# Change in the UV-VIS Absorbance of Amino Acids as a Result of Femtosecond Laser Irradiation

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Problems such as genetic damage should be considered in the medical application of lasers. Photochemical damage to living tissue is caused by high-photon energy light. Changes in the UV-VIS absorbance of amino acids by femtosecond laser irradiation are investigated in the present study. Ultraviolet absorption spectra of amino acids were measured following their irradiation by femtosecond laser. The results indicate that changes in ultraviolet absorption appeared at a peak intensity above 70 GW/cm$^2$, suggesting that a change in the chemical structure was caused by multiphoton absorption during the femtosecond laser irradiation.

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**Keywords:** amino acid, femtosecond laser, ultraviolet absorption, photon energy, multiphoton absorption

## 1. Introduction

The femtosecond laser is a candidate for various laser therapies [1-3]. Femtosecond laser radiation may be used to achieve clear and precise cutting of biological tissue [1]. Tissue incision by femtosecond laser is caused by multiphoton absorption, laser-induced break-down, and photothermal ablation [4].

In the case of multiphoton absorption, the tissue is photochemically damaged and denaturation during the laser irradiation because the photon energy of laser radiation is comparable to the binding energy of several molecules contained in the tissue [5]. The wavelength of the Ti:sapphire laser, which is a typical femtosecond laser, is nominally 800 nm, which corresponds to a photon energy of 1.6 eV. For three-photon absorption, the photon energy is approximately 4.8 eV, which breaks the chemical bonds. Unlike their use in material processing, there are a number of problems peculiar to the medical application of the femtosecond laser because of the nature of living tissue.

In the present study, the influences of femtosecond laser irradiation on soft tissue were examined. Amino acid solutions were chosen as samples. Ultraviolet (UV) absorption spectra have been measured to examine the changes in the structures of amino acids as a result of femtosecond laser irradiation.

## 2. Experiment

The experimental arrangement used in the present study is shown in Fig. 1. The femtosecond laser system, which consists of a fiber laser oscillator and a Ti:sapphire amplifier, was operated at a repetition rate of 100 Hz. The laser beam was plane polarized after passing through the polarizer in the laser system. The pulse width was 150 fs at a nominal wavelength of 775 nm, which was estimated using a background-free second-order autocorrelator.

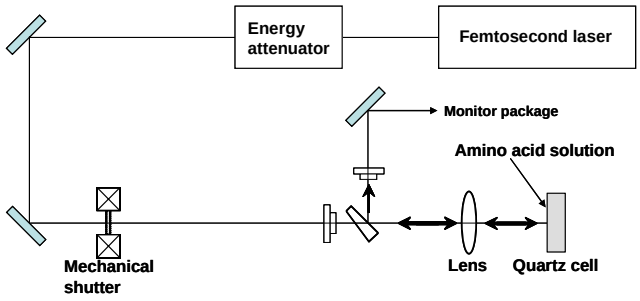
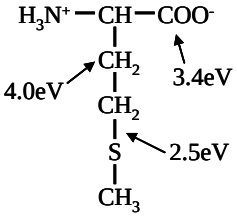
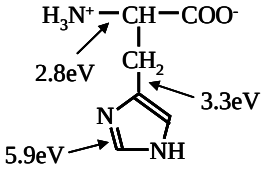



**Fig. 1** Schematic diagram of the experimental arrangement.

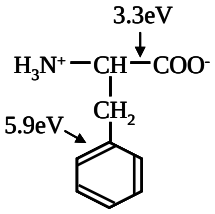
A histidine, a phenylalanine, and a methionine, which are essential amino acids, were adopted as samples. They were dissolved in the distilled water at pH 7. The concentration of a histidine, a phenylalanine, and a methionine solutions were adjusted to be 0.05, 0.005, and 0.05 mol/kg, respectively. Each solution was used to fill a quartz cell that has no absorption at the laser radiation wavelength of 775 nm (photon energy of 1.6 eV). Figure 2 shows the structure of the three amino acids, (a) methionine, (b) phenylalanine, and (c) histidine. The energies shown in these figures indicate the binding energy estimated in the single bond [6]. At the low-intensity region, 1.6-eV laser radiation has no effect on the bonding. The multiphoton absorption at the higher intensity region would affect the bonding, which causes dissociation of bonds and denaturation of the amino acid.




(a) methionine




(c) histidine



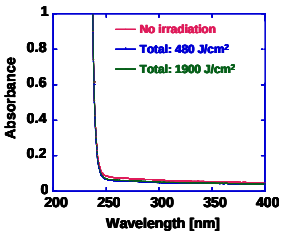

(b) phenylalanine

**Fig. 2** Structure of amino acid for (a) histidine, (b) phenylalanine, and (c) methionine.

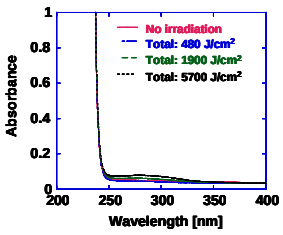
The laser radiation is incident into the energy attenuator in order to control the energy reaching the sample. The laser is then focused through a lens having a focal length of 100 mm. A quartz cell filled with an amino acid solution was placed at the quasi far-field in order to suppress the air break-down due to the high-intensity field in the air. The monitor package shown in Fig. 1 consists of image relay optics and a CCD camera and can measure the laser beam profile on the quartz cell. The laser energy fluence was adjusted to be 8, 11, and 16 mJ/cm$^2$•pulse, corresponding to peak intensities of 53, 74, and 106 GW/cm$^2$, respectively. Throughout the experiments, the energy fluence was measured within an uncertainty of ±5%. After laser irradiation, UV absorption spectra were measured using a SHIMADU UV-160 spectrometer.

## 3. Results and discussion

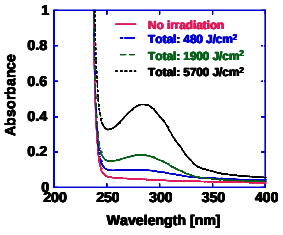
Figure 3 shows the UV absorption spectra of histidine for (a) 8 mJ/cm$^2$•pulse (53 GW/cm$^2$), (b) 11 mJ/cm$^2$•pulse (74 GW/cm$^2$), and (c) 16 mJ/cm$^2$•pulse (106 GW/cm$^2$). The fluences shown in these figures denote the accumulated irradiation energy. The UV spectra of non-irradiated histidine are also shown for comparison. In Fig. 3(c), the absorption between 250 to 320 nm clearly appeared through femtosecond laser irradiation. This absorption increased with increasing accumulated irradiation energy fluence. In Fig. 3(b), UV spectra were slightly changed by laser irradiation. A remarkable difference in the UV absorption spectra was observed between Figs. 3(b) and 3(c) for the case of the accumulated irradiation energy of 5,700 J/cm$^2$. This suggests that the change in UV absorption is not related to the total irradiation energy and is an intensity-dependent phenomenon. However, no changes in the UV spectra were observed for the low-intensity irradiation case shown in Fig. 3(a).




(a) 8 mJ/cm²•pulse (53 GW/cm²•pulse)

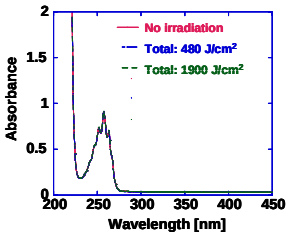



(b) 11 mJ/cm²•pulse (74 GW/cm²•pulse)

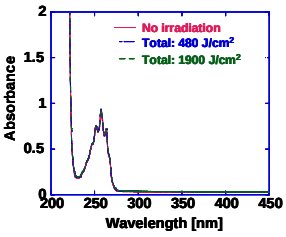



(c) 16 mJ/cm²•pulse (106 GW/cm²•pulse)

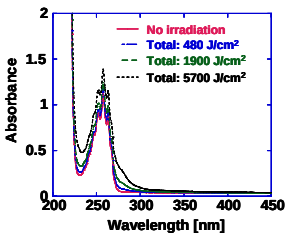
**Fig. 3** Ultraviolet absorption spectra of histidine for (a) 8 mJ/cm$^2$pulse (53 GW/cm$^2$pulse), (b) 11 mJ/cm$^2$pulse (74 GW/cm$^2$pulse), (c) 16 mJ/cm$^2$pulse (106 GW/cm$^2$pulse)




(a) 8 mJ/cm²•pulse (53 GW/cm²•pulse)




(b) 11 mJ/cm²•pulse (74 GW/cm²•pulse)




(C) 16 mJ/cm²•pulse (106 GW/cm²•pulse)

**Fig. 4** Ultraviolet absorption spectra of phenylalanine for (a) 8 mJ/cm$^2$pulse (53 GW/cm$^2$pulse), (b) 11 mJ/cm$^2$pulse (74 GW/cm$^2$pulse), (c) 16 mJ/cm$^2$pulse (106 GW/cm$^2$pulse)

The UV absorption spectra of phenylalanine are shown in Fig. 4 for (a) 8 mJ/cm$^2$•pulse (53 GW/cm$^2$), (b) 11 mJ/cm$^2$•pulse (74 GW/cm$^2$), and (c) 16 mJ/cm$^2$•pulse (106 GW/cm$^2$). The absorption around 250 nm is due to the aromatic component in the phenylalanine. The enhancement of this absorption was induced at the higher intensity irradiation in Fig. 4(c). On the other hand, no difference for the cases before and after laser irradiation was observed in Figs. 4(a) and 4(b). For the methionine solution, the UV absorption spectra did not change for the highest intensity (106 GW/cm$^2$) in the present study.

The intensity dependence of the absorption at the wavelength of 280 nm for histidine and phenylalanine is shown in Fig. 5. This indicates the accumulated irradiation energy fluence of 1,900 J/cm$^2$. The absorption enhancements are

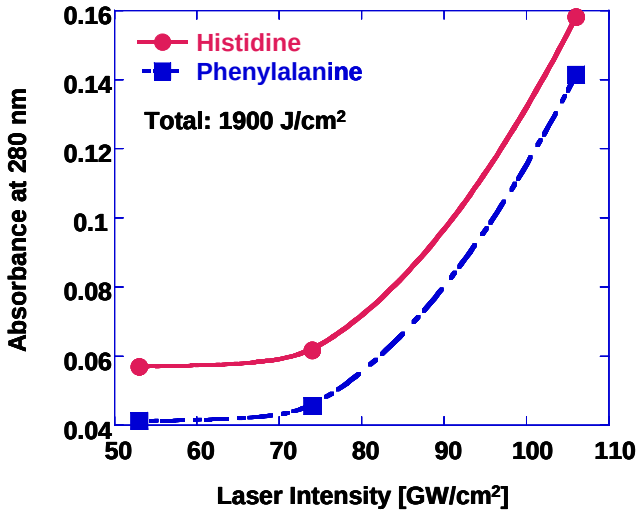



**Fig. 5** Intensity dependence of the absorption at the wavelength of 280 nm.

nonlinear, so that the multiphoton absorption affects the UV spectrum.

These results demonstrate the change in UV absorption caused by femtosecond laser irradiation, which depends on the peak intensity. A particularly dramatic change in the spectra was observed for the histidine. The primary purpose of the present study is to examine the change in amino acids caused by multiphoton absorption.

The histidine, which has an imidazole ring, has a weak absorption caused by the n to $\pi^*$ transition in the UV region. The form of the UV absorption spectrum after laser irradiation is similar to that affected by the oxidation of an imidazole ring. The mechanism of chemical reaction would be a cycloaddition of excited oxygen to the imidazole group, which result in an unstable cyclic peroxide [7-8]. Then the aromatic system would be reformed via proton rearrangement. This reaction influences the absorption in the near UV region [8]. The increase of absorption at 280 nm is almost proportional to the accumulated energy fluence, suggesting that the amount of oxygen consumed is determined by the peak intensity of laser pulse. The multiphoton absorption due to high-intensity femtosecond laser irradiation is thought to cause the change of imidazole ring.

For phenylalanine, the enhancement of the absorption around 250 nm can be observed after laser irradiation. The aromatic component is influenced by multiphoton absorption. The change in the UV spectra is slight. The UV spectra should be measured in order to investigate the detailed mechanism under a region of higher intensity than that investigated in the present experiment.

## 4. Summary

UV absorption spectra of femtosecond laser-irradiated amino acids have been measured to examine the change in chemical structure caused by multiphoton absorption. Dramatic changes in the spectra, which depended on the peak intensity of the femtosecond laser, were observed in the histidine. The oxidation of a heterocyclic component affects the UV absorption spectra. Actual medical application of femtosecond laser requires a higher energy and a higher intensity than that examined in the present study. Problems concerning the breaking of chemical bonds through multiphoton absorption should be discussed further.

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