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Where's the Charge? Protonation Sites in Gaseous Ions Change with Hydration

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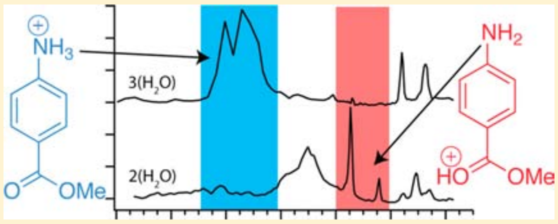
Supporting Information

ABSTRACT: The role of water in stabilizing sites of protonation in small gaseous ions is investigated using electrospray ionization (ESI) coupled with infrared photodissociation spectroscopy and computational chemistry. Protonation of *p*-aminobenzoic acid (PABA) and *p*-aminobenzoic acid methyl ester (PABAOMe) occurs at the carbonyl oxygen atom both in isolation and when one water molecule is attached. However, protonation occurs at the amine nitrogen atom, which is the most favorable site in aqueous solution, for $PABAOMeH^+\cdot(H_2O)_3$ and for a significant fraction of $PABAH^+\cdot(H_2O)_6$. Fewer water molecules are necessary to stabilize the solution-phase isomer of $PABAOMeH^+$ (3) than for $PABAH^+$ (≥6), indicating that the favorable hydrogen bonding in $PABAH^+$ is a more important factor than the higher gas-phase basicity of $PABAOMeH^+$ in stabilizing protonation at the carbonyl oxygen atom. Relative Gibbs free energies (133 K) calculated using B3LYP and MP2 with the 6-311++G** basis set were significantly different from each other, and both are in poor agreement with results from the experiments. ωB97X-D/6-311++G**, which includes empirical dispersion corrections, gave results that were most consistent with the experimental data. The relative stabilities of protonating at the carbonyl oxygen atom for $PABAH^+\cdot(H_2O)_{0-6}$ and $PABAOMeH^+\cdot(H_2O)_{0-2}$ can be rationalized by resonance delocalization. These findings provide valuable insights into the solvent interactions that stabilize the location of a charge site and the structural transitions that can occur during the ESI desolvation process.



INTRODUCTION

The structure of a molecule in solution depends on the intrinsic properties of the molecule itself as well as the interactions between the molecule and its surrounding solvent environment. Solvents with high dielectric constants are especially effective at stabilizing charges on molecules. For example, amino acids adopt zwitterionic structures in aqueous solutions within a wide range of pH, but in the gas phase, the most stable form of the naturally occurring amino acids is nonzwitterionic.[1−3] The zwitterionic forms can be stabilized by the presence of adjacent charges,[4−11] and salt bridges can occur even in small protonated dipeptides[12] as well as larger peptides[13,14] and proteins.[15,16] Solvent also affects the relative ordering of the acid−base properties of molecules. The gas-phase basicity (GB) of methylamines follows the order $NH_3 < MeNH_2 < Me_2NH < Me_3N$, increasing monotonically with an increasing number of methyl groups attached to the nitrogen atom.[17,18] In contrast, the pK_a values in water follow the order $NH_3 < Me_3N < MeNH_2 \approx Me_2NH$.[19−21] Additional methyl groups can stabilize charge due to their polarizability,[17] but less sterically hindered charge sites can be more favorably solvated.[19] These two effects result in the different ordering of the gas- and solution-phase basicities of these molecules.

Electrospray ionization (ESI) can gently transfer intact molecules from solution into the gas phase, and this method has been widely used to produce gaseous ions ranging in size from individual atomic species[22] up to analytes with molecular weights in excess of a MDa.[23,24] Ions are typically desolvated completely prior to mass analysis or characterization by other structural methods, but extensively hydrated ions can also be formed and investigated.[25−30] Structural changes to analytes can occur during the ion desolvation process that takes place in ESI and in the gas phase. Many studies have shown that protein ions can retain a "memory" of their solution structures,[31−37] indicating that large gaseous ions can be kinetically trapped in higher-energy structures during the desolvation process. This kinetic trapping makes it possible in some cases to deduce information about solution-phase structures from gas-phase experiments.

Recent results show that the solvent used in ESI can also affect the structures of small gaseous ions.[38−41] Deprotonated *p*-hydroxybenzoic acid ($[PHBA\text{-}H]^-$) was found to predominantly adopt a phenoxide structure when sprayed from CH_3OH/H_2O solutions, whereas the carboxylate was predominantly produced from CH_3CN containing solutions.[38] In contrast, the opposite behavior was observed when the structure of deprotonated PHBA was investigated with different methods and apparatus.[38,39] These results suggest that the form of the ion that is produced by ESI can depend on both the solvent used and the experimental conditions.

Received: May 21, 2012
Published: September 6, 2012

dx.doi.org/10.1021/ja304929h | *J. Am. Chem. Soc.* 2012, 134, 15805−15813

Related studies on protonated *p*-aminobenzoic acid ($PABAH^+$, Figure 1) show that the ESI solvent can also affect the structure of the gaseous protonated ions. In aqueous solution, the amine group of PABA is the most basic site for protonation to occur,[42] but both calculations[43] and results from recent experiments[40,41] show that protonation of the carbonyl oxygen atom is more favorable in the gas phase. The lowest-energy gas-phase isomer was formed exclusively from a CH_3OH/H_2O solution, but a population of ions adopting the lowest-energy aqueous-solution structure was reported to be formed from a solution containing CH_3CN.[40,41]

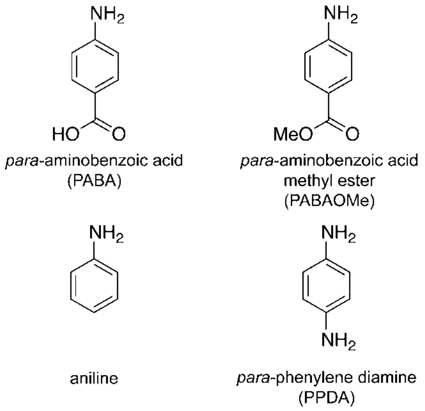


Figure 1. Structures and abbreviations for *p*-aminobenzoic acid and related molecules.

Here, the structures of $PABAH^+$ and structurally related molecules are investigated using infrared photodissociation (IRPD) spectroscopy and theory to determine how water stabilizes protonation of these molecules and to determine what structural changes occur during the desolvation process. Unlike previous studies that used mixed solvent systems, the results presented here are from aqueous solutions so that the solvent environment is unaffected by preferential evaporation of a more volatile component. These results indicate that protonated PABA and *p*-aminobenzoic acid methyl ester ($PABAOMeH^+$) are in the lowest-energy gas-phase form when one water molecule is attached, but the more stable solution-phase form is observed when just a few more water molecules are bound. Calculations using B3LYP, *ω*B97X-D, and MP2 and the 6-311++G** basis set gave widely contrasting results, with *ω*B97X-D being mostly closely consistent with the experimental data.

EXPERIMENTAL SECTION

IRPD Spectroscopy. A 2.75 T Fourier-transform ion cyclotron resonance (FT/ICR) mass spectrometer was used to measure the IRPD spectra of hydrated, protonated PABA as well as structurally related molecules: PABAOMe, aniline, and *p*-phenylene diamine (PPDA), (Figure 1). A description of the experimental apparatus is reported elsewhere.[5] All samples were obtained from Sigma-Aldrich (St. Louis, MO) and were electrosprayed from water, purified by a Milli-Q purification system (Millipore, Billerica, MA), at a concentration of 4−5 mM using borosilicate capillaries pulled to an inner tip diameter of ~2 *μ*m. A platinum wire in contact with the solution in the capillary is held at a potential between ~700 and ~1000 V relative to a heated metal capillary of the nanoelectrospray interface. Electrostatic lenses guide the ions through five stages of differential pumping and into the ion cell of the mass spectrometer. The ion cell temperature is controlled by a copper jacket that is cooled to 133 K with a regulated flow of liquid nitrogen.[44] A pulse of dry nitrogen gas is introduced into the vacuum chamber at a pressure of $\sim10^{-6}$ Torr for ~5 s to improve ion trapping and thermalization and is followed by a ~7 s pumpdown to reduce the pressure inside the cell to $<10^{-8}$ Torr. A stored waveform inverse Fourier transform is subsequently used to mass select precursor clusters prior to photodissociation.

The 1064 nm fundamental of a Nd:YAG laser (Continuum Surelight I-10, Santa Clara, CA) is pulsed at a 10 Hz repetition rate to pump the OPO/OPA (LaserVision, Bellevue, WA) used to photodissociate mass-selected clusters. Irradiation times from 5−60 s are used to induce substantial but not complete photodissociation of the precursor. First-order photodissociation rate constants are obtained from the precursor and product ion abundances after irradiation as a function of laser frequency.[45] These rate constants are then corrected for frequency-dependent variations in laser power as well as BIRD, which occurs as a result of absorption of blackbody photons from the 133 K ion cell and cell jacket.

Computational Chemistry. A Monte Carlo conformational search for $PABAH^+$ with up to six water molecules attached was performed by using Macromodel 9.1 (Schrödinger, Inc., Portland, OR) to generate at least 300 geometries for the amino-protonated and for the carbonyl-protonated isomers. Separate conformational searches were carried out for $PABAOMeH^+$ and anilinium$^+$ with up to three water molecules, and $PPDAH^+$ with three water molecules. A selection of the low-energy conformers as well as structures obtained from chemical intuition were used to create isomer geometries that represent different hydrogen bonding patterns. Q-Chem 3.1[46] (Q-Chem, Inc., Pittsburgh, PA) was then used to perform a geometry optimization at the B3LYP/6-31+G** level of theory prior to vibrational frequency and intensity calculations at the same level of theory. For calculated spectra, vibrational frequencies were scaled by 0.955 and convolved with a 100 and 15 cm^{-1} fwhm Lorentzian for the 2900−3100 and 3100−3900 cm^{-1} regions, respectively. Zero-point energies, enthalpy, and entropy corrections at 133 K were calculated for these structures using unscaled B3LYP/6-31+G** harmonic oscillator vibrational frequencies. Additional optimizations were performed for $PABAOMeH^+\cdot(H_2O)_{2,3}$ and $PABAH^+\cdot(H_2O)_6$ using the B3LYP, MP2, and *ω*B97X-D methods and the 6-311++G** basis set. Zero-point energy, enthalpy, and entropy corrections for these structures were calculated using frequencies obtained at the B3LYP/6-311++G** level of theory.

RESULTS

Hydration of $PABAH^+$. Protonation of isolated PABA occurs at the carbonyl oxygen atom of the carboxylic acid,[40,41] but protonation of the amine group is most favorable in aqueous solution.[42] To investigate how water solvates the protonated molecule in the gas phase and to determine how many water molecules are required to make protonation at the amine site favorable, IRPD spectra of $PABAH^+\cdot(H_2O)_{1-6}$ were measured in the spectral region between 2600 and 3900 cm^{-1} (Figure 2). The bands in the spectrum of $PABAH^+\cdot(H_2O)_1$ at 3443 and 3553 cm^{-1} correspond to the free N−H symmetric stretch (s.s.) and asymmetric stretch (a.s.), respectively, of the neutral amine group. These same two bands persist in the spectrum of $PABAH^+$ with up to six water molecules attached. The presence of these bands indicates that there is a population of ions that is not protonated at the amine group, and therefore this population must be protonated at the carbonyl oxygen atom. The assignment of these bands as free N−H stretches of a protonated amine can be ruled out based on the spectra of structurally related ions (see below).

The spectrum of $PABAH^+\cdot(H_2O)_6$ has a broad and intense band near 3100 cm^{-1} corresponding to a hydrogen bonded (HB) N−H stretch of a protonated amine. In comparison, this feature appears in the spectra of hydrated, protonated Val,[47] Phe,[48] and Pro[49] between 2900 and 3200 cm^{-1}. These results indicate that a significant fraction of the ion population is

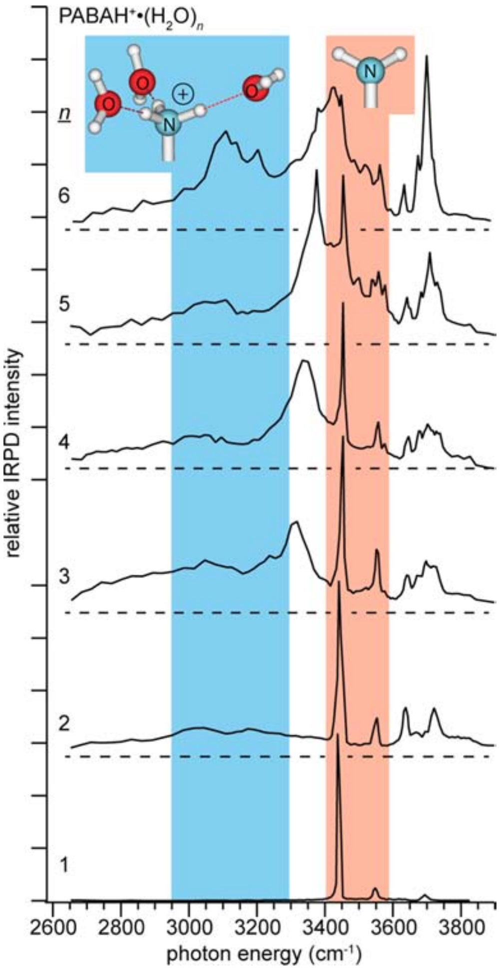


Figure 2. IRPD spectra of PABAH$^+$·$(H_2O)_{1-6}$ at 133 K. Bands associated with a fully hydrated protonated amine or unhydrated neutral amine group are designated by the blue and red regions, respectively.

protonated and hydrated at the amine group for PABAH$^+$·$(H_2O)_6$. Dissociation below 3200 cm^{-1} is also observed for PABAH$^+$·$(H_2O)_{2-5}$, but this feature is much weaker and broader than the HB N−H stretches that appear in the spectrum of PABAH$^+$·$(H_2O)_6$. The difference in the width of these two features suggests that they originate from two different vibrational modes. In addition, the absence of a free N−H band of a protonated amine from the spectrum of PABAH$^+$·$(H_2O)_2$ (which is shown below to occur between 3250 and 3350 cm^{-1}) is further evidence that this feature does not correspond to the N−H stretch of a protonated amine. Therefore the broad dissociation observed for PABAH$^+$·$(H_2O)_{2-5}$ is attributed to the HB O−H stretches of the protonated carboxylic acid. Because this feature overlaps with the HB N−H stretches associated with a protonated amine group, it is difficult to unambiguously conclude whether a small population of the solution-phase isomer contributes to the signal below 3200 cm^{-1} for PABAH$^+$·$(H_2O)_{3-5}$.

In addition to the free N−H features, there are several bands in the spectra of PABAH$^+$·$(H_2O)_{2-4}$ between 3300 and 3900 cm^{-1} that indicate protonation and hydration occurs predominantly at the carboxylic acid. The bands near 3650 and 3730 cm^{-1} in each of these spectra correspond to a single-acceptor (SA) water s.s. and a.s. New bands appear in the spectra of PABAH$^+$·$(H_2O)_{3,4}$ at 3340 and 3705 cm^{-1} corresponding to the HB O−H and free O−H stretch of an acceptor−donor (AD) water molecule. The appearance of bands associated with an AD water molecule indicates that the number of solvating water molecules exceeds the number of acidic H-atoms at the charge site to which water can bind. The absence of these bands for doubly hydrated PABAH$^+$, but not for triply hydrated PABAH$^+$, suggests that the charge site has two H-atoms where water can bind, consistent with a protonated carboxylic acid. In contrast, a protonated amine group has three H-atoms to which water can directly coordinate, and these sites are more favorable for water to bind to than the formation of a second hydration shell with the third water molecule. The appearance of an outer solvation shell has been reported for NH_4^+·$(H_2O)_4$, where the number of water molecules equals the number of hydration sites, but such isomers are higher in energy.[50] A second solvation shell has also been reported for ValH$^+$·$(H_2O)_4$, where one water molecule hydrogen bonds to the carboxylic acid and two water molecules hydrogen bond to the protonated nitrogen atom. The fourth water molecule forms a second solvation shell to the amine and is stabilized by forming a HB to the carbonyl oxygen atom of the C-terminus, an interaction that is not possible for hydrated PABAH$^+$.[47]

The spectrum of PABAH$^+$·$(H_2O)_1$ has just one band in the free O−H region at 3697 cm^{-1}, which is too low in energy to be a SA water a.s. Therefore, this band is assigned to a double-acceptor (DA) water a.s. The DA water s.s. is not observed, but this may be due to weak absorption typical of this mode.[51,52] The presence of a DA water molecule indicates that the water molecule forms HBs to both H-atoms of the protonated carboxylic acid. These results indicate that with up to five water molecules, PABAH$^+$ is predominantly protonated and hydrated at the carboxylic acid site, but a transition to the more favorable protonation site in aqueous solution occurs when this ion is hydrated by six or more water molecules.

Hydration of PABAOMeH$^+$. The IRPD spectra of PABAOMeH$^+$·$(H_2O)_{1-3}$ are shown in Figure 3. The spectra of PABAOMeH$^+$·$(H_2O)_{1,2}$ have bands near 3450 and 3555 cm^{-1}, corresponding to the free N−H s.s. and a.s., respectively, of a neutral amine group, consistent with protonation and hydration at the carbonyl oxygen of the ester group. The signature of a DA and a SA water molecule in the spectra of the

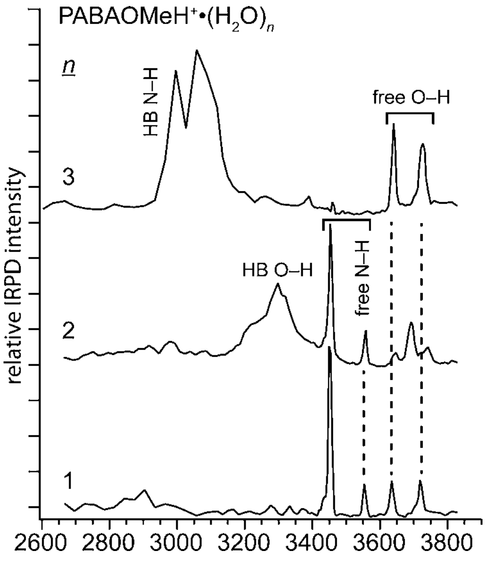


Figure 3. IRPD spectra of PABAOMeH$^+$·$(H_2O)_{1-3}$ at 133 K.

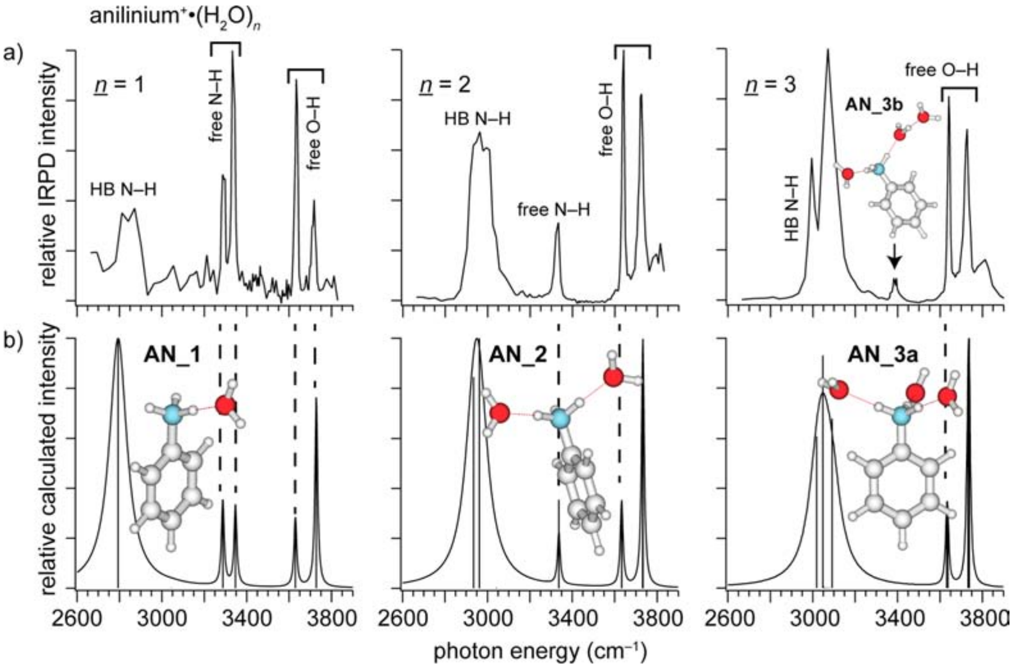


Figure 4. (a) IRPD spectra at 133 K and (b) calculated structures and spectra for lowest-energy structures of anilinium$^+$·$(H_2O)_{1-3}$ at the B3LYP/6-31+G** level of theory.

single hydrates of PABAH$^+$ and PABAOMeH$^+$, respectively, is consistent with the inability of a water molecule to form two HB in the latter ion. The free N−H stretches are largely absent in the spectrum of PABAOMeH$^+$·$(H_2O)_3$, and two bands appear between 2930 and 3200 cm^{-1}, consistent with the HB N−H stretches of a protonated amine, indicating that protonation and hydration occurs at the amine. These results suggest that PABAOMeH$^+$ is protonated at the carbonyl oxygen atom when singly and doubly hydrated, but protonation occurs at the amine group when three water molecules are attached.

The appearance of a significant population of the solution-phase isomer occurs at a lower hydration state for PABAOMeH$^+$ (three water molecules) than it does for PABAH$^+$ (6 water molecules). Although there is evidence for both isomers in the spectrum of PABAH$^+$·$(H_2O)_6$, the IRPD spectrum for triply hydrated PABAOMeH$^+$ indicates that the vast majority of the ion population is the amino-protonated isomer. The origin for the different behavior these two molecules is discussed below.

Confirmation of Band Assignments. Here, we confirm the assignments of all the bands in the spectra of hydrated PABAH$^+$ and PABAOMeH$^+$ from comparisons to the IRPD spectra of structurally related compounds, shown in Figure 1, and by comparisons to calculated spectra. Hydrated anilinium$^+$ is a model for protonation and hydration at the amine, and hydrated *p*-phenylene diamine (PPDAH$^+$) is a reference for the N−H stretches of an unprotonated amine. The spectra of these hydrated ions are discussed below.

Hydration of Anilinium$^+$. The spectra of anilinium$^+$·$(H_2O)_{1-3}$ (Figure 4a) can be readily interpreted based on previous results for other ions as well as from a comparison to calculated spectra of lowest-energy structures (Figure 4b). The spectrum of singly hydrated anilinium$^+$ has a band at 2841 cm^{-1} that blue shifts to 3054 cm^{-1} and increases in intensity for anilinium$^+$·$(H_2O)_3$. This band is too low in energy to be an O−H or a free N−H stretch. It is therefore assigned to the HB N−H stretch of a protonated amine group. This assignment is supported by calculations, which remarkably predict frequencies for these bands to within 50 cm^{-1} of the observed frequencies despite the harmonic oscillator approximation and the single scaling factor used in these calculations. This band also appears in the spectra of anilinium$^+$·$(H_2O)_{4-6}$ (Figure 5), although it is blue-shifted to 3100 cm^{-1}. This HB N−H stretch band occurs in the same region as a similar band in the spectrum of PABAH$^+$·$(H_2O)_6$, confirming the presence a population of the amino-protonated isomer for these ions. This HB N−H stretch band in the spectrum of anilinium$^+$·$(H_2O)_3$ (Figure 4 top right) is nearly the same as the corresponding band in the spectrum of PABAOMeH$^+$·$(H_2O)_3$ (Figure 3 top), confirming that the amine in the latter ion is both protonated and hydrated.

The calculated spectra of the lowest-energy isomers of anilinium$^+$·$(H_2O)_{1-3}$ (Figure 4b) have two, one and no free N−H stretches, respectively, between 3250 and 3350 cm^{-1} that closely match in frequency with the corresponding bands in the measured spectra. The appearance of a comparatively low-intensity band in this region for anilinium$^+$·$(H_2O)_3$ indicates that there is also a small population of ions where water forms a second solvation shell, i.e., only two of the three water molecules HB to the protonated amine, as seen with structure **AN_3b**. The structure with all three water molecules coordinating to the protonated amine group (**AN_3a**) is calculated to be the lowest-energy structure, but **AN_3b** is only 4.9 kJ/mol higher in energy, consistent with the appearance of a minor population of this ion in the measured spectrum. The bands associated with the free N−H stretch of a protonated amine are absent from the spectrum of PABAH$^+$·$(H_2O)_2$, supporting the assignment of the dissociation below 3200 cm^{-1} to the HB O−H stretches of the carboxylic acid.

The bands in the HB O−H and free O−H regions can be assigned based both on calculations and on previous results for hydrated ammonia.[50,53] The spectra of anilinium$^+$·$(H_2O)_{1-3}$ have bands near 3640 and 3720 cm^{-1} corresponding to a SA

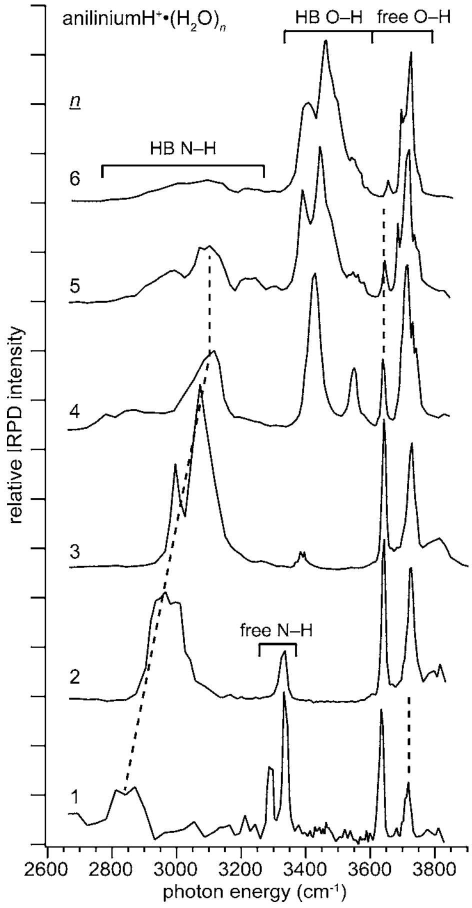


Figure 5. IRPD spectra of anilinium$^+$·$(H_2O)_{1-6}$ at 133 K.

water s.s. and a.s., respectively, and these frequencies are also accurately predicted by theory to within 15 cm^{-1} (Figure 4). New bands appear in the spectra of anilinium$^+$·$(H_2O)_{4-6}$ (Figure 5) between 3340 and 3600 cm^{-1}, consistent with HB O−H stretches also observed for NH_4^+·$(H_2O)_{5-7}$.[50] Additionally, the band near 3717 cm^{-1} increases in intensity due to the appearance of an AD water free O−H stretch that overlaps with the SA water a.s. Interestingly, bands associated with the formation of a second solvation shell for anilinium$^+$·$(H_2O)_4$ occur at 3430 and 3552 cm^{-1}, potentially overlapping with the free N−H stretches of a neutral amine group. However, these bands can be distinguished by their widths. The neutral amine N−H stretch bands have a fwhm of less than 20 cm^{-1}, whereas the HB O−H stretch features have a fwhm of greater than 30 cm^{-1}. The greater widths of the HB O−H stretch bands can be attributed to the effects of anharmonicity and dynamics associated with HBs that are not present for the free N−H stretches.[54]

Hydration of PPDAH$^+$. PPDAH$^+$ has both a protonated and a neutral amine group. Thus, the spectrum of PPDAH$^+$·$(H_2O)_3$ provides information about where the N−H stretches of an uncharged amine in PABAH$^+$ and PABAOMeH$^+$ should occur, and the IRPD spectrum of this ion as well as that of anilinium$^+$·$(H_2O)_3$ are shown in Figure 6a. The bands for the HB N−H stretches of a protonated amine group and SA water O−H stretches appear in both spectra, indicating an ion structure where all of the water molecules solvate the protonated amine for PPDAH$^+$·$(H_2O)_3$. The band near 3400 cm^{-1} indicates that a small fraction of the population has a water molecule in the outer solvation shell. In addition to the bands observed for anilinium$^+$·$(H_2O)_3$, the spectrum of PPDAH$^+$·$(H_2O)_3$ also has bands at 3458 and 3557 cm^{-1}. These bands can be confidently assigned to the free N−H stretches of an unhydrated neutral amine group and support the assignments for the corresponding peaks in the spectra of PABAH$^+$·$(H_2O)_{1-6}$, and PABAOMeH$^+$·$(H_2O)_{1,2}$.

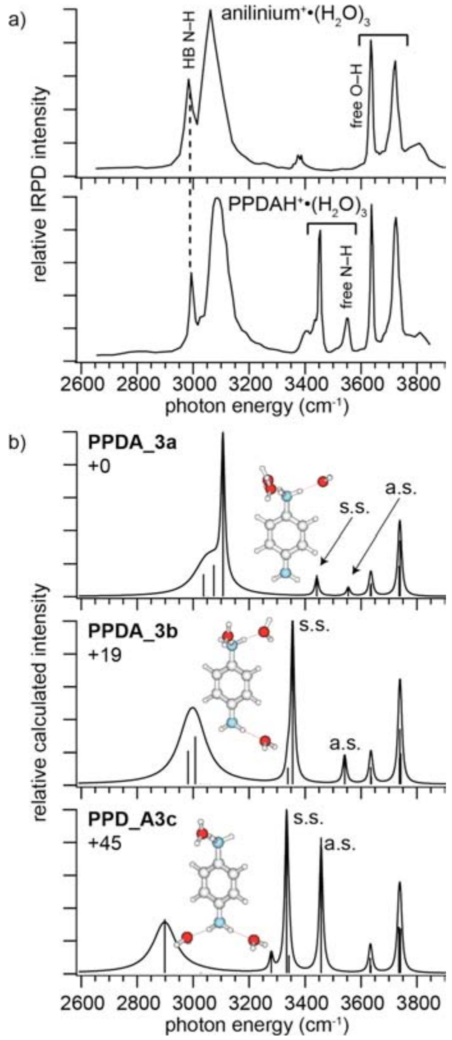


Figure 6. (a) IRPD spectra of anilinium$^+$·$(H_2O)_3$ and PPDAH$^+$·$(H_2O)_3$ at 133 K. (b) Calculated spectra and 133 K relative Gibbs free energies (in kJ/mol) of low-energy structures (the symmetric stretch (s.s.) and asymmetric stretch (a.s.) of the neutral N−H stretches are indicated on the spectra). Structures, spectra, and relative Gibbs free energies are calculated at the B3LYP/6-31+G** level of theory.

Figure 6b shows calculated structures and spectra of low-energy conformers along with calculated relative 133 K Gibbs free energies. The lowest-energy structure, **PPDA_3a**, has a fully solvated, protonated amine group and an unsolvated, neutral amine group. The calculated spectrum for **PPDA_3a** is a close frequency match to the IRPD spectrum, supporting the above assignments for these bands. The structure that has two water molecules solvating the protonated amine and one water molecule solvating the neutral amine, **PPDA_3b**, is a poor frequency match with the experimental spectrum and is 19 kJ/mol higher in energy than **PPDA_3a**. Attaching a water molecule to the neutral amine group causes a red shift in the neutral amine s.s. to ~3350 cm^{-1}. The neutral amine a.s., however, remains relatively unperturbed. The addition of a second water molecule to the neutral amine group, shown in **PPDA_3c**, causes a red shift in the neutral amine a.s. to ~3450 cm^{-1}, while the frequency of the s.s. exhibits only a subtle red shift in comparison to the spectrum of **PPDA_3b**. **PPDA_3c** is a poor frequency match with the experimental spectrum, and is calculated to be 45 kJ/mol higher in energy. Thus, it is unlikely that the ion population contains a significant amount of this isomer. These results indicate that the ion population is predominantly **PPDA_3a**.

■ DISCUSSION

Gas-Phase Basicity vs Hydrogen Bonding. In the absence of solvent, PABAH$^+$ is protonated at the carboxylic acid[40,41] whereas in aqueous solution, protonation of the amine is more favorable.[42] The GB of PABAOMe is 20.7 kJ/mol greater than that of PABA,[55] which should make protonation of the carbonyl oxygen of PABAOMeH$^+$ even more favorable. So why are fewer water molecules required for PABAOMeH$^+$ to adopt its solution-phase structure (3) than for PABAH$^+$ (≥6)? PABAOMeH$^+$ has one fewer H-atom at this site that can form a HB to water, making it somewhat less favorable to adduct water molecules to the ester. Evidently, protonation of a carbonyl oxygen atom is better stabilized by more favorable solvation of the protonated carboxylic acid of PABAH$^+$ than by the higher basicity of the methyl ester for PABAOMeH$^+$.

Stability of a Protonated Carboxylic Acid. The higher basicity of the carboxylic acid compared to the amine for isolated PABA can be attributed to resonance stabilization. The GB of aniline is 18.3 kJ/mol greater than that of PABA and 60.5 kJ/mol greater than that of benzoic acid.[55] Thus, the basicity at each group is significantly affected by the presence of the other group. A neutral amine can donate electron density into the conjugated π system to better stabilize the positive charge associated with the protonated carboxylic acid (Figure 7a). If the amine is protonated, the carboxylic acid withdraws electron density due to the electronegativity of the oxygen atom, which destabilizes the positive charge at the protonated amine group (Figure 7b). This conjugation between the two functional groups results in the carboxylic acid of a neutral PABA molecule being the most basic site in the gas phase. Similarly, the stability of the phenoxide form of deprotonated PHBA was attributed to resonance stabilization.[39] Based on the measured GB values, conjugation stabilizes protonation of the carboxylic acid by as much as ~42 kJ/mol, and the protonation of the amine is destabilized by more than ~18 kJ/mol.

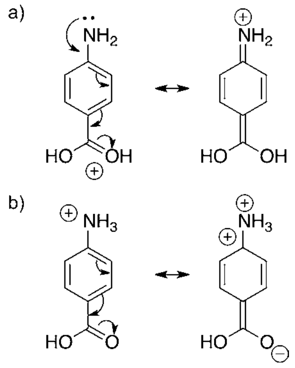


Figure 7. Resonance contributors for PABAH$^+$ protonated at (a) the carboxylic acid or at (b) the amine.

Kinetic Trapping? Kass and co-workers reported that the gaseous structures of PABAH$^+$ depend on the electrospray solvent used, indicating that kinetic trapping of high-energy structures occurs.[40,41] In order to determine whether the structures observed in these experiments are the lowest-energy structures, or if they are higher-energy structures that are kinetically trapped by evaporation of water molecules from more extensively hydrated ions, the structures of these ions and their hydrates were investigated using computational chemistry. The relative Gibbs free energies for PABAH$^+$·(H$_2$O)$_{0-3,6}$ and PABAOMeH$^+$·(H$_2$O)$_{0-3}$ at 133 K were calculated at the B3LYP/6-31+G** level of theory. Protonation at the carbonyl oxygen atom is lower in energy than protonation at the amine nitrogen by 35 and 41 kJ/mol for isolated PABA and PABAOMe, respectively (structures in Figures S1 and S2, top). The value for isolated PABA is consistent with a previously reported 298 K enthalpy difference of 33 kJ/mol at this same level of theory.[40] An enthalpy difference of 17 kJ/mol was calculated with the G3 method, although the difference in enthalpy decreased to less than 12 kJ/mol if this value was corrected for deviations in computed and experimental proton affinities of aniline and benzoic acid.[41] Protonation at a carbon atom in the ring of PABA is at least 40 kJ/mol higher in energy (Figure S3), consistent with previous calculations.[40]

Protonation of the carbonyl oxygen atom is favored by 36 and 32 kJ/mol for PABAH$^+$·(H$_2$O)$_1$ and PABAOMeH$^+$·(H$_2$O)$_1$, respectively (low-energy structures are shown in Figures S1 and S2). Although the difference in energy decreases with increasing hydration state, protonation of the carbonyl oxygen atom is still calculated to be lower in energy by 22 and 19 kJ/mol for PABAH$^+$·(H$_2$O)$_6$ and PABAOMeH$^+$·(H$_2$O)$_3$, respectively. Protonation at the carbon atom in the ring is even higher in energy when these ions are hydrated (Figure S3). These results indicate that the most stable structures for PABAH$^+$·(H$_2$O)$_{1-6}$ and PABAOMeH$^+$·(H$_2$O)$_{1-3}$ are protonated at the carbonyl oxygen atom, and that amino-protonated structures should not be observed in these experiments. In striking contrast, the experiments indicate that protonation at the amine occurs for the most extensively hydrated ions studied. To the extent that the energetic values obtained from these calculations are accurate, the solution-phase isomers that are observed in these experiments at higher hydration states must be formed by solvent evaporation and kinetic trapping from more extensively hydrated ions for which the solution-phase structure is most stable.

To evaluate the accuracy of these calculations, additional calculations were performed for PABAOMeH$^+$·(H$_2$O)$_{2,3}$ and PABAH$^+$·(H$_2$O)$_6$, and these results are summarized in Table 1 (structures shown in Figure 8). PABAOMeH$^+$·(H$_2$O)$_{2,3}$ is potentially an excellent benchmark for theory because of the distinct transition from a carbonyl-protonated structure for PABAOMeH$^+$·(H$_2$O)$_2$ to an amino-protonated structure for PABAOMeH$^+$·(H$_2$O)$_3$ indicated by the experimental data. For PABAOMeH$^+$ with two and three water moleculues, B3LYP/6-31+G** indicates that protonation of the carbonyl oxygen atom is 22 and 19 kJ/mol more favorable, respectively. With the 6-311++G** basis set, these differences are reduced slightly to 19 and 13 kJ/mol, respectively. In sharp contrast, MP2/6-311+G** indicates that amine protonation is favorable by 14 and 33 kJ/mol for this ion with two and three water molecules

Table 1. Calculated Relative Gibbs Free Energies at 133 K in kJ/mol of Selected Structures

	B3LYP/6-31+G**	B3LYP/6-311++G**	ωB97X-D/6-311++G**	MP2/6-311++G**
PABAOMe_S_2a	22	19	6.6	0
PABAOMe_G_2a	0	0	0	14
PABAOMe_S_3a	19	13	0	0
PABAOMe_G_3a	0	0	1.1	–
PABAOMe_G_3b	14	10	12	33
PABA_S_6a	22	20	0	0
PABA_G_6a	0	0	2.5	29

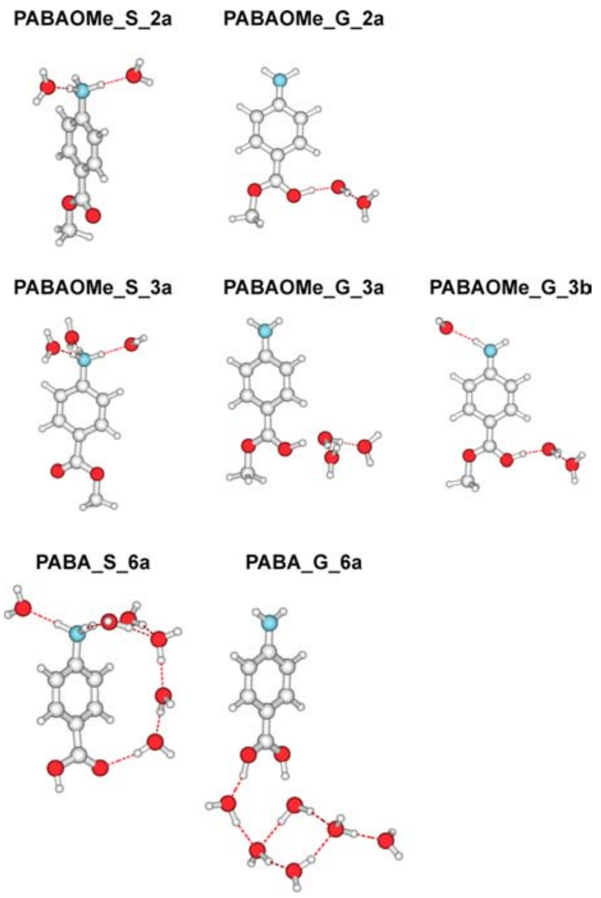


Figure 8. Low-energy structures for PABAOMeH$^+\cdot$(H$_2$O)$_{2,3}$ and PABAH$^+\cdot$(H$_2$O)$_6$ minimized at the B3LYP/6-31+G** level of theory. With the exception of **PABAOMe_G_3a**, structures obtained at higher levels of theory are nearly identical.

attached, respectively. With MP2, structure **PABAOMe_G_3a** (Figure 8) is not stable, and this structure underwent a proton transfer to form neutral PABAOMe and a hydronium ion (Figure S4). The relative B3LYP and MP2 energies calculated with the 6-311++G** basis set for O vs N protonation for PABAOMeH$^+$ with two and three water molecules differ by 33 and 46 kJ/mol, respectively.

The ωB97X-D functional, which includes empirical atom−atom dispersion corrections, was recently introduced and should be well suited for systems where noncovalent interactions, such as hydrogen bonding, are significant.[56] With ωB97X-D/6-311++G**, protonation at the carbonyl oxygen atom of PABAOMeH$^+\cdot$(H$_2$O)$_2$ is lower in energy by 6.6 kJ/mol compared to protonation at the amine nitrogen. For PABAOMeH$^+$ with three water molecules attached, protonation at the amine nitrogen is favored, although it is predicted to be nearly isoenergetic (to within ∼1 kJ/mol) with protonation of the carbonyl oxygen atom. Thus, the change in structure from O to N protonation for PABAOMeH$^+$ with two and three water molecules observed in the experiment is correctly predicted by ωB97X-D/6-311++G**.

A similar comparison between different levels of theory for PABAH$^+\cdot$(H$_2$O)$_6$ (Table 1), where a mixture of both the gas- and solution-phase isomers were observed in the IRPD spectrum, was performed. Increasing the basis set with B3LYP has a relatively small effect on the relative energies, and protonation of the carbonyl oxygen atom is favored by 20 kJ/mol with B3LYP/6-311++G**. By contrast, protonation of the amine nitrogen is 29 kJ/mol *lower* in energy with MP2/6-311++G**. This is a 49 kJ/mol difference in the relative energies predicted by these two methods. With ωB97X-D/6-311++G**, protonation of the carbonyl oxygen atom is only 2.5 kJ/mol higher in energy, consistent with the observed presence of both isomers. As was the case for PABAOMeH$^+\cdot$(H$_2$O)$_{2,3}$, the ωB97X-D/6-311++G** level of theory is in closest agreement with the experimental data.

To the extent that these ωB97X-D/6-311++G** calculations are accurate, this indicates that ions are not kinetically trapped in higher-energy structures in these experiments. For PABAH$^+\cdot$(H$_2$O)$_6$, **PABA_S_6a** (Figure S1), in which the water molecules form a bridge between the two protonation sites, is the lowest-energy structure at the MP2/6-311++G** and ωB97X-D/6-311+G** levels of theory. This and similar structures should make proton transfer between the two protonation sites facile, reducing the possibility of kinetic trapping. A water bridge between two charges sites is unfavorable for PABAOMeH$^+\cdot$(H$_2$O)$_{2,3}$, yet the experimental results show that these ions are protonated at different sites. The excellent agreement between experiment and the ωB97X-D/6-311++G** calculations for both ions suggests that low-energy structures are formed in these experiments even without water bridging. Additional higher level calculations will ultimately be required to more definitively answer this question.

■ CONCLUSION

IRPD spectra of hydrated, protonated PABA were measured in the region from 2600 to 3900 cm^{-1} in order to study the relative stabilities of the preferred isolated gas- and solution-phase isomers as a function of hydration state. The preferred gas-phase isomer was observed for PABAH$^+\cdot$(H$_2$O)$_{1-5}$, but both the solution- and gas-phase isomers are present for PABAH$^+\cdot$(H$_2$O)$_6$. For PABAOMeH$^+$, protonation of the carbonyl oxygen atom occurs with one and two water molecules attached, but protonation of the amine occurs with three water molecules attached. The spectral assignments for these ions were confirmed by measuring IRPD spectra of structurally related compounds. Results from calculations depend strongly on the method used. B3LYP and MP2 with the 6-311++G** basis set give widely opposing results, neither of which is in line with results from these experiments. In contrast, the ωB97X-D/6-311++G** level of theory gave relative energies that are much more consistent with the experimental data.

Fewer water molecules are necessary to stabilize protonation at the amine of PABAOMeH$^+$ compared to PABAH$^+$, indicating that the more favorable HB in the latter ion is more important in stabilizing protonation of a carboxyl group than the higher GB of this site in PABAOMe. Protonation of the carbonyl oxygen atom in the isolated ion is favored due to the conjugation between the carboxyl and amine groups. When the carboxyl group is protonated, the neutral amine donates electron density into the conjugated π system, delocalizing and stabilizing the positive charge associated with a protonated carbonyl oxygen atom.

This is the first study to report a change in protonation from the most favorable site in the gas phase to the most favorable site in aqueous solution with increasing hydration state. These results provide insight into how water stabilizes protonation sites in molecules and structural transitions that can occur during the desolvation process in ESI.

■ ASSOCIATED CONTENT

S Supporting Information

Complete ref 46; calculated low-energy structures and energies for selected ions. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the National Science Foundation (Grants CHE-1012833 and OISE-730072) for generous financial support of this research and for the support of the University of California, Berkeley Molecular Graphics and Computation Facility (CHE-0233882, and CHE-0840505). The authors also thank Maria Demireva and Dr. Jeremy T. O'Brien for helpful discussions.

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