Chair Bioorganic and Biophysical Chemistry

2.3.11 Laboratory : Applied Structural Biology

Member:	Professor	Mikami, Bunzo, D. Agric.Sci
	Assistant Professor	Takahashi, Nobuyuki, D. Agric. Sci
	Assistant Professor	Mizutani, Kimihiko, D. Agric. Sci
	Doctor's program	2
	Master's Program	3
	Undergraduate	3

A. Research Activities (2010.4-2011.3)

A-1. Main Subjects

a) Structure Determination of Proteins and Enzymes

Using X-ray crystallographic analysis, we have determined 3D structures of many proteins (Egg white proteins, plant seed proteins, lectins, and so on) and enzymes (amylase, pullulanase, polysaccharide lyase, and so on). Furthermore, proteins forming good crystals such as ovotransferrin could be applied for subatomic resolution X-ray crystallography and neutron crystallography to determine the positions of hydrogen atoms. The enzyme mechanisms of transglutaminase and protein glutaminase are studying by structural analyses of their mature and proenzyme forms.

b) Functional Analysis and Protein Engineering based on Structure Analysis 1.

Industrially utilized enzymes such as β -amylase, a-amylase and pullulanase are trying to be improved on their enzymatic functions by protein engineering based on their structural analyses. The optimal pH and product specificity of β -amylase are modified by site-directed mutagenesis of a few amino acid residues around the catalitic site including a flexible loop of the enzyme based on their crystallographic models. The product specificity of pullulanase is proved to be engineered by site-directed mutagenesis on the loop adjacent to its active site.

c) Functional Analysis and Protein Engineering based on Structure Analysis 2.

Ovalbumin, a majour component of egg white is going to be modified by rational design based on its 3D structure: The protein does not have inhibitory activity, although it belongs to a superfamily of serine proteinase inhibitors (SERPIN), which exert physiologically important roles in vertebrate by a conformational change called loop-insertion. The crystallographic data along with successful productions for mutants with an increased loop-insertion rate strongly suggested that the acquisition of the serpin inhibitory activity is possible for ovalbumin. Except for ovalbumin, the structure of neuloserpin has analysed by X-ray crystallography.

d) Development of high-throughput construction method of protein expression system for X-ray crystallography

We are developing high-throughput construction method of eukaryotic membrane proteins and secretory proteins using yeast (Saccharomyces cerevisiae and Pichia pastoris) for efficient and high-throughput X-ray crystallography of proteins.

A-2.Publications and presentations

a) Publications

Original Papers(including book-reviews)

- Ochiai A., Yamasaki M., Mikami B., Hashimoto W., Murata K.: :Crystal structure of exotype alginate lyase Atu3025 from Agrobacterium tumefaciens. J. Biol. Chem., 285, 24519-24528 2010.

- Chu H.N., Kobayashi J., Yoshikane Y., Mikami B., Yagi T. :Crystallization and preliminary X-ray analysis of SDR-type pyridoxal dehydrogenase from Mesorhizobium loti. Acta Crystallogr. Sect F, 66, 718-720. 2010.

- Takahashi N., Maeda M., Yamasaki M., Mikami B. :Protein-engineering study of contribution of conceivable D-serine residues to the thermostabilization of ovalbumin under alkaline conditions. Chem. Biodivers., 7, 1634-1643 2010.

- Maruyama Y., Ochiai A., Itoh T., Mikami B., Hashimoto W., Murata K.. :Mutational studies of the peptidoglycan hydrolase FlgJ of Sphingomonas sp. strain A1.J. Basic. Microbiol., 50, 311-317 2010.

- Mizutani K., Toyoda M., Sagara K., Takahashi N., Sato A., Kamitaka Y., Tsujimura S., Nakanishi Y., Sugiura T., Yamaguchi S., Kano K., Mikami B. :X-ray analysis of bilirubin oxidase from Myrothecium verrucaria at 2.3 Å resolution using a twinned crystal. Acta Crystallogr. Sect F, 66, 765-770 2010.

- Toyoda M., Jitsumori K., Mikami B., Wackett L.P., Kurihara T., Esaki N. :Crystallization and preliminary X-ray analysis of L-azetidine-2-carboxylate hydrolase from Pseudomonas sp. strain A2C. Acta Crystallogr. Sect F, 66, 801-804 2010.

- Nakagawa M., Ueyama M., Tsuruta H., Uno T., Kanamaru K., Mikami B., Yamagata H. :Functional analysis of the cucumisin propeptide as a potent inhibitor of its mature enzyme. J. Biol. Chem., 285, 29797-29807 2010.

- Takase R., Ochiai A., Mikami B., Hashimoto W., Murata K. :Molecular identification of unsaturated uronate reductase prerequisite for alginate metabolism in Sphingomonas sp. A1.Biochim. Biophys. Acta., 1804, 1925-1936 2010.

- Takehara S., Zhang J., Yang X., Takahashi N., Mikami B., Onda M. :Refolding and polymerization pathways of neuroserpin. J. Mol. Biol., 403, 751-762 2010.

- Tandang-Silvas M.R., Carrazco-Peña L., Barba de la Rosa A.P., Osuna-Castro J.A., Utsumi S., Mikami B., Maruyama N. :Expression, purification and preliminary crystallization of amaranth 11S proglobulin seed storage protein from Amaranthus hypochondriacus L. Acta Crystallogr. Sect F, 66, 919-922 2010.

- Shutov A.D., Prak K., Fukuda T., Rudakov S.V., Rudakova A.S., Tandang-Silvas M.R., Fujiwara K., Mikami B., Utsumi S., Maruyama N.: Soybean basic 7S globulin subunit heterogeneity and molecular evolution.. Biosci. Biotechnol. Biochem., 74, 1631-1634 2010.

- Masuda T., Goto F., Yoshihara T., Mikami B. :The universal mechanism for iron translocation to the ferroxidase site in ferritin, which is mediated by the well conserved transit site. Biochem. Biophys. Res. Commun., 400, 94-99 2010.

- Maeda Y., Doubayashi D., Ootake T., Oki M., Mikami B., Uchida H. :Crystallization and preliminary Xray analysis of formate oxidase, an enzyme of the glucose-methanol-choline oxidoreductase family. Acta Crystallogr. Sect F, 66, 1064-1066. 2010.

- Cabanos C., Urabe H., Masuda T., Tandang-Silvas M.R., Utsumi S., Mikami B., Maruyama N. :Crystallization and preliminary X-ray analysis of the major peanut allergen Ara h 1 core region. Acta Crystallogr. Sect F, 66, 1071-1073 2010.

- Nakamichi Y., Maruyama Y., Mikami B., Hashimoto W., Murata K.: :Structural determinants in streptococcal unsaturated glucuronyl hydrolase for recognition of glycosaminoglycan sulfate groups. J. Biol. Chem. 286, 6262-6271 2010.

- Mizutani K., Yoshioka S., Mizutani Y., Iwata S., Mikami B.. :High-throughput construction of expression system using yeast Pichia pastoris, and its application to membrane proteins. Protein Expr. Purif, 77, 1-8 2010.

- Maruyama Y., Ochiai A., Mikami B., Hashimoto W., Murata K. :Crystal structure of bacterial cell-surface alginate-binding protein with an M75 peptidase motif. Biochem. Biophys. Res. Commun., 405, 411-416. 2011.

- Maruyama Y., Chuma A., Mikami B., Hashimoto W., Murata K. :Heterosubunit composition and crystal structures of a novel bacterial M16B metallopeptidase. J. Mol. Biol., 407, 180-192 2011.

- Chu H.N., Kobayashi J., Mikami B., Yagi T. :The crystal structure of SDR-type pyridoxal 4dehydrogenase of Mesorhizobium loti. Biosci. Biotechnol. Biochem., 75, 388-390. 2011.

Reports, others

- Mikami, B., Moriya, T, Tandang, M., Masuda, T., Utsumi S. : X-Ray crystallographic analysis of mung bean 8S globulin. SPring-8 User Experiment Report 2010A1309, 2010.

- Mikami, B., Tanabe, A., Hashizume, R., Yoshimura, T. : X-Ray crystallographic analysis of mutant betaamylase/maltose complex.-Titration of two loop conformations of V99N by maltose- SPring-8 User Experiment Report 2010A1489, 2010.

- Mikami, B., Urabe, H., Tandang, M., Masuda, T., Maruyama, N., Utsumi S. : X-Ray crystallographic analysis of peanut conarachin. Spring-8 User Experiment Report 2010A6538, 2010.

- Mikami, B., Tanabe, A., Adachi, M., Okumura, T. : X-Ray crystallographic analysis of mutant betaamylase/maltose complex.-Titration of two loop conformations of K295A by maltose-. SPring-8 User Experiment Report 2010B1181, 2010.

- Mikami, B., Yamazaki, Y., Maruyama, N., Tandang, M., Masuda, T., Itoh, T., Utsumi S. : X-Ray crystallographic analysis of a mutant mung bean 8S globulin. SPring-8 User Experiment Report 2010B1456, 2010.

- Mikami, B., Maruyama, N., Utsumi S. : X-Ray crystallographic analysis of vacuolar sorting receptor (VSR) from soybean cells . SPring-8 User Experiment Report 2010B6538, 2010.

b) Conference and seminar papers presented

- The 2011 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry : 11 papers

- The 2010 Annual Meeting of Japanese Biochemical Society : 3 papers

- The 2010 Annual Meeting of Protein Science Society of Japan : 3 papers

A-3.Off-campus activities 1

Membership in academic societies

- Mikami, Bunzo, D. Agric .Sci : The Japanese Society of Applied Glycoscience (an editoryal board member)

A-3.Off-campus activities 2

Research grants

1. Grants-in-aid for Scientific Research(KAKENHI)

- General Scientific Research (B) : Mikami, B. : Elucidation of loop function of food related enzymes

2. Other Research Grants

- National Project on Target Proteins : Collaborator Mikami, B., Leader Hashimoto, W. : Structural biology of bacterial super-biosystem for import and degradation of polysaccharides and its application to food and environmental areas

- Program for Promotion of Basic Research Activities for Innovative Biosciences : Collaborator Mikami, B., Leader Murata, K.: Production of ethanol from marine biomass (alginate)

A-4.International cooperation and overseas activities 1

International joint research, overseas research surveys

- Tertiary structure of bacterial enzymes. Mikami B. Seoul National University, Korea

A-4.International cooperation and overseas activities 2

Visiting Research Scholars

- Scholar 1 (Indonesia)

B.Educational Activities(2010.4-2011.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level :	Laboratory Course in Applied Life Science (Mikami, Takahashi, Mizutani), Structural Biology (Mikami). Introduction of AppliedLife Science I (Mikami)
- Graduate level :	Applied Structural Biology (Mikami), Applied Structural Biology Seminar (Mikami,, Takahashi, Mizutani), Experimental Course of Applied Structural Biology (Mikami, Takahashi, Mizutani)

B-2.Off-campus teaching etc.

Part-time lecturer

- Mikami, B.: Faculty of Medical Life Science, Doshisha University (Structural Biology)

- Takahashi, N.: Dep. of Food Sci. and Nutr., School of Human Environ. Sci., Mukogawa Women's University (Biochemistry), Faculty of Engineering, Kyoto Sangyo University (Biophysics)

Open lectures, etc.

- Mikami, B.: Uji, Open Campus seminar, Uji campus

C.Other Remarks

- Mikami, B.: Japan atomic energy agency, Council for neutron beam application