VIII-Ⅱ-1. Project Research

Project 3

PR3-1 Structural Investigation of the 20S Proteasome and its Inhibitor

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INTRODUCTION: Proteasomes are widely distributed in eukaryotes, ranging from human to yeasts. In higher eukaryotes, proteasomes are known to act as antigen-processing enzymes responsible for the generation of peptide ligands presented by major histocompatibility complex (MHC) class I molecules. Proteasomes have molecular masses of approximately 750 kDa and sedimentation coefficients of approximately 20S. They are barrel-like particles formed by the axial stacking of four rings made up of two outer α-rings and two inner β-rings, being associated in the order of αββα Furthermore, some reagents, which are small aromatic compounds, inhibit the proteasome activities, and its assay shows apoptosis of myeloma cells. How the mechanism of such inihibitor reaction into the proteasome structure with atomic resolution, we have tried a structural analysis of the 20S proteasome and its inhibitors.

EXPERIMENTS: Yeast 20S proteasome tagged with affinity peptides was prepared in yeast. Cells were homogenized by glass beads and the crude extracts purified by M2 affinity chromatography and Mono-Q anion exchange one. Vapor diffusion method was applied. Crystals are isomorphous as described in the previous paper and belong to the space group $P2_1$. Initial phases were determined by molecular replacement method, and the structure model without ligand was refined by *Refmac*. The crystal structure of the deubiquitinating enzyme (UCH37) including N-terminal domain has been determined, and a conjugating site into the 20S core particle is examined. The UCH37 including C-terminal domain could be expressed as a GST fusion protein and purified using affinity chromatographic method.

RESULTS: A total of 237 amino acids out of 329 residues was solved at 2A resolution, but the conjugating site was undetermined because of the C-terminal deletion. C-terminal UCH protein, however, are poorly obtained and crystals are not available for structure analysis. Otherwise, crystals of 20S whole particle are obtained and determined by 2.5A resolution. Electron density maps depict suitable and available for enzymatic inihibition scheme by added reagents. Such density maps exist inside or outside of the particle. Inhibitors can attack surface of the particle via solvent region, but inside compound, especially inside wall of the particle, could not reach there by various amino acid residues.

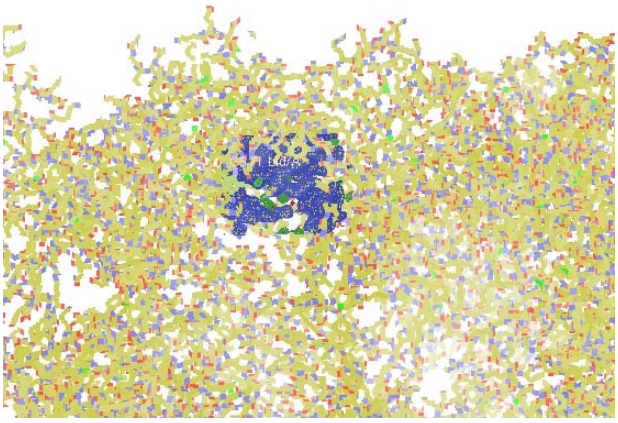

Fig.1. Electron densities for inihibitor compound inside 20S particle.

It is very important that how the UCH enzyme or inhibitor does to bind 20S core particle, and where and what happened on the 20S subunits. We hope to clarify a quaternary structure of the 20S subunits with the enzyme. Therefore we have planning to examine the quaternary structure analysis and related other protein molecules. Even deuterated compounds are available, neutron densities have just distinguished location of such compounds and/or UCH enzyme.

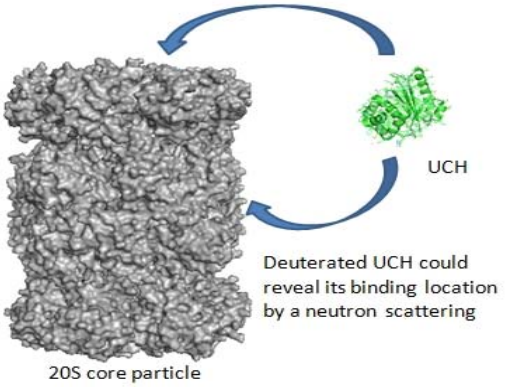

Fig.2. A schematic drawing of a 20S proteasome and a UCH enzyme, how to bind through the two ways.

Since the deuterium effect on the neutron scattering could amplify those characteristics according to an H/D exchange in a solution, particle components or binding proteins would be made them to admit location. Such a technique will be powerful to demonstrate a formation or combination of subunits in a protein complex research.

PR3-2 Structural Deformation of Eye Lens Protein (crystalline) by γ-ray Irradiation

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INTRODUCTION: It is supposed that abnormal aggregation, which means huge aggregation (larger than normal aggregated proteins) by denatured proteins, induces a serious disease, such as variant Creutzfeldt-Jakob disease (by abnormal prion), Alzheimer's disease (by β-amyloid proteins), and so on. *Cataract* is induced by aggregated crystallins in eye lens, and then is considered as one of those abnormally protein-aggregated diseases. Because there is no metabolic system in eye lens, the denatured proteins by external stresses accumulate in whole life and then the denatured protein aggregates up to the huge size, namely abnormal aggregation occurs.

Eye lens mainly consists of three crystallin proteins, α-, β-, γ-crystallin. Among them, α-crystallin with the largest molecular weight of ca 800 kDa has a chaperone activity to prevent from anomalous huge aggregation of the crystallins in the human eye lens, and therefore maintain the transparency.

In our previous study, it was clarifies that there are two steps in the initial stage of abnormal aggregation of α-crystallin under UV-irradiation. Here, we are interesting of the aggragation mechanics of the other crystallins by external stress and the chaperon activity of α-crystallin for the other protein. In this report, we show the structural deformation of βB_2-crystallin by γ-ray irradiation, observing with small-angle x-ray scattering (SAXS).

EXPERIMENTS: Human βB_2-crystallin expressed by *E.Coli* was used as a sample. The concentrations of samples were tuned to be 3.0 mg/ml and the solvent was 20 mM Tris/HCl (pH 7.8) + 150 mM NaCl. One sample solution was γ-ray irradiated with 10Gy.

The SAXS experiments were carried out at room temperature with a SAXS apparatus (SAXES) installed at BL10C of Photon Factory in Institute of Materials Structure Science (IMSS), High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. An X-ray beam (1.488 Å in wavelength) was used as a light source of SAXES and the intensity distribution of the scattered X-ray was measured by a one-dimensional position sensitive proportional counter. The magnitude of the scattering vector ($q = (4\pi/\lambda)\sin(\theta/2)$, where λ is the wavelength and θ is the angle of scatter) ranged from 7.0×10^{-3} to 1.5×10^{-1} Å $^{-1}$. The observed X-ray intensity was corrected for the buffer scattering and absorption, and then normalized with respect to the thickness of the sample (1 mm) and irradiation beam intensity. Typical irradiation time for sample was 1800 sec.

RESULTS: Figure 1 shows SAXS profiles of βB_2-crystallins with and without γ-ray irradiation. It is clearly observed the difference in the low *q*-region, indicated that γ-ray irradiated βB_2-crystallin has slightly larger size than no-irradiated one. It means that 10Gy γ-ray irradiation makes initial abnormal aggregation on βB_2-crystallin. In the next step, we will examine if there is the interaction between normal αA-crystallin and denatured βB_2-crystallin.

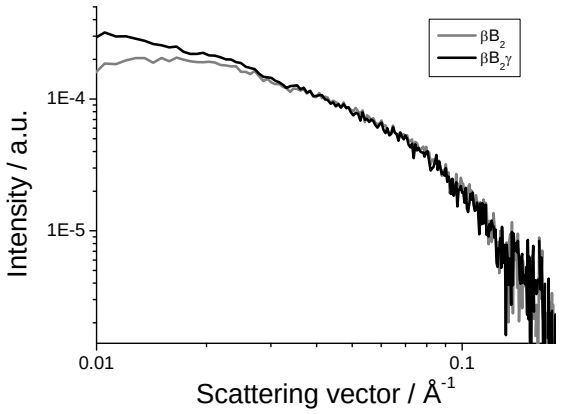

Fig. 1. SAXS profiles of βB_2-crystallins with and without γ-ray irradiation.

採択課題番号 22P3-2　階層性の観点から見たタンパク質の凝集過程・機構の解析　プロジェクト（京大・原子炉）杉山正明

PR3-3 High Substrate Selectivity of Mammalian D-Aspartyl Endopeptidase

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INTRODUCTION

The formation and accumulation of D-Aspartate residue (D-Asp) in proteins caused by oxidative stress leads to dysfunction and/or denaturation of proteins, and is consequently responsible for aging-related misfolding diseases such as cataracts, prion disease, and Alzheimer's disease. We sought to identify that an unknown protease selectively degrades the noxious D-Asp-containing protein, namely D-aspartyl endopeptidase (DAEP), and finally identified it from the inner mitochondrial membrane of mouse liver [1]. In order to analyze the substrate stereoselectivity of DAEP, we synthesized a peptide corresponding to 55-65 (Thr-Val-Leu-Asp-Ser-Gly-Ile-Ser-Glu-Val-Arg) of human αA-crystallin and its corresponding diastereoisomers in which L-α-Asp was replaced with L-β-, D-α- or D-β-Asp residue at position 58.

EXPERIMENTS

Sample preparation: The purification of DAEP from mouse (DDY) liver was previously described [1]. Purification factor of the purified DAEP used in this study was above 200. Human αA-crystallin peptide: Thr^{55}-Val-Leu-Asp-Ser-Gly-Ile-Ser-Glu-Val-Arg^{65}, and its corresponding diastereoisomers in which L-α-Asp was replaced with L-β-, D-α- or D-β-Asp residue at position 58 were synthesized by Fmoc (9-fluorenylmethoxycarbonyl) solid phase chemistry in dimethylformamide [2].

Analysis of the altered Asp-containing αA-crystallin peptide isomers hydrolyzed by DAEP: 10 μg of each αA-crystallin peptide containing normal (L-α-Asp) or abnormal isomers (L-β-, D-α- and D-β-Asp) was mixed and incubated with the DAEP assay buffer (10 mM Tris-HCl (pH 8.5), 200 mM NaCl, 3 mM $MnCl_2$) including 0.1 μg of purified mouse DAEP (total volume: 200 μl) at 37°C. Each reaction mixture following 0-, 1-, 3-, 9- and 24-hour incubation was independently applied to reverse-phase HPLC, and eluted with a linear gradient of 0-40% acetonitrile in 0.1% TFA. The molecular masses of digested peptides (peaks indicated by arrowheads in Fig.1 were determined using MALDI-TOF mass spectrometers, AXIMA-TOF^2 (Shimadzu Biotech, Kyoto, Japan).

RESULTS & DISCUSSION

Following incubation of D-α-Asp^{58}-contanining αA-crystallin peptide with DAEP, the peptide was degraded at D-α-Asp^{58} and new two fragments were produced as incubating time advanced (Fig.1). Mass spectrometry analysis of the peaks, indicated by white and black arrowheads, reveals that the molecular masses correspond to those of the peptide fragments: Thr^{55}-Val-Leu-Asp^{58} and Ser^{59}-Gly-Ile-Ser-Glu-Val- Arg^{65}, respectively. However, L-α-, L-β- and D-β- Asp^{58}-contanining peptides could not be degraded by DAEP even after 24-hour incubation. These results indicate that DAEP recognizes the internal D-α-Asp residue of the substrate in a stereoselective manner and specifically degrades it. DAEP therefore seems to physiologically serve as the quality control system against the noxious D-α-Asp-containing protein in the long life span of mammals. To investigate the coordination of DAEP with other quality-control proteases will provide a basis for developing treatments for protein-misfolding diseases caused by isomerization of Asp residue in protein.

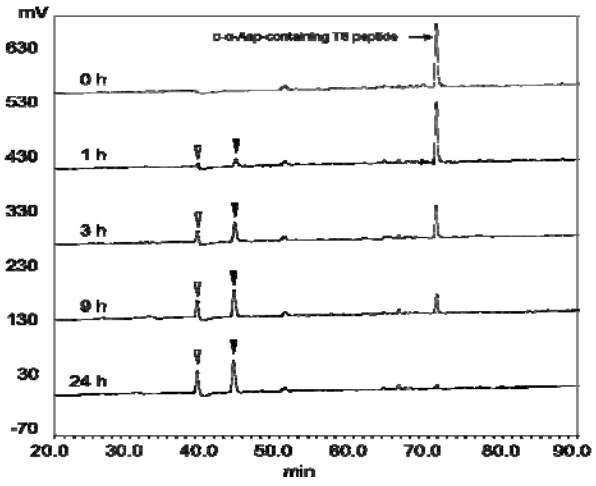

Fig.1. Chromatograms of of D-α-Asp^{58}-contanining αA-crystallin peptide hydrolyzed by DAEP.

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採択課題番号 22P3-3 D-アスパラギン酸含有蛋白質の蓄積モデルの構築とそれに特異的な分解酵素による品質管理機構の研究 プロジェクト

（京大・原子炉）木野内忠稔、藤井紀子

PR3-4 Damage to Biological Molecules Induced by Ionizing Radiation and Biological Defense Mechanisms III

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INTRODUCTION: Some bacteria show considerably high resistance to ionizing radiation [1]. A common feature of these bacteria is that they contain red carotenoid pigments [1, 2, 3]. Because colorless mutants of these radioresistant bacteria are more sensitive than wild types to gamma irradiation, the red carotenoid pigments are thought to contribute to the bacteria's defense mechanisms against ionizing radiation [1]. It is well known that biological effects induced by low-LET ionizing radiation are caused mainly by radicals that are generated by radiolysis. Carotenoid pigments possess high radical scavenging activity. And carotenoid pigments are localized within the lipids of the cell surfaces in procaryote. From these facts, we consider that red carotenoid pigments in radioresistant bacteria must defend lipids of the cell surfaces of these bacteria against ionizing radiation.

In considering the biological defense mechanism of these radioresistant bacteria against ionizing radiation, it is important to elucidate the process through which lipids are damaged by ionizing radiation. There are two main kinds of lipid damage: oxidative degradation and peroxidation induced by radical reaction. In this study, the dose-effect relationship of lipid damage in gamma irradiation to linolenic acid, a fatty acid that is the simplest biological lipid, was analyzed.

EXPERIMENTS: Sample Preparation: Linolenic acid was dissolved in benzene at a final concentration of 5.0 x 10^{-1} M. Gamma Irradiation: The prepared solutions were irradiated with ^{60}Co gamma rays at a dose rate of 30 kGy/h. Assay of the Oxidative Degradation of Linolenic Acid: The modified method of Buege and Aust was used [4]. TCA-TBA-BHT-HCl regent containing 15% trichloroacetic acid, 0.38% thiobarbituric acid, 0.04% butylated hydroxytoluene, and 0.25 N hydrochloric acid was prepared. The gamma-irradiated sample was diluted 50 times with benzene, and 3 ml of the diluted solution was evaporated in vacuo. The residue was then dispersed in 9 ml of PBS(-) by a sonicator. One ml of the dispersed solution was combined with 2.0 ml of TCA-TBA-BHT-HCl reagent and mixed thoroughly, and the mixed solution was heated for 15 min in a boiling water bath. The absorption at 532 nm of the reaction solution was measured. The amount of generated malondialdehyde (MDA) was calculated using the molar absorption coefficient (ε) of the color substance generated by a TBA reaction, which is 1.56 x 10^5 at 532 nm. In this study, the level of the oxidative degradation of linolenic acid was evaluated by the amount of generated MDA. Assay of the Linolenic Acid Peroxidation: The modified method of Pryor and Castle was used [5]. The gamma-irradiated sample was diluted 1,000 times with benzene, and then 5 ml of the diluted solution was evaporated in vacuo. The residue was dissolved in 5 ml of *n*-hexane, and the average absorbance from 230-236 nm of this solution, which is derived from the generated conjugated diene, was measured. In this study, the level of linolenic acid peroxidation was evaluated by the amount of generated conjugated diene.

RESULTS: The results of this study indicate that the oxidative degradation reaction and the peroxidation reaction of linolenic acid progress dose-dependently under 0-90 kGy gamma irradiation. The oxidative degradation reaction and the peroxidation reaction of lipids, which are the main types of lipid damage induced by radical reactions, are thought to proceed through different reaction processes. This study reveals that there exists a similar dose-effect relationship between different types of lipid damage in gamma irradiation. These results suggest that high-dose gamma irradiation causes various types of damage to biological lipids *in vivo*.

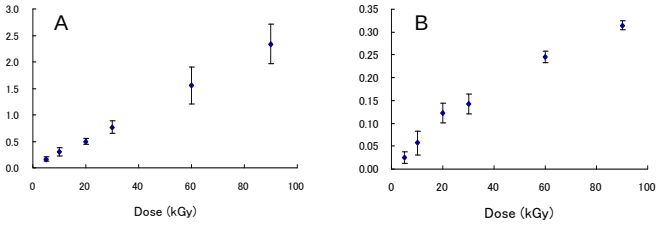

Fig. 1. Damage to linolenic acid induced by gamma irradiation. A: Oxidative degradation damage. B: Peroxidation damage.

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PR3-5 Comparison of Rate Constant of Beta-Linkage Isomerization and Stereoconversion of Specifc Aspartyl Residues in Recombinant Human AlphaA-crystallin Protein

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INTRODUCTION: The stereoconversion of aspartyl residue arise through intramolecular rearrangement, such as *via* a succinimide intermediate (see Fig. 1). 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. A small amount of L-succinimide intermediate undergoes reversible stereoconversion, and also produced the mixture of D-Asp and D-isoAsp residue in a same ratio above.

In this study, we compared the rate constants for the beta-linkage isomerization and stereoconversion to D-form in the typtic hydrolyzed peptides and recombinant human alphaA-crystalline.

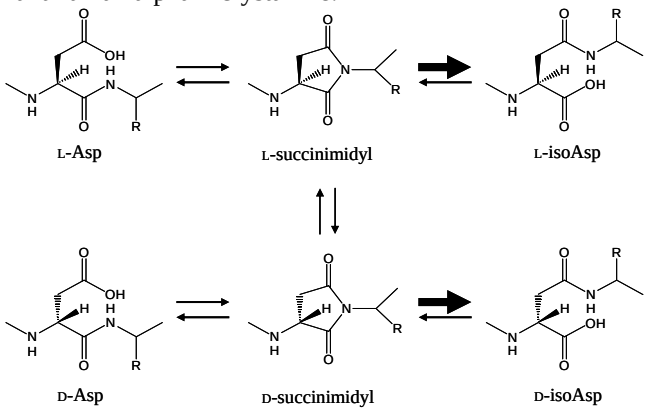

Fig. 1. The beta-linkage isomerization and stereoinversion to D-form of aspartyl residue

EXPERIMENTS: The peptide fragments and recombinant protein of alphaA-crystalline were prepared by the methods described in [1]. The rates of beta-linkage isomerization were determined by isocratic HPLC analyses described in [1]. The data of sterocoversion were referred by [2,3]. Kinetic studies of beta-linkage isomerization of Asp residue were carried out using the following eqn.

$$-\mathrm{d[isoAsp]} / \mathrm{d}t = k[\mathrm{Asp}] - k'[\mathrm{isoAsp}]$$

Rate constants for the isomerization reaction (alpha to beta) and for the reverse reaction (beta to alpha) are represented by k and k', respectively. We assume $k:k' = 3.6:1$ because the isoAsp/Asp ratio in the T6 peptide was shown to reach 3.6 at equilibrium state.

RESULTS: The beta-linkage isomerization of Asp58 was drastically suppressed in the recombinant protein by conformational effect, therefore the order of rate at 50°C constant was Asp58 < Asp151, which were not suppressed (Table 1). In higher temperature at 90°C, the order of rate constant in the protein was same as that in the peptide. Although the order of rate constants between beta-linkage isomerization and stereoconversion were different each other in the peptide at both examined temperatures, the both orders in the recombinant protein were same each other. The order was Asp58 < Asp151 at 50°C, however the order was reversed at 90°C, i.e. Asp58 > Asp151. These results suggest that both beta-linkage isomerization and stereoconversion were affected equally by conformational effect of recombinant protein. Since the rate constant of Asp151 in the recombinant protein were almost same as that in the peptide, the Asp151 residue of alphaA-crystallin is located in the flexible region of the protein, which was also showed by X-ray structural analysis of truncated bovine alphaA-crystallin [4]. Since alphaA-crystallin has long half-lives, the large amount of isoAsp is accumulated spontaneously at Asp151 residue of alphaA-crystallin. Structural altered Asp residues were reported to induce the drastic conformational change of protein. The conformational alteration of alphaA-crystallin protein may be induced by structural alteration of specific Asp residue, which may create the chiral reaction field in the aged alphaA-crystallin protein.

Table 1. Comparison of rate constants between beta linkage isomerization and stereoconversion

		beta-linkage isomerization	stereoconversion
50 °C	In peptide	Asp58 > Asp151	Asp58 < Asp151 *
	In protein	Asp58 < Asp151	Asp58 < Asp151 *
90 °C	In peptide	Asp58 > Asp151	Asp58 < Asp151 *
	In protein	Asp58 > Asp151	Asp58 > Asp151 *

* Data from Int. J. Peptide Protein Res. 48, 118- (1996) and Biochim. Biophy. Acta 1764, 800- (2006)

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採択課題番号 22P3-5　　タンパク質中のアスパラギン酸残基付近の放射線による構造と機能変化の解析　　プロジェクト

（鈴鹿医療科学大学・薬学部）定金 豊、（京大・原子炉）藤井紀子

PR3-6 L-Tryptophan Synthetic Pathway from D-Serine on Tyrptophanase

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INTRODUCTION: Tryptophanase, an enzyme with extreme absolute stereospecificity for optically active stereoisomers, catalyzes the synthesis of L-tryptophan from L-serine and indole through a β-substitution mechanism of the ping-pong type, and has no activity on D-serine. We previously reported that tryptophanase changed its stereospecificity to degrade D-tryptophan in highly concentrated diammonium hydrogen phosphate, $(NH_4)_2HPO_4$ solution. The present study provided the same stereospecific change seen in the D-tryptophan degradation reaction also occurs in tryptophan synthesis from D-serine. Tryptophanase became active to D-serine to synthesize L-tryptophan in the presence of diammonium hydrogen phosphate. This reaction has never been reported before.

EXPERIMENTS: Reaction mixtures included 0.2 mM of pyridoxal 5'-phosphate, 4.8 mM of L-serine or D-serine, and 2.7 mM of indole; 0.23 μM of tryptophanase. Saturation concentration of diammonium hydrogen phosphate was prepared from 0 to 60 %. 20% saturation concentration was used to analyze tryptophan synthesized from D-serine with HPLC. The effect of the different salts on tryptophanase activity toward D-serine was examined at 20% saturation concentration with each salt. A total volume of the reaction mixture was 2 mL per tube. Reactions were conducted at 37°C for 6 hr in a Dry Thermo Unit DTU-1B (Taitec, Tokyo, Japan). The reaction was stopped by adding 2 mL of *n*-butanol for thin layer chromatographic analyses or by icing at 0°C for high pressure liquid chromatographic analyses. The former was vigorously mixed, and then immediately centrifuged at approximately 1,000*g* for 10 min. After centrifugation, the reaction mixture was separated into two phases. The supernatant was extracted, dried over several days at 60°C in the oven to concentrate the tryptophan produced. The concentrated aliquot was applied for tryptophan analyses on cellulose thin layer chromatography. The reactant products were also analyzed on a Crownpack CR (+) Resolution HPLC column (Daicel Industry Ltd., Tokyo, Japan) with UV and CD monitor. The sample cooled at 0°C with ice was filtered through Ultrafree-MC 5000 NMWL Filter Unit (Millipore Co. Bedford, MA, USA), immediately injected into the HPLC column, and resolved at a flow rate of 1.0 mL/min with the eluting buffer running with an HPLC pump (635 Liquid Chromatogram, Hitachi, Ltd., Tokyo, Japan) through a Degasser (DG-980-50, Jasco, Tokyo, Japan). The eluant was monitored with an UV and CD monitor (CD-1595 detector, Jasco, Tokyo, Japan) at a wavelength of λ = 233 nm. UV absorbance, which was detected at a wavelength of λ = 280 nm, was expressed in arbitrary unit. Ellipticity of the eluting buffer was set up to zero at a wavelength of λ= 233 nm, so that the ellipticity of D-Trp and L-Trp had a negative and positive peak, respectively. Ellipticity [θ] was expressed in units of mdeg. The retention time of D- and L-tryptophan was 11.4 and 14.6 min, respectively. Tryptophanase activity on tryptophan synthesis from L- or D-serine was calculated from a peak area of L-tryptophan on HPLC chromatogram, compared against saturation concentration of diammonium hydrogen phosphate.

RESULTS: The only active enantiomeric form of tryptophan and serine is the L type in α-, β-eliminations and β-substitution reactions of tryptophanase under ordinary physiological saline concentrations because the enzyme has absolute stereospecificity. However, concentrated diammonium hydrogen phosphate can change tryptophanase stereoselectivity. When D-serine is added in a tryptophan synthesis reaction in the presence of diammonium hydrogen phosphate, it can serve as substrate to be synthesized into L-tryptophan. Although absolute stereoselectivity is generally displayed in a synthesis reaction more so than in a degradation reaction, the present result also reconfirms that synthesis has less efficient activity than degradation. In addition we speculate L-tryptophan is synthesized from D-serine by tryptophanase *via* enzyme-bounded α-aminoacylate intermediate, which plays a key role in L-tryptophan synthesis from L-serine. In other words, D-serine seems to undergoβ-replacement *via* an enzyme-bonded α-aminoacylate intermediate to yield L-tryptophan. It appears reasonable to think the tryptophanase stereoselectivity is more flexible than we always image. Perhaps the exclusive stereoselective mechanism of L-amino acid from D-amino acid will be driven by the slight difference in stereostructure. The mechanism of changing stereospecificity on tryptophanase has still remained ambiguous, but we are confident that it will offer valuable information and clue to the origin of homochirality.

SUMMARY: When D-serine is added for tryptophan synthetic reaction in the presence of diammoniumhydrogen phosphate, it can serve as substrate to be synthesized into L-tryptophan. L-tryptophan seems to be produced from D-serine by β-replacement reaction.

採択課題番号 22P3-6　　ガンマー線照射による酵素の立体構造の変化の測定　　プロジェクト
（筑波大・生命環境科学研究科）島田秋彦　（京大・原子炉）藤井紀子、齋藤　毅

PR3-7 UV-B-irradiation Enhances the Racemization and Isomerizaiton of Aspartyl Residues and Advanced Glycation End Products in the Same Proteins of Skin

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IINTRODUCTION: UV-B irradiation is one of the risk factors, which causes the age-related diseases. We have reported that biologically uncommon D-beta-Asp residues accumulated in proteins from sun-exposed elderly human skin. On the other hand, a previous study reported that advanced glycation end products (AGEs) which are induced from the oxidation of glucose, also increased by UV-B irradiation. The formation of D-beta-Asp and AGEs were reported as the alteration of proteins in UV-B irradiated skin, independently. In this study, in order to clarify the relationship between the formation of D-beta-Asp and AGEs, immunohistochemical analysis using anti-peptide 3R and anti-CML (carboxymethyl lysine; one of the AGEs) antibodies was performed in the UV-B irradiated mouse and the modified proteins in the UV-B irradiated skin were identified by proteomic analysis.

EXPERIMENTS: Normal back skin of HR-1 mice (5 week-old) received UV-B irradation at a total dose of 200 mJ/cm^2. After 3 days, skin samples were taken by punch biopsy before and after the irradiation, embedded in paraffin, then subjected to immnohistochemical examination using the anti-peptide 3R and anti-CML antibodies. The proteins were extracted from the UV-B irradiated mouse skin with 8 M urea, 2 M thiourea, 3% CHAPS solution and then analyzed by 2-D PAGE followed by western blotting using anti-peptide 3R and anti-CML antibodies. Then the modified proteins were identified by MALDI-TOF-MS analysis.

RESULTS: The D-beta-Asp and CML modified proteins were co-localized to the same section of epidermis and dermis of sun-exposed skin (Fig. 1). Western blot analyses of the proteins isolated from UV-B irradiated skin demonstrated that proteins of 50-70 kDa were immunoreactive towards antibodies for both D-beta-Asp containing peptide and CML. These proteins were identified by proteomic analysis as members of the keratin families including keratin-1, 6B, 10 and 14 (Fig. 2)

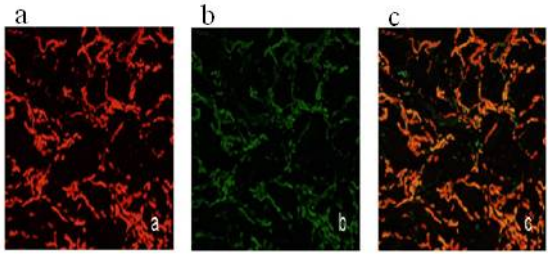

Fig 1. Double immunofluorescence labeling of sun-damaged skin (80-year-old glabellar skin) using the antibodies for D-beta-Asp containing peptide (peptide 3R) and CML. Cryostat sections were incubated with polyclonal antibody for peptide 3R (a) or monoclonal antibody for CML (b) or both (c).

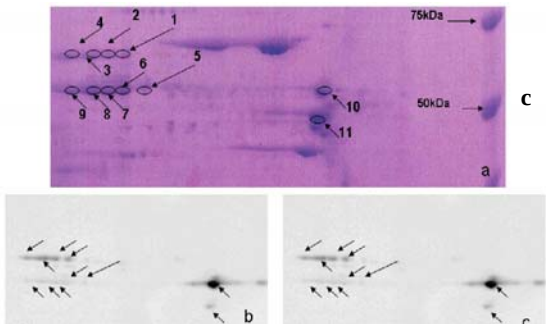

Fig. 2. 2-D PAGE and western blot analysis of the proteins extracted 3 days after 200 mJ/cm^2 UV-B-irradiated mouse skin. Detection was performed using (a) Coomassie brilliant blue-staining, (b) anti-peptide 3R antibody and (c) anti-CML antibody. Eleven proteins indicated by arrows in Fig. 2, clearly showed common reactivity with the anti-peptide 3R antibody and anti-CML antibody.

CONCLUSION: The present result shows that the racemization of Asp residues and glycation simultaneously proceed in keratin-1, 6B, 10 and 14 proteins of the mouse skin by UV-B irradiation.

採択課題番号 22P3-7　　放射線や紫外線照射によるタンパク質の異常凝集とアミノ酸のラセミ化　　プロジェクト

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PR3 Project Research on the Abnormal Aggregation of Proteins by UV and Gamma-ray-irradiation 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 irradiation such as gamma-ray and UV and protein function. We also investigate the repair mechanism for the damaged protein by irradiation. This research program has started in 2008. In this year, the 7 research subjects were carried out. The allotted research subjects (ARS) are as follows;

ARS-1: Structural Investigation of the 20S Proteasome and Its Inhibitor.
(Y. Morimoto and K. Nishio)

ARS-2: Structural Deformation of Eye Lens Protein (crystalline) by γ-ray Irradiation.
(M.Sugiyama, N. Fujii and N. Fujii)

ARS-3: High Substrate Selectivity of Mammalian D-Aspartyl Endopeptidase (T. Kinouchi and N. Fujii)

ARS-4: Damage of Biological Molecules Induced by Ionizing Radiation and Biological Defense Mechanisms III (T. Saito and N. Fujii)

ARS-5:Comparison of Rate Constant of Beta-linkage Isomerization and Stereoconversion of Specifc Aspartyl Residues in Recombinant Human alphaA-crystallin Protein. (Y. Sadakane and N. Fujii)

ARS-6: L-tryptophan Synthetic Pathway from D-Serine on Tyrptophanase.
(A. Shimada, N. Fujii and T. Saito)

ARS-7: UVB-irradiation Enhances the Racemization and Isomerizaiton of Aspartyl Residues and Advanced Glycation End-products (AGE) in the Same Proteins of Skin. (N. Fujii, Y. Mori, K. Aki, N. Fujii, M. Watanabe, T. Kiouchi and Y. Kaji)

Main Results and Contents of This Project

ARS-1: Morimoto, *et.al.* have investigated structural studies for a yeast 20S proteasome and its inhibitor compounds or deubiquitinating enzyme with atomic resolution. A proteasome ubiquitin system which functions on a degradation of un-needed proteins is focused in the structural characteristics. A crystal structure of the 20S proteasome and inihibitor complex has been determined at 2.5Å resolution, and the 26S whole structure was investigated by small-angle scattering with no other structural information. A complex structure has revealed some specific amino acid residues against inihibitor compounds according to electron density maps interact with them by hydrogen or electrostatic interaction and bondings.

ARS-2: Sugiyama *et al*. investigated gamma-ray irradiation effect on beta B2-crystallin. The beta B2-crystallins with or without 10 Gy gamma-ray irradiation were observed with Small-angle X-ray Scattering (SAXS). The SAXS profile with the gamma-ray irradiation shows the upturn in the low q-range, indicating that the 10 Gy gamma-ray irradiation makes an initial aggregation of beta B2-crystallin.

ARS-3: Kinouchi and Fujii analyzed the substrate stereoselectivity of mammalian D-Aspartylendopeptidase (DAEP). Our results indicate that DAEP recognizes the internal D-α-Asp residue of the substrate in a stereoselective mannerand seems to physiologically serve as the quality control system against the noxious D-α-Asp-containing protein in the long life span of mammals.

ARS-4: Saito *et al.* showed that there exists a similar dose-effect relationship between different types of lipid damage in gamma irradiation. These results suggest that high-dose gamma irradiation causes various types of damage to biological lipids *in vivo*.

ARS-5: Sadakane *et al*. analyzed the rate constant of beta-linkage isomerization of specific aspartyl residues in recombinant human alpha-crystallin protein by using our developed method with RP-HPLC, and revealed that effect of conformation on the beta-linkage isomerization was different from that on stereoconversion to D-form.

ARS-6**:** Shimada *et al*. demonstrated that when D-serine was added for tryptophan synthetic reaction in the presence of diammoniumhydrogen phosphate, it could serve as a substrate to be synthesized into L-tryptophan. Additionally they elucidated L-tryptophan was produced from D-serine by β-replacement reaction.

ARS-7: Fujii *et al*. showed that the racemization of Asp residues and glycation simultaneously proceed in keratin-1, 6B, 10 and 14 proteins from the mouse skin 3 days after 200 mJ/cm^2 UVB irradiation.

採択課題番号 22P3　　放射線や紫外線照射によるタンパク質の異常凝集と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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