FULL PAPER

Kinetic Method Analysis of the Effect of Halogenation on Relative Proton Affinity of Tyrosine

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Relative proton affinities (PAs) of tyrosine, 3-chlorotyrosine, and 3-iodotyrosine were obtained using the kinetic method approach. The measured mean values are 922.5, 912.9, and 917.9 kJ/mol, respectively, with ± 0.1 kJ/mol standard deviation, indicating that halogenation of tyrosine decreases its PA. In general, PA of a molecule increases as its isotropic polarizability increases, but no such correlation has been found for the three molecules investigated in this study. Our findings show that PA decreases with increasing electronegativity of the modifying atom for a halogenated molecule, further supporting computational results of previous work [5].

Keywords: Mass spectrometry, Kinetic method, Proton affinity, Density functional theory calculations, Protonated amino acid clusters

Introduction

Several researchers [1 – 9] have investigated the effect of chemical or structural modification on proton affinities (PAs) of biologically relevant molecules. In general, there is a positive correlation between intermolecular hydrogen-bonding interaction or polarizability and PA [3 – 9]; the excess positive charge is strongly bound to the molecule through attractive hydrogen-bonding interactions, and increased polarizability provides additional stabilization to the electronic structure of the protonated molecule.

Rodgers et al. [5] predicted, based on density functional theory calculations, that fluorination of uracil lowers its PA, and suggested a correlation between the electronegativity of the halogen atom and PA of the molecule. This prediction is supported experimentally in previous studies [1][10][11], where the PAs of chloroacetonitrile, bromoacetonitrile, and iodoacetonitrile are lower than that of acetonitrile, although no correlation between PA and halogenation is established therein. In this work, we report PA values of tyrosine, 3-chlorotyrosine, and 3-iodotyrosine (*Fig. 1*) obtained using *Cooks*' kinetic method [12], and explicitly provide further evidence that for a halogenated molecule, there is an inverse relation between the electronegativity of the modifying atom and PA. We compare our result to those [1][10][11] for acetonitrile and halogenated acetonitrile.

Results and Discussion

In order to establish the effect of halogenation on the PA of tyrosine, we first measure PA of the molecule and compare it to those of halogenated species. Collision-induced dissociation tandem mass spectrometry (CID MS2) results of $[Tyr{\bullet}A_{ref}]{\bullet}H^+$ (A_{ref} = Ile, Thr, Phe, and Met) clusters are shown in *Fig. 2a – d*. The precursor clusters dissociate to yield $Tyr{\bullet}H^+$ and $A_{ref}{\bullet}H^+$ as primary products, with their mass spectral intensities representing relative PAs of tyrosine and the reference molecules. The PAs of isoleucine, threonine, and phenylalanine are lower than that of tyrosine, whereas the PA of methionine is higher. The kinetic method analysis (*Fig. 2e*) shows that the tyrosine PA is 922.5 kJ/mol (all measured proton affinities are shown in *Table*), which is very close to those [10][13] obtained by similar experimental approaches. Upon chlorination and iodination of the molecule on the phenol ring, the PA sharply decreases to 912.1 and 917.9 kJ/mol, respectively, as obtained from CID MS2 results for 3-chlorotyrosine and 3-iodotyrosine (*Figs. 3* and *4*). Qualitatively similar observations have been made in previous studies [1][10], where the PAs of chloroacetonitrile, bromoacetonitrile, and iodoacetonitrile are 745.6, 752.3, and 765.3 kJ/mol [1][10], respectively. These values are lower than that of acetonitrile at 787.4 kJ/mol [11], suggesting a strong correlation between PA and the overall electronic properties of a molecule.

It should be noted that uncertainties in the reference PA values are typically ± 8 kJ/mol [2][14]. Thus, the PAs obtained in this work, while reproducible to ± 0.1 kJ/mol standard deviation, may not be very close to true PAs of tyrosine and the halogenated species. However, considering that nearly identical sets of references are used, the three molecules have essentially the same structure, and that the mean values are clearly separated, differences

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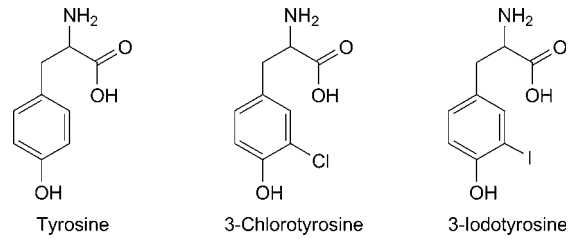

Fig. 1. Schematic structures of tyrosine, 3-chlorotyrosine, and 3-iodotyrosine.

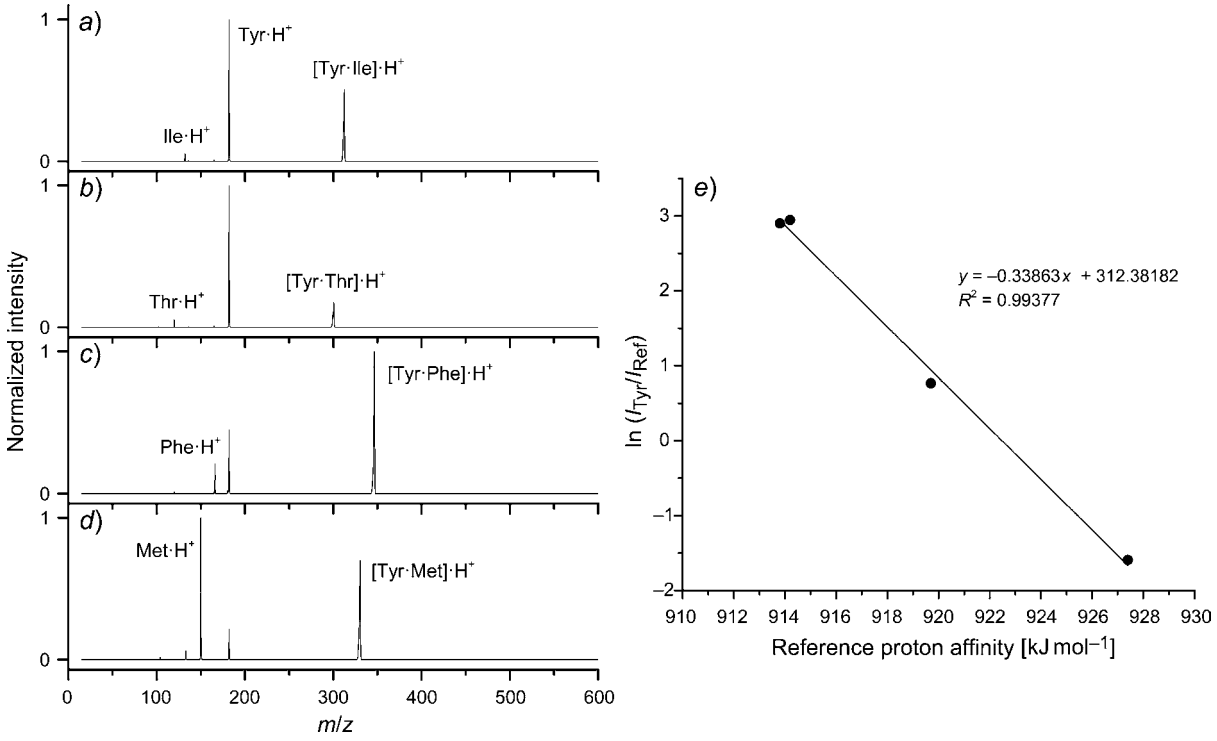

Fig. 2. CID Tandem mass spectra of (*a*) [Tyr•Ile]•H$^+$, (*b*) [Tyr•Thr]•H$^+$, (*c*) [Tyr•Phe]•H$^+$, and (*d*) [Tyr•Met]•H$^+$, and (*e*) a plot of $\ln(I_{Tyr}/I_{Ref})$ *vs.* PAs of reference molecules [14] with linear regression. Resonance excitation voltage of 0.35 V is used.

between the individual measurements should reflect the true PA differences among the three molecules investigated.

Our X3LYP computational results based on experimentally determined structures [15][16] of neutral and protonated tyrosine are consistent with the current measurements, where the PAs for tyrosine, 3-chlorotyrosine, and 3-iodotyrosine are 918.0, 909.3, and 914.1 kJ/mol, respectively (all computational results are shown in *Table*). X3LYP structures of neutral and protonated tyrosine and halogenated tyrosine are shown in *Fig. 5*. Molecules with higher polarizability usually have higher PAs [4][8][9]. Thus, polarizabilities *should* decrease in the order of tyrosine > 3-iodotyrosine > 3-chlorotyrosine because lower PA implies reduced proton stabilization through intramolecular charge separation [9]. However, PBE1PBE polarizabilities of the molecules do not follow this trend, with the values of 125.77, 137.37, and 157.29 Bohr^3 for tyrosine, 3-chlorotyrosine, and 3-iodotyrosine, respectively. This result shows that there is no positive correlation between polarizability and PA of molecules modified through halogenation. However, one can use electronegativity of the modifying halogen atom to provide a better quantitative measure of relative PA, as has been suggested previously [5]. As electronegativity is a measure of the propensity of an atom to pull a negative charge, electron density of the molecule will shift toward the modifying atom and decrease the attractive interaction between the molecule and H-atom. This would be less pronounced if the electronegativity of the atom is lower. Thus, since the electronegativities of hydrogen, iodine, and chlorine atoms are 2.1, 2.5, and 3.0 [17], respectively, the PA should decrease in the order of tyrosine > 3-iodotyrosine > 3-chlorotyrosine, as observed in

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Table. Relative proton affinities (PAs) and isotropic polarizabilities of tyrosine, 3-chlorotyrosine, and 3-iodotyrosine. Experimental PAs are averages of four individual measurements. Calculated PAs are X3LYP values, and isotropic polarizabilities are PBE1PBE values. 6-311 + G (2d,2p) is used for all atoms except for H, Br, and I at the C3 position, for which MiDiX is used. The electronegativity values are from reference [20]

	PA[a]) Exper. value [kJ/mol]	PA[b]) X3LYP [kJ/mol]	Polarizability[b]) PBE1PBE [Bohr^3]	Electronegativity[c]) of the atom at C(3) position
Tyrosine	922.5	918.0	125.77	2.1 (H)
3-Chlorotyrosine	912.9	909.3	137.37	3.0 (Cl)
3-Iodotyrosine	917.9	914.1	157.29	2.5 (I)

[a]) The values are averages of four measurements with ± 0.1 kJ/mol standard deviation. Error in each measurement based on the analysis of propagation of uncertainty is ± 0.7 kJ/mol or less [18]. [b]) 6-311 + G(2d,2p) is used for all atoms except for H, Br, and I at the C(3) position, for which MiDiX is used. [c]) From [17].

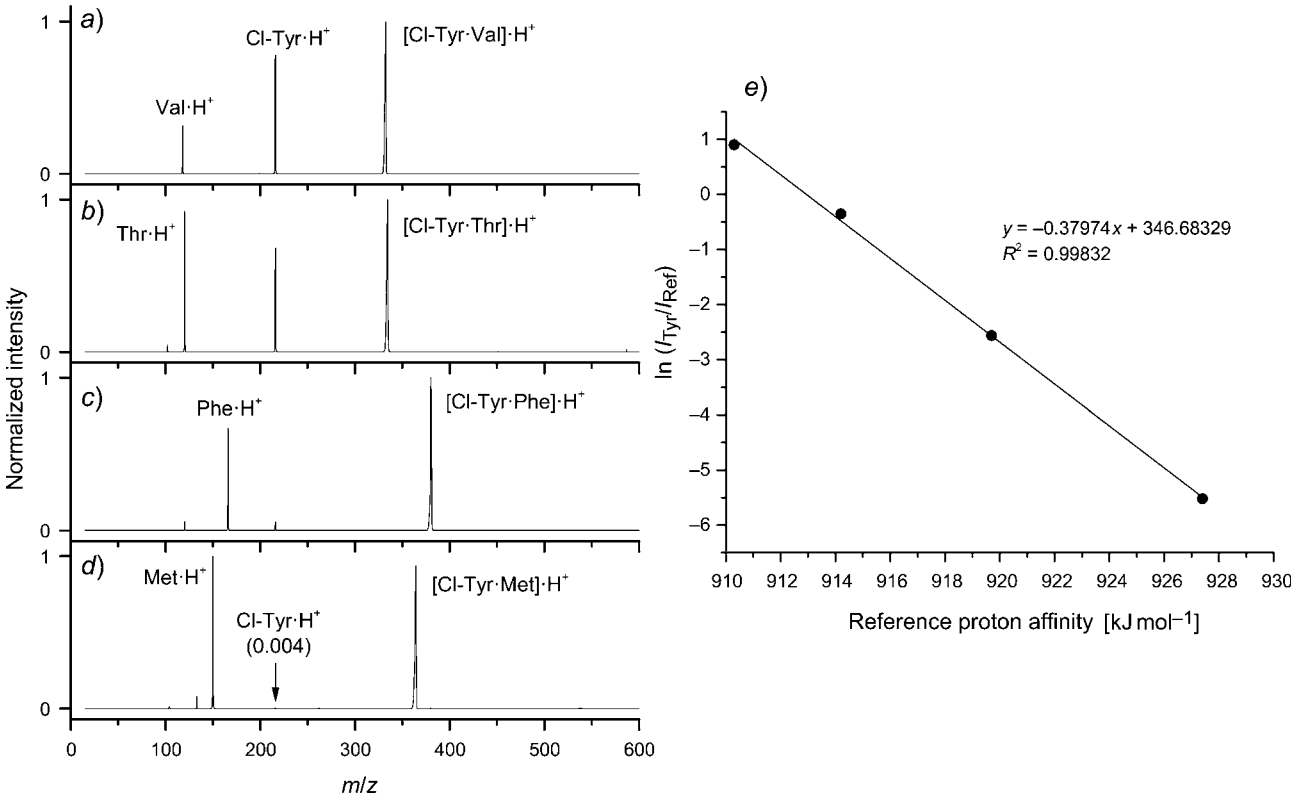

Fig. 3. CID Tandem mass spectra of (*a*) [Cl-Tyr•Val]•H$^+$, (*b*) [Cl-Tyr•Thr]•H$^+$, (*c*) [Cl-Tyr•Phe]•H$^+$, and (*d*) [Cl-Tyr•Met]•H$^+$, and (*e*) a plot of ln ($I_{Cl\text{-}Tyr}/I_{Ref}$) *vs.* PAs of reference molecules [14] with linear regression. Resonance excitation voltage of 0.35 V is used.

this work. The trend is also apparent for the acetonitrile and iodo-, bromo-, and chloroacetonitrile series, where the electronegativity of bromine is 2.8 [17], and the measured [1][10][11] PA decreases in the order of acetonitrile > iodoacetonitrile > bormoacetonitrile > chloroacetonitrile. Our DFT calculation results for the latter group of molecules also support this conclusion: while the calculated PA decreases in the same order (787.4, 765.3, 752.3, and 745.6 kJ/mol, respectively), its correlation with polarizability is clearly not established (27.78, 53.43, 43.76, and 36.72 Bohr^3, respectively), in contrast to the cases of nonhalogenated molecules [4][5][8][9].

Conclusions

We have used the kinetic method [12] to investigate the effect of halogenation on the PA of tyrosine. The PAs of tyrosine, 3-chlorotyrosine, and 3-iodotyrosine are 922.5, 912.9, and 917.9 kJ/mol, respectively, clearly demonstrating that halogenation decreases the molecule–proton interaction strength and supporting previous computational prediction [5]. The change in PA upon halogenation is correlated with electronegativity of the modifying atoms, where increasing electronegativity decreases PA. This trend is also readily observed [1][10][11] in PAs of

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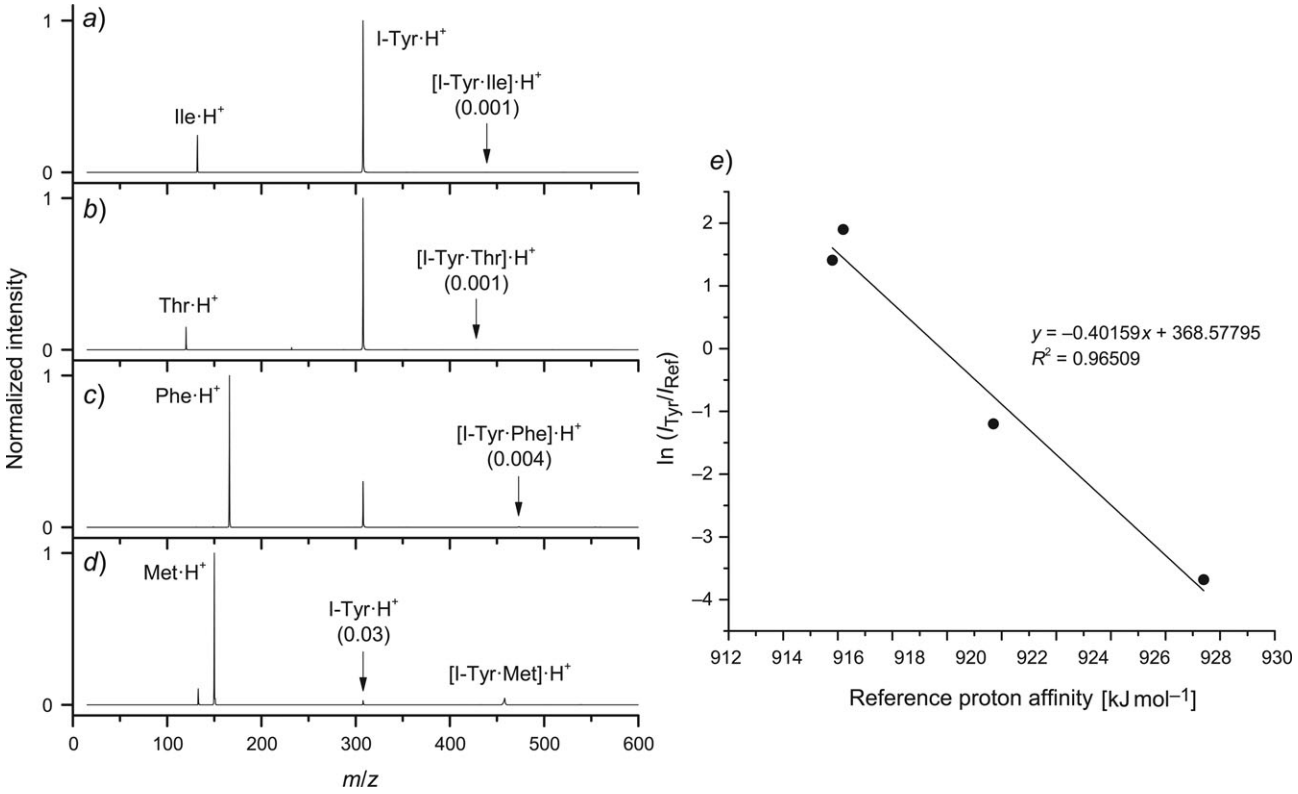

Fig. 4. CID Tandem mass spectra of (*a*) [I-Tyr•Ile]•H$^+$, (*b*) [I-Tyr•Thr]•H$^+$, (*c*) [I-Tyr•Phe]•H$^+$, and (*d*) [I-Tyr•Met]•H$^+$, and (*e*) a plot of $\ln(I_{\text{I-Tyr}}/I_{\text{Ref}})$ *vs.* PAs of reference molecules [14] with linear regression. Resonance excitation voltage of 0.35 V is used.

acetonitrile and the halogenated species. These results suggest that electronegativity rather than polarizability is a better diagnosis for quantifying PA changes for halogenated molecules.

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Experimental Part

General

Tyrosine, 3-chlorotyrosine, or 3-iodotyrosine and a reference amino acid (isoleucine, threonine, phenylalanine, or methionine for tyrosine and 3-iodotyrosine; valine, threonine, phenylalanine, or methionine for 3-chlorotyrosine) were dissolved in an aqueous solution (1:1 mixture of water and methanol with 1% acetic acid) to the concentration of 0.025M. Several drops of concentrated hydrochloric acid were added to each sample mixture in order to facilitate dissolution of the amino acids. Each sample solution was perpendicularly electrosprayed (0.3 ml/h) into the desolvation capillary (250 °C) of an ion-trap mass spectrometer (MSD Trap XCT, *Agilent Technologies*, Santa Clara, CA, USA), with N_2 as the nebulizer gas (40 psi) and drying gas (5 l/min). [Tyr•A$_{\text{ref}}$]•H$^+$, [Cl-Tyr•A$_{\text{ref}}$]•H$^+$, and [I-Tyr•A$_{\text{ref}}$]•H$^+$ (A$_{\text{ref}}$ = reference amino acid) precursor clusters were isolated in the $\Delta(m/z) = 2.0$ window and were subjected to CID at the resonance excitation voltage of 0.35 V. The CID MS2 spectra are normalized with respect to the base peak.

As detailed in previous studies [3][4][6][8][12][14], CID of precursor clusters yield two product ions

$$[A_0 \cdot A_i] \cdot H^+ \xrightarrow{k_0} A_0 \cdot H^+ + A_i \tag{1a}$$

$$[A_0 \cdot A_i] \cdot H^+ \xrightarrow{k_i} A_0 + A_i \cdot H^+ \tag{1b}$$

where A_0 is the molecule with unknown PA (tyrosine, 3-chlorotyrosine, or 3-iodotyrosine), A_i is the reference molecule with known PA (valine, isoleucine, threonine, phenylalanine, or methionine), and k is the dissociation rate constant. PA of A_0 is obtained using the linear equation [3][4][6][8][12]

$$\ln\frac{I_0}{I_i} = -\frac{PA_i}{RT_{\text{eff}}} + \frac{PA_0}{RT_{\text{eff}}} \tag{2}$$

where I is the mass spectral intensity, R is the ideal gas constant, and T_{eff} is the effective temperature of the dissociating precursor. The assumption [4][12][14] in this equation is that the ratio of mass spectral intensities I_0/I_{i} represents the ratio of rate constants k_0/k_{i}.

PAs reported in this study are averages of four measurements made over four consecutive days, and

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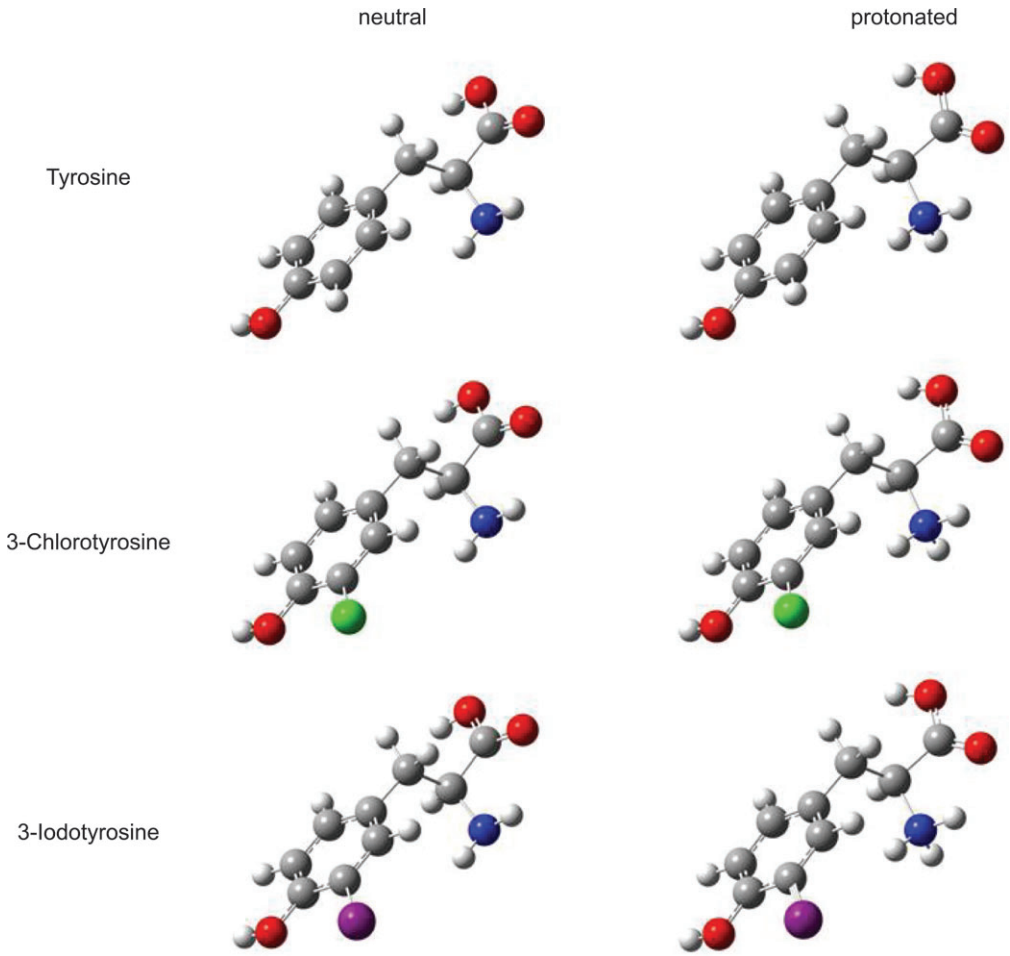

Fig. 5. X3LYP Structures of neutral and protonated tyrosine, 3-chlorotyrosine, and 3-iodotyrosine

are reproducible to within ± 0.1 kJ/mol standard deviation. Error in each measurement based on the analysis of propagation of uncertainty [18] was 0.7 kJ/mol or less. Reference PA values are adopted from the work of *Tabet et al.* [13]. This reference scale is used because it was established [13] using PAs of serine, leucine, threonine, methionine, and tryptophan, which are consistent in various measurements. Entropic effect was not taken into consideration as it has been suggested [13] that the presence of side chains results in similar entropy change regardless of the amino acid structure; our attempt, using 3-chlorotyrosine as a pilot molecule, to observe entropic effect by employing different CID energies produced negative results; we did not observe any appreciable changes in the PA as the collision energy was varied. This suggests that the entropic effect is negligible within the uncertainty of the measurement and/or the sensitivity limit of the instrument under the current experimental condition. For consistency, the original kinetic method [12] was used to derive relative PAs of tyrosine and the halogenated species. Differences between PAs of the three molecules should reflect PA shifts associated with halogenation of tyrosine.

Computational Part

Structure optimization, harmonic frequency analysis, and PA calculations for tyrosine, 3-chlorotyrosine, and 3-iodotyrosine were performed at the X3LYP level using Gaussian09 [19], and isotropic polarizabilities were calculated at the PBE1PBE level. The 6-311 + G(2d,2p) basis set was used for all atoms except for hydrogen, chlorine, and iodine atoms at the C(3) position of the phenol ring; split valence basis sets are not available for iodine, and the MidiX basis set was used for the three atoms. The X3LYP and PBE1PBE levels of theory describe inter- and intramolecular interaction [20] [21], and isotropic polarizability [9] with high degrees of accuracy, respectively. The calculated PAs were zero-point corrected.

Density functional theory (DFT) calculation results for acetonitrile, chloroacetonitrile, bromoacetonitrile, and iodoacetonitrile are also presented for comparison.

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