

Enantiomer-Selective Photolysis of Cold Gas-Phase Tryptophan in L-Serine Clusters with Linearly Polarized Light

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Abstract Photostability of cold gas-phase tryptophan (Trp) enantiomers in L-serine (L-Ser) clusters at 8 K as a model for interstellar molecular clouds was examined using a tandem mass spectrometer containing a cold ion trap to investigate the hypothesis that homochirality in gas-phase Ser clusters promotes the enantiomeric enrichment of other amino acids via enantiomer-selective photolysis with linearly polarized light. In the UV excitation of heterochiral H^+ (L-Ser)₃(D-Trp), the CO₂-eliminated product in the cluster was observed. In contrast, the photodissociation mass spectrum of homochiral H^+ (L-Ser)₃(L-Trp) showed that photolysis of amino acids in the cluster did not occur due to the evaporation of L-Ser molecules. In the spectra of the homochiral H^+ (L-Ser) (L-Trp) and heterochiral H^+ (L-Ser) (D-Trp), the evaporation of L-Ser was the primary reaction pathway, and no difference between the L- and D-enantiomers was observed. The findings confirm that when 3 L-Ser units are present in the cluster, the photolytic decomposition of Trp is enantiomerically selective.

Keywords Photolysis · Amino acid · Gas-phase cluster · Enantiomeric enrichment · Homochirality

Introduction

The origin of life is one of the most interesting subjects in science, and a number of hypotheses for the origin of homochirality in biomolecules have been proposed based on several experimental and analytical studies (Bonner 1991; Ruiz-Mirazo et al. 2014). One such hypothesis is the extraterrestrial origin of biomolecules, which proposes that biological molecules were formed in the interstellar space and delivered to the Earth. This proposal was examined by studying the abiotic formation of amino acids under simulated extraterrestrial conditions and by searching for the enantiomeric enrichment of amino acids in meteorites.

Chemical processes in interstellar molecular clouds occur at low temperatures (10–100 K), at low densities (10^2 – 10^4 cm⁻³), and in intense radiation fields. Previous work has shown that

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ultraviolet (UV) irradiation of interstellar molecules such as H₂O, CH₃OH, NH₃, and HCN, condensed on a cold solid surface, formed several amino acids such as serine and alanine as racemates (Bernstein et al. 2002; Caro et al. 2002). These results suggest the possibility of the abiotic formation of amino acids under extraterrestrial conditions. However, no enantiomeric selectivity was observed.

L-Enantiomer-enriched amino acids found in the Murchison meteorites indicated an extraterrestrial origin of homochirality (Cronin and Pizzarello 1997). The observation of the circular polarization of light in star-formation regions suggested that enantiomer-selective photodestruction could induce the enantiomeric enrichment of amino acids in the interstellar space (Bailey et al. 1998; Meinert et al. 2011). On the basis of these results, a hypothesis for the extraterrestrial origin of homochirality in biomolecules was formulated, suggesting a process whereby the abiotic formation of racemic amino acids occurs in the interstellar molecular clouds, and is followed by the enantiomer-selective photodestruction of the D-enantiomer with circularly polarized light.

Laboratory studies on amino acids in the interstellar molecular cloud analogues have been limited to the condensed phase because of the difficulty in generating temperature-controlled hydrogen-bonded clusters containing biological molecules in the gas phase. To understand chemical processes in the interstellar molecular clouds, it is necessary to study the structure and reactivity of mass-selected and temperature-controlled gas-phase clusters consisting of interstellar and biological molecules. We proposed a hypothesis for the origin of enantiomeric enrichment based upon enantiomer-enriched amino acids found in meteorites (Cronin and Pizzarello 1997) and enantiomer selectivity of organic chiral molecules on surface (Soai et al. 1999; Hazen et al. 2001; Orme et al. 2001); according to this hypothesis, enantiomer-selective photolysis of amino acids on chiral substances with linearly polarized light could induce enantiomeric enrichment of L-amino acids. In a previous work, we examined UV photolysis of gas-phase amino acid–chiral molecular complexes at low temperatures with linearly polarized light using a tandem mass spectrometer containing a temperature-variable ion trap, and detected enantiomer-selective photolysis of cold gas-phase protonated tryptophan (TrpH⁺) with a chiral crown ether (Fujihara et al. 2014). These results show that enantiomer-selective photolysis of amino acids on chiral substances with linearly polarized light was important in understanding the origin of the enantiomeric enrichment of amino acids.

In this study, we examined the UV photolysis of cold gas-phase tryptophan (Trp) enantiomers in L-serine clusters as interstellar molecular cloud analogues. Serine (Ser) forms magic-number cluster ions composed of eight amino acid units in the gas phase, and the clusters show a remarkable preference for homochirality (Cooks et al. 2001; Nanita and Cooks 2006). On the basis of the results, we discussed the possibility that the stability and preference for homochirality of gas-phase Ser clusters induces the enantiomeric enrichment of other amino acids via enantiomer-selective photolysis with linearly polarized light.

Methods

L-Ser, L-Trp, and D-Trp were obtained from Sigma–Aldrich. A solution containing 0.5 mM of Trp and 2 mM of L-Ser in a mixture of water and methanol (50/50) with 1 % acetic acid was used.

Details of the experimental apparatus have been described previously (Fujihara et al. 2014). Hydrogen-bonded cluster ions were generated by electrospray ionization and transferred to the gas phase through a capillary and skimmer. The ions were guided by an octopole, and mass-selected by a quadrupole mass filter. The mass-selected ions were decelerated and refocused by

a stack of cylindrical electrodes into a temperature-variable 22-pole ion trap (8–350 K). In the trap, the ions were thermalized by multiple collisions with the He buffer gas contained within a copper housing at a well-defined temperature, analogous to a thermal bath (Gerlich 1995). The temperature-controlled ions were extracted from the trap and then irradiated with a photo dissociation laser pulse. The fragment ions were orthogonally accelerated to 2.8 keV by two-stage pulsed electric fields, and then mass-analyzed by a reflectron time-of-flight (TOF) mass spectrometer. The repetition rate of the experimental cycle was 10 Hz, and the trapping time of the ions was ~80 ms. The fourth harmonic of a Nd: YAG laser (Minilite II, Continuum) was used as the photodissociation light. The typical laser fluence was around 2 mJ/cm².

Results and Discussion

Mass Spectra

Figure 1 shows a typical TOF mass spectrum of protonated water clusters $\text{H}^+(\text{H}_2\text{O})_n$ generated by electrospray ionization and with the temperature controlled to 8 K. Electrospray ionization involves a direct transfer of charged droplets from solutions to the gas phase. Our apparatus could generate mass-selected and temperature-controlled hydrogen-bonded cluster ions in the gas phase. The mass spectrum exhibits intensity enhancements at $n = 21$ and 28. The ion intensity distribution for $\text{H}^+(\text{H}_2\text{O})_n$ agreed with the observations reported by several groups, in which $\text{H}^+(\text{H}_2\text{O})_n$ was generated in the gas phase using supersonic expansion (Nagashima et al. 1986). The closed cage structures of $\text{H}^+(\text{H}_2\text{O})_{21}$ and $\text{H}^+(\text{H}_2\text{O})_{28}$ were reported using infrared (IR) photo dissociation spectroscopy (Miyazaki et al. 2004, Wu et al. 2005).

Figure 2 shows typical TOF mass spectra of homochiral $\text{H}^+(\text{L-Ser})_n(\text{L-Trp})_m$ and heterochiral $\text{H}^+(\text{L-Ser})_n(\text{D-Trp})_m$ clusters at 8 K. The (n, m) values for major cluster ions are indicated in the spectrum. $\text{H}^+(\text{L-Ser})_4$ and $\text{H}^+(\text{L-Ser})_8$ were stable magic-number clusters, as reported previously (Nanita and Cooks 2006). In addition to protonated serine clusters, substitutions of L-Ser with L-Trp in clusters, producing $\text{H}^+(\text{L-Ser})_n(\text{L-Trp})$ and $\text{H}^+(\text{L-Ser})_n(\text{L-Trp})_2$, were observed. Protonated clusters with $(n+m)$ even numbers of amino acids were mainly generated, indicating that the clusters consisted of amino acid dimer subunits (Cooks et al. 2001). A noticeable difference between the homochiral and heterochiral clusters was not detected in the mass spectra shown in Fig. 2a and b.

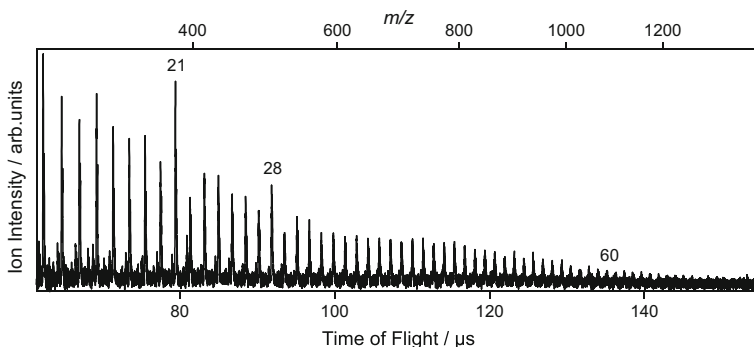


Fig. 1 Typical TOF mass spectrum of $\text{H}^+(\text{H}_2\text{O})_n$ at 8 K

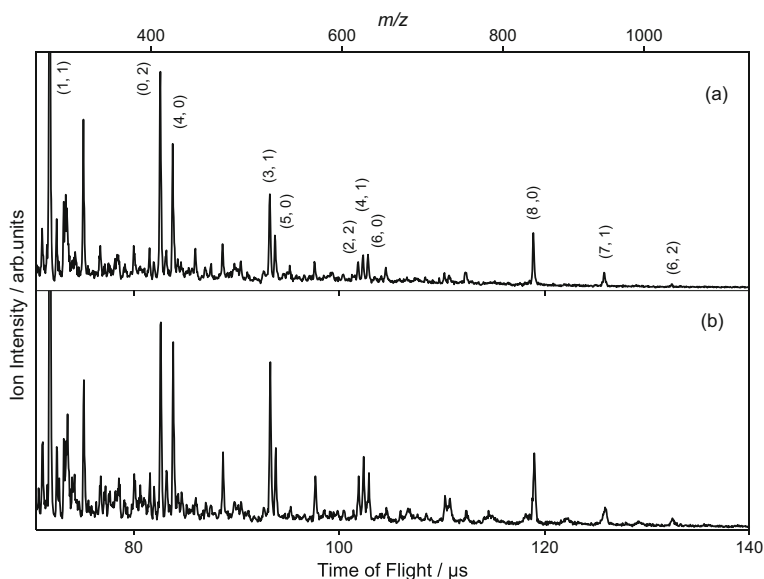


Fig. 2 Typical TOF mass spectra of (a) $\text{H}^+(\text{L-Ser})_n(\text{L-Trp})_m$ and (b) $\text{H}^+(\text{L-Ser})_n(\text{D-Trp})_m$ at 8 K. The (n, m) values for major cluster ions are indicated

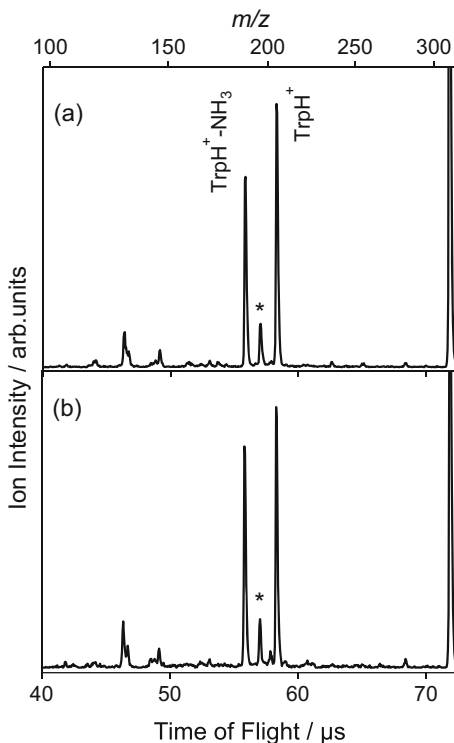
Evaporation of Molecules

To examine the photochemical and photophysical properties of cold gas-phase TrpH^+ enantiomers in L-amino acid clusters, UV photodissociation experiments were performed. The irradiation wavelength was 266 nm, which excites the indole ring of tryptophan to the $\pi\pi^*$ excited state (Grégoire et al. 2009).

Figure 3 shows the photodissociation mass spectra of homochiral $\text{H}^+(\text{L-Ser})(\text{L-Trp})$ and heterochiral $\text{H}^+(\text{L-Ser})(\text{D-Trp})$ (m/z 310) at 8 K, which are $n=m=1$ clusters. In the homochiral $\text{H}^+(\text{L-Ser})(\text{L-Trp})$ shown in Fig. 3a, evaporation of L-Ser, forming $\text{H}^+(\text{L-Trp})$ (m/z 205), was the primary photodissociation pathway. The proton in $\text{H}^+(\text{L-Ser})(\text{L-Trp})$ remained associated with L-Trp after the photo-induced evaporation. This is because the proton affinity of Trp is larger by 26.4 kJ/mol compared to that of Ser (Bojesen and Breindahl 1994). Fragment ions resulting from NH_3 loss, $(\text{NH}_3+\text{CH}_2\text{CO})$ loss, $(\text{NH}_3+\text{CO}_2)$ loss, NHCHCOOH loss, and $\text{NH}_2\text{CH}_2\text{COOH}$ loss from free $\text{H}^+(\text{L-Trp})$, corresponding to m/z 188, 146, 144, 132, and 130, respectively, are observed in the spectrum. The peak marked with an asterisk is assigned to the NH_3 -eliminated product from free L-TrpH^+ in the field-free region of the reflectron TOF mass spectrometer. These fragment ions were also formed in photo-induced dissociation (PID) and low-energy collision-induced dissociation (CID) experiments of free TrpH^+ (Grégoire et al. 2009, Aribi et al. 2004). The observations indicate that the fragment ions observed in the spectrum of $\text{H}^+(\text{L-Ser})(\text{L-Trp})$ were formed by a sequential reaction via the evaporation of an L-Ser molecule. The photodissociation mass spectrum of heterochiral $\text{H}^+(\text{L-Ser})(\text{D-Trp})$, shown in Fig. 3b, is similar to that of homochiral $\text{H}^+(\text{L-Ser})(\text{L-Trp})$ shown in Fig. 3a. Therefore, no enantioselectivity of the photolysis process was observed in the 1:1 clusters.

Figure 4 shows the photodissociation mass spectra of homochiral $\text{H}^+(\text{L-Ser})_3(\text{L-Trp})$ and heterochiral $\text{H}^+(\text{L-Ser})_3(\text{D-Trp})$ (m/z 520) at 8 K. In the spectrum of homochiral $\text{H}^+(\text{L-Ser})_3(\text{L-Trp})$ shown in Fig. 4a, evaporation of $(\text{L-Ser})_2$ forming $\text{H}^+(\text{L-Ser})(\text{L-Trp})$ was the main

Fig. 3 Photodissociation mass spectra of (a) $\text{H}^+(\text{L-Ser})(\text{L-Trp})$ and (b) $\text{H}^+(\text{L-Ser})(\text{D-Trp})$ (m/z 310) at 8 K. The peaks marked with asterisks are assigned to the NH_3 -eliminated product from free TrpH^+ in the field-free region of the reflectron TOF mass spectrometer

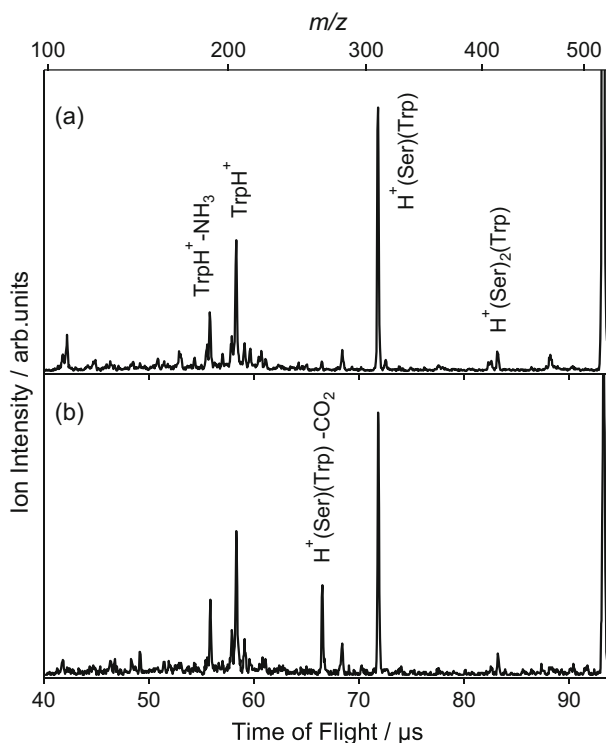


photodissociation pathway, and $\text{H}^+(\text{L-Trp})$ and NH_3 -eliminated product from free L-TrpH^+ (m/z 188) were observed. The m/z 188 product is considered to be formed by a sequential reaction via the evaporation of L-Ser molecules. The results that $\text{H}^+(\text{L-Ser})(\text{L-Trp})$ formed via evaporation of $(\text{L-Ser})_2$ was the main product and $\text{H}^+(\text{L-Ser})_2(\text{L-Trp})$ was the minor peak indicated that $\text{H}^+(\text{L-Ser})_3(\text{L-Trp})$ consisted of amino acid dimer subunits (Cooks et al. 2001), which was consistent with the observations of the mass spectrum shown in Fig. 2a. Evaporation of L-Ser molecules was the main process in UV excitation of homochiral $\text{H}^+(\text{L-Ser})_3(\text{L-Trp})$. Photolysis of amino acid molecules in the clusters was not observed in $\text{H}^+(\text{L-Ser})_3(\text{L-Trp})$, $\text{H}^+(\text{L-Ser})(\text{L-Trp})$, and $\text{H}^+(\text{L-Ser})(\text{D-Trp})$. This is because the energy absorbed in the Trp molecule was released through the evaporation of L-Ser molecules.

Photolysis in Cluster

In the photodissociation mass spectrum of heterochiral $\text{H}^+(\text{L-Ser})_3(\text{D-Trp})$ shown in Fig. 4b, $\text{H}^+(\text{L-Ser})(\text{D-Trp})$ and $\text{H}^+(\text{D-Trp})$ formed via evaporation of L-Ser molecules, and the NH_3 -eliminated product from free D-TrpH^+ was observed, as in the case of homochiral $\text{H}^+(\text{L-Ser})_3(\text{L-Trp})$ shown in Fig. 4a. The clusters also consisted of amino acid dimer subunits. In addition to these product ions, the CO_2 -eliminated product from $\text{H}^+(\text{L-Ser})(\text{D-Trp})$ (m/z 266) was observed, which corresponded to the photolysis product of D-Trp molecule in the L-Ser cluster. In contrast, fragmentations induced by proton transfer, such as NH_3 loss, $(\text{NH}_3 + \text{CH}_2\text{CO})$ loss, $(\text{NH}_3 + \text{CO}_2)$ loss, NHCHCOOH loss, and $\text{NH}_2\text{CH}_2\text{COOH}$ loss from $\text{H}^+(\text{L-Trp})$, which were the main fragmentation pathway of free TrpH^+ (Grégoire et al. 2009, Aribi et al. 2004), were not observed in the cluster.

Fig. 4 Photodissociation mass spectra of (a) $\text{H}^+(\text{L-Ser})_3(\text{L-Trp})$ and (b) $\text{H}^+(\text{L-Ser})_3(\text{D-Trp})$ (m/z 520) at 8 K



In the case of UV photolysis of cold gas-phase TrpH^+ enantiomers with crown ethers, CO_2 loss was observed, whereas the fragmentations induced by proton transfer from the NH_3^+ group of TrpH^+ were suppressed due to the hydrogen bonding of the NH_3^+ group with the crown ether (Fujihara et al. 2014). The CO_2 loss from amino acids was previously reported to be the dominant photoproduct under UV irradiation using standard matrix isolation techniques, in which the amino acids were non-zwitterionic, having both NH_2 and COOH groups (Ehrenfreund et al. 2001). Therefore, the proton in $\text{H}^+(\text{L-Ser})_3(\text{D-Trp})$ was assumed to localize in the cluster, and CO_2 loss was the main photolysis process in the heterochiral cluster under UV irradiation. Photolysis in clusters was not observed in the spectra of $\text{H}^+(\text{L-Ser})_3(\text{L-Trp})$, $\text{H}^+(\text{L-Ser})(\text{L-Trp})$, and $\text{H}^+(\text{L-Ser})(\text{D-Trp})$ shown in Fig. 3a and b, Fig. 4a, respectively, due to the evaporation of L-Ser molecules. The CO_2 loss in cluster observed only in the spectrum of heterochiral $\text{H}^+(\text{L-Ser})_3(\text{D-Trp})$ shown in Fig. 4b confirms the enantiomer-selective photolysis of D-Trp in the L-Ser cluster. However, a Ser: Trp ratio of at least 3:1 is needed for this phenomenon to occur. In the chiral specific reaction, multiple-point interactions in a polydentate complex are required, even if only transiently. The UV excitation of heterochiral $\text{H}^+(\text{L-Ser})_3(\text{D-Trp})$ induces the evaporation of (L-Ser)₂ and CO_2 loss, forming the chiral-specific CO_2 -eliminated product from $\text{H}^+(\text{L-Ser})(\text{D-Trp})$.

These results indicate that homochirality in gas-phase Ser clusters could promote the enantiomeric enrichment of other amino acids, and suggest that enantiomer-selective photolysis with linearly polarized light in gas-phase clusters plays an important role in the enantiomeric enrichment step. It is necessary to study the structure and reactivity of gas-phase clusters consisting of interstellar and biological molecules as a function of cluster size and temperature in understanding chemical processes in interstellar molecular clouds.

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