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High-energy collisions of protonated enantiopure amino acids with a chiral target gas

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ABSTRACT

We have studied the fragmentation of the singly protonated L- and D-forms of enantiomerically pure phenylalanine (Phe), tryptophan (Trp), and methionine (Met) in high-energy collisions with chiral and achiral gas targets. (S)-(+)-2-butanol, racemic (±)-2-butanol, and argon were used as target gases. At center-of-mass frame collision energy of 1 keV, it was found that all of the ions exhibit common fragmentation pathways which are independent of target chirality. For all projectile ions, the elimination of NH_3 and H_2O+CO were found to be the main reaction channels. The observed fragmentation patterns were dominated by statistically driven processes. The energy deposited into the ions was found to be sufficient to yield multiple fragment ions, which arise from decomposition via various competitive reaction pathways.

1. Introduction

The current drive in pharmaceutical research has shifted toward the development of single enantiomer-based drugs rather than racemic mixture of both enantiomeric forms [1–3]. The fundamental reason for this is the highly specific nature of drug/target interactions which often depend on stereochemistry. The enantiomers of the same drug can produce different biological responses [4]. A shocking but demonstrative example of this phenomenon is the tragedy of birth defects linked with the active use of racemic thalidomide for the treatment of morning sickness in pregnant women in the late 1950s and early 1960s [5,6].

The increasing demand for optically pure pharmaceuticals calls for efficient enantioselective analytical methods to be developed. The fast and sensitive performance of mass spectrometry (MS) places it among the most promising techniques in drug analysis. However, despite significant success in the development of MS-based chiral recognition methods [7–15], many gas-phase processes which lie behind this recognition are still poorly understood.

Furthermore, these methods of chiral recognition often involve specifically modified instruments, require enantiomerically pure reference compounds, involve time-consuming building of calibration curves, and may depend on complex data analysis of fragmentation patterns. Further research efforts as well as an understanding of the ion physics and chemistry involved are urgently needed in order to overcome these limitations.

In the late 1960s and early 1970s, McLafferty and co-workers performed in-depth studies of keV collisional activation fragmentation as a function of various parameters [16]. From these studies, it was concluded that the nature of the collision gas has little influence on the product fragment intensity ratios [16,17]. Though, in recent studies with other molecules, the nature of the collision gas was observed to influence the fragmentation [18–20]. Chiral projectile ions, however, were not studied in high-energy collision experiments with chiral target gases.

Here we report the results of high-energy collisional activation of ions of enantiomerically pure amino acids with chiral and achiral target gases. Due to its suitable vapor pressure and commercial availability in enantiopure form, 2-butanol was selected to be used as a volatile chiral target molecule in our measurements. Among the additional reasons for choosing this particular gas was its successful utilization as a volatile chiral dopant for enantioselective separations by ion mobility spectrometry [21,22]. The mechanism of enantioselectivity that lay behind those gas-phase chiral discriminations is still not fully understood.

This study represents, therefore, the first examination performed by our group directed to the understanding of the ion chemistry and physics behind gas-phase chiral separations based

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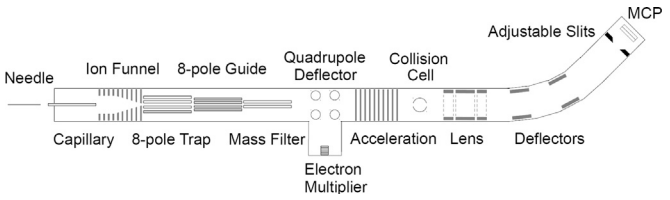


Fig. 1. Sketch of the experimental apparatus used in the experiments.

on the use of a chiral target gas. The possibility of stereochemically dependent interactions with keV activation under single collision conditions was addressed in this particular study. A separate manuscript devoted to eV collisions of enantiomeric ions and molecules is under preparation.

2. Experimental

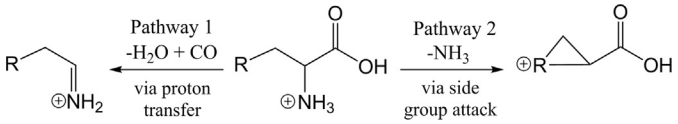
2.1. Electrospray ion source platform

An electrospray ion source platform, developed and constructed at the Department of Physics at Stockholm University [18], was used in this investigation. A sketch of the experimental apparatus is shown in Fig. 1. In every measurement, a continuous molecular ion beam of protonated amino acids was generated in an electrospray ion source. After ionization, the ions entered the vacuum system through a heated capillary and were focused in a radio-frequency ion funnel, mass selected in a quadrupole mass filter, and accelerated to the intended collision before entering a collision cell containing the target cell. The typical pressure-range in the 4-cm-long collision cell during the experiments was 0.5–1 mTorr. After passing through the cell, horizontal deflector plates and a cylindrical lens were used to analyze the primary molecular ion beam and the fragments according to their kinetic energy to charge ratio by guiding them to a position-sensitive microchannel plate detector. The position on the detector and the corresponding voltages on the deflector plates were used to obtain the fragment mass distribution.

2.2. Collision gases and reagents

The L- and D-forms of enantiomerically pure tryptophan (Trp), phenylalanine (Phe), and methionine (Met) were supplied by Sigma–Aldrich; their structures are shown in Scheme 1. Purity of all the amino acids was ≥98%. The chiral target (S)-(+)-2-butanol (99%) and the racemate of (±)-2-butanol (99.5%) were purchased from Sigma–Aldrich. Methanol (>99.8%) and acetic acid (>99.8%) were obtained from Fluka. Finally, argon (99.998%) was purchased from Air Liquide.

Stock solutions of all of the amino acids were prepared at a concentration of 0.05 M in a solvent mixture – methanol:water:acetic acid (49:49:2). Just prior to the experiments, the stock solutions were diluted to a concentration of 0.005 M using the same solvent system. The sample solutions were introduced into electrospray source by using a syringe pump (Harvard Apparatus) at a typical flow rate 1.5 μL/min. A stable electrospray of protonated amino acid ions was achieved in the positive ion mode under the following instrument settings: needle voltage, 3.5–4.8 kV; capillary voltage, 200–300 V; capillary temperature, 110 °C. Each new measurement was preceded by a thorough cleaning of the injection system using the above mentioned solvent mixture.



Scheme 2. Main fragmentation pathways for protonated amino acids (R-side chain group).

3. Results and discussion

3.1. High-energy collisional activation

In this work, we examined the influence of the nature of the collision gas (chiral, achiral) on the dissociation yield of different fragmentation products resulting from the dissociation of enantiopure amino acid ions following 1 keV collisions (center-of-mass frame).

The collision induced dissociation (CID) spectra measured after collisions of the protonated amino acids with argon, S-(+)-2-butanol and (±)-2-butanol are discussed in Sections 3.2–3.4. In all spectra, the intensity of the parent ion is normalized to 1.0 and the intensity ratios of individual fragments are determined with respect to the parent ion.

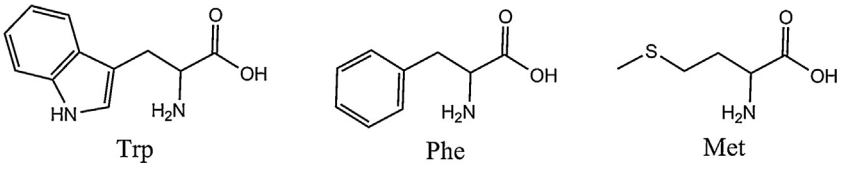
At present, two major concepts are used to explain the fragmentation mechanisms of protonated amino acid ions: the mobile proton model [23,24] and the model involving side chain groups for facilitation of cleavage reactions [24,25] (Scheme 2). Nearly all our CID results are consistent with these models and could be rationalized by them.

3.2. $PheH^+$ in collisions with Ar, (S)-(+)-2-butanol, and (±)-2-butanol

The CID pattern of protonated phenylalanine colliding with Ar is shown in Fig. 2a. Our results agree with previous reports [26–29]: the most common fragment appears at $m/z = 120$ and corresponds to the iminium ion, formed by the loss of H_2O and CO from the protonated parent. Following the work by Shoeib et al. [26], it is currently believed that this fragment is due to H_2O and CO loss (Scheme 2, Pathway 1) rather than the loss of dihydroxycarbene, as was suggested earlier. The iminium ion is typically observed in the fragmentation of singly protonated amino acids [26–29].

The significant abundances of product ions other than iminium indicate that a collision energy of 1 keV (center-of-mass frame) is high enough for the initiation of competing fragmentation channels. The reaction competing with proton transfer and the combined release of $H_2O + CO$ is the loss of NH_3 (Fig. 2a, $m/z = 149$; Scheme 2, Pathway 2). Decay of the fragment ions formed after NH_3 elimination was observed to proceed via the loss of H_2O ($m/z = 131$), $H_2O + CO$ ($m/z = 103$), etc.

The fragmentation patterns of L- and D-Phe after collisions with racemic and enantiomerically pure 2-butanol are plotted in Fig. 2b and c, respectively. Comparison among the spectra plotted



Scheme 1. Structures of the amino acids used in the collision induced dissociation study.