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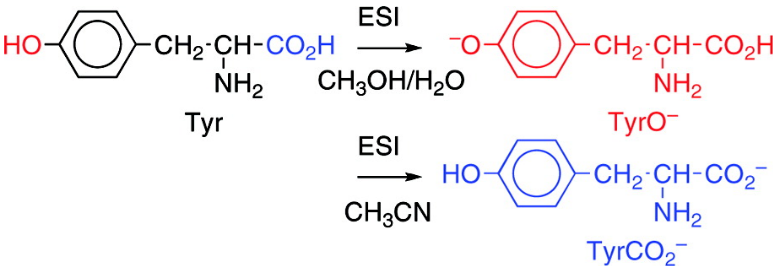

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Does Electrospray Ionization Produce Gas-Phase or Liquid-Phase Structures?

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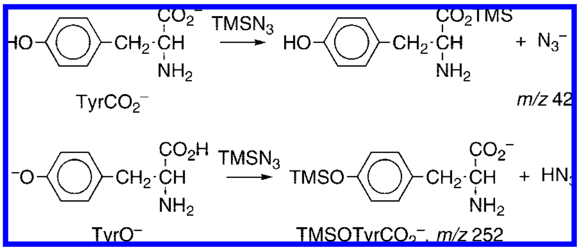
Electrospray ionization (ESI) mass spectrometry has become a routine and widely used tool for the analysis of a diverse range of compounds including peptides, nucleotides, and carbohydrates.[1] These listed species have more than one acidic site, but it is uniformly assumed that the same isomer present in solution is produced in the gas-phase. This raises the question, what happens when the most stable ionic structure in aqueous media and the gas-phase is different? More specifically, will ESI produce the liquid-phase structure, the gas-phase species, or a combination of the two? This is a critical issue for the myriad of experiments which are carried out on ions generated by ESI. In this paper, we report that ESI can be used to selectively form either the phenoxide or carboxylate M − H ion of tyrosine and *p*-hydroxybenzoic acid.

In aqueous solution, the most acidic sites in tyrosine are the carboxyl position and the protonated α-amine.[2] As a result, the conjugate base is a carboxylate ion. In the gas-phase, phenol and acetic acid have the same acidities (i.e., $\Delta H^\circ_{acid} = 348.0 \pm 1.0$ and 348.2 ± 1.4 kcal mol^{-1}, respectively),[3] so it is not immediately obvious what the preferred structure is for deprotonated tyrosine. To address this issue, we developed a chemical probe for differentiating these anions. More specifically, the carboxylate ($TyrCO_2^-$) was independently prepared in a Fourier transform mass spectrometry by reacting the ethyl ester of tyrosine with a mixture of amide and hydroxide ions. The desired ion was produced in small abundance (~5%), but was isolated so that its reactivity could be probed. Upon reaction with trimethylsilyl azide ($TMSN_3$) only N_3^- (*m*/*z* 42) was observed (Scheme 1). This was expected because azide ion is not basic enough to deprotonate phenol (i.e., (ΔH°_{acid} (HN_3) = 345.8 ± 2.1 kcal mol^{-1})).[4] In contrast, the phenoxide ion ($TyrO^-$), which was generated by reacting F^- with tyrosine trimethylsilyl ether (TyrOTMS),[5] only gives an addition $-HN_3$ product at *m*/*z* 252. This pathway takes place because N_3^- is basic enough to abstract a proton from the carboxyl group of phenylalanine (i.e., ΔH°_{acid} ($C_6H_5CH_2CH(NH_2)CO_2H$) = 338.9 ± 4.3 kcal mol^{-1}).[6] A secondary reaction between $TMSOTyrCO_2^-$ and $TMSN_3$ subsequently occurs to afford N_3^-, but this process can be differentiated from the primary reaction by monitoring the product distribution as a function of time. These findings indicate that trimethylsilyl azide is a suitable reagent for differentiating the two tyrosine M − H ions, and that the phenoxide/carboxylate ratio can be determined from the *m*/*z* 252: *m*/*z* 42 abundances.

Tyrosine was deprotonated with a HO^-/H_2N^- mixture in the gas-phase under conditions where tyrosine was allowed to equilibrate with the resulting M − H ion. The composition of the conjugate base was determined using $TMSN_3$ and was found to be a 70:30 phenoxide/carboxylate mixture.[7] This indicates that the phenoxide ion is the more stable species, but the energy difference ($\Delta\Delta G^\circ$) between the two anions is only 0.5 ± 0.3 kcal mol^{-1} or 0.4 p*K* units. Consequently, the structure of deprotonated tyrosine undergoes a change in going from aqueous media to the gas-phase. As a result, tyrosine is a suitable substrate for addressing the title question and probing the isomeric composition of the ESI-generated M − H ion.

Scheme 1. Tyrosine Carboxylate ($TyrCO_2^-$) and Phenoxide ($TyrO^-$) Reaction Products with Trimethylsilyl Azide



When a basic solution of tyrosine was sprayed from a 3:1 (v:v) CH_3OH/H_2O mixture containing Li^+, NH_4^+, or n-Bu_4N^+ as the counterion, the resulting M − H ion was found to consist of a 70:30 phenoxide/carboxylate mixture. This corresponds to the gas-phase equilibrium composition and does not reflect the structure of the ion in solution. These observations indicate that the counterions have no effect on the isomeric composition in CH_3OH/H_2O, and that the M − H ion undergoes an isomerization during the desolvation process. A similar result was reported for cysteine in that a thiolate is formed in the gas-phase, as opposed to the carboxylate which is present in solution.[8,9] In the latter instance, however, there is a small unimolecular barrier for the interconversion of the two anions which enables them to interchange and presumably precludes their independent preparation at room temperature.

Both methanol and water are hydrogen bond donors and may serve as acid-catalysts for the $TyrCO_2^-$ to $TyrO^-$ conversion. Therefore, the tyrosine M − H ion produced from an aprotic solvent was examined. Acetonitrile was used for this purpose because it probably is the most commonly employed solvent aside from water/methanol mixtures. The lithium salt of tyrosine, however, is not very soluble in anhydrous acetonitrile, so the tetra-*n*-butyl-ammonium salt was used instead. Interestingly, this led to the almost exclusive formation of the carboxylate ion (i.e., ~95%). To the best of our knowledge this represents the first report that the isomeric structure of an ion can be altered by the choice of the solvent from which it is sprayed, and that an ion can be trapped in its solution phase structure.[10]

The effect of added water or methanol to acetonitrile up to 1:1 (v:v) mixtures were subsequently explored. Water was found to have no effect on the tyrosine M − H phenoxide/carboxylate ion ratio (Table 1), which indicates that it is not responsible for the structural conversion upon ESI. This implicates methanol as the culprit, and in accord with this inference, the addition of small amounts leads to greatly altered phenoxide/carboxylate ion ratios. It seems reasonable to attribute this behavior to the greater gas-phase acidity of methanol compared to water (i.e., $\Delta H^\circ_{acid} = 381.9$

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Table 1. Deprotonated Tyrosine Phenoxide vs Carboxylate Ion Ratios Produced via ESI[a]

solvent mixtures (v:v)	product ratio phenoxide/carboxylate
CH_3CN	7:93
CH_3CN: H_2O (99:1)	5:95
CH_3CN: H_2O (50:50)	7:93
CH_3CN: CH_3OH (99:1)	57:43
CH_3CN: CH_3OH (98:2)	58:42
CH_3CN: CH_3OH (96:4)	63:37
CH_3CN: CH_3OH (50:50)	69:31
CH_3OH: H_2O (75:25)	70:30
gas-phase equilibrium ratio	70:30

[a] Product ratios were determined from the reaction of the tyrosine M − H ion with $TMSN_3$.

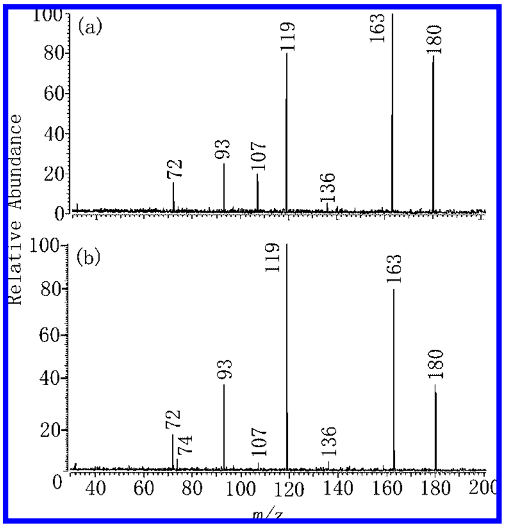

Figure 1. Collision-induced dissociation spectra of the tyrosine M − H ion (*m/z* 180). (a) The *m/z* 180 ion was formed from the lithium salt of tyrosine in a 3:1 (v/v) CH_3OH/H_2O solution. (b) The *m/z* 180 ion was formed from the tetra-*n*-butylammonium salt of tyrosine in anhydrous CH_3CN.

± 0.6 and 390.27 ± 0.02 kcal mol^{-1}, respectively),[11] and suggests that a relay mechanism[12] in which CH_3OH protonates the carboxylate and simultaneously abstracts the phenolic proton maybe operating. An alternative stepwise process involving two sequential proton transfers can be ruled out given the large gas-phase acidity difference between methanol and tyrosine (i.e., $\Delta\Delta H°_{acid} = 44.2 \pm 2.7$ kcal mol^{-1})[6,11] unless a more acidic methanol cluster ($(CH_3OH)_n$, $n \geq 2$) is involved.

Our results indicate that spraying compounds from different solvent systems can lead to the formation of isomeric species. The reactivity and fragmentation behavior attributed to a given ion may vary as a result. To further illustrate this point, the collision-induced dissociation spectra of the M − H ion of tyrosine generated from CH_3OH/H_2O and neat CH_3CN are given in Figure 1. The two spectra are similar but are not the same, and the differences are quite reproducible. For example, the *m/z* 107 ion is nearly absent in the latter spectrum whereas the *m/z* 74 ion is missing in the former one. These two ions correspond to *p*-methylphenoxide (*p*-$CH_3C_6H_4O^-$) and deprotonated glycine ($NH_2CH_2CO_2^-$),[13] and presumably arise from $TyrO^-$ and $TyrCO_2^-$, respectively. Both fragments can be attributed to the homolytic cleavage of the weak $ArCH_2{-}CH$ bond.

Similar results were obtained for *p*-hydroxybenzoic acid, which is known to give a phenoxide ion upon deprotonation in the gas-phase and a carboxylate anion in solution.[14] When this compound was sprayed from CH_3CN, the M − H ion underwent 1 H/D exchange with CF_3CH_2OD and lost CO_2 upon CID, whereas the species formed from CH_3OH/H_2O (3:1) did not incorporate deuterium ($k < 1 \times 10^{-12}$ cm^3 $molecule^{-1}$ s^{-1}) or fragment under the same experimental conditions. These findings indicate that the gas-phase structure is produced from CH_3OH/H_2O whereas the liquid-phase species is generated from CH_3CN. This leads us to suggest that similar results will be uncovered when cationic systems are probed. Our data also can be viewed as a novel form of ion suppression,[15] and a detailed study of ESI variables on the formation of isomeric structures is warranted.

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Supporting Information Available: Figure S1 and a description of the experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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