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CHIRALITY

Enantioselective Collision-Activated Dissociation of Gas-Phase Tryptophan Induced by Chiral Recognition of Protonated L-Alanine Peptides

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Abstract Enantioselective dissociation in the gas phase is important for enantiomeric enrichment and chiral transmission processes in molecular clouds regarding the origin of homochirality in biomolecules. Enantioselective collision-activated dissociation (CAD) of tryptophan (Trp) and the chiral recognition ability of L-alanine peptides (L-Ala_n; n = 2–4) were examined using a linear ion trap mass spectrometer. CAD spectra of gas-phase heterochiral H^+(D-Trp)(L-Ala_n) and homochiral H^+(L-Trp)(L-Ala_n) noncovalent complexes were obtained as a function of the peptide size n. The H_2O-elimination product was observed in CAD spectra of both heterochiral and homochiral complexes for n = 2 and 4, and in homochiral H^+(L-Trp)(L-Ala_3), indicating that the proton is attached to the L-alanine peptide, and H_2O loss occurs from H^+(L-Ala_n) in the noncovalent complexes. H_2O loss did not occur in heterochiral H^+(D-Trp)(L-Ala_3), where NH_3 loss and (H_2O + CO) loss were the primary dissociation pathways. In heterochiral H^+(D-Trp)(L-Ala_3), the protonation site is the amino group of D-Trp, and NH_3 loss and (H_2O + CO) loss occur from H^+(D-Trp). L-Ala peptides recognize D-Trp through protonation of the amino group for peptide size n = 3. NH_3 loss and (H_2O + CO) loss from H^+(D-Trp) proceeds via enantioselective CAD in gas-phase heterochiral H^+(D-Trp)(L-Ala_3) at room temperature, whereas L-Trp dissociation was not observed in homochiral H^+(L-Trp)(L-Ala_3). These results suggest that enantioselective dissociation induced by chiral recognition of L-Ala peptides through protonation could play an important role in enantiomeric enrichment and chiral transmission processes of amino acids.

Keywords Enantiomeric enrichment · Chiral transmission · Proton transfer · Fragmentation · Mass spectrometry

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Introduction

Polypeptides consist of L-amino acids linked through peptide bonds, and can recognize chiral molecules with high sensitivity in proteins. For example, one enantiomer of a chiral drug is highly toxic, while the other is medically effective. The origin of homochirality in biomolecules and the chiral recognition phenomena are key problems in science, and a number of hypotheses for the origin of life have been proposed regarding molecular evolution (Bonner 1991).

Miller synthesized amino acids from simple compounds under conditions that simulated primitive Earth's atmosphere (Miller 1953). Amino acids were formed by ultraviolet (UV) irradiation of interstellar molecules condensed on a cold surface, suggesting an abiotic formation of amino acids under extraterrestrial conditions (Bernstein et al. 2002; Caro et al. 2002). Dipeptides were also formed in interstellar model ices (Gontareva et al. 2009; Kaiser et al. 2013). However, no enantiomeric enrichment was observed.

A hypothesis for the extraterrestrial origin of biomolecules has been proposed on the basis of several experimental and analytical studies, in which biological molecules were formed in interstellar space and reached Earth (Meinert et al. 2011). The excess L-amino acids found in the Murchison meteorite suggest an extraterrestrial origin of homochirality (Cronin and Pizzarello 1997; Engel and Macko 1997). Observation of the circular polarization of light in star-formation regions implies that enantiomer-selective photodestruction with circular polarized light could induce the excess L-amino acids in interstellar space (Bailey et al. 1998).

The chiral preference of gas-phase serine and proline clusters has been reported using mass spectrometry (Nanita and Cooks 2006; Holliday et al. 2013). We have proposed that the reactivity of size-selected and temperature-controlled gas-phase clusters, consisting of interstellar and biological molecules, is important for understanding the chemical processes in interstellar molecular clouds. We have previously examined the UV photodissociation of temperature-controlled gas-phase tryptophan (Trp) enantiomers, both on a chiral crown ether (Fujihara et al. 2014a, 2015a) and in L-serine (L-Ser) clusters (Fujihara et al. 2014b, 2015b), as a model for interstellar molecular clouds.

Enantioselective photodissociation of gas-phase protonated tryptophan (H^+Trp) enantiomers, having NH_3^+ and COOH groups, on the chiral crown ether has been examined as a function of cluster temperature (Fujihara et al. 2014a, 2015a). The photo-induced hydrogen atom transfer preceded the C_α–C_β bond cleavage of cold H^+(D-Trp) on the chiral crown ether. Above 170 K, the C_α–C_β bond cleavage of H^+(D-Trp) was suppressed gradually with increasing temperature, and no difference in reactivity was observed between the D- and L-enantiomers of H^+Trp at 300 K. These temperatures of mass-selected gas-phase cluster ions correspond to those of interstellar to atmospheric molecular clouds. The results indicated that enantioselective photodissociation proceeds at low temperatures, and that hydrogen atom transfer plays an important role in it. The UV photodissociation study of cold gas-phase Trp enantiomers in protonated L-serine clusters H^+(L-Ser)$_m$, as a function of cluster size m, showed that when 3 L-Ser units are present in the cluster, the photolytic decomposition of Trp is enantioselective (Fujihara et al. 2014b). These enantioselective dissociations are the transmission of chirality from one type of molecule to another via photodissciation, suggesting that enantioselective photodissociation of gas-phase amino acids on chiral substances could induce enantiomeric enrichment and chiral transmission. Furthermore, we have applied enantioselective photodissociation to quantitative chiral analysis, in which the optical purity of amino acids can be determined by measuring the relative abundance ratio of two fragment ions in a single photodissociation mass spectrum (Fujihara et al. 2015a, b).

In this work, we have examined the enantioselective dissociation and chiral recognition from two viewpoints with respect to molecular evolution. One is the possibility of enantioselective collision-activated dissociation (CAD) of amino acids, as collisional activation is a universal process and enantioselective dissociation is important to formulate a hypothesis for the origin of homochirality in biomolecules. The other is the chiral recognition ability of L-alanine peptides (L-Ala$_n$), because Ala has a large helix propensity, and a helix with 3.6 residues per turn is a common short-range secondary structure (O'Neil and DeGrade 1990; Scholtz and Baldwin 1992; Myers et al. 1997; Solov'yov et al. 2006; Jarrold 2007). In order to examine enantioselective CAD of amino acids and chiral recognition of L-Ala$_n$, CAD experiments of gas-phase noncovalent complexes of Trp enantiomers with H^+(L-Ala$_n$) were performed as a function of peptide size n, using a linear ion trap mass spectrometer. This was because Trp when noncovalently complexed with the appropriate chiral substances, demonstrated enantioselective photodissociation, as previously reported by our group (Fujihara et al. 2014a, b; Fujihara et al. 2015a, b). On the basis of these results, we have discussed the relationship between enantioselective dissociation of amino acids and chiral recognition of L-alanine peptides.

Methods

D-Trp and L-Trp (purity of both being >98 %) were obtained from Sigma–Aldrich. L-Dialanine (L-Ala-Ala; L-Ala$_2$), L-trialanine (L-Ala-Ala-Ala; L-Ala$_3$), and L-tetraalanine (L-Ala-Ala-Ala-Ala; L-Ala$_4$) were obtained from Bachem. A solution containing 1 mM Trp and 0.5 mM L-Ala$_n$ was made in a mixture of water and methanol (50/50, *v*/v).

A linear ion trap mass spectrometer (LTQ XL, Thermo Fisher) with a dual conversion dynode detector was used in the CAD experiments of mass-selected ions. Gas-phase noncovalent complexes of Trp enantiomers with protonated L-alanine peptides, H^+(D-Trp)(L-Ala$_n$) and H^+(L-Trp)(L-Ala$_n$), were generated using nanoelectrospray ionization, where a voltage of 2.5 kV was applied. The ions were transferred to the gas phase through a heated capillary and ion guides. The capillary temperature and applied voltage were 200 °C and 14 V, respectively. The voltage of the tube lens was 70 V. The ions were mass-selected, dissociated, and mass-analyzed using a linear ion trap (Schwartz et al. 2002). CAD experiments using He collision gas at room temperature were carried out at an activation q_z of 0.25 and an activation time of 30 ms. Collision energies were 15 % for n = 2 and 3, and 20 % for n = 4 (normalized collision energy given by the instrument), where the supplemental AC voltages applied across the rods were below 1 V. Other instrumental parameters were multipole 1 offset of −7.6 V, intermultipole lens 1 of −9.4 V, multipole 2 offset of −9.4 V, intermultipole lens 2 of −11.8 V, gate lens of −40.0 V, multipole 3 offset of −15.5 V, front lens of −14.4 V, front section offset of −9.0 V, center section offset of −12.0 V, back section offset of −7.0 V, and back lens of −0.1 V.

Results and Discussion

Figure 1 shows the CAD spectra of heterochiral H^+(D-Trp)(L-Ala$_2$) and homochiral H^+(L-Trp)(L-Ala$_2$) (m/z 365). H^+Trp (m/z 205) and H^+(L-Ala$_2$) (m/z 161), observed in the spectra, are formed by evaporation of L-Ala$_2$ and Trp, respectively. H_2O-elimination and CH_3OH-elimination products are observed at m/z 347 and m/z 333, respectively, which are dissociation

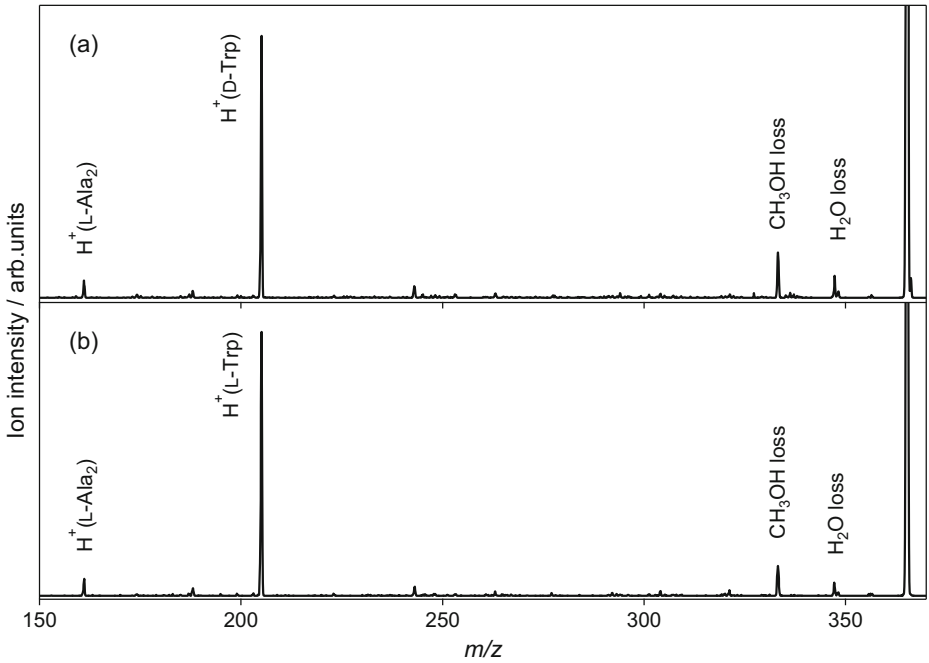

Fig. 1 Collision-activated dissociation spectra of **a** H^+(D-Trp)(L-Ala_2) and **b** H^+(L-Trp)(L-Ala_2) (*m*/*z* 365)

pathways in the noncovalent complexes. The CAD spectra at $n = 2$ show no difference between the D- and L-enantiomers of Trp.

Figure 2 shows the CAD spectra of heterochiral H^+(D-Trp)(L-Ala_3) and homochiral H^+(L-Trp)(L-Ala_3) (*m*/*z* 436). H^+(L-Ala_3), formed by evaporation of Trp, is observed at *m*/*z* 232 in both spectra. In the heterochiral complex, NH_3 loss, CO loss, and (H_2O + CO) loss are observed at *m*/*z* 419, 408, and 390, respectively, as shown in Fig. 2a. These dissociations in the complex do not occur at $n = 2$. In contrast to the heterochiral complex, H_2O loss, CO loss, and CO_2 loss are observed at *m*/*z* 418, 408, and 392, respectively, in the CAD spectrum of homochiral H^+(L-Trp)(L-Ala_3) as shown in Fig. 2b. The product ion at *m*/*z* 231, observed in the heterochiral H^+(D-Trp)(L-Ala_3), is assigned to L-$Ala_3{}^+$.

In the CAD spectra of heterochiral H^+(D-Trp)(L-Ala_4) and homochiral H^+(L-Trp)(L-Ala_4) (*m*/*z* 507) shown in Fig. 3, H_2O-elimination and CH_3OH-elimination products are observed at *m*/*z* 489 and *m*/*z* 475, respectively, as with $n = 2$ complexes. The product ion at *m*/*z* 377 is assignable to the C_α–C_β bond cleavage of Trp in the complexes.

The H_2O-elimination product is observed in the CAD spectra of both heterochiral and homochiral complexes of $n = 2$ and 4, and homochiral H^+(L-Trp)(L-Ala_3). In contrast, H_2O loss from the complex does not occur in heterochiral H^+(D-Trp)(L-Ala_3), where NH_3 loss and (H_2O + CO) loss are the main dissociation pathways in the complex, as shown in Fig. 2a. H_2O loss is the main dissociation pathway of protonated polyalanines in CAD experiments using a quadrupole ion trap mass spectrometer, in which the NH_3-elimination product is not observed (Rivera-Tirado and Wesdemiotis 2011). The dehydration takes place at the carbonyl group or amino bond of protonated polyalanines. NH_3 loss and (H_2O + CO) loss are the primary dissociation pathways in the CAD of protonated tryptophan (*m*/*z* 205), in which the H_2O-elimination product (*m*/*z* 187) is not observed (Lioe and O'Hair 2004; Aribi et al. 2004). The

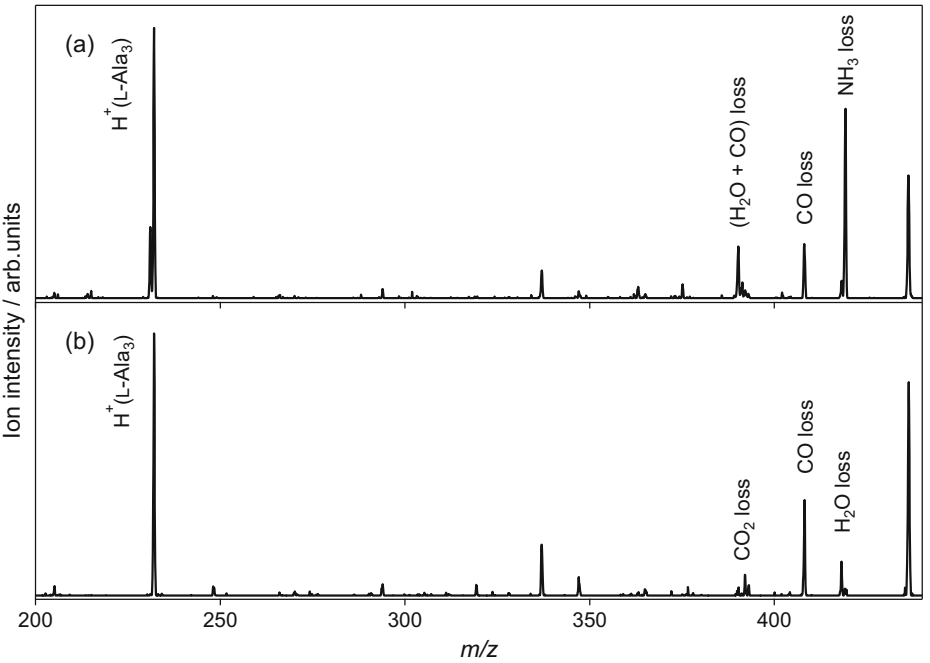

Fig. 2 Collision-activated dissociation spectra of **a** H^+(D-Trp)(L-Ala_3) and **b** H^+(L-Trp)(L-Ala_3) (*m/z* 436)

protonation site of protonated tryptophan is the amino group, and the dissociations are induced by proton transfer from the NH_3^+ group. In collisional activation and photoexcitation, CO_2 loss is the dominant dissociation pathway of non-zwitterionic amino acids that have NH_2 and COOH groups (Tao et al. 2000; Ehrenfreund et al. 2001; Fujihara et al. 2015b).

The observations of the H_2O-elimination product in the CAD spectra indicate that the proton is attached to the L-alanine peptide in both heterochiral and homochiral complexes of n = 2 and 4, and homochiral H^+(L-Trp)(L-Ala_3). H_2O loss occurs from H^+(L-Ala_n) in the noncovalent complexes. Only in heterochiral H^+(D-Trp)(L-Ala_3), the protonation site is the amino group of D-Trp, and NH_3 loss from H^+(D-Trp) is seen to occur in CAD. L-Alanine peptides recognize D-Trp through the protonation of the amino group, at peptide size n = 3. This chiral recognition through protonation could play an important role in the enantiomeric enrichment and chiral transmission processes. NH_3 loss and (H_2O + CO) loss from H^+(D-Trp) proceeds via enantioselective CAD of heterochiral H^+(D-Trp)(L-Ala_3) at room temperature. In contrast, no dissociation of L-Trp is observed in homochiral H^+(L-Trp)(L-Ala_3). The enantiomeric excess of L-Trp via enantioselective CAD of D-Trp induced by the chiral recognition of protonated L-Ala_3 in the experimental condition is 30 %, which was derived from the ion intensities shown in Fig. 2. The enantioselective CAD of gas-phase Trp is not a real prebiotic start-up scenario for enantiomeric enrichment due to the presence of L-Ala_3. It is an accidental chiral effect, where the chiral influence involves the transmission of chirality from one type of molecule to another via CAD. This type of chiral transmission was also observed in UV photodissociation, as mentioned in the introduction, in which the intramolecular hydrogen atom transfer precedes the enantioselective photodissociation at low temperature (Fujihara et al. 2014a).

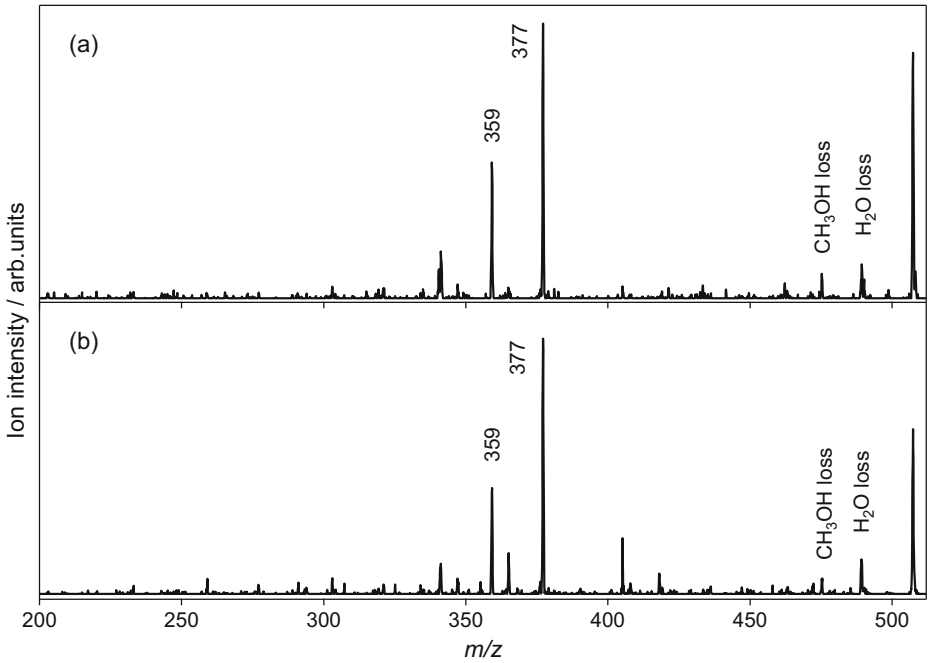

Fig. 3 Collision-activated dissociation spectra of **a** H^+(D-Trp)(L-Ala_4) and **b** H^+(L-Trp)(L-Ala_4) (*m/z* 507)

In the preliminary CAD experiments for n = 5 and 6, H_2O-elimination and CH_3OH-elimination products were observed for both heterochiral and homochiral complexes, as is shown for n = 4 (Fig. 3). NH_3 loss did not occur in either hetero- or homochiral complexes having n = 5, or in homochiral H^+(L-Trp)(L-Ala_6). In the CAD spectrum of heterochiral H^+(D-Trp)(L-Ala_6), the NH_3-elimination product was observed at one-third of the ion intensity of the H_2O-elimination product. The observation of both H_2O loss and NH_3 loss in the CAD of heterochiral H^+(D-Trp)(L-Ala_6) suggests that the proton is attached to L-Ala_6 and D-Trp in the noncovalent complex. However, it could not be determined whether there was a coexistence of isomers or if a proton share had occurred between L-Ala_6 and D-Trp in the noncovalent complex.

The geometrical structures, in which the proton is attached to the amino group of D-Trp, could be stabilized only in the heterochiral n = 3 noncovalent complex. The structures determined by inter- and intra-molecular hydrogen bonds in the noncovalent complex are important to understand the chiral recognition through protonation and the enantioselective CAD. To reveal the geometrical structures relating to the chiral recognition mechanism, it is necessary to perform photodissociation spectroscopy using a variable wavelength laser system and make direct comparisons with the theoretical calculations for size-selected and temperature-controlled gas-phase noncovalent complexes.

In conclusion, enantioselective CAD, induced by chiral recognition of L-alanine peptides through protonation, could play an important role in enantiomeric enrichment and chiral transmission processes of amino acids in molecular clouds. To understand the origin of homochirality in biomolecules, it is important to investigate enantioselective reactions of biological molecules in gas-phase noncovalent complexes.

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