Effects of UV-B irradiation on the stability of amino acids and peptides

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Abstract

Ultraviolet (UV) light has been reported to influence on various biological components. In this study, we examined the stability of amino acids and peptides against the irradiation of UV-B, the light with 320-400 nm wavelength range. The experiments using 15 types of peptides suggested that the peptides containing five amino acids, Trp, His, Met, Phe, and Tyr were easily reduced by UV-B irradiation. Both stabilities of amino acid and peptide against UV-B irradiation were differed by the presence of coexistent of other amino acids. The amino acid, Met was drastically reduced by UV-B irradiation only in the case of coexistent of other amino acids, and the peptides located in the αA-crystallin protein also were easily degraded by the irradiation. These results may suggest that amino acids, which easily absorb UV light, had an effect on the stabilities of other amino acid and some peptide fragments.

Key words : ozone hole, HPLC, eye
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Introduction

Ultraviolet (UV) light is electromagnetic radiation with a wavelength shorter than that of visible light, but longer than x-rays. The electromagnetic spectrum of UV light is subdivided; the light with 320 - 400 nm and 280 - 320 nm wavelength ranges are named as UV-A and UV-B, respectively. Both UV-A and UV-B are reached to the Earth's surface, but almost all reached UV radiation is UV-A due to absorption in the atmosphere's ozone layer. However, recent ozone layer depletion is expected to increase surface UV-B level.

Overexposure of UV-B light has been reported to influence on various biological components. For example, UV-B damages collagen fibers and vitamin A in the skin and thereby accelerates aging of the skin[1]. Overexposure of UV-B also damage DNA with the formation of thymine dimmers, which results in the formation of mutations and inducing cancers. Some amino acids such as tryptophan (Trp) and tyrosine (Tyr) can absorb UV-B light and thereby it is possible that the UV-B irradiation may alter the structure or function of the peptides or proteins containing such amino acids. Some reports show that UV-B altered secondary conformation in protein and induced the stereoinversion of specific amino acid[2,3]. In this study, we investigated the stabilities of amino acids and peptides against UV-B irradiation and found that the stability of amino acids and peptides altered by their situation, that is, the stability of amino acid, Met was drastically reduced in the amino acid mixture and the stabilities of some peptide fragments were also drastically reduced when UV light was irradiated to the original protein.

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Materials and Methods

1. UV treatment

The peptides and amino acids were dissolved in the 50 μM phosphate buffer at final concentration to 100 μM, and the protein, αA-crystallin was dissolved at final concentration to 2 mg/mL. The specimens were placed in the transparent glass sample tube and degassed. The tubes were made airtight, and placed on the UV lumps attached in the UV irradiation apparatus AB-1500 (ATTO) at 4 ℃for 24 h.

2. Amino acid analysis

The amino acids were separated by HPLC with an ion-exchange column (MCI GEL AFR2PC, 6 mm i.d. x 50 mm, Mitsubishi Chemicals), detected as *o*-phthalaldehyde derivatives, and monitored with JASCO FP-920 (Nippon Bunko) with the excitation and the emission wavelengths of 344 nm and 443 nm, respectively.

3. Peptide synthesis and protein preparation

The peptides used in the study were synthesized by standard Fmoc chemistry using a Shimadzu PSSM-8 peptide synthesizer (Shimadzu). Fmoc-protected amino acids were purchased from Watanabe Chemical Co. Ltd. The synthesized peptides were purified by reversed-phase HPLC and confirmed by mass spectrometry. The recombinant αA-crystallin was prepared according to the methods described in the previous manuscript[4].

4. HPLC separation

To determine the remaining rates of various peptides, the peptides were separated by reversed-phase HPLC using a JASCO HPLC system 880 (Nippon Bunko) with a C18 column, Develosil ODS-UG-5 (4.6 mm i.d. x 150 mm, Nomura Chemical) with appropriate concentration of acetonitrile containing 0.1 % , with monitoring at 215 nm. The tryptic hydrolysate of αA-crystallin was also separated by reversed-phase HPLC with a C18 column with a linear gradient of 0 － 30 % acetonitrile containing 0.1 % TFA, over 30 min at a flow rate of 1 mL/min, with monitoring at 215 nm.

Results and Discussion

1. *Effect of UV-B irradiation on the stability of various peptides.*

We firstly investigated the stability of various peptides against UV-B irradiation. The peptides were irradiated with the UV-B light in aqueous solution for 24 h at 4 ℃, and these amounts were determined by reversed phase HPLC (Fig. 1). In the examined 15 types of peptides, the amount of four types of peptides named B (RW), C (VW), F (SLHTLF), and L (HFSPEDLTVK) were drastically reduced by UV-B irradiation. The amount of three types of peptides named K (DRVYIHPF), N (IQTGLDATHAER),andO(IMDGEADAMSLDGGFVYIAGK) also reduced by UV-B irradiation. Comparing the sequences of reduced peptides and unaffected peptides, we notices five amino acids, Trp, histidine (His) , methionine (Met), phenylalanine (Phe), and Tyr because the reduced peptides commonly contained these amino acids. (These amino acids were underlined in each peptide described above.)

2. *Effect of UV-B irradiation on the stability of amino acids.*

We examined the stability of five amino acids, Trp, His, Met, Phe, and Tyr against UV-B irradiation. These five amino acids and one control amino acid, leucine (Leu) were irradiated independently with UV-B light for 24 h and the amounts of amino acids were determined by amino acid analyzer (Fig. 2). UV-B irradiation reduced the amount of amino acids, Trp, Tyr and His, but not that of amino acids, Met, Phe and Leu. We further examined the stabilities of amino acids on the condition other amino acids coexisted. The mixture containing fifteen amino acids, aspartate (Asp), threonine (Thr), serine (Ser), glutamate (Glu), glycine (Gly), alanine (Ala), valine (Val). Met, isoleucine (Ile), Leu, Tyr, Phe, lysine (Lys), His, and arginine (Arg) were irradiated with UV-B for 24 h. The amounts of remaining amino acid in the mixtures were analyzed by amino acid analyzer. The HPLC profiles of the mixtures before and after UV-B irradiation were shown in Fig. 3A and B, respectively. The quantitative results of Fig. 3 are shown in the Fig. 4, which showed that the amounts of amino acids, Met,

Tyr and His were drastically decreased, and Phe was not reduced by UV-B irradiation. These results show that the actions of three amino acids, Tyr, His and Phe are not different between the conditions that UV-B was irradiated to sole amino acid and to the mixture of amino acid. However, the action of Met was different between two conditions. This result suggests that coexisting amino acids has an effect on the stability of Met against UV-B irradiation.

3. *Effect of UV-B irradiation to the original protein on the peptide stability*

We also examined the stability of peptide fragments on the condition that other amino acids coexisted. UV-B was irradiated to the protein, αA-crystallin, which included the three peptide fragments named L (HFSPEDLTVK), M (TVLDSGISEVR) and N (IQTGLDATHAER), and these peptide fragments were isolated and quantified by reversed phase HPLC. Fig. 6A shows that the amounts of all three isolated peptides were drastically reduced when UV-B was irradiated to the protein. The remaining rates of the isolated peptide fragments were nearly zero. We further examined the effect of irradiation of UV-A, the energy of which is lower than that of UV-B, to the αA-crystallin protein on the stability of three peptide fragments (Fig. 6B). Contrary to our expectation, the UV-A irradiation to the protein also drastically reduced the remaining rates of all three peptide. The remaining rates were almost same as that with UV-B irradiation, whereas the stabilities of the peptides were not affected when UV-A was irradiated to the peptide fragment directly. The αA-crystallin used in the study includes the Trp residue, which may be able to absorb the UV-A light because the UV-A light apparatus irradiates wide range of wavelength. The presence of Trp residue may give explanation for the degradation of the peptides when UV-A is irradiated to the protein. Anyway, in this study we found that peptide fragments were very fragile against UV irradiation in αA-crystallin protein. Since αA-crystallin is major protein composing eye lens, the exposure to even UV-A light should be avoided.

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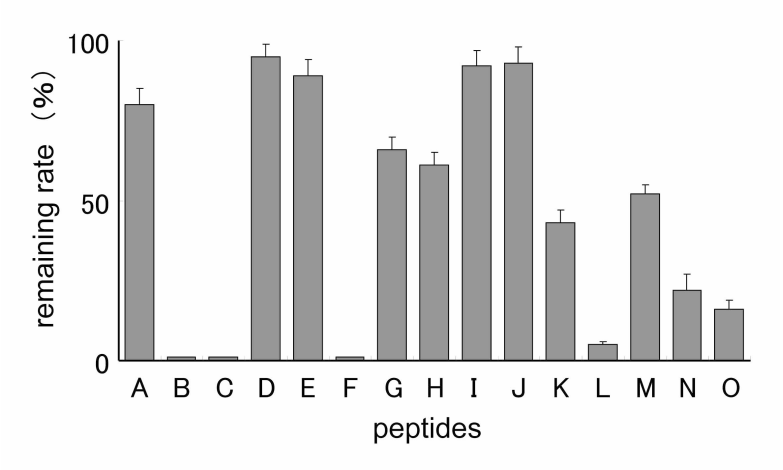

Fig. 1. Effect of UV-B irradiation on the stability of the various peptides. Each peptide was dissolved in the phosphate buffer at final concentration to 100 μM, and irradiated with UV-B light for 24 h at 4 ℃. The amount of each peptide was determined by reversed phase HPLC and the remaining rates were calculated by the comparison with initial amount of the peptide. The amino acid sequence of each peptide was as follows. A, FL; B, RW; C, VW D, LIY, E, MDAK, F, SLHTLF; G, GLVDAY; H, KYLEIAA; I, VVSVLT; J, FQNAL; K, DRVYIHPF; L, HFSPEDLTVK; M, TVLDSGISEVR; N, IQTGLDATHAER; O, IMDGEADAMSLDGGFVYIAGK. Data are expressed as means ± SD (n = 3).

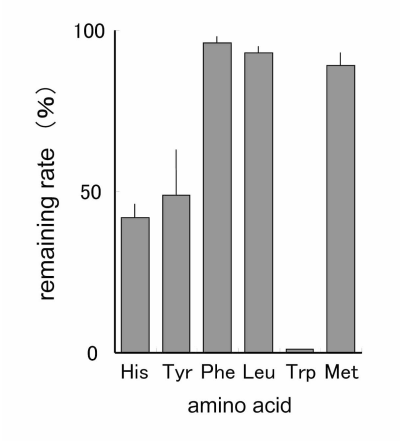

Fig. 2. Effect of UV-B irradiation on the stability of the various amino acids. Each amino acid was dissolved in the phosphate buffer at final concentration to 100 μM, and irradiated with UV-B light for 24 h at 4℃. The amount of each amino acid was determined by amino acid analyzer and the remaining rates were calculated in comparison with initial amount. Data are expressed as means ± SD (n = 3).

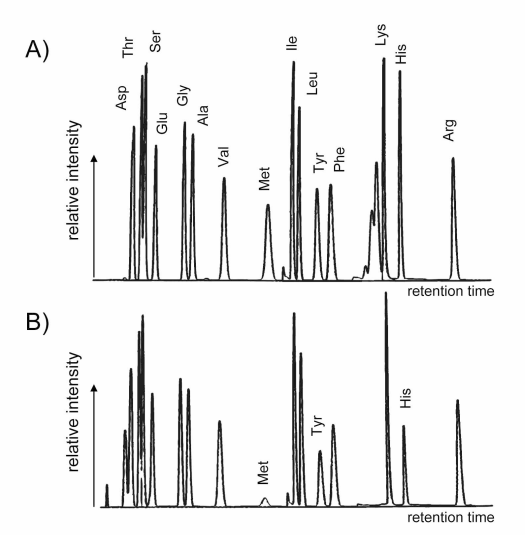

Fig. 3. HPLC profiles analyzing amino acid contents in the mixture before (A) and after (B) UV-B irradiation. The amino acid is indicated on the top of each peak, and detected with the fluorescence as *o*-phthalaldehyde derivative (excitation 344 nm, emission 443 nm).

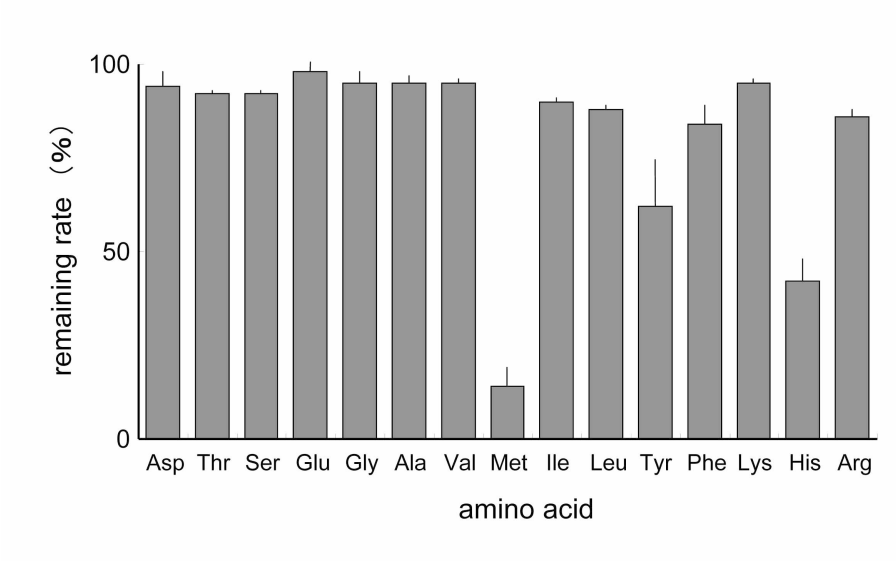

Fig. 4. The quantitative results of Fig. 3. The remaining rate in each amino acid was calculated in comparison to the amount of non-treated amino acid. Data are expressed as means ± SD (n = 3).

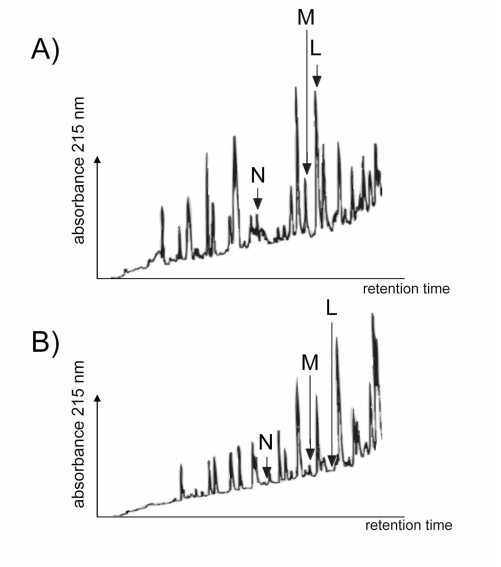

Fig. 5. HPLC profiles of tryptic digestion of αA-crystallin treated without (A) or with (B) UV-B irradiation. Recombinant αA-crystallin was dissolved in phosphate buffer and irradiated with UV-B light for 24 h. The specimen was digested with trypsin and the peptide fragments were separated by reversed phase HPLC. The marks, L, M and N indicates the peptide fragments, HFSPEDLTVK, TVLDSGISEVR and IQTGLDATHAER; respectively.

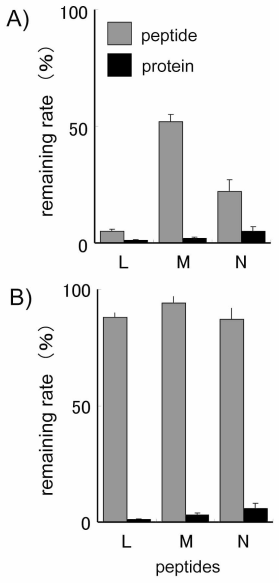

Fig. 6. Effect of irradiation with UV-B (A) and UV-A (B) on the stability of three peptide fragments, L (HFSPEDLTVK), M (TVLDSGISEVR) and N (IQTGLDATHAER). The UVs were irradiated to the peptide fragments or αA-crystallin protein for 24 h. The peptide fragments of αA-crystallin were isolated and quantified by HPLC. Data are expressed as means ± SD (n = 3).

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紫外線（UV-B）がアミノ酸とペプチドの安定性に及ぼす影響

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日本語要旨

紫外線は様々な生体分子に影響を与えることが知られている．今回，我々はUV-B紫外線に対するアミノ酸およびペプチドの安定性を調べた．15種類のペプチドにUV-Bを照射した実験結果から，Trp，His，Met，Phe，Tyrの5種類のアミノ酸を含むペプチドの安定性が低いことがわかった．アミノ酸およびペプチドの安定性は共存するアミノ酸の有無で大きく変化することも明らかになった．Metは共存するアミノ酸があるときのみUV-B照射で減少し，αAクリスタリンを構成するペプチド断片は，タンパク質に紫外線を照射したときに容易に分解した．これらの結果は紫外線を吸収しやすいアミノ酸が他のアミノ酸やペプチド断片の安定性に影響を与えていることを示唆している．

キーワード：オゾンホール，HPLC，眼