

Resonant Electron Capture by Amino Acids and Peptides

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Abstract:

Resonant electron capture mass spectrometry (REC-MS) has been used to study gas-phase resonant reactions of molecules of amino acids, small peptides, and their methyl esters with nearly mono-energetic electrons over the energy range 0-12 eV. The formation and decomposition of negative ion (NI) resonant states have been examined and no long-lived molecular anions were observed from all compounds studied. The most prominent decomposition channel of the resonant interactions of amino acids with electrons is [M-H]⁻ ions occurring at the energies 1.2-1.3 eV and having a carboxylate anion structure. Depending on the electron capturing center of a particular peptide, the resonances have been established to decay into negatively-charged fragments and their neutral counterparts via the break of peptide or N-C_α bonds with localization of the charge either on C- or N-termini. With increasing size of peptides, the energetic onset of the decomposition reactions have been found to move towards lower energies.

Introduction:

Amino acids are subunits of the proteins that make up the majority of the "machinery" of living organisms and by studying low-energy interactions of electrons with isolated peptides and amino acids, it should be possible to understand the structure and functions of proteins as well as mechanisms of elementary charge transfer reactions that occur in the species. At present, there are many highly effective mass spectrometric techniques to study proteins, peptides, and amino acids that normally deal with positive ions in the compounds, but there has been only limited study of the NIs of proteins and amino acids.

Results:

Figure 1 shows the NI mass spectrum of glycine as recorded by the REC-MS and the NI mass spectrum of glycine methyl ester (Figure 2) is shown for comparison. In both spectra the formation of the carboxylate anion (*m/z* = 74) at energies 1.2-1.3 eV is the most intense peak and the effective yield curves for the NIs are shown in comparison (Figure 3), where the native amino acid and its methyl ester are depicted in blue and in red, respectively. One of the least understood NIs in amino acids is that with the nominal mass of 16, that can be either O⁻ or NH₂⁻. From comparison with earlier published data [4,5], we believe that these ions are mainly NH₂⁻ ions due to the lack of high energy resonance peaks in their yield curves that we attribute to O⁻ from water in previous work [4,5]. Table 1 shows the complete REC-MS mass spectra with possible ion formula assignment, relative intensities, and energy maximum (a) for glycine.

The REC-MS mass spectrum of phenylalanine and its methyl ester are characterized by intense NIs at two energy ranges associated with the loss of the side chains. However, the most intense ion peaks in the mass spectra are again those assigned to [M-H]⁻ and [M-Me]⁻ anions from phenylalanine (Table 2) and its ester, respectively. It is worth noting that fragment NIs generated in aromatic amino acids from resonance states associated with molecular orbitals (MO) located mainly in the carboxyl group have similar abundances and effective yield curves as their counterparts in aliphatic amino acids. The lower energy resonance (ca. 1.2 eV) is associated with a shape resonance formed by electron capture into the *n_u*^{*} MO (see Figure 3 for [M-H]⁻ and [M-Me]⁻ from glycine and Figure 4 for corresponding NIs with *m/z* 164 from phenylalanine), whereas higher energy resonance energies 2-4 eV are believed to be a Feshbach resonance generated via electron capture by electronically-excited states *n_u*^{*} or *n_u*⁺, i.e. again associated with the carboxyl group. The typical examples are fragment NIs OH⁻ and OCH₂⁻ (Figure 5) and *m/z* = 16 (Figure 6) from phenylalanine and its ester with blue being the native amino acid and red the methyl ester.

The REC-MS mass spectrum of the methyl ester of the pentamer of glycine is typical for other peptides studied. Effective yield curves of some representative NIs from the peptide are shown in Figure 7. NIs with *m/z* = 74 (depicted in pink in Fig. 7) are formed via initial generation of carboxylate anions followed by the first peptide cleavage at the C-terminus (*y*-type ions) and hydrogen attachment from its neutral counterpart. The NIs with *m/z* = 131 (blue curve) and *m/z* 186 (green curve) are analogues ions formed by the cleavage of the two following bonds. The complete REC-MS mass spectrum of the pentamer of glycine is shown in Table 3.

Figure 1: The MS of glycine summarized over the 0-12 eV energy range.

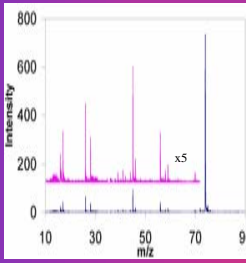


Figure 3: Comparison of relative cross-section for the (M-H)⁻ from glycine (blue) and the (M-Me)⁻ from the glycine methyl ester (red).

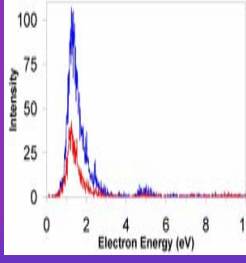


Figure 2: The MS of glycine methyl ester summarized over the 0-12 eV energy range.

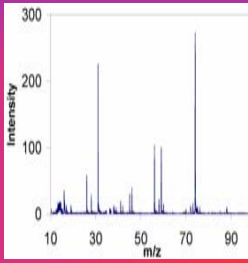


Table 1: List of peaks (*m/z*) from glycine and the possible formula (ions), relative intensity (RI), and energy maxima (E_{max}).

| <i>m/z</i> | Ion | RI (%) | E _{max} (eV) |
|------------|---|------------------|-----------------------|
| 74 | [M-H] ⁻ | 100 | 1.28 |
| 75 | [M-H] ⁻ | 5.6 | 5.35 |
| 99 | [M-NH ₂] ⁻ | 2.4 | 1.9 |
| 99 | [M-NH ₂] ⁻ | 1.1 | 1.9 |
| 58 | [M-NH ₂] ⁻ [OH] ⁻ | 0.7 | 5.5 |
| 12 | CH ₂ O | 1.2 | 5.2 |
| 46 | CH ₂ NO | 5.0 | 6.0 |
| 46 | CH ₂ NO | 1.7 | -0.1 |
| 45 | CHO | 2 | 5.5 |
| 45 | CHO | 1.2 | 2.8 |
| 45 | CHO | 0 | 5.7 |
| 44 | OH ⁻ | 1.1 | 5.7 |
| 42 | C ₂ H ₃ N/C ₂ H ₃ O/CNO | 1.3 ^a | -0.1 |
| 41 | C ₂ H ₃ N | 1.2 | 1.5 |
| 41 | C ₂ H ₃ N | 0.05 | 6 |
| 39 | C ₂ H ₃ N | 1.2 | 1 |
| 28 | CH ₂ N | 1.1 | 6.1 |
| 28 | CH ₂ N | 4.4 | 6 |
| 27 | CN | 1.9 | 1.85 |
| 16 | OH ⁻ | 0.9 | 10 |
| 16 | OH ⁻ | 4.4 | 6 |
| 16 | O ⁻ | 1.9 | 10 |
| 16 | O ⁻ | 10 | 6.2 |

^a RI means relative intensity and E_{max} means electron energy at which an ion has its maximum yield from a resonance. ^b Intensity of the ion at thermal energies (< 0.1 eV) depends on thermal decomposition of the sample. ^c Intensity of ion depends on the presence of water vapor in the sample.

Table 2: List of peaks (*m/z*) from phenylalanine and the possible formula (ions), relative intensity (RI), and energy maxima (E_{max}).

| <i>m/z</i> | Ion | RI (%) | E _{max} (eV) |
|------------|---|--------|-----------------------|
| 164 | [M-H] ⁻ | 100 | 1.16 |
| 149 | [M-NH ₂] ⁻ | 3.3 | 1.71 |
| 149 | [M-NH ₂] ⁻ | 1.1 | 5.7 |
| 148 | [M-NH ₂] ⁻ [OH] ⁻ | 1.3 | 5.5 |
| 147 | [M-NH ₂] ⁻ | 1.1 | 5.7 |
| 146 | [M-OH-R] ⁻ | 0.7 | 6 |
| 118 | [M-COOH-R] ⁻ | 2 | 6.7 |
| 105 | C ₆ H ₅ -CH ₂ - | 2 | 1.8 |
| 91 | C ₆ H ₅ -CH ₂ - | 2 | 1.7 |
| 91 | C ₆ H ₅ -CH ₂ - | 2.7 | 6.3 |
| 74 | [M-C ₆ H ₅ CH ₂] ⁻ | 1.3 | 1.05 |
| 73 | [M-C ₆ H ₅ CH ₂] ⁻ | 10.7 | 1.2 |
| 73 | [M-C ₆ H ₅ CH ₂] ⁻ | 5.3 | 6.8 |
| 46 | CH ₂ NO | 4 | -0.1 |
| 45 | CHO | 2 | 6.3 |
| 42 | C ₂ H ₃ N/C ₂ H ₃ O/CNO | 2.6 | 6.5 ^b |
| 39 | C ₂ H ₃ N | 1.2 | 6.5 |
| 28 | CH ₂ N | 1.1 | 1.5 |
| 28 | CH ₂ N | 1.2 | 5.5-6 |
| 28 | CN | 4 | 1.8 |
| 17 | OH ⁻ | 1.5 | 7 |
| 17 | OH ⁻ | 9.5 | 6 |
| 16 | O ⁻ | 4.6 | 6.6 |

Figure 5: Comparison of relative cross-section for the ion *m/z* = 16 from phenylalanine (blue) and from the phenylalanine methyl ester (red).

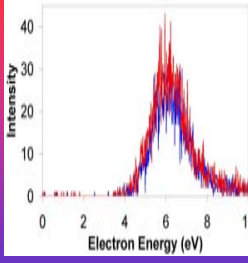


Figure 4: Comparison of relative cross-section for the (M-H)⁻ from phenylalanine (blue) and the (M-Me)⁻ from the phenylalanine methyl ester (red).

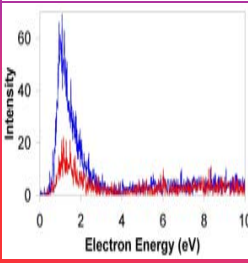


Figure 6: Comparison of relative cross-section for the (OH)⁻ from phenylalanine (blue) and the (OMe)⁻ from the phenylalanine methyl ester (red).

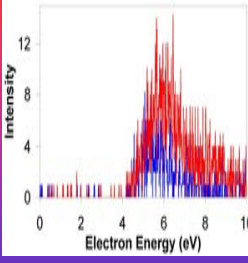


Figure 7: Comparison of relative cross-section for the (M-H)⁻ from glycine (blue) and the (M-Me)⁻ from the glycine methyl ester (red).

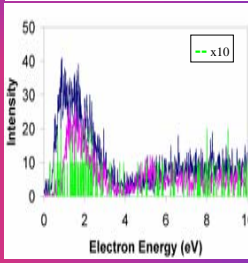


Table 3: List of peaks (*m/z*) from the peptide pentaglycine methyl ester and the possible formula (ions), relative intensity (RI), and energy maxima (E_{max}).

| Pentamer of glycine methyl ester: <i>m/z</i> = 101 | | | |
|--|--------|------------|-----------------------|
| <i>m/z</i> | RI (%) | <i>m/z</i> | E _{max} (eV) |
| 209 | 100 | 0.7 | 1.8 |
| 231 | 2.9 | 1.3 | 1.8 |
| 208 | 0.2 | 1.8 | 4.8 |
| 188 | 2.4 | 1.8 | 4.2 |
| 170 | 10.6 | 1.2 | 1.8 |
| 169 | 5.2 | non clear | 1.8 |
| 168 | 4.2 | 1.2 | 1.7 |
| 167 | 4.2 | 1.2 | 1.7 |
| 165 | 10.6 | 1.4 | 2.7 |
| 151 | 2.8 | -0.1 | 1.8 |
| 146 | 1.9 | 1.9 | 1.8 |
| 145 | 6.9 | -0.1 | 1.8 |
| 145 | 3.1 | 1.9 | 4.6 |
| 133 | 1.2 | 1.4 | 2.1 |
| 132 | 11.8 | 1.7 | 4.5 |
| 131 | 109 | 1 | 4.4 |
| 130 | 5.1 | 1.8 | 4.2 |
| 129 | 1.2 | 1.8 | 4.2 |
| 127 | 3.5 | non clear | 4.1 |
| 119 | 7.6 | 1.8 | 31 |
| 114 | 10 | 5.4 | 7 |
| 113 | 10.6 | 1.8 | 28 |
| | 13.0 | 5.6 | 26 |
| | 7.1 | 7.1 | 18.4 |
| 110 | 5.2 | 1.7 | 13.9 |
| 88 | 3.5 | 0.8 | 9.7 |
| 40 | 4.0 | 5.4 | 16 |
| 37 | 1.9 | 1.8 | 11.1 |
| | 3.8 | 5.4 | 11.1 |

^a RI means relative intensity and E_{max} means electron energy at which an ion has its maximum yield from a resonance. ^b Intensity of the ion at thermal energies (< 0.1 eV) depends on thermal decomposition of the sample. ^c Intensity of ion depends on the presence of water vapor in the sample.

Conclusions:

1. Methyl esterification does not dramatically affect the electronic structure of the parent negative ions of amino acids. Aliphatic amino acids and their methyl esters form a temporary resonant state over the energy range 1-2 eV associated with the electron attachment into *n_u*^{*} molecular orbital (shape resonance). An additional resonant state which shows up in the dissociative electron attachment spectra through a variety of fragment anions observed is Feshbach resonance also associated with the carboxyl group of the compounds when the electron capture process is accompanied by *n_u*^{*} or *n_u*⁺ excitation. Aromatic amino acids and their esters are characterized by the presence of lower energy resonant states originating in the aromatic rings.

2. The main difference in resonant electron capture spectra of amino acids and their methyl esters is the dramatic decrease in cross section formation of the [M-Me]⁻ fragment anions in esters with respect to the [M-H]⁻ anions in amino acids. However, both of these ions are formed from the same resonance located in the energy range 1-2 eV and have carboxylate anion structures.

3. Increasing peptide size results in a change of the character of fragmentation pathways of parent negative ions over the energy range 1-2 eV when amino acids associated with the peptide bond cleavage become more intense with respect to the decay processes associated with the carboxyl group. Depending on the electron-capturing center, the peptide fragmentation can favor the production of either C- or N-terminal negative ions.

Methods:

Unmodified amino acids and peptides were purchased from Sigma Aldrich (St. Louis, MO) and derivatized to their methyl esters by Fischer esterification. The compounds were volatilized by heating in a sealed inert probe to form a molecular beam that was directed into the ionization chamber of a time-of-flight REC-MS spectrometer (ToF-REC-MS) [7]. The ToF-REC-MS produces a near mono-energetic electron beam with an energy resolution of ± 80 meV (electron current is ca. 10 nA) that is ramped in the energy range from 0 to 15 eV. The electron and molecular beams are crossed at right angle to produce NIs that are extracted and analyzed by a ToF mass analyzer and 3D data are generated (intensity vs. *m/z* vs. electron energy).

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