Gas-Phase Deprotonation of *p*-Hydroxybenzoic Acid Investigated by IR Spectroscopy: Solution-Phase Structure Is Retained upon ESI

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Electrospray ionization (ESI) has become an essential tool in mass spectrometry. While the degree to which electrosprayed peptides and proteins maintain their solution-phase structure is still under much debate (see, e.g., refs 1–3), it is generally assumed that small molecular ions will relax to their gas-phase global minimum structure. Thus, it is also assumed that the gas-phase structure of a molecular ion produced by electrospray is independent of the conditions of the ESI process. However, recent negative ion ESI studies on species with multiple deprotonation sites, such as *p*-hydroxybenzoic acid and tyrosine, suggest that deprotonation occurs on either the carboxylic acid or the phenol group, depending on the ESI solvent used.[4,5] For deprotonated tyrosine, this conclusion is based on different products formed in ion–molecule reactions of carboxylate and phenoxide model compounds (the two [Tyr − H]⁻ isomers are virtually isoenergetic[5,6]). Remarkably, photoelectron (PE) spectroscopy[5] and IR multiple photon dissociation (IRMPD) spectroscopy[7] suggest a carboxylate structure for the [Tyr − H]⁻ anion irrespective of the ESI solvent used.

While gas-phase acidities are routinely determined using mass spectrometry, the deprotonation site is not as easily identified. The conjugate base of *para*-hydroxybenzoic acid (HO−C₆H₄−COOH, *p*-HBA) forms a classical example.[8,9] While even in the gas phase benzoic acid is more acidic than phenol,[10] the hydroxy proton of *p*-HBA is more acidic than the carboxy proton, due to resonance stabilization in the phenoxide structure resulting in a high degree of charge delocalization[9,11] (see Figure S1). The carboxylate isomer, however, is favored in aqueous solution[8] due to stabilization by electrostatic, H-bonding interactions between the charge-localized carboxylate and the protic solvent. It is important to note here that such stabilizing interactions are largely absent in aprotic solvents, so that the anion likely adopts its gas-phase structure.[9,12] These issues raise the question of isomerization upon desolvation in ESI. Using collision induced dissociation (CID) and H/D exchange, Kass and co-workers investigated the deprotonation site of [*p*-HBA − H]⁻ produced from different ESI solvents and concluded that an acetonitrile solution produces the carboxylate isomer, whereas a methanol solution yields the phenoxide isomer.[5] PE spectra of deprotonated *p*-HBA reported in the same paper, however, suggest the opposite assignment.

This unexplained discrepancy and the lack of a satisfactory explanation for the observations call for further investigation. Here, we determine the gas-phase structure of the conjugate base of *p*-HBA and the influence of ESI solvent by IR spectroscopy. An IRMPD spectrum is recorded using the Fourier-transform ion cyclotron resonance (FTICR) mass spectrometer coupled to the infrared free electron laser FELIX.[13,14] Ions are produced by ESI from two different solutions, 2 mM *p*-HBA with 2 mM KOH added

in 90:10 MeOH/H₂O and in 90:10 CH₃CN/H₂O, using a Micromass Z-spray source. Ions are accumulated in a hexapole trap for 3 s before transfer to the FTICR mass spectrometer, where they are stored for 1 s before irradiation. The IR frequency was scanned between 550 and 1850 cm⁻¹. Resonance with vibrational modes in deprotonated *p*-HBA (*m/z* 137) induces dissociation to form C₆H₅O⁻ (*m/z* 93) via CO₂ loss. Vibrational spectra for each isomer are calculated at the B3LYP/6-311++G(2d,2p) level of theory using Gaussian03.[15] Harmonic frequencies are scaled by 0.98 and convoluted with a Gaussian line shape of 20 cm⁻¹ fwhm.
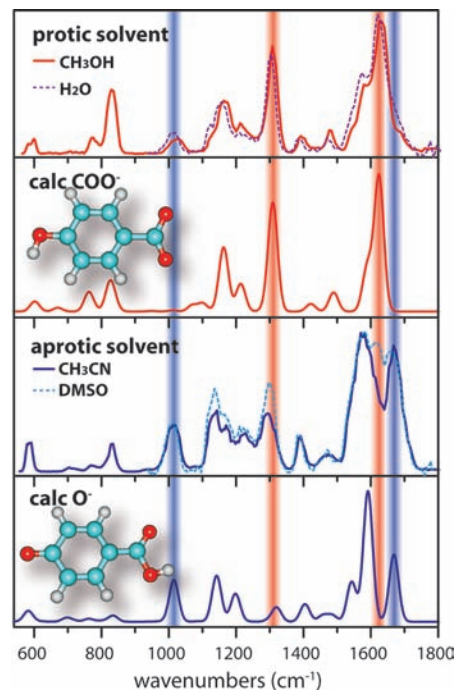
Figure 1. IRMPD spectra of deprotonated *p*-HBA generated by ESI from protic (methanol and water) and aprotic solvents (acetonitrile and DMSO) compared to calculated spectra of the carboxylate (COO⁻) and phenoxide (O⁻) isomers. The red (blue) bars indicate diagnostic carboxylate (carboxylic acid) bands.

The IRMPD spectra of the conjugate base of *p*-HBA sprayed from methanol and from acetonitrile, shown in Figure 1, demonstrate clear differences indicating that different structures are indeed produced. We note however that the IRMPD dissociation channel (CO₂ loss) and efficiency were similar for both ESI solutions, in contrast to the large difference in CID efficiencies reported in ref 5 (which was not reproduced on our instrument; see Figure S2). Even more remarkable is the unmistakable observation of a free carboxylate spectral signature[16] for the sample sprayed from the methanol solution, with strong symmetric and antisymmetric

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carboxylate stretch modes at 1305 and 1630 cm^{-1}, respectively. In contrast, the spectrum of [p-HBA − H]$^-$ sprayed from an acetonitrile solution shows a distinctive carboxylic acid C=O stretch at 1680 cm^{-1} and an in-plane carboxylic acid COH bending mode at 1015 cm^{-1}, indicating that deprotonation has occurred on the phenol rather than on the carboxylic acid functional group. Comparison of experimental and calculated spectra further confirms the assignment of a carboxylate structure to the sample sprayed from methanol and of a phenoxide structure to the sample sprayed from acetonitrile. Hence, our assignment of structures is opposite to that reported in ref 5 based on CID and H/D exchange.

The observation of different structures obtained from the different spray solvents can be rationalized by the solution-phase structures. In a protic solvent (e.g., methanol) the anion adopts a charge-localized carboxylate structure due to stabilizing H-bonding interactions with the solvent.[9] Upon ESI, the carboxylate structure is apparently retained even though the phenoxide structure is now lower in energy. In contrast, the charge-delocalized phenoxide structure is favored in aprotic solvents,[9,12] such as acetonitrile, and hence the phenoxide structure is observed upon ESI. To further corroborate this hypothesis, alternate protic and aprotic solvents (H$_2$O and DMSO, respectively) were used, which consistently gave a phenoxide structure for the aprotic solvents and a carboxylate structure for the protic solvents (see dashed curves in Figure 1).

Note that a small admixture of the alternate isomer is present in each of the spectra. Using the computed band intensities of the two isomers, a roughly 4:1 ratio of isomers is estimated by least-squares fitting the two contributing spectra to the experimental ones (see Supporting Information, Figure S3).
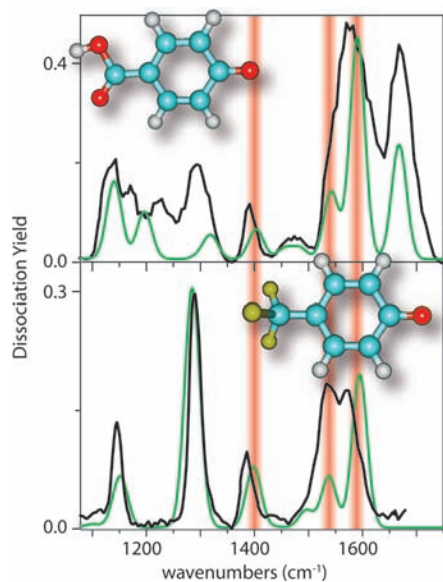
Figure 2. IRMPD spectrum of deprotonated p-HBTF (lower panel) compared to the IRMPD spectrum of deprotonated p-HBA sprayed from acetonitrile (upper panel). Green traces represent scaled DFT calculations for the structures shown. The red lines indicate the positions of vibrational bands with substantial phenoxide CO stretch character (from [p-HBA]$^-$ calculation).

While carboxylate and carboxylic acid vibrational modes are well-known, the gas-phase infrared signature of a phenoxide moiety has not been reported, so that comparison with literature values is not possible. Hence, the IRMPD spectrum of the conjugate base of *para*-hydroxybenzotrifluoride (CF$_3$C$_6$H$_4$OH, p-HBTF) was recorded, as shown in Figure 2 along with that of [p-HBA − H]$^-$

produced from the acetonitrile solution. Experimental and computed spectra of deprotonated p-HBTF agree reasonably well. The band observed at 1530 cm^{-1} appears too intense, perhaps due to the adjacent absorption band to the blue, which upon activation could shift into resonance with the laser, a common effect in IRMPD spectra.[17] The calculations show that the three bands near 1390, 1530, and 1580 cm^{-1} (see red lines in Figure 2) common to both anions are due to phenoxide CO stretching coupled to ring CC stretching. Such unusually high frequencies for a formally single-bond CO stretch is indicative of conjugation between the negatively charged oxygen atom and the aromatic ring,[9] giving partial double bond character to the CO bond. Similarly, the extremely low frequency (1680 cm^{-1}) of the free carboxylic acid C=O stretch in phenoxide [p-HBA − H]$^-$ (compare, e.g., to ~1760 cm^{-1} in benzoic acid) is also a consequence of the two resonant structures. A full study of phenoxide IR spectra including other compounds is in progress.

In conclusion, the present IR spectra confirm that the conjugate base of p-HBA adopts different isomeric structures depending on the ESI solvent used; however, the assignment of structures is reversed from that reported on the basis of CID and H/D exchange.[5] Interestingly, our assignment agrees with that based on PE spectra also reported in ref 5. IR spectra obtained using different protic and aprotic ESI solvents suggest that p-HBA is a special case where the solution-phase structure is conserved upon ESI. No isomerization to the lowest-energy gas-phase structure takes place, leading to a kinetically trapped structure when using protic solvents.

Supporting Information Available: Structures. SORI-CID mass spectra of p-HBA. Spectral fit yielding estimated proportions of isomeric mixture. Full reference for ref 15. This material is available free of charge via the Internet at http://pubs.acs.org.

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