CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.201301831

Photoreaction of *rac*-Leucine in Ice by Circularly Polarized Synchrotron Radiation: Temperature-Induced Mechanism Switching from Norrish Type II to Deamination

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Abstract: The delivery of extraterrestrial organics to primitive Earth is considered to have triggered the origin and subsequent evolution of life. Indeed, enantiomerically enriched amino acids of nonterrestrial origin have been found in carbonaceous meteorites, and enantioselective photodecomposition by circularly polarized light (CPL) in outer space has been proposed to have played some role in the initial enantiomeric bias. To experimentally examine this possibility and elucidate the photoreaction mechanisms, we have studied the photolysis of racemic leucine (*rac*-Leu) in acidic and neutral ice/water media at 21–298 K with left- and right-CPL in an attempt to detect enantiomerically enriched D- and L-Leu, respectively. Comprehensive product analyses revealed that the CPL-induced deracemization of Leu proceeds in both acidic and neutral ice matrices even at 21 K, and that the main mechanism switches from Norrish-type II γ-hydrogen abstraction to S_Ni deamination on lowering the temperature. The potential role of the CPL-induced photodecomposition of amino acids as a source of the enantiomer imbalance in meteorites is discussed.

Keywords: amino acids · chirality · photolysis · reaction mechanism · temperature effects

Introduction

The origin of homochirality in the biosphere is one of the most crucial issues in chemical evolution, for which a variety of biotic and prebiotic theories and chemical and physical mechanisms have hitherto been proposed.[1–10] Recent meteorite studies have revealed that carbonaceous meteorites carry biologically important organics, including aliphatic amino acids.[2,3] Crucially, both the natural and unnatural amino acids found in meteorites are not racemic but enriched enantiomerically as well as isotopically in ^{13}C and ^{15}N, which indicates a nonterrestrial origin of the enantiomer imbalance.[3]

In the 1980s, Rubenstein, Bonner et al. proposed a cosmic scenario in which circularly polarized synchrotron radiation from electrons orbiting neutron stars enantioselectively photodecomposed racemic amino acids in an icy comet to leave enantiomerically enriched antipodes that were subsequently delivered to the primitive Earth and triggered biomolecular homochirality.[4] However, circularly polarized light (CPL) has never been detected in the vicinity of a neutron star and thus the emission of CPL from neutron stars was questioned.[5] However, the star-forming region of the Orion molecular cloud was found to emit CPL in the IR region,[6] presumably provoked by Mie scattering on aligned interstellar dust grains.[6,7] The proposal and discovery of such interstellar CPL sources stimulated the investigation to elucidate the plausibility of the cosmic scenario,[4,6,8,9] including the photochemical enantiomer enrichment of amino acids by CPL in outer space.[1a,b,9] Indeed, a variety of (racemic) aliphatic amino acids are known to be produced upon vacuum ultraviolet (VUV) irradiation of a matrix of H_2O, CH_3OH, CO_2, CO, and NH_3 at 12 K under high vacuum.[10] Nevertheless, the subsequent process, that is, the enantioselective photodecomposition of racemic amino acids by CPL, has not been experimentally proven or disproven under interstellar conditions.

The principle of enantiomer enrichment upon CPL photolysis of a racemic substrate[11] rests on the relative difference in molar extinction coefficients (ε) of an enantiomer pair under left- or right-CPL (*l*- or *r*-CPL), which leads to unequal rates of excitation and subsequent reaction. The anisotropy factor (g), a measure of preferential excitation, is defined as the normalized difference in ε of an enantiopure

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.201301831.

compound towards *l*- and *r*-CPL at a given wavelength: $g=(\varepsilon_l-\varepsilon_r)/\varepsilon=\Delta\varepsilon/\varepsilon$, in which $\varepsilon=(\varepsilon_l+\varepsilon_r)/2$ and hence $0\leq g<2$.[11,12]

It is crucial that the Kuhn–Condon zero-sum rule[13] does not apply to the CPL photolysis of aliphatic amino acids in water/ice media simply because the media absorb the VUV CPL (<200 nm) and only the UV CPL of 200–250 nm is absorbed by amino acids.[12]

In 1977, Bonner and co-workers reported that CPL photolysis of *rac*-Leu in 0.1 M HCl at 212.8 nm led to 2–2.5 % enantiomeric excess (*ee*) in the remaining Leu at 59–75 % conversion, although no photodegradation mechanism was proposed.[14]

In our recent study,[12] *rac*-Leu and *rac*-alanine (Ala) were photolyzed with CPL at 215 nm in acidic and neutral aqueous solutions at room temperature to give enantiomerically enriched Leu and Ala, irrespective of the solution pH. However, the mechanism was significantly pH-dependent, switching from Norrish-type II γ-hydrogen abstraction in acidic solutions to the less efficient deamination to an α-lactone by intramolecular nucleophilic substitution (S_Ni) in neutral solutions,[12,15] as a result of the change in the excited chemical species from carboxylic acid to carboxylate; for the detailed mechanisms in acidic and neutral solutions, see Scheme 1.

Meierhenrich et al. performed the CPL photolysis of a thin film of pure *rac*-Leu at 182 nm to obtain enantiomerically enriched L- and D-Leu in 0.88 ± 0.28 and 2.60 ± 0.16 % *ee*, respectively, upon *l*- and *r*-CPL irradiation, presumably at ambient temperatures.[16a] More recently, the same group reported the results of the CPL photolysis of a 2:1:1 mixture of H_2O, CH_3OH, and NH_3.[16b] The mixture was first irradiated at 80 K by CPL at 187 nm to produce radical species trapped in the low-temperature matrix, which was allowed to thaw and react to afford a mixture of stable products containing amino acids. The resulting mixture was subjected to a second CPL irradiation at room temperature to enantioselectively photodecompose the racemic amino acids produced in the first irradiation; L- and D-enriched Ala were obtained in 0.71 ± 0.30 and 1.34 ± 0.40 % *ee* upon *l*- and *r*-CPL irradiation at 187 nm, respectively.[16b] Takano et al. reported that an aqueous extract of high-molecular-weight complex organics (of up to 3000 Da), prepared by irradiating a gaseous mixture of CO, NH_3, and H_2O with 3 MeV protons, gave L- and D-Ala in 0.65 ± 0.23 and 0.44 ± 0.31 % *ee* upon *l*- and *r*-CPL irradiation at >200 nm and subsequent hydrolysis by acid.[17]

However, none of our experiments,[12] nor those of Meierhenrich[16] or Takano,[17] has directly proven the occurrence of the enantioselective decomposition of racemic amino acids or identified the products derived therefrom under conditions similar to interstellar ice (10–20 K).

In this study to elucidate the feasibility and the detailed mechanism of the photoreaction of amino acids in ice matrices and hopefully to obtain some insight into the cosmic scenario, we chose *rac*-Leu as a representative aliphatic amino acid substrate for the following reasons: (1) Enantiomerically enriched Leu has been found in carbonaceous meteorites,[3a] 2) VUV irradiation of a mixture of simple starting materials (H_2O, CH_3OH, CO_2, CO, and NH_3) produces *rac*-Leu,[10] and 3) Leu photodecomposes efficiently, at least at ambient temperatures.[12] The photolysis of *rac*-Leu was performed in neutral and acidic water and ice media at temperatures ranging from 298 to 21 K to elucidate the effects of temperature and pH on the photoreaction mechanism of amino acids by using unpolarized UV light (214 nm) from a Y-line lamp and circularly polarized synchrotron radiation (215 nm) from a polarizing undulator[18a] installed in the electron storage ring UVSOR-II.[18b]

Results and Discussion

Temperature- and pH-dependent anisotropy factor: In the CPL photolysis experiment, the anisotropy factor ($g=\Delta\varepsilon/\varepsilon$) of the chiral substrate plays a decisive role in determining the efficiency of photochemical enantiomer enrichment. Hence we first examined the chiroptical properties of L-Leu in acidic and neutral aqueous solutions at 268–318 K.

Aliphatic L-amino acids are known to show weak n,π* transitions ($\varepsilon=45\text{–}70\,\mathrm{M^{-1}\,cm^{-1}}$) with a positive Cotton effect ($\Delta\varepsilon=1\text{–}2\,\mathrm{M^{-1}\,cm^{-1}}$) at around 210 nm and an extremely weak negative Cotton effect ($|\Delta\varepsilon|\leq 0.001\,\mathrm{M^{-1}\,cm^{-1}}$) at 250–255 nm in aqueous solutions at pH 1.[12,19] This is exactly the case with L-Leu. As shown in Figure 1, the anisotropy spectrum (Figure 1c) is broader than the UV and CD spectra (Figure 1a,b), affording a relatively large positive *g* factor of around 0.03 at 210–220 nm and a much smaller negative *g* factor of around −0.002 at 255 nm.

CD spectral analysis and product study: The Leu samples photolyzed by circularly polarized and unpolarized light in acidic and neutral media at various temperatures were subjected to CD spectral analysis and then to qualitative and quantitative product analyses.[16] The CD spectral changes caused by CPL irradiation at 21 and 81 K are shown in Figure 2 and Figure S3 (in the Supporting Information), respectively, and the nonvolatile and volatile products (see Figures S4–S16) and their yields obtained upon photolysis in the temperature range 21–298 K are summarized in Tables 1 and 2, respectively.

Because the *g* factor is dimensionless and hence independent of the volume change caused by varying the solution temperature, it is convenient to examine precisely the effects of temperature on the chiroptical properties. On cooling the acidic Leu solution, the *g* factor was appreciably enhanced at 210–220 nm at the expense of that at 255 nm (Figure 1c), which has been attributed to the shift in the equilibrium of amino acid conformers and hence is reversible in nature.19b Upon further cooling, the aqueous Leu solution solidified to give an ice matrix, which was glassy but not completely transparent and hence slightly scattered incident light. Although the frozen Leu samples were not suitable for spectral analysis, it is likely that the conformer com-

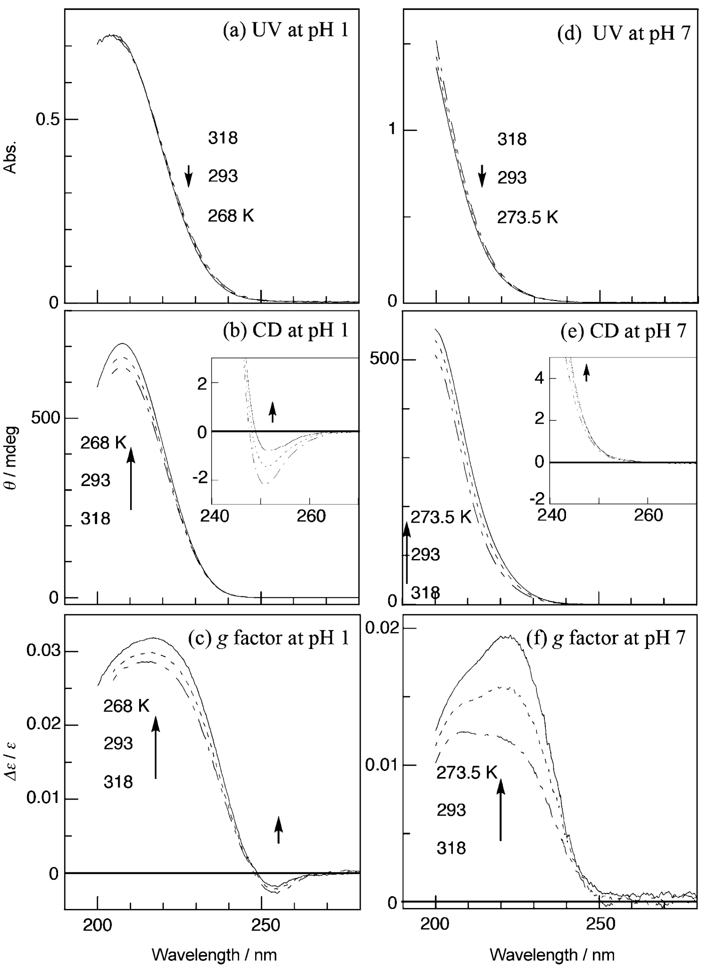


Figure 1. UV (top), CD (middle), and anisotropy spectra (bottom) of acidic (left) and neutral (right) aqueous solutions of L-Leu (10.6 mM for the acidic solution and 106 mM for the neutral solution) at 318–268 K. The UV and CD spectra at 318 and 293 K are corrected for the volume changes caused by temperature variation, whereas the anisotropy factor is inherently free from the errors arising from volume change as $g = \Delta\varepsilon/\varepsilon$.

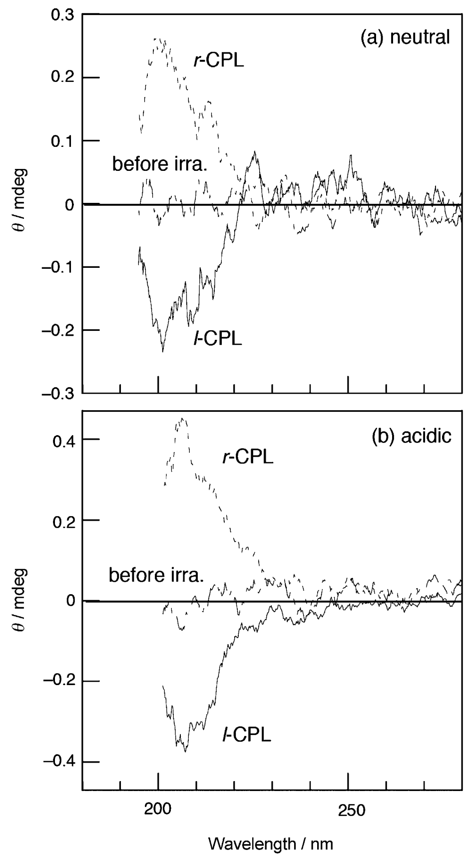


Figure 2. CD spectra of *rac*-Leu solutions recorded at 296 K before and after CPL irradiation at 215 nm in an ice matrix at 21 K. (a) Neutral samples ([*rac*-Leu] = 7.0 mM, $pH_{298K} = 7$), in which the conversions upon irradiation of *l*- and *r*-CPL (dose: 860 mA h) were 21 and 17 %, respectively. (b) Acidic samples ([*rac*-Leu] = 30.5 mM, $pH_{298K} = 1$), in which the conversions upon irradiation of *l*- and *r*-CPL (dose: 400 mA h) were 6 and 7 %, respectively.

position, and therefore the *g* factor, near the freezing point (268 K) are preserved or at least not significantly altered in the ice matrix.

In a neutral solution at pH 7, zwitterionic L-Leu shows only a structureless absorption tail at 200–250 nm in both the UV and CD spectra (Figure 1 d,e), whereas the anisotropy spectrum (Figure 1 f) shows a broad positive peak at 210–230 nm that is greatly enhanced up to 0.02 on cooling the solution to near the freezing temperature, that is, 273.5 K.

From a technical point of view, it was convenient that the anisotropy spectra are relatively flat and the maximal values of 0.02–0.03 are reached in the same wavelength region for both the acidic and neutral solutions. This allowed us to exploit the optimal conditions for CPL photolysis without fine-tuning the irradiation wavelength (which is achieved only by altering the energy of electron acceleration in the synchrotron).

Figure 2a illustrates the CD spectra of the neutral Leu samples irradiated at 21 K with *l*- and *r*-CPL; the spectra are consistent in shape with those of D- and L-Leu, respectively, which indicates preferential photodecomposition of the antipode (L- and D-Leu), as anticipated by the CD sign at 215 nm (Figure 1d). Naturally, no appreciable CD signals developed upon irradiation with linearly polarized light (LPL). The optical purity (which is normally equivalent to the *ee* but determined from the optical rotation) of the enantiomerically enriched Leu was determined from the induced ellipticity to be −0.3 and +0.3 % (at 17 and 21 % conversion) for *l*- and *r*-CPL, respectively. The acidic *rac*-Leu samples irradiated at 21 K with *l*- and *r*-CPL also exhibit mirror-image CD spectra with extrema at 200–210 nm (Figure 2b). From the ellipticity induced, the optical purity was determined to be −0.1 and +0.1 % (at 6 and 7 % conversion) for *l*- and *r*-CPL irradiation, respectively.

Upon irradiation of Leu in an acidic aqueous solution at 298 K, Gly was produced as the major product in 47 % yield (based on the consumed Leu), together with a small amount of ammonia (13 % yield). In contrast, the photolysis of a neutral solution of Leu afforded α-hydroxyisocaproic acid (38 % yield) and ammonia (73 % yield) as the major prod-

Table 1. Nonvolatile products detected in the *rac*-Leu samples irradiated at 214 nm with a Y-line lamp (YL) or at 215 nm with left-circularly polarized synchrotron radiation (*l*-CPL) in acidic and neutral aqueous solutions and ice matrices at various temperatures.[a]

T [K]	Light source	pH_{298K}	Irradiation Time [h]	Irradiation Dose [mA h][b]	Leu [mM][c]	Absorbance[d]	Conversion [mM (%)]	Product yield [mM (%)][e] Gly	NH_3	$i\text{BuCH}_2\text{NH}_2$	$i\text{BuCH(OH)CO}_2\text{H}$
298	YL	1	1.5		28.1	0.24[e]	11.8 (41.9)	5.48 (46.5)	1.5 (13)	0.1 (1)	<0.1
		7	5		8.12	0.05[e]	1.24 (14.8)	0	0.9 (73)	0.1 (8)	0.47 (38)
268	YL	1	1.5		28.1	0.24	10.6 (37.8)	3.86 (36.3)	0.9 (8)	0.1 (1)	0
238	YL	1	4		29.4	0.28	7.48 (25.5)	1.11 (14.8)	1.8 (24)	<0.1	0
		7	5		6.75	0.05	0.84 (12.4)	0	0.4 (47)	<0.1	0
183	YL	1	4		29.4	0.28	4.54 (15.5)	0.82 (18.1)	0.9 (20)	<0.1	0
		7	5		6.75	0.05	0.78 (11.6)	0	0.3 (38)	0.1 (13)	0
123	YL	1	4		29.4	0.28	2.76 (9.4)	0.19 (6.9)	0.5 (18)	<0.1	0
		7	5		6.75	0.05	0.77 (11.4)	0	0.3 (39)	0.1 (13)	0
81	*l*-CPL	1		200	30.5	0.29	1.75 (5.7)	0.14 (8.0)	0.6 (34)	0.1 (6)	0
		7		860	7.08	0.05	1.41 (19.9)	0	0.6 (43)	0.1 (7)	0
77	YL	1	4		29.4	0.28	0.72 (2.5)	0	0.2 (28)	<0.1	0
			12		29.4	0.28	1.54 (5.2)	0	0.5 (32)	<0.1	0
		7	10		6.75	0.05	0.89 (13.2)	0	0.2 (22)	<0.1	0
21	*l*-CPL	1		400	30.5	0.29	1.75 (5.7)	0	0.6 (34)	0.1 (6)	0
		7		860	6.95	0.05	1.30 (18.7)	0	0.5 (38)	0.1 (8)	0

[a] Irradiated at 298–123 K in a Unisoku USP-203 cryostat, at 81 K in a continuous-flow liquid N_2 cryostat, at 77 K in a Suprasil Dewar vessel filled with liquid N_2, and at 21 K in a continuous-flow liquid helium cryostat. [b] Light intensity defined as the accumulated electron beam current of the electron storage ring UVSOR-II. [c] Lower concentrations were employed for neutral samples, due to the lower solubility of zwitterionic Leu. [d] Absorbance calculated from the concentration and the effective pathlength (1.8 mm), unless noted otherwise. [e] Product yield based on the consumed Leu. In all irradiated samples, trace amounts of CH_3CO_2H and HCO_2H were detected. Effective pathlength: 1.5 mm.

Table 2. Gaseous and ether-extracted products detected upon gas chromatographic analyses of the *rac*-Leu samples photolyzed at 215 nm with left-circularly polarized synchrotron radiation (*l*-CPL) in acidic and neutral ice matrices at 81 and 21 K.[a]

T [K]	pH_{298K}	Dose [mA h][b]	Conc. [mM]	Absorbance[c]	Conversion [mM (%)]	Product yield [mM (%)][d] CO	CO_2	*i*BuCHO	$CH_3CH{=}CH_2$	$(CH_3)_2C{=}CH_2$
81	1	200	30.47	0.28	1.75 (6.4)	–[e]	–[e]	0.07 (4)	–[e]	–[e]
	7	860	6.95	0.05	1.23 (17.7)	–[e]	–[e]	0.09 (7)	–[e]	–[e]
21	1	400	30.47	0.28	2.05 (6.8)	>0.04 (>2)	>0.07 (>3)	0.12 (6)	>0.01 (>0.1)	<0.01
	7	860	6.95	0.05	1.38 (19.9)	>0.04 (>3)	>0.12 (>9)	0.16 (12)	>0.01 (>0.1)	<0.01

[a] Irradiated in a continuous-flow liquid N_2 (81 K) or He (21 K) cryostat. [b] Light intensity defined as the accumulated electron beam current of UVSOR-II. [c] Absorbance calculated from the concentration and the effective pathlength (1.8 mm). [d] Product yield based on the consumed Leu. [e] Yield not determined.

ucts. The rate of photodecomposition, or conversion, in general, was reduced by lowering the irradiation temperature, but the effect was more significant for the acidic samples (Table 1). As shown in Figure 3a, a dramatic change was observed for the Gly yield, which suddenly dropped at the freezing point (indicated by the vertical broken line in Figure 3a) and gradually faded out upon further cooling, finally disappearing at 77 K. The sudden drop in Gly yield at the freezing point may be ascribed to the reduction of conformational freedom upon freezing, which hinders the access of the excited carbonyl oxygen to the γ-hydrogen and subsequent abstraction (Scheme 1). It is interesting, however, that some γ-hydrogen abstraction still occurs even in ice matrices, at least at temperatures higher than 77 K, for which two independent mechanisms (one static and one dynamic) may be responsible. It is reasonable to assume that (1) part of the Leu molecules in the ice matrix are frozen in conformation(s) that are suitable for γ-hydrogen abstraction and (2) there still remains some partial mobility available for the excited carbonyl moiety to approach and abstract the γ-hydrogen atom. It is likely that both of these mechanisms may operate in ice matrices at higher temperatures but become structurally and/or energetically unfeasible at temperatures below 77 K.

In contrast, the ammonia yield is much less sensitive to temperature and in fact increases at lower temperatures at the expense of Gly (Figure 3a). This indicates that the S_Ni attack of the excited carboxylate moiety on the α-carbon atom, leading to deamination, is conformationally more tolerant and energetically more feasible.

Leu in neutral ice matrices shows very different photobehavior. As shown in Figure 3b, the conversion and the ammonia yield only moderately decrease on lowering the temperature from 298 (solution) to 238 K (ice), whereas the formation of α-hydroxyisocaproic acid, the main product of the solution-phase irradiation, is completely suppressed in ice, even at 238 K. This result is not unexpected because the attack of water on the intermediate α-lactone, leading to α-hydroxyisocaproic acid, is not feasible in ice matrices, as discussed below (Scheme 1).[12,19a,20a]

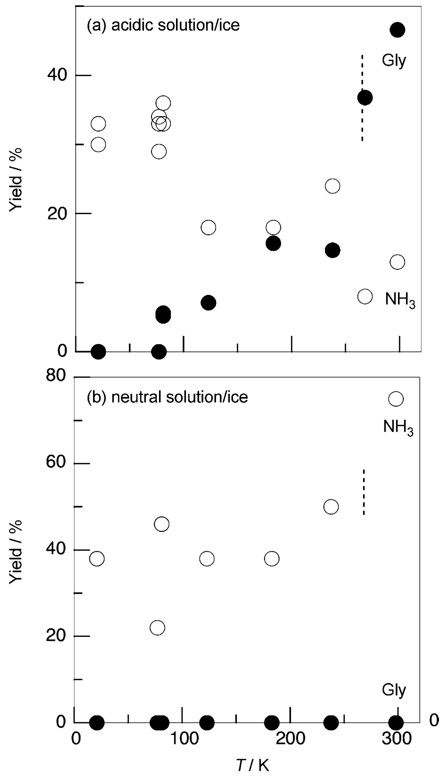


Figure 3. Chemical yields of ammonia (○) and Gly (●) obtained upon irradiation of (a) acidic and (b) neutral *rac*-Leu samples in aqueous solutions at 268–298 K and in ice matrices at 21–238 K. The vertical broken line indicates the freezing point of each solution.

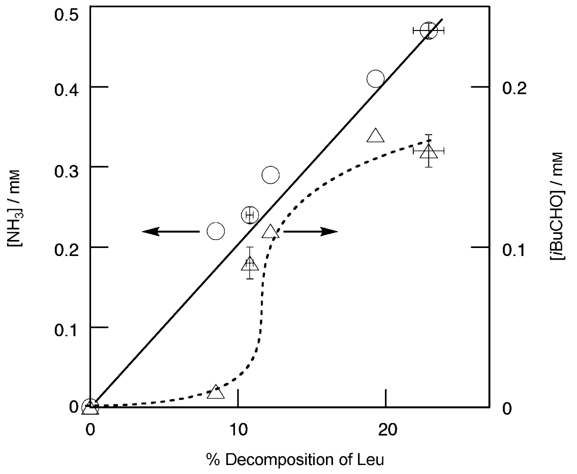
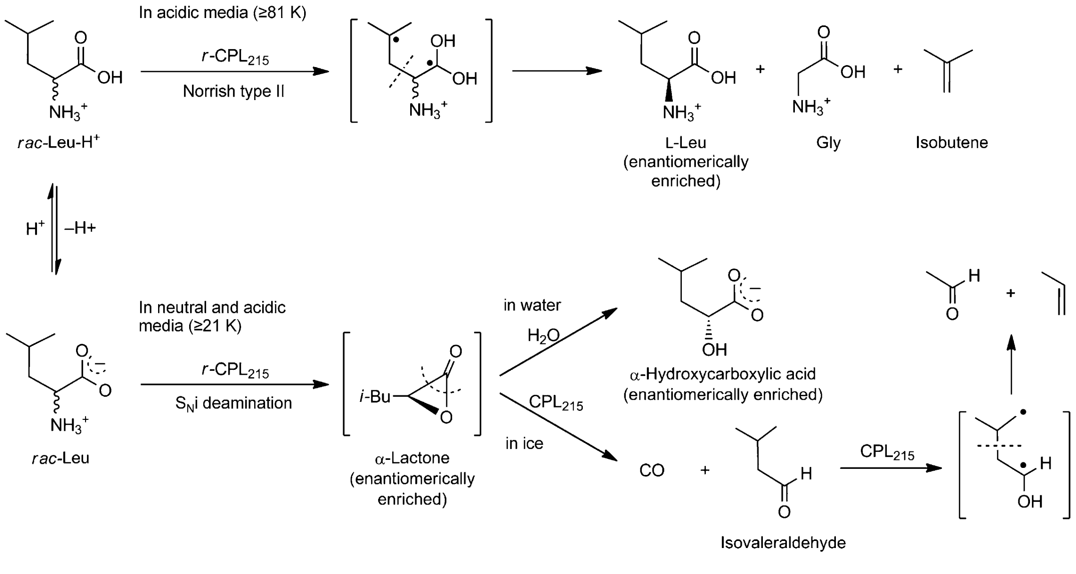


Figure 4. Yields of ammonia (○) and isovaleraldehyde (*i*BuCHO, △) as a function of the photodecomposition of neutral Leu (6.63–6.95 mM) upon irradiation by *l*-CPL of varying doses (100–860 mA h) at 21 K.

Gas chromatographic (GC) analysis revealed that the gaseous products collected from the irradiated neutral and acidic Leu samples contained CO in >2–3 % yield and CO_2 in >3–9 % yield, whereas the diethyl ether extract contained isovaleraldehyde in 7–12 % yield for the neutral sample and in 4–6 % yield for the acidic sample (Table 2). Crucially, the formation of isovaleraldehyde was not proportional to the period of irradiation, but showed an induction period, which is in sharp contrast to the linear dependence of the ammonia yield (Figure 4). This indicates that isovaleraldehyde is a product derived from a secondary photoreaction of the initial product. We propose isobutyl-α-lactone as the most plausible intermediate for the formation of isovaleraldehyde upon irradiation in ice; this lactone, which is derived from the photochemical S_Ni deamination of Leu, is highly reactive and immediately attacked by water to give α-hydroxyisocaproic acid in aqueous solutions.[12,20] In low-temperature ice matrices, the α-lactone produced is likely to be trapped intact and suffer secondary photodecomposition to isovaler-



Scheme 1. Photochemical reactions of *rac*-Leu in acidic and neutral aqueous solutions and ice matrices.

aldehyde and CO (Scheme 1, bottom row). Experimentally, the detection of propene, a photodecomposition product from isovaleraldehyde, in the photolyzed solution (Table 2) may support this mechanism (Scheme 1, middle right).

Conclusion

In this study, we have identified the major volatile and nonvolatile products obtained upon direct irradiation of Leu in aqueous solutions and also in ice matrices. Based on the results of the product study, we have discussed the photochemical behavior of Leu (as a representative aliphatic amino acid) in acidic and neutral media at temperatures ranging from 298 to 21 K to reveal that the photoreaction mechanism and the major products derived therefrom are critical functions of the phase, temperature, and pH of the media.

In acidic media, the overall photoreaction is greatly decelerated by lowering the irradiation temperature, particularly below the freezing point, and the main photoreaction mechanism switches from the Norrish-type II γ-hydrogen abstraction, which affords Gly and isobutene, to S_Ni deamination to give isobutyl-α-lactone, which is trapped in situ and subsequently photodecarbonylated to isovaleraldehyde in ice matrices.

In neutral media, the photoreaction proceeds consistently by the S_Ni deamination mechanism to give the α-lactone, which is followed by the nucleophilic attack of water leading to α-hydroxyisocaproic acid in aqueous solutions at ambient temperatures or alternatively by photodecarbonylation to isovaleraldehyde in ice matrices at cryogenic temperatures. The overall efficiency of the photoreaction is surprisingly insensitive to the irradiation temperature (and medium), maintaining the same level of conversion throughout the temperature range examined (21–298 K). This suggests that the S_Ni deamination survives as the major photoreaction process under interstellar conditions (10–20 K).

Intriguingly, the photoreaction mechanism converges to the deamination process in both acidic and neutral media at temperatures below 77 K. This indicates that the amino acid exists in its zwitterionic form in ice matrices at lower temperatures, irrespective of the original pH at 298 K, because the deamination occurs only upon excitation of the carboxylate moiety of a zwitterionic amino acid.[12,19a,20a] In this connection, it is crucial to point out that the dissociation of HCl adsorbed on an ice surface is discouraged at low temperatures[21a,b] and HCl on amorphous ice at 70 K is totally molecular[21a,c] whereas around 85 % of HCl is molecular in ice nanocrystals at 60 K.[21d] This means that HCl becomes more molecular, or less acidic, at lower temperatures and that it is difficult to protonate zwitterionic amino acid in ice matrices. This shift in equilibrium rationalizes the switching of the major photoreaction mechanism from the γ-hydrogen abstraction by an excited carboxylic acid to the S_Ni deamination by an excited carboxylate (Scheme 1).

On the other hand, we are less certain about the potential roles of different ice phases in controlling the photobehavior as we cooled the sample solutions at a constant rate to the designated temperatures and no literature information is available on the phases of ice matrices containing 7–30 mM *rac*-Leu (and HCl). However, the experimental results obtained in this study (i.e., no clear jump is observed in conversion or product yield at the phase transition temperatures) suggest that the photobehavior is not significantly affected by the phase transitions from metastable phase I cubic (I_c) to phase I hexagonal (I_h) and then to phase XI observed upon cooling pure ice,[22] or more likely the ice matrices containing Leu and/or HCl are amorphous in nature and hence experience no clear phase transition.

From the point of view of the chemical evolution, it is crucial that not only the CPL photoreaction of *rac*-amino acids in ice has been experimentally proven to proceed under interstellar conditions, but also that the major photoreaction mechanisms and products have been elucidated. These results substantiate the cosmic scenario in which interstellar ice analogues containing simple starting materials irradiated by VUV light produce a mixture of racemic amino acids[10] that subsequently photodecompose enantioselectively by CPL radiated from the star-formation region en route to primitive Earth.

However, the enantiomeric excesses obtained in the CPL photolysis of aliphatic amino acids are generally low (0.1–2.6 % *ee*),[12,16a,b,17] including the present case. It has been shown that the enantioselectivity obtained by CPL photodecomposition of a racemic mixture is highly conversion-dependent and can be enhanced to 90 % *ee* and even to 99 % *ee* at 88 and 96 % conversion, respectively, if the *g* factor is 1.0.[11] In reality, the *g* factors of aliphatic amino acids, including Leu (Figure 1), are typically 0.02 or slightly higher at the irradiation wavelength (215 nm).[23] This means that an *ee* of 4.6 % will be achieved at 99 % conversion and furthermore that the last single molecule will have statistically 48 % *ee* on average even when one mole of an amino acid with $g=0.02$ is subjected to CPL photolysis.[11] Hence, the unexpectedly high *ee* values of up to 50–60 % reported recently by Pizzarello and co-workers[3] for nonterrestrial isoleucine and *allo*-isoleucine extracted from the Murchison and other meteorites, and also by Glavin et al.[3m] for nonterrestrial proteinogenic aspartic acid and glutamic acid from two out of the three examined fragments of the Tagish Lake meteorite, are evidently unexplainable by the CPL photodecomposition mechanism alone and indicate the operation of other mechanisms such as preferential crystallization to conglomerates[24] during the aqueous alteration in a meteorite parent body.[3m] Nevertheless, it is also worth noting that essentially all of the proteinogenic amino acids found in the Tagish Lake meteorite are L-enriched,[3m] which suggests a certain seeding or biasing mechanism to trigger the enantiomeric imbalance in which the CPL photodecomposition would have played some role.

Experimental Section

Materials: *rac*-Leu ($\geq$99 % pure), D-Leu (99 % pure, 97 % *ee*), and L-Leu ($\geq$98 % pure, 99 % *ee*) were purchased from Wako and used without further purification.

Sample preparation: Acidic Leu solutions (28.1–30.5 mM) were prepared by dissolving *rac*-, D-, or L-Leu in an aqueous 0.1 M HCl solution (pH_{298K} 1.03) for photoirradiation or in an aqueous 0.5 M HCl solution for UV and CD spectral measurements, whereas neutral Leu solutions (6.8–8.1 mM) at pH_{298K} 6.78 were prepared by dissolving *rac*-, D- and L-Leu in distilled water. The acidic and neutral Leu solutions, with a thickness of 1.5–1.8 mm, were relatively transparent at 215 nm, showing absorbances of 0.24–0.29 and approximately 0.05, respectively. Immediately after their preparation, each Leu solution of 0.7 mL volume was placed in a regular (10×10×40 mm) or thin (2×10×40 mm) quartz cell, degassed by three freeze–pump–thaw cycles, sealed under high vacuum ($<10^{-5}$ Pa), and then subjected to spectral examination and photoirradiation at ambient and lower temperatures.

Spectroscopy: UV and CD spectra of the acidic and neutral L-Leu solutions prepared above were recorded at 268–318 K on a JASCO V-550 spectrophotometer fitted with an HMC-358 temperature controller and on a J-720WI spectropolarimeter fitted with a PMH-354WI temperature controller. Spectral analysis at temperatures lower than the freezing point was not feasible or the result was not reproducible due to surface roughness and scattering of the frozen samples.

Photoirradiation: The Leu samples prepared above were irradiated at 214 nm by using unpolarized light from an Ushio Y-line lamp in solution at 268–298 K and also in ice at 77–238 K.

The frozen samples for unpolarized light irradiation were prepared as follows. A quartz cell containing 0.7 mL of the Leu solution was gradually cooled to 238 K by horizontally placing the cell in a cooling bath. Once frozen, the icy sample was quickly transferred to a Unisoku USP-203 cryostat (with three optical windows for spectral examination and irradiation) and further cooled to the desired temperature of $\geq$123 K at a rate of 10 $K\,min^{-1}$ or directly to 77 K by immersing the sample cell in liquid N_2, and then irradiated by a Y-line lamp.

For CPL irradiation, a quartz cell containing the Leu solution was fixed on a brass sample holder, which was placed horizontally in a vacuum chamber connected to the polarizing undulator beam-line and cooled to 21 or 81 K first at a rate of 2–5 $K\,min^{-1}$ (298–220 K) and then at 10–20 $K\,min^{-1}$ ($<$220 K) in a continuous-flow helium or nitrogen cryostat with temperature monitored by a thermocouple attached to the cell surface. The quartz cell containing a frozen Leu sample of around 1.5–1.8 mm thickness was rotated to the upright position and irradiated at 21 ± 3 or 81 ± 2 K with CPL at 215 nm generated by the polarizing undulator installed in UVSOR-II at the Institute for Molecular Science (see Figures S1 and S2 in the Supporting Information).[18]

Product analyses: The irradiated samples were warmed to ambient temperature in a plastic bag with a septum cap. The volatile products were sampled by using a gas-tight syringe, and an aliquot of the aqueous solution was extracted with diethyl ether, both of which were analyzed by gas chromatography (GC). The rest of the solution was subjected to qualitative and quantitative analyses as described below.[12]

The gaseous inorganic and organic products collected in a gas-tight syringe were analyzed by GC on a Shimadzu GC-14A instrument (with a TCD detector) fitted with a column (2.1 m×3 mm i.d.) of molecular sieves (5 Å, 60/80 mesh). The ether extract was subjected to GC-MS analysis on an HP-6890GC/5973MSD instrument fitted with a CP-PoraBOND Q column (25 m×0.25 mm i.d.) to characterize and quantify the organic products. The yields obtained were corrected for the distribution coefficients of the analytes between the ether (2 mL) and Leu solution (0.2 mL), which were determined on a Shimadzu GC-14A with a J&W DB wax column (30 m×0.25 mm i.d.). Nonvolatile products in the photolyzed Leu samples were characterized and quantified by using the amino acid analysis, ion chromatography, and capillary electrophoresis, as reported previously.[12]

CD spectral analysis of the CPL-irradiated samples was carried out to determine their optical purity (op) at 296 K in a cylindrical cell (13 mm i.d.×2 mm thickness) on a JASCO J-725 spectropolarimeter with a modified optical system for higher light intensity and sensitivity. The optical purities were calculated by using the equation $op=\theta_{215}/(33\Delta\varepsilon_{215}[Leu])$, in which θ_{215} is the ellipticity at 215 nm of a CPL-irradiated sample, $\Delta\varepsilon_{215}$ is the molar CD of enantiopure Leu at the same wavelength, and [Leu] the concentration of Leu remaining in the photolyzed solution.[12]

Acknowledgements

This work was supported by the Joint Studies Program of the Institute for Molecular Science and the Japan Society for the Promotion of Science through a Grant-in-Aid for Scientific Research (A, grant no. 21245011) both of which are gratefully acknowledged. We thank the members of the UVSOR facility, Institute for Molecular Science, in particular Mr. Eiken Nakamura and Mr. Takayuki Murakami, for the operation of the linear accelerator and the continuous flow liquid He/N_2 cryostat. We also thank Ushio Inc. for the use of a Y-line lamp.

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[1] a) W. A. Bonner, *Top. Stereochem.* **1988**, *18*, 1; b) W. A. Bonner, *Origins Life Evol. Biosphere* **1991**, *21*, 59; c) J. Alain, C. Corinne, *Origins Life Evol. Biosphere* **2002**, *32*, 129; d) U. J.; Meierhenrich, G. M. Muños Caro, W. A. Schutte, B. Barbier, A. A. Segovia, H. Rosenbauer, W. H. P. Thiemann, A. Brack, *European Space Agency Special Publication* **2002**, *SP-518*, 25; e) C. Meinert, J. H. Bredehoft, J.-J. Filippi, Y. Baraud, L. Nahon, F. Wien, N. C. Jones, S. V. Hoffmann, U. J. Meierhenrich, *Angew. Chem.* **2012**, *124*, 4562; *Angew. Chem. Int. Ed.* **2012**, *51*, 4484.

[2] a) J. Podlech, *Angew. Chem.* **1999**, *111*, 501; *Angew. Chem. Int. Ed.* **1999**, *38*, 477; b) S. Pizzarello, *Acc. Chem. Res.* **2006**, *39*, 231; c) U. J. Meierhenrich, J.-J. Filippi, C. Meinert, P. Vierling, J. P. Dworkin, *Angew. Chem.* **2010**, *122*, 3826; *Angew. Chem. Int. Ed.* **2010**, *49*, 3738; d) J. E. Hein, D. G. Blackmond, *Acc. Chem. Res.* **2012**, *45*, 2045.

[3] a) M. H. Engel, B. Nagy, *Nature* **1982**, *296*, 837 (see also ref. [3f] for further discussion); b) M. H. Engel, S. A. Macko, J. A. Silfer, *Nature* **1990**, *348*, 47; c) S. Pizzarello, J. R. Cronin, R. V. Krishnamurthy, S. Epstein, *Geochim. Cosmochim. Acta* **1991**, *55*, 905; d) J. R. Cronin, S. Pizzarello, *Science* **1997**, *275*, 951; e) M. H. Engel, S. A. Macko, *Nature* **1997**, *389*, 265; f) S. Pizzarello, J. R. Cronin, *Geochim. Cosmochim. Acta* **2000**, *64*, 329; g) M. A. Sephton, *Nat. Prod. Rep.* **2002**, *19*, 292; h) S. Pizzarello, M. Zolensky, K. A. Turk, *Geochim. Cosmochim. Acta* **2003**, *67*, 1589; i) S. Pizzarello, Y, Huang, M. R. Alexandrer, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 3700; j) D. P. Glavin, J. P. Dworkin, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 5487; k) D. P. Glavin, M. P. Callahan, J. P. Dworkin, J. E. Elsila, *Meteorit. Planet. Sci.* **2010**, *45*, 1948; l) S. Pizzarello, D. L. Schrader, A. A. Monroe, D. S. Lauretta, *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 11949; m) D. P. Glavin, J. E. Elsila, A. S. Burton, M. P. Callahan, J. P. Dworkin, R. W. Hilts, C. D. K. Herd, *Meteorit. Planet. Sci.* **2012**, *47*, 1347.

[4] a) E. Rubenstein, W. A. Bonner, H. P. Noyes, G. S. Brown, *Nature* **1983**, *306*, 118; b) W. A. Bonner, E. Rubenstein, *Biosystems* **1987**, *20*, 99.

[5] J. A. Roberts, *Nature* **1984**, *308*, 318.

[6] a) J. Bailey, A. Chrysostomou, J. H. Hough, T. M. Gledhill, A. MaCall, S. Clark, F. Mènard, *Science* **1998**, *281*, 672; b) M. Buschermöhle, D. C. B. Whittet, A. Chrysostomou, J. H. P. Hough, W. Lucas, A. J. Adamson, B. A. Whitney, M. J. Wolff, *Astrophys. J.* **2005**, *624*, 821.

[7] a) K. Kobayashi, T. Kasamatsu, T. Kaneko, J. Koike, T. Oshima, T. Saito, T. Yamamoto, H. Yanagawa, *Adv. Space Res.* **1995**, *16*, 21; b) B. T. Draine, *Astrophys. J.* **2003**, *598*, 1017.

[8] J. Oró, *Nature* **1961**, *190*, 389.

[9] U. Meierhenrich, B. Barbier, R. Jacquet, A. Chabin, C. Alcaraz, L. Nahon, A. Brack, *European Space Agency Special Publication* **2001**, *SP-496*, 167.

[10] a) G. M. Muñoz Caro, U. J. Meierhenrich, W. A. Schutte, B. Barbler, A. A. Segovia, H. Rosenbauer, W. H. P. Thiemann, A. Brack, J. M. Greenberg, *Nature* **2002**, *416*, 403; b) M. P. Bernstein, J. P. Dworkin, S. A. Sandford, G. W. Cooper, L. J. Allamandola, *Nature* **2002**, *416*, 401; c) C. Meinert, J.-J. Filippi, P. Marcellus, L. L. S. Hendecourt, U. J. Meierhenrich, *ChemPlusChem* **2012**, *77*, 186.

[11] a) W. Kuhn, E. Knopf, *Z. Phys. Chem., Abt. B* **1930**, *7*, 292; b) G. Balavoine, A. Moradpour, H. B. Kagan, *J. Am. Chem. Soc.* **1974**, *96*, 5152; c) H. Rau, *Chem. Rev.* **1983**, *83*, 535; d) H. Nishino, A. Nakamura, Y. Inoue, *J. Chem. Soc., Perkin Trans. 2* **2001**, 1693.

[12] H. Nishino, A. Kosaka, G. A. Hembury, F. Aoki, K. Miyauchi, H. Shitomi, H. Onuki, Y. Inoue, *J. Am. Chem. Soc.* **2002**, *124*, 11618.

[13] S. F. Mason, *Nature* **1997**, *389*, 804.

[14] J. J. Flores, W. A. Bonner, G. A. Massey, *J. Am. Chem. Soc.* **1977**, *99*, 3622.

[15] A. D. Mclaren, D. Shugar, *Photochemistry of Proteins and Nucleic Acids*, Pergamon Press, Oxford, **1964**.

[16] a) U. J. Meierhenrich, L. Nahon, C. Alcaraz, J. H. Bredehöft, S. V. Hoffmann, B. Barbier, A. Brack, *Angew. Chem.* **2005**, *117*, 5774; *Angew. Chem. Int. Ed.* **2005**, *44*, 5630; b) P. de Marcellus, C. Meinert, M. Nuevo, J.-J. Filippi, G. Danger, D. Deboffle, L. Nahon, L. Le Sergeamt d'Hendecourt, U. J. Meierhenrich, *Astrophys. J.* **2011**, *727*, L27.

[17] Y. Takano, J. Takahashi, T. Kaneko, K. Marumo, K. Kobayashi, *Earth Planet. Sci. Lett.* **2007**, *254*, 106.

[18] a) S. Kimura, M. Kimada, H. Hama, X. Maréchal, T. Tanaka, H. Kitamura, *J. Electron Spectrosc. Relat. Phenom.* **1996**, *80*, 437; b) M. Katoh, K. Hayashi, T. Honda, Y. Hori, M. Hosaka, T. Kinoshita, S. Kouda, Y. Takashima, J. Yamazaki, *Nucl. Instrum. Methods Phys. Res. Sect. A* **2001**, *467/468*, 68.

[19] a) I. Mandl, A. D. McLaren, *J. Am. Chem. Soc.* **1951**, *73*, 1826; b) R. Håkansson in *Chemistry of Acid Derivatives, Vol. 2* (Ed. S. Patai), Wiley, Chichester, **1992**, Pt. 1.

[20] a) O. L. Chapman, P. W. Wojtkowski, W. Adam, O. Rodriguez, R. Rucktäschel, *J. Am. Chem. Soc.* **1972**, *94*, 1365; b) O. L. Chapman, *Pure Appl. Chem.* **1974**, *40*, 511.

[21] a) L. Delzeit, B. Rowland, J. P. Devlin, *J. Phys. Chem.* **1993**, *97*, 10312; b) N. Uras, M. Rahman, J. P. Devlin, *J. Phys. Chem. B* **1998**, *102*, 9375; c) H. Kang, T. H. Shin, S. C. Park, I. K. Kim, S. J. Han, *J. Am. Chem. Soc.* **2000**, *122*, 9842; d) J. P. Devlin, N. Uras, J. Sadlej, V. Buch, *Nature* **2002**, *417*, 269.

[22] A. Leadbetter, R. C. Ward, J. W. Clark, P. A. Tucker, T. Matsuo, H. Suga, *J. Chem. Phys.* **1985**, *82*, 424.

[23] H. Nishino, A. Kosaka, G. A. Hembury, K. Matsushima, Y. Inoue, *J. Chem. Soc., Perkin Trans. 2* **2002**, 582.

[24] a) C. Viedma, *Origins Life Evol. Biosphere* **2001**, *31*, 501; b) A. Alvarez Rodrigo, H. Lorenz, A. Seidel-Morgenstern, *Chirality* **2004**, *16*, 499; c) C. Viedma, J. E. Ortiz, T. de Torres, T. Izumi, D. Blackmond, *J. Am. Chem. Soc.* **2008**, *130*, 15274.

Received: May 13, 2013
Revised: July 7, 2013