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Mechanism of pH-Dependent Photolysis of Aliphatic Amino Acids and Enantiomeric Enrichment of Racemic Leucine by Circularly Polarized Light

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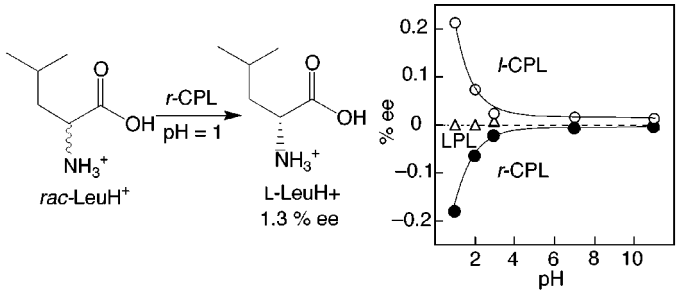
ABSTRACT



It has been proposed that the origin of biological homochirality may be the result of irradiation of a racemic sample of amino acids by circularly polarized light (CPL). To determine the mechanism of enantiomeric enrichment, the irradiation of aliphatic amino acids by CPL was undertaken. An enantiomerically enriched sample (e.g., L isomer enrichment from *r*-CPL) was found to result from the preferential excitation/decomposition of one enantiomer over another via a Norrish Type II mechanism (leucine, valine, and isoleucine), with the enantiomeric excess dependent on the degree of protonation of the amino/carboxylic acid moiety.

A variety of biotic and prebiotic theories have been proposed to explain the origin of the homochirality in biomolecules, and a number of mechanisms have been investigated.[1] One of the most probable hypotheses is that amino acid enantiomeric enrichment arose from interstellar circularly polarized light (CPL) irradiation of a racemic amino acid sample, via an "absolute asymmetric synthesis" (AAS) process. The principle of AAS is based on the different molecular absorption coefficients of an optically pure compound toward left- and right-handed circularly polarized light (*l*- and *r*-CPL), thus giving rise to different photoreaction rates between enantiomers. The degree of preferential excitation is determined by the anisotropy factor (*g* factor), $g = (\epsilon_l - \epsilon_r)/\epsilon = \Delta\epsilon/\epsilon$, where $\epsilon = (\epsilon_l + \epsilon_r)/2$ and $0 \leq g < 2$ (ϵ is the molar extinction coefficient).[2−6]

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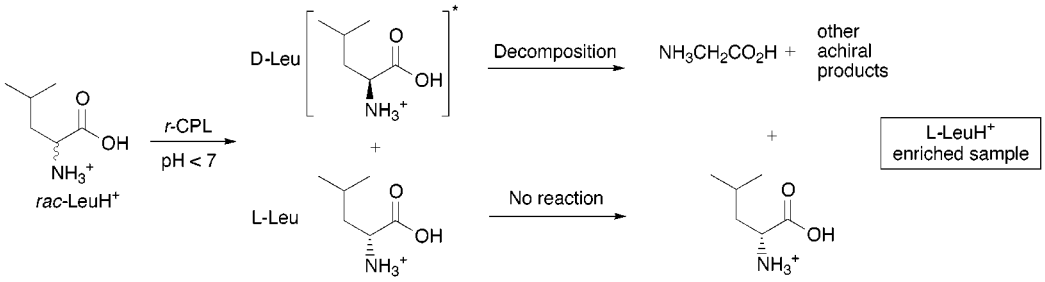
Scheme 1. Representation of the Major Pathway for the Enantioenrichment of *rac*-Leu[a]



[a] The perferential excitation of D-Leu and its resulting decomposition leads to an L isomer enriched sample. The minor pathway (excitation/decomposition of L-Leu) is not shown.

The enantiomeric enrichments of some amino acids have previously been obtained by CPL irradiation,[2,7−9] e.g., the AAS of leucine (Leu) at pH 1 by laser irradiation.[8] However, the pH dependence of the % ee generated by CPL irradiation has not been determined, although it is expected that the amphoteric character of amino acids will influence the outcome of such reactions. Indeed, even the detailed pH dependence of the amino acid *g* factor has not been reported. The mechanism by which these AAS reactions occur has been, as yet, unresolved, key to which has been the poor identification of the photoproducts.[10−12] Although various photochemical mechanisms have been proposed, these are not able to adequately explain the photoproducts detected.[12]

Here we first examine the pH dependence of the chiroptical properties of Leu.[13] Then on irradiation of a racemic Leu (*rac*-Leu) sample by *l*- or *r*-CPL (generated by a polarizing undulator installed in an electron storage ring) we show how pH controls the efficiency of the AAS and thus enantiomeric enrichment. Subsequently, the photodecomposition products of several aliphatic amino acids are identified, verifying the pH dependence of the % ee. Then, crucially, it is revealed that the mechanism of the ee generating photodecomposition of aliphatic amino acids on CPL irradiation is via the preferential excitation and subsequent decomposition of one enantiomer over another (Scheme 1). These results reveal an insight into the conditions necessary for significant enantioenrichment of a racemic amino acid sample and, consequently, a further clue as to the origin of homochirality in biomolecules.

Figure 1 shows the variation of the *g* factor for D- and L-Leu at different pHs. Although the *g* factor of leucine at pH 1 has been reported,[14] Figure 1 shows the first comprehensive analysis of the pH dependence of the *g* factor of an amino acid. On decreasing the pH, the intensity of the *g* factor increases at all wavelengths, but particularly in the 215−220 nm region, although the profile at pH 11 shows some deviation. This is crucial to understanding the possible mechanism by which an excess of one enantiomer may be produced in the CPL irradiation process, as it is the magnitude of the *g* factor that dictates the efficiency of the enantiodifferentiating process, and thus the available ee. At pH 1 the carboxyl group of Leu is in the carboxylic acid state and the amino group is in the ammonium state, with the UV and CD bands at 208 nm arising from the n,π^* transition of the carboxyl group. As the pH changes from 1 to 7, the amino/carboxyl moiety changes to the zwitterionic state. At pH 11 Leu exists as the aminocarboxylate anion. The UV and CD changes are in agreement with the reported literature[15,16] and also show a dependency on the ionic state (and thus pH) of the amino/carboxyl moiety ($pK_{a1} = 2.33$, $pK_{a2} = 9.74$).[17]

Figure 2 shows the final CD spectra of *rac*-Leu irradiated at 215 nm by CPL at various pHs. At pHs 7 and 11 the

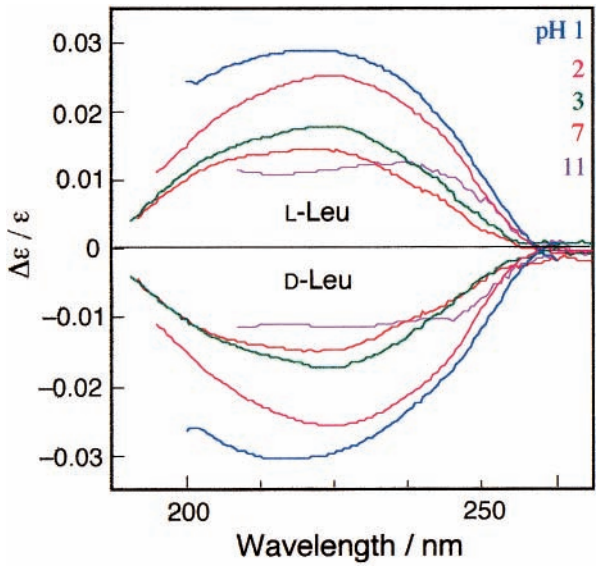


Figure 1. *g* factor of D- and L-Leu at various pHs.

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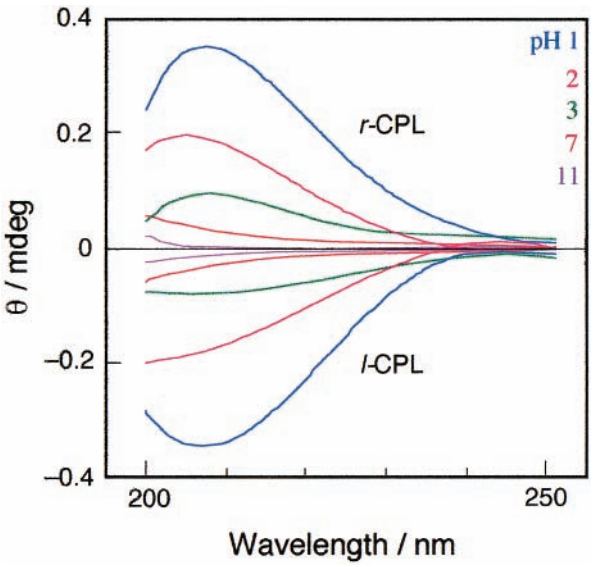

Figure 2. CD spectra of Leu irradiated at various pHs with CPL (dose: 60.3 ± 0.3 mA/h).

intensities of the CD absorptions are nearly equal to zero, but as the pH is reduced, corresponding increases in the intensity of the CD signal are observed, revealing greater enantiomeric enrichment. When Leu was irradiated at pHs 1 and 2, the CD_{max} values were 208 and 205 nm, respectively, with the CD absorptions at pH 1 and 2 closely reflecting the corresponding molar CD spectra.[13] The signs of the observed CDs mean that the preferential excitation and decomposition of D-Leu proceeds by *r*-CPL irradiation and that of L-Leu is by *l*-CPL irradiation.

Figure 3 depicts the pH dependence of the % optical purity (op) of Leu.[13] Each op was estimated using the equation op = $\{\theta_{215}/33(\Delta\epsilon_{215}$ of D- or L-Leu)$\}$/[Leu]. However, for this study, as all the photoproducts are known and (apart from

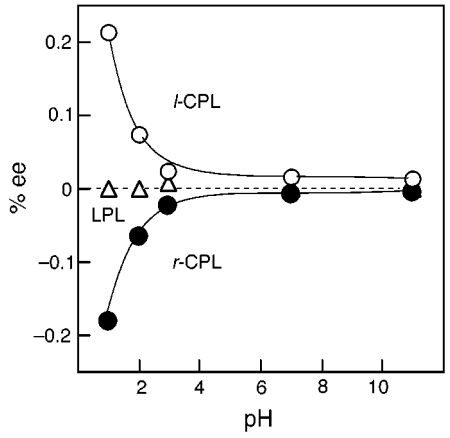

Figure 3. pH dependence of the % ee of Leu irradiated with CPL at 215 nm (dose: 60.3 ± 0.3 mA/h): (○) *l*-CPL, (●) *r*-CPL, (△) LPL.

D- and L-Leu) are achiral (Scheme 1), the % op is equivalent to the % enantiomeric excess (ee). As the solution pH changes from 1 to 11, the ee of Leu becomes smaller and almost zero at pHs 7 and 11.

According to calculations using Kagan's equation, which describes the theoretical relationship between the ee, the % conversion, and the *g* factor,[2,3,14] the observed ee at pH 1 is in good agreement with that predicted.[13]

However, according to Kagan's theory, the ee should remain at 0.1% above pH 7, but the observed % ee was significantly smaller and approaching zero. Even though at pHs 7 and 11 there is almost no ee, *rac*-Leu still undergoes significant achiral photochemical decomposition on CPL and linearly polarized light (LPL) irradiation (% decompositions: pH 1 = 15.4−14.7; pH 2 = 14.7−12.3; pH 3 = 9.9−9.8; pH 7 = 7.5−6.5; pH 11 = 7.3−5.9).[18] Although the degree of photodecomposition becomes smaller as the solution pH changes from pH 1 to 11, Leu is clearly decomposed over the entire pH range.

To rationalize the generation of a significant ee at low pH, but the generation of achiral photoproducts at high pH, product analysis of several aliphatic amino acids irradiated with LPL at pHs 1 and 7 was undertaken (Table 1). From

Table 1. Results of Product Analysis of Aliphatic Amino Acids Irradiated with LPL at pH 1 and 7. Doses Were pH 1, 60.0−60.8 mA/h, and pH 7, 80.1−81.2 mA/h

				products (% yield)	
amino acid	pH	concn (mM)	% convn	Gly	other amino acid
Gly	1	12.4	3.9	0	0
Ala	1	5.7	14.5	0	0.9
Val	1	5.7	23.6	12.4	3.7
Leu	1	5.2	17.4	8.0	0
Ile	1	5.4	22.8	11.8	0
Gly	7	12.3	4.9	0	0
Ala	7	5.4	3.8	0	0
Val	7	5.6	6.4	0	0
Leu	7	5.2	8.1	0	0
Ile	7	5.6	3.3	0	0

product analysis, Gly was detected in the photodecomposition products of *rac*-Ala, *rac*-Leu, *rac*-Val, and *rac*-Ile. In the cases of *rac*-Leu, *rac*-Val, and *rac*-Ile at pH 1, Gly was the main product, with the amount of Gly reaching approximately 50% of the total amount of photodecomposition product. Crucially though, in terms of the reaction mechanism, Gly was not detected in the case of Ala, in disagreement with reported literature.[11] The inference that can be made from this is that the ee generating mechanism (resulting

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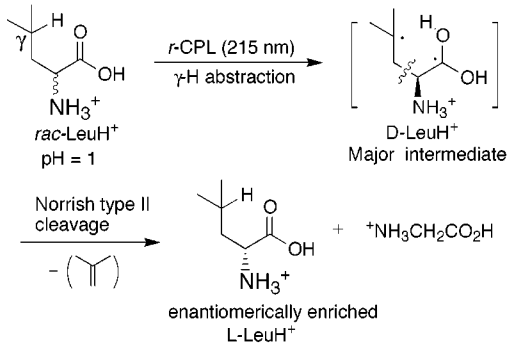
in Gly production) requires the presence of a hydrogen in the γ-position, which is not present in Ala.

These results further indicate that it is the n,π^* transition of the carboxyl group of Leu that governs the pH dependence of the ee and the decomposition; in light of the pH dependence of the ionic composition of Leu,[13] this idea is thought to be reasonable. It can thus be clearly seen that all the different aspects of the reaction such as the ee, % decomposition, and yield of Gly correspond directly with each other and to the ionic state of Leu.

On the basis of the products detected by GC-MS[13] and the production of Gly (nonproduction for Ala), it can be inferred that the photodecomposition of Leu, Val, and Ile at pH 1 proceeds initially via preferential excitation to the biradical of one enantiomer over another (D by *r*-CPL, and vice versa). Both excited enantiomers then decompose via hydrogen abstraction by the excited carboxyl group from the γ-position in a Norrish Type II reaction (Scheme 2).[18] Thus,

Scheme 2. Proposed Norrish Type II Leu Enantioenrichment Mechanism at low pHs



in the case of *r*-CPL irradiation of *rac*-Leu, an ee of the L isomer is obtained. The excited carboxyl group abstracts a hydrogen from the γ-position, resulting in cleavage of the C2−C3 bond, giving Gly and isobutylene. The isobutylene then decomposes to the detected products by radical and/or ionic processes. This demonstrates that the photodecomposition begins via the excitation of the carboxyl group, which is why the *g* factor determines the ee. This enantioselective activation of the carboxyl group, in principle, should be applicable to the other aliphatic amino acids which possess a γ-hydrogen and have similar chiroptical properties (such as Val and Ile).

Irrespective of the solution pH if a significant *g* factor exists at the irradiation wavelength, and the photoreaction proceeds via the excited state, an ee should be observed. The reason this is not observed at high pHs is because in the delocalized carboxylate form the biradical necessary for γ-hydrogen abstraction cannot form. Therefore, it is speculated that some part of the decomposition at pHs 7 and 11 proceeds via a thermal process and/or an indirect photochemical process.

To summarize, the irradiation of *rac*-Leu by *l*- or *r*-CPL results in an enantiomerically enriched sample, which occurs via the differential decomposition of the excited state D and L isomers via a Norrish Type II mechanism, forming the biologically important molecule glycine as the main product. The occurrence of this enantiomeric enriching mechanism (and the magnitude of the ee generated) was found to be entirely dependent on the ionic state (and thus pH) of the amino/carboxylic acid moiety, as indicated by the variation of the *g* factor with pH, as well as the % decomposition. Even though Leu absorbs light between 190 and 240 nm in the zwitterionic and carboxylate forms, irradiation by CPL did not result in the formation of enantiomeric excess, while an ee of 1.3% was obtained at pH 1 for 55% decomposition,[13] clearly revealing the necessity of the amino acid to be in the carboxylic acid form for successful enantiomeric enrichment by CPL irradiation. These results indicate that for successful enantioenrichment of a meteorite born amino acid sample, the carboxylic acid form is required,[19] which is as yet an unresolved issue. Further, this mechanism is thought to be applicable to the other aliphatic amino acids, an issue that is currently under investigation.

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Supporting Information Available: The chiroptical properties of D- and L-Leu at various pHs ranging from 1 to 11, the pH dependence of the decomposition of Leu and the yield of Gly, and the results of product analysis of aliphatic amino acids irradiated with LPL irradiation at pH 1 and 7. This material is available free of charge via the Internet at http://pubs.acs.org.

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