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Entropy Drives an Attached Water Molecule from the C- to N-Terminus on Protonated Proline

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Abstract: Results from infrared photodissociation (IRPD) spectroscopy and kinetics of singly hydrated, protonated proline indicate that the water molecule hydrogen bonds preferentially to the formally neutral carboxylic acid at low temperatures and at higher temperatures to the protonated N-terminus, which bears the formal charge. Hydration isomer populations obtained from IRPD kinetic data as a function of temperature are used to generate a van't Hoff plot that reveals that C-terminal binding is enthalpically favored by 4.2−6.4 kJ/mol, whereas N-terminal binding is entropically favored by 31−43 J/(mol K), consistent with a higher calculated barrier for water molecule rotation at the C-terminus.

Interactions with water molecules are crucial in regulating the function of many biomolecules, including proteins. A number of techniques, including NMR,[1] X-ray and neutron diffraction,[2] and femtosecond spectroscopy,[3] indicate that some water molecules may be vital to protein structure and activity, whereas others, especially those at the protein surface, interact with the protein only slightly more strongly than with other solvent water molecules. Understanding these hydration interactions is thus vital in unraveling the complexities of protein folding, structure, and function. Because a large variety of adjacent functional groups in a protein can cooperate or compete to bind the same water molecule, it can be very challenging to determine their intrinsic water binding affinities. In the gas phase, the competitive hydration of various functional groups can be studied in detail using mass-selected model ions of known structure.

Infrared photodissociation spectroscopy results for singly hydrated, protonated valine indicate that the water molecule is attached at the N-terminus, which carries the formal charge.[4] In contrast, results for singly hydrated, protonated phenylalanine and its *N*-methyl derivatives indicate that a substantial population of these ions have a water molecule attached at the formally neutral C-terminus.[5] Competition between the charge site and the C-terminus for hydration is supported by calculations,[5,6] and the attractiveness of the C-terminus has been attributed to the favorable orientation of the attached water molecule's dipole moment with the N-terminal formal charge,[6] although this charge−dipole interaction is yet more favorable when the water molecule binds to the N-terminus itself.

We recently introduced an IRPD kinetic method to determine the relative thermal populations of hydration isomers of ions with high isomerization barriers.[5] Briefly, isolated hydrated ions are irradiated with IR photons resonant with only one isomer, which dissociates substantially faster than the other isomer. Slow dissociation of the latter isomer reflects off-resonance absorption or gradual conversion to the resonant isomer, resulting in overall biexponential dissociation kinetics. The relative isomer populations and, hence, relative Gibbs free energies are obtained from the biexponential kinetic data. Whereas the other protonated aliphatic and aromatic amino acids can have three or more competitive hydration sites, potentially complicating direct comparison of individual hydration isomer populations, $ProH^+$ has only two strongly competitive hydration sites, making it an ideal target for studying the temperature-dependence of the isomer populations.

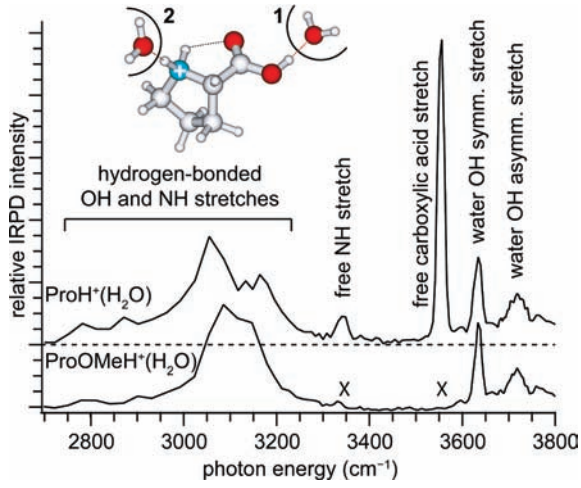

Figure 1. IRPD spectra of $ProH^+(H_2O)$ and $ProOMeH^+(H_2O)$ measured at 238 K. Band assignments are indicated on the spectra. Inset: schematic structures for two low-energy isomers of $ProH^+(H_2O)$, where the water molecule can attach to the hydroxyl group at the neutral C-terminus (site **1**) or to the free NH group at the charged N-terminus (site **2**). The interaction between the other NH group and the carbonyl oxygen atom, which makes this site less favorable for water molecule binding, is indicated by a dashed line.

Here, IRPD spectra and kinetics of $ProH^+(H_2O)$ are measured as a function of temperature, and the role of enthalpy and entropy in C-terminal versus N-terminal hydration is discussed. All experimental data are acquired using a 2.75 T Fourier transform ion cyclotron resonance mass spectrometer coupled to a tunable 10 Hz OPO/OPA laser system that generates light in the ∼2500−4000 cm^{-1} range.[7] Ions are generated with nanoelectrospray from ∼2.5 mM solutions of Pro adjusted to a pH of ∼5 with HCl. The ion cell is surrounded by a copper jacket with an adjustable temperature regulated by a controlled flow of liquid nitrogen.[8] Computational chemistry was performed using MacroModel 9.7 to generate initial low-energy structures using the MMFFs force field, and final structures were optimized using Q-Chem 3.2 or Gaussian 09. Thermochemical values for these computed structures were obtained using unscaled vibrational frequencies.

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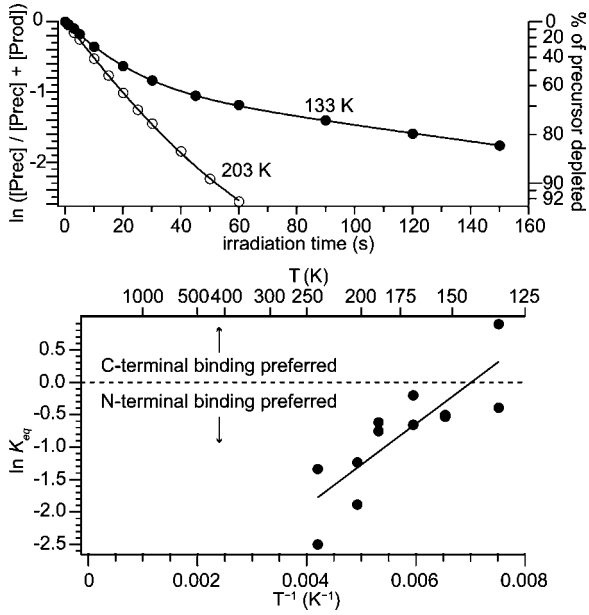

Figure 2. (Top) Representative IRPD kinetic data measured at 3557 cm^{-1} for $ProH^+(H_2O)$ (circles), corresponding to the free carboxylic acid stretch (resonant for water molecule binding at site **2**), with biexponential fits (lines). Note that the *y*-axis is logarithmic to make clear the biexponential character of the kinetic data. (Bottom) van't Hoff plot for $ProH^+(H_2O)$ at 3557 cm^{-1} at ion cell temperatures between 133 and 238 K, where K_{eq} is defined as the ratio of the ion population with a C-terminally bound water molecule to that with an N-terminally bound water molecule, with least-squares fit line.

Two hydrogen atoms of $ProH^+$ compete for the attachment of a water molecule (Figure 1, inset): the carboxylic acid hydrogen atom (site **1**) and one hydrogen atom at the protonated N-terminus (site **2**). The other hydrogen atom at the N-terminus is less competitive because it interacts strongly with the C-terminal carbonyl oxygen atom, analogously to results reported for other protonated amino acids.[4−6] All bands in the IRPD spectrum of $ProH^+(H_2O)$ can be assigned based on prior results and the IRPD spectrum of singly hydrated, protonated proline methyl ester (spectra at 238 K shown in Figure 1).[4,5] The spectrum of $ProH^+(H_2O)$ indicates two hydration isomer populations with water binding to either site. The free NH stretch at 3340 cm^{-1} corresponds to a water molecule binding to site **1**, and the strong free carboxylic acid stretch band at 3557 cm^{-1} corresponds to a water molecule binding to site **2**. These assignments are confirmed by the disappearance of both of these isomer-specific bands in the spectrum of $ProOMeH^+(H_2O)$, where water binds to site **2** and the absence of a free-NH band rules out a second competitive binding site at the N-terminus. Because IRPD band intensities can depend nonlinearly on one-photon absorption cross sections, and because experimental spectra for the isolated isomers have not been measured, the intensities of these two bands are an unreliable measure of relative isomer populations. Instead, the pre-exponential factors from biexponential IRPD kinetics at 3557 cm^{-1} (on resonance with structures hydrated at the N-terminus) are used to obtain isomer populations, as illustrated in Figure 2 (top) for data acquired at 133 and 203 K. Poor overlap of the ion cloud with the laser beam can be eliminated as the source of the biexponential kinetics, because IRPD kinetic experiments at 3557 cm^{-1} for $Li^+(H_2O)_5$ and $Cs^+(H_2O)_4$, for which multiple isomers with low interconversion barriers are present[9] and which bracket the mass of and have comparable binding energies[10,11] to $ProH^+(H_2O)$, dissociate with linear kinetics ($R^2 > 0.998$) to >96% depletion of the precursor population under these conditions. Equilibrium constants, K_{eq}, are obtained from these populations as a function of temperature, and the resulting van't Hoff analysis (Figure 2, bottom) provides the differences in enthalpy and entropy for a water molecule binding to the two sites in $ProH^+$. Although there is significant scatter in these data owing to the well-known difficulty of accurately fitting biexponential data, the values for $\Delta H°$ and $\Delta S°$ fall within a remarkably narrow range: binding at the C-terminus is favored enthalpically by 4.2−6.4 kJ/mol and entropically at the N-terminus by 31−43 J/(mol K).

The relative Gibbs free energy difference between these two hydration isomers is calculated to be small over a wide temperature range (<7 kJ/mol from 0 to 300 K for all levels of theory used here), but site **1** is enthalpically favored by 0.9 (0.04) kJ/mol at the B3LYP/6-31+G** (6-311++G(2d,2p)) level, whereas site **2** is favored enthalpically by 4.2 (3.7) kJ/mol at the MP2(full)/6-31+G** (6-311++G(2d,2p)) level. Water binding to the N-terminus is favored entropically by between 3.3 and 9.8 J/(mol K) depending on the level of theory. Better qualitative agreement is observed between experiment and the B3LYP results, and these experimental values should serve as a stringent benchmark for theory.

Why, then, is water molecule adduction at the formally neutral C-terminus enthalpically favored, whereas it is entropically favored at the protonated N-terminus, which carries the formal charge? The nearly planar configuration of the water molecule and the C-terminus in structure **1** suggests that there may be a very weak second hydrogen bond with the carbonyl oxygen atom. Natural bond order (NBO) analysis of the MP2/6-311++G(2d,2p) structures indicates a significantly greater (158 vs 88 kJ/mol) stabilization energy due to the hydrogen bond between the water molecule oxygen atom and the C-terminal hydroxyl group as compared to that at the N-terminus. Evidently, the greater charge−dipole attraction in structure **2** only partially compensates for its weaker hydrogen bond. MP2/6-311++G(2d,2p) transition state calculations indicate that there is a slightly higher barrier for rotation of the water molecule at site **1** (2.2 kJ/mol at 0 K) than at site **2** (0.1 kJ/mol at 0 K), consistent with NBO results that indicate that the hydrogen bond strength is lowered by rotation of the water molecule at site **1** but not at site **2**. The greater stability of the hydrogen bond at site **1** results in the enthalpic preference of the C-terminal binding site, making this the most favorable site below ∼142 K, and the lower barrier for rotation of the N-terminally bound water molecule at site **2** makes this site more favorable at higher temperature. These results indicate that interactions of water molecules with protonated biomolecules, as occur in H/D exchange experiments used to infer information about gaseous protein conformation, can be favorable at sites other than those carrying a formal charge. These results also indicate that water molecules may interact as strongly with uncharged acidic residues as with charged residues in solution, where entropic effects should be less significant due to interactions with other water molecules.

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Supporting Information Available: Full citations for Q-Chem 3.2 and Gaussian 09, dissociation kinetics at 3557 cm^{-1} for $Li^+(H_2O)_5$ and

$Cs^+(H_2O)_4$ at 133 K, and IRPD spectrum of $ProH^+(H_2O)$ at 133 K. This material is available free of charge via the Internet at http://pubs.acs.org.

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