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Influence of the Side Chain in the Structure and Fragmentation of Amino Acids Radical Cations

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Abstract: The conformational properties of ionized amino acids (Gly, Ala, Ser, Cys, Asp, Gln, Phe, Tyr, and His) have been theoretically analyzed using the hybrid B3LYP and the hybrid-meta MPWB1K functionals as well as with the post-Hartree Fock CCSD(T) level of theory. As a general trend, ionization is mainly localized at the $-NH_2$ group, which becomes more planar and acidic, the intramolecular hydrogen bond in which $-NH_2$ acts as proton donor being strengthened upon ionization. For this reason, the so-called conformer IV(+) becomes the most stable for nonaromatic amino acid radical cations. Aromatic amino acids do not follow this trend because ionization takes place mainly at the side chain. For these amino acids for which ionization of the side chain prevails over the $-NH_2$ group, structures III(+) and II(+) become competitive. The $C_\alpha-X$ fragmentations of the ionized systems have also been studied. Among the different decompositions considered, the one that leads to the loss of $COOH^\bullet$ is the most favorable one. Nevertheless, for aromatic amino acids fragmentations leading to $R^\bullet$ or R^+ start being competitive. In fact, for His and Tyr, results indicate that the fragmentation leading to R^+ is the most favorable process.

Introduction

Protein, peptide, and amino acid radicals may play an important role in several biological processes. One of them is the oxidative damage of proteins, which is related to pathological disorders[1,2] and subsequent development of diseases such as Alzheimer[3−9] or glaucoma.[10] Since this effect is mainly due to reactions that take place in amino acids, the knowledge of their structure and reactivity upon ionization is of great importance. Moreover, their study is also important to understand the role of transient species involved in protein radical catalysis.[11] On the other hand, gas-phase studies have shown that radical cations of some oligopeptides can be produced by collision induced dissociation of $[Cu^{II}(dien)M]^{\bullet 2+}$ complex ions.[12] Their dissociation behavior is very rich and differs considerably from that of protonated peptides, which make them very attractive for peptide sequentiation. Because of that, in the past few years, the properties of different amino acid and derived radicals have attracted considerable attention, both from an experimental and theoretical point of view.[12−50]

Amino acids usually present intramolecular hydrogen bonds which are crucial to understand their structure and reactivity. However, these hydrogen bonds can be largely modified upon ionization. Previous studies have shown that removing an electron from such a system modifies both the acidity and the basicity of the groups involved in the hydrogen bond, in such a way that it is difficult to establish how this interaction would be affected by oxidation.[27,51−54] For glycine the observed changes in intramolecular hydrogen bonds have been related to the nature of the electron hole in different electronic states.[27] On the other hand, oxidized species can also lead to intermolecular spontaneous proton-

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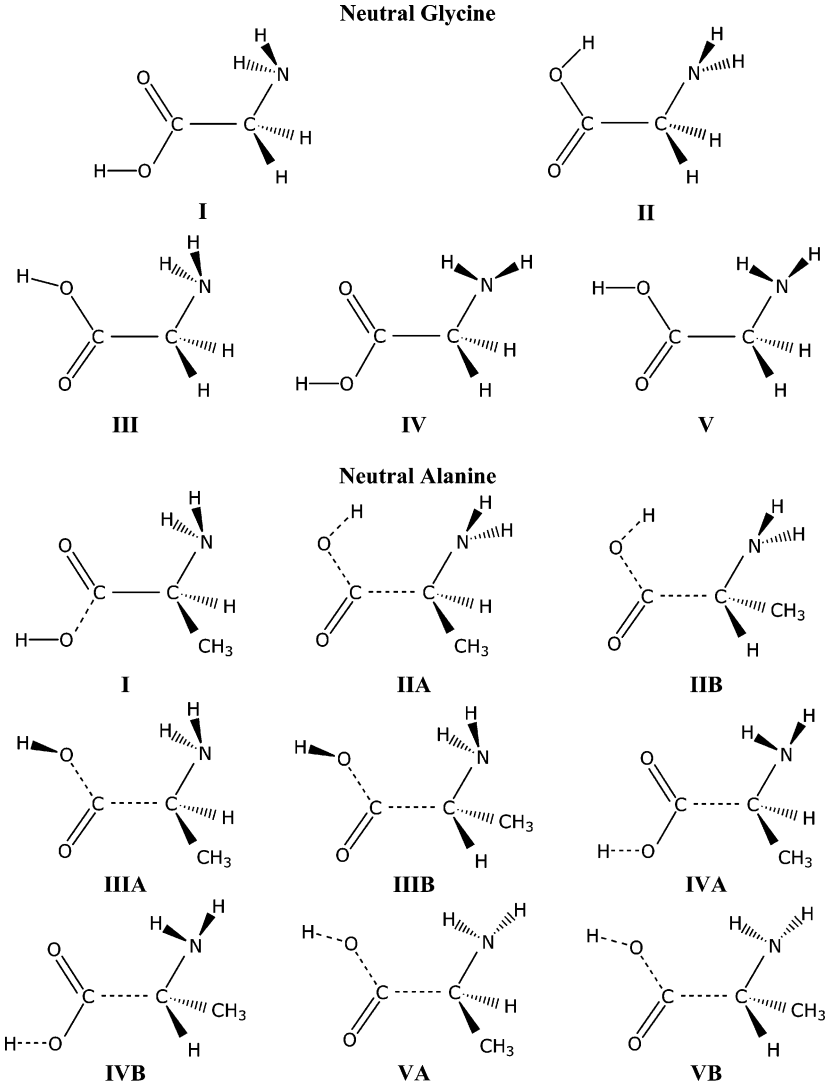
Scheme 1



transfer processes in solution. Rega et al.[26] have observed that the main product after glycine ionization in solution is the glycyl radical $[NH_2CHCOOH]^{\bullet}$, even at low pH, due to the large acidity of the $-CH_2$ group[55,56] in ionized species.

Glycine is the simplest amino acid and consequently an important model compound, which has been the subject of many experimental and theoretical investigations.[13−16,19,20,26−30,38,50] However, most of the studies have focused their attention on the structure and magnetic properties of the C-centered glycyl $[NH_2CHCOOH]^{\bullet}$ radical, one of the radiation products of glycine in solution. Glycyl radical has also been generated in the gas phase[57,58] by collisional neutralization of the stable glycyl cation $[NH_2CHCOOH]^+$, which is obtained by dissociative ionization of several amino acids such as phenylalanine or serine. Unimolecular decompositions are then studied by reionization mass spectrometry experiments. Moreover, photoion mass spectrometry studies of different amino acids in the 6−22 eV photon energy region have provided new information about their dissociative ionization products.[17,18] It has been shown that for the glycine radical cation, the most intense peak is due to the aminomethyl cation, $NH_2CH_2^+$, in complete agreement with a previous study,[28] where the loss of the COOH radical was calculated to be the lowest-energy ion fragmentation. This result was confirmed later on by Lu et al.[30]

Fewer conformational studies have been performed for the other amino acids[25,31−37,39−49] due to their higher conformational complexity. Their study, however, is interesting, because it introduces the influence of the side chain on the stability of the conformations as well as on the preference for any possible fragmentations upon ionization. This work reports an exhaustive gas-phase conformational study for 9 amino acids belonging to different groups (nonpolar, polar, acidic, basic, or aromatic) in their ionized forms. The studied amino acids are as follows: glycine, alanine, serine, cysteine, aspartic acid, glutamine, phenylalanine, tyrosine, and histidine. We expect that this exhaustive conformational study as well as the unimolecular decomposition analysis will help to explain the role of the side chain in oxidative processes of amino acids and to interpret mass spectrometry experiments.

Methods

It is well-known that amino acids can exist in a large number of conformations due to many single-bond rotamers. Given the conformational complexity introduced by the side chain,

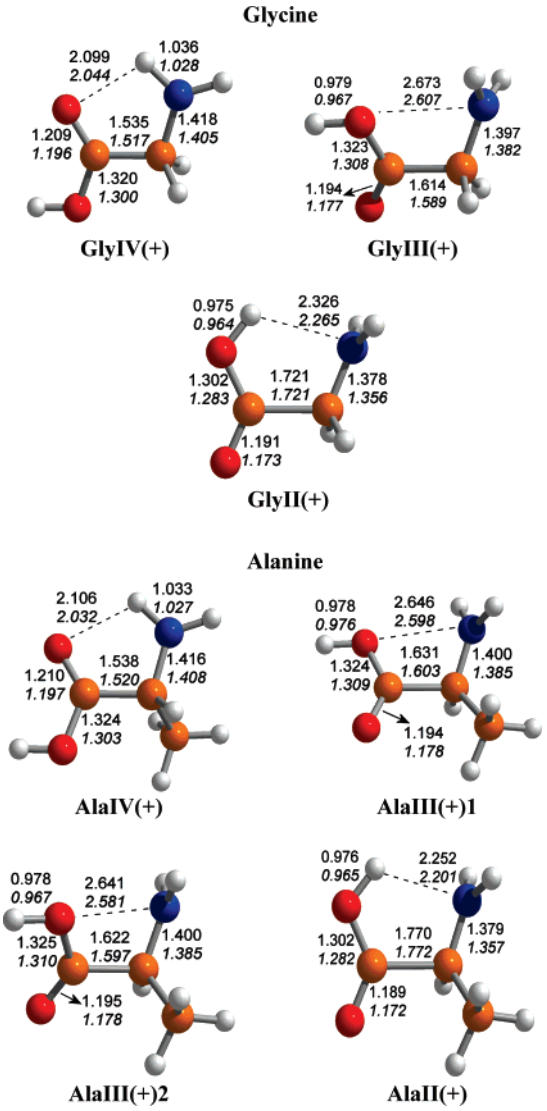


Figure 1. Optimized geometries for the lowest-energy conformer of Gly and Ala radical cations, at the B3LYP and MPWB1K/6-31++G(d,p) levels of theory. Distances are in angstroms.

the following strategy has been applied to find the lowest-energy conformations of each amino acid. First, we have performed a Monte Carlo Multiple Minimum (MCMM) conformational search[59,60] with the MMFF94s force field.[61,62] All plausible structures within an energy window of 50 kJ mol^{-1} were selected for subsequent quantum chemical optimizations of the neutral systems. Radical cation structures were then obtained by ionizing and reoptimizing each neutral conformation.

Optimized geometries and harmonic vibrational frequencies have been obtained using the hybrid B3LYP[63−65] and hybrid-meta MPWB1K[66] functionals with the 6-31++G(d,p) basis set. Geometry optimizations at the MP2/6-31++G(d,p) level of theory have been performed as well, and for all systems except tyrosine, which is very similar to phenylalanine, single-point calculations at the CCSD(T)/6-31++G(d,p) level have also been carried out. All valence electrons were correlated at the MP2 and CCSD(T) levels of theory. Mean average deviations of the used functionals as well as MP2 with respect to CCSD(T) with the 6-31++G(d,p) show that for these radical cation species MPWB1K tends to give results in much better agreement with CCSD(T) than B3LYP or MP2, the MPWB1K, B3LYP, and MP2 average deviations being 0.6, 1.6, and 2.1 kcal mol^{-1}, respectively (see the Supporting Information). On the other hand, the effect of further enlarging the basis set has been analyzed for glycine and alanine by performing calculations with the augmented aug-cc-pVDZ and aug-cc-pVTZ basis sets.[67]

Net atomic charges and spin densities have been obtained using the natural population analysis of Weinhold et al.[68] All DFT calculations and post Hartree−Fock MP2 and CCSD(T) with the small 6-31++G(d,p) basis set have been performed with the Gaussian 03 package,[69] and open-shell systems have been treated with an unrestricted formalism. CCSD(T) with the aug-cc-pVXZ (X = D and T) sets have been performed with the MOLPRO program and were based on a restricted Hartree−Fock reference wave function.[70] A Monte Carlo Multiple Minimum (MCMM) conformational search has been performed with the Macromodel 7.0 package.[71]

Results and Discussion

Removing an electron from neutral amino acids induces significant structural changes that vary depending on the starting conformation. In order to understand the influence of ionization in all amino acids, we will first analyze in detail the structural features of the radical cations of the two simplest amino acids: glycine (Gly) and alanine (Ala).

Table 1. Relative Energies (ΔE) in kcal mol^{-1} at Different Levels of Theory

structure	B3LYP 6-31++G(d,p)	MPWB1K 6-31++G(d,p)	CCSD(T)// MPWB1K/6-31++G(d,p) 6-31++G(d,p)	aug-pVDZ	aug-pVTZ
			Glycine		
GlyIV(+)	0.0	0.0	0.0	0.0	0.0
GlyIII(+)	−3.0	1.3	1.1	1.9	1.6
GlyII(+)	6.2	11.7	12.1	11.0	10.1
			Alanine		
AlaIV(+)	0.0	0.0	0.0	0.0	0.0
AlaIII(+)1	−3.1	0.4	0.9	1.0	0.8
AlaIII(+)2	−2.5	1.5	1.4	1.5	1.4
AlaII(+)	5.6	10.4	11.5	-	-

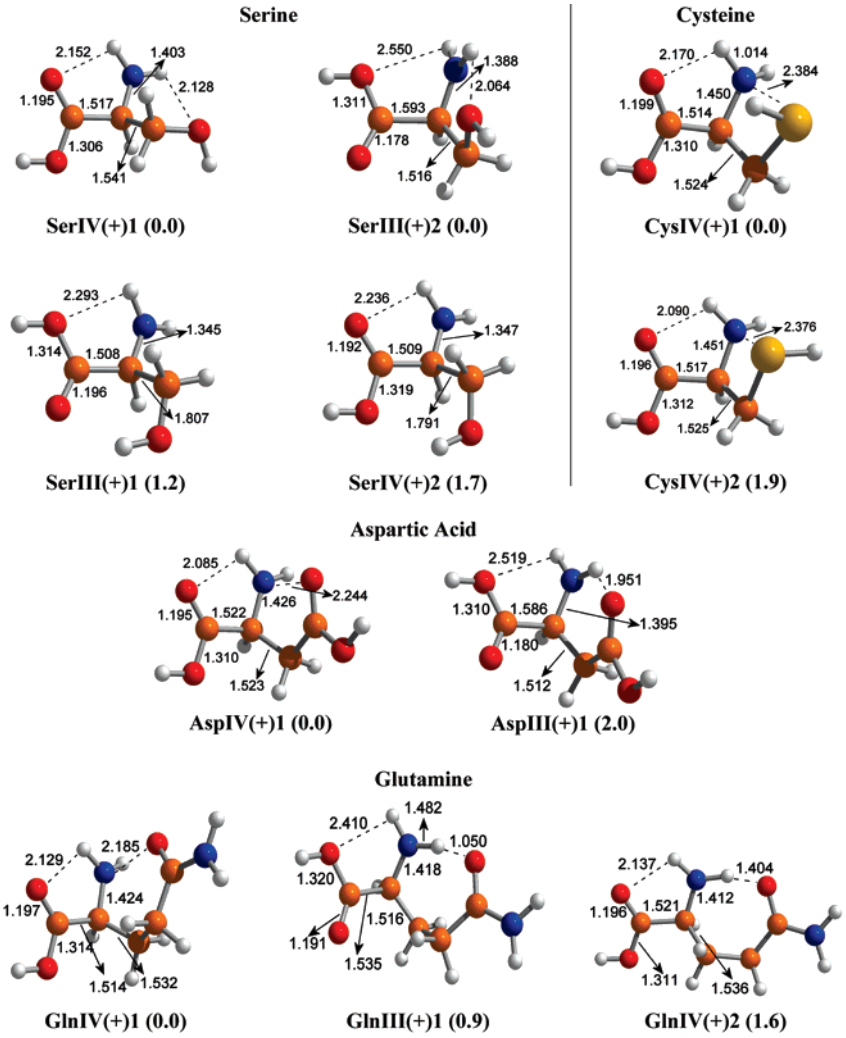


Figure 2. Optimized geometries and relative energies (ΔE) for the lowest-energy conformers of Ser, Cys, Asp, and Gln radical cations at the MPWB1K/6-31++G(d,p) level of theory. Distances are in angstroms and energies are in kcal mol^{-1}.

Second, we will consider the influence of ionizing serine (Ser), cysteine (Cys), aspartic acid (Asp), and glutamine (Gln) amino acids, which contain acidic and basic sites in their side chains that can be involved in intramolecular hydrogen bonds. Next, we will present the results corresponding to the aromatic amino acids phenylalanine (Phe), tyrosine (Tyr), and histidine (His), which have an easy ionizable side chain. Finally, all possible C_α cleavages of the ionized amino acids will be analyzed.

Structural Changes. *Gly and Ala*. Previous accurate theoretical studies have identified eight minimum energy conformers of neutral glycine.[50] Among them, five conformers present relative energies which are less than 1000 cm^{-1} (2.86 kcal mol^{-1}), the relative energies of the three remaining conformers being larger than 4.52 kcal mol^{-1} with respect to the ground-state structure. For alanine 13 conformers have been identified as minima on the potential energy surface.[32] However, only 9 present relative energies lower than 1000 cm^{-1}. The five major structures of neutral glycine and nine major structures of alanine are shown in Scheme 1. Notation used has been taken from refs 32 and 50. It can be observed that the increase in the number of stable conformers for Ala is due to the doubling of conformers for structures II, III, IV, and V because of the loss of symmetry plane. Nevertheless, upon ionization of these neutral conformers only three stable structures are found for glycine radical cation and four for alanine radical cation. Optimized geometries are shown in Figure 1, whereas relative energies ΔE at different levels of theory are given in Table 1. These conformers have been labeled as II(+), III(+), and IV(+), in analogy to the notation used for the neutral systems, which is related to the nature of intramolecular hydrogen bonds, but we have added a (+) symbol to indicate that it refers to the radical cation species.

Table 2. MPWB1K/6-31++G(d,p) Charge (Spin Density) from Natural Population Analysis for the Lowest-Energy Conformer of Gly, Ala, Ser, Cys, Asp, Gln, Phe, Tyr, and His Radical Cations

amino acid	NH_2	COOH	R	CH
GlyIV(+)	0.64 (0.90)	0.12 (0.00)	0.34 (0.06)	−0.10 (0.04)
AlaIV(+)	0.62 (0.88)	0.11 (0.00)	0.17 (0.08)	0.10 (0.04)
SerIV(+)1	0.63 (0.87)	0.12 (0.01)	0.16 (0.06)	0.10 (0.06)
CysIV(+)1	0.19 (0.40)	0.08 (0.00)	0.62 (0.60)	0.10 (0.00)
AspIV(+)1	0.51 (0.75)	0.10 (0.00)	0.27 (0.24)	0.11 (0.01)
GlnIV(+)1	0.46 (0.69)	0.08 (0.00)	0.36 (0.31)	0.10 (0.00)
PheII(+)1	−0.06 (0.03)	0.23 (0.20)	0.72 (0.72)	0.11 (0.03)
TyrII(+)1	−0.07 (0.02)	0.14 (0.11)	0.83 (0.84)	0.10 (0.03)
HisIII(+)1	−0.10 (0.00)	0.08 (0.03)	0.89 (0.96)	0.12 (0.01)

Moreover, an additional number has been included after (+) to distinguish between conformers with the same amino/carboxylic intramolecular hydrogen bond pattern.

First, it can be noted that structures I(+) and V(+) are not found to be a minima on the potential energy surface since ionization of I or V leads to structure III(+). On the other hand, structures IIA and IIB and IVA and IVB of alanine collapse to conformers II(+) and IV(+), respectively, which reduces significantly the number of stable conformers for the radical cation. This is not surprising considering that structures A and B differ on the relative orientation the carboxylic group with respect to the CH_3 side chain. For example, the OCCN dihedral angles for structures IIA and IIB are 169° and −167°, respectively. However, ionization introduces a positive charge that leads to a unique minimum with a dihedral angle of 179°.

As a general trend, it is observed that both for Gly and Ala ionization is localized at the $-NH_2$ group, and, thus, the hydrogen bonds that involve this group are modified. That is, the amino group becomes more planar, $-NH_2^+$ increases its acidity, and consequently the intramolecular hydrogen bonds in which $-NH_2$ acts as proton donor are strengthened. For this reason structure IV(+) becomes largely stabilized for glycine and alanine radical cations. In contrast, structure II(+) in which $-NH_2$ acts as proton acceptor becomes the most unstable one due to the decrease of basicity of $-NH_2$ upon ionization.

It can be observed in Figure 1 that the optimized geometries with the two functionals are quite similar. However, the computed relative energies largely depend on the functional used, the one that better compares to the CCSD(T) method being the hybrid-meta MPWB1K (see Table 1). That is, for both Gly and Ala at the MPWB1K level of theory, structure IV(+) is predicted to be the global minimum in agreement with the CCSD(T) calculations. However, at the B3LYP level a III(+)-like structure is determined to be the most stable one. It should be mentioned that the CCSD(T) values are almost the same regardless of whether we use the B3LYP or MPWB1K optimized geometries to perform the single-point CCSD(T) calculations. The discrepancy between both functionals is not surprising considering that structures III(+) present a two-center/three-electron bond between N and O, which has been shown to be overstabilized by the B3LYP functional, due to an overestimation of the self-interaction part of the exchange energy because of the delocalized nature of the electron hole.[72,73] These studies showed also that the admixture of exact exchange energy reduces the error, as found here with MPWB1K, which includes a 44% of exact exchange. On the other hand, it can be observed in Table 1 that the influence of further enlarging the basis set at the CCSD(T) level is much smaller, the values with the largest aug-cc-pVTZ basis set being in quite good agreement with the MPWB1K/6-31++G(d,p) results, which validates this latter level of theory as a cost-effective one for studying these systems. Because of that in the following sections, and in order to facilitate the discussion, only the MPWB1K results will be reported.

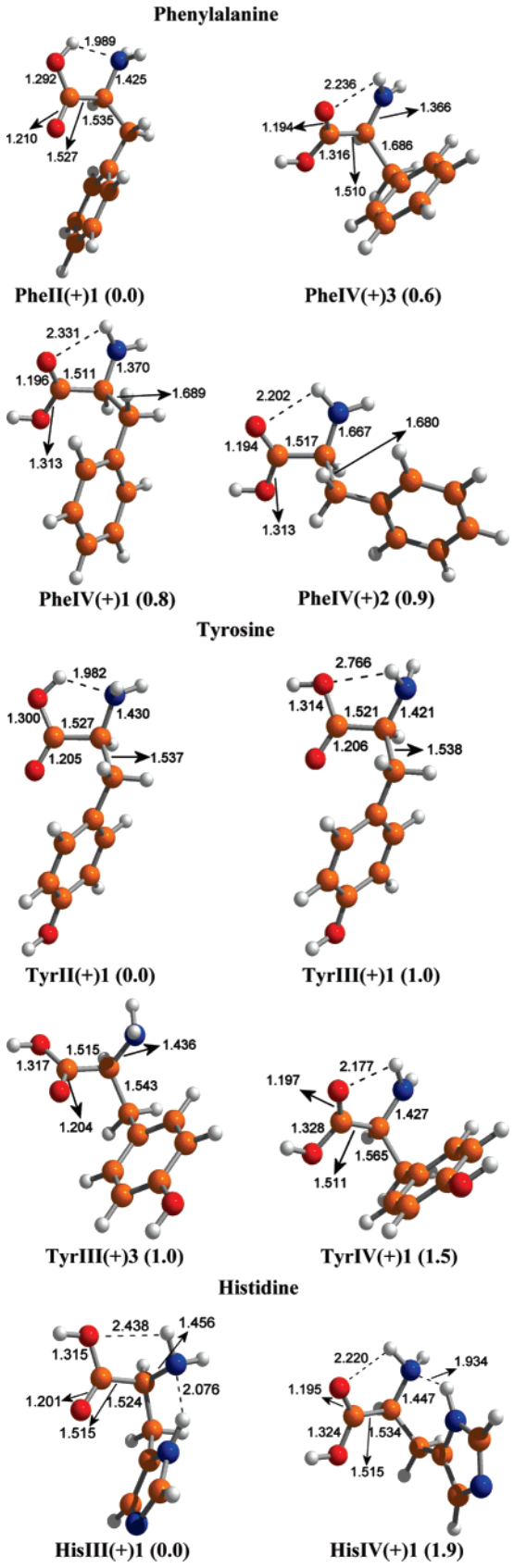


Figure 3. Optimized geometries and relative energies (ΔE) for the lower energy conformers of Phe, Tyr, and His at the MPWB1K/6-31++G(d,p) level of theory. Distances are in angstroms and energies are in kcal mol^{-1}.

Ser, Cys, Asp, and Gln. Let us now consider those amino acids that contain hydrocarbon side chains with acidic and

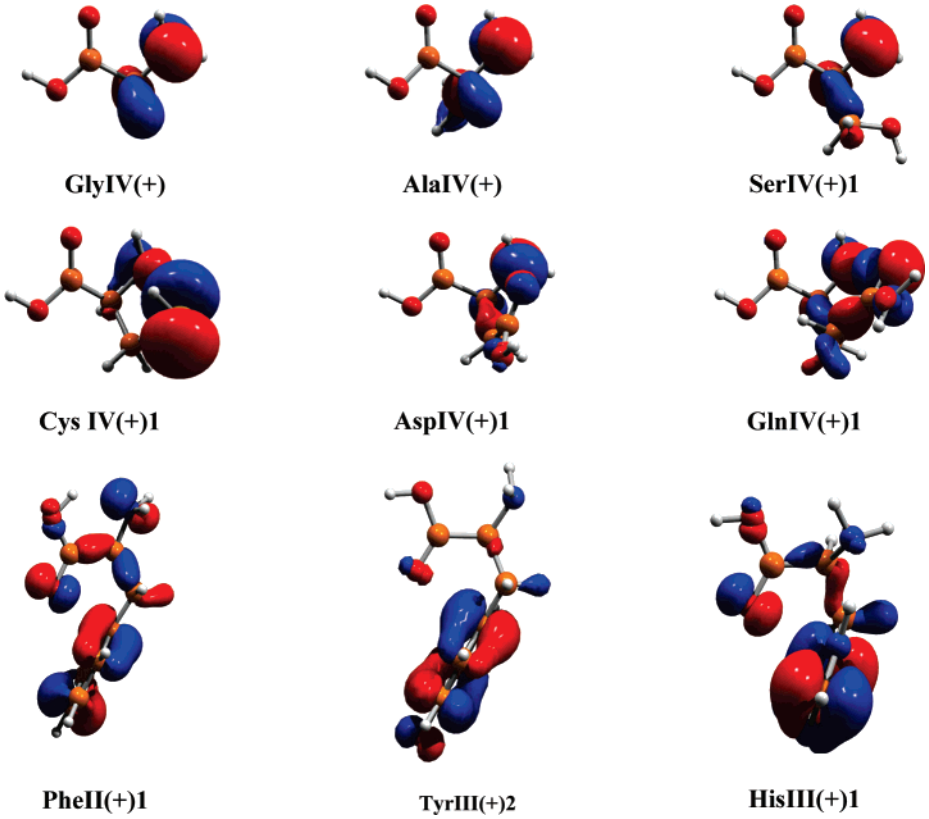


Figure 4. Single occupied molecular orbital of the lowest conformer of Gly, Ala, Ser, Cys Asp, Gln, Phe, Tyr, and His radical cations.

Table 3. MPWB1K/6-31++G(d,p) Internal Energies of Reaction (ΔU_{0K}) for the Unimolecular Decompositions of Gly, Ala, Ser, Cys, Asp, Gln, Phe, Tyr, and His Radical Cations (kcal mol^{-1})[a]

	$GX^{\cdot+} \rightarrow G^+ + X^{\cdot}$	Gly	Ala	Ser	Cys	Asp	Gln	Phe	Tyr	His
(1)	$[NH_2CHRCOOH]^{\cdot+} \rightarrow [NH_2CRCOOH]^+ + H^{\cdot}$	33.0	21.8	23.5	31.8	25.8	27.5	26.5	39.5	50.3
(2)	$[NH_2CHRCOOH]^{\cdot+} \rightarrow [CHRCOOH]^+ + [NH_2]^{\cdot}$	79.0	71.2	42.9	45.6	56.5	28.1	40.3	46.9	42.5
(3)	$[NH_2CHRCOOH]^{\cdot+} \rightarrow [NH_2CHR]^+ + [COOH]^{\cdot}$	23.5	12.5	22.6	22.4	16.7	18.6	20.5	27.3	37.4
(4)	$[NH_2CHRCOOH]^{\cdot+} \rightarrow [NH_2CHCOOH]^+ + R^{\cdot}$	33.0	28.2	28.2	29.9	28.3	47.0	26.4	37.2	38.4
	$GX^{\cdot+} \rightarrow G^{\cdot} + X^+$	Gly	Ala	Ser	Cys	Asp	Gln	Phe	Tyr	His
(5)	$[NH_2CHRCOOH]^{\cdot+} \rightarrow [NH_2CRCOOH]^{\cdot} + H^+$	180.3	181.8	185.8	189.7	186.2	199.1	193.0	204.5	209.8
(6)	$[NH_2CHRCOOH]^{\cdot+} \rightarrow [CHRCOOH]^{\cdot} + [NH_2]^+$	163.7	163.3	169.9	172.1	172.0	185.1	175.7	186.7	191.1
(7)	$[NH_2CHRCOOH]^{\cdot+} \rightarrow [NH_2CHR]^{\cdot} + [COOH]^+$	67.8	70.7	72.9	76.7	73.9	89.4	82.7	92.7	94.6
(8)	$[NH_2CHRCOOH]^{\cdot+} \rightarrow [NH_2CHCOOH]^{\cdot} + R^+$	180.3	88.8	37.1	40.8	68.1	66.1	27.2	25.8	34.4

[a] R = H, CH_3, CH_2OH, CH_2SH, CH_2COOH, $CH_2CH_2CONH_2$, $CH_2C_6H_5$, $CH_2C_6H_4OH$, $CH_2C_3N_2H_4$ for Gly, Ala, Ser, Cys, Asp, Gln, Phe, Tyr, and His, respectively.

basic groups such as Ser, Cys, Asp, and Gln for which R = $-CH_2OH$, $-CH_2SH$, $-CH_2COOH$, and $-CH_2CH_2CONH_2$, respectively. Because now the number of stable conformers is much larger due to the presence of many single-bond rotamers, the following strategy has been applied to find the lower conformers of each amino acid. Starting from the major structures of glycine (see Scheme 1) a Monte Carlo Multiple Minimum (MCMM) conformational search[59,60] with the MMFF94s force field[61,62] has been performed allowing only the internal rotations of the side chain. All plausible structures within an energy window of 50 kJ mol^{-1} were selected for subsequent quantum chemical optimizations of the neutral systems. Structures of radical cations were then obtained by reoptimizing these structures after removing one electron from the system. We expect that with this strategy the main conformers of the radical cations of these amino acids have been localized. Optimized geometries and relative energies of these low-lying conformers (up to 2 kcal mol^{-1} at the MPWB1K level) are shown in Figure 2. The remaining structures as well as their relative energies at different levels of theory are given in the Supporting Information.

It can be observed that, as found for Gly and Ala, the low-lying energy conformer of these amino acid radical cations are either of type III(+) or IV(+). In all cases the initially pyramidalized $-NH_2$ group becomes more planar in the radical cation species due to the fact that ionization mainly takes place at $-NH_2$. This is confirmed by natural population analysis which indicates that the spin density

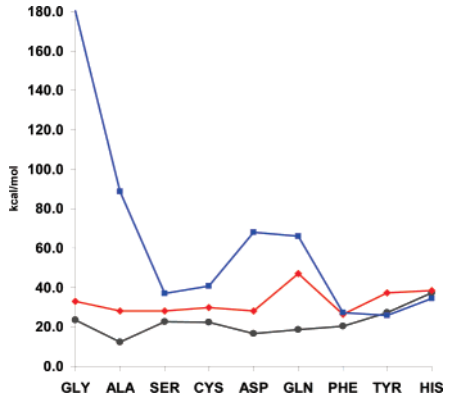


Figure 5. Reaction energies ΔU_{0K} for (●) $[NH_2CHRCOOH]^{\bullet+} \rightarrow [NH_2CHR]^+ + [COOH]^{\bullet}$, (◆) $[NH_2CHRCOOH]^{\bullet+} \rightarrow [NH_2CHCOOH]^+ + R^{\bullet}$, and (■) $[NH_2CHRCOOH]^{\bullet+} \rightarrow [NH_2CHCOOH]^{\bullet} + R^+$ for each amino acid at the MPWB1K/6-31++G(d,p) level of theory.

mainly lies at this $-NH_2$ group (see Table 2). Thus, ionization increases the $-NH_2$ acidity, which favors intramolecular hydrogen bond interactions in which this group acts as proton donor. For this reason structure IV(+) becomes the lowest-energy conformer in all cases. Nevertheless, for Ser we have located an almost degenerate conformer of type III(+) in which the $-NH_2$ establishes a quite strong hydrogen bond interaction with the OH group of the side chain.

For Cys, the more stable conformers, CysIV(+)1 and CysIV(+)2, present a two-center/three-electron hemibond interaction between the $-NH_2$ and the $-SH$ group of the side chain. For Ser, however, we have not been able to locate a conformation with such an interaction with the side chain, in agreement with the fact that the $-OH$ group prefers to establish hydrogen bonds interaction than two-center/three-electron hemibonds.[51,53] In contrast, for Asp the most stable IV(+) structure presents a stabilizing hemibond interaction between the carbonyl oxygen of the side chain and the $-NH_2$ group, which shows that the existence or not of such hemibond interactions in radical cations results from a subtle balance between the stabilization gained by this hemibond interaction and the possibility of establishing intramolecular hydrogen bonds. In fact for glutamine we have located two structures IV(+), one in which the carbonyl oxygen of the side chain forms an intramolecular hydrogen bond with the NH_2 group and another one in which it establishes a two-center/three-electron interaction, the relative energies between them being 1.6 kcal mol^{-1}. It should be noted that for those structures that show a two-center/three-electron bond between the $-NH_2$ group and a basic site of the side chain (CysIV(+)1, AspIV(+)1, and GlnIV(+)1) natural population analysis indicates that the spin density is delocalized between the two interacting groups (see Table 2)

Glutamine is a particularly interesting amino acid since in many cases ionization leads to a spontaneous proton transfer from the C_α to the CO group of the side chain. In fact, the most stable structural isomer of glutamine radical cation corresponds to a diol $[NH_2C(CH_2CH_2CONH_2)C(OH)_2]^{\bullet+}$ species, which lies 27.0 kcal mol^{-1} below the most stable nonproton transferred structure shown in Figure 2. As found for glycine radical cation,[29] this diol structure is largely stabilized by captodative effects in the glycyl like species formed. However, in contrast to glycine, further studies[74] have confirmed that glutamine easily evolves to a diol structure due to its long side chain with basic groups which allow it to act as a proton-acceptor and also as a solvent assistant catalyst.

His, Phe, and Tyr. Optimized geometries and relative energies of the low-lying conformers of His, Phe, and Tyr radical cations are shown in Figure 3. For Tyr we have only included one of the two (almost degenerate) conformers associated with the rotation of the OH of the side chain. Aromatic amino acids do not follow the trends found for the previous amino acids because ionization mainly takes place at the side chain. This is in agreement with the spin density values and the nature of the open-shell orbital shown in Table 2 and Figure 4, respectively. Note that for Tyr and His the spin density at the side chain is 0.8−0.9 and that the open-shell orbital is mainly centered at the aromatic ring. For Phe the spin density is more delocalized although it still has its major contribution at the ring. For these amino acids for which ionization of the side chain prevails over ionization of the $-NH_2$ group, structures type III(+) and II(+) become competitive. In fact, the most stable structure for His is a distorted structure III(+) in which the NH group of the imidazole ring forms a hydrogen bond with the $-NH_2$ group. This imidazole NH group is more acidic due to the ionization of the side chain. On the other hand, for Phe and Tyr, structures derived from II(+) become the ground-state structures, which shows the importance of the side-chain nature in the effects of ionization.

Unimolecular Decompositions. In addition to the changes observed in intramolecular hydrogen bonds, other major geometry changes occur upon ionization, which can determine the fragmentations that will be observed in mass spectrometry experiments.

Table 3 shows the internal energy of reaction (ΔU_{0K}) corresponding to the different fragmentation processes of Gly, Ala, Ser, Cys, Gln, Asp, Phe, Tyr, and His radical cations. Four different $C_\alpha-R$ bond cleavages can be considered: $C_\alpha-COOH$, $C_\alpha-H$, $C_\alpha-NH_2$, and $C_\alpha-R$. Such cleavages can be produced in two different ways: that is, by losing a neutral radical ($COOH^{\bullet}$, $H^{\bullet}$, $NH_2^{\bullet}$,$R^{\bullet}$) or by losing a cation ($COOH^+$, H^+, NH_2^+, R^+). Thus, eight different reactions have been considered, the computed reaction energies being collected in Table 3.

Among the four decompositions that involve the loss of a neutral radical, the loss of $[COOH]^{\bullet}$ is the most favorable process for all amino acids. This fact, previously observed for Gly, Ala, Ser, and Cys,[46] is also true for Gln, Asp, and Phe and is in very good agreement with their mass spectra,[75] since the most intense peaks at $m/z = 30$, $m/z = 44$, $m/z = 60$, $m/z = 76$, and $m/z = 88$, respectively, can be assigned to the $[NH_2CH_2]^+$, $[NH_2CHCH_3]^+$, $[NH_2CHCH_2OH]^+$, $[NH_2CHCH_2SH]^+$, and $[NH_2CHCH_2COOH]^+$ ions formed by loss of the $[COOH]^{\bullet}$ radical. Phe mass spectra also present an intense peak at $m/z = 120$, corresponding to the decomposition: $[NH_2CHRCOOH]^{\bullet+} \rightarrow [NH_2CHR]^+ + [COOH]^{\bullet}$.

As the side chain increases, the loss of $R^{\bullet}$ (eq 4) starts to be a competitive process. This fact, observed for Ser, Cys,

Table 4. MPWB1K/6-31++G(d,p) Adiabatic Ionization Energy of Each Amino Acid and the Different Fragments Formed (kcal mol^{-1})[a]

	Gly	Ala	Ser	Cys	Asp	Gln	Phe	Tyr	His
$NH_2CHRCOOH$	207.1	202.6	201.1	196.0	199.4	185.0	192.5	181.6	181.8
$[NH_2CRCOOH]^{\bullet}$	164.7	152.0	149.8	154.1	151.6	140.4	145.5	147.0	152.5
$[CHRCOOH]^{\bullet}$	204.5	197.1	162.3	162.8	173.8	132.3	153.8	149.4	140.6
$[NH_2CHR]^{\bullet}$	143.8	129.8	137.7	133.7	130.8	117.2	125.8	122.6	130.8
$[NH_2CHCOOH]^{\bullet}$	164.7	164.7	164.7	164.7	164.7	164.7	164.7	164.7	164.7
$H^{\bullet}$	312.0	312.0	312.0	312.0	312.0	312.0	312.0	312.0	312.0
$[NH_2]^{\bullet}$	289.3	289.3	289.3	289.3	289.3	289.3	289.3	289.3	289.3
$[COOH]^{\bullet}$	188.0	188.0	188.0	188.0	188.0	188.0	188.0	188.0	188.0
$[R]^{\bullet}$	312.0	225.3	173.6	175.6	204.5	183.8	165.4	153.2	160.7

[a] R = H, CH_3, CH_2OH, CH_2SH, CH_2COOH, $CH_2CH_2CONH_2$, $CH_2C_6H_5$, $CH_2C_6H_4OH$ and $CH_2C_3N_2H_4$ for Gly, Ala, Ser, Cys, Asp, Gln, Phe, Tyr, and His, respectively.

Table 5. MPWB1K/6-31++G(d,p) Cα−X Dissociation Energies (D_0)[a] for Neutral Gly, Ala, Ser, Cys, Asp, Gln, Phe, Tyr, and His (in kcal mol^{-1})

$GX \rightarrow G^{\bullet} + X^{\bullet}$	Gly	Ala	Ser	Cys	Asp	Gln	Phe	Tyr	His
$[NH_2CHRCOOH] \rightarrow [NH_2CRCOOH]^{\bullet} + H^{\bullet}$	75.4	72.4	74.9	73.7	73.6	72.1	73.5	74.1	79.6
$[NH_2CHRCOOH] \rightarrow [CHRCOOH]^{\bullet} + [NH_2]^{\bullet}$	81.5	76.7	81.7	78.8	82.1	80.8	78.9	79.1	83.6
$[NH_2CHRCOOH] \rightarrow [NH_2CHR]^{\bullet} + [COOH]^{\bullet}$	86.8	85.3	85.9	84.7	85.3	86.4	87.2	86.3	88.4
$[NH_2CHRCOOH] \rightarrow [NH_2CHCOOH]^{\bullet} + R^{\bullet}$ [b]	75.4	66.2	64.6	61.2	62.9	67.3	54.2	54.1	55.5

[a] Zero point energy computed from harmonic vibrational frequencies. [b] R = H, CH_3, CH_2OH, CH_2SH, CH_2COOH, $CH_2CH_2CONH_2$, $CH_2C_6H_5$, $CH_2C_6H_4OH$, $CH_2C_3N_2H_4$ for Gly, Ala, Ser, Cys, Asp, Gln, Phe, Tyr, and His, respectively.

and aromatic Phe, Tyr, and His amino acids, is in very good agreement with the mass spectra of Ser, Cys, and Phe which show a very intense peak at $m/z = 74$ corresponding to the fragment $[NH_2CHCOOH]^+$. The third process that can compete with the loss of $[COOH]^{\bullet}$ and $[R]^{\bullet}$ (as the side chain increases) is the glycyl formation; that is, the loss of the cationic side chain. It can be observed in Table 3 that, among the four reactions (eqs 5−8) leading to the loss of a cation fragment, the C_α−R cleavage is the most favorable process for all amino acids except for Gly and Ala which prefer the loss of $COOH^+$. This is not surprising considering that the larger the side chain is, the better the positive charge is delocalized. Internal energy changes (ΔU_{0K}) of these three decompositions are shown in Figure 5. It can be observed how the loss of R^+ becomes very important for aromatic amino acids, this reaction becoming even the preferred unimolecular decomposition for Tyr and His. In fact, these two amino acids present a very intense peak in their mass spectra, $m/z = 81$ and $m/z = 107$, respectively, corresponding to the R^+ fragment.

As noted previously,[28,46] a simple thermodynamic cycle allows us to decompose the internal energy of reaction in

$$\Delta U_{0K}(-X^{\bullet}) = -\mathrm{IE}(GX) + D_0(GX) + \mathrm{IE}(G^{\bullet})$$

or

$$\Delta U_{0K}(-X^{+}) = -\mathrm{IE}(GX) + D_0(GX) + \mathrm{IE}(X^{\bullet})$$

depending on whether the radical cation loses a neutral or a cationic fragment.

D_0(GX) corresponds to the homolytic dissociation energy of the neutral amino acid, IE(GX) corresponds to the adiabatic ionization energy of the considered amino acid, and IE($G^{\bullet}$) and IE($X^{\bullet}$) correspond to the ionization energy of each fragment. That is, the internal energy of reaction (ΔU_{0K}) of an unimolecular decomposition depends on three different parameters: (i) the ionization energy of the corresponding amino acid, (ii) the dissociation energy of the neutral compound, and (iii) the ionization energy of each fragment. Ionization energies are given in Table 4, whereas D_0(GX) values are shown in Table 5. Since we are interested in analyzing which is the most favorable fragmentation within an amino acid, the first parameter, its ionization energy, remains constant and, thus, will not be discussed further in the text.

For each fragmentation let us start to analyze the preference in the loss of the neutral radical fragment $X^{\bullet}$ or cation fragment X^+. From the energy decomposition scheme it can be noted that for each amino acid this preference only depends on the $X^{\bullet}$ and $G^{\bullet}$ relative ionization energies. As the ionization energies of the fragment that contains C_α (IE($G^{\bullet}$)) is lower than IE($X^{\bullet}$), the loss of $X^{\bullet}$ is, in general, the most favorable process. For example, for Ala the ionization energy of $[NH_2CHCH_3]^{\bullet}$ (IE=129.8 kcal mol^{-1}) is lower than that of $[COOH]^{\bullet}$ (IE=188.0 kcal mol^{-1}), which makes the loss of neutral $[COOH]^{\bullet}$ the most favorable process. The same conclusion can be reached for each pair of fragmentations of all nine amino acids. The only exception comes when the decomposition process implies the loss of R^+ or $R^{\bullet}$ because for Phe, Tyr, and His the IE of $R^{\bullet}$ (165.4, 153.2, and 160.7 kcal mol^{-1}, respectively) is very similar to that of the glycyl radical, $[NH_2CHCOOH]^{\bullet}$, which is 164.7 kcal mol^{-1}. This fact explains why the loss of cationic side chain ($[NH_2CHRCOOH]^{\bullet+} \rightarrow [NH_2CHCOOH]^{\bullet} + R^+$) is preferred over the loss of the neutral radical ($[NH_2CHRCOOH]^{\bullet+} \rightarrow [NH_2CHCOOH]^+ + R^{\bullet}$) in the case of Tyr and His.

When different fragmentations are compared, the dissociation energy of the involved bond of the neutral amino acids (D_0(GX)) needs to be taken into account. That is, the

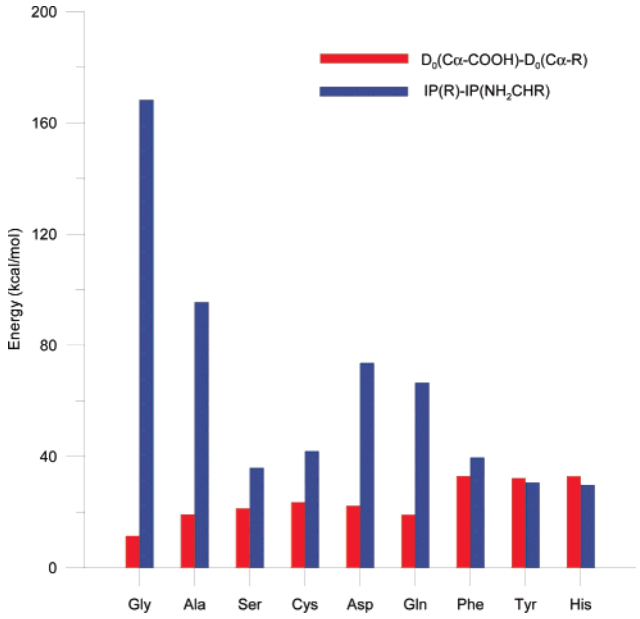


Figure 6. $D_0(C\alpha-COOH)-D_0(C\alpha-R)$ and $IE(R)-IE(NH_2-CHR)$ in kcal mol^{-1}.

preference for one reaction or another will depend not only on the ionization energy of the fragment that will finally support the positive charge but also on the energy required to break the corresponding bond. Since differences on the dissociation energies are rather small compared to the variations on ionization energies, usually the dominant term is the ionization energy in such a way that the preferred process is the one that leaves the positive charge in the fragment with the lower ionization energy; that is, reaction 3 $[NH_2CHRCOOH]^{\bullet+} \rightarrow [NH_2CHR]^+ + [COOH]^\bullet$ (see Table 3). Nevertheless, as the side chain becomes more voluminous, the $C_\alpha-R$ becomes weaker (see Table 5), in such a way that the $C_\alpha-R$ dissociation energy for aromatic amino acids (54−55 kcal mol^{-1}) becomes significantly smaller than the $C_\alpha-COOH$ (84−88 kcal mol^{-1}), $C_\alpha-NH_2$ (78−83 kcal mol^{-1}), or the $C_\alpha-H$ (72−79 kcal mol^{-1}) dissociation energies. Therefore, the loss of the side chain, particularly R^+, $[NH_2-CHRCOOH]^{\bullet+} \rightarrow [NH_2CHCOOH]^\bullet + R^+$ reaction 8, becomes competitive for Phe, Tyr, and His. This can be clearly seen in Figure 6 where the difference on neutral dissociation energies ($D_0(C_\alpha-COOH)-D_0(C_\alpha-R)$) and ionization energies of the two cationic fragments ($(IE([R]^\bullet)-IE([NH_2-CHR]^\bullet))$ corresponding to these competing reactions are represented for each amino acid. These two quantities act in an opposite way; that is, the larger the first one is, the more favorable becomes reaction 8 (loss of R^+), whereas the larger the second term is, the more favorable becomes reaction 3 (loss of $[COOH]^\bullet$). It can be observed that for aromatic amino acids the two columns become almost equal so that the two fragmentation processes become energetically similar.

Summary

This work provides a theoretical study of the conformational behavior of nine ionized amino acids by means of the hybrid B3LYP and meta-hybrid MPWB1K functional as well as by means of post-Hartree Fock calculations at the CCSD(T) level of theory. Different kinds of amino acids have been chosen in order to study the effect of the side chain on the reorganization and fragmentation processes upon ionization. In almost all cases ionization of these amino acids takes place at the amino group, which becomes more planar and acidic. As a consequence NH···OC hydrogen bonds are strengthened, and conformer IV(+) is largely stabilized for the ionized species. In fact for all amino acids except the aromatic ones, a IV(+)-like conformer is the ground-state structure, the side chain being involved in additional intramolecular hydrogen bonds or in two-center/three-electron interactions with the ionized $-NH_2$ group. However, for Phe, Tyr, and His aromatic amino acids ionization takes place mainly at the aromatic ring. Because of that, for Phe and Tyr, structures II(+) become the most stable ones. In the case of His ionization increases the acidity of the imidazole −NH− group in such a way that it tends to form a hydrogen bond with the lone pair of the $-NH_2$ leading to a distorted structure III(+). Finally, among the different $C_\alpha-X$ fragmentation processes, the one that leads to the loss of $[COOH]^\bullet$ is the most favorable one. Nevertheless, for amino acids with an increasing size chain, fragmentations leading to R^+ or $R^\bullet$ start being competitive. In fact, for the aromatic amino acids Tyr and His, the fragmentation leading to R^+ is the most favorable process. This is important because it leads to the formation of glycyl radical, which is known to be involved in different protein radical processes.

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Supporting Information Available: Low-lying energy conformers of Gly, Ala, Ser, Cys, Asp Gln, Phe, Tyr, and His radical cations and relative energies at different levels of theory. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Berlett, B. S.; Stadtman, E. R. *J. Biol. Chem.* **1997**, *272*, 20313.

(2) Stadtman, E. R. *Ann. Rev. Biochem.* **1993**, *62*, 797.

(3) Butterfield, D. A.; Boyd-Kimbal, D. *Biochim. Biophys. Acta* **2005**, *1703*, 149.

(4) Hou, L.; Shao, H.; Zhang, Y.; Li, H.; Menon, N. K.; Neuhaus, E. B.; Brewer, J. M.; Byeon, I.-J. L.; Ray, D. G.; Vitek, M. P.; Iwashita, T.; Makula, R. A.; Przybyla, A. B.; Zagorski, M. G. *J. Am. Chem. Soc.* **2004**, *126*, 1992.

(5) Clementi, M. E.; Martorana, G. E.; Pezzotti, M.; Giardina, B.; Misiti, F. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 2066.

(6) Misiti, F.; Martorana, G. E.; Nocca, G.; Di Stasio, E.; Giardina, B; Clementi, M. E. *Neuroscience* **2004**, *126*, 297.

(7) Butterfield, D. A.; Bush, A. I. *Neurobiol. Aging* **2004**, *25*, 563.

(8) Ciccosto, G. D.; Barnham, K. J.; Cherny, R. A.; Masters, C. L.; Bush, A. I.; Curtain, C. C.; Cappai, R.; Tew, D. *Lett. Pept. Sci.* **2003**, *10*, 413.

(9) Barnham, K. J., et al. *J. Biol. Chem.* **2003**, *278*, 42959.

(10) Kantorow, M.; Hawse, J. R.; Cowell, T. L.; Benhamed, S.; Pizarro, G. O.; Reddy, V. N.; Hejtmanicik, J. F. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 9654.

(11) Stubbe, J.; van der Donk, W. A. *Chem. Rev.* **1998**, *98*, 705.

(12) Bagheri-Majdi, E.; Ke, Y.; Orlova, G.; Chu, I. K.; Hopkinson, A. C.; Siu, K. W. M. *J. Phys. Chem. B* **2004**, *108*, 11170.

(13) Chis, V.; Brustolon, M.; Chis, V.; Morari, C.; Cozar, O.; David, L. *J. Mol. Struct.* **1999**, *482*, 283.

(14) Brustolon, M.; Chis, V.; Maniero, A. L.; Brunel, L. C. *J. Phys. Chem. A* **1997**, *101*, 4887.

(15) Sanderud, A.; Sagstuen, E. *J. Phys. Chem. B* **1998**, *102*, 9353.

(16) Bonifačic, M.; Štefanić, I.; Hug, G. L.; Armtrong, D. A.; Asmus, K. D. *J. Am. Chem. Soc.* **1998**, *120*, 9930.

(17) Jochims, H. W.; Schwell, M.; Chotin, J. L.; Clemino, M.; Dulieu, F.; Baumgärtel, H.; Leach, S. *Chem. Phys.* **2004**, *298*, 279.

(18) Lago, A. F.; Coutinho, L. H.; Marinho, R. R. T.; Naves de Brito, A.; De Souza, G. G. B. *Chem. Phys.* **2004**, *307*, 9.

(19) Messer, B. M.; Cappa, C. D.; Smith, J. D.; Wilson, K. R.; Gilles, M. K.; Cohen, R. C.; Saykally, R. J. *J. Phys. Chem. B* **2005**, *109*, 5375.

(20) Kumar, S.; Rai, A. K.; Singh, V. B.; Rai, S. B. *Spectrochim. Acta, Part A* **2005**, *61*, 2741.

(21) Snoek, L. C.; Robertson, E. G.; Kroemer, R. T.; Simons, J. P. *Chem. Phys. Lett.* **2000**, *321*, 49.

(22) Huang, Y.; Kenttämaa, H. *J. Am. Chem. Soc.* **2005**, *127*, 7952.

(23) Wilson, K. R.; Belau, L.; Nicolas, C.; Jimenez-Cruz, M.; Leone, S. R.; Ahmed, M. *Int. J. Mass. Spectrom.* **2006**, *249/250*, 155.

(24) Kovačevic, B.; Rožman, M.; Klansinc, L.; Srzić, D.; Maksić, Z. B.; Yáñez, M. *J. Phys. Chem. A* **2005**, *109*, 8329.

(25) Rauk, A.; Yu, D.; Armstrong, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 8848.

(26) Rega, N.; Cossi, M.; Barone, V. *J. Am. Chem. Soc.* **1998**, *120*, 5723,

(27) Rodríguez-Santiago, L.; Sodupe, M.; Oliva, A.; Bertran, J. *J. Phys. Chem. A* **2000**, *104*, 1256.

(28) Simon, S.; Sodupe, M.; Bertran, J. *J. Phys. Chem. A* **2002**, *106*, 5697.

(29) Simon, S.; Sodupe, M.; Bertran, J. *Theor. Chem. Acc.* **2003**, *111*, 217.

(30) Lu, H. F.; Li, F. Y.; Lin, S. H. *J. Phys. Chem. A* **2004**, *108*, 9233.

(31) Gronert, S.; O'Hair, R. A. J. *J. Am. Chem. Soc.* **1995**, *117*, 2071.

(32) Császár, A. G. *J. Phys. Chem.* **1996**, *100*, 3541.

(33) Stepanian, S. G.; Reva, I. D.; Radchenko, E. D.; Adamowicz, L. *J. Phys. Chem. A* **1998**, *102*, 4623.

(34) Lambie, B.; Ramaekers, R.; Maes, G. *J. Phys. Chem. A* **2004**, *108*, 10426.

(35) Noguera, M.; Rodríguez-Santiago, L.; Sodupe, M.; Bertrán, J. *J. Mol. Struct. (THEOCHEM)* **2001**, *537*, 307.

(36) Blanco, S.; Lesarri, A.; López, J. C.; Alonso, J. L. *J. Am. Chem. Soc.* **2004**, *126*, 11675.

(37) Lakard, B. *J. Mol. Struct. (THEOCHEM)* **2004**, *681*, 183.

(38) Ai, H.; Bu, Y.; Li, P.; Li, Z. *J. Chem. Phys.* **2004**, *120*, 11600.

(39) Pecul, M.; Ruud, K.; Rizzo, A.; Helgaker, T. *J. Phys. Chem. A* **2004**, *108*, 4269.

(40) Jeon, I. S.; Ahn, D. S.; Park, S. W.; Lee, S.; Kim, B. *Int. J. Quantum Chem.* **2005**, *101*, 55.

(41) Miao, R.; Jin, C.; Yang, G.; Hong, J.; Zhao, C.; Zhu, L. *J. Phys. Chem. A* **2005**, *109*, 2340.

(42) Gong, X.; Zhou, Z.; Du, D.; Dong, X.; Liu, S. *Int. J. Quantum Chem.* **2005**, *103*, 105.

(43) Pecul, M. *Chem. Phys. Lett.* **2006**, *418*, 1.

(44) Kushwaha, P. S.; Mishra, P. C. *J. Photochem. Photobiol., A* **2000**, *137*, 79.

(45) Kushwaha, P. C.; Mishra, P. C. *J. Mol. Struct. (THEOCHEM)* **2001**, *549*, 229.

(46) Simon, S.; Gil, A.; Sodupe, S.; Bertran, J. *J. Mol. Struct. (THEOCHEM)* **2005**, *727*, 191.

(47) Dehareng, D.; Dive, G. *Int. J. Mol. Sci.* **2004**, *5*, 301.

(48) Zhang, M.; Huang, Z.; Lin, Z. *J. Chem. Phys.* **2005**, *122*, 134313.

(49) Huang, Z; Yu, W.; Lin, Z. *J. Mol. Struct. (THEOCHEM)* **2006**, *801*, 7.

(50) Császár, A. G. *J. Am. Chem. Soc.* **1992**, *114*, 9568.

(51) Sodupe, M.; Oliva, A.; Bertran, J. *J. Am. Chem. Soc.* **1994**, *116*, 8249.

(52) Sodupe, M.; Oliva, A.; Bertran, J. *J. Phys. Chem. A* **1997**, *101*, 9142.

(53) Sodupe, M.; Oliva, A.; Bertran, J. *J. Am. Chem. Soc.* **1995**, *117*, 8416.

(54) Rodríguez-Santiago, L.; Sodupe, M.; Oliva, A.; Bertran, J. *J. Am. Chem. Soc.* **1999**, *121*, 8882.

(55) Gil, A.; Bertran, J.; Sodupe, M. *J. Am. Chem. Soc.* **2003**, *125*, 7462.

(56) Gil, A.; Sodupe, M.; Bertran, J. *Chem. Phys. Lett.* **2004**, *27*, 395.

(57) Tureček, F.; Carpenter, F. H.; Polce, M. J.; Wesdemiotis, C. *J. Am. Chem. Soc.* **1999**, *121*, 7955.

(58) Turečec, F.; Carpenter, F. H. *J. Chem. Soc., Perkin Trans. 2* **1999**, 2315.

(59) Chang, G.; Guida, W. C.; Still, W. C. *J. Am. Chem. Soc.* **1989**, *111*, 4379.

(60) Saunders, M.; Houk, K. N.; Wu, Y. D.; Still, W. C.; Lipton, M.; Chang, G.; Guida, W. C. *J. Am. Chem. Soc.* **1990**, *112*, 1419.

(61) Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 720.

(62) Halgren, T. A. *J. Comput. Chem.* **1999**, *20*, 730.

(63) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.

(64) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B* **1988**, *37*, 785.

(65) Stephens, P. J.; Devlin, F. J.; Chablowski, C. F.; Frisch, M. J. *J. Phys. Chem.* **1994**, *98*, 11623.

(66) Zaho, Y.; Truhlar, D. G. *J. Phys. Chem. A* **2004**, *108*, 6908.

(67) Kendall, R. A.; Dunning, T. H., Jr.; Harrison, R. J. *J. Chem. Phys.* **1992**, *96*, 6796.

(68) Reed, A. E.; Curtiss, L. A.; Weinhold, *Chem. Rev. (Washington, D. C.)* **1988**, *88*, 899.

(69) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03, Revision D.01*; Gaussian, Inc.: Wallingford, CT, 2004.

(70) MOLPRO is a package of ab initio programs written by H.-J. Werner, P. J. Knowles, R. Lindh, F. R. Manby, M. Schütz, P. Celani, T. Korona, G. Rauhut, R. D. Amos, A. Bernhardsson, A. Berning, D. L. Cooper, M. J. O. Deegan, A. J. Dobbyn, F. Eckert, C. Hampel, G. Hetzer, A. W. Lloyd, S. J. McNicholas, W. Meyer, M. E. Mura, A. Nicklass, P. Palmieri, R. Pitzer, U. Schumann, H. Stoll, A. J. Stone, R. Tarroni, and T. Thorsteinsson.

(71) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440.

(72) Sodupe, M.; Bertran, J.; Rodríguez-Santiago, L.; Baerends, E. J. *J. Phys. Chem. A* **1999**, *103*, 166.

(73) Braïda, B.; Hiberty, P. C.; Savin, A. *J. Phys. Chem. A* **1998**, *102*, 7872.

(74) Gil, A.; Simon, S.; Sodupe, M.; Bertran, J. *Theor. Chem. Acc.* **2007**, *118*, 589.

(75) Stein, S. E. NIST Mass Spectrometry Data Center, 2006.

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