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Mass Spectrometry

Gas-Phase versus Liquid-Phase Structures by Electrospray Ionization Mass Spectrometry**

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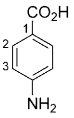
Electrospray ionization (ESI) is routinely used for the analysis of a wide variety of compounds which possess more than one ionization site.[1] In cases where the most acidic or basic site differs in the gas-phase from that in solution an interesting question arises: Which species is produced? More specifically, is the liquid-phase ion structure preserved or does an isomerization take place during the evaporation and desolvation process?

We recently communicated that the $[M{-}1]^-$ ions of tyrosine and *p*-hydroxybenzoic acid are formed with their gas-phase structures when these compounds are sprayed from a 3:1 (v/v) CH_3OH/H_2O mixture using a commercial ESI source.[2,3] In both cases phenoxide ions are produced. In contrast, when acetonitrile is used as the solvent the carboxylate ions are formed. Most mass spectrometry studies, however, are carried out with positive ions. Consequently, herein we present our findings on the $[M{+}1]^+$ ion of *p*-aminobenzoic acid produced by ESI.

In water, the most basic site in *p*-aminobenzoic acid (**1**) is the amino group,[4] whereas the preferred protonation position in the gas-phase has not been determined nor is it immediately obvious. Aniline is more basic than benzoic acid by 61.5 ± 11.7 kJ mol^{-1} (i.e., $\mathrm{PA(PhNH_2)} = 882.4 \pm 8.4$ and $\mathrm{PA(PhCO_2H)} = 820.9 \pm 8.4$ kJ mol^{-1}, PA = proton affinity),[5] but this does not guarantee that the amino group is the most basic site in **1**. The effect of each substituent (amino and carboxy) on the proton affinity of the other must be considered. Undoubtedly the carboxy group diminishes the basicity of the amine, but the amino group facilitates protonation at the carboxy position. Given that the experimentally determined proton affinity of **1** is 864.8 ± 8.4 kJ mol^{-1}, which lies between the values for aniline and benzoic acid,[5] the preferred protonation site is unclear. Semiempirical computations are divided on this issue as the AM1 calculations[6] predict that the amino group is more basic, whereas the PM3 Hamiltonian[7] indicates that the carboxy group is the preferred location for protonation.

To try and resolve this issue, we carried out Becke 3-parameter hybrid exchange and Lee–Yang–Parr correlation density functional (B3LYP)[8] calculations along with the 6-31+G(d,p) basis set (Table 1). Protonation at the carbonyl oxygen atom is predicted to be favored by 32.9 kJ mol^{-1}, but decreases to 20.8 kJ mol^{-1} if the computed errors in the proton affinities of aniline and benzoic acid are corrected. This value (20.8 kJ mol^{-1}) is in excellent accord with a high-level Gaussian (G3)[9] prediction of 17.2 kJ mol^{-1}. Protonation at the hydroxy oxygen atom and the ring carbon atoms *ortho* (C3) and *para* (C1) to the amino group were also considered,[10] but these species are predicted to be less stable than the ammonium ion. Protonation of the carboxy group therefore seems to be the preferred site in the gas phase, which is not the case in solution.

Table 1: Computed proton affinities of *p*-aminobenzoic acid, aniline, and benzoic acid, and the relative energies of their conjugate acids.[a]

Cmpd	Protonated Site	E_{Relative}		PA		
		B3LYP[b]	G3	B3LYP[b]	G3	Expt.[c]
	C=*O*	0.0	0.0	887.0	874.5	864.8 ± 8.4
	NH_2	32.9	17.2			
	*O*H	51.0	41.1			
	C1	50.6	39.3			
	C3	44.6	42.7			
$PhNH_2$				881.2	882.0	882.4 ± 8.4
$PhCO_2H$				831.8	824.7	820.9 ± 8.4

[a] All values are in kJ mol^{-1}. [b] B3LYP = B3LYP/6-31+G(d,p). [c] See ref. [5].

In accordance with these computational predictions, self-CI (chemical ionization) of **1** under conditions where equilibration can take place by intermolecular proton transfer leads to the carboxy-protonated isomer. This structural assignment is based upon the collision-induced dissociation (CID) spectrum of the $[M{+}1]^+$ ion, which loses H_2O to produce a *m*/*z* 120 ion as the most abundant fragment. This result is consistent with the behavior of protonated carboxylic acids,[11] and strongly suggests that the gas-phase protonation of **1** occurs preferentially at the carboxy group. It also indicates that the protonation site of **1** depends upon the medium, which led us to carry out ESI experiments on this compound.

When *p*-aminobenzoic acid was sprayed using 3:1 (v/v) CH_3OH/H_2O and 1:1 CH_3CN/H_2O solutions, $[M{+}1]^+$ ions were formed. Their CID spectra, however, are quite different (Figure 1). In the CH_3OH/H_2O case, the most abundant fragment corresponds to the loss of H_2O (*m*/*z* 120), whereas in the CH_3CN/H_2O instance the base peak is due to the loss of

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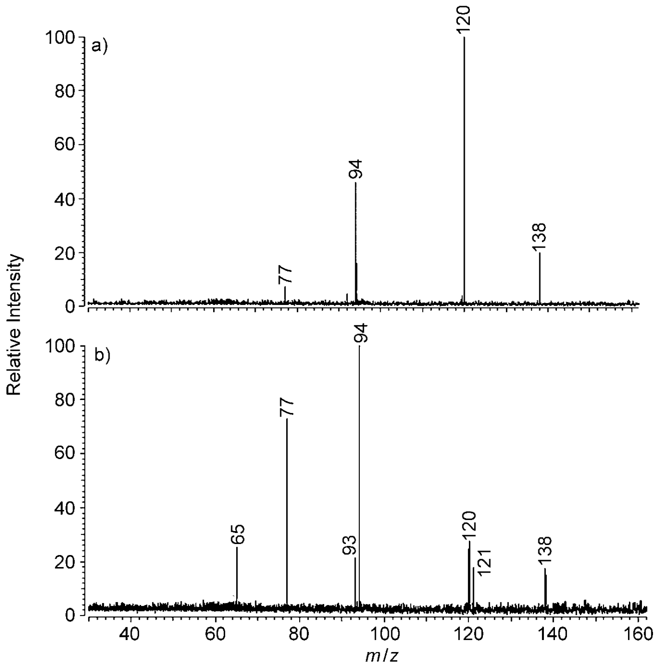


Figure 1. CID spectra of the $[M+1]^+$ ions (m/z 138) of *p*-aminobenzoic acid using sustained off-resonance irradiation (4.0 V, 1.0 s) at a static argon pressure (1.9×10^{-7} Torr). The m/z 138 ions were generated by ESI from a) 3:1 (v/v) CH_3OH/H_2O and b) 1:1 (v/v) CH_3CN/H_2O mixtures.

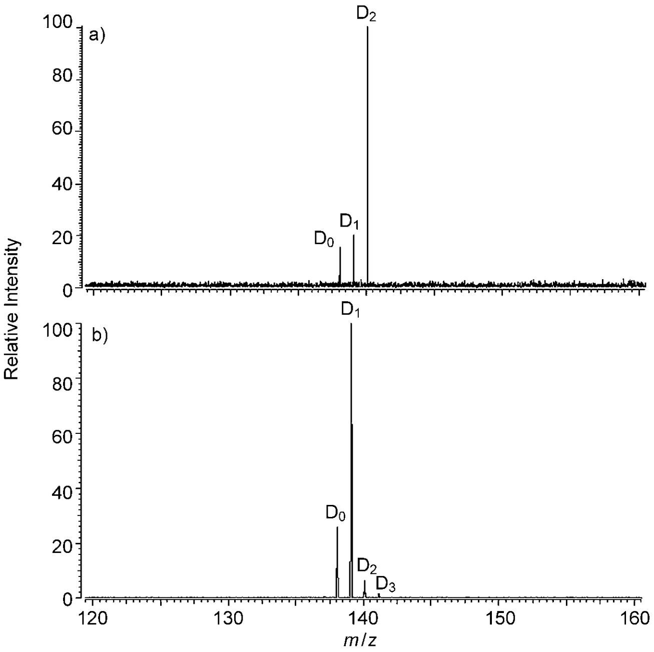
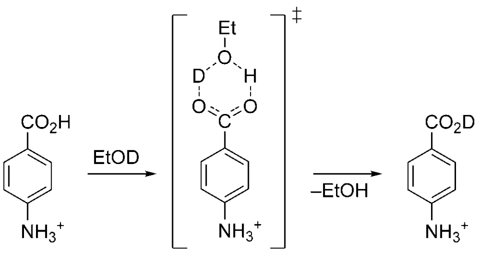


Figure 2. H/D exchange of protonated *p*-aminobenzoic acid with EtOD. The D_0 $[M+1]^+$ ion at m/z 138 was produced by ESI from a) MeOH/H_2O (v/v, 3/1) and b) CH_3CN/H_2O (v/v, 1/1). In (a), it was allowed to react with 1.5×10^{-7} Torr of EtOD for 4.0 s, whereas in (b) 1.3×10^{-6} Torr of EtOD and a reaction time of 10.0 s were employed.

CO_2 (m/z 94). These results clearly indicate that two different isomers are formed when **1** is sprayed from a CH_3OH/H_2O or a CH_3CN/H_2O solvent mixture. The CH_3OH/H_2O solution leads to the O-protonated $[M+1]^+$ ion, which is the more stable gas-phase structure, and indicates that an isomerization takes place during the evaporation and desolvation process. The CH_3CN/H_2O mixture on the other hand produces the liquid-phase species (i.e., N-protonation). Both of these observations are consistent with the findings for the $[M-1]^-$ ions of tyrosine and *p*-hydroxybenzoic acid.[2,3]

To confirm the present results, hydrogen–deuterium (H/D) exchange experiments were carried out on both $[M+1]^+$ ions generated from **1**. It was anticipated that the O- and N-protonated $[M+1]^+$ ions would lead to different H/D exchange behavior as they have two and three labile hydrogen atoms, respectively. In accordance with this expectation, their reactivity was observed to be different (Figure 2). The $[M+1]^+$ ion generated from **1** dissolved in the CH_3OH/H_2O mixture underwent two relatively rapid H/D exchanges upon reaction with EtOD ($k=(1.9\pm0.5)\times 10^{-10}\,\mathrm{cm^3\,molecule^{-1}\,s^{-1}}$). This observation is consistent with the known behavior of protonated carboxy ions, and our assignment of the gas-phase structure for this species (i.e., O-protonation). In contrast, the $[M+1]^+$ ion obtained from the CH_3CN/H_2O mixture primarily underwent one H/D exchange, and the reaction occurred at a much slower rate [$k=(8.3\pm0.6)\times10^{-12}\,\mathrm{cm^3\,molecule^{-1}\,s^{-1}}$]. This result clearly indicates that a different ion is formed when CH_3CN/H_2O is used as the solvent, and it is reasonable to conclude that the N-protonated species is formed.

The H/D exchange behavior of the ammonium ion can be explained by invoking a charge-remote six-centered flip-flop mechanism,[12] in which the carboxy hydrogen atom is replaced by a deuterium atom [Eq. (1)].



(1)

Molecular modeling supports this proposal in that the transition structure is computed to be 28.2 kJ mol^{-1} more stable than the separated reactants (i.e., the ($[M+1]^+$ ion + EtOD)) at the B3LYP/6-31+G(d,p) level. This process is energetically accessible, but given the unfavorable entropy for such a transformation, it is not surprising that it takes place relatively slowly. As for the small amount of the D_2 and D_3-ions that are observed, they may arise from the exchange of aromatic hydrogen atoms *ortho* to the NH_3^+ particularly as G3 calculations predict this carbon atom to be only 25.5 kJ mol^{-1} less basic than the amine in **1**.

These findings show that the isomeric identity of a positive ion produced by ESI can be altered by the solvent in which the sample is dissolved. A few related studies have been carried out on organometallic systems,[13,14] but the factors that are responsible for these observations still remain to be elucidated.

Experimental Section

Gas-Phase Experiments: An IonSpec (now Varian) data system and electrospray cart equipped with a Z-Spray (Micromass) ESI source was used in conjunction with a 3 T superconducting magnet. The resulting Fourier transform mass spectrometer (FTMS) was operated using Omega Ver. 8.0.294 software. CH_3OH/H_2O or CH_3CN/H_2O solutions containing circa 200 μM *p*-aminobenzoic acid and a small amount of acetic acid to make the mixture slightly acidic were injected into the system at a flow rate of 10 μL min^{-1}. The resulting $[M+1]^+$ ions were cooled with a pulse of argon and isolated using an arbitrary waveform. The resulting ions were then fragmented by CID or allowed to react with EtOD (99.5+ atom % D) for variable times. In the latter case the instrument was treated with the deuterated reagent for several hours before the H/D exchange experiments were carried out so as to increase the effective deuterium content to $\geq 95\%$.

Computations: B3LYP optimized structures and vibrational frequencies were obtained using the 6-31+G(d,p) basis set. In some cases G3 energies also were computed as described in the literature.[9] All of the resulting energetics are reported as enthalpies at 298 K, and small vibrational modes, which contribute more than $^1/_2$(RT) to the thermal energy were replaced by 1.2 kJ mol^{-1}. These calculations were performed on workstations at the Minnesota Supercomputer Institute for Advanced Computational Research using Gaussian 03.[15]

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