How the site of ionisation influences side-chain fragmentation in histidine radical cation

Adrià Gil a,*, Sílvia Simon b,*, Mariona Sodupe a, Juan Bertrán a

a Departament de Química, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain
b Institut de Química Computacional, Departament de Química, Universitat de Girona, 17071 Girona, Spain

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Abstract

Based on MPWB1K/6-31++G(d,p) calculations, we have performed a charge and spin density analysis for the most representative structures of Hisd(+) and Hise(+) radical cations. This analysis shows that depending on the site where ionisation takes place, fragmentation of the side-chain can occur via R + elimination to give the m/z 81 peak in the 70 eV EI-MS spectrum, or via the most favoured RHþ/C5 elimination to give the most intense m/z 82 peak. The mechanism for RHþ elimination involves a proton-transfer from the amino group to the free N61 of the imidazole ring.

1. Introduction

Histidine is one of the 20 most common natural amino acids present in proteins. This aromatic amino acid has an imidazole ring at the side-chain which is able to produce tautomerism as shown in Scheme 1. These tautomers, being the N52–H tautomer more common than the N61–H one, exist in an equilibrium described by the constant KF [1]. Nitrogen atoms of the imidazole ring have different properties. One of them is bound to hydrogen, the remaining electron pair being delocalised in the aromatic ring, thus, the N–H is slightly acidic. However, the other nitrogen participates with one electron to the aromatic ring and has a free lone pair thus, being basic. Most proteins take advantage of these properties in different ways [2–7].

In a previous study [8], in which we studied the Cα–X fragmentation processes of 9 amino acids belonging to different groups (non-polar, polar, acidic, basic or aromatic) in their radical cation forms, we found that among the different Cα–X fragmentation processes, the one that leads to the loss of [COOH]+ is the most favourable one. Nevertheless, for amino acids with increasing side chain, fragmentations leading to R+ or R + start being competitive and for the aromatic amino acids Tyr and His, the fragmentation leading to R+ is the most favourable process. This fact is in very good agreement with the most intense peaks in their 70 eV EI-MS spectra [9]. However, for histidine radical cation, although the peak corresponding to R+ (m/z 81) is very intense, the most important peak in the 70 eV EI-MS spectrum is localised at m/z 82 and corresponds to the positively charged 4-methylene-1H-imidazole species (RH+). This peak has been also observed recently by Barlow et al. [10] in an elegant study in which mass spectrometry experiments by means of collision induced dissociation (CID) spectra of histidine complexes and theoretical calculations were carried out.

In this Letter, we present a charge and spin density analysis for the most representative conformers of histidine radical cation taking into account the existence of N52–H and N61–H tautomers. This analysis will allow us to understand the presence of these important peaks (m/z 81 and m/z 82) in the 70 eV EI-MS spectrum and will lead also to an interpretation of peak m/z 82 by means of proton-transfer.
2. Methods

Since amino acids can exist in a large number of conformations due to single-bond rotamers, the following strategy has been applied in order to find the lowest energy conformations of histidine amino acid. Starting from the most stable glycine conformations shown in Scheme 2 (I–IV), a Monte Carlo Multiple Minimum (MCMM) conformational search [11,12] with the MMFF94 force field [13,14] has been performed allowing only the internal rotations of the side-chain. Moreover, we also considered the N–H tautomerisation of the side-chain for the conformational search. All plausible structures within an energy window of 12 kcal mol\(^{-1}\) were selected for full geometry optimisations using the non-local meta-hybrid MPWB1K density functional approach [15] with the 6-31++G(d,p) basis set [16,17]. Radical cation structures were obtained after ionisation and reoptimisation of the minima found for each neutral conformation.

The MPWB1K density functional has been shown to perform reasonably well for this kind of systems compared to coupled-cluster single-double and perturbative triple excitation calculations [8]. Moreover, radical cations are open-shell systems, and there are some examples in the literature that show that hybrid density functionals with small percentage of exact exchange tend to overstabilise structures with a too delocalised electron hole [18,19]. Recent studies have shown that MPWB1K hybrid meta-GGA functional, which includes 44% of exact exchange, performs well for open-shell systems [20,21]. MPWB1K calculations present a low spin contamination (\(S^2\) being between 0.755 and 0.767), very similar to the widely-used B3LYP functional [8].

Thermodynamic corrections were computed assuming an ideal gas, unscaled harmonic vibrational frequencies and the rigid-rotor approximation by standard statistical methods [22]. Net atomic charges and spin densities were obtained using the natural population analysis (NPA) of Reed et al. [23] All calculations were performed with the Gaussian 03 software [24].

3. Results and discussion

As found in previous studies for different amino acids, ionisation significantly modifies the relative stability of the I–IV conformers shown in Scheme 2 [8,25,26]. For neutral systems the lowest energy structures correspond to conformers I and II whereas for ionised systems, the lowest energy ones are mainly the IV(+) structures. This is due to the fact that ionisation is produced mainly on the NH\(_2\) amino group of the backbone, which becomes flatter and increases its acidity. Consequently, the intramolecular hydrogen bonds in which NH\(_2\) acts as proton-donor are strengthened and thus, structures IV(+) are stabilised. On the other hand, structures II(+) in which NH\(_2\) acts as a proton acceptor become the least stable ones due to the decrease of basicity of NH\(_2\) upon ionisation. Nevertheless, as the side-chain increases, ionisation of the side-chain becomes important [8,26]. This is the case of aromatic amino acids as histidine, in which ionisation takes place mainly on the side-chain in most of the conformers and other kind of structures like those derived from II(+) and III(+) become competitive upon ionisation.

Table 1 shows the relative energies of the most stable structures for ionised histidine within an energy window of \(\Delta G_{298K} = 3\) kcal mol\(^{-1}\) taking into account \(\delta\) and \(\epsilon\) tautomers. Each structure has been labelled with a roman number according to Scheme 2, the letter \(\delta\) or \(\epsilon\) depending on where the NH group is localised in the imidazole ring and another natural number according to the relative Gibbs free energy order. In agreement to previous studies [8], II(+) and III(+) histidine conformers in Table 1 are
close in energy to the IV(+) ones, some of them being even more stable. This is the case of the distorted HisIII\(d\)(+)1 and HisII\(e\)(+)1, which are the absolute minima for \(d\) and \(e\) tautomers, respectively.

Fig. 1 shows the optimised geometries of the most representative \(d\) and \(e\) histidine structures with their intramolecular hydrogen bond interactions. It is observed that all His\(d\)(+) conformations have an NH…NH\(_2\) hydrogen bond between the side-chain and the NH\(_2\) group of the backbone, the basic N\(_d\) atom being very far from the backbone. Thus, structures His\(d\)(+) are not appropriate for explaining the mechanism of the RH\(^+\)/C\(_5\) elimination. On the other hand, structures His\(e\)(+) prefer 2-center-3-electron interactions mainly between the basic N\(_d\) atom of the side-chain and the different groups of the backbone. Only HisIV\(_e\)(+)2 shows a NH…N hydrogen bond between the NH\(_2\) group of the backbone and the basic N\(^{61}\) of the side-chain. This conformation could easily evolve through a proton-transfer or a hydrogen-atom abstraction \([10]\) from the backbone to the side-chain. Moreover, as observed in Fig. 1, this latter hydrogen bond is stronger than the formers for His\(d\)(+) (1.670 Å for HisIV\(_e\)(+)2 vs. 2.076, 1.934, 1.899 and 1.919 Å for the rest of His\(d\)(+) structures), even though in His\(d\)(+) structures some cooperative effects can be expected \([27]\). All these hydrogen bond interactions as well as 2-center-3-electron ones have been corroborated by AIM theory \([28]\).

Table 2 shows the charge and spin density for each functional group of the most important histidine conformations and the different groups of the backbone.

### Table 1

<table>
<thead>
<tr>
<th>System</th>
<th>Energy (kcal mol(^{-1})) with respect to HisIII(d)(+)1 conformation at MPWB1K/6-31++G(d,p) level of calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(\Delta E)</td>
</tr>
<tr>
<td>HisIII(d)(+)1</td>
<td>0.0 (0.2)</td>
</tr>
<tr>
<td>HisIV(d)(+)1</td>
<td>1.9 (1.6)</td>
</tr>
<tr>
<td>His(d)(+)1</td>
<td>2.5 (1.8)</td>
</tr>
<tr>
<td>HisII(e)(+)1</td>
<td>1.0 (1.0)</td>
</tr>
<tr>
<td>HisIV(e)(+)1</td>
<td>1.1 (0.1)</td>
</tr>
<tr>
<td>HisIII(d)(+2)</td>
<td>3.0 (2.3)</td>
</tr>
<tr>
<td>HisIV(e)(+2)</td>
<td>3.9 (0.8)</td>
</tr>
<tr>
<td>HisIV(e)(+3)</td>
<td>2.4 (1.6)</td>
</tr>
<tr>
<td>HisIII(e)(+)1</td>
<td>3.2 (0.0)</td>
</tr>
</tbody>
</table>

Values in parentheses corresponds to the B3LYP/6-31++G(d,p) ones and in this case are referred to HisIII\(e\)(+)1.

### Table 2

<table>
<thead>
<tr>
<th>System</th>
<th>NH(_2)</th>
<th>COOH</th>
<th>R</th>
<th>CH</th>
</tr>
</thead>
<tbody>
<tr>
<td>HisIII(d)(+)1</td>
<td>0.10 (0.00)</td>
<td>0.08 (0.03)</td>
<td>0.90 (0.96)</td>
<td>0.12 (0.01)</td>
</tr>
<tr>
<td>HisIV(d)(+)1</td>
<td>0.08 (0.00)</td>
<td>0.05 (0.00)</td>
<td>0.91 (0.98)</td>
<td>0.12 (0.02)</td>
</tr>
<tr>
<td>His(d)(+)1</td>
<td>0.08 (0.00)</td>
<td>0.06 (0.00)</td>
<td>0.91 (0.99)</td>
<td>0.11 (0.01)</td>
</tr>
<tr>
<td>HisII(e)(+)1</td>
<td>0.06 (0.02)</td>
<td>0.16 (0.15)</td>
<td>0.79 (0.80)</td>
<td>0.12 (0.03)</td>
</tr>
<tr>
<td>HisIV(e)(+)1</td>
<td>0.42 (0.65)</td>
<td>0.08 (0.00)</td>
<td>0.38 (0.35)</td>
<td>0.11 (0.00)</td>
</tr>
<tr>
<td>HisIII(d)(+2)</td>
<td>0.08 (0.00)</td>
<td>0.06 (0.00)</td>
<td>0.92 (0.99)</td>
<td>0.10 (0.01)</td>
</tr>
<tr>
<td>HisIV(e)(+2)</td>
<td>0.58 (0.89)</td>
<td>0.10 (0.00)</td>
<td>0.22 (0.07)</td>
<td>0.10 (0.07)</td>
</tr>
<tr>
<td>HisIV(e)(+3)</td>
<td>0.06 (0.22)</td>
<td>0.05 (0.00)</td>
<td>0.78 (0.78)</td>
<td>0.01 (0.00)</td>
</tr>
<tr>
<td>HisIII(e)(+)1</td>
<td>0.04 (0.03)</td>
<td>0.10 (0.11)</td>
<td>0.81 (0.82)</td>
<td>0.14 (0.03)</td>
</tr>
</tbody>
</table>

Values in parentheses corresponds to the B3LYP/6-31++G(d,p) ones and in this case are referred to HisIII\(e\)(+)1.

Fig. 1. Optimised geometries of the lowest energy conformers of histidine radical cations (\(d\) and \(e\) tautomers) at the MPWB1K/6-31++G(d,p) level of calculation. Distances are in Å.
and Fig. 2 shows the SOMO orbitals. For His\(\delta^{(+)}\) structures, it is observed that ionisation mainly takes place at the side-chain. On the other hand, for the His\(\varepsilon^{(+)}\) conformers, two different behaviours are obtained: for HisII\(\varepsilon^{(+)}\)1, HisIV\(\varepsilon^{(+)}\)3 and HisIII\(\varepsilon^{(+)}\)1 structures ionisation takes also place at the side-chain but for HisIV\(\varepsilon^{(+)}\)1 and HisIV\(\varepsilon^{(+)}\)2 ionisation is mainly localised at the NH\(_2\) group of the backbone. For the formers, it is possible to think in a hydrogen-atom abstraction from the amine to the side-chain. However, from the geometries (see Fig. 1), the free nitrogen atom of the side-chain appears to be far and geometry reorganisation would be necessary for such reaction. For the latters, since ionisation mainly takes place at the amino group, the hydrogen-atoms become more acidic and the process could be carried out by means of a proton-transfer reaction instead of a hydrogen-atom abstraction. Moreover, HisIV\(\varepsilon^{(+)}\)2 has the perfect orientation for the transfer, and can be obtained from HisIV\(\varepsilon^{(+)}\)1 just rotating the side-chain. Thus, in order to study the proton-transfer process which leads to the RH\(^+\)/C\(_5\) fragmentation, we will take into account HisIV\(\varepsilon^{(+)}\)2 conformer.

The calculated energy profile for the proton-transfer process and subsequent RH\(^+\) fragmentation is depicted in Fig. 3. Relative energies including entropic effects are also collected in Fig. 3. The computed mechanism involves two main steps. The first step corresponds to a proton-transfer from the NH\(_2\) group to the basic N\(_d\) of the imidazole group in order to produce a protonated-imidazole ring in the side-chain and the second step involves the fragmentation of the C\(_x\)–RH\(^+\) bond. Energy as sum of the free fragments is added at the end of the profile in Fig. 3. The final RH\(^+\) fragment has a distonic character since the charge mainly lies at the imidazole ring (0.83) whereas spin density mainly lies at the C\(_b\) (0.72).

The first step is very favoured thermodynamically, since the generated proton-transferred species His\(_{\text{transfer}}\) is stabilised by 6.7 kcal mol\(^{-1}\), in terms of Gibbs free energy, with respect to its direct precursor. Moreover, the transition state corresponding to this step has an energy barrier of only 0.6 kcal mol\(^{-1}\) from potential energies but −0.8 kcal mol\(^{-1}\) considering Gibbs free energy and thus, the step can be considered barrierless (see Fig. 3). Hydrogen bond distances for these structures are shown in Fig. 3 and it is observed that the associated transition structure is an early transition state, since the NH distance in the amino group is only 0.123 Å longer than that for the precursor HisIV\(\varepsilon^{(+)}\)2. Thus, as said above, all generated HisIV\(\varepsilon^{(+)}\)2 structures will become proton-transferred structures since such process is favoured thermodynamically as well as kinetically. NPA calculations confirmed that the transferred H species in this process has a proton nature, since the charge of this atom in the TS\(_{\text{transfer}}\) species is 0.5 and the spin density 0.0. An important consequence of this step is that Histransfer is the most stable species, becoming 4.4 kcal mol\(^{-1}\) lower in energy than the initially considered lowest-lying conformer HisIII\(\delta^{(+)}\)1. In the second and final step the fragmentation of the protonated side-chain is produced. This last step is not favoured, neither thermodynamically nor kinetically.

Comparing to the results of Ref. [10], similar energy profile is observed. However, the structure corresponding to the origin of energies has different geometry. We took into account HisIV\(\varepsilon^{(+)}\)2 as origin of energies, whereas in Ref. [10] the geometry of the structure is similar to...
our HisIIIe(+)
1. In fact, we carried out some B3LYP/6-31++G(d,p) calculations within the set of Table 1 and gave this structure as the most stable one in terms of Gibbs free energy (see Table 1). However, it is known that B3LYP tends to overestimate the stability of structures with 2-center-3-electron interactions [18] like HisIIIe(+)
1 or HisIIe(+)
1, which are the most stable structures at B3LYP level (see Table 1). Moreover, B3LYP tends to give more delocalised distribution of charge and spin density. In fact, B3LYP natural population analysis for HisIVe(+)
2 gives values of 0.33 (NH2) and 0.45 (R) for the charge and 0.51 (NH2) and 0.43 (R) for the spin density. However, the MPWB1K results are 0.58 (NH2) and 0.22 (R) for charge and 0.89 (NH2) and 0.07 (R) for the spin density, a situation that is clearly less delocalised than the one obtained with B3LYP. Consequently, according to B3LYP results the formation of Histransfer could be considered a hydrogen-atom transfer from NH2 to R instead of proton-transfer as suggested from the MPWB1K. Thus, depending on the functional used one could achieve different conclusions about the nature of the H-transferred species.

In a recent study, Sutherland et al. [29] have found that His+ structures, in which H-transfer from Cα to the imidazole ring leads to the most stable isomer of His+. These structures are stabilised by captodative effects in which the spin is delocalised on the N-Cα-COOH moiety. However, Cα-RH+ fragmentation from such structures are less favourable from a thermodynamical point of view since the fragments remain ΔG^0_{298K} = 31.8 kcal mol⁻¹ above the HisIVe(+)
2 asymptote. Moreover, since these structures are very stable, fragmentation barriers from such structures

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Fig. 3. Gas-phase electronic energy profile (kcal mol⁻¹) for the proton-transfer and subsequent fragmentation of the His(+) radical cation. Values in boldface include thermal corrections to Gibbs free energies. Distances are in Å.
are very high, energetic profile of Fig. 3 being the most probable. Another possibility would be that RH⁺ were generated via 1,2 H⁻ migration between C₆ and C₇. This would result in the 4-methyl-imidazole radical cation. Calculations indicate that this species is about ΔG₂⁹⁸K = 14.5 kcal mol⁻¹ less stable than the 4-methylene-imidazole radical cation.

As observed, RH⁺ fragmentation is a very favourable process for histidine radical cation (ΔG₂⁹⁸K = 1.7 kcal mol⁻¹), which becomes ΔG₂⁹⁸K = 4.0 kcal mol⁻¹ if the energy is referred to the lowest-lying conformer HisIIIΔ(+)1). Among the 8 different cleavages of C₅ analysed in a previous study [8], R⁺ fragmentation was the most favourable process for histidine radical cation (ΔG₂⁹⁸K = 21.6 kcal mol⁻¹ referred to HisIIIΔ(+)1) but compared to the RH⁺ fragmentation this latter is clearly favoured from a thermodynamic point of view. However, fragmentation via RH⁺ elimination needs some structural and electronic rearrangements, which require an energy barrier of 25.2 kcal mol⁻¹ (with respect to Histransfer, see Fig. 3), 20.8 kcal mol⁻¹ referred to HisIIIΔ(+)1 (see Table 1). Even though, compared to the 21.6 kcal mol⁻¹ for the R⁺ elimination barrier, fragmentation via RH⁺ is still slightly more favourable. On the other hand, formation of Histransfer seems to be possible only from Hisz(+) structures in which ionisation takes place on the NH₂ group. The rest of structures HisΔ(+) and Hisz(+) in which ionisation takes place mainly on the side-chain, the preferred fragmentation will be R⁺. Thus, one may conclude that depending on the ionisation site, either RH⁺ or R⁺ elimination will be favoured. Consequently, it is not surprising that the most important peaks in the 70 eV EI-MS spectrum of histidine radical cation are m/z 81 and m/z 82. RH⁺ fragmentation for giving the m/z 82 is clearly the most favoured process but it needs some structural and electronic requirements. Therefore, conformations without these requirements will cleave via R⁺ elimination, giving the m/z 81 peak.

4. Conclusions

In this Letter, a charge and spin density analysis was performed for the most representative conformers of histidine radical cation. Calculations were carried out at MPWB1K level after a previous conformational search taking into account the different δ and ε tautomers. The results of this analysis show that depending on the site of ionisation, which is related to the δ/ε tautomerism and intramolecular hydrogen bonds, the side-chain fragmentation of histidine radical cation can evolve via R⁺ elimination or via the most favourable RH⁺ one. Both eliminations are related to the m/z 81 and m/z 82 peaks in the 70 eV EI-MS spectrum of histidine respectively, the last one also found in CID experiments of histidine complexes and derivatives. RH⁺ elimination was found to be a process more favourable than the R⁺ fragmentation. However, formation of Histransfer from which RH⁺ is eliminated, is only possible for certain Hisz conformers for which ionisation is localised on the NH₂ group. The mechanism for such process implies a proton-transfer from the NH₂ to the Nδ¹ basic nitrogen of the imidazole ring and subsequent fragmentation of the side-chain. The choice of B3LYP functional, however, could lead to a different interpretation of the nature of the H-transfer, due to its trend to delocalise charge and spin density.

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References