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	1
	Master's Program	3
	Undergraduate	3

A. Research Activities (2009.4-2010.3)

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 sub-atomic 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

Industrially utilized enzymes such as β-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. Furthermore, 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 ovoalbumin,the structure of neuloserpin has analysed by X-ray crystallography.

c) 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

Books

- Bunzo, Mikami, N. Maruyama, S. Utsumi: Globulin proteins. Daizu no subete (Kitamura, K. ed) p. 116-121. Sience Forum, Tokyo 2010.

Original Papers

- Sakurama H, T. Takita, B. Mikami, T. Itoh, K. Yasukawa, K. Inouye: Two crystal structures of lysyl-tRNA synthetase from Bacillus stearothermophilus in complex with lysyladenylate-like compounds: insights into the irreversible formation of the enzyme-bound adenylate of L-lysine hydroxamate. J. Biochem., 145 (5); 555-563, 2009.
- Ochiai A, T. Itoh, B. Mikami, W. Hashimoto, K. Murata: Structural determinants responsible for substrate recognition and mode of action in family 11 polysaccharide lyases. J. Biol. Chem., 284 (15):10181-10189, 2009.
- Takehara S, M. Onda, J. Zhang, M. Nishiyama, X. Yang, B. Mikami, D.A. Lomas; The 2.1-Å crystal structure of native neuroserpin reveals unique structural elements that contribute to conformational instability. J. Mol. Biol., 388 (1): 11-20 2009.
- Hashimoto W., A. Ochiai, K. Momma, T. Itoh, B. Mikami, Y. Maruyama, K. Murata: Crystal structure of the glycosidase family 73 peptidoglycan hydrolase FlgJ. Biochem. Biophys. Res. Commun., 381 (1):16-21 2009.
- Hashimoto W., A. Ochiai, J. He, T. Itoh, B. Mikami, K. Murata: Crystallization and preliminary crystallographic analysis of the cell-surface alginate-binding protein Algp7 isolated from Sphingomonas sp. A1. Acta Crystallogr. Sect. F, 65 (Pt 5): 515-517, 2009.
- Maruyama Y., Y. Nakamichi, T. Itoh, B. Mikami, W. Hashimoto, K. Murata: Substrate specificity of streptococcal unsaturated glucuronyl hydrolases for sulfated glycosaminoglycan. J. Biol. Chem., 284 (27):18059-18069, 2009.

- Matsuda S., N. Yokochi, Y. Yoshikane, J. Kobayashi, C.N. Huy , S. Baba, S. Kuramitsu, B. Mikami, T. Yagi: Crystallization and preliminary X-ray analysis of 4-pyridoxolactonase from Mesorhizobium loti. Acta Crystallogr. Sect F, 65 (Pt 9); 886-889, 2009.
- Ogura K., M. Yamasaki, T. Yamada, B. Mikami, W. Hashimoto, K. Murata K: Crystal structure of family 14 polysaccharide lyase with pH-dependent modes of action. J. Biol. Chem., 284 (51); 35572-35579, 2009.
- Masuda T., F. Goto, T. Yoshihara, B. Mikami: Crystal structure of plant ferritin reveals a novel metal binding site that functions as a transit site for metal transfer in ferritin. J. Biol. Chem., 285 (6):4049-4059, 2010.
- Tsuruta H., B. Mikami, T. Higashi, Y. Aizono: Crystal structure of cold-active alkaline phosphatase from the psychrophile Shewanella sp. Biosci. Biotechnol. Biochem., 74 (1): 69-74 2010.
- Mizutani K., K. Hashimoto, N. Takahashi, M. Hirose, S. Aibara, B. Mikami: Structural and functional characterization of recombinant human serum transferrin secreted from Pichia pastoris. Biosci. Biotechnol. Biochem., 74 (2):309-315 2010.

Reports

- Masuda, T., S. Kigo, K. Ohta, B. Mikami,N. Kitabatake: Structural biology on the function of taste related proteins. SPring-8 User Experiment Report 2009A1096, 2009.
- Mikami, B., H. Urabe, T. Yoshimura, R. Hashizume, N. Maruyama, S. Utsumi: X-Ray crystallographic analysis of peanut conarachin. SPring-8 User Experiment Report 2009A1165, 2009.
- Mikami, B., Y. Toyoda, T. Saito, M. Adachi, S. Utsumi: X-ray crystallographic analysis of mutant b-amylase/maltose complex. -Titration of two loop conformations of G97A by maltose-. SPring-8 User Experiment Report 2009A1168, 2009.
- Takase, R., A. Ochiai, Y. Nakamichi, T. Itoh, K. Ogura, B. Mikami, W. Hashimoto, K. Murata: X-ray Crystal Structure of Sphingomonas sp. A1 α-Keto Acid Reductase for Alginate Metabolism. SPring-8 User Experiment Report 2009A1179, 2009.
- Masuda, T., F. Goto, T. Yoshihara, T. Yoshimura, B. Mikami: X-ray crystallographic analysis of phytoferritin subunit from soybean. SPring-8 User Experiment Report 2009A1249, 2009.
- Maruyama, Y., A. Chuma, A. Ochiai, K. Ogura, Y. Nakamichi, B. Mikami, W. Hashimoto, K. Murata: X-ray structural analysis of the metallopeptidase family M16 enzyme. SPring-8 User Experiment Report 2009A1329, 2009.

- N. Kishi, B. Mikami, F. Goto, T. Yoshihara: X-Ray crystallographic analysis of soybean ferritin variant E173A. SPring-8 User Experiment Report 2009A6935, 2009.
- Takase, R., A. Ochiai, T. Itoh, K. Ogura, B. Mikami, W. Hashimoto, K. Murata: Structural biology of bacterial enzymes for production of bioetanol from polysaccharide of plant cell walls. SPring-8 User Experiment Report 2009B1177, 2009.
- Maruyama, Y., A. Chuma, A. Ochiai, K. Ogura, Y. Nakamichi, B. Mikami, W. Hashimoto, K. Murata: Crystal structure of the metallopeptidase family M16 enzyme from Sphingomonas sp. strain A1. SPring-8 User Experiment Report 2009B1192, 2009.
- Nakamichi, Y., Y. Maruyama, T. Itoh, A. Ochiai, B. Mikami, W. Hashimoto, K. Murata: X-ray crystal analysis of unsaturated gluchydrolase acting glycosaminoglycan from bactera and its medical application. SPring-8 User Experiment Report 2009B1192, 2009.
- Masuda, T., S. Kigo, B. Murata, N. Kitabatake: High resolution structural analysis of the function of sweat taste proteins SPring-8 User Experiment Report 2009B1379, 2009.
- Mikami, B., M. Tandang, T. Yoshimura, R. Hashizume, T. Masuda, N. Maruyama: X-Ray crystallographic analysis of pro legumin from amaranth. SPring-8 User Experiment Report 2009B1299, 2009.
- Mikami, B., M. Adachi, A. Tanabe, R. Hashizume, T. Yoshimura, S. Utsumi: X-ray crystallographic analysis of loop-less b-amylase/maltose complex. SPring-8 User Experiment Report 2009B1461, 2009.
- Mikami, B., M. Tandang, N. Maryuama, T. Itoh, S. Takehara, S. Utsumi: X-Ray crystallographic analysis of plant seed globulins. SPring-8 User Experiment Report 2009B6935, 2009.

A-3.Off-campus activities

Membership in academic societies

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

Research grants

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

- Scientific Research (B) : Mikami, B. : Elucidation of loop function of food related enzymes
- Scientific Research (B) : Takahasi, N. : Molecular basis preventing amiloid formation: The role of inter b structure among the polymer of ovalubumin

2.Other Research Grants

- National Project on Target Proteins : Leader Hashimoto, W.(Collaborator Mikami, B.): 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 : Leader Murata, K (Collaborator Mikami, B.) : Production of ethanol from marine biomass (alginate)

A-4.International cooperation and overseas activities

International joint research, overseas research surveys

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

B.Educational Activities(2009.4-2010.3)

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, Aibara, Takahashi, Mizutani)

B-2.Off-campus teaching etc.

Part-time lecturer

- Mikami, B.: Faculty of Agriculture, Kobe U (Chemistry of Macromolecular Function), Faculty of Agriculture, Shimane U (Food Biochemistry), Faculty of Agriculture, Kinki U (Basic Life Science), Faculty of Medical Life Science, Doshisha U (Structural Biology)
- Takahashi, N.: Mukogawa Women's University; Dep. of Food Sci. and Nutr., School of Human Environ. Sci. (Biochemistry)

C.Other Remarks

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