

ENANTIOENRICHMENTS OF α -AMINO ACIDS WITH HIGH-INTENSITY EXCIMER LASER

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It has been found that the irradiation with highly intense circularly polarized light (CPL) from an XeF (351 nm) laser can induce the enantiodifferentiation of racemic methionine with reasonable enantiocontrol by switching the sense of the CPL. Also, a similar result was obtained for threonine and it was newly revealed that the enantioenrichment is largely affected by the chemical structure, especially the functional groups, of α -amino acids, to cause photodecomposition such as decarboxylation and deamination. Furthermore, it was shown for the first time that the irradiation of a proline (Pro)-valine (Val) mixture with the CPL induces their enantioselective decomposition simultaneously, suggesting the occurrence of intermolecular interaction between Pro and Val.

Keywords: Enantioenrichment; α -amino acids; excimer laser; circularly polarized light; high-intensity; enantioselective photodecomposition

INTRODUCTION

α -Amino acids, which are the basic constituent substances of peptides and proteins, are very important compounds biologically. Since they are chiral molecules and usually comprise only one enantiomer in life, an efficient asymmetric synthesis is essential for their preparation [1]. In the preparation of enantiomers, although the absolute asymmetric synthesis is one important reaction, enantiomeric enrichment from a

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racemate plays a very important role because the synthesis of a racemate is often much easier than that of an enantiomer. It is well known that a photochemical method using circularly polarized light (CPL) is also available to prepare enantiomers [2], and much attention is being paid to this photochemical method in connection with the origin of the optically active biomolecules in Nature. However, to date, reports on the enantioenrichment of α -amino acids by this method have been limited to only a few compounds such as alanine and glutamic acid [3], leucine [4], and tyrosine [5]. Also, Norden reported that the photochemical method (Xe lamp light) can not induce the enantioenrichment of proline [3]. Furthermore, it is well known that experiments of this nature using CPL are largely dependent on the anisotropy factor g (the factor to determine the reactivity of the asymmetric photochemical reactions) of substrates, and they have a low limit on the possible extent of enantioenrichment [2]. On the other hand, as reported already [6], we recently demonstrated that such a method using a high-intensity laser can efficiently induce the enantioenrichment of tartaric acid (TA). We therefore attempted to expand this finding to amino acid systems to achieve an efficient synthesis of the enantiomer.

Both methionine (Met) and threonine (Thr) taken up here are essential amino acids and very important materials for medicines and food additives [7]. Also, since the latter has both OH and COOH groups as does TA, besides an NH_2 group, a comparison of the reactivity with TA is of great interest. Moreover, it is well known that several of the derivatives of cysteine, which are a sulfur-containing amino acid like Met, act as a sort of chemical protecting agent against radiation damage [8]. Thus, these chemical and structural characteristics also prompted us to choose the compounds as models of α -amino acids. In this paper, we describe the first report regarding enantiomeric enrichment of both Met and Thr by a photochemical method using a highly intense right (r)- and left (l)-CPL from an XeF (351 nm) laser.

On the other hand, as described previously, proline (Pro), which is an α -amino acid, had not been enantioenriched with Xe lamp light irradiation [3]. In fact, in this regard also, the result of our attempt using a highly intense laser in place of a lamp source was the same as that of Norden. Thus, it has been shown that Pro can not induce the enantioenrichment by a photochemical method even if a highly intense laser is used as a light source. However, it is well known biochemically that in the presence of certain bacteria, both amino acids are subject

to degradation, the so-called Stickland reaction [9, 10], when Pro is available together with valine (Val), similarly to an α - and essential amino acids. This fact also prompted us to choose Val together with Pro as a model of a new enantioenrichment means of α -amino acids which are not enriched individually by a photochemical method. In addition, this pair was chosen as a better one by considering the good solubility in H_2O at room temperature and the good separation-determination in HPLC analyses of these enantiomers. In the last part in this paper, we describe the results for enantioselective and simultaneous photodecomposition of a Pro-Val mixture by irradiation with a focused r-CPL from an XeF laser.

EXPERIMENTAL

Gaseous product analyses were performed using gas chromatographs (Shimadzu GC-3BT, column: molecular sieve 5A; GC-7A, column: Porapak Q and GC-4C, column: Porapak N). High-performance liquid chromatographic analysis was performed using a Shimadzu LC-6A instrument. UV spectra were recorded on a Shimadzu UV-2100 spectrophotometer. IR-spectra were recorded on a Perkin-Elmer FT-IR spectrometer model 1720X using zinc selenide optics.

All the racemates and the enantiomers of α -amino acids (Met, Thr, Pro, and Val) of guaranteed reagent grade used in this study were obtained from Tokyo Kasei.

The XeF laser beam (Lumonics Hyper EX-460, initial power: 2.6 W, frequency: 16 Hz, pulse duration: 12–15 ns, beam shape: $9 \times 34 \text{ nm}^2$) was converted into r-CPL (initial power: 1.3 W at 16 Hz) by passing it through a polarizer ($60 \times 30 \text{ mm}^2$, 5 mm thick) arranged at an angle of 56.5° and a quarter-wave plate ($40 \times 40 \text{ mm}^2$, 0.6 mm thick) orientated with an angle of $+45^\circ$ to the plane of the incident beam by being mounted on a rotatable stage. The l-CPL was also obtained by switching the sense of the r-CPL by rotation of the quarter-wave plate through 90° . Both r-CPL and l-CPL were focused at the center (initial fluence: $3.8 \text{ J cm}^{-2} \text{ pulse}^{-1}$) of the reaction vessel (light path-length: 50 mm) with a condenser lens (φ 60 mm, focal length: 120 cm).

The irradiation with the focused r- and l-CPL was carried out in a Pyrex glass cylindrical reaction vessel (58.5 cm^3) with a Suprasil window (3 mm thick) for the incidence of the light [6]. The aqueous

solutions {[**1**] = 8.59×10^{-2} ($c = 1.28$ g/100 ml), [**2**] = 1.34×10^{-1} ($c = 2$ g/100 ml), [**4**] = 4.20×10^{-1} ($c = 5$ g/100 ml), [**6**] = 2.10×10^{-1} ($c = 2.5$ g/100 ml), [**7** + **8**], [**9** + **10**] = 4.22×10^{-2} mol dm⁻³} were placed in the vessel, respectively, and well-bubbled with N₂. These concentrations were mainly employed on the basis of both the concentration of a standard solution for the measurement of the specific rotations and the solubility for H₂O, respectively [11]. The N₂-saturated solution was stirred magnetically, and irradiated with the light in a thermostatic chamber at ca. 20°C. Furthermore, the temperature of the solution was maintained at 20°C by using an oscillating fan additionally during the irradiation. Also, the temperature was measured with a thermometer inserted in the vessel through the port. The quantity of light was determined using a calorimeter (Sciencetech 38-4UV5). The calorimeter was also placed behind the reaction vessel to determine precisely the quantity of light absorbed in the solution during the irradiation.

Analyses of the irradiated solutions were carried out with high-performance liquid chromatograph [column: SUMICHIRAL OA-6100, column size: 4.6×150 mm, mobile phase: 1 mmol dm⁻³ CuSO₄ (II) in H₂O/CH₃CN (95/5), flow rate: 1.0 ml min⁻¹, column temp.: r. t., detector: UV 254 nm for Met and Thr; column: SUMICHIRAL OA-5000, column size: 4.6×150 mm, mobile phase: 1 mmol dm⁻³ CuSO₄ (II) in H₂O, flow rate: 1.0 ml min⁻¹, column temp.: r. t., detector: UV 254 nm for Pro and Val] by referring to the authentic samples (Tokyo Kasei) in various concentrations. The peaks of **1**–**10** were separated satisfactorily under these analytical conditions.

RESULTS AND DISCUSSION

Simple Systems

Enantioenrichment of Met

Figure 1 shows the concentration of *D*-Met **2** in a solution of *DL*-Met **1** irradiated with a focused r-CPL. The concentration of **2** decreases with increasing absorbed energy. The decrease at an irradiation dose of about 1×10^4 J was ca. 3.6%. On the other hand, the concentration of *L*-Met **3** did not decrease and was almost constant throughout the

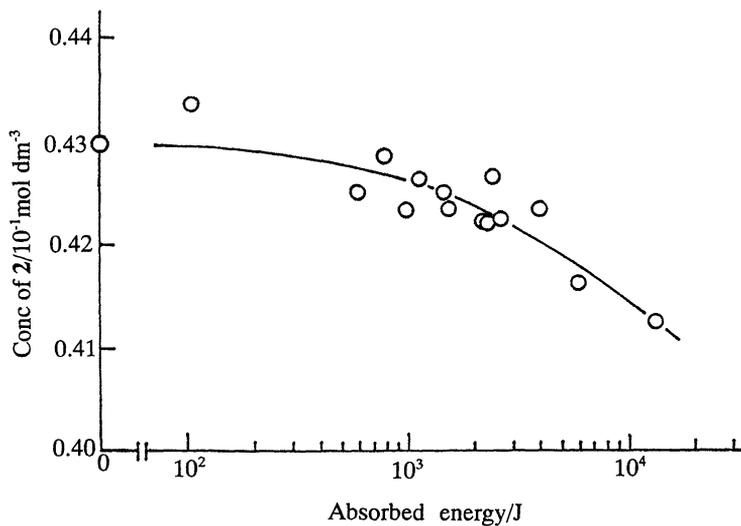


FIGURE 1 The concentration of 2 in the solution of 1 ($8.59 \times 10^{-2} \text{ mol dm}^{-3}$) irradiated with a focused r-CPL.

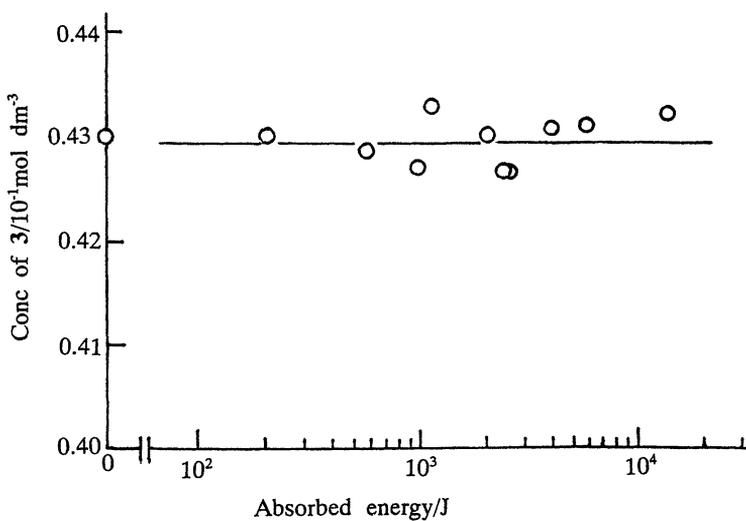


FIGURE 2 The concentration of 3 in the solution of 1 ($8.59 \times 10^{-2} \text{ mol dm}^{-3}$) irradiated with a focused r-CPL.

irradiation of **1** as shown in Figure 2, unlike **2**. Thus, it was clearly revealed that **3** can enrich from the racemate by irradiation with a focused r-CPL from and XeF laser.

In sharp contrast to the r-CPL irradiation, when **1** was irradiated with a focused l-CPL, a decrease in the concentration of **3** was observed as shown in Figure 3, and it decreased with increasing absorbed energy. The decrease at an irradiation dose of about 1×10^4 J was ca. 4.3% in agreement with the case of **2** by the r-CPL irradiation in Figure 1. Similarly, the decrease of **3** was also observed when **3** was irradiated with the l-CPL, supporting the result of Figure 3. On the other hand, as shown in Figure 4, the concentration of **2** remains almost constant throughout the irradiation of **2** with a focused l-CPL. These results clearly indicate that **2** also can be enriched by irradiation with a focused l-CPL from an XeF laser. The enantiomeric enrichment (ee) of **2** estimated from the decrease of **3** in Figure 3 was ca. 2.2% at an irradiation dose of about 1×10^4 J. Although this ee is low, the value is exactly equal to that (2.0~2.5%) [4] in the case of leucine of the same α -amino acids. Furthermore, in the case of Figures 1, 3 and the irradiation for **3** with the l-CPL, gaseous products such as CO, CO₂, H₂, and NH₃ *etc.* were detected as shown in

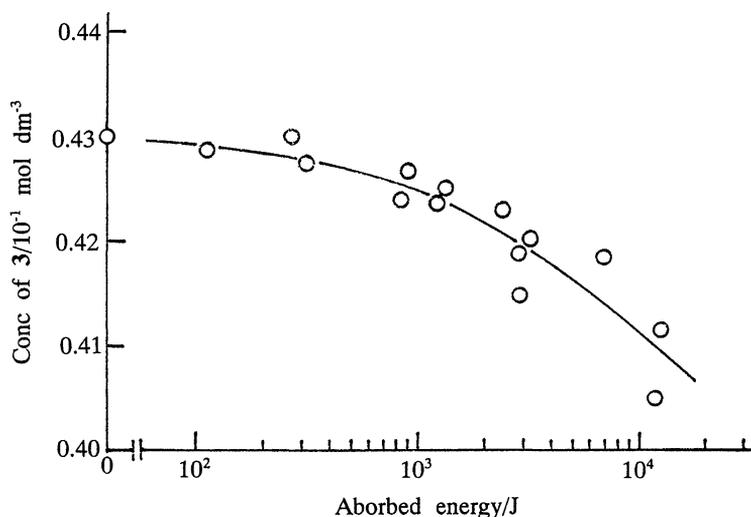


FIGURE 3 The concentration of **3** in the solution of **1** ($8.59 \times 10^{-2} \text{ mol dm}^{-3}$) irradiated with a focused l-CPL.

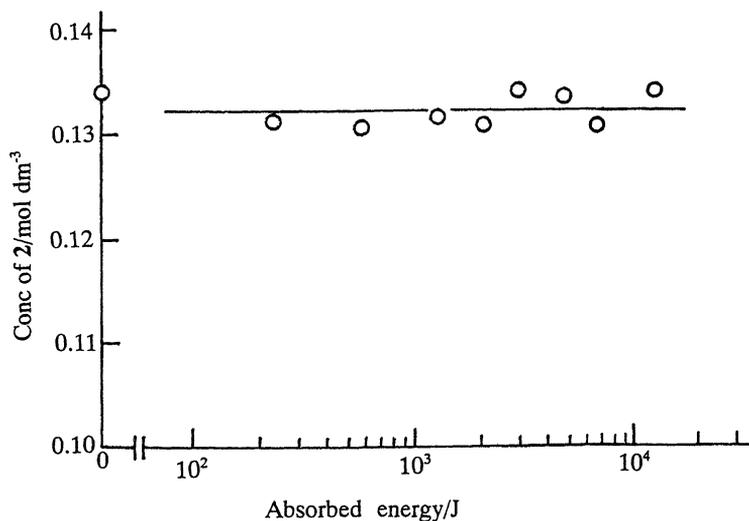


FIGURE 4 The concentration in the solution of **2** ($1.34 \times 10^{-1} \text{ mol dm}^{-3}$) irradiated with a focused l-CPL.

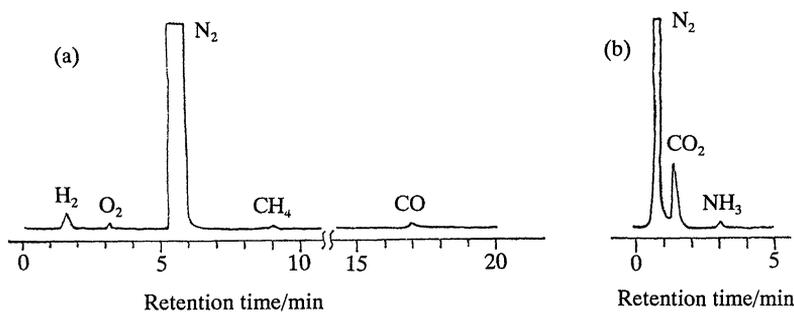
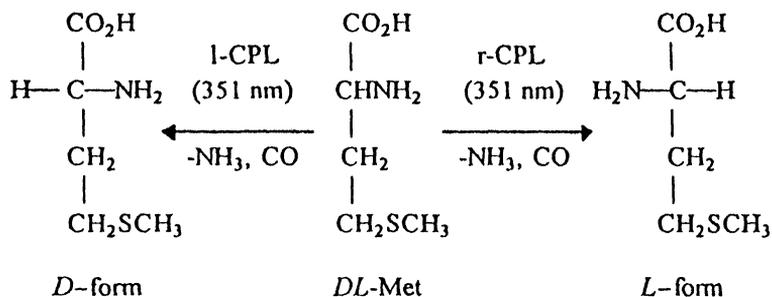


FIGURE 5 Gas chromatograms of evolved gaseous products by the irradiation ($2 \times 10^3 \text{ J}$) of the solution of **1** ($8.59 \times 10^{-2} \text{ mol dm}^{-3}$) with a focused r-CPL. (a) column: molecular sieve 5A 80/100 mesh, detector: TCD, carrier gas: Ar, column temp., 70°C ; (b) column: Porapak N 80/120 mesh, detector: TCD, carrier gas: He, column temp., 120°C .

Figure 5, suggesting photodecomposition had occurred. On the other hand, photoracemization did not occur in this system.

From these results, it can be concluded that high-intensity CPL irradiation can induce the enantiodifferentiating reaction of racemic methionine by switching the sense of the CPL (Scheme 1). It is postulated



SCHEME 1

that the enantiodifferentiating reaction mainly proceeds through the photodecomposition such as decarboxylation [6], as judged from the gaseous products, together with deamination [3] because the height of IR absorption peak at about 550 cm^{-1} of Met after irradiation ($1 \times 10^4 \text{ J}$) was almost the same as that before irradiation, indicating that the scission at the C—S bond did not occur in this system. Furthermore, Met scarcely has absorption at a wavelength (351 nm, 3.5 eV) of the XeF laser light. Accordingly, this reaction would proceed *via* a multiphoton absorption process, similar to that of TA [6]. For this process, we assume that excitations of the carboxyl and amino chromophores in Met occur and that the C—CO₂H, O—H and C—NH₂ bonds are cleaved by the absorption of two photons. In fact, it is probable that this assumption applies to the present reaction: $2 h\nu (\text{XeF}) = 7.1 \text{ eV}$; $E_d(\text{C—C}) = 4.2 \text{ eV}$; $E_d(\text{O—H}) = 4.4 \text{ eV}$; $E_d(\text{C—N}) = 3.6 \text{ eV}$ [12]. On the other hand, the enrichment ($ee = 2.2\%$) were much smaller than that ($ee = 7.5\%$) [6] of TA though the *g*-factor of Met [13] is nearly equal to that of TA [13]. The reason may be considered to be that the stabilization [8] *via* the scavenging by the S atom of the intermediate radical is mainly responsible for such lower enrichment, compared with TA, which mainly causes the decomposition of Met through decarboxylation and deamination, formed on the COOH or NH₂ group by the CPL irradiation.

Enantioenrichment of Thr

As shown in Figure 6, a detectable decrease in the concentration of *D*-Thr 5 was observed when *DL*-Thr 4 was irradiated with a focused

r-CPL, like Met, and it decreased with increasing absorbed energy. Such a decrease of **5** was also observed when **5** was irradiated with the CPL. In contrast, the concentration of *L*-Thr **6** remained almost constant when **6** was irradiated with the CPL as shown in Figure 7. Thus, these results suggest that the enantiomeric system of D-Thr

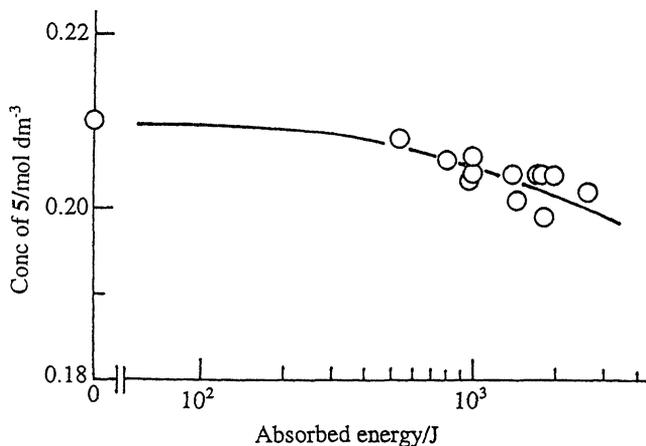


FIGURE 6 The concentration of **5** in the solution of **4** ($4.20 \times 10^{-1} \text{ mol dm}^{-3}$) irradiated with a focused r-CPL.

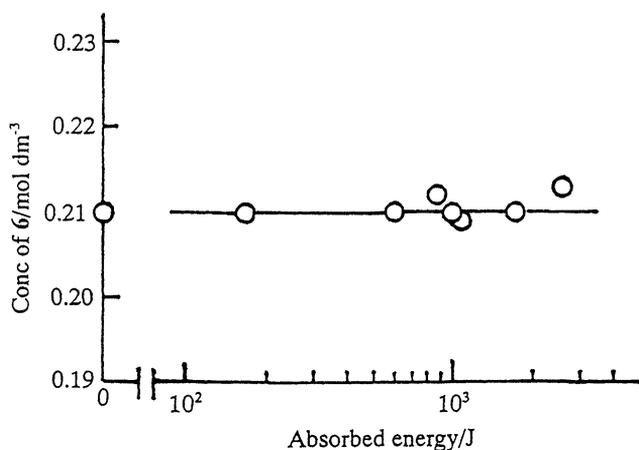


FIGURE 7 The concentration in the solution of **6** ($2.10 \times 10^{-1} \text{ mol dm}^{-3}$) irradiated with a focused r-CPL.

decreases with a highly intense r-CPL from an XeF laser, which results in an enrichment of the *L*-form, like Met.

Binary System

Enantioselective Photodecomposition of Pro-Val Mixture

As shown in Figure 8, the concentration of *D*-Pro **7** by the r-CPL irradiation for the mixture with *L*-Val **8** decreased with increasing absorbed energy, and also that of **8** decreased simultaneously. This is in sharp contrast to the result of Pro alone as mentioned below. The decrease in concentration of **7** was determined to be ca. 5.8% at an irradiation dose of about 2×10^3 J. During this irradiation, gaseous products such as CO, CO₂, H₂, NH₃ *etc.* were detected in analogy with the case of Met shown in Figure 5, indicating that decarboxylation and

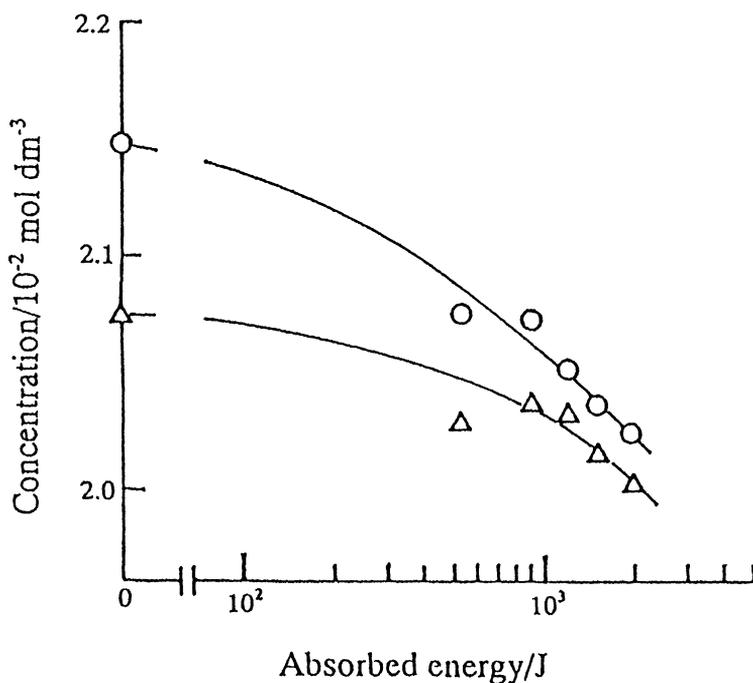


FIGURE 8 The concentrations of both **7** (o) and **8** (Δ) in the mixed solution ($4.22 \times 10^{-2} \text{ mol dm}^{-3}$, fraction of **7** = 50.8 mol%) irradiated with a focused r-CPL.

deamination had occurred to cause decomposition. Accordingly, it can be considered that these decreases by the CPL irradiation are attributable to the photodecomposition of both **7** and **8**. Furthermore, it is particularly noteworthy that Val is simultaneously photodecomposed in this mixed system although there is not yet even a preliminary report on attempt of the enantioenrichment by a photochemical method.

In contrast to the results of Figure 8, as shown in Figure 9, all the concentrations of both *L*-Pro **9** and *D*-Val **10** were hardly changed even by mixing them, in addition to the results for the respective substrate alone at the r-CPL irradiation. Thus, it was evidently revealed that the enantioselective photodecomposition of both Pro and Val proceeds by the CPL irradiation in the mixed aqueous solution.

The irradiation (2×10^3 J) of r-CPL for either only **7** or only **8** did not cause any decomposition. In sharp contrast, in binary system, the photodecomposition of both **7** and **8** was largely affected by the mixture ratio. Moreover, it should be noted that the behavior of the

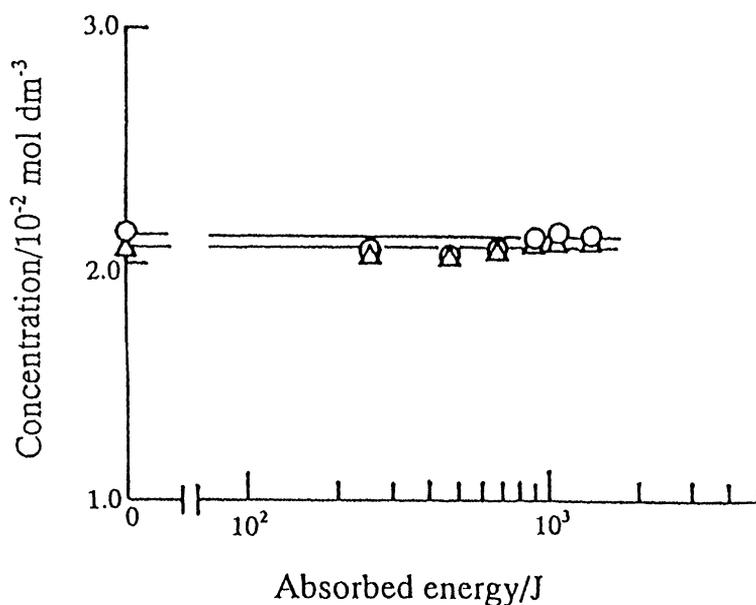


FIGURE 9 The concentrations of both **9** (o) and **10** (Δ) in the mixed solution (4.22×10^{-2} mol dm^{-3} , fraction of **9** = 50.8 mol%) irradiated with a focused r-CPL.

decomposition for **7** and **8** on the mixture ratio is very similar. This result suggests strongly the occurrence of intermolecular interaction between **7** and **8**.

Since the gaseous products similar to those of simple systems were detected, in the binary system as well, the reaction would proceed by their photodecompositions *via* intermolecular interaction between the two α -amino acids. As an example of this interaction, it may be considered that the interaction of the Stickland reaction type [9] where Pro acts as a hydrogen acceptor, while Val as a hydrogen donor, and the two molecules lead to the formation of a complex of some sort. As reported already [6], as the enantioenrichment by a photochemical method using the CPL irradiation with high-intensity laser is achieved through the enantioselective decomposition such as decarboxylation at the COOH site in racemate. Moreover, there has been decomposition *via* deamination at the NH₂ site for amino acids of this case, and then the experimental data described above would show a good possibility of enantioenrichments of both Pro and Val by a photochemical method. Also, this result may provide a new enantioenrichment means for α -amino acids which are not enantioenriched individually by a photochemical method.

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