Isomeric effects in ion-induced fragmentation of α- and β-alanine

P Sobocinski1, S Bari1, J Postma1, F Alvarado1, R Hoekstra1, B Manil2, J Rangama2, V Bernigaud2, B A Huber2 and T Schlathölter1

1 KVI, Rijksuniversiteit, Zernikelaan 25, 9747AA Groningen, The Netherlands
2 CIRIL-GANIL, CEA-CNRS-ENSICaen-UCBN,14070 Caen Cedex 05, France

E-mail: sobocinski@kvi.nl

Abstract. We have investigated the dissociation of α- and β-alanine following impact of slow multicharged ions, namely He+, He2+, O5+ and Xe20+ at 10 keV per charge unit. The collision products were analyzed using a reflectron-type time-of-flight mass spectrometer. In general, for a given projectile, ionization of both alanine-isomers gives rise to similar fragmentation patterns. However, significantly different peak intensities are observed for fragments with mass over charge ratios (m/q) of 30 and 44 amu. These differences can be explained in terms of isomer geometry and fragment stability. By analyzing the fragment kinetic energies, we found that protons as well as ionic oxygen fragments are sufficiently energetic (up to 200 eV for Xe20+) to induce further damage in a biological environment.

1. Introduction

When an ionizing particle interacts with human tissue, along its track it generates a large amount of secondary ions and electrons. These secondary particles in turn can interact with the surrounding biological medium. The action of the secondary particles can thus lead to formation of double strand breaks or even clustered damage in the surrounding DNA molecules, inducing severe biological damage [1]. Up to 70% of the total damage created in the tissue can be due to the action of secondary electrons or ions [1].

Low energy electrons are the most abundant secondary species. They are known to induce single and double strand breaks in DNA [2,3] mainly via dissociative electron attachment to nucleobases [4-8]. Degradation processes of DNA by secondary ions remain more ambiguous. However, Lacombe et al have investigated single and double strand break formation in plasmid DNA by keV Ar+ impact [9]. A very recent study shows that damage to plasmid DNA induced by slow carbon ions depends on both the kinetic and potential energies of the ion [10]. In addition, ions with kinetic energies as low as 10 eV can induce strand breaks in plasmid DNA [11,12].

In this context, amino-acids are relevant since they are the building blocks of the histone proteins, around which the DNA is wound. These histones and therefore also amino acids can be regarded as “sources” for radiation induced generation of secondary particles. The study of amino-acid ionization and dissociation is of importance for biological radiation damage. Several studies have been devoted to electron and photon-induced fragmentation of amino-acids by mass spectrometric techniques. For instance, in the 6-22 eV photon energy range, fragmentation patterns, ionization energies and ion appearance energies were reported for several amino-acids [13] and compared to results of electron
impact ionization [14]. However, to our knowledge ion-induced fragmentation of amino acids has not been studied before.

In this paper, we discuss the fragmentation dynamics of two isomers of alanine occurring after collisions with $\text{He}^+$, $\text{He}^{2+}$, $\text{O}^+$ and $\text{Xe}^{20+}$ ions at 10 keV/q. The two main questions, to be clarified, are: i) How does the isomer geometry affect the fragmentation process? ii) Can the produced fragments cause subsequent biological damage?

In the following, we will first compare the mass spectra obtained for both isomers and in the second part we will discuss the issue of fragment kinetic energies.

2. Experimental setup
The $\text{He}^+$, $\text{He}^{2+}$, $\text{O}^+$ and $\text{Xe}^{20+}$ ions were extracted from the KVI 14 GHz electron cyclotron resonance (ECR) ion source floating on a 10 kV potential. This way, projectile ions acquire a kinetic energy of 10 keV per charge unit. Selection of ion mass/charge ratios was accomplished by deflection of the beam in a 110° magnet. The ion beam was then chopped by means of $\approx$200 V pulses periodically applied to the chopper plates. Ion pulses of a few ns duration were generated.

In the collision chamber the base pressure does not exceed $\approx 2\times10^{-8}$ mbar. In order to keep the collision chamber free from residual gases a stainless steel plate, mounted close to the collision region, was kept at liquid nitrogen temperature and served as a cryotrap. After interaction of the ion beam with gas-phase alanine evaporated from an oven (at $\approx 150$ °C), positively charged ions were extracted by means of a static electric field ($\approx 150$ V/cm) into a reflectron time-of-flight spectrometer. The acquisition system allows measurements in a single stop mode, giving rise to conventional mass spectra, as well as in a coincidence mode. A more detailed description of the setup is given elsewhere [15,16].

3. Results and discussion
The two isomers $\alpha$- and $\beta$-alanine ($\text{C}_3\text{H}_7\text{NO}_2$) differ by the position of the amino ($\text{NH}_2$) group (Figure 1). In $\alpha$-alanine this group is attached to the middle carbon atom, while it is bound to the end of the carbon chain in $\beta$-alanine. In the case of $\beta$-alanine, the nitrogen and the three carbon atoms form a single chain.

Figure 2 displays mass spectra of $\beta$-alanine measured with $\text{He}^+$, $\text{He}^{2+}$, $\text{O}^+$ and $\text{Xe}^{20+}$ projectiles. The peak assignment follows that given by Jochims et al [13]. The mass spectra obtained with the first three projectiles are similar and exhibit three clearly defined groups of peaks corresponding to fragments containing 1, 2 and 3 heavy atoms (C, N, O) and a variable number of hydrogen atoms. In the case of the $\text{Xe}^{20+}$ projectile we observe complete disintegration into light, to a large extent even atomic fragments. Several peaks corresponding to doubly and triply charged O and C fragments appear. Note the systematic presence of a peak at $m/q = 2$ amu due to the $\text{H}_2^+$ ion, which can only be formed by fast bond rearrangement following electron removal or ionization. Even small yields of $\text{H}_3^+$ are observed. The absence of fragments with masses comparable to that of the parent molecule (89 amu) indicates that alanine loses at least three heavy atoms (for example the $-\text{COOH}$ group) when it dissociates.

Figure 1. Geometries of $\alpha$- and $\beta$-alanine.
Figure 3 shows a comparison between the mass spectra obtained for both isomers in the case of the O$^{5+}$ projectile. While being qualitatively similar on a logarithmic scale, quantitatively the spectra differ strongly for a number of fragments. A large difference is observed for the fragments at m/q = 30 amu and 44 amu, whose yields drastically change when going from α- to β-alanine (Figure 3). The formation of the NH$_2$CH$_2$+ ion (m/q = 30 amu) in the case of α-alanine requires the dissociation of two bonds and the migration of a hydrogen whereas in the case of β-alanine this fragment is easily formed by simple bond rupture [13]. The peak at m/q = 30 amu therefore dominates the spectrum for β-alanine but is suppressed for the α-isomer. The peak at m/q = 44 is attributed to simple bond rupture for both isomers. The different yields observed at m/q = 44 are explained in terms of fragment stability. Indeed, the NH$_2$CH$_3$CH+ ion in α-alanine is more stable than the diradical ion fragment NH$_2$(CH$_2$)$_2$+ originating from β-alanine dissociation [17]. Another difference is observed for the parent ion peak (m/q = 89 amu): while a small intensity is found in the case of β-alanine (for He$^{2+}$ and O$^{5+}$ projectiles), the parent ion is totally absent from the mass spectra obtained with α-alanine. This indicates a higher stability of β-alanine upon ion impact.

In the time-of-flight spectra, the peak width contains information on the fragment ion kinetic energies. Two identical fragments of mass m, emitted along the detection axis but in opposite directions, will be detected with a time difference $\Delta T$. The fragment kinetic energy $\varepsilon$ is given by [18]:

$$\varepsilon = \frac{\Delta T^2 q^2 E^2}{8m}$$

(1)

where $q$ and $E$ are the fragment charge state and the extraction field, respectively. Thus, by using the above equation the mass spectra can be transformed into fragment energy distributions. Here, the discussion will be restricted to protons, nitrogen and oxygen fragments. Heavy (polyatomic) fragments
Figure 3. Mass spectra of β-alanine (top) and α-alanine measured following 50 keV-O⁵⁺ impact. The dashed arrows located at m/q = 30, 44 and 89 amu indicate the most pronounced differences.

are usually emitted with small kinetic energies. Carbon ions stem to some extent from sites surrounded by heavy atoms, which prevents them from acquiring a significant velocity during dissociation [15].

Figure 4 shows the kinetic energies distributions for protons and oxygen fragments in the case of both isomers. Note that no differentiation is made between the different fragmentation channels: all H⁺ and O⁺ fragments are taken into account independently of the formation process. The energy distributions are found practically identical for α and β-alanine. For a given projectile the fastest fragments are protons. Similarly to the trend already observed in C⁹⁺ + Thymine collisions [19], the fragment kinetic energies increase with the projectile charge state. For instance, the proton energy distributions are peaked at 4 eV with He⁺ projectiles and at 15 eV with O⁵⁺ projectiles. In the case of Xe²⁰⁺ impact, proton and O⁺ energy distributions extend up to 200 and ~30 eV, respectively. Even with the singly charged He⁺ projectile H⁺ and O⁺ fragments with more than 10 eV are observed. This finding is of particular interest in the context of biological damage since cations with energies as low as 10 eV can induce lesions in DNA [11]. In other words, the fragments produced are fast enough to cause subsequent biological damage.

More detailed information can be obtained from the coincidence data which allow to study each fragmentation channel separately. For instance, the proton kinetic energies vary from ~8 eV for the H⁺-N⁺ fragmentation channel to ~16 eV when protons are repelled by C⁺ ions. For the same fragmentation channel kinetic energies can differ by a factor of 1.5 between α- and β-alanine. For instance, protons originating from the H⁺-O⁺ repulsion have an average energy of 15 eV in the case of α-alanine, but only 10 eV for β-alanine. Thus, the different response of the two isomers to ion impact also manifests itself in terms of fragment kinetic energies.
Figure 4. Fragment energy distribution (left column: H\(^+\) fragments, right
column: O\(^+\) fragments) following impact of He\(^+\), He\(^{2+}\), O\(^{5+}\) and Xe\(^{20+}\)
projectiles on \(\alpha\)- and \(\beta\)-alanine.

4. Conclusion

We have studied ion-induced dissociation of \(\alpha\)- and \(\beta\)-alanine by means of mass spectrometry over
projectile charge states ranging from 1 to 20. Our results are very promising in the sense that they
reveal a strong geometry-dependence of the fragmentation process. In particular, when going from \(\alpha\)-
to \(\beta\)-alanine, the mass spectra exhibit significantly different peak intensities at m/q = 30 and 44 amu.
The different behavior of the two isomers can also be observed in the fragment energy distributions.
Thus, the energy of a given fragment depends not only on the fragmentation channel but also on the
isomer geometry. However, the fragment energy distributions exhibit some common features. In
particular, O\(^+\) and H\(^+\) energies largely exceed 30 eV, which indicates that fragmentation of amino-
acids generates secondary ions which can, in turn, induce severe biological damage.

Highly charged ion impact might appear as an useful new tool for protein sequencing, since
fragmentation involves also bonds unaffected by conventional techniques. In this context, we are
planning measurements involving clusters of alanine as well as alanine embedded in water molecules
in order to investigate the influence of a biological medium on the fragmentation dynamics of a single
amino-acid.
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