Amine vs. carboxylic acid protonation in ortho-, meta-, and para-aminobenzoic acid: An IRMPD spectroscopy study

Adam P. Cismesia; Georgina R. Nicholls; and Nicolas C. Polfer*

Department of Chemistry, University of Florida, P.O. Box 117200, Gainesville, FL 32611-7200, U.S.A.

***Corresponding Author**

Phone: 352-392-0492

Fax: 352-392-0872

E-mail: polfer@chem.ufl.edu

Notes

The authors declare no competing financial interest.

Keywords

IRMPD, gas phase, protonation, substitution, aromatic, isomers

Abstract

Infrared multiple photon dissociation (IRMPD) spectroscopy and computational chemistry are applied to the ortho-, meta-, and para- positional isomers of aminobenzoic acid to investigate whether the amine or the carboxylic acid are the favored sites of proton attachment in the gas phase. The NH and OH stretching modes yield distinct patterns that establish the carboxylic acid as the site of protonation in para-aminobenzoic acid, as opposed to the amine group in ortho- and meta-aminobenzoic acid, in agreement with computed thermochemistries. The trends for para- and meta-substitutions can be rationalized simplistically by inductive effects and resonant stabilization, and will be discussed in light of computed charge distributions based from electrostatic potentials. In ortho-aminobenzoic acid, the close proximity of the amine and acid groups allow a simultaneous interaction of the proton with both groups, thus stabilizing and delocalizing the charge more effectively, and compensating for some of the resonance stabilization effects.

Introduction

Understanding acid-base chemistry in aromatic molecules is intimately associated with resonance stabilization and inductive effects by electron-donating and withdrawing groups on the ring. The structural isomers of amino-benzoic acid (ABA), ortho-aminobenzoic acid (OABA), meta-aminobenzoic acid (MABA) and para-aminobenzoic acid (PABA) present an intriguing case, given that both basic (i.e., amino) and acidic (i.e., carboxylic acid) groups are present.

Table 1: Literature values for pKa's (for $[ABA+H^+] \rightarrow [ABA]$), and proton affinities (PAs) for PABA, MABA, and OABA.^{1,2}

Compound	pKa	PA (KJ/mol)
<i>PABA</i>	2.38	864.7
<i>MABA</i>	3.07	864.7
<i>OABA</i>	2.05	901.5

In solution, the published pKa values (see Table 1) show that OABA is the strongest acid, followed by PABA and MABA. Another way to phrase this is that neutral OABA is the weakest base, as it requires the lowest pH to be protonated. The loss of the proton from the protonated form $[ABA+H^+]$ can occur from the protonated amine, or alternatively, from the acid group. Given the competition between the amine and the carboxylic acid, as well as the influence of the solvent, understanding these effects in condensed phase on a fundamental level is not trivial.

When studying molecules in the gas phase, the problem can be simplified to a characterization of the favored (de)protonation site. Previous kinetic method experiments have shown that OABA is most basic (in notable contrast to solution), followed by MABA and PABA, which have identical proton affinities.² The role of carboxylic acid as an acidic or basic site in the gas phase is in fact more convoluted. In

negative ion mode, the carboxylic acid can be readily deprotonated,³ or may compete with other putative sites (e.g., phenol).⁴⁻⁷ In positive ion mode, some studies on metal-adducted amino acids and peptides have shown that carboxylic acids can be de-protonated, and hence act as acidic moieties.⁸⁻¹⁰ Conversely, studies on protonated PABA have shown that the carboxylic acid site can be protonated, and thus act as a basic site.¹¹⁻¹⁴

For an aromatic system like ABA, resonance stabilization is expected to play an important role whether the amino or carboxylic acid groups are the favored sites of protonation. Scheme 1 illustrates that in PABA, the electron-donating amino group can couple the electron density of its lone pair into the ring, and further onto the carboxylic acid, thereby making the carboxylic acid group more basic, and consequently the amino group less basic. A similar effect would be expected for OABA, but not for MABA, as the enhanced electron density cannot couple with meta substitutions. At first sight, it is therefore surprising that MABA and PABA have identical proton affinities (Table 1), even if many more resonance structures of the neutral and ionized forms would have to be considered to establish definite trends.

Scheme 1: Some resonance structures for PABA.

As a complicating factor, it has recently been shown that when ionizing protonated PABA or the related benzocaine in electrospray ionization (ESI), the favored solution-phase structure, protonated on the amine group, can be kinetically trapped into the gas phase, especially when aprotic solvents are employed.^{11,14,15} Conversely, an isomerization to the favored gas-phase structure, protonated on the carboxylic acid group, is observed for protic solutions (and higher temperatures in the ESI source).¹² While the exact mechanism for this isomerization process has not been proven, it has been hypothesized that a protic bridge mechanism could facilitate the proton transfer from the amine to the carboxylic acid.^{11,12} Infrared photodissociation spectroscopy and computational studies on partially hydrated protonated PABA¹⁶ and MABA¹⁷ show that in the case of PABA the addition of several water molecules can affect the energetics of proton attachment.

In this study, we employ protic solvents in order to prevent trapping of solution-phase structures, and in an effort to determine the favored sites of protonation of these substitution isomers in the gas phase. Thus, the inherent role of the substitution patterns, and thereby the respective effects of inductive, resonance and charge solvation effects, can be studied in the absence of water or other solvents.

Experimental

Sample Preparation:

A 100 μ M solution of tryptophan (Sigma Aldrich, St. Louis, MO) was made up in methanol/water/formic acid (70:29:1). 1 μ M solutions of PABA, MABA, and OABA (Sigma Aldrich, St. Louis, MO) were made up in methanol/water/formic acid (70:29:1). All sample vials were centrifuged for 10 min (6000 RPMs) to remove particulates which may clog the emitter needle (50 μ m ID/360 μ m OD).

Instrumentation:

All IRMPD spectra were recorded on a custom quadrupole mass filter-quadrupole ion trap-time of flight (QMF-QIT-ToF) mass spectrometer, previously described.¹⁸ Ions were generated in a modified electrospray ionization (ESI) source (Analytica, Branford, CT) equipped with a heated metal capillary to aid desolvation, and an rf ion funnel to increase ion transmission. Ions were first accumulated in a hexapole, and then guided via a QMF for mass selection, followed by gas-assisted trapping in a QIT. After a pump down delay (100 ms), the ions were irradiated with the tunable output from an optical parametric oscillator/amplifier (OPO/A) (LaserVision, Bellevue, WA) pumped by a Surelite III, unseeded 1064 nm Nd:YAG laser (Continuum, San Jose CA) to induce infrared multiple photon dissociation (IRMPD).¹⁹⁻²¹ The IR beam was focused by a $\varnothing$ 1" CaF₂ plano-convex lens (250 mm focal length) through a $\varnothing$ 1" BaF₂ window (Thor Labs). A shutter, controlled by a delay generator, allowed a single (or multiple) pulse(s) from the OPO/A to irradiate the ions. For PABA and OABA, a single IR pulse was sufficient to induce abundant IRMPD yield; for MABA the ion population was subjected to three IR pulses to enhance the (lower) IRMPD yield. In some additional experiments, following a brief delay (100 ns), the ions were irradiated with a second IR pulse (20-100 ms) from a fixed-wavelength CO₂ laser (10.6 μ m) to enhance the IRMPD yield. The remaining precursor and photofragment ions were then pulsed into the ToF for mass analysis.

IRMPD Measurements:

Unless otherwise stated, all IRMPD spectra were collected from 2800 cm⁻¹ to 3800 cm⁻¹. The output frequency was increased by steps of 2 cm⁻¹ and 20 mass spectra were averaged per each wavenumber analyzed. An IRMPD spectrum of protonated tryptophan (from 3450 cm⁻¹ to 3600 cm⁻¹, not shown) served as a control experiment for calibration, as that spectrum had previously been recorded using a wavemeter.²² The IRMPD yield for each wavenumber step was expressed on a linear scale, using the following equation: $IRMPD\ Yield = \frac{\Sigma(Fragment\ Ions)}{\Sigma(Parent+Fragment\ Ions)}$

The IRMPD spectra shown in the main part of the manuscript were not corrected for OPO pulse energy to allow for a more facile visual comparison of the experimental and theoretically predicted band positions. The pulse energy-corrected IRMPD spectrum for MABA is shown in the supporting information (Fig S1) as an example of how some of the NH bands become barely visible following a pulse energy correction. These results suggest that the IRMPD yield does not respond linearly with pulse energy, with OH bands being considerably more intense than NH bands, in contrast to the computed linear absorption spectra. Our analysis here will focus exclusively on band positions rather than IRMPD intensities.

Calculations: Calculated structures and conformations of protonated OABA, MABA and PABA were generated with chemical intuition in Gabedit 2.4.8.²³ Gaussian 09²⁴ was then utilized to perform DFT calculations for energy optimizations and IR frequencies for each conformation at B3LYP/cc-pVTZ. The electronic energies were corrected for zero-point vibrational energies, which are reported here as ZPE (zero-point energy) corrected energies (without scaling). The latter energies served as a measure for the relative thermochemical stabilities of these conformations. Gabedit and/or Avogadro²⁵ were used for visualization of the geometries and inspection of the IR modes. All spectra were broadened with a Gaussian linewidth fwhm (full-width half-maximum) = 5 cm⁻¹ and scaled by 0.960²⁶ for more facile

comparison with experiment. GaussView²⁷ was employed to generate the electrostatic potential (ESP) maps using the Gaussian utility cubegen. All ESP maps used the same isovalue (0.0004 au) and surface potential ranges (0.11 au (red) – 0.18 au (blue)).

Results and discussion:

The experimental IRMPD spectra for PABA, MABA and OABA, from 3100 – 3700 cm⁻¹, are shown in Figure 1, and the experimental band positions are summarized in Table 2. Note that the full IRMPD spectra, for the entire 2800 – 3800 cm⁻¹ range are depicted in the Supplementary Material (Figure S2), but that all measured spectral bands are contained in Figure 1. At first sight, the spectra for MABA and OABA look most similar, both having at least two peaks in the 3200 – 3400 cm⁻¹ range, and a single feature in the 3500-3600 cm⁻¹ region. Conversely, the IRMPD spectrum for PABA exhibits a single peak in the 3400 – 3450 cm⁻¹ region, but a doublet in the 3500 – 3600 cm⁻¹ range. The spectral differences between PABA on the one hand, and MABA and OABA on the other, strongly suggest different protonation sites for either set. As protonated PABA has been previously studied,^{11,13,14,16} and is the subject of a parallel study on trapping of solution-phase structures from ESI,¹² we will begin our structural interpretation with that positional isomer.

Figure 1: Experimental IRMPD spectra for protonated OABA, MABA and PABA (bottom to top) from 3100 – 3700 cm^{-1} . Inset shows magnified 3250 – 3475 cm^{-1} region for MABA.

Table 2: Experimental band positions for the IRMPD spectra for protonated PABA, MABA and OABA.

Positional Isomer	Band Positions (cm ⁻¹)
PABA	3590, 3550, 3435
MABA	3565, 3295, 3250
OABA	3550, 3335, 3280

Para-aminobenzoic acid (PABA)

A computational investigation of the conformations for carboxylic acid- and amine-protonated PABA show that the carboxylic acid is the more favored protonation site in the gas phase. The lowest-energy conformations are depicted in Figure 2. For the sake of simplicity, we will refer to these conformations as structures 2A, 2B, etc., as they appear in Figure 2. The computed spectra for each of these conformers are compared to the experimental IRMPD spectrum in Figure 3. Based on the NH and OH vibrations, it is apparent that the carboxylic acid-protonated structures match best, which is also in agreement with previous computational and experimental studies.^{11,12,14}

Figure 2: The lowest-energy conformations for PABA calculated at the B3LYP/cc-pVTZ level of theory with relative ZPE-corrected energies for carboxylic acid-protonated (left) and amine-protonated (right). The ESP maps for the respective two lowest-energy conformers, 2A and 2D, are depicted.

Various metrics about the conformations in Figure 2 are summarized in Table S1, including their protonation sites, computed energies, dipole moments, and their symmetries. For the carboxylic acid-protonated conformations, depending on the orientations of the hydrogens in the CO₂H₂⁺ group, C_s and C_{2v} symmetries are possible. The lower C_s symmetry (2A) is energetically favored, presumably by virtue of forming an unimpeded hydrogen bond between a hydroxyl H and a hydroxyl O. The amine-protonated conformations surprisingly do not adopt a C_s symmetry, due to a non-symmetric orientation of the NH₃⁺ group with respect to the plane of the aromatic ring (for the case of 2D), and an additional rotation of the COOH moiety with respect to the plane of the aromatic ring (for the case of 2E) (see Supporting Information, Figure S3). Once again, the H-bonded structure (2D) is favored vis-à-vis the structure devoid of this H-bond (2E).

While the agreement between experiment and theory is far from a perfect match, it does appear that the lowest-energy structure, structure 2A, gives the most compelling match. Nonetheless, a breaking of the carboxylic acid H-bond would lead to an interconversion to structures 2B or 2C, and neither 2B nor 2C can be completely excluded based on the IRMPD spectrum. Table S2 summarizes the predicted modes and band positions for these OK structures. Note that for the protonated carboxylic acid (CO_2H_2^+) moiety, discrete OH stretches are labeled as ν_{OH} , whereas for coupled modes, symmetric (sym. ν_{OH}) and antisymmetric (asym. ν_{OH}) are distinguished. In terms of a general assignment of the experimental bands that are observed, the 3435 cm^{-1} feature is unambiguously assigned to the symmetric NH_2 stretching mode; 3550 cm^{-1} is likely due to the a CO_2H_2^+ moiety OH stretching mode, which cannot be resolved from the asymmetric NH_2 stretching mode; the 3590 cm^{-1} band arises from a higher-frequency CO_2H_2^+ OH stretching mode.

As opposed to the gas phase, the favored protonation site in solution is on the amine group, as verified by NMR measurements in polar — both protic and aprotic — solvents.¹¹ It is highly likely that the very large dipole moment, up to almost 16(!) Debye, must play an important role when stabilizing the amine-protonated structure in a polar solvent. Computations on protonated PABA-water clusters had shown that sequential solvation makes the amine-protonated structure progressively competitive in terms of thermochemistry, indicating that 6 water molecules would lead to an isoenergetic situation where amine and carboxylic acid protonation are equally favorable.¹⁶ It is yet unclear to what extent different intermolecular interactions, including H-bonds between PABA and solvent molecules, dipole-dipole interactions, and even van der Waals interactions contribute to this stabilization.

For the gas phase, the results presented here suggest that the simple resonance stabilization picture in scheme 1 correctly predicts the higher proton affinity of the carboxylic acid moiety compared to the amine. Moreover, the C_s symmetry of structure 2A, having the NH_2 group in-plane with the aromatic ring, is consistent with an sp^2 hybridization for the amine N, also in agreement with the resonant

stabilization in scheme 1. The calculated ESP maps for the protonated carboxylic acid (structure 2A) and protonated amine (structure 2D) are shown in Figure 2, depicting more negative regions (in red) and more positive regions (in blue). It is clear from these distributions that the charge is much more evenly distributed in the case of the carboxylic acid-protonated isomer, effectively meaning that the lone pair electron density is coupled throughout the aromatic ring and the CO_2H_2^+ group, thus balancing the positive charge from the proton. In contrast, there is an extreme imbalance in charge for the amine-protonated isomer, with the net negative charge adjacent on the carboxylic acid, and the positive charge on the protonated NH_3^+ group, thus also accounting for the large dipole moment in this molecule. These trends are also compatible with the strong electron-donating properties of amines, as opposed to the moderately electron-withdrawing properties of carboxylic acid. In other words, the carboxylic acid group is not expected to donate electron density to the protonated amine, to balance the charges.

Figure 3: Experimental IRMPD spectrum of PABA (in gray) overlaid with the calculated vibrational spectra of structures 2A (0 kJ/mol), 2B (10.4 kJ/mol), 2C (20.1 kJ/mol), 2D (40.7 kJ/mol), and 2E (81.6 kJ/mol). Calculated stick spectra (in red) convoluted with a Gaussian function fwhm=5 cm⁻¹ (in black).

Meta-aminobenzoic acid (MABA)

The conformations for protonated MABA are shown in Figure 4. The two lowest-energy structures, 4A and 4B, are both amine-protonated structures, and are close to isoenergetic, as they merely differ in the rotation of the NH_3^+ group. The third amine-protonated structure, 4C, incurs a substantial energetic penalty, due to the loss of the hydrogen bond on the carboxylic acid. Structures 4D and 4E are carboxylic acid-protonated species. It is interesting to note that 4D is only 6.2 kJ/mol higher in energy than 4A, in principle suggesting that both protonation sites are possible. Nonetheless, given the large dipole for amine-protonated structures (see Table S1), the amine-protonated structures would likely be even more favored in solution. This means that the carboxylic acid-protonated structures are effectively disfavored in solution and the gas phase, and are therefore unlikely to be observed here. The higher-energy carboxylic acid-protonated structures yet again display different hydrogen atom arrangements around the CO_2H_2^+ moiety. For the sake of completeness, the energies, dipoles, and symmetries for these structures are listed in Table S1, and the mode assignments are given in Table S2.

Figure 4: The lowest-energy structures for MABA calculated at the B3LYP/cc-pVTZ level of theory with zero-point corrected electronic energies for amine-protonated (left) and carboxylic acid-protonated (right). The ESP maps for the respective two lowest-energy conformers, 4A and 4D, are depicted.

The computed spectra for these conformations are compared to the experimental data for protonated MABA in Figure 5. The experimental IRMPD spectrum shows two prominent peaks at 3565 cm^{-1} and 3250 cm^{-1} and a possibly suppressed peak at 3295 cm^{-1} (Table 2). The calculated vibrational spectra for the amine-protonated species, 4A-C, all have three predicted NH bands in the $3200 - 3350\text{ cm}^{-1}$ region, as well as one band in the OH stretching region. Again, the agreement between experiment and theory is far from ideal, yet the vibrational peaks are largely consistent with this pattern. Conversely, the carboxylic acid-protonated structures predict a distinct symmetric NH_2 stretching mode at 3450 cm^{-1} and additional features in the $3500 - 3600\text{ cm}^{-1}$ range, neither of which are reproduced.

As a general observation, the IRMPD yield for MABA was suppressed compared to PABA and OABA under identical conditions. This is illustrated by the fact that three OPO/A pulses were required to generate the IRMPD spectrum in Figure 1, as opposed to merely one for PABA and OABA. This observation suggests that the photofragmentation pathways in MABA involve higher barriers, which will be discussed in more detail later. This barrier is particularly a problem for vibrational modes that are anharmonic. We hypothesize that upon IR activation, the NH_3^+ stretching modes at $\sim 3300\text{ cm}^{-1}$ are redshifted, thus precluding absorption of further photons, and consequently leading to a lower IRMPD yield. For the lower-frequency NH_3^+ mode at 3250 cm^{-1} , this is presumably not a problem, as the higher frequency modes will shift into resonance at higher internal energies.^{20,28} By conducting the IRMPD experiment for protonated MABA under different conditions, the intensity of the weaker 3295 cm^{-1} peak could be enhanced relative to background (see paragraph on “Further Analysis of Suppressed MABA Mode” and Figure S4, Supplementary Material), lending further credence to the claim that this feature is real.

In contrast to PABA, MABA is identified to be protonated on the amine. This is again consistent with the simple resonance stabilization picture in scheme 1, as the electron-donating amine group cannot couple its electron density to the carboxylic acid group. This effect is nicely visualized in the ESP map of structure 4D (Figure 4), where the charge remains unevenly distributed over the molecule, in marked contrast to PABA structure 2A.

Figure 5: Experimental IRMPD spectrum of MABA (in gray) and the calculated vibrational spectra of structure 4A (0 kJ/mol), structure 4B (0.2 kJ/mol), structure 4C (37.9 kJ/mol), structure 4D (6.2 kJ/mol), structure 4E (8.4 kJ/mol), and structure 4F (9.8 kJ/mol). Calculated stick spectra (in red) convoluted with a Gaussian function $\text{fwhm}=5\text{ cm}^{-1}$ (in black).

Ortho-aminobenzoic acid (OABA)

The final question to address is how does OABA protonate? By just taking the inductive effect and resonant stabilization into consideration, OABA should behave similarly to PABA, as the nitrogen lone pair can transfer charge onto the ring and carboxylic acid. However, due to the proximity of the amine and carbonyl groups, the proton is likely shared between both sites. By visual comparison, OABAs IRMPD spectrum matches that of MABA more closely than that of PABA in Figure 1, making the protonation site on the amine group more probable.

Figure 6: The lowest-energy structures OABA calculated at the B3LYP/cc-pVTZ level of theory with zero-point corrected electronic energies for amine-protonated (left) and carboxylic acid-protonated (right). The ESP maps for the respective two lowest-energy conformers, 6A and 6C, are depicted.

The calculated structures for protonated OABA are shown in Figure 6. Computations strongly suggest that amine-protonation is favorable (by 37 kJ/mol). For the two lower-energy conformations (6A and 6B), a considerable energy penalty is observed for the loss of the hydrogen bond on the carboxylic acid.

Figure 7: Experimental IRMPD spectrum of OABA (in gray) and the calculated vibrational spectra of structure 6A (0 kJ/mol), structure 6B (29.9 kJ/mol), structure 6C (37.4 kJ/mol), and structure 6D (52.5 kJ/mol). Calculated stick spectra (in red) convoluted with a Gaussian function fwhm = 5 cm^{-1} (in black).

Figure 7 illustrates the comparison of the IRMPD spectrum of protonated OABA to the calculated vibrational spectra for the structures shown in Figure 6. From a visual comparison, the three experimental peaks in the OABA IRMPD spectrum seem to be in good agreement with the three modes of the amine-protonated structure 6A, while none of the diagnostic carboxylic acid-protonated modes are verified. It is interesting to note that unlike all of the other computed spectra presented in this paper, there are only three OH and NH modes for the amine-protonated spectra of OABA in this region of the spectrum. An intense NH stretching mode is predicted at much lower frequency, at 2584 cm^{-1} and 2387 cm^{-1} for structures 6A and 6B, respectively. The molecular structure for 6A illustrates the lengthened N-H bond ($1.07\text{ \AA}$) for the hydrogen pointing to the carbonyl O ($\text{H}\cdots\text{O}$ distance = $1.58\text{ \AA}$), consistent with a hydrogen bonding ($<2\text{ \AA}$) interaction. This hydrogen bonding results in a large redshift of this NH stretch, which could unfortunately not be verified in the experiment, due to reduced OPO/A power $<2800\text{ cm}^{-1}$. Note that previous studies have shown that the potential energy surfaces of shared protons are often very anharmonic, and can exhibit modes in a broad range of frequencies, depending on the relative proton affinities of the competing attachment sites.²⁹

The harmonic calculations presented here seem to adequately describe the higher-frequency NH_3^+ stretching modes, as demonstrated by the match of the doublet around 3300 cm^{-1} with theory. Nonetheless, little experimental insight is obtained into how the proton is shared between both groups, and the calculated thermochemistries may have to be treated with some caution. Finally, the calculated dipoles and ESP maps (and reduced dipoles) for both amine- and carboxylic acid-protonation indicate less extreme charge separation, which is compatible with a sharing of the proton between both sites.

Photofragmentation chemistry

The lower IRMPD yields for MABA, as opposed to OABA and PABA, are seen very clearly in the raw IRMPD mass spectra at selected OPO/A frequencies in Figure S5. The m/z 120 and 94 photofragment mass channels are well known, corresponding to H_2O (18 amu) and CO_2 (44 amu) losses, respectively. The H_2O loss channel requires the proton to be associated with the carboxylic acid group. It is striking that the H_2O loss is very abundant for OABA, somewhat abundant for PABA, but almost undetectable for MABA. This observation is consistent with the molecular picture that the proton is either already on the carboxylic acid group (in PABA), or at least in very close vicinity (in OABA). In the case of MABA, the proton would have to migrate from the amine to the carboxylic acid group, which due to distance, introduces a considerable kinetic barrier. These trends are compatible with a higher dissociation barrier for protonated MABA, and thus a lower IRMPD yield.

Summary and conclusions

The amino and carboxylic acid sites in aminobenzoic acid present competing sites for proton attachment in the gas phase. An IRMPD spectroscopy study combined with computations at the density functional theory level show that:

- Para-aminobenzoic acid (PABA) is protonated on the carboxylic acid site, whereas meta-aminobenzoic acid (MABA) is protonated on the amine, in agreement with calculated gas-phase thermochemistries. Ortho-aminobenzoic acid (OABA) is predicted to be protonated on the amine, in agreement with the IRMPD spectrum and computed thermochemistry, even if the proximity of the amino and acid groups make this a more special case for a sharing of the proton.
- The trends for (de)stabilizing protonation on the amine versus the carboxylic acid can be rationalized by resonance stabilization. In the para-substituted form, the amino group donates its electron density to the ring and carboxylic acid group, thereby reducing its proton affinity, and making the latter a better site of proton attachment. In the meta-form, this stabilization is not possible, meaning that the amino site remains the most basic site. These trends can also be visualized by ESP maps, showing a very even electron density for carboxylic acid-protonated PABA, as opposed to an uneven charge distribution for (carboxylic acid-protonated) MABA.
- The different protonation sites for PABA (i.e., carboxylic acid) and MABA (i.e., amine) are somewhat at odds with their reported identical proton affinities (PAs).¹ Given the recent discovery of kinetically trapped solution-phase structures in the gas phase,^{11,12} it is conceivable those PA measurements are also (partially) based on solution-phase conformers.

Acknowledgements

The project was financially supported by the United States National Science Foundation (NSF) under grant number CHE-1403262 and the United States National Institutes of Health (NIH) under grant number R01GM110077.

References

- (1) Jencks, W. P.; Regenstein, J.: Ionization Constants of Acids and Bases. In *Handbook of Biochemistry and Molecular Biology, Fourth Edition*; CRC Press, 2010; pp 595-635.
- (2) Hunter, E. P. L.; Lias, S. G.: Evaluated Gas Phase Basicities and Proton Affinities of Molecules: An Update. *Journal of Physical and Chemical Reference Data* **1998**, *27*, 413-656.
- (3) Wende, T.; Wanko, M.; Jiang, L.; Meijer, G.; Asmis, K. R.; Rubio, A.: Spectroscopic Characterization of Solvent-Mediated Folding in Dicarboxylate Dianions. *Angewandte Chemie International Edition* **2011**, *50*, 3807-3810.
- (4) Steill, J. D.; Oomens, J.: Gas-Phase Deprotonation of p-Hydroxybenzoic Acid Investigated by IR Spectroscopy: Solution-Phase Structure Is Retained upon ESI. *Journal of the American Chemical Society* **2009**, *131*, 13570-13571.
- (5) Tian, Z.; Kass, S. R.: Does Electrospray Ionization Produce Gas-Phase or Liquid-Phase Structures? *Journal of the American Chemical Society* **2008**, *130*, 10842-10843.
- (6) Schröder, D.; Buděšínský, M.; Roithová, J.: Deprotonation of p-Hydroxybenzoic Acid: Does Electrospray Ionization Sample Solution or Gas-Phase Structures? *Journal of the American Chemical Society* **2012**, *134*, 15897-15905.
- (7) Almasian, M.; Grzetic, J.; van Maurik, J.; Steill, J. D.; Berden, G.; Ingemann, S.; Buma, W. J.; Oomens, J.: Non-Equilibrium Isomer Distribution of the Gas-Phase Photoactive Yellow Protein Chromophore. *The Journal of Physical Chemistry Letters* **2012**, *3*, 2259-2263.
- (8) Kapota, C.; Lemaire, J.; Maître, P.; Ohanessian, G.: Vibrational Signature of Charge Solvation vs Salt Bridge Isomers of Sodiated Amino Acids in the Gas Phase. *Journal of the American Chemical Society* **2004**, *126*, 1836-1842.
- (9) Jockusch, R. A.; Lemoff, A. S.; Williams, E. R.: Effect of Metal Ion and Water Coordination on the Structure of a Gas-Phase Amino Acid. *Journal of the American Chemical Society* **2001**, *123*, 12255-12265.
- (10) Dunbar, R. C.; Polfer, N. C.; Oomens, J.: Gas-Phase Zwitterion Stabilization by a Metal Dication. *Journal of the American Chemical Society* **2007**, *129*, 14562-14563.
- (11) Schmidt, J.; Meyer, M. M.; Spector, I.; Kass, S. R.: Infrared Multiphoton Dissociation Spectroscopy Study of Protonated p-Aminobenzoic Acid: Does Electrospray Ionization Afford the Amino- or Carboxy-Protonated Ion? *The Journal of Physical Chemistry A* **2011**, *115*, 7625-7632.
- (12) Patrick, A. L.; Cismesia, A. P.; Tesler, L. F.; Polfer, N. C.: Effects of ESI conditions on kinetic trapping of the solution-phase protonation isomer of p-aminobenzoic acid in the gas phase. *International Journal of Mass Spectrometry* **2016**, submitted.
- (13) Tian, Z.; Kass, S. R.: Gas-Phase versus Liquid-Phase Structures by Electrospray Ionization Mass Spectrometry. *Angewandte Chemie International Edition* **2009**, *48*, 1321-1323.

- (14) Campbell, J. L.; Le Blanc, J. C. Y.; Schneider, B. B.: Probing Electrospray Ionization Dynamics Using Differential Mobility Spectrometry: The Curious Case of 4-Aminobenzoic Acid. *Analytical Chemistry* **2012**, *84*, 7857-7864.
- (15) Warnke, S.; Seo, J.; Boschmans, J.; Sobott, F.; Scrivens, J. H.; Bleiholder, C.; Bowers, M. T.; Gewinner, S.; Schöllkopf, W.; Pagel, K.; von Helden, G.: Protomers of Benzocaine: Solvent and Permittivity Dependence. *Journal of the American Chemical Society* **2015**, *137*, 4236-4242.
- (16) Chang, T. M.; Prell, J. S.; Warrick, E. R.; Williams, E. R.: Where's the Charge? Protonation Sites in Gaseous Ions Change with Hydration. *Journal of the American Chemical Society* **2012**, *134*, 15805-15813.
- (17) Chang, T. M.; Chakrabarty, S.; Williams, E. R.: Hydration of Gaseous m-Aminobenzoic Acid: Ionic vs Neutral Hydrogen Bonding and Water Bridges. *Journal of the American Chemical Society* **2014**, *136*, 10440-10449.
- (18) Gulyuz, K.; Stedwell, C. N.; Wang, D.; Polfer, N. C.: Hybrid quadrupole mass filter/quadrupole ion trap/time-of-flight-mass spectrometer for infrared multiple photon dissociation spectroscopy of mass-selected ions. *Review of Scientific Instruments* **2011**, *82*, 054101.
- (19) Bagratashvili, V. N.; Letokhov, V. S.; Makarov, A. A.; Ryabov, E. A.: *Multiple Photon Infrared Laser Photophysics and Photochemistry*; Harwood Academic Publishers, 1985.
- (20) Oomens, J.; Sartakov, B. G.; Meijer, G.; von Helden, G.: Gas-phase infrared multiple photon dissociation spectroscopy of mass-selected molecular ions. *International Journal of Mass Spectrometry* **2006**, *254*, 1-19.
- (21) Fridgen, T. D.: Infrared consequence spectroscopy of gaseous protonated and metal ion cationized complexes. *Mass Spectrometry Reviews* **2009**, *28*, 586-607.
- (22) Mino, W. K.; Gulyuz, K.; Wang, D.; Stedwell, C. N.; Polfer, N. C.: Gas-Phase Structure and Dissociation Chemistry of Protonated Tryptophan Elucidated by Infrared Multiple-Photon Dissociation Spectroscopy. *The Journal of Physical Chemistry Letters* **2011**, *2*, 299-304.
- (23) Allouche, A.-R.: Gabedit—A graphical user interface for computational chemistry softwares. *Journal of Computational Chemistry* **2011**, *32*, 174-182.
- (24) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, H. P.; Izmaylov, A. F.; Bloino, J.; Zheng, G.; Sonnenberg, J. L.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, J. J. A.: *Gaussian 09 Revision A.1*; Gaussian Inc.: Wallingford CT, 2009.
- (25) Hanwell, M. D.; Curtis, D. E.; Lonie, D. C.; Vandermeersch, T.; Zurek, E.; Hutchison, G. R.: Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. *Journal of Cheminformatics* **2012**, *4*, 1-17.
- (26) Lanucara, F.; Chiavarino, B.; Scuderi, D.; Maitre, P.; Fornarini, S.; Crestoni, M. E.: Kinetic control in the CID-induced elimination of H₃PO₄ from phosphorylated serine probed using IRMPD spectroscopy. *Chemical Communications* **2014**, *50*, 3845-3848.
- (27) Dennington, R.; Kieth, T.; Millam, J.: *Semichem Inc.* **2009**.
- (28) Parneix, P.; Basire, M.; Calvo, F.: Accurate Modeling of Infrared Multiple Photon Dissociation Spectra: The Dynamical Role of Anharmonicities. *The Journal of Physical Chemistry A* **2013**, *117*, 3954-3959.
- (29) Roscioli, J. R.; McCunn, L. R.; Johnson, M. A.: Quantum Structure of the Intermolecular Proton Bond. *Science* **2007**, *316*, 249-254.

Graphical Abstract

$^+\text{H}_2\text{O}_2\text{C} \cdots \text{NH}_2$

$\text{HO}_2\text{C} \cdots \text{NH}_3^+$