Ion Fragmentation

DOI: 10.1002/anie.200703327

Combined Experimental and Theoretical Study on the Nature and the Metastable Decay Pathways of the Amino Acid Ion Fragment $[M-H]^-$**

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Electron attachment to molecules in the gas phase, clusters, and condensed phase has been studied on a multitude of molecular systems in recent decades.[1] The majority of these systems are halogenated compounds, for which studies were originally triggered by their use as gas-phase insulators, their role in plasma etching, and as pollutants in the atmosphere. A number of non-halogenated compounds have also been studied and in recent years the attention has moved towards biologically relevant molecules.[2] The main reason for this interest in low-energy-electron interaction with biologically relevant molecules is the increasing amount of ionizing radiation we are exposed to in modern society. The interaction of ionizing radiation with living organisms is complex, and though the direct damage through radiation of sufficient energy may be lethal to the cell, the production of secondary reactive species along its track is believed to play the dominant role in radiation damage.[3] One of the most abundant of these secondary species are electrons with kinetic energies below 20 eV.[4] These electrons may react with molecules by dissociative electron attachment (DEA), causing fragmentation directly along a repulsive state, or the fragmentation may be preceded by considerable redistribution of the excess energy or even through rearrangement within the molecular anion. Such predissociation processes are commonly associated with an initial occupation of a π^* orbital, as has been observed for the biologically relevant amino acids.[5–7]

Though DEA has been studied on a large variety of molecules, little attention has been given to secondary processes, that is, the further (metastable) decay of the anionic fragments. In a recent DEA study on valine, it was shown that the most abundant product is $[Val-H]^-$, the ion formed by loss of hydrogen as H· from the molecular ion.[6] This ion is observed over two distinct resonances; the first at 1.2 eV is assigned to a temporary occupation of the $\pi^*_{C=O}$ orbital, and the second, at about 5.3 eV, is assigned to be a core-excited resonance.[6] The same observation has been made for the other aliphatic amino acids glycine and alanine.[5,7,8] These studies do not, however, reveal the nature of these anions, that is, from where within the molecule the hydrogen loss takes place, nor do they reveal any information on the stability of these anions with respect to further dissociation.

Herein we present three different experimental approaches as well as ab initio molecular dynamics calculations to reveal the nature of $[Val-H]^-$ and the pathways of its decay. We compare the metastable decay of this anion formed upon DEA through the different resonances with collision-induced dissociation (CID) of the same anion and the metastable decay of the $[Val-H]^-$ fragment formed by deprotonation of the neutral molecule in matrix-assisted laser desorption ionization (MALDI).

Metastable decay upon DEA was measured on a double-focusing sector-field mass spectrometer (Innsbruck).[9] The molecular beam is crossed with an electron beam of 1 eV resolution. Metastable dissociation in the field-free region between the magnetic and electric sector and the resulting kinetic energy release distribution (KERD) is investigated by mass-analyzed ion kinetic energy scans (MIKE).[10] The parent anion passes the magnetic sector in about 14 μs after its formation and needs another 13 μs to cross the field-free region. CID is performed by collision of the mass-selected ions with stagnant N_2 gas in the field-free region. The kinetic energy in the center of mass is about 500 eV.

Metastable decay of the ions formed by deprotonation was determined with a reflectron-type UV-MALDI-TOF instrument RFLEX IV (Bruker, Germany; measured at the University of Iceland). All experiments were carried out in post-source decay mode (PSD) as described in detail elsewhere.[11] The ions are formed by MALDI of valine embedded in a 4′,6-diamidino-2-phenylindole matrix. After a linear flight of about 13 μs the ions are reaccelerated with an ion mirror to acquire mass spectra of their fragmentation products.

Figure 1 compares a MIKE scan of $[Val-H]^-$ formed upon DEA to valine through the higher-energy resonance with the metastable decay spectrum of deprotonated valine

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[**] This work has been supported by The Icelandic Centre for Research (RANNIS), the FWF, Wien and the EU-Projekt ITS-LEIF. H.D.F. acknowledges support for a visit to Innsbruck from the COST Action P9 (Radiation Damage in Biomolecular Systems; RADAM).

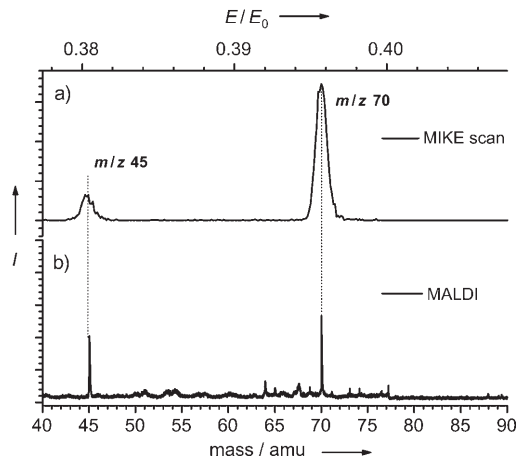

Figure 1. Comparison of the metastable decay pathways of $[Val-H]^-$ when formed a) through DEA (MIKE scan) and b) through MALDI.

formed through MALDI. In both cases, two distinct fragments are observed; the first at 45 amu, the second at 70 amu. We assign the first to the carboxyl group; however the constitution of the second can not be unambiguously identified. The different intensity ratios in the MALDI and the MIKE scan result from the different timeframes of the instruments and the actual lifetime distribution of the parent ion with regards to the different fragments. The small additional MALDI peaks arise from the instrument and from secondary matrix fragments.

Figure 2 shows an electron energy scan of the $[Val-H]^-$ formation upon DEA and the electron energy dependence of the metastable decay of this anion to form the *m*/*z* ratios 45 and 70.

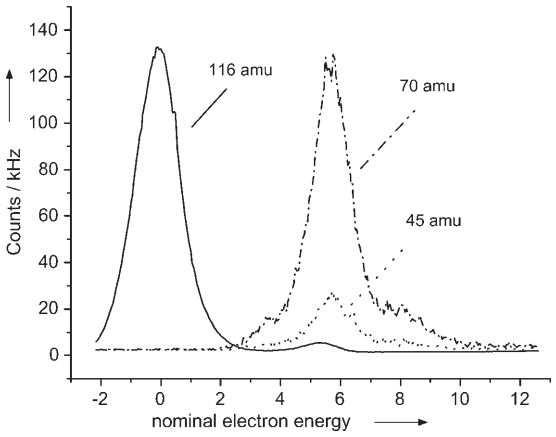

Figure 2. Electron energy scan of $[Val-H]^-$ formation through DEA and the formation of the metastable decay products *m*/*z* 45 and 70.

It is clear from Figure 1 and 2 that a) the metastable decay of $[Val-H]^-$ within this timeframe is independent of the initial formation of the precursor ion $[Val-H]^-$, that is, the ion has no recollection of whether it was formed by neutral hydrogen loss upon DEA or by deprotonation in the MALDI process, and b) metastable decay of the precursor ion $[Val-H]^-$ proceeds only through the higher energy resonance. The KERD for both fragments are of the usual Maxwell–Boltzmann form with the average being 34.0 and 16.5 meV for *m*/*z* 45 and 70, respectively. From the KERD and the fact that the ion formation is independent of the origin of the anion, it is clear that the metastable decay of $[Val-H]^-$ may be understood as a statistical reaction, with randomization of the excess energy E^* over all vibrational degrees of freedom.

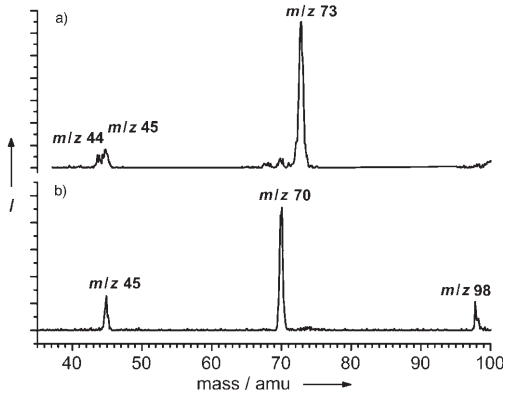

Figure 3. CID spectra of $[Val-H]^-$ when the parent ion is formed through a) the lower energy resonance, and b) the higher energy resonance.

Figure 3 shows CID spectra of $[Val-H]^-$ when the parent ion is formed through the lower- and the higher-energy resonance. For a statistical reaction, the same fragmentation pattern would be expected for both resonances as is observed in metastable decay of the higher-energy resonance and eventually some new fragments. This is the case for the higher-energy resonance, for which CID leads to the same fragments as observed from the metastable decay of the parent ion and additionally to the fragment *m*/*z* 98. CID of the low-energy resonance, on the other hand, leads to a totally different fragmentation pattern. The dominant fragment is *m*/*z* 73 which most likely corresponds to the loss of the side chain, but also the fragment *m*/*z* 44 and 45 are formed. For a statistical process this should not be the case, unless the precursor ions from the low-energy resonance are different in nature from those formed at higher energies.

The theoretically estimated value of the energy threshold for H· abstraction from the hydroxy group and the α carbon is 1.04 eV and 2.20 eV, respectively.[6] At the same level of theory we calculate the threshold for the abstraction from the amino group to be 2.85 eV. Thus the low-energy resonance is expected to constitute the hydrogen loss from the hydroxy group. The higher-energy resonance on the other hand, may constitute the hydrogen loss from the α carbon or from the amino group as well. To try to identify possible fragmentation channels for the three different precursor anions, if formed through the higher-energy resonance, we have conducted molecular dynamics (MD) simulations of the dissociation process, assuming the ion is stable enough to reach the thermally equilibrated ground electronic state before frag-

mentation. The calculations where carried out using density functional theory with the PW91 functional and the VASP code.[12,13] First, a geometric energy minimization of the three different valine anions was carried out. The lowest-energy structures were then heated to 400 K to account for the sublimation temperature in the DEA experiments. Ten geometric snapshots were then taken for each ion and to account for the initial electron energy an internal energy of 5 eV was added to the system by scaling the atomic velocities. Constant energy trajectories were then calculated for up to one ps. Of the ten trajectories simulated for each of the three ions, no fragmentation was observed if the hydrogen had been removed from the hydroxy group or the α carbon atom. However, if the hydrogen had been removed from the amino group, fragmentation leading to the formation of $COOH^-$ was observed in eight of the ten trajectories. In the remaining two, the ion was stabilized by proton transfer from the hydroxy to the amino group.

This behavior is consistent with our experimental observations and indicates that the DEA fragments $[Val-H]^-$ are stable towards further dissociation if the hydrogen extraction proceeds from the hydroxy group or the α carbon atom, but may fragment further if the hydrogen abstraction proceeds from the amino group. Considering the resonant nature of the DEA process, it is reasonable to presume that only one distinct fragment ion is formed through each resonance. To verify this hypothesis, we have carried out a DEA study on *N*,*N*-dimethylglycine, from which hydrogen abstraction from the amino group is blocked. In contrast to glycine, which behaves very similarly to valine with regards to DEA, *N*,*N*-dimethyl-glycine shows near-quantitative quenching of the higher-energy resonance.

In conclusion, we have assigned the two dehydrogenation resonances generally observed in DEA to amino acids to the selective hydrogen loss from specific sites. The dominant low-energy resonance at about 1 eV represents hydrogen loss from the hydroxy group while the high-energy resonance around 5–6 eV leads to the hydrogen loss from the amino group. Furthermore, $[Val-H]^-$ formed by hydrogen loss from the amino group through DEA at 5.3 eV undergoes further decay in the metastable timeframe, leading to two lower-mass anions. These are the same fragments we observe for $[Val-H]^-$ formed through deprotonation in MALDI, showing that the molecular ion loses recollection of its formation mechanism. The experimental results are in excellent agreement with our ab initio MD calculations, showing that the secondary fragmentation channels we observe upon DEA and in MALDI are due to an initial hydrogen abstraction from the amino group, followed by further fragmentation into $COOH^-$ after thermal equilibration.

Received: July 24, 2007
Published online: September 14, 2007

Keywords: amino acids · mass spectrometry · metastable compounds · molecular dynamics

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