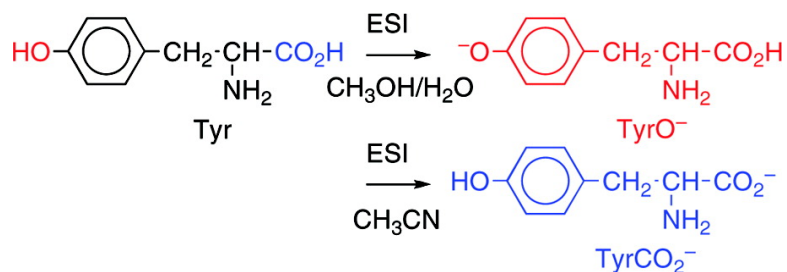


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## Does Electrospray Ionization Produce Gas-Phase or Liquid-Phase Structures?

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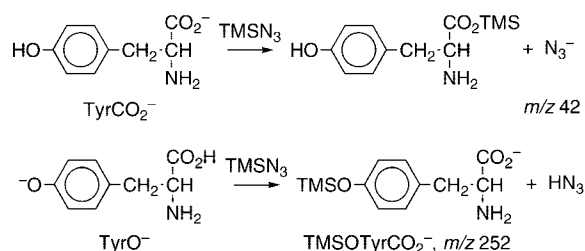
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Electrospray ionization (ESI) mass spectrometry has become a routine and widely used tool for the analysis of a diverse range of compounds including peptides, nucleotides, and carbohydrates.<sup>1</sup> These listed species have more than one acidic 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  $\alpha$ -amine.<sup>2</sup> As a result, the conjugate base is a carboxylate ion. In the gas-phase, phenol and acetic acid have the same acidities (i.e.,  $\Delta H^\circ_{\text{acid}} = 348.0 \pm 1.0$  and  $348.2 \pm 1.4$  kcal mol<sup>-1</sup>, respectively),<sup>3</sup> 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<sub>2</sub><sup>-</sup>) 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<sub>3</sub>) only N<sub>3</sub><sup>-</sup> (*m/z* 42) was observed (Scheme 1). This was expected because azide ion is not basic enough to deprotonate phenol (i.e., ( $\Delta H^\circ_{\text{acid}}(\text{HN}_3) = 345.8 \pm 2.1$  kcal mol<sup>-1</sup>).<sup>4</sup> In contrast, the phenoxide ion (TyrO<sup>-</sup>), which was generated by reacting F<sup>-</sup> with tyrosine trimethylsilyl ether (TyrOTMS),<sup>5</sup> only gives an addition -HN<sub>3</sub> product at *m/z* 252. This pathway takes place because N<sub>3</sub><sup>-</sup> is basic enough to abstract a proton from the carboxyl group of phenylalanine (i.e.,  $\Delta H^\circ_{\text{acid}}(\text{C}_6\text{H}_5\text{CH}_2\text{CH}(\text{NH}_2)\text{CO}_2\text{H}) = 338.9 \pm 4.3$  kcal mol<sup>-1</sup>).<sup>6</sup> A secondary reaction between TMSOTyrCO<sub>2</sub><sup>-</sup> and TMSN<sub>3</sub> subsequently occurs to afford N<sub>3</sub><sup>-</sup>, 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<sup>-</sup>/H<sub>2</sub>N<sup>-</sup> 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<sub>3</sub> and was found to be a 70:30 phenoxide/carboxylate mixture.<sup>7</sup> 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  $0.5 \pm 0.3$  kcal mol<sup>-1</sup> or 0.4 pK units. Consequently, the structure of deprotonated tyrosine undergoes a change in going from aqueous media to the gas-phase.

**Scheme 1.** Tyrosine Carboxylate (TyrCO<sub>2</sub><sup>-</sup>) and Phenoxide (TyrO<sup>-</sup>) Reaction Products with Trimethylsilyl Azide



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.

When a basic solution of tyrosine was sprayed from a 3:1 (v:v) CH<sub>3</sub>OH/H<sub>2</sub>O mixture containing Li<sup>+</sup>, NH<sub>4</sub><sup>+</sup>, or *n*-Bu<sub>4</sub>N<sup>+</sup> 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<sub>3</sub>OH/H<sub>2</sub>O, 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.<sup>8,9</sup> 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.

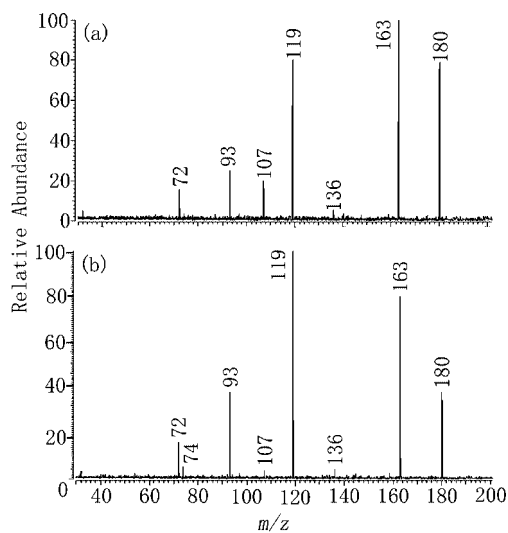
Both methanol and water are hydrogen bond donors and may serve as acid-catalysts for the TyrCO<sub>2</sub><sup>-</sup> to TyrO<sup>-</sup> conversion. Therefore, the tyrosine  $M - H$  ion produced from an aprotic solvent was examined. Acetonitrile was used for this purpose because it probably is the 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.<sup>10</sup>

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.,  $\Delta H^\circ_{\text{acid}} = 381.9$

**Table 1.** Deprotonated Tyrosine Phenoxide vs Carboxylate Ion Ratios Produced via ESI<sup>a</sup>

solvent mixtures (v:v)	product ratio phenoxide/carboxylate
CH <sub>3</sub> CN	7:93
CH <sub>3</sub> CN: H <sub>2</sub> O (99:1)	5:95
CH <sub>3</sub> CN: H <sub>2</sub> O (50:50)	7:93
CH <sub>3</sub> CN: CH <sub>3</sub> OH (99:1)	57:43
CH <sub>3</sub> CN: CH <sub>3</sub> OH (98:2)	58:42
CH <sub>3</sub> CN: CH <sub>3</sub> OH (96:4)	63:37
CH <sub>3</sub> CN: CH <sub>3</sub> OH (50:50)	69:31
CH <sub>3</sub> OH: H <sub>2</sub> O (75:25)	70:30
gas-phase equilibrium ratio	70:30

<sup>a</sup> Product ratios were determined from the reaction of the tyrosine M – H ion with TMSN<sub>3</sub>.



**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<sub>3</sub>OH/H<sub>2</sub>O solution. (b) The  $m/z$  180 ion was formed from the tetra-*n*-butylammonium salt of tyrosine in anhydrous CH<sub>3</sub>CN.

$\pm 0.6$  and  $390.27 \pm 0.02$  kcal mol<sup>-1</sup>, respectively),<sup>11</sup> and suggests that a relay mechanism<sup>12</sup> in which CH<sub>3</sub>OH 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.,  $\Delta\Delta H_{\text{acid}}^{\circ} = 44.2 \pm 2.7$  kcal mol<sup>-1</sup>)<sup>6,11</sup> unless a more acidic methanol cluster ((CH<sub>3</sub>OH)<sub>*n*</sub>, *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<sub>3</sub>OH/H<sub>2</sub>O and neat CH<sub>3</sub>CN 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<sub>3</sub>C<sub>6</sub>H<sub>4</sub>O<sup>-</sup>) and deprotonated glycine (NH<sub>2</sub>CH<sub>2</sub>CO<sub>2</sub><sup>-</sup>),<sup>13</sup> and presumably arise from TyrO<sup>-</sup> and TyrCO<sub>2</sub><sup>-</sup>, respectively. Both

fragments can be attributed to the homolytic cleavage of the weak ArCH<sub>2</sub>–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.<sup>14</sup> When this compound was sprayed from CH<sub>3</sub>CN, the M – H ion underwent 1 H/D exchange with CF<sub>3</sub>CH<sub>2</sub>OD and lost CO<sub>2</sub> upon CID, whereas the species formed from CH<sub>3</sub>OH/H<sub>2</sub>O (3:1) did not incorporate deuterium ( $k < 1 \times 10^{-12}$  cm<sup>3</sup> molecule<sup>-1</sup> s<sup>-1</sup>) or fragment under the same experimental conditions. These findings indicate that the gas-phase structure is produced from CH<sub>3</sub>OH/H<sub>2</sub>O whereas the liquid-phase species is generated from CH<sub>3</sub>CN. 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,<sup>15</sup> and a detailed study of ESI variables on the formation of isomeric structures is warranted.

**Acknowledgment.** Dr. Lev Lis prepared the tetra-*n*-butylammonium salt of tyrosine used in this work and helpful discussions with Mr. Matthew Meyer are acknowledged. We also thank Prof. Richard O’Hair for bringing ref 10a to our attention. Financial support from the National Science Foundation is gratefully acknowledged too.

**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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## **Supplemental Information**

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

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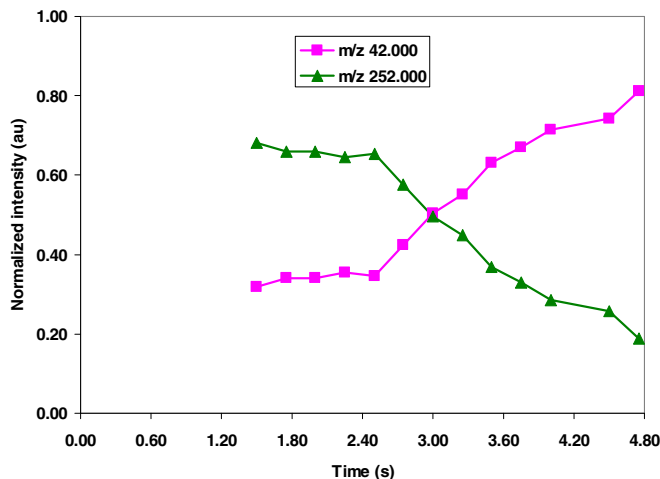
Contribution from the Department of Chemistry, University of Minnesota, Minneapolis,  
MN 55455

## Experimental

All of the experiments were carried out in a dual cubic-cell Fourier transform mass spectrometer (FTMS) or a single cylindrical cell instrument with an electrospray ionization source. Both FTMS's were equipped with 3 Tesla superconducting magnets and were controlled by Omega data systems supplied by IonSpec (now Varian). In the latter instrument the M – H ion of tyrosine was generated from a solution of the amino acid containing LiOH or NH<sub>4</sub>OH dissolved in a combination of solvents (i.e., CH<sub>3</sub>OH, H<sub>2</sub>O, and CH<sub>3</sub>CN). Alternatively, TyrCO<sub>2</sub><sup>-</sup>N(*n*-Bu)<sub>4</sub><sup>+</sup> was used in some instances. The resulting solutions were approximately 250 μM, had pH's of about 7, and were sprayed at 10 μl min<sup>-1</sup> using a Z-Spray ionization source (Micromass). Negative ions were accumulated in a hexapole to build their intensity before being transferred to the ion cyclotron resonance cell via a rf-only quadrupole ion guide. A pulse of argon up to a pressure of ~10<sup>-5</sup> Torr was admitted into the cell to facilitate the trapping of the ions, and to thermalize them before fragmenting them via energetic collisions (i.e. collision-induced dissociation) or probing their reactivity with trimethylsilyl azide (TMSN<sub>3</sub>). Very low pressures of TMSN<sub>3</sub> were employed in these studies (~10<sup>-9</sup> – 10<sup>-10</sup> Torr) so as to minimize the amount of reaction which occurs during the transfer process, and to slow down a subsequent secondary transformation. A typical plot of the data is illustrated in Fig. 1S and corresponds to a *m/z* 252 : *m/z* 42 (phenoxide/carboxylate) ratio of 66 : 34.

The gas-phase equilibrium phenoxide : carboxylate ratio of deprotonated tyrosine also was determined, and these experiments were carried out in our dual cell instrument. A mixture of hydroxide and amide ions was generated by electron ionization (7.0 eV) of a pulse of ammonia vapor containing some water. The resulting ions were allowed to react

**Fig. 1S.** Product ratio versus time for the reaction of the tyrosine M – H ion with trimethylsilyl azide.<sup>a</sup>



<sup>a</sup> The product at  $m/z$  252 is due to the phenoxide ion whereas  $m/z$  42 results from the carboxylate anion.

with tyrosine, which was admitted into the system via a heated solid inlet probe. After allowing the ion composition to equilibrate for 1 s the resulting ions were transferred to the second cell and the M – H ion of tyrosine was isolated using a CHIRP excitation (S1). Its reactivity with  $\text{TMSN}_3$  ( $\sim 1\text{-}2 \times 10^{-8}$  Torr) was then monitored as a function of time.

The authentic phenoxide and carboxylate ions also were generated in our dual cell instrument. In the former case, electron ionization of a pulse of  $\text{NF}_3$  (7.0 eV) produced  $\text{F}^-$ , and this ion was allowed to react with the trimethylsilyl ether of tyrosine (TyrOTMS) (S2). The resulting M – TMS ion at  $m/z$  180 was formed in small amounts ( $\sim 1\%$ ) (the major product is due to proton abstraction), but still could be isolated and allowed to react with  $\text{TMSN}_3$ . Alternatively, an  $\text{NH}_2^-/\text{OH}^-$  mixture was allowed to react with tyrosine

ethyl ester to afford TyrCO<sub>2</sub><sup>-</sup> (~5%) and the M – H ion. The former species was isolated and transferred to the second cell where its reactivity with TMSN<sub>3</sub> was probed.

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