Hydration of Gas-Phase Proteins: A Special Hydration Site on Gas-Phase BPTI

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Water is known to play a critical role in determining the tertiary structure of proteins,[1−3] but there is little detailed quantitative information available about the interaction of water with proteins. Here we report the first measurements of the enthalpy and entropy changes for the initial steps in the hydration of a gas-phase protein. Our results reveal the presence of a special hydration site on gas-phase bovine pancreatic trypsin inhibitor (BPTI) which may be related to a unique structural water molecule observed for this protein in solution.[3,4] The relative importance of solvent and intramolecular interactions in determining the three-dimensional structure of a protein has been the object of extensive discussion for many years. Previously, thermodynamic information about the interaction of water with proteins has been obtained from studies of the hydration of protein films.[1,2] But hysteresis effects make these values unreliable, and only average quantities can be obtained. Recently, the development of new ionization methods[5] has made it possible to use mass spectrometry based techniques to study the properties of protein ions in the gas phase.[6−8] However, there has been surprisingly little work on the hydration of gas-phase biomolecules.[9−12] Kebarle and collaborators have measured free energy changes at 293 K for the first steps in the hydration of some small peptides, the largest being Gly_4H^+.[10] In the work described here, we have studied the hydration of the 6+ charge state of BPTI as a function of temperature and determined enthalpy and entropy changes as a function of the number of adsorbed water molecules. BPTI was selected for these studies because it is a small, well-characterized protein. It has 58 residues and three disulfide bridges that partially lock the backbone in place and make BPTI very resistant to denaturation. Molecular dynamics simulations performed for BPTI in vacuum[13,14] suggest that the time-averaged structure is near, but not identical to, the crystal structure.

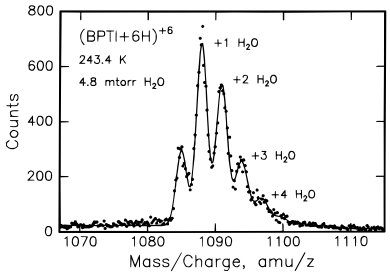

Figure 1. Mass spectrum measured for the 6+ charge state of BPTI with 4.8 mTorr of water and with a drift tube temperature of 243.4 K. Peaks due to the addition of up to four water molecules are apparent. The points are the measured data, and the line is a least-squares fit using Gaussian functions.

Equilibrium constants for the hydration reactions

$$(\mathrm{BPTI} + 6\mathrm{H})^{6+}(\mathrm{H_2O})_n + \mathrm{H_2O} \rightleftarrows (\mathrm{BPTI} + 6\mathrm{H})^{6+}(\mathrm{H_2O})_{n+1}$$

were measured using an injected ion drift tube apparatus equipped with an electrospray source.[8,11] A solution of ~5 × 10^{-5} M BPTI (Sigma Chemical Co.) in a 50:50 mixture of water and methanol was electrosprayed at 5 kV in air. Ions enter the vacuum chamber through a 130 μm diameter aperture, pass through a desolvation region, and are focused into a quadrupole mass spectrometer. Electrospray ionization of disulfide-intact BPTI generates predominantly the 5+ to 7+ charge states, where the charge results from protonation. The 6+ charge state was injected into the drift tube at 1200 eV. The drift tube was operated with an electric field of 13.2 V cm^{-1}, and a total pressure of water vapor (2−240 mTorr, corrected for thermal transpiration[15]) and helium of around 5 Torr. The temperature of the drift tube was regulated with a precision of <1 K over the 223−273 K range employed. The ions spend 1−2 ms traveling through the drift tube and then exit through a small aperture. After being mass analyzed in a second quadrupole mass spectrometer, the ions are detected.

Figure 1 shows a mass spectrum recorded for the 6+ charge state of BPTI with a drift tube temperature of 243.4 K and with 4.8 mTorr of water vapor. The unsolvated 6+ charge state is at m/z ~1085 amu, and peaks due to the addition of up to four water molecules are present. The line in Figure 1 is a least-squares fit to the experimental data using Gaussian functions. Equilibrium constants for the hydration reactions were derived from

$$K = \frac{I[\mathrm{BPTI} + 6\mathrm{H})^{6+}(\mathrm{H_2O})_{n+1}]}{I[(\mathrm{BPTI} + 6\mathrm{H})^{6+}(\mathrm{H_2O})_n]\ P[\mathrm{H_2O}]}$$

where $P[H_2O]$ is the water vapor pressure in atmospheres and $I[(\mathrm{BPTI} + 6\mathrm{H})^{6+}(\mathrm{H_2O})_{n+1}]$ and $I[(\mathrm{BPTI} + 6\mathrm{H})^{6+}(\mathrm{H_2O})_n]$ are the intensities from the fits to the mass spectra. Some measurements were performed with the drift field reduced by a factor of 2, so that the ions spend twice as long in the drift tube. Mass spectra measured with different drift times were not significantly different, which demonstrates that equilibrium is established. However, the equilibrium constants show a small decrease as the water vapor pressure is increased. This nonideal behavior probably results from the presence of several closely related conformations with slightly different hydration properties. Previous ion mobility measurements for the 6+ charge state of BPTI show a single peak, with a mobility close to that predicted for the native conformation.[8] However, the peak is slightly broader than expected for a single conformation, which indicates that more than one conformation is present. Three slightly different crystal structures have been found for BPTI[16]

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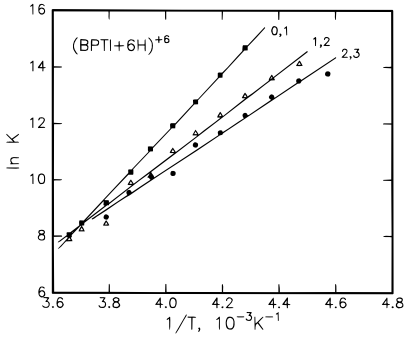

Figure 2. Plot of ln K against $1/T$ for addition of the first three water molecules to $(BPTI + 6H)^{6+}$. The points are an average of up to 14 independent measurements, and the lines are least-squares fits used to extract values for $\Delta H°$ and $\Delta S°$.

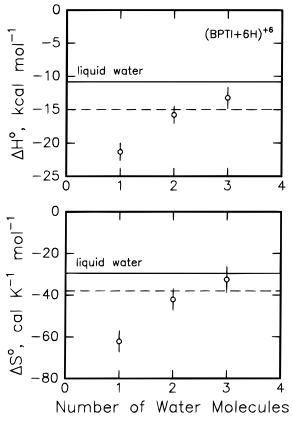

Figure 3. Plot of the enthalpy and entropy changes for adsorption of the first three water molecules onto $(BPTI + 6H)^{6+}$. The error bars are two standard deviations from the linear regression plus the estimated uncertainty from the small pressure dependence of the equilibrium constants. The solid lines show the enthalpy and entropy changes for transferring a water moleucle from the gas phase to liquid water. The dashed lines show average values for $\Delta H°$ and $\Delta S°$ for the initial stage in the hydration of protein films.

and the dispersion in the cross sections for these structures is comparable to the distribution observed in the ion mobility measurements.[17]

Figure 2 shows a plot of ln K against $1/T$ for the first three water molecules adsorbed by $(BPTI + 6H)^{6+}$. The points are an average of up to 14 independent measurements, and the lines are least-squares fits. The slope of the lines gives $-\Delta H°/R$, and the intercept gives $\Delta S°/R$. Figure 3 shows $\Delta H°$ and $\Delta S°$ plotted against the number of adsorbed water molecules. The error bars represent two standard deviations from the linear regression plus the estimated uncertainty associated with the slight pressure dependence of the equilibrium constants. $\Delta H°$ for the addition of the first water molecule is -21.3 ± 1.0 kcal mol^{-1}. For subsequent water molecules the enthalpy change is significantly less negative. The entropy changes follow a similar trend. $\Delta S°$ for the addition of the first water molecule is -62 ± 5 cal K^{-1} mol^{-1}. We have determined $\Delta H°$ and $\Delta S°$ for up to the sixth water molecule adsorbed. However, equilibrium constants for the fourth to sixth water molecules have only been measured over a relatively narrow temperature range so that the values derived for $\Delta H°$ and $\Delta S°$ have a large uncertainty associated with them. $\Delta H°$ and $\Delta S°$ for the fourth to sixth water molecules appear to lie between those determined for the second and third water molecules. The solid lines in Figure 3 show the enthalpy and entropy changes for transferring a water molecule from the gas phase to the liquid.[18] The dashed lines show average values for $\Delta H°$ and $\Delta S°$ determined for the initial stage in the hydration of protein films, $\Delta H° = -15.0$ kcal mol^{-1} and $\Delta S° = -38$ cal K^{-1} mol^{-1}.[1] $\Delta H°$ and $\Delta S°$ for the second and subsequent water molecules adsorbed on $(BPTI + 6H)^{6+}$ are close to these values.

The entropy change for hydration has contributions due to the loss of the translational and rotational entropy of the water molecule and the increase in the vibrational entropy of the product. The translational and rotational entropies can be calculated, but the vibrational contribution is difficult to estimate because the frequencies are not known. Using vibrational entropies deduced for water adsorption on ice and for the first few steps in the hydration of H_3O^+,[19] an entropy change of -25 to -35 cal K^{-1} mol^{-1} should be expected for the hydration reactions. The entropy change observed for the first water molecule is clearly much more negative than predicted. This indicates that the entropy of the protein must decrease by 25−35 cal K^{-1} mol^{-1} when the first water molecule is adsorbed. This entropy must be derived from the remaining configurational entropy of the protein. The large loss of configurational entropy probably results from the water molecule locking two parts of the peptide chain together. This behavior can occur with structural water molecules. Four structural water molecules have been identified for BPTI and these are conserved in all three crystal structures[16] and have been observed by NMR in solution.[4] One of the structural water molecules is hydrogen bonded in a pocket to Cys38, Cys14, and Thr11, the other three form a hydrogen-bonded cluster of water molecules that are hydrogen-bonded to Pro8, Tyr10, Asn43, Lys41, and Asn44. The isolated structural water molecule is unusual in that it forms four hydrogen bonds to the protein. Calculations suggest that this is the most strongly bound hydration site on BPTI,[20] with a hydration energy of ≈25 kcal mol^{-1}. Thus this is a reasonable candidate for the site of the first water molecule adsorbed in the gas phase. One concern with this assignment is that Cys14 and Cys38 are already linked by a disulfide bridge, so it is not obvious that binding a water in this site should lead to such a large entropy change. However, the hydrogen bond to Thr11 will constrain the protein, and Thr11 is in turn hydrogen bonded to Gly36, so introducing the water may cause the whole pocket to order. The configurational entropy of an unfolded protein is enormous, around 720 cal K^{-1} mol^{-1} for BPTI in vacuum.[21] So it is not unreasonable that a small fraction of the configurational entropy persists in the folded gas-phase protein and that this entropy is lost when water molecules adsorb and order the structure. The adsorbed water may also favor one of the many possible ways of arranging the protons among the basic sites, and this would also contribute to the lowering of the configurational entropy. The large enthalpy change observed for the first water molecule adsorbed could also be explained by this water solvating one of the charge sites. However, the charge sites are expected to be shielded by carbonyl oxygens,[7] and charge solvation could not account for the remarkably large entropy change observed for the first water molecule adsorbed.

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