VIII-Ⅱ-1. Project Research

Project 3

PR3 Project Research on the Abnormal Aggregation of Proteins by Post-Translational Modifications, and Study of Repair Mechanism

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Objectives and Allotted Research Subjects:

The aim of this project research is to elucidate the correlation between the change of the protein structure induced by various post-translational modifications with UV irradiation, gamma-irradiation, aging and protein function. We also investigate the repair mechanism for the damaged protein by irradiation. This research program has started in 2011. In this year, the 6 research subjects were carried out. The allotted research subjects (ARS) are as follows;

ARS-1: Detection of D-aspartyl endopeptidases activity in plants. (T. Kinouchi and N. Fujii)

ARS-2: Damage to biological molecules induced by ionizing radiation and biological defense mechanisms provided by radical scavengers III. (T. Saito and N. Fujii)

ARS-3: Expression of protein L-isoaspartyl methyltransferase (PIMT) and analysis of substrate specificity using prion peptide fragments. (Y. Sadakane and N. Fujii)

ARS-4: Analysis of environmental stress-related hearing loss in mice. (N. Ohgami and N. Fujii)

ARS-5: Change in enantioselectivity of γ-tryptphan synthase. (A. Shimada, N. Fujii and T. Saito)

ARS-6: UV-B exposure leads to simultaneous photo-oxidation of tryptophan/tyrosine and racemization of neighboring aspartyl residues in peptides. (N. Fujii, S. Cai, N. Fujii and T. Saito)

Main Results and Contents of This Project

ARS-1: *Kinouchi et al.* searched for D-aspartyl-endopeptidase (DAEP) in plants. As a result, the DAEP activity was detected in radish cultivar (*Raphanus sativus var. sativus*). Since plants are also exposed to various and severe stresses, DAEP-like system is supposed to have especially developed in plants to maintain the protein homeostasis.

ARS-2: Saito *et al.* showed that astaxanthin does not affect gamma radiation-induced peroxidation of α-linolenic acid, suggesting that carotenoid affects radical reactions that proceed after the lipid peroxidation reaction in the biological defense mechanism *in vivo*.

ARS-3: Sadakane *et al.* prepared a repair enzyme for aged proteins, protein L-isoaspartyl methyltransferase (PIMT) by *E. coli* expression system, and analyzed the substrate specificity of its recombinant enzyme using prion peptide (106–126) bearing L-Asp, L-isoAsp, D-Asp or D-isoAsp residue. Our recombinant PIMT only catalyzes the peptide bearing L-isoAsp but not one bearing D-Asp, which is substrate for PIMT of other species.

ARS-4: Ohgami *et al*. showed that barium administered by drinking water specifically accumulates in inner ears resulting in severe impairments of hearing with degeneration of inner ears in mice. We are investigating whether hearing loss involves aggregation of a specific protein in the auditory neurons in inner ears.

ARS-5: Shimada *et al.* investigated the difference of the enantioselectivity between γ-TSase and γ-tryptophanase that synthesize L-tryptophan from L-serine and indole. γ-TSase activity showed a sharp depression different from tryptophanase. This indicates that TSase enantioselectivity is more susceptible to γ-ray irradiation than tryptophanase.

ARS-6: Fujii et al. investigated that isomerization of Asp residues is affected by the existence of UV absorbable tryptophan or tyrosine residue in the peptide. The results clearly indicated that D-Asp was generated by UV-B exposure when there is tryptophan or tyrosine nearby aspartyl residue in peptide sequence.

採択課題番号 25P3　放射線照射や酸化的ストレスによるタンパク質中のアミノ酸残基の修飾が誘起するタンパク質の異常凝集―その防御及び修復機構に関する研究　プロジェクト

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PR3-1 Detection of D-Aspartyl Endopeptidases Activity in Plants

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INTRODUCTION: D-isomer of Asp (D-Asp) residue is often detected in abnormally aggregated proteins causing age-related diseases (i.e., cataract, prion disease and Alzheimer's disease), and it is strongly suggested that formation of D-Asp in human proteins is potentially noxious for metabolic turnover. The D-aspartyl endopeptidase (DAEP), we have discovered from mammals and have been characterizing [1–3], stereoselectively degrades its substrate at the internal D-Asp residue, and seems to physiologically serve as a scavenger against the noxious D-Asp containing-protein. Since oxidative stress causes a severe conformational change of a native protein by D-Asp formation, DAEP is supposed to have especially developed in long life span animals such as mammals to maintain the protein homeostasis.

On the other hand, DAEP is not confined to mammals: for example, with African clawed frog (*Xenopus laevis*), high DAEP activity was detectable in its testes, ovaries and unfertilized eggs. However, the distribution of DAEP in plants is not as clear as in animals. Since plants are also exposed to various and severe stresses such as UV, it is no wonder that plants have DAEP-like system. We therefore started examining the distribution of DAEP in plants used for biological experiments.

EXPERIMENTS: Plant Materials and Growth Conditions> Seeds of radish cultivar (*Raphanus sativus var. sativus*) and wild-type micro-tom （*Solanum lycopersicum* L.）, a dwarf cultivar of tomato, were purchased from Takii Seed (Kyoto, Japan) and Inplanta Innovations Inc., respectively. Those seeds were sown on vermiculite moistened with deionized water and incubated at 22°C under a 16-h light/8-h dark cycle in a 60%-humidified growth chamber. Germinated seeds were then transplanted into hydroponic media containing major nutrients (1 mM $Ca(NO_3)_2$, 1 mM KCl, 0.5 mM $MgSO_4$, 0.25 mM $(NH_4)_2HPO_4$, and 0.18 mM Fe(III)-EDTA) and micronutrients (46 μM H_3BO_3, 9 μM $MnCl_2$, 0.8 μM $ZnSO_4$, 0.3 μM $CuSO_4$, and 0.08 μM $(NH_4)_6Mo_7O_{24}$), and grown in the chamber under the same conditions.
Extraction of Crude Enzyme> Radish taproots and tomato fruits were harvested after 20 and 45 days of hydroponic culture, respectively, and immediately homogenized by a Polytron agitator on ice. The homogenate was centrifuged at 10,000 g for 20 min at 4°C. Because those supernatants were crude enzyme mixtures that might include not only DAEP but also other proteases, the appropriate dose of protease inhibitor cocktail for plant cell extracts (purchased from Sigma-Aldrich, Inc) was added into the supernatant.
Measurement of DAEP activity> We developed an assay system for DAEP activity using the synthetic D-Asp containing substrate, Succinyl-D-Aspartic acid α-(4-methyl-coumaryl-7-amide) (Suc-D-Asp-MCA) [1]. Supernatant of the above biological materials was mixed and incubated with 0.1 mM Suc-D-Asp-MCA and the assay buffer (10 mM Tris-HCl (pH 8.5), 200 mM NaCl, 3 mM $MgCl_2$) at 25°C. The fluorescence of aminomethylcoumarin liberated from Suc-D-Asp-MCA by DAEP was measured at λex = 380 nm and λem = 460 nm.

RESULTS & DISCUSSION: As a result of searching for DAEP activity in plants, radish taproots were shown to have the DAEP activity but in quite low amounts. On the other hand, the DAEP activity in tomato fruits was not detectable because of the spontaneous background fogging. Several kinds of pigments in the extract of tomato fruits would disturb the measurement of the DAEP activity.

As we reported, mammalian and aquatic animal DAEPs have common specific features: the molecular weight is up to 600 kDa, and the activity is strongly inhibited by Zn^{2+} and a synthesized DAEP inhibitor (benzyl-L-Arg-L-His-D-Asp-CH_2Cl), which we developed [1–3]. It is not clear at present whether or not DAEP activity detected in radish results from the homologue in animals. To purify the enzyme indicating the DAEP activity from radish taproots will provide the common feature of DAEP in animals and plants.

Table. Specific activities of DAEP in various samples.

Samples (tissue)	Specific activity (% of max in mouse)
Mouse (liver)	100
Radish (taproot)	2.5
Tomato (fruit)	Not detectable

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[1] T. Kinouchi *et al.*, Biochem. Biophys. Res. Commun., **314** (2004) 730–736.
[2] T. Kinouchi *et al.*, Chem. Biodivers., **7** (2010) 1403–1407.
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採択課題番号 25P3-1　D-アスパラギン酸含有蛋白質に特異的な分解酵素の研究　プロジェクト
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PR3-2 Damage to Biological Molecules Induced by Ionizing Radiation and Biological Defense Mechanisms Provided by Radical Scavengers III

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INTRODUCTION: Some bacteria exhibit extreme resistance to ionizing radiation [1]. A common feature of these bacteria is that they contain red carotenoid pigments [1, 2, 3]. Colorless mutants of these radioresistant bacteria are more sensitive to gamma irradiation than wild types [1]. Therefore, carotenoids are thought to be involved in the bacterial defense mechanisms against ionizing radiation [1]. Biological effects induced by low-linear energy transfer ionizing radiation are mainly attributed to radicals generated by radiolysis. Carotenoids have high radical scavenging activity, and they are localized in cell surface lipids in prokaryotes. These facts indicate that carotenoids are likely to defend the cell surface lipids of radioresistant bacteria against ionizing radiation.

When considering the biological defense mechanism of these radioresistant bacteria against ionizing radiation, it is important to elucidate the effects of carotenoids on damage to biological molecules, especially biological lipids. In this study, we analyzed the effect of astaxanthin, a typical carotenoid, on gamma radiation induced peroxidation of α-linolenic acid, a type of fatty acid.

EXPERIMENTS: Sample Preparation: α-Linolenic acid was dissolved in benzene at a final concentration of 5.0×10^{-1} M, and astaxanthin was added at a final concentration of 5.0×10^{-8} to 5.0×10^{-4} M. Gamma Irradiation: The prepared solutions were irradiated with ^{60}Co gamma rays at a dose of 30 kGy and a dose rate of 400 Gy/min. Analysis of Peroxidation of α-Linolenic Acid: The method described by Kennedy and Liebler was used with some modifications [4]. The gamma-irradiated samples were diluted 600-fold with *n*-hexane, and then 5 mL of this solution was evaporated under reduced pressure. The resulting residue was dissolved in 5 mL of *n*-hexane, and the average absorbance from 230–236 nm of this solution, which was derived from the conjugated diene formed, was measured. In this study, the level of peroxidation of α-linolenic acid was evaluated by determining the relative amount of conjugated diene formed.

RESULTS: Under the experimental conditions used, astaxanthin had no significant effect on gamma radiation-induced peroxidation of α-linolenic acid, at any of the concentrations (Fig. 1). We have previously reported that astaxanthin affects the oxidative degradation reaction of α-linolenic acid induced by gamma irradiation using an analysis in which the level of the oxidative degradation of linolenic acid was evaluated by the amount of malondialdehyde formed [5]. During the process of radical damage to α-linolenic acid, peroxidation occurs as the initial reaction, followed by oxidative degradation involving radical reactions [6]. These facts indicate that during the process of radical damage to α-linolenic acid induced by gamma irradiation, astaxanthin have no effect on the peroxidation reaction, but have a significant effect on the subsequent oxidative degradation.

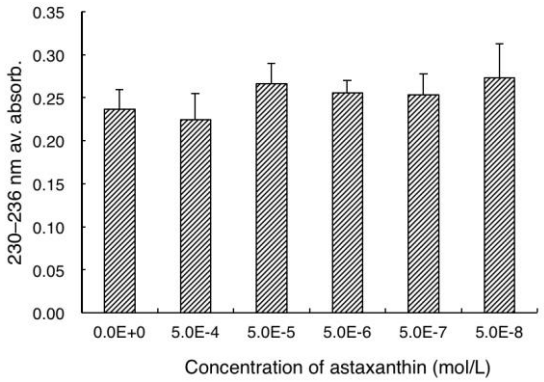

Fig. 1. Effect of astaxanthin on gamma radiation-induced peroxidation of α-linolenic acid. The peroxidation of α-linolenic acid was evaluated by measuring the average absorbance of the *n*-hexane solution of the irradiated sample in the 230–236 nm range as an index. The horizontal axis shows the concentration of astaxanthin and the vertical axis shows the average absorbance in the 230–236 nm range. Each data set is presented as the mean ± SD.

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採択課題番号 25P3-2　　放射線照射による生体分子の損傷とラジカルスカベンジャーによる生体防御機構　　プロジェクト

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PR3-3

Expression of Protein L-Isoaspartyl Methyltransferase (PIMT) and Analysis of Substrate Specificity Using Prion Peptide Fragments

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INTRODUCTION: The stereoconversion of aspartyl (Asp) residue arise through intramolecular rearrangement, such as *via* a succinimide intermediate. The native L-Asp residue in the protein was converted to the L-succinimide intermediate, and it is quickly hydrolyzed and produced the mixture containing L-Asp and L-isoAsp residue in a ratio of approximately 1:3. Protein L-isoaspartyl methyltransferase (PIMT) catalyzes repair of L-isoAsp peptide bonds in aged proteins by transferring a methyl group from S-adenosylmethionine to a α-carboxyl group of L-isoAsp residue (Fig. 1). The PIMT-deficient mice, in which isomerized Asp residues are accumulated at a level several times higher than in wild-type mice, undergo several tonic-clonic seizures, and die at a mean age of only 42 days.

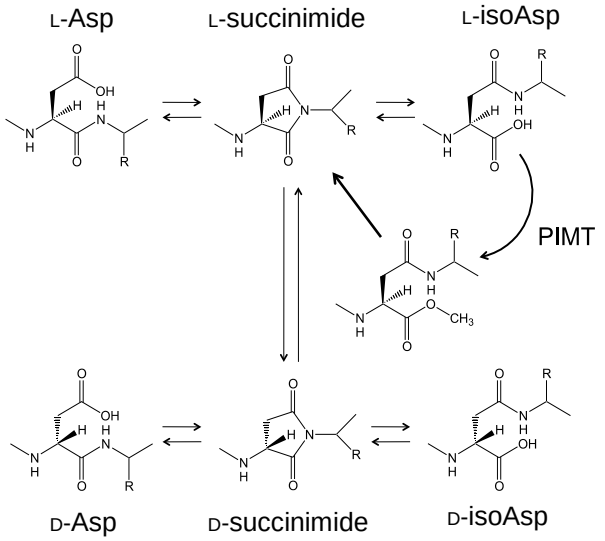

Fig. 1 The β-linkage isomerization and stereoinversion to D-form of aspartyl residue and PIMT repair system.

In this study, we prepared PIMT by *E. coli* expression system, and analyzed the substrate specificity of its recombinant enzyme using prion peptide (106–126) bearing L-Asp, L-isoAsp, D-Asp or D-isoAsp residue.

EXPERIMENTS: The recombinant PIMT protein was prepared by His-tag conjugated *E. coli* expression system, and the peptide fragments of prion 106–126 were synthesized using Fmoc amino acids, and purified by HPLC. We synthesized four peptides bearing L-Asp, L-isoAsp, D-Asp or D-isoAsp at 108th residue. The isomerization of Asp was determined by reversed-phase HPLC as described in [1].

RESULTS: The four types of prion peptides bearing various Asps were incubated in recombinant PIMT with co-substrate S-adenosylmethionine for 2 hr. Then, the specimen was analyzed by reversed phased HPLC with appropriate concentration of acetonitrile solving in pH 5.0 phosphate buffer (Fig. 2).

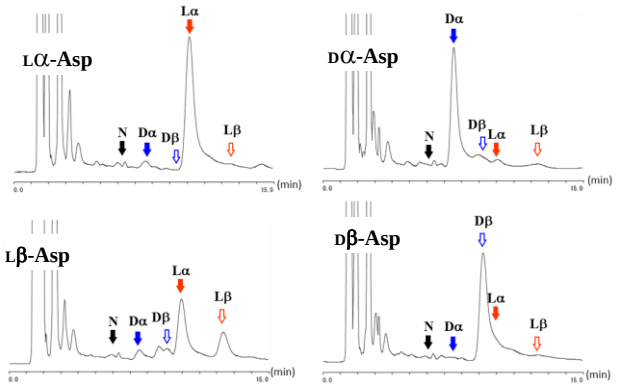

Fig. 2 HPLC profiles of the prion peptides incubated with recombinant PIMT for 2 hr. The initial peptide was described at the upper left in each profile, and the retention times of isomerized peptides were indicated by N (Asn), Lα(L-Asp), Lβ(L-isoAsp), Dα(D-Asp) and Dβ(D-isoAsp).

The HPLC profile of the peptide bearing L-isoAsp residue was drastically changed after the incubation with the recombinant PIMT. This result shows that the peptide with L-isoAsp is good substrate for the PIMT.

Our recombinant PIMT only catalyzes the peptide bearing L-isoAsp but not one bearing D-Asp, which is substrate for PIMT of other species.

REFERENCE:

[1] Y. Sadakane, K. Konoha, M. Kawahara and K. Nakagomi,Chem. Biodivers., **7** (2010) 1371–1319.

採択課題番号 25P3-3　タンパク質中のアスパラギンおよびアスパラギン酸残基の異性化と機能変化に関する研究　プロジェクト
（鈴鹿医療大・薬）定金　豊　（京大・原子炉）　藤井紀子

PR3-4 Analysis of Environmental Stress-Related Hearing Loss in Mice

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INTRODUCTION: It has been shown that barium can be detected in tube well drinking water and foods including seaweeds or nuts. Thus, we ordinary eat or drink barium contained in drinking water and foods [1]. However, it has not been recognized that the intake of barium contained in food and water can be a potential risk to our health. A previous study has been shown that exposure to barium affects physiological impairments including blood pressure [2]. In an *ex vivo* study, direct administration of barium to inner ears has been shown to affect physiological abnormalities in inner ears [3]. However, the influence of ingestion of barium via drinking water on hearing levels has not been clarified in experimental animals. This study aimed at analyzing the influence of ingestion of barium on hearing levels and morphology of inner ears in mice.

EXPERIMENTS: Barium chloride ($BaCl_2$) dissolved in drinking water at 0.7 and 7.0 ppm was orally administered to wild-type ICR mice for 2 weeks. This study used 3-week-old wild-type female mice (ICR). We regularly monitored the amount of drinking water and food ingested by the mice and body weights during the administration period. All experiments were permitted by the Institutional Animal Care and Use Committee in Chubu University (approval number: 2510053) and followed the Japanese Government Regulations for Animal Experiments. Barium concentrations in various tissues were measured with inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500cx) [4]. Morphological analyses of inner ears were performed as described previously [5]. After perfusion fixation by Bouin's solution, inner ears from mice were immersed in the same solution for 3 days to 1 week at 4˚C. Kluver-Barrera's staining was performed with paraffin serial sections. We further performed morphological analysis of inner ears transmission electron microscope after fixation with a mixture of 2% paraformaldehyde and 2% glutaraldehyde in 0.3 M HEPES-buffer (pH 7.4). After 2-week ingestion of $BaCl_2$ dissolved in drinking water, auditory brain stem responses (ABR) of mice were measured to determine hearing levels.

RESULTS: $BaCl_2$ dissolved in drinking water severely affected hearing levels, especially in higher frequency in mice. Mice administered with $BaCl_2$ showed neurodegeneration of inner ears including inner and outer hair cells, stria vascularis and spiral ganglion neurons. Meanwhile, mice administered with $BaCl_2$ and those without barium showed no significant difference in intake of both food and water and body weights. Mice administered with $BaCl_2$ significantly showed higher levels of barium in inner ears than those without barium, while barium levels in other tissues including cerebrum, heart and liver were undetectably low in both groups.

CONCLUSIONS: Results obtained in this study suggest that barium administered by drinking water specifically accumulates in inner ears resulting in severe impairments of hearing with degeneration of inner ears in mice. A previous study has shown that exposure to heavy metals can cause aggregation of proteins resulting in neurodegeneration [6]. Further studies will be needed to investigate whether exposure to barium causes a protein aggregation in inner ears to elucidate a mechanism of degeneration of auditory neurons.

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採択課題番号 25P3-4　騒音ストレスによる内耳タンパク質中のアスパラギン酸残基の異性化の解析　プロジェクト

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PR3-5 Change in Enantioselectivity of γ-Tryptphan Synthase

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INTRODUCTION: We have been researching to characterize tryptophanase enantioselectivity. We also are searching other enzyme with the same enantioselectivity as tryptophanase. Tryptophan synthase (TSase) is known as an enzyme that has the same reaction and enantioselectivity as tryptophanase. TSase has been studied extensively as the subject of great interest, too. It is interesting to compare the stereoselectivity of TSase with that of tryptophanase. TSase is commonly found in Eubacteria, Archaebacteria, Protista, Fungi, and Plantae. However, it is absent from Animalia. TSase is an enzyme that catalyzes the final two steps in the biosynthesis of tryptophan. It is the first enzyme that has two catalytic capabilities *via* substrate channeling. It is typically found as an $\alpha_2\beta_2$ tetramer (existing as an α-ββ-α complex.). The α and β subunits have molecular masses of 27 and 43 kDa, respectively. The α subunits catalyze the reversible formation of indole and glyceraldehyde-3-phosphate (G3P) from indole-3-glycerol phosphate (IGP). The β subunits catalyze the irreversible condensation of indole and serine to form tryptophan in a pyridoxal 5'-phosphate (PLP) dependent reaction. Each α active site is connected to a β active site by a 25 angstrom long hydrophobic channel contained within the enzyme. This facilitates the diffusion of indole formed at α active sites directly to β active sites in a process known as substrate channeling. Their assembly into a complex leads to structural changes in both subunits resulting in reciprocal activation. There are two main mechanisms for intersubunit communication. First, the COMM domain of the β-subunit and the α-loop2 of the α-subunit interact. Additionally, there are interactions between the α-Gly181 and β-Ser178 residues. The active sites are regulated allosterically and undergo transitions between open, inactive, and closed, active, states. The rate limiting step is the isomerization of IGP in α subunit reaction which catalyzes the formation of indole and G3P from a retro-aldol cleavage of IGP. On the other hand, β subunit reaction catalyzes the β-replacement reaction in which indole and serine condense to form tryptophan in a PLP dependent reaction. However, the exact mechanism has not been conclusively determined. In this research, we irradiated γ-ray against TSase to compare with tryptophanase.

EXPERIMENTAL: Generally speaking, γ-ray irradiated enzyme reduces its activity because γ-ray irradiation gives damaging tertiary conformational change with irreversible denaturation. However, the irradiation of lower level provides just few data on the activity of TSase. The present study aims to investigate the influence that lower dose gives to its activity. TSase was purchased from Sigma Chem. Co., prepared to a concentration of 200 μg/ml in 100 mM potassium phosphate buffer solution with 20 % saturation concentration of diammoniumhydrogen phosphate (DAP) and 380 mM pyridoxal 5'-phosphate. The enzyme was exposed to γ-rays, for which a cobalt-60 source was used with a dose rate of 3 Gy/sec. TSase was irradiated at doses of 30–5400 Gy. The activity of γ-TSase was assayed as below. Reaction mixture was composed of 20% saturation DAP, 380 mM pyridoxal 5'-phosphate, 100 mM L-serine, 6 mM indole, 1 μM γ-TSase (pH7.8). Reaction time and temperature was 6 h and 60°C, respectively. After the reaction, the aliquot was resolved on a CROWN PACK CR(+) column to determine L-tryptophan synthesis. The activity of γ-TSase was compared with that of TSase to analyze how a low irradiation of γ-rays influenced on the enantioselectivity of tryptophan synthase.

RESULTS AND DISCUSSION: As generally-accepted notion, γ-enzyme increasingly deactivates with irradiation dose. Tryptophanase gradually decreased in response to increasing γ-ray dose, too. γ-Tryptophanase did not almost change between 0 and 600 Gy. It was quite amazingly that γ-tryptophanase could exceed tryptophanase by several percent in some cases. Tryptophanase linearly deactivated in response to increasing γ-ray dose from 600 to some 2000 Gy. Its reduction rate slowed down over 2000 Gy, almost stopping between 4000–5400 Gy. Based on this result, we irradiated γ-rays from 30 to 5400 Gy against TSase. Prior to experiment, we forecasted γ-TSase steadily declined in the same feature as γ-Tryptophanase. However, when TSase was irradiated from 30 to 5400 Gy, its activity showed sharp decrease, different from a reduction aspect of tryptophanase. TSase was more susceptible to γ-ray than tryptophanase. TSase has a reaction mechanism different from tryptophanase such as substrate channeling between α and β subunits. TSase is of higher importance than tryptophanase in allosteric higher-order structure change for enantioselectivity as well as reciprocal activation. Small structural change will lead to this sharp decreased tryptophan-synthetic activity even in lower dose of γ-rays. The higher order conformation is significant for enzyme to encourage it to step into catalysis when substrate is reversibly bound to its active sit.

採択課題番号 25P3-5　　ガンマー線照射によるトリプトファンシンターゼの活性変化の追跡　　プロジェクト

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PR3-6 UV-B Exposure Leads to Simultaneous Photo-Oxidation of Tryptophan/tyrosine and Race-mization of Neighboring Aspartyl Residues in Peptides

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INTRODUCTION: Our previous studies indicate that the racemization of aspartyl (Asp, D) residues in proteins occurs by UV-B exposure [1]. However, Asp could not absorb UV-B light since there is no aromatic group in its chemical structure. We anticipate that isomerization of Asp residues is affected by the existence of UV absorbable tryptophan (Trp, W) or tyrosine (Tyr, Y) residue in the peptide. In addition, the isomerization is supposed to be promoted by the absorbed energy when Trp/Tyr residue is close to aspartyl residue in peptide sequence.

To certify this conjecture, we synthesized the peptides within Trp/Tyr residue closing to Asp residue and irradiated these peptides with UV-B light. After that, D/L ratio of Asp and photo-oxidation of Trp/Tyr were measured.

EXPERIMENTS: A partial peptide (IQTGLD^{151}ATHAER) of human lens αA-crystallin was selected as a model to synthesize peptides (a-g) of which residues nearby D^{151} were replaced by W or Y.

a IQTGLDATHAER (M.W. 1310.67): corresponding to residues 146–157 of the human eye lens αA-crystallin.

b IQTWLDATHAER (M.W. 1439.72): a peptide in which Gly was replaced with Trp at position 149 of peptide **a**.

c IQTGWDATHAER (M.W. 1383.66): a peptide in which Leu was replaced with Trp at position 150 of peptide **a**.

d IQTGLDWTHAER (M.W. 1425.71): a peptide in which Ala was replaced with Trp at position 152 of peptide **a**.

e IQTGLDAWHAER (M.W. 1395.70): a peptide in which Thr was replaced with Trp at position 153 of peptide **a**.

f IQTGYDATHAER (M.W. 1360.64): a peptide in which Leu was replaced with Tyr at position 150 of peptide **a**.

g IQTGLDYTHAER (M.W. 1402.69): a peptide in which Ala was replaced with Tyr at position 152 of peptide **a**.

All synthesis peptides were irradiated by UV-B (0–69 J/cm^2 for peptides within W; 0–346 J/cm^2 for peptides within Y). After irradiation, the peptides were hydrolyzed and the D/L ratio of resulting amino acids was determined by RP-HPLC. Photo-oxidation of Trp and Tyr was detected by TOF-MS.

RESULTS and DISCUSSION: After UV-B irradiation, peptides within Trp/Tyr were photo-oxidized at the position of Trp/Tyr residue. D-Asp was generated by UV-B exposure when there is Trp or Tyr nearby Asp residue in peptide sequence (Table 1). According to this result, we suppose that UV-B irradiation could lead to simultaneous photo-oxidation of Trp/Tyr and racemization of Asp residue closing to Trp/Tyr in peptide sequence. The effect to racemization by Trp was much more significant than Tyr since the UV-B absorption of Trp is much higher. In addition, Trp on the C-terminus side neighboring to Asp residue could lead to the greatest promotion to racemization since the isomerization process is possibly occurred by the –NH group of the residue on the right (C-terminus) side next to Asp residue in peptide sequence [2].

Table 1 D/L ratios of Asp residues in the peptides a–e

Peptide \ Dose*	0	17	35	69	Growth rate (%)
a	0.041	0.041	0.041	0.043	5
b	0.046	0.051	0.054	0.058	26
c	0.033	0.035	0.042	0.047	42
d	0.033	0.049	0.052	0.058	76
e	0.037	0.038	0.042	0.045	22

Dose* : J/ cm^2

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