Chair Food Life Sciences

2.7.1 Laboratory : Enzyme Chemistry

Member:	Professor	Inouye, Kuniyo, PhD	
	Associate Professor	Yasukawa, Kiyoshi, PhD	
	Assistant Professor	Takita, Teisuke, PhD;	Kojima, Kenji, M
	Doctor's program	2	
	Master's Program	8	
	Undergraduate	4	
	Other	4	
	Program-Specific Resea	a 2	
	Researcher	1	

A. Research Activities (2010.4-2011.3)

A-1. Main Subjects

a) Studies on proteolytic enzymes.

(i) Molecular mechanism of the activity of thermolysin. We found that the activity of thermolysin, a typical thermophilic proteinase, is greatly enhanced up to over 20 times in the presence of high concentration (2-5 M) of neutral salts. Thermal stability of the enzyme is also enhanced in the presence of the salts. We expect that the molecular mechanism of the activity of thermolysin can be revealed by understanding the halophilicity of this enzyme. Involvement of tyrosyl residues at the active site and charged groups on the surface of the enzyme in the enhancement of the enzyme activity has been suggested. Site-directed mutagenesis and chemical modification have been applied to reveal the roles of tyrosyl, tryptophyl, lysyl, aspartic, and glutamiac residues in the halophilicity of thermolysin.

(ii) Enzymatic properties of MMP-7. The metalloproteinase MMP-7, which contains a zinc ion essential for enzyme activity, plays an important role in tumor invasion and metastasis with proteolysis of extracellular matrix proteins. We have compared enzymatic properties of MMP-7 with those of thermolysin. In addition, we have studied on naturally occurring MMP-7 inhibitors which could be useful for cancer therapy.

b) Studies on aminoacyl-tRNA synthetases.

Reaction mechanism of lysyl-tRNA synthetase (LysRS) of mesothermophilic bacteria. AminoacyltRNA synthetases guarantee the fidelity of translation of the genetic information into the structure of a protein by their substrate recognition mechanisms. We purified LysRS to homogeneity from Bacillus stearothermophilus. Interactions of the substrates (L-lysine and ATP) and their analogues with LysRS were studied by a combination of several enzyme-activity assays, fluorescence titration, equilibrium dialysis, stopped-flow method etc. The order of binding of the substrates to LysRS and some features of substrate recognition by the enzyme were revealed. We cloned the LysRS gene of Bacillus stearothermophilus, deduced total amino acid sequence, and established the overexpression system by using E. coli. For further details of the recognition mechanism of LysRS, we have applied site-directed mutagenesis to the LysRS gene and tried to evaluate the 3D-structure by X-ray crystallographic analysis. In addition, we have cloned aminoacyl-tRNA synthetase cDNA from hyperthermophilic archaeon Aeropyru pernix Kl and tried its expression in E. coli.

c) Studies on carbohydrate hydroxylases and their inhibitors.

Stabilization of bacterial amylases against thermal denaturation has been examined by mutagenesis and improvement of the reaction conditions. The thermal stability was much improved by introducing negatively-charged residues into the calcium-ion binding sites. In the cases of Bacillus amylases, enhancement in the stabilization has been desired for glucose-production industry, whereas attenuation in the stability for the baking industry. The results obtained might be useful for these purposes. On the other hand, we have purified a protein amylase inhibitor named 0.19AI from the albumin fraction of wheat proteins, and examined the inhibition against porcine pancreas alpha-amylase (PPA). It was revealed that a single electrostatic interaction is essential for the interaction between 0.19AI and PPA. Currently, we are in progress for identifying the residues involved in the electrostatic interaction by chemical modification. The fruits of this study might be useful for prevention and therapy of obesity and diabetes

d) Studies on application of monoclonal antibodies.

(i) The application to immunoassays. Monoclonal antibodies have been used widely in diagnoses and analysis of bioactive substances. There are some points to be improved in enhancement of the sensitivity and simplification in the operation. We would solve these points by the use of active fragments, and bispecific antibodies in place of the native monoclonal antibodies. Liposome assay and fluorometric assay might be examined for development of homogeneous enzyme immunoassays. In order to increase a sensitivity of the enzyme immunoassays, we have developed an assay system using synchronization of multiple enzymes containing an alkaline phosphatase conjugated with a second antibody. We have also studied an enzyme immunoassay for histamine, which is important in food analysis and a test for allergy.

(ii) Catalytic antibodies. Monoclonal antibodies which catalyze the hydrolysis of ester derivatives of chloramphenicol are examined from the view of enzyme kinetics and spectrophotometric analysis.

e) Studies on the application of soy proteins and whey proteins.

Soy proteins especially defatted ones are not utilized well. In this project, a potentiality of the soy proteins as food staffs are examined. We have developed a method of deodorization of soybean proteins by physicochemical processing with hydrophobic resins. In addition, we have developed a new method to make a bean curd with proteinases. We are studying the aggregation process of soy proteins induced by various proteinases with physicochemical methods. On the other hand, whey protein especially gluten is also an important food protein but it has unique amino acid composition so that its proteolytic digestion in vitro and in vivo proceeds hardly. Presently, we are in process to develop an effective method for whey protein degradation to amino acids.

f)Studies on reverse transcriptase

Reverse transcriptase (RT) is an enzyme which is indispensable as a tool for research in molecular biology and diagnosis of RNA virus. Although RTs from avian myeloblastosis virus (AMV) and Moloney murine leukaemia virus (MMLV) have been the most extensively used due to their high catalytic activity, thermal stability, and fidelity, those with higher activity and stability have been desired. We are in process to improve their activities and thermal stabilities by site-directed mutagenesis.

a) Publications

Books

- Inouye, K. and Hashida, Y.: Kinetic analysis-general remarks. Handbook of enzyme technology (Supervised Editor: Komiyama M.), p. 51-55, NTS, Tokyo, 2010 (Japanese)

- Inouye, K. and Yasukawa, K.: Synthesis of aspartame. Handbook of enzyme technology (Supervised Editor: Komiyama M.), p. 632-636, NTS, Tokyo, 2010 (Japanese)

- Inouye, K. (Co-translator) : Biochemistry - Tje molecular Basis of Life (Fourth Edition) (T. McKee and J. R. McKee)(Japanese translated edition), pp. 801, Kagakudojin, Kyoto, 2010 (Japanese)

Original Papers

- Inouye, K., Yasumoto, M., Tsuzuki, S., Mochida, S., and Fushiki, T. The optimal activity of a pseudozymogen form of recombinant matriptase under the mildly acidic pH and low ionic strength conditions. J. Biochem., 147; 485-492, 2010

- Kusano, M., Yasukawa, K., and Inouye, K. Synthesis of N-carbobenzoxy-L-aspartyl-Lphenylalanine methyl ester catalyzed by thermolysin variants with improved activity. Enzyme Microb. Technol., 46; 320-325, 2010 - Kusano, M., Yasukawa, K., and Inouye, K. Effects of the mutational combinations on the activity and stability of thermolysin. J. Biotechnol., 147; 7-16, 2010

- Miyake, Y., Yasumoto, M., Tsuzuki, S., Fushiki, T., and Inouye, K. The role of asparagine-linked glycosylation site on the catalytic domain of matriptase in its zymogen activation. Biochim. Biophys. Acta, 1804; 156-165, 2010

- Miyake, Y., Tsuzuki, S., Fushiki, T., and Inouye, K. Matriptase does not require hepatocyte growth factor activator inhibitor type-1 for activation in an epithelial cell expression model. Biosci. Biotechnol. Biochem., 74; 848-850, 2010

- Tsukiyama, T., Lee, J., Okumoto, Y., Teraishi, M., Tanisaka, T., and Inouye, K. Gene cloning, bacterial expression, and purification of a novel rice (Oryza sativa L.) ubiquitin-related protein, RURM1. Biosci. Biotechnol. Biochem., 74; 430-432, 2010

- Mizuno, M., Yasukawa, K., and Inouye, K. Insight into the mechanism of the stabilization of Moloney murine leukaemia virus reverse transcriptase. Biosci. Biotechnol. Biochem., 74; 440-442, 2010

- Yasukawa, K., Agata, N., and Inouye, K. Detection of cesA mRNA from Bacillus cereus by RNA-specific amplification. Enzyme Microb. Technol., 46; 391-396, 2010

- Narita, Y. and Inouye, K. Kinetic analysis and mechanism on the inhibition of chlorogenic acid and its components against porcine pancreas a-amylase isozymes I and II. J. Agric. Food Chem., 57; 9218-9225, 2010 - Yasukawa, K., Konishi, A., and Inouye, K.: Effects of organic solvents on the reverse transcription reaction catalyzed by reverse transcriptases from avian myeloblastosis virus and Moloney murine leukaemia virus. Biosci. Biotechnol. Biochem., 74: 1925-1930, 2010

- Mochida, S., Tsuzuki, S., Inouye, K., and Fushiki, T.: A recombinant catalytic domain of matriptase induces detachment and apoptosis of small-intestinal epithelial IEC-6 cells cultured on laminin-coated surface. J. Biochem., 148: 721-732, 2010

- Yasukawa, K., Mizuno, M., Konishi, A., and Inouye, K.: Increase in thermal stability of Moloney murine leukaemia virus reverse transcriptase by site-directed mutagenesis. J. Biotechnol., 150: 299-306, 2010

- Inouye, K., Tsuzuki, S., Yasumoto, M., Kojima, K., Mochida, S., and Fushiki, T.: Identification of the matriptase second CUB domain as the secondary site for interaction with hepatocyte growth factor activator inhibitor type-1. J. Biol. Chem., 285: 33394-33403, 2010

- Tsuzuki, S., Murai, N., Miyake, Y., Inouye, K., and Fushiki, T.: The structural requirements of matriptase in its ectodomain release in polarized epithelial cells. Biosci. Biotechnol. Biochem., 74: 1295-1297, 2010

- Muta, Y., Yasui, N., Matsumiya, Y., Kubo, M., and Inouye, K.: Expression in Escherichia coli, refolding, and purification of the recombinant mature form of human matrix metalloproteinase 7 (MMP-7). Biosci. Biotechnol. Biochem., 74: 2515-2517, 2010

- Menach, E., Yasukawa, K., and Inouye, K.: Effects of site-directed mutagenesis of the loop residue of the N-terminal domain of Gly117 of thermolysin on its catalytic activity. Biosci. Biotechnol. Biochem., 74: 2457-2462, 2010

- Ueda M., Goto T., Nakazawa M., Miyatake K., Sakaguchi M., and Inouye K.: A novel coldadapted cellulase complex from Eisenia foetida: characterization of a multienzyme complex with carboxymethylcellulase, beta-glucosidase, beta-1,3 glucanase, and beta-xylosidase. Comp. Biochem. Physiol. B Biochem. Mol. Biol., 157: 26-32, 2010

- Sekiguchi, S., Kohno, H., Yasukawa, K., and Inouye, K.: Chemiluminescent enzyme immunoassay for the measuring of leptin. Biosci. Biotechnol. Biochem., 75: 752-756, 2011

- Narita Y.and Inouye K.: Inhibitory effects of chlorogenic acids from green coffee beans and cinnamate derivatives on the activity of porcine pancreas α -amylase isozyme I. Food Chemistry, 127: 1532-1539, 2011

- Muta, Y. and Inouye, K.: Tyr219 of human matrix metalloproteinase 7 (MMP-7) is not critical for catalytic activity, but is involved in the broad pH-dependence of the activity J. Biochem., in press.

- Sekiguchi, S., Hashida Y, Yasukawa, K., and Inouye, K.: Effects of amines and aminoalcohols on bovine intestine alkaline phosphatase activity. Enzyme Microb. Technol., in press

- Miyake, T., Yasukawa, K., and Inouye, K.: Analysis of the mechanism of inhibition of human matrix metalloproteinase 7 (MMP-7) activity by green tea catechins. Biosci. Biotechnol. Biochem., in press

- Konishi, A., Nemoto, D., Yasukawa, K., and Inouye, K..: Comparison of the thermal stabilities of the ab heterodimer and a subunit of avian myeloblastosis virus reverse transcriptase. Biosci. Biotechnol. Biochem., in press

Reviews

- Inouye, K. (Co-translator). Biochemistry, 4th Edition. The Molecular Basis of Life (T. McKee and J. McKee), p. 171-120, Kagaku-Dojin, Kyoto (2010)

Patents

- Yasukawa, K, and Inouye, K. Reverse transcriptase variants, nucleic acid that encode reverse transcriptase variants, production method of reverse transcriptase variants, method to improve thermostabilities of nucleic acid-related enzymes, method for reverse transcription, reverse transcriptation reaction kits, and test kits: 2010-181471 (August 13, 2010)

- Inouye, K., Murakami, Y., and Kanaya, M.: Preparative method of water-soluble hemicellulose, 2009-169711 (disclosure: Jan. 21, 2010)

b) Conference and seminar papers presented

- 57th Annual Meeting of Kinki Branch of the Japanese Biochemical Society: 4 papers

- Meeting of Kansai Branch of the Japan Society for Bioscience, Biotechnology, and Agrochemistry, 3 papers

- 10th Annual Meeting of the Food Enzyme Chemistry Forum: 1 paper

- 62th Annual Meeting of the Japanese Society for Biotechnology: 1 paper

- 33th Annual Meeting of the Molecular Biology Society of Japan and 83th Annual Meeting of Japanese Biochemical (BMB 2010): 4 papers

- 468th Meeting of Kansai Branch of the Japan Society for Bioscience, Biotechnology, and Agrochemistry: 1 paper

- Annual Meeting of the Japan Society (2011) for Bioscience, Biotechnology, and Agrochemistry: 15 papers

A-3.Off-campus activities 1

Membership in academic societies

- Inouye, Kuniyo, PhD : The Japanese Biochemical Society (Councilor, Councilor of the Kinki Branch; Annual Meeting Advisory Member), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Director, Chief of the Kansai Branch, Councilor of the Kansai Branch), The Japanese Society for Food and Technology (Councilor of the Kansai Branch), Japanese Association of Animal Cell Technology (Councilor), Academic Meeting of the Food Enzyme Chemistry Forum (Chair), Japanese Association of Food Analysis (Councilor), The Japanese Society for Proteases in Pathophysiology (Councilor)

- Yasukawa, Kiyoshi, PhD : Japan Society for Bioscience, Biotechnology, and Agrochemistry (Member of Committees of Symposium and Program of Annual Meeting, Organizer of General Affairs of Kansai buranch, Member of Article Judging Committee) A-3.Off-campus activities 2

Research grants

Categories

- Inouye, Kuniyo, PhD : Protein engineering and reaction control engineering of thermolysin aiming at expansion of use of thermolysin in food industry : Research (C)

- Yasukawa, Kiyoshi, PhD : Evaluation of the cDNA synthesis and RNA amplification with reverse transcriptase with high performances and expansion of their use :

2.Other Research Grants

- NEDO Grants for Innovation Practicing Support: Inouye, Kuniyo, PhD: Development of a carriable immuno-analyzing system

A-4.International cooperations and overseas activities 1

Membership in academic societies

- Inouye, Kuniyo, PhD: Biotechnology Annual Review (Elsevier) Vols. 1-15 (Editor), New Biotechnology (Elsevier) (Review Editor), Enzyme and Microbial Technology (Elsevier) (Editor), Food Science and Biotechnology (International Editorial Board Member)

International meetings(country,roles)

- Inouye, Kuniyo, PhD: 2nd International Conference on Novel Enzymes. University of Exeter, Exeter, UK. (Invited speaker) (April, 2010), 20thInternational Congress of Fibrinolysis and Proteolysis (ISFP 2010), Amsterdam, The Netherlands (Poster) (August, 2010), 14th International Biotechnology Symposium and Exhibition (IBS 2010). Rimini, Italy (Speaker) (Septemper, 2010), 6th Meeting of East Asia for Mushroom Science 2010. Gyeongyu, Korea, (Poster) (November, 2010), 2010 International Chemical Congress of Pacific Basin Societies (Pacifichem 2010), (Invited speaker) (December, 2010)

International joint research, overseas research surveys

- Proteases on bacterial infection, diagnosis, and therapy, Inouye, Kuniyo, PhD (University of Michigan, USA)

- Characterization of soy and wheat protein, Inouye, Kuniyo, PhD (University of Wageningen, the Netherlands)

- Role of MMPs in digestive diseases, Inouye, Kuniyo, PhD (University of Leiden and University of Groningen, the Netherlands)

- Reaction mechanism of MMPs, Inouye, Kuniyo, PhD (Imperial College, London, UK)

- Biotechnology of enzymes and antibodies, Inouye, Kuniyo, PhD (University of Tromso, Norway)

- Research on structure-function relationship of thermophilic enzyme, Inouye, Kuniyo, PhD (Seoul National University, Yonsei University and Gwanju Institute of Technology, Korea)

- Enzyme reaction mechanism, Inouye, Kuniyo, PhD (Warwick and Exceter, UK)

- Research on reaction mechanism of amylase and proteinase and their application to food science and technology, Inouye, Kuniyo, PhD (Seoul, Korea)

- Application of amylases and proteinases, Inouye, Kuniyo, PhD (University of Guelph, Canada)

- Collaborative study on the stabilization of proteins, Inouye, Kuniyo, PhD (Graz Technical University, BOKU, and Austrian Research Center for Science and Technology, Austria)

B.Educational Activities (2010.4-2011.3)

B-1.On-campus teaching

a) Courses given

- Undergraduate level : I	Introduction of the Molecular Cell Biology (Inouye, Yasukawa), Food
]	Biochemistry II (Yasukawa), Food Biochemistry II (Yasukawa), Enzymes:
]	Function and Application (Inouye, Yasukawa), Enzyme Chemistry (Inouye,
•	Yasukawa), Introduction to Research I (Inouye, Yasukawa, Takita, Kojima),
Laboratory Course in Enzyme Chemistry and Biochemistry (Yasukawa	
r	Takita, Kojima).

- Graduate level: Advanced Course of Enzyme Chemistry (Inouye, Yasukawa), Enzyme Chemistry Seminar (Inouye, Yasukawa, Takita, Kojima), Experimental Course of Enzyme Chemistry (Inouye, Yasukawa, Takita, Kojima).

B-2.Off-campus teaching etc.

Part-time lecturer

- Inouye, Kuniyo, PhD: Iwate University, Faculty of Agriculture

- Inouye, Kuniyo, PhD: Prefectural University of Hiroshima, Graduate School of Life and Environment; Special Lecture (2010, June)

- Inouye, Kuniyo, PhD: Toyo University, Faculty of Life Sciences; Special Lecture (2010, October)

Open lectures, etc.

- Inouye, Kuniyo, PhD: Fukui Future-Technology Creation Network Seminar Lecturer

- Inouye, Kuniyo, PhD: Niigata University Region-collaborative Food Science Center Seminar (Niigata, 2011, January)

- Inouye, Kuniyo, PhD: Fukuyama University Green Science Seminar (Fukuyama, 2011, February)

- Inouye, Kuniyo, PhD: Kyoto Industry-Academia-Public Collaboration Organization International Symposium, Panel Discussion Paneller (Kyoto, 2011, March)

B-3.Overseas teaching 1

International students

- International students : Master 3 (Kenya 1, Zimbabwe 1, China 1) Research Students 4 (China 3, Ethiopia 1)

C.Other Remarks

- Inouye, Kuniyo, PhD: Member of the Institute of Science and Technology Policy of the Ministry of Education, Culture, Sports, Science and Technology, Kyoto Municipal Bio-industrial Business Promotion Forum, Member of Advisory Committee for Kyoto Municipal Project of the Cooperation of Medicine, Engineering, and Life Sciences, Member of the Judging Committee for Member of the Ministry of Economy, Trades, and Industry, Member of the Judging Committee for Industrialization by Minor Enterprises, Member of the Science Committee of the Iijima Foundation, Visiting Professor of Toyo University, Visiting Researcher of the Nippon University Biotechnology Research Center, Member of the Judging Committee for JSPS Fellows of the Japan Society for the Promotion of Science, Member of the Kansai Science Forum Working Group

- Inouye, Kuniyo, PhD: Deputy Director-General of the University Office of Society-Academia Collaboration for Innovation (SACI), Member of the Committee for the Operation of the Office of SACI, Member of the Committee for the Operation of the Innovative Collaboration Center, Member of the Plan for the Future of the Graduate School of Agriculture, Member of the Committee for the Building of the Graduate School of Agriculture, Member of the Committee for Undergraduate Education of the Graduate School of Agriculture, Cooperative-researcher of the Research Institute of Humanosphere, Kyoto University, Member of the Committee for the Operation of Endowed Chair of the the Graduate School of Agriculture, Kyoto University Science-Technology-Area Invention Evaluation Committee, Chief Member, Kyodai Venture Fund Investment-Ethics Evaluation Committee, Member

- Yasukawa, Kiyoshi, PhD: Member of the Committee for Student's Life of the Graduate School of Agriculture