. This configuration is energetically favorable: the average potential energy of BPTI $(M + 6H)^{6+}$ is around 63 kJ mol^{-1} lower. There must be an activation barrier associated with this process since it does not occur in MD simulations started from the crystal structure. However, the conformational changes that result in the increased self-solvation destroy the W122 pocket. The water does not stay in the pocket now due to a lack of hydrogen-bonding partners. We searched for other possible internal water sites using an approach similar in spirit to the GRID algorithm.[27] For each position of the water oxygen atom (inside and around the protein) the positions of hydrogen atoms were optimized by a conjugate gradient method. Another favorable internal site was found for the water not far from the W122 site. Like W122 in the crystal structure, this water is tetrahedrally coordinated, and the cavity is accessible from the outside. In this new site the water is hydrogen-bonded to THR11, ARG20, PHE33, and ASN44. There is not a water in this site in any of the crystal structures because the residues are not close enough together. $(S_{BPTI·H_2O} - S_{BPTI})_{vib}$ for this site is −46 J K^{-1} mol^{-1} and thus $\Delta S°$ is −232 J K^{-1} mol^{-1}. A negative value of $(S_{BPTI·H_2O} - S_{BPTI})_{vib}$ indicates that the vibrational entropy of BPTI $(M+6H)^{6+}·H_2O$ is *less* than for BPTI $(M+6H)^{6+}$ even though the complex has six additional vibrational degrees of freedom. Figure 1 shows a plot of the cumulative entropy change as a function of frequency for hydration in the new cavity. The decrease in the vibrational entropy results from a stiffening of the low-frequency vibrational modes that involve the global motion of the entire protein.

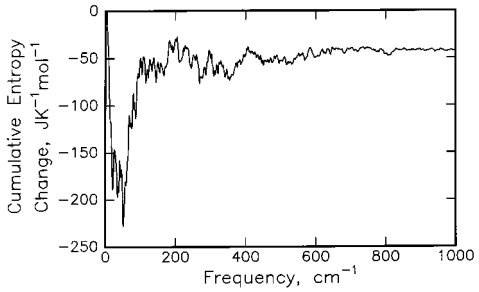

Figure 1. The cumulative vibrational entropy change for hydration at the tetrahedrally coordinated hydration site of BPTI $(M+6H)^{6+}$.

While the calculated $\Delta S°$ *(−232 J K^{-1} mol^{-1}) for hydration of this cavity site is close to the measured* $\Delta S°$ for the first water (−259 ± 21 J mol^{-1} K^{-1}) the agreement between the measured (−89 ± 4 kJ mol^{-1}) and calculated (−54 kJ mol^{-1}) $\Delta H°$ is less good. The small calculated $\Delta H°$ results because hydrogen bonds in the cavity must be disrupted in order to incorporate the water. The sizable discrepancy between the measured and calculated $\Delta H°$ may indicate that this particular hydration site in this particular structure is not the site responsible for the anomalous behavior of the first water molecules adsorbed on BPTI. But the conclusion that a tetrahedrally coordinated water molecule is responsible for the anomalously large $-\Delta S°$ appears to be valid. We found one more example of tetrahedral coordination. In unprotonated BPTI the water stayed in the W122 cavity and retained the tetrahedral coordination that exists in the crystal structure. $(S_{BPTI·H_2O} - S_{BPTI})_{vib}$ is −26 J mol^{-1} K^{-1}, the negative value again resulting from a stiffening of the low-frequency vibrational modes. On the other hand, we found many examples of 3-fold coordinate water. In all cases, $(S_{BPTI·H_2O} - S_{BPTI})_{vib}$ was positive. Two examples are shown in Table 1. When bound to the protonated arginines the water is usually 2-coordinate. It is clear from Table 1 that only the tetrahedrally coordinated sites can account for the large $-\Delta S°$ observed experimentally. The calculated $-\Delta S°$ for the tetrahedral site slightly underestimates the measured quantity. This may be because we have used a harmonic approximation to calculate the entropies.[28] If anharmonic effects are included, $(S_{BPTI·H_2O} - S_{BPTI})_{vib}$ and $\Delta S°$ are expected to become even more negative.

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