

2.7 DIVISION OF FOOD SCIENCE AND BIOTECHNOLOGY

Division of Food Science and Biotechnology established on April 2001 comprises three basic chairs: Food Life Sciences, Food and Health Science, and Food Production Technology. Food is the vital alimentary material for human to sustain life and to promote wellness, therefore, must be highly acceptable for human consumption. The challenges of this century are to overcome worldwide problems of food production and prevailed life style-related diseases.

To establish fundamental concept of foods for improving quality of life from various points of view, we take a multidisciplinary approach including sciences related to natural resources, environment, culture, social, life sciences, and information technology. We have the education and research programs of studying food materials at chemical, biological and physiological level using the updated information and technology about rapidly-advancing bioscience. We are developing a new methodology for food production using bioengineering and gene technology.

Division of Food Science and Biotechnology includes 8 laboratories, in which located at three basic chair, Enzyme Chemistry, Food and Environmental Sciences, Organic Chemistry in Life Science, Nutrition Chemistry, Molecular Function of Food, Physiological Function of Food, Bioengineering, and Basic and Applied Molecular Biotechnology. Currently, for the graduate program, 61 first year students are working towards master's degree, and 31 students toward Ph.D. including 13 international students. In undergraduate program, 33 freshmen, 34 sophomores, 38 juniors, and 31 seniors are enrolled.

Chair of Food Life Sciences

2.7.1 Laboratory of Enzyme Chemistry

Staff *Professor* : Inouye, Kuniyo, Dr. Agric. Sci. (Kyoto Univ.)
 Associate Professor: Yasukawa, Kiyoshi, Dr. Med. Sci. (Osaka Univ.) (From 10/1)
 Assistant Professor : Nakatani, Hiroshi, Dr. Sci. (Kyoto Univ.)
 Assistant Professor : Takita, Teisuke, Dr. Agric. Sci. (Kyoto Univ.)

Students and research fellows

Doctor's program: (7)
Master's program: (12)
Undergraduate : (3)
Research fellow : (1)

A. Research Activities (2004.4-2005.3)

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 glutamic 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.

(i) Reaction mechanism of lysyl-tRNA synthetase (LysRS) of mesothermophilic bacteria. Aminoacyl-tRNA 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 *Aeropyrum pernix* K1 and tried its expression in *E. coli*.

c) Studies on carbohydrate hydroxylases and glucanotransferases.

(i) Identification of catalytic amino acid residues from pK and heat of ionization using statistical analysis. The cluster analysis, one of statistical classification methods, was applied to identify catalytic amino acid residues of enzymes using pK and heat of ionization. It was confirmed that model organic compounds containing carboxyl, imidazole, thiol and amino groups are classified exactly into four clusters, irrelevantly with calculation methods to estimate mutual distance between two components or clusters. Representative data of which ionic residues had been well identified were at first examined whether they are classified into the correct groups. The analysis was carried out adding one additional datum of an enzyme into the model organic compounds, to search in which cluster the added datum belongs. Carboxyl and imidazole groups in lysozyme, trypsin and chymotrypsin were classified into the correct groups with one of the calculation methods (weighted pair-group method). The cluster analysis using the weighted pair-group method was applied to 13 catalytic ionic amino acid residues in 7 enzymes. The results were quite reasonable except one datum. The cluster analysis is reliable for the identification of catalytic amino acid residues from thermodynamic data since catalytic residues locate usually around the surface of enzymes.

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d) Studies on oxidoreductases.

(i) Studies on structure-function relationship of microsomal cytochromes P450. We have examined metabolism of dibenzo-p-dioxins (PCDDs) by twelve forms of human cytochrome p450 (CYP) and rat CYP1A1. Multiple forms of CYPs showed remarkable metabolism of 0 to 3-chlorinated PCDDs. Substrate specificity and reaction specificity of the CYPs towards PCDDs were significantly different from each other. Although human CYP1A1, rat CYP1A1 and human CYP1A2 showed no detectable activity towards 2,3,7,8-TCDD which was known to be most toxic among PCDDs, they showed notable activities towards 2,3,7-TCDD. To generate a 2,3,7,8-TCDD-metabolizing enzyme, more than 20 mutants of rat CYP1A1 were constructed by

site-directed mutagenesis. Among the mutants, F228A, F240A, F319A and F385A showed catalytic activity towards 2,3,7,8-TCDD.

(ii) Reaction mechanism of mammalian vitamin D₃ hydroxylases. we have succeeded in the expression of human vitamin D₃ 25-hydroxylase (CYP27A1), 1 α -hydroxylase (CYP27B1) and 24-hydroxylase (CYP24) cDNAs in *Escherichia coli* and revealed their enzymatic properties. Missense mutants of CYP27B1 derived from patients with rickets and mutants of CYP27A1 derived from patients with cerebrotendinous xanthomatosis were analyzed to reveal structure-function relationships of both CYP27B1 and CYP27A1. Human and rat CYP24 showed remarkable metabolism of 1 α , 25-dihydroxyvitamin D₃. It was noted that a clear difference was observed between human and rat CYP24 on the metabolism of 1 α , 25-dihydroxyvitamin D₃ and its analogs.

e) 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.

(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.

f) Studies on the uses of soyproteins.

Soyproteins especially defatted ones are not utilized well. In this project, a potentiality of the soyproteins as food stuffs 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.

A-2. Publications and presentations

a) Publications

Books

Inouye, K.: Anticipation to Food Enzyme Chemistry. In: Food Enzyme Chemistry, its Most Up-to-date Technology and Application and Perspectives to Food Proteomics (Editor: Inouye, K.) p.1-12, CMC, Tokyo, 2004 (Japanese)

Oneda, H. and Inouye, K.: Wheat proteinaceous α -amylase inhibitor . In: Food Enzyme Chemistry, its Most Up-to-date Technology and Application and Perspectives to Food Proteomics (Editor: Inouye, K.) p.34-39, CMC, Tokyo, 2004 (Japanese)

Inouye, K.: Thermolysin. In: Food Enzyme Chemistry, its Most Up-to-date Technology and Application and Perspectives to Food Proteomics (Editor: Inouye, K.) p.105-114, CMC, Tokyo, 2004 (Japanese)

Inouye, K. (Editor): Biotechnology Annual Review, Vol. 10, Elsevier, Amsterdam, the Netherlands, 2004

Inouye, K. (Editor): Food Enzyme Chemistry, its Most Up-to-date Technology and Application and

Perspectives to Food Proteomics (Supervised Editor: Inouye, K.) p.1-243, CMC, Tokyo, 2004 (Japanese)

Inouye, K. and Oneda, H.: Chapter I-5, Higher-Structural Analysis. In: Instrumental Analysis in Biosciences (Editor in Chief: Murao, S.. Editor: Shin, T.), p.132-150, Sankyo Publishing, Tokyo, 2004 (Japanese)

Original papers

Kamao, M., Tatematsu, S., Hatakeyama, S., Sakaki, T., Sawada, N., Inouye, K., Ozono, K., Kubodera, N., Reddy, G. S., and Okano, T.: C-3 epimerization of vitamin D3 metabolites and further metabolism of C-3 epimers: 25-hydroxyvitamin D3 is metabolized to 3-epi-25-hydroxyvitamin D3 and subsequently metabolized through C-1 α or C-24 hydroxylation. *J. Biol. Chem.* 279; 15897-15907, 2004

Matsumiya, Y., Nishikawa, K., Aoshima, H., Inouye, K., and M. Kubo: Analysis of autodegradation sites of thermolysin and enhancement of its thermostability by modifying Leu¹⁵⁵ at an autodegradation site. *J. Biochem.* 135; 547-553, 2004

Sawada, N., Kusudo, T., Sakaki, T., Hatakeyama, S., Hanada, M., Abe, D., Kamao, M., Okano, T., Ohta, M., and Inouye, K.: Novel metabolism of 1 α , 25-dihydroxyvitamin D3 with C24-C25 bond cleavage catalyzed by human CYP24A1. *Biochemistry* 43; 4530-4537, 2004

Yamamoto, K., Matsuno, H., Sawada, N., Sakaki, T., Inouye, K., Ishiguro, M., and Yamada, S.: Homology modeling of human 25-hydroxyvitamin D3 1 α -hydroxylase (CYP27B1) based on the crystal structure of rabbit CYP2C5. *J. Steroid Biochem. Mol. Biol.* 89-90; 167-171, 2004

Oneda, H., Lee, S., and Inouye, K.: Inhibitory effect of 0.19 AI α -amylase inhibitors from wheat kernel on the activity of porcine pancreas α -amylase and its thermal stability. *J. Biochem.* 135; 421-427, 2004

Lee, S.-B., Nam, K.-c., Lee, S.-J., Lee, J.-H., Inouye, K., and Park, K.-H.: Antioxidative effects of glucosyl-ascorbic acids synthesized by maltogenic amylase to reduce lipid oxidation and volatiles production in cooked chicken meat. *Biosci. Biotechnol. Biochem.* 68; 36-43, 2004

Okumura, S., Akao, T., Higuchi, K., Saitoh, H., Mizuki, E., Ohba, M., and Inouye, K.: *Bacillus thuringiensis* serovar shandongiensis strain 89-T34-22 produces multiple cytotoxic proteins with similar molecular masses against human cancer cells. *Lett. Appl. Microbiol.* 39; 89-92, 2004

Tsumura, K., Saito, T., Kugimiya, W., and Inouye, K.: Selective proteolysis of the glycinin and β -conglycinin fractions in a soy protein isolate by pepsin and papain with controlled pH and temperature. *J. Food Sci.* 69; C363-367, 2004

Sawada, N., Sakaki, T., Yoneda, S., Kusudo, T., Shinkyo, R., Ohta, M., and Inouye, K.: Conversion of vitamin D3 to 1 α ,25-dihydroxyvitamin D3 by *Streptomyces griseolus* cytochrome P450SU-1. *Biochem. Biophys. Res. Commun.* 320; 156-164, 2004

Nagai, K. and Inouye, K.: Insights into the reaction mechanism of the coagulation of soy protein isolates induced by subtilisin Carlsberg. *J. Agric. Food Chem.* 52; 4921-4927, 2004

Kasai, N., Sakaki, T., Shinkyo, R., Ikushiro, S., Iyanagi, T., Kamao, M., Okano, T., Ohta, M., and Inouye, K.: Sequential metabolism of 2,3,7-trichlorodibenzo-p-dioxin (2,3,7-triCDD) by cytochrome P450 and UDP-glucuronosyltransferase in human liver microsomes. *Drug Metab. Dispos.* 32; 870-875, 2004

Oneda, H., Muta, Y., and Inouye, K.: Substrate-dependent activation of thermolysin by salt. *Biosci.*

- Biotechnol. Biochem. 68; 1811-1813, 2004
- Kusudo, T., Sakaki, T., Abe, D., Fujishima, T., Kittaka, A., Takayama, H., Hatakeyama, S., Ohta, M., and Inouye, K.: Metabolism of A-ring diastereomers of 1 α , 25-dehydroxyvitamin D3 by CYP24A1. Biochem. Biophys. Res. Commun. 321; 774-82, 2004
- Muta, Y., Oyama, S., Umezawa, T., Shimada, M., and Inouye, K.: Inhibitory effects of lignans on the activity of human matrix metalloproteinase 7 (matrilysin). J. Agric. Food Chem. 52; 5888-5894, 2004
- Uchida, E., Kagawa, N., Sakaki, T., Urushino, N., Sawada, N., Kamakura, M., Ohta, M., Kato, S., and Inouye, K.: Purification and characterization of mouse CYP27B1 overproduced by an Escherichia coli system coexpressing molecular chaperonins GroEL/ES. Biochem. Biophys. Res. Commun. 323; 505-511, 2004
- Oneda, H. and Inouye, K.: Detection of antigen-antibody reaction using fluorescent probe and its application to homogeneous competitive-type immunoassay for insulin. Biosci. Biotechnol. Biochem. 68; 2190-2192, 2004
- Sinkyo, R., Sakaki, T., Kamakura, M., Ohta, M., and Inouye, K.: Metabolism of vitamin D by human microsomal CYP2R1. Biochem. Biophys. Res. Commun. 324; 451-457, 2004
- Sulistyaningdyah, W.T., Ogawa, J., Li, Q.S., Shinkyo, R., Sakaki, T., Inouye, K., Schmid, R.D., and Shimizu, S.: Metabolism of polychlorinated dibenzo-p-dioxins by cytochrome P450 BM-3 and its mutant. Biotechnol. Lett. 26; 1857-1860, 2004
- Muta, Y., Oneda, H., and Inouye, K.: Inhibitory effects of alcohols on the activity of human matrix metalloproteinase 7 (matrilysin). Biosci. Biotechnol. Biochem. 68; 2649-2652, 2004
- Inouye, K. and Nagai, K.: Development of a new convenient method for proteolytic activity assay using turbidity change in soyprotein solutions. Soy Protein Research, Japan 7: 48-51, 2004 (Japanese)

Patents

- Application filing no. 2004-253995: A novel preparation method for proteins from *Bacillus thuringiensis*. Inventors: Okumura, S., Wasano, N., Saitoh, H., and Inouye, K., Applicant: Okumura, S., Application date: Sept. 1, 2004

Reviews

- Inouye, K.: Biosensing and Nanotechnology in the Post-genomic Era. Nippon-Nougei-Kagakaishi 78; 112, 2004

b) Conference and seminar papers presented

- Annual Meeting of the Japan Society (2004) for Bioscience, Biotechnology, and Agrochemistry: 20 papers
- 77th Annual meeting of the Japanese Biochemical Society: 11 papers
- Annual Meetings of Kansai Branch and Nishi-Nippon Branche of the Japan Society for Bioscience, Biotechnology, and Agrochemistry: 7 papers
- Annual Meeting of the Japan Society for Food Science and Technology: 1 paper
- Annual Meeting of the Japanese Society for Biotechnology : 1 paper
- Annual Meeting of the Japanese Society for Biophysics: 1 paper
- Annual Meeting of the Japanese Vitamin Society: 1 paper
- Annual Meeting of the Food Enzyme Chemistry Forum: 1 paper

A-3. Off-campus activities

Membership in academic societies (roles)

Inouye, K.: The Japanese Biochemical Society (Councilor, Editorial board of J. Biochem, Member of Article Judging Committee), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Director, Councilor of the Kansai Branch, Member of Article Judging Committee), Japanese Association of Animal Cell Technology (Councilor), The Japanese Society for Food and Technology (Councilor of the Kansai Branch), Japanese Association of Food Analysis with Enzymes (Councilor)

Research grants

Monbu-Kagaku-sho Research Grant, Research (C) (2): "Metabolism of vitamin D analogs by human enzymes" (Sakaki, representative)

Monbu-Kagaku-sho Research Grant for Encouragement of Young Scientist (A): "Molecular mechanism and alteration of the substrate-specificity of class II aminoacyl-tRNA synthetase. (Takita, representative)

Health Science Research Grants from the Ministry of Health Labour and Welfare of Japan: "Studies on metabolism of dioxins and mechanism of their toxicity" (Inouye, Sakaki cooperative)

A-4. International cooperations and overseas activities

International meetings (roles)

Inouye, K.: Korea-Japan Symposium on Food Enzyme Chemistry (Organizer)

Inouye, K.: Biotechnology Annual Review (Series), Elsevier, Amsterdam, the Netherlands (International Editor).

Inouye, K.: International COE Symposium, Wakayama (Coauthor)

Inouye, K.: World Rice Research Conference, Tsukuba (Coauthor)

Inouye, K.: 8th International Conference on Biology and Synchrotron Radiation, Himeji (Coauthor)

Inouye, K.: 10th International Conference on the Crystallization of Biological Macromolecules, Beijin (Coauthor)

International joint researches, overseas research surveys

Inouye, K.: Multilateral studies on bispecific antibodies (the Netherlands) Biotechnology of enzymes and antibodies (Norway) Research on structure-function relationship of thermophilic enzyme (Korea) Japan-Korea Core Universities Joint Research on the Energy Science between Kyoto University and Seoul National University (Korea) Research on reaction mechanism of amylase and proteinase and their application to food science and technology (Korea)

Editorial work for international journals (roles)

Inouye, K.: Biotechnology annual Review, Vol8, 2002, Elsevier (editor).

Inouye, K.: Applied Microbiology and Biotechnology (editor)

Inouye, K.: Journal of Biochemistry (associate editor)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Food Basic Biology I (Inouye), Food Molecular Biology (Inouye), Enzyme chemistry (Inouye, Yasukawa), Introduction to research I (Inouye, Yasukawa), Laboratory work of enzyme chemistry (Inouye, Yasukawa)

Graduate level: Enzyme chemistry (Advanced course) (Inouye, Yasukawa), Food Life Science (Advanced course) (Inouye), Seminar in enzyme chemistry (Inouye, Yasukawa), Laboratory course in enzyme chemistry (Inouye, Yasukawa).

B-2. Off-campus teaching, etc.

Part-time lecturer

Inouye, K.: Ritsumeikan University, Graduate School of Science and Technology (Biological Reaction Technology); Soujou University, Graduate School of Technology (Biological Functions); University of the Ryukyus, Graduate School of Agriculture (Applied Biological Chemistry)

Open seminar, etc.

Inouye, K.: Industry-Academia Cooperation Meeting based on the Agribio-technology of Kyoto University. Tokyo

B-3. Overseas teaching

Students and research fellows from abroad

Korea (2 graduate students)

C. Other remarks

Committees

Inouye, K.: Member of the Kyoto University Student Administrative Office; Member of the Committee for Development of Industry-University Government Cooperation in Kyoto University; Member of Judging Committee for Industrialization by Minor Enterprises; Member of the Kyoto Municipal Bio-industrial Business Promotion Forum

Takita, T.: Member of the Committee for the Protection of Radiation Damage of the Graduate School of Agriculture

2.7.2 Laboratory of Food and Environmental Sciences

Staff Professor : Kitabatake, Naofumi, D. Agric. Sci.

Associate Professor: Tani, Fumito, D. Agric. Sci.

Assistant Professor : Masuda, Tetsuya, M. Agric. Sci.

Students and research fellows

Doctor's program : (3)

Master's program : (5)

Undergraduate : (4)

Research fellow : (2)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

- a) Functional properties of food proteins: The major component of milk whey protein, β -lactoglobulin, was mixed and heated together with the major components of egg white, ovalbumin and lysozyme. Molecular complex was observed by heating at neutral and acidic pH. Binding mechanism and molecular interaction have been analyzed. This hetero-complex of these protein would develop new type of food materials
- b) Astringent taste at acidic pH induced by milk whey protein and its reduction by novel technique: Milk whey protein shows an astringent taste at acidic pH. In this study the characteristics of this astringency induced by protein were analyzed.
- c) Studies on the taste-eliciting activity of proteins: Protein has usually no taste and flavor. However, we found some kinds of protein elicit sweet and/or astringent taste. The mechanism of taste-eliciting activity by proteins have been studied. Egg white lysozyme is one of sweet-tasting proteins. Its taste-eliciting mechanism has been analyzed using chemical modification and site-directed mutagenesis method.
- d) Digestion and physiological properties of food polysaccharides: Degradation and digestibility of starch have been analyzed in vitro and in vivo experiments using mice and humans.
- e) Studies on the responses of innate immunity to changing environments:

The gastrointestinal tract, where we encounter with a variety of beneficial food constituents as well as harmful antigens including pathogens, accommodates many immune cells at the boundary environment. Macrophages, dendritic cells, B cells, and NK cells are the essential components of the innate immune system and sentinels to sense danger signals of invaders in the mucosal system.. We are studying the immunological role on macrophages and B cells of heat shock protein 70 that is the major intracellular component of all kinds of living cells and functions as an immunoregulatory molecule, to establish the food informatics for regulating the physiological functions of gut-associated lymphoid tissues. When hsp70s derived from mouse, spinach plant, *Lactobacillus acidophilus* and *Escherichia coli* were compared, we recently found that the distinct mechanism required for hsp70 recognition works in between macrophages and B cells, depending on the sequence diversity at the C-terminal region of hsp70.

A-2. Publications and presentations

a) Publications

Original papers

- Ohno M, Kitabatake N, Tani F: Role of the C-terminal region of mouse inducible Hsp72 in the recognition of peptide substrate for chaperone activity. *FEBS Letter* **576**, 381-386 (2004)
- Masuda T., Ueno Y., and Kitabatake N.: High yield secretion of the sweet-tasting protein lysozyme from the yeast *Pichia pastoris*. *Protein Expr. Purif.* **39**, 35-42 (2005)
- Tani F. Shirai N., Nakanishi Y., Yasumoto K., and Kitabatake N.: Role of the carbohydrate chain and two phosphate moieties in the heat-induced aggregation of hen ovalbumin. *Biosci. Biotechnol. Biochem.* **68**, 2466-2476 (2004)
- Hirose J. Kitabatake N., Kimura A., and Narita H.: Recognition of native and /or thermally induced denatured forms of the major food allergen, ovomucoid, by human IgE and mouse monoclonal IgG antibodies. *Biosci. Biotechnol. Biochem.* **68**, 2490-2497 (2004)
- Masuda T., Ide N., and Kitabatake, N.: Effects of chemical modification of lysine residues on the sweetness of lysozyme. *Chemical Senses.* **30**, 253-264 (2005)
- Sano H., Egashira T., Kinekawa Y., and Kitabatake N.: Astringency of bovine milk whey protein. *J. Dairy Sci.* **88**, 2312-2317 (2005)

Reports

- Masuda T. and Kitabatake N.: The San-Ei Gen Foundation for Food Chemical Research ANNUAL REPORT, 2004
- Masuda T. and Kitabatake N.: Effects of Salt on the Taste Quality of Sweet protein Thaumatin, Salt Research Foundation 2004

b) Conference and seminar papers presented

- The 50th Annual Meeting of Japanese Society of Food Science and Technology 2004: 2 papers
- The annual meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry 2004: 7 papers
- The annual meeting of Kansai branch of the Japan Society for Bioscience, Biotechnology and Agrochemistry: 3 paper

A-3. Off-campus activities

Membership in academic societies

- Kitabatake, N: Japan Society for Bioscience, Biotechnology, and Agrochemistry (Board in Kansai Branch), The Japanese Society for Food Science and Technology (Board), Biosci. Biotechnol. Biochem. Editorial board, International Food Science and Technology Research Editorial board

Membership in Science Council of Japan, etc.

- Committee member of nutrition and food science division, Science Council of Japan. (Kitabatake)

Research grants

- Grant from the Ministry of Agriculture, Forestry and Fisheries of Japan. (Kitabatake)
- Grant-in-Aid for Scientific Research (B)(2) "Studies on taste-eliciting activity of proteins." (Kitabatake)
- Grant-in-Aid for Scientific Research (S)(2) "Integrated studies on sustainable development of rural area in Africa through regional fieldworks." (Kitabatake)
- Grant-in-Aid for Exploratory Research "Principles for the molecular mechanism that creates the

diversity in biological responses .” (Tani)
Grant-in-Aid for Scientific Research (Young scientist B) “Studies on taste-eliciting mechanism of egg white lysozyme” (Masuda)
Grant from Japan Society of Promotion of Science. (Masuda)
Grant from San-Ei Gen F.F.I.,Inc. (Masuda)

A-4. International cooperations and overseas activities

a) International meeting

Kitabatake N., Kaneko R., Masuda T.: Structure-sweetness relationship in thaumatin.
ISOT/JASTS 2004 Kyoto July 5-9.

Masuda T and Kitabatake N.: Sweetness of lysozyme ISOT/JASTS 2004 Kyoto July 5-9.

b) Other remarks (Co-operative studies)

Kitabatake N.: JICA Expert “SUA Centre for Sustainable Rural Development” Project (Tanzania).

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Introduction to the Molecular Cell Biology III (Tani), Introduction and Practice in the Department of Food Science and Biotechnology I (Kitabatake), Food Safety I (Kitabatake, Tani), Food and Environmental Studies (Kitabatake), Laboratory Course in Chemical Engineering (Tani), Seminar in Information Processing Basics (Tani)
Graduate level: Food and Environmental Sciences (Kitabatake), Food and Environmental Sciences Seminar (Kitabatake), Food and Environmental Sciences Seminar (Kitabatake, Tani), Experimental Course in Food and Environmental Sciences (Kitabatake, Tani)

B-2. Off-campus teaching, etc.

Part-time lecturer

Kitabatake N.: Shiga Prefecture University, Okayama University, Kyoto Women’s University, Nagasaki University

B-3. Overseas teaching

Students and research fellows from abroad

Doctor’s program: (1, China)

C. Other remarks

Kitabatake N.: Adviser of Center for Medium and Small Companies of Kyoto Prefecture

Kitabatake N.: Adviser of Wakayama Prefecture

Kitabatake N.: Adviser of Shiga Prefecture

2.7.3 Laboratory of Organic Chemistry in Life Science

Staff Professor : Ohigashi, Hajime, Dr. Agric. Sci.

Associate Professor: Irie, Kazuhiro, Dr. Agric. Sci.

Assistant Professor : Murakami Akira, Dr. Agric. Sci.

Students and research fellows

Doctor's Program: (6)

Research Fellow: (1)

Postdoctoral Fellow: (2)

Master's Program: (10)

Undergraduate : (3)

Research Student: (1)

Collaborative Laboratory (IIC)

Professor: Hirai, Nobuhiro, Dr. Agr. Sci.

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Studies on the regulation of life style-related disease

We recently showed that zerumbone, a sesquiterpene found in subtropical ginger, suppresses colonic tumor marker formation in rats and induces apoptosis in colon cancer cell lines. In this study, the anti-tumor initiating and promoting activities of zerumbone in mouse skin were evaluated using a conventional two-stage carcinogenesis model. A single topical pretreatment to mouse skin (2 micromol) 24 hours prior to application of dimethylbenz[a]anthracene (0.2 micromol) markedly suppressed tumor incidence by 60% and the number of tumors by 80% per mouse. Repeated pretreatment (16 nmol) twice weekly during the post-initiation phase reduced the number of 12-O-tetradecanpylphorbol-13-acetate (TPA, 1.6 nmol)-induced tumors by 83% as well as their diameter by 57%. Multiple reverse transcription-polymerase chain reaction experiments revealed that zerumbone (2 micromol) enhanced the mRNA expression level of manganese superoxide dismutase, glutathione peroxidase-1, glutathione S-transferase-P1, and NAD(P)H quinone oxidoreductase in the epidermis, however, not that of cytochrome P450 1A1 or 1B1. Further, it diminished TPA-induced cyclooxygenase-2 protein expression and phosphorylation of extracellular signal-regulated kinase 1/2, while pretreatment(s), in either the priming or activation stage or both, reduced double TPA application-induced hydrogen peroxide formation and edema induction by 29% to 86%, respectively. Histological examination revealed that pretreatment(s) with zerumbone suppressed leukocyte infiltration and reduced proliferating cell nuclear antigen-labeling indices. Together, our results indicate that zerumbone is a promising agent for the prevention of both tumor initiating and promoting processes, through induction of anti-oxidative and phase II drug metabolizing enzymes as well as attenuation of proinflammatory signaling pathways.

b) Chemistry of tumor promoter and protein kinase C (PKC)

Protein kinase C (PKC) isozymes are major receptors of tumor-promoting phorbol esters. Recent investigations discovered nonkinase-type phorbol ester receptors, RasGRP1-4, chimaerins, and Unc13s. Phorbol ester binding occurs at the cysteine-rich sequences of about 50 residues in the C1 domains of these receptors. Fifty-one-residue RasGRP C1 peptides except for RasGRP2 showed significant phorbol 12,13-dibutyrate (PDBu) binding, but the K_d values of the RasGRP1 and RasGRP3 C1 peptides were about 10-fold larger than those for the corresponding whole enzymes. Addition of the C-terminal basic amino acid cluster decreased their K_d values about 10-fold, suggesting that the positive charges of these C1 peptides play an important role in the

PDBu binding in the presence of negatively-charged phosphatidylserine. The 51-mer chimaerin C1 peptides showed potent PDBu binding, while the Unc13 and Munc13-1 C1 peptides without sufficient positive charges hardly bound PDBu. By the rapid screening system using this C1 peptide library along with the PKC C1 peptides recently developed, 5-prenyl-indolactam-V was identified as a promising lead for the novel protein kinase C isozyme (PKC δ , ϵ , η and θ) selective ligands.

c) Chemistry of Alzheimer's β peptide

The aggregation of 42-mer amyloid β (A β 42) plays a central role in the pathogenesis of Alzheimer's disease. Our recent research on proline mutagenesis of A β 42 suggested that the formation of a turn structure at positions 22 and 23 could play a crucial role in its aggregative ability and neurotoxicity. Since E22K-A β 42 (Italian mutation) aggregated more rapidly and with more potent neurotoxicity than wild-type A β 42, the tertiary structure at positions 21-24 of E22K-A β 42 fibrils was analyzed by solid-state NMR using dipolar assisted rotational resonance (DARR) to identify the 'malignant' conformation of A β 42. Two sets of chemical shifts for Asp-23 were observed in a ratio of about 2.6 : 1. The 2D DARR spectra at the mixing time of 500 ms suggested that the side chains of Asp-23 and Val-24 in the major conformer, and those of Lys-22 and Asp-23 in the minor conformer could be located on the same side, respectively. These data support the presence of a turn structure at positions 22 and 23 in E22K-A β 42 fibrils. The formation of a salt bridge between Lys-22 and Asp-23 in the minor conformer might be a reason why E22K-A β 42 is more pathogenic than wild-type A β 42.

d) Ecological chemistry of plants

The bean plant-herbivory mite-predatory mite system is a good model to investigate interaction between plant and insect. In response to herbivore damage, the bean plant emits volatiles that attract natural predatory mite of the attacking herbivores. The presence of elicitors which induce volatiles in the leave has been suggested. We examined the possibility of abscisic acid (ABA) as the elicitor, but ABA did not show clear activity to induce the volatiles, showing that ABA would not be the elicitor.

Chimpanzees in the Mahale Mountain National Park in Tanzania are known to use some plants as medicines. On the basis of a screening study of such possible chimpanzee's medicinal plants or medicinal foods (20 species) for their bioactivities, *Trema orientalis* exhibited a high antischistosomal activity. Having explored the bioactive components using a brine shrimp assay, we isolated and identified 2 compounds, linoleic acid and β -sitosterol. Linoleic acid was proven to possess antischistosomal activity. The activity of β -sitosterol is now ongoing.

e) Molecular technology of a plant hormone, abscisic acid

Abscisic acid (ABA) is an important plant hormone that induces adaptative responses in plants upon water stress and low temperature. Inhibitors of the ABA degrading enzyme, ABA 8'-hydroxylase, could increase the ABA level, and result in increasing resistance against water deficiency through suppression of the ABA inactivation. Triazole type compounds known as P-450 were screened for inhibitors of the hydroxylase using the hydroxylase expressed with the insect cells, and uniconazole-P was found to be a strong inhibitor of the hydroxylase with K_i 8.0 nM. Uniconazole-P not only increased ABA level along with decrease in its metabolite, phaseic acid, but also increased resistance against water deficiency in *Arabidopsis thaliana*. These findings suggest that inhibitors of the hydroxylase could be used for cultivation of crops in semi-arid area.

A-2. Publications and presentations

a) Publications

Books

- Ohigashi, H.: Chapter 2, Present status and perspective of cancer prevention, In: New aspects of the development of cancer preventive food factors. Ed. Osawa, T., CMC, pp. 13-17, 2005 (In Japanese)
- Ohigashi, H.: Editor's postscript, In Nutrition and body-response in genetical and Immunological systems, pp.139-140, 2004 (In Japanese)
- Ohigashi, H.: On the functional foods. In: Toward the development of food function, New Food Creation Technology Group, pp. 21-24, 2004 (In Japanese)
- Murakami, A. and Ohigashi, H.: Molecular mechanisms of carcinogenesis and the effects of dietary factors, In: Physiological functions of dietary factors (Ed., Yamada, K.), Asakura, pp.30-41, 2004 (in Japanese)
- Murakami, A.: Chapter 10, Terpenes, In: New aspects of the development of cancer preventive food factors. Ed. Osawa, T., CMC, pp.191-197, 2005 (in Japanese)
- Murakami, A. and Ohigashi, H.: Polymethoxy flavonoids: Cancer preventive and therapeutic potentials derived from anti-inflammatory and drug metabolism – modifying properties. In: Phytochemicals in Health and Disease (Yongping, B. and Fenwick, R. eds.), Marcel Dekker Inc., New York, pp. 187-211, 2004
- Murakami, K., Irie, K., Morimoto, A., Masuda, Y., Ohigashi, H., Nagao, M., Fukuda, H., Shimizu, T. and Shirasawa, T.: New aggregation model of amyloid β by the systematic proline replacement. Peptide Science 2004, Shimohigashi, Y. (Ed.), The Japanese Peptide Society, pp.179-182, 2005

Original papers

- Kawabata, K., Murakami, A. and Ohigashi, H.: Nobiletin, a citrus flavonoid, down-regulates matrix metalloproteinase-7 (matrilysin) expression in HT-29 human colorectal cancer cells. Biosci. Biotechnol. Biochem. 69 (2); 307-314, 2005
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- Kwon, K. H., Murakami, A., Tanaka, T. and Ohigashi, H.: Suppressive activity of a fermented grain food product on dextran sulfate sodium-induced experimental colitis in mice. Biofactors. 21 (1-4); 179-184, 2004
- Nakamura, Y., Yoshida, C., Murakami, A., Ohigashi, H., Osawa, T. and Uchida, K.: Zerumbone, a tropical ginger sesquiterpene, activates phase II drug metabolizing enzymes. FEBS Lett. 572 (1-3); 245-250, 2004
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Patents

Open patents

- No.2004-359732 "Anti-oxidants": Ohigashi, H., Murakami, A., Inafuku, M., Yonaha, M., 12/24/04, Ryukyu Bioresource Co. Ltd.
- No.2005-8594 "Anti-inflammatory drugs and NO generation suppressants", Uenakai, K., Murakami, A., Nishikawa, Y. Minamida, K., 01/13/05, Tamanoi Vineger Co. Ltd.
- No.2005-53826 "Anti-inflammatory drugs", Ohigashi, H., Murakami, A., Aniya, Y., Inafuku, M., Nakamoto, K., Ryukyu Bioresource Co. Ltd., Nakazen Medicinal Plant Farm.

Patent pending

- No.2005-087184 "Screening method of medicines or preventive agents for Alzheimer's disease using β-amyloid peptides", Irie, K., Murakami, K., Ohigashi, H., Nagao, M., Shirasawa, T. and Shimizu, T., 03/24/05, Kyoto University and Tokyo Metropolitan Institute of Gerontology.

Reviews

- Ohigashi H, Murakami, A.: Cancer prevention with food factors: alone and in combination. *Biofactors* 22(1-4): 49-55, 2004
- Ohigashi H, Murakami, A.: Regulation of oxidative stress by dietary factors and their possible application to cancer preventive compound, *Science and Industry*, 78(4); pp.182-187, 2004 (In Japanese)
- Ohigashi, H.: Exploitation of physiologically functional plant-food sources in tropical and subtropical reagrions: a case of cancer prevention research. *Jpn Food Sci.*, 43 (12), pp. 33-37, 2004 (In Japanese)
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- Murakami, A., Ohigashi, H.: Dual faces of green tea catechins, *Journal of Bioscience and*

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- Irie, K., Nakagawa, Y. and Ohigashi, H.: Indolactam and benzolactam compounds as new medicinal leads with binding selectivity for C1 domains of protein kinase C isozymes. Curr. Pharm. Design 10 (12); 1371-1385, 2004
- Hirai, N.: Deuterium-labeled phaseic acid and dihydrophaseic acids for internal standards. Nihon Nougai Kagaku Kaishi, 78; 996-997, 2004

Reports

- Ohigashi, H. and Irie, K.: Development of new agents that regulate cellular signal transduction. Report (2004) for Monbu-kagakusho Research Grant [Scientific Research (A) (2)]
- Ohigashi, H.: Analysis of physiological function of non-nutritious components in food. Report (2004) for Monbu-Kagakusho Special Coordination Funds
- Ohigashi, H.: Elucidation of physiological functions of food factors and their application. Report (2004) for funds from the ministry of Agriculture, Forestry, and Fisheries of Japan
- Irie, K.: Structural analysis of β -amyloid fibrils using solid-state NMR. Report (2004) for Monbu-kagakusho Research Grant [Scientific Research (B) (1)]
- Irie, K.: Analysis of the non-covalent ligand-receptor interactions by the electrospray ionization mass spectrometry. Report (2004) for Monbu-kagakusho Research Grant [Germination]
- Murakami, A.: Elucidation of physiological functions of food factors and their applications. Report (2004) for funds from the ministry of Agriculture, Forestry, and Fisheries of Japan
- Murakami, A.: The role of inflammation in carcinogenesis. Report (2004) for funds from the ministry of Health, Labor and Welfare
- Hirai, N.: Regulation mechanism of the resistance induced by insects in plants. Reports (2002-2004 and 2004) for Japan Science and Technology Corporation, Core Research for Evolutionary Science and Technology
- Hirai, N.: Elucidation of the biosynthetic pathway of abscisic acid in fungi by organic chemistry and molecular analysis. Report (2002-2003) for Monbu-kagakusho Research Grant [Scientific Research (B) (1)]
- Hirai, N.: Carbon dioxide fixation program and development of effective techniques. Report (2004) for Research Institute of Innovative Technology for the Earth (RITE).
- b) Conference and seminar papers presented
- The 2004 Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Sapporo): general papers (11)
- The 4th Meeting of AOB research: 1 general paper
- The 11th Conference of Japanese Society for Cancer Prevention: general papers (4)
- The 63rd Annual Meeting of the Japanese Cancer Association: general papers (5)
- The 9th Forum of Japanese Society for Food Factors: general papers (4)
- The 19th Annual Meeting of Spice Research: 1 general paper
- 39th Summer School on Natural Products Chemistry (Awajishima): 1 general paper
- 46th Symposium on the Chemistry of Natural Products (Hiroshima): 1 general paper
- 2004 Symposium on Molecular Structures (Tokyo): 1 general paper
- 37th Summer School on Peptide Science (Kyoto): 1 invited presentation
- 43rd Symposium on NMR (Tokyo): 1 general paper

438th Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in Kansai branch: 1 general paper

114h Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry in Chubu branch: 1 general paper

The 39th Annual Meeting of the Japanese Society for Chemical Regulation of Plants: 4 general papers

The 2004 Annual Meeting of the Japanese Society of Plant Physiologist: 3 general papers

The 48th Annual Meeting of Terpenes, Essential Oil, and Aroma Chemistry: 1 general paper

The 2nd Annual Meeting of Sangaku-Renkei Society: 1 general paper

A-3. Off-campus activities

Membership in academic societies (roles)

Ohigashi, H.: Japan Society for Bioscience, Biotechnology, and Agrochemistry (trustee, head of the Kansai Branch, advisory board), The Japan Society of Analytical Chemistry (councilor of Kinki Branch), The Japanese Society of Food Science and Technology (councilor of Kansai Branch), The Society of Chemical Regulation of Plants (councilor), Japanese Society for Food Factors (councilor), Spice Research Forum of Japan (councilor), Forum of Clinical Pathology on Free Radicals (organizer), Forum on Oxidative Stress Marker (supervisor), Japan Society for Preventive Medicine (trustee), The Japanese Society of Lipid Peroxide and Free Radical Research (councilor), Japanese Association for Animal Cell Technology (councilor)

Hirai, N.: The Japanese Society for Chemical Regulation of Plants (general secretary)

Murakami, A.: Japanese Society for Food Factors (advisory board)

Murakami, A.: Food Science Forum (councilor)

Research grants

Monbu-Kagakusho Research Grant

Scientific Research (A) (2): Development of new agents that regulate cellular signal transduction (Ohigashi, H., Head, Irie, K., Cooperator)

Scientific Research (B) (1): Structural analysis of β -amyloid fibrils using solid-state NMR (Irie, K., Head)

Germination: Analysis of the non-covalent ligand-receptor interactions by the electrospray ionization mass spectrometry (Irie, Head)

Scientific Research (C) (2) The effect of lauric acid on enhancement of the inflammatory mediators production: A risk assessment (Murakami, A., Head)

Monbu-Kagakusho Special Coordination Funds: Analysis of physiological function of non-nutritious components in food (Ohigashi, H., Cooperator)

Funds from the Ministry of Agriculture, Forestry, and Fisheries of Japan: Elucidation of physiological functions of food factors and their applications (Ohigashi, H., Cooperator; Murakami, A., Cooperator)

Grant-in-aid from the Ministry of Health, Labour, and Welfare: Roles of inflammation in carcinogenesis and chemopreventive compounds (Murakami, A., Cooperator)

Japan Science and Technology Corporation, Core Research for Evolutionary Science and Technology: Regulation mechanism of the resistance induced by insects in plants (Hirai, N., Cooperator)

Grant from Research Institute of Innovative Technology for the Earth (RITE): Mechanism of triazole type compounds inducing resistance to water deficiency, and development of new compound (Hirai, N., Head)

A-4. International cooperations and overseas activities

International meetings (roles)

Ohigashi, H.: 9th Asian Congress of Nutrition (New Delhi, India) (plenary lecture)

Murakami, A., Ohigashi, H.: Combination of anti-oxidants: a strategy for chemoprevention. 'Dietary Factors and Cancer Prevention: Current Premises and Future Promises'. (Rochester, MN) (1 invited presentation)

Murakami, A., Ohigashi, H.: The 2nd biannual meeting of Asia Pacific Organization for Cancer Prevention (Seoul, Korea) (1 invited presentation)

Murakami, A., Ohigashi, H.: WorldNutra 2004 (San Francisco) (1 invited presentation)

Ohigashi, H. Murakami, A.: The 229th ACS National Meeting (San Diego) (1 invited presentation)

Irie, K.: 1st Asia-Pacific International Peptide Symposium, 41st Japanese Peptide Symposium (Fukuoka, 1 general paper)

Irie, K.: XXI International Conference on Magnetic Resonance in Biological Systems (Andhara Pradesh, 1 general paper)

Hirai, N.: The 18th International Congress on Plant Growth Substances (Canberra, 5 general papers)

Editorial work for International journals

Ohigashi, H.: Journal of Medicinal Food (editor), Asian Pacific Journal of Cancer Prevention (editor), Journal of Agricultural and Food Chemistry (advisory board), BioFactors (editor)

Membership in international academic societies

Ohigashi, H. and Murakami, A.: American Association for Cancer Research (member)

Irie, K.: American Chemical Society (member)

Hirai, N.: International Plant Growth Substances Association (member)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Seminar on Food Science and Biotechnology (Ohigashi, H.), Organic Chemistry in Food Science I (Irie, K.), Organic Chemistry in Food Science II (Irie, K.), Organic Chemistry in Food Science III (Ohigashi, H. and Irie, K.), Organic Chemistry in Life Science (Ohigashi, H. and Irie, K.), Laboratory Course in Organic Chemistry (Irie, K., Hirai, N. and Murakami, A.), Introduction and Practice in the Department of Food Science and Biotechnology I, II (Ohigashi, H., Irie, K., Hirai, N. and Murakami, A.)

Graduate level: Advanced Course of Life Sciences in Food (Ohigashi, H.), Organic Chemistry in Life Science (Ohigashi, H., Irie, K., and Hirai, N.), Seminar of Organic Chemistry in Life Science (Ohigashi, H. and Irie, K.), Experimental Course of Organic Chemistry in Life Science (Ohigashi, H. and Irie, K.)

B-2. Off-campus teaching, etc.

Part-time lecturer

Ohigashi, H.: Graduate School of Kyoto women's University (Food Science), Graduate School of Osaka City University (Special Lecture)

Hirai, N.: Faculty of Textile Science, Kyoto Institute of Technology (Phytochemistry)

Open seminars

Ohigashi, H.: 1st Forum on Foods and Health in Kagoshima (lecture), Symposium on Analysis of physiological function of non-nutritious components in food (Monbu-Kagakusho Special Coordination Funds) (speaker), Research meeting between the University researchers and Company researchers (Agribio Technology)(lecture), Seminar on Establishment of New Agribio Business (lecture), Ifia Japan (lecture), Analysis of physiological function of non-nutritious components in food 9th Symposium on the Regionally Originated Science and Technology Originated (plenary lecture), Food Factor Forum in Kyoto 2004 (organizer), 2004 Symposium of the Plant Research Foundation (organizer)

Murakami, A.: Meeting for Food New Technology, 'Functional Food Factors as Double-edged Sword: An Approach from Basic Research' (lecture), Technological seminar in Glico, Co. Ltd. 'Studies on Food Functionality: the State-of-Art, Methodology, and Perspectives' (lecture), Danon DIJ Conference 'Establishment of Novel Bioassay Systems Based on Bioavailability and Metabolism of Food Chemicals' (lecture)

B-3. Overseas teaching

Students and research fellows from abroad

Doctors Program: 3 (Korea), JSPS Research Fellow: 1 (Tanzania), Research Fellow: 1 (China)

C. Other remarks

Ohigashi, H.: Steering Committee of the African Area Studies of Kyoto University (member), Administrative Committee of Counseling of Kyoto University (member), 170 Committee on Redox Life Science of the Japan Society for the Promotion of Science (member), Society for Order-made Disease Prevention of Kyoto Prefecture (coordinator), Food Safety Council of Kyoto City (chairperson), Plant Research Foundation (trustee), Executive Committee of the Health Research Foundation (member), Nomination Committee for the Financial Support of the Tea Science Foundation of Kyoto Prefecture (member)

Irie, K.: Safeguard Committee of Faculty of Agriculture for Radioisotopes and Radiation (member), Environment Preservation Center (member of KYS), Plant Research Foundation (councilor)

Hirai, N.: Dowa and Human Right Problems Committee of Kyoto University (member), Plant Research Foundation (councilor), Steering committee of the 2004 Symposium of the Kinki branch of the Science Council of Japan (member), Coordination of research meetings between the University researchers and Company researchers.

Chair of Food Bioscience

2.7.4 Laboratory of Nutrition Chemistry

Staff Professor : Fushiki, Tohru, Dr. Agric. Sci.

Associate Professor: Kawada, Teruo, Dr. Agric. Sci. (until 6/30)

Assistant Professor : Inoue, Kazuo, Dr. Agric. Sci.

Assistant Professor : Tsuzuki, Satoshi, Dr. Agric. Sci.

Students and research fellows

Doctor's program: (6)

Master's program: (13)

Undergraduate : (4)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Palatability of foods; Nutrition, Physiology and Brain science

It is important conception in the field of research of food science that new food resources are palatable and acceptable in human. To get this final goal, new foods are required to have a good sensitive nature, a good nutritional property, safety and many other good properties. We want to eat delicious foods. It can not be coped with a close food crisis that the development of food resources are accepted only by starving people. In this point of view, we studied what properties of food resources have high acceptability in human. Especially, we study on fat taste and the traditional "umami" taste by the analyses of interaction mechanisms on tongue of experimental animals.

b) Control of gastrointestinal epithelial turnover and the regulation by food components, and regulatory mechanism of gastrointestinal hormone secretion

The mucosal surface of the intestine comprises epithelial monolayer cells that are critical for the absorption of nutrients and defense. Aging epithelial cells must be rapidly replaced by younger cells for the maintenance of these functions. However, details of the underlying mechanism governing the rapid turnover of intestinal epithelial cells have remained unknown. We found a novel enzyme, designated membrane-type serine protease 1 (MT-SP1), and suggested that this enzyme is involved in the control of intestinal epithelial turnover under physiological conditions. Furthermore, we found that the activities of MT-SP1 and granzyme A (GrA), which is likely to induce apoptosis of abnormal intestinal epithelial cells, are regulated by food components. Our studies have revealed that some food components can regulate the turnover of the intestinal epithelium.

c) Development of special foods to increase endurance capacity.

Long-distance runners have broken many world records in recent years. Because they apparently ingested special foods to increase their endurance capacity, these exogenous substances and their effects on endurance capacity have been brought into the light. We devised an adjustable-current swimming pool for the evaluation of endurance capacity of mice. Our apparatus provides for the reliable and reproducible evaluation of the endurance capacity of mice. By using our apparatus, we studied the detecting and mechanism of the effects of dietary

differences and drug pretreatment on the endurance capacity. In addition, we investigate the relation to central fatigue induced by brain TGF-beta with endurance capacity in order to clarify whether food stuff that has effects on endurance capacity also modifies the manifestation of tiredness.

d) Mechanisms of manifestation of central fatigue and TGF-beta in brain

Intracerebroventricular administration of cerebrospinal fluid (CSF) from exercise-fatigued rats elicited the decrease in spontaneous motor activity of sedentary mice, as though they were exhausted. There was no such effect in the CSF from sedentary rats. Those mice administered the CSF from fatigued-rats seemed to occur the feeling of fatigue and lose their willingness to move.

We thought that the substance which involved in this phenomena was the factor that cause the feeling of fatigue. With various experiments we clarified that transforming growth factor-beta (TGF- β) was the responsible substance, because 1) the concentration of active TGF- β in CSF from fatigued-rats increased, 2) treatment of CSF from fatigued-rats with anti-TGF- β antibody eliminated the effect of decreasing spontaneous motor activity of mice, 3) elevating exercise load on rats increased both the concentration of active TGF- β in CSF and the inhibitory effect on spontaneous motor activity on mice, and 4) purified TGF- β dose-dependently depressed the spontaneous motor activity of mice. These results strongly suggested that active TGF- β in brain elicited the manifestation of central fatigue and depression in willingness to move.

In addition, we showed the administration of TGF- β into brain could augment the ratio of utilization of fatty acid in whole body and the preference for sweet taste. These indicated that active TGF- β in brain not only caused feeling of fatigue, but affected to peripheral tissues (via autonomic nervous system) and involved in the mechanisms which changed metabolic state to the one during/after exercise.

A-2. Publications and presentations

a) Publications

Original papers

- Mizunoya W, Haramizu S, Shibakusa T, Okabe Y, Fushiki T: Dietary conjugated linoleic acid increases endurance capacity and fat oxidation in mice during exercise. *Lipids*. Mar; 40(3): 265-71, 2005
- Lim K, Ryu S, Suh H, Ishihara K, Fushiki T: (-)-Hydroxycitrate ingestion and endurance exercise performance. *J Nutr Sci Vitaminol (Tokyo)*. 2005 Feb; 51(1): 1-7
- Tsuzuki S, Murai N, Miyake Y, Inouye K, Hirayasu H, Iwanaga T, Fushiki T: Evidence for the occurrence of membrane-type serine protease 1/matrilysin on the basolateral sides of enterocytes. *Biochem J*. Jan 26; 2005
- Takii H, Takii Nagao Y, Kometani T, Nishimura T, Nakae T, Kuriki T, Fushiki T: Fluids containing a highly branched cyclic dextrin influence the gastric emptying rate. *Int J Sports Med*. 2005; 26(4): 314-9.
- Shimura T, Imaoka H, Okazaki Y, Kanamori Y, Fushiki T, Yamamoto T: Involvement of the Mesolimbic System in Palatability-induced Ingestion.. *Chem Senses*. 30 Suppl 1: i188-i189. 2005
- Fushiki T, Kawai T: Chemical reception of fats in the oral cavity and the mechanism of addiction to dietary fat. *Chem Senses*. 2005 Jan; 30 Suppl 1: i184-i185.

- Park JY, Kawada T, Han IS, Kim BS, Goto T, Takahashi N, Fushiki T, Kurata T, Yu R.: Capsaicin inhibits the production of tumor necrosis factor alpha by LPS-stimulated murine macrophages, RAW 264.7: a PPARgamma ligand-like action as a novel mechanism. *FEBS Lett.* 2004 Aug 13; 572(1-3): 266-70.
- Son C, Hosoda K, Ishihara K, Bevilacqua L, Masuzaki H, Fushiki T, Harper ME, Nakao K.: Reduction of diet-induced obesity in transgenic mice overexpressing uncoupling protein 3 in skeletal muscle. *Diabetologia* 47(1): 47-54 JAN 2004
- Manabe Y., Utunomiya H., Gotoh K., Kurosu T., Fushiki T.: Relationship between the preference for sake (Japanese rice wine) and the movements of metabolic parameters coinciding with sake intake. *Biosci. Biotechnol. Biochem.* 68(4) 796-802 (2004)
- Toyoda T., Hayashi T., Miyamoto L., Yonemitsu S., Nakano M., Tanaka S., Ebihara K., Masuzaki H., Hosoda K., Inoue G., Otaka A., Sato K., Fushiki T., Nakao K.: Possible Involvement of the α 1 Isoform of 5'AMP-Activated Protein Kinase in Oxidative-Stress-Stimulated Glucose Transport in Skeletal Muscle *Am. J. Physiol. (Endocrinol & Metab)* 287, E166-173, 2004
- Mizunoya W., Oyaizu S., Hirayama A., Fushiki T.: Effects of physical fatigue in mice on learning performance in a water maze. *BIOSCI BIOTECH BIOCH* 68(4): 827-834 (2004)
- Takii H., Kometani T., Nishimura T., Kuriki T., Fushiki T.: A sports drink based on highly branched cyclic dextrin generates few gastrointestinal disorders in untrained med bicycle exercise. *Food. Sci. Technol. Res.* 10(4), 428-431 (2004)

Reviews

- Fushiki, T. : Is oil the sixth taste? In search of the mechanism of its reception. *Microscopia.* 21 (4), 10-15, 2004
- Lim K, Ryu S, Suh H, Ishihara K . and Fushiki T.: (-)-hydroxycitrate ingestion and endurance exercise performance. *J. Nutr. Sci. Vitaminol. (Tokyo)*, 51 (1), 1-7, 2005
- Inoue K, Matsumura S, and Fushiki T: Manifestation of central fatigue by TGF- β in brain. *Molecular Medicine.* 41: 1258-1263, 2004
- Tsuzuki S and Fushiki T: Molecular nutritional studies on serine proteases and their inhibitors that regulate cell turnover of intestinal mucosal epithelium. *J. Jpn. Soc. Nutr. Food Sci.* 57: 277-282, 2004

b) Conference and seminar papers presented

- Annual meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (5 papers)
- Annual meeting of Japanese Society of Nutrition and Food Science (6 papers)
- Annual meeting of Physiological Society of Japan (2 papers)
- Annual meeting of the Japan Neuroscience Society (2 papers)
- Annual meeting of the Japanese Association for the Study of Taste and Smell (2 papers)
- Annual meeting of Japan Society for the Study of Obesity (3 papers)
- Annual meeting of Japan Society for Spice Research (2 papers)

A-3. Off-campus activities

Membership in academic societies (roles)

- Fushiki, T.: Japanese Society of Nutrition and Food Science (Councilor), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor of Kansai branch), Japanese Society of Biochemistry (Councilor), Japan Society of Spice Study (President), Japanese

Association for the Study of Taste and Smell(Councilor)

Kawada, T.: Japan Society for the Study of Obesity (Councilor), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch general affair).

Research grants

Fushiki, T: Monbusho Research Grant: Scientific Research (B) “Research for the physiological function of non-pungent principle hot pepper and application for now functional material” (Fushiki, representative).

Kawada, T: Scientific Research (B) “The signal transduction of Dietary fat and energy metabolism via nuclear receptors” (Kawada, representative).

Inoue, K: Scientific Research (C) “Elucidation of mechanisms of manifestation of central fatigue”. (Inoue, representative)

Tsuzuki, S: Scientific Grant (C) “Elucidation of the role for granzyme A, a binding molecule of monitor peptide, in the peptide-mediated CCK release

Other grant: Grant from Bio-Orineted Technology research Program for Promotion of Basic research Activities for Innovative Biosciences (Kawada, cooperative).

A-4. International cooperation and overseas activities

Fushiki T: International Symposium on Olfaction and Taste 2004, Kyoto, Japan (Vice President), US Conference on Chemical Senses, FL Sarasota, USA (presentation)

Inoue K: International Conference on Fatigue Science, Nagano, Japan (Organization Committee, invited speaker), ISOT 2004, Kyoto, Japan (Chair person)

Editorial work for international journals

Fushiki, T.: Journal of Nutritional Science and Vitaminology (Vice Editor), American Journal of Physiology (nominated as a part time reviewer),

Kawada, T.: B.B.A.(referee) , Life Science (referee), Lipid (referee), J. Agric. Food Chem.(referee)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Nutrition Chemistry (Fushiki), Animal and Plant Nutrition Laboratory (Kawada), Biochemistry in Foods (Kawada), Introduction of Research (Fushiki, Kawada)

Graduate level: Nutrition Chemistry (Advanced course) (Fushiki), Laboratory Course in Nutrition Chemistry (Fushiki, Kawada)

B-2. Off-campus teaching etc.

Enlightenment lecture

Fushiki, T: IUFoST-Japan/FT-Japan symposium, “New Aspect of Food Research through compound eye”. (Tokyo), F&H Laboratory Lecture, “What the tongue instruct” (Tokyo), Forum on meat and health, “Taste of meat”, (Tokyo), Lecture at Society for Science of Microstructure of Food, “The Structure and taste of food”, (Tokyo), Lecture at Society for Research on Palatable Food and Culture, “Preference of animals and human” (Kyoto), 20th anniversary of Society for Research on Food Culture in Kyoto, “Kyoto, Food and Health” (Kyoto), 19th Seminar on Nursery Canteen of Japan, Basic Lecture, (Kyoto)

Inoue K: Annual Meeting of Japanese Society of Physical Fitness and Sports Medicine,

C. Other remarks

Fushiki: Nutrition and food science committee responsible for Science Council of Japan (member), Health, Labour and Welfare Ministry-led council for food sanitation (provisional member), Ministry of Education, Technology and Science council (expert member), Advisory Board for the Committee of Kyoto wholesale market, Active strategy for establishment of base for food culture. Advisory Board for Kyoto University Radioisotope Research Center (member), Advisory Board for the Committee of Diabetes Diet in the Japan Diabetes Society (member).

Kawada: Advisory Board for the Committee of Animal Experiment in Kyoto University Radioisotope Research Center (member), Kinki Agricultural-High School Committee (Chair of Food division).

2.7.5 Laboratory of Molecular Function of Food

Staff *Professor* : *Kawada, Teruo, Dr. Agric. Sci.*
 Associate Professor: Urade, Reiko, Dr. Agric. Sci.
 Assistant Professor : Moriyama, Tatsuya, Dr. Agric. Sci.

Students and research fellows

Doctor's Program : (1)
Master's Program : (3)
Undergraduate : (4)
Postdoctoral fellow : (2)
Research fellow : (1)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Genomic regulatory science on lipid metabolism and obesity

Overweight is superfluous formation of the fat which constitute an adipose tissue. It has been pointed out that the factor secreted from a fat cell as a key factor of a lifestyle-related diseases of recent years. Multiplication of a fat cell, specialization, and secretion of the various factor further related to development of symptoms are strongly influenced by the food ingredient to daily take. As a result of analyzing the transcriptional regulation mechanism over the target gene of PPARs (peroxysome proliferators activated receptors) which are the master regulator of specialization of a fat cell differentiation. And then we found out that CREB binding protein (CBP) was the indispensable factor of fat cell specialization. Furthermore, the activation factor of PPARs which promotes carbohydrate metabolism and lipid metabolism using this system was found out to natural occurring materials, especially a medicinal herb, or plants.

b) Basic and applied studies on lipid metabolism aimed at prevention of hyperlipidemia.

Initiation and progression of the life-style related disease are involved in the lipid

metabolism in the various organs including liver and adipocyte. For understanding and prevention of these diseases, we are performing the basic studies about mammalian lipid metabolism and applied studies about food factors regulating lipid metabolism properly. Our current research topics are 1) the mechanism of regulated degradation of HMG-CoA reductase, which acts at rate-limiting step of cholesterol. 2) basic and applied studies about microsomal triglyceride transfer protein (MTP) in liver, small intestine and brain. 3) novel lipase-like protein which might be involved in generation of lipid mediators. 4) biochemical studies about insulin-involved metabolism. 5) mechanism of lipid lowering effect of soybean fractions. 6) screening of food factors regulating lipid metabolism. 7) effect of soybean protein on the physiological response. 8) development of novel methods or experimental tools for lipid research.

c) Basic and applied studies on food allergies.

Food allergy is one of the serious nutritional problem both in children and adults, since a number of patients with atopic dermatitis are considered to be suffering from food hypersensitivity mediated by allergen-specific IgE antibodies. There is no effective medical treatment, so it is an urgent demand for food scientists to define the food components responsible for the allergies to develop hypoallergenic foods for the allergic patients. We identified several major allergenic proteins from soybean, one of which is demonstrated to be highly homologous to house dust mite allergen, Der p 1. The purpose of this research project is to clarify the nature of allergenic proteins and to establish a strategy for reducing their allergenicity and to produce hypoallergenic processed foods for the soybean-sensitive patients using biotechnological methods.

d) Studies on folding and quality control of protein in endoplasmic reticulum.

The endoplasmic reticulum (ER) is the site of synthesis and posttranslational modifications of secretory and membrane proteins. The ER also plays an important role in folding and quality control of nascent polypeptides. The nascent polypeptides translocated into the ER are folded with assistance of molecular chaperones and many enzymes, which are localized in the ER. The unfolded and misfolded proteins are removed from the transport pathway to the Golgi and then degraded by ER-associated degradation systems. Our primary research goal of this project is to clarify the interactions between nascent polypeptide, molecular chaperones and enzymes during folding of proteins and its regulatory mechanism. We are also studying the roles of ER chaperones and enzymes on the regulation of VLDL secretion from liver, which is an important factor affecting serum triacylglycerol level. In addition, we are interested in the folding mechanism of soybean storage proteins. We are actively studying the characteristics and expression profiles of soybean ER-resident chaperons and their interactions with storage proteins during maturing of cotyledon.

e) Basic and applied studies on dietary lipids and health.

Intensive studies using bacteria, plant and animal culture cells, rat heart and platelets have been performed focusing how dietary lipids consisting of various fatty acids influence on the structure and function of biological membrane. Previously, the toxic effects of saturated fatty acids, erucic acid and trans-fatty acids on animal cells have been revealed. We also have demonstrated that linoleic acid is an essential component for respiratory enzyme, cytochrome c oxidase in heart mitochondria. Moreover, arachidonoyl molecular species of phosphatidylinositol was shown to play a primary role on the signal transduction for activation of platelets by using a method for phospholipid molecular species analysis, which was progressed by our laboratory. Based on those studies on the physiological roles of dietary lipids at the molecular levels, a

reasonable way to intake fatty acids for health is being designed.

A-2. Publications and presentations

a) Publications

Original Papers

- Yu R, Kim C.-S, Kawada T, Kwon T.-W, Lim T.-H, Kim Y.-W and Kwon B.-S: Involvement of leukotactin-1, a novel CC chemokine, in human atherosclerosis. *Atherosclerosis* 174: 35-42, 2004
- Tsuda T, Ueno Y, Aoki H, Koda T, Horio F, Takahashi N, Kawada T, Osawa T: Anthocyanin enhances adipocytokine secretion and adipocyte-specific gene expression in isolated rat adipocytes. *Biochem Biophys Res Commun.* 316: 149-157, 2004
- Jun-Young Park, Teruo Kawada, In-Seob Han, Byung-Sam Kim, Tsuyoshi Goto, Nobuyuki Takahashi, Tohru Fushiki, Tadao Kurata, and Rina Yu: Capsaicin, a spicy component of hot pepper, inhibits the production of tumor necrosis factor alpha by LPS-stimulated murine macrophage Raw 264.7: a PPARgamma ligand-like action of capsaicin as a novel mechanism. *FEBS Letters.* 572: 266-270, 2004
- Kim CS, Park WH, Park JY, Kang JH, Kim MO, Kawada T, Yoo H, Han IS, Yu R: Capsaicin, a spicy component of hot pepper, induces apoptosis by activation of the peroxisome proliferator-activated receptor gamma in HT-29 human colon cancer cells. *J Med Food.* 7: 267-273, 2004
- Urade R, Okudo H, Kato H, Moriyama T, Arakaki Y: ER-60 domains responsible for interaction with calnexin and calreticulin. *Biochemistry.* 43: 8858-8868, 2004
- Qiu W, Kohen-Avramoglu R, Rashid-Kolvear F, Au CS, Chong TM, Lewis GF, Trinh DK, Austin RC, Urade R, Adeli K: Overexpression of the endoplasmic reticulum 60 protein ER-60 downregulates apoB100 secretion by inducing its intracellular degradation via a nonproteasomal pathway: evidence for an ER-60-mediated and pCMB-sensitive intracellular degradative pathway. *Biochemistry.* 43: 4819-4831, 2004
- MaCormick, L. M., Urade, R., Arakaki, Y., Schwartz, A. L., and Bu, G.: Independent and cooperative roles of N-glycans and molecular chaperones in the folding and disulfide bond formation of the low-density lipoprotein (LDL) receptor-related protein. *Biochemistry* 44: 5794-5803, 2005
- Moriyama T, Kishimoto K, Nagai K, Urade R, Ogawa T, Utsumi S, Maruyama N, Maebuchi M: Soybean beta-conglycinin diet suppresses serum triglyceride levels in normal and genetically obese mice by induction of beta-oxidation, downregulation of fatty acid synthase, and inhibition of triglyceride absorption. *Biosci Biotechnol Biochem.* 68: 352-359, 2004
- Jeon T, Hwang SG, Hirai S, Matsui T, Yano H, Kawada T, Lim BO, Park DK: Red yeast rice extracts suppress adipogenesis by down-regulating adipogenic transcription factors and gene expression in 3T3-L1 cells. *Life Sci.* 75:3195-3203, 2004

Reviews

- Kawada T, Takahashi N: Adipocyte formation and differentiation. *Adiposcience.* 1: 8-18, 2004
- Kawada T: Molecular nutrition and its application. *Nutrition for Aging* 122: 11-17, 2004
- Kusudo T, Moriyama T, Takahashi N, Kawada T: Obesity and Genome; the stand point from genomic pharmacology

b) Conference and seminar (paper presented)

The 2005 Annual Meeting of Japan Society for Bioscience, Biotechnology and Biochemistry (7)

The 56th Annual Meeting of Japan Society for Nutrition and Food Science (6)

The 46th Annual Meeting of Japanese Conference on the Biochemistry of Lipids (1)

The 2003 Annual Meeting of Japan Society for Nutrition and Food Science, Kinki Branch (4)

The 74th Annual Meeting of the Japanese Biochemical Society (1)

A-3. Off-campus activities

Board in academic societies (roles)

Kawada, T.: Japan Society for the Study of Obesity (Councilor), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch general affair), The Japan Endocrine Society (General affair)

Research grants

Monbusho Research Grant: Scientific Research on Priority Areas “Adipomics; Analysis of molecular mechanism that hypertrophy of adipocytes” (Kawada, representative), Scientific Research (B) “The signal transduction of Dietary fat and energy metabolism via nuclear receptors” (Kawada, representative). Scientific Research (Young researcher B) (Moriyama, representative)

A-4. International cooperation and overseas activities

International Joint Research

Kawada: Studies on cytokine and chemokine from adipose tissue and obesity related diseases (South Korea, University of Ulsan)

Urade: Studies on roles on interaction of RAP and ER-60 (Washington University of Medicine; United States), Studies on the roles of ER-60 on regulatory degradation of ApoB-100 (Toronto University, Canada)

Editorial work for international journals

Kawada, T.: J. of Medicinal Food (editorial board), B.B.A.(referee), Life Science (referee), Lipid (referee), J. Agric. Food Chem.(referee)

B. Educational activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Molecular Function of Food (Kawada and Urade), Introduction and practice in the department of food science and biotechnology I (Urade), Introduction and practice in the department of food science and biotechnology II (Kawada and Urade), Seminar in Information Processing Basics (Urade), Seminar in Food Proteins (Urade), Food biochemistry II (Kawada), Food biochemistry I (Urade), Laboratory Course in Enzyme Chemistry and Biochemistry (Urade)

Graduate level: Molecular Function of Food Constituents (Ogawa and Urade), Seminar on Molecular Function of Food Constituents (Kawada and Urade), Experimental Course in Molecular Function of Food Constituents (Kawada and Urade)

B-2. Off-campus activities

Part-time lecturer

Kawada, Doshisha Women's University Faculty of Home Economics (Enzyme Science)

2.7.6 Laboratory of Physiological Function of Food

Staff *Professor: Yoshikawa, Masaaki, D. Agric. Sci.*

Lecturer : Ohinata, Kousaku, D. Agric. Sci.

Students and research fellows

Doctor's program : (1)

Master's program : (5)

Postdoctoral fellow: (1)

A. Research Activities (2004.4-2005.3)

A-1. Main Subjects

- a) Mode of action and production in genetically modified soy bean of novokinin (RPLKPW), a multifunctional peptide

RPLKPW, which has been obtained by designing a vasorelaxing peptide RADHPF (ovokinin(2-7)) derived from ovalbumin, showed hypotensive activity after oral administration at a dose of 0.1 mg/kg in spontaneously hypertensive rats (SHR). This peptide was named novokinin. The vasorelaxing and hypotensive activity of novokinin were blocked by PD123318, an AT₂ receptor antagonist, suggesting that these were mediated by angiotensin AT₂ receptor. Both of them were blocked by indomethacin, an inhibitor for cyclooxygenase suggesting that they are mediated by prostaglandin. Both of them were also blocked by ONO-AE₃-240A, a selective antagonist for EP₃ receptor. These suggest that PGE₂ released downstream of AT₂ receptor binds EP₃ receptor. Novokinin stimulated hair growth in shaved black mice after oral administration at a dose of 0.3 mg/kg for 2 weeks. Novokinin also prevented alopecia induced by etoposide, an anti-cancer agent, after oral administration in rat neonatal rats at a dose of 3.0 mg/kg for 6 days. These were blocked by AH23848, a selective antagonist for EP₄ receptor. These suggest that novokinin act through EP₄ receptor in hair follicles.

- b) Mechanism for anti-alopecia by a soy-derived immunostimulating peptide soymetide and related peptides.

Soymetide-4 (MITL) stimulates phagocytosis by polymorphonuclear leukocytes through formyl peptide receptor. Previously we found that an alopecia in neonatal rats induced by etoposide, a cancer chemotherapy agent, could be prevented by 300 mg/kg (po.) of soymetide-4. The anti-alopecia effect was inhibited by indomethacin, a cyclooxygenase inhibitor, and AH23845, an antagonist selective for EP₄ receptor, suggesting that prostaglandin E₂ released downstream of formyl peptide receptor binds EP₄ receptor. Pyrilamine, a histamine H₁ antagonist, which blocked anti-alopecia effect of intraperitoneously given fMLP did not block that of orally given soymetide-4. This means antialopecia might be different according to the application route. On the other hand, Gly-Leu-Phe, a phagocytosis-stimulating peptide of which receptor is different

from that for soymetide-4, also prevented etoposide-induced alopecia at a dose of 300 mg/kg (po.) .

c) Multifunctional properties of Leu-Ser-Arg derived from bovine κ -casein and soy glycinin.

We found previously that Leu-Ser-Arg (LSR) derived from bovine lowerd serum cholesterol in mice fed high-cholesterol diet. It also stimulated bile acid secretion after intraduodenal administration in rats at a dose of 100 mg/kg. The stimulation of bile acid secretion by LSR was blocked by atropine and L-NAME suggesting that acetylcholine and nitric oxide are involved in the mechanism. In spontaneously hypertensive rats (SHR), LSR lowered blood pressure after oral administration at a dose of 10 mg/kg. LSR was released from soy glycinin, as well as κ -casein, after digestion with thermolysin and trypsin.

d) Localization of prostaglandin D₂ receptor (DP) regulating food intake in the brain

We found that ICV administered prostaglandin D₂ (PGD₂) stimulated food intake in mice via neuropeptide Y (NPY). The prostaglandin D₂ receptor (DP)-like immunoreactivity was localized in the median eminence of the hypothalamus in mouse brain. The mRNA expression of the DP was also observed in median eminence and almost all cells of the arcuate nucleus, where the NPY-positive neurons were present. Probably the DP neurons in median eminence or arcuate nucleus innerve NPY neuron in energy homeostasis. The immunoreactivity of Lipocalin-type PGD synthase (L-PGDS), which is responsible for production of PGD₂ from PGH₂ in the CNS was also present in the median eminence. The PGD₂ system in the hypothalamus may, at least in part, transfer peripheral signals in the regulation of food intake.

A-2. Publications and Presentations

a) Publications

Books

Yoshikawa, M., M. Yokoo, A. Ito, C. Matsumoto, T. Tsuruki and H. Usui: RPLKPW, a designed hypotensive peptide relaxes artery and stimulates hair growth via angiotensin AT₂ receptor. "Peptide Science 2004" (Ed. by Y. Shimohigashi), p.99-102, The Japanese Peptide Society, Osaka, 2005

Takeda, S., R. Yamauchi, H. usui, Y. Kawamura and M. Yoshikawa: β -Lactotensin, a neurotensin NT₂ agonist, stimulates bile acid secretion. "Peptide Science 2004" (Ed. by Y. Shimohigashi), p.259-262, The Japanese Peptide Society, Osaka, 2005

Tsuruki, T. and M. Yoshikawa: Anti-alopecia mechanism of soymetide-4. "Peptide Science 2004" (Ed. by Y. Shimohigashi), p.263-266, The Japanese Peptide Society, Osaka, 2005

Ohinata, K., T. Shimano, S. Sonoda, Y. Takenaka and M. Yoshikawa: Human enterostatin (APGPR) stimulates learning performance. "Peptide Science 2004" (Ed. by Y. Shimohigashi), p.267-270, The Japanese Peptide Society, Osaka, 2005

Fujita, H. and M. Yoshikawa: Chapter 4.5, Amino Acids and Peptides, "Biotechnological Applications of Marine Natural Substances" (edited by N. Fusetani), p.228-237, CMC Publishing CO.,LTD., Tokyo, 2005 (in Japanese)

Yoshikawa, M: Chapter 2.2 Function of food proteins. (Eds. S. Kaminogawa and M. Tanokura) p.34-38, Tokyo Kagakudojinn, Tokyo, 2005 (in Japanese)

Original papers

Tsuruki, T., A. Ito, K. Takaha, and M. Yoshikawa: FPRL1 receptor agonist peptides prevent etoposide-induced alopecia in neonatal rats. J. Invest. Dermatol. 123; 242-243, 2004

Tsuruki, T. and M. Yoshikawa: Design of soymetide-4 derivatives to potentiate the anti-alopecia

- effect. *Biosci. Biotechnol. Biochem.* 68; 1139-1141, 2004
- Tsuruki, T., K. Takahata and M. Yoshikawa: A soy-derived immunostimulating peptide inhibits etoposide-induced alopecia in neonatal rats. *J. Invest. Dermatol.* 122; 848-850, 2004
- Ohinata, K., T. Shimano, R. Yamauchi, S. Sakurada, K. Yanai and M. Yoshikawa: The anorectic effect of neurotensin is mediated via a histamine H₁ receptor in mice. *Peptides.* 25; 2135-2138, 2004
- Takenaka, Y., N. Doyama, N. Maruyama, S. Utsumi and M. Yoshikawa: Introduction of DPR, an enterostatin fragment peptide, into soybean β -conglycinin α' subunit by site-directed mutagenesis. *Biosci. Biotechnol. Biochem.* 68; 253-256, 2004
- Onishi, K., N. Matoba, Y. Yamada, N. Doyama, N. Maruyama, S. Utsumi and M. Yoshikawa: Optimal designing of β -conglycinin to genetically incorporate RPLKPW, a potent anti-hypertensive peptide. *Peptides* 25; 37-43, 2004
- Yang, Y.J., E.D. Marczak, H. Usui, Y. Kawamura and M. Yoshikawa: Antihypertensive properties of spinach leaf protein digests. *J. Agric. Food Chem.* 52; 2223-2225, 2004
- Lipkowski, A.W., D.B. Carr, V.J. Hruby and M. Yoshikawa: Is RNA-laden p24 capsid protein a vector for HIV? *Med. Hypotheses* 62; 919-921, 2004
- Choi, S.K., M. Adachi, M. Yoshikawa, N. Maruyama and S. Utsumi: Soybean glycinin A1aB1b subunit has a molecular chaperone-like function to assist folding of the other subunit having low folding ability. *Biosci. Biotechnol. Biochem.* 68; 1991-1994, 2004
- Sonoda, S. and M. Yoshikawa: Studies on Interaction between Soy Constituents and Central Nervous System, *Soy Protein Research (Japan)* Vol. 7 p.75-78, 2004 (in Japanese)

Patents

- Patent pending/applied for Patent application no. 2005-60624 'Soybean containing functional peptides', inventor: Yoshikawa, M., applicant: Oike, K., the president of Kyoto Univ., application date: Mar. 4, 2005
- Patent pending/applied for Patent application no. 2004-196613 'Learning stimulant', inventor: Yoshikawa, M., applicant: Oike, K., the president of Kyoto Univ., application date: Jul. 2, 2004
- Patent pending/applied for Patent application no. 2004-157848 'Novel hair stimulant', inventor: Yoshikawa, M., T. Tsuruki and A. Ito, applicant: Oike, K., the president of Kyoto Univ., application date: May. 27, 2004
- Patent pending/applied for Patent application no. 2004-145143 'Learning stimulant', inventor: Yoshikawa, M., applicant: Oike, K., the president of Kyoto Univ., application date: May. 14, 2004

Reviews

- Hujita, H. and M. Yoshikawa: Angiotensin-Converting Enzyme Inhibitory Peptides Derived from Food Proteins, *The FFI Journal* Vol. 209, 2004 (in Japanese)
- Yoshikawa, M., E. Marczak and H. Fujita: Roles of Peptides in Regulation of Blood Pressure, *FOOD Style* 21, 8(7), p.45-50, 2004 (in Japanese)
- b) Conference and seminar papers presented
- The 49th Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry: 8 papers
- Kansai Regional Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry: 2 papers

The Japanese Dairy Science Annual Symposium: 1 paper

The 1st Asia-Pacific International Peptide Symposium and the 41st Japanese Peptide Symposium: 4 paper

The 51st Annual Meeting of the Japanese Society for Food Science and Technology: 1 paper

A-3. Off-campus activities

Roles in academic societies

Yoshikawa: Japan Society for Bioscience, Biotechnology and Agrochemistry Japan (Councilor), Japanese Society for Food Science and Technology (Trustee), Japanese Society for Nutrition and Food Science (Councilor), The Japanese Dairy Science Association (Councilor, editorial board), The Japanese Association for Animal Cell Technology (Councilor), Japanese Narcotic Research Conference (Organizer)

Research grants

Grant-in-Aid for Scientific Research (B)(2): Novel function and mode of action of receptor subtypes for neurotensin and angiotensin. (Yoshikawa, representative), Grant-in-Aid for Young Scientists (B): Studies on disturbances in food intake regulation in zinc deficiency (Ohinata, representative)

Grant from Ministry of Agriculture, Forestry, and Fisheries: Production system for useful substance by use of plants, animals and insects. (Yoshikawa, collaborator), Studies on physiological functions and safety of foods. (Yoshikawa, collaborator)

Basic Research Grant from PROBRAIN: Production of food materials to prevent life style-related diseases based on genomic information. (Yoshikawa, representative)

Grant from Japan Science and Technology Agency: Studies on peptides regulating plants. (Yoshikawa, representative)

Grant from Japan Dairy Science Association: Studies to promote powdered skim milk utilization. (Yoshikawa, representative)

Grant from The Food Science Institute Foundation: Studies on physiological function of food proteins. (Yoshikawa, representative)

Grant from Chubu Feeds: Studies on peptides stimulating plant growth. (Yoshikawa, representative)

A-4. International cooperation and overseas activities

International meetings (roles)

Yoshikawa: An International Multidisciplinary Symposium PEPTIDE Receptors, Montreal (oral presentation)

Yoshikawa: The 19th International Dairy Science Forum, Tokyo (invited lecture)

Yoshikawa: Bioactive Peptides Derived from Food Protein, Shinchu (invited lecture)

Yoshikawa: International Symposium for Opioid and Pain-related Peptide, Sendai (chairman)

Ohinata: International Narcotics Research Conference 2004, Kyoto (poster presentation)

International joint researches, overseas research surveys

Yoshikawa: Drug design based on natural products (Research Institute for Industrial Chemistry, Poland)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Introduction to the molecular cell biology (Yoshikawa), Physiological functions of foods (Yoshikawa), KUINEP Program (Yoshikawa), Introduction to experimental food bioscience I and II (Yoshikawa, Ohinata), Basic data processing (Ohinata), Laboratory course in food and nutrition chemistry (Ohinata)

Graduate level: Over view of Agriculture in Japan (Yoshikawa), Advanced course in Physiological functions of foods (Yoshikawa, Ohinata), Seminar in Physiological functions of foods (Yoshikawa, Ohinata), Experimental course in Physiological functions of foods (Yoshikawa, Ohinata)

B-2. Off-campus teaching

Part-time lecture

Yoshikawa: Osaka University of Pharmaceutical Sciences (Graduate School of Pharmaceutical Science), Nagoya University (Graduate School of Life Science and Agriculture), Fukui Prefectural University (Graduate School of Bioresources)

Open seminar, etc

Yoshikawa: Educational Program for Dietician, lecturer “On Physiological Function of Foods” 2005, 7, 10, Saga

B-3. Overseas teaching

Students and research fellows from abroad

Visiting scientists: 2 (Poland, Israel)

C. Other Remarks

Yoshikawa: Member of Specialist Committee on Foods for Specified Health Use. Trustee of the Japanese Society for Dairy Technology. Trustee of NPO Association Sakigake

Chair of Food Production Technology

2.7.7 Laboratory of Bioengineering

Staff *Professor* : Adachi, Shuji, Dr. Agric. Sci.
 Associate Professor: Kimura, Yukitaka, Dr. Agric. Sci.
 Assistant Professor : Shima, Motohiro, Dr. Agric. Sci.
 Part-time Secretary: Kamiya, Rumiko
 Part-time Secretary: Furuta, Chisa (until 8/31)

Students and research fellows

Doctor's program: (4) *Research student*: (1)
 Master's program: (8)
 Undergraduate : (3)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Development of novel food processing using subcritical water:

The dielectric constant of water decreases to a value approximately equal to that of organic solvent and the ion product of water increases as temperature and pressure increase. We intend to develop novel food processing procedures utilizing such properties of water under subcritical conditions. The solubility of lipids in subcritical water has been measured, and hydrolyses of esters and saccharides by the water have also been investigated.

b) Analysis of characterization of nano-emulsion:

Nanoemulsions, in which the diameter of oil droplets is several dozen nanometers, are expected to possess unique properties and to be usable as new food materials. However, the properties have not fully been elucidated. From the viewpoint of the lipid oxidation the effect of the diameter of oil droplets in nano- and microemulsions on the oxidation is now under investigation.

c) Biochemical reaction engineering on construction of bioreactors:

To design reasonably the reactor systems using biocatalysts such as enzymes and microorganisms, it is important to understand both the characteristics of biochemical reaction and phenomena occurring in the reactors. The following enzymatic reactions are investigated: production of edible surfactants and modification of polyunsaturated fatty acids to retard their autooxidation.

d) Food chemical engineering on development of food materials with high functionalities:

Rapid dehydration of emulsions consisting of liquid lipid and a dense solution of edible polymer produces microcapsules in which small lipid droplets are covered with the dehydrated polymer layer. This technology is called lipid microencapsulation. Microencapsulation of liquid lipid provides the lipid some novel functionalities such as retardation of oxidation and carrier function to fat-soluble physiologically active substances from the mouth to the intestinal tract. The factors affecting the functionalities are investigated to establish designing methods for reasonably preparing the encapsulated lipid. We have proposed a novel food material,

microcapsule of W/O/W emulsion, which acts as a carrier for hydrophilic bioactive substances to the intestine, and have realized the microcapsules.

A-2. Publications and presentations

a) Publications

Books

Adachi S.: 2.2 Food processing using subcritical water. Beneficial properties of water and their utilization –Application in agriculture, food, and medicine–. p.183-190, NTS Co., Ltd., Tokyo, 2005

Adachi S.: Chapter 10 Lipase-catalyzed condensation in an organic solvent. Handbook of Biocatalysis (ed. by C. T. Hou). p. 10-1 – 10-15, Taylor & Francis Books, 2005

Original papers

Oomori, T., S. Haghighat Khajavi, Y. Kimura, S. Adachi, and R. Matsuno: Hydrolysis of disaccharides containing glucose residue in subcritical water. *Biochem. Eng. J.* **18**(2); 143-147, 2004

Khuwijitjaru, P., T. Fujii, S. Adachi, Y. Kimura, and R. Matsuno: Kinetics on the hydrolysis of fatty acid esters in subcritical water. *Chem. Eng. J.* **99**; 1-4, 2004

Watanabe, Y., X. Fang, S. Adachi, H. Fukami, and R. Matsuno: Oxidation process of 6-*O*-arachiconoyl L-ascorbate microencapsulated with a polysaccharide by spray-drying. *Lebensm.-Wissens. u. Technol.* **37**(4); 395-400, 2004

Adachi, S., H. Imaoka, H. Ashida, H. Maeda, and R. Matsuno: Preparation of microcapsules of W/O/W emulsions containing a polysaccharide in the outer aqueous phase by spray-drying. *Eur. J. Lipid Sci. Technol.* **106**; 225-231, 2004

Shima, M., Y. Kobayashi, Y. Kimura, S. Adachi, and R. Matsuno: Effect of the hydrophilic surfactants on the preparation and encapsulation efficiency in course and fine W/O/W type emulsions. *Colloids and Surfaces A: Physicochem. Eng. Aspects* **238**; 83-90, 2004

Khuwijitjaru, P., Y. Kimura, R. Matsuno, and S. Adachi: Preparation of finely dispersed O/W emulsion from fatty acid solubilized in subcritical water. *J. Colloid Inter. Sci.* **278**; 192-197, 2004

Khuwijitjaru, P., Y. Kimura, R. Matsuno, and S. Adachi: Solubility of oleic and linoleic acids in subcritical water. *Food Sci. Technol. Res.* **10**(3); 261-263, 2004

Piao J. and S. Adachi: Enzymatic preparation of fatty acid esters of sugar alcohols by condensation in acetone using a packed-bed reactor with immobilized *Candida actarctica* lipase. *Biocatal. Biotransform.* **22**(4); 269-274, 2004

Shima, M., M. Tanaka, Y. Kimura, S. Adachi, and R. Matsuno: Enhancement in transport of a hydrophilic marker through intestinal epithelial cell (Caco-2) monolayer by W/O/W multiple emulsion containing C8TG. *Food Hydrocolloids* **19**; 321-328, 2005

Chen, J., Y. Kimura, and S. Adachi: Continuous synthesis of 6-*O*-linoleoyl hexose using a packed-bed reactor system with immobilized lipase. *Biochem. Eng. J.* **22**; 145-149, 2005

Haghighat Khajavi, S., Y. Kimura, T. Oomori, R. Matsuno, and S. Adachi: Kinetics on sucrose decomposition in subcritical water. *Lebensm.-Wissens. u. Technol.* **38**; 297-302, 2005

Kuwabara, K., Y. Watanabe, S. Adachi, and R. Matsuno: Stability of saturated acyl L-ascorbates in aqueous solution. *J. Food Sci.* **70**; E7-E11, 2005

Reviews

- Adachi, S. and Y. Matsumura: Suppression of lipid oxidation by its interaction with a food polymer (in Japanese). *Nippon Shokuhin Kagaku Kogaku Kaishi* **51**(5); 221-228, 2004
- Adachi, S. and Y. Kimura: Utilization of subcritical water in food processing (in Japanese). *Industrial Information* **32**, 16-20, 2004
- Adachi, S.: Synthesis of emulsifiers through lipase-catalyzed condensation in organic solvent and their properties (in Japanese). *Foods & Food Ingredients Journal of Japan* **209**; 482-489, 2004
- Adachi, S.: Food engineering studies for providing lipids new functionalities (in Japanese). *Japan J. Food Eng.* **5**(4); 223-233, 2004
- Kobayashi, T. and S. Adachi: Reaction equilibrium for lipase-catalyzed condensation in organic solvent systems. *Biotechnol. Lett.* **26**; 1461-1468, 2004
- Adachi, S.: Stability of nanoemulsion and lipid oxidation in the emulsion (in Japanese). *Foods & Food Ingredients Journal of Japan* **209**; 955-960, 2004
- Adachi, S. and T. Kobayashi: Synthesis of esters by immobilized-lipase-catalyzed condensation reaction of sugars and fatty acids in water-miscible organic solvent. *J. Biosci. Bioeng.* **99**(2); 87-94, 2005

Reports and Proceedings

- Piao, J. and S. Adachi: Enzymatic synthesis of fatty acid esters of sugar alcohols and their emulsifier properties. *Proceedings of the 10th Asian Pacific Confederation of Chemical Engineering, Kitakyushu, Paper No. 186*, 2004
- Kimura, Y., S. Haghighat Khajavi, T. Oomori, R. Matsuno, and S. Adachi: Kinetics on disaccharide decomposition in subcritical water. *Proceedings of the 10th Asian Pacific Confederation of Chemical Engineering, Kitakyushu, Paper No. 9008*, 2004
- Adachi, S.: Continuous synthesis of acyl ascorbates with antioxidative and emulsifying ability and their properties. *Urakami Foundation Memories* **12**; 21-27, 2004
- Adachi, S.: Microencapsulation of multiple emulsion containing a bioactive substance (in Japanese). *Food Plant Manage* **Dec.**; 24-25, 2004
- Adachi, S.: Unique water –subcritical water– (in Japanese). *Food Plant Manage* **Jan.**; 16-17, 2005
- Adachi, S.: Why can ion exchangers separate saccharides? –reconsideration of conventional separation process– (in Japanese). *Food Plant Manage* **Feb.**; 18-19, 2005
- Adachi, S.: Enzymatic reaction in organic solvent –synthesis of esters– (in Japanese). *Food Plant Manage* **Mar.**; 32-33, 2005
- Adachi, S.: Deterioration kinetics of oil and fat by autooxidation and deep frying (in Japanese). *Sugiyama Chemical & Industrial Laboratory Annual Report*; 59-75, 2005
- b) Conference and seminar papers presented
- 95th AOCS Annual Meeting (1 paper)
- The 2nd seminar for Leading Biotechnology (2 papers)
- Annual Meeting of Japan Society for Food Engineering, 2004 (6 papers)
- Meeting of the Society of Chemical Engineers in Shimonoseki (2 papers)
- Meeting of Kansai Branches of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 2004 (1 paper)
- 10th the Asian Pacific Confederation of Chemical Engineering Congress (2 papers)
- The 8th China-Japan-Korea joint Symposium on Enzyme Engineering (1 paper)

The 52th Meeting of the Japan Society of Enzyme Engineering (1 paper)

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 2005 (2 papers)

A-3. Off-campus activities

Membership in academic societies

Adachi, S.: The Japan Society for Food Engineering (director), The Japanese Society on Enzyme Engineering (secretary), Japan Oil Chemists' Society (secretary of Kansai branch).

Research grants

Adachi, S.: Monbukagakusho Research Grant, Grant-in-Aid for Exploratory Research: Feasibility for synthesizing food materials in water-organic solvent mixture under subcritical condition (representative); Nanotechnology project, the Ministry of Agriculture, Forestry and Fisheries: Mass transfer on the surface of nano- and microparticles and its control (co-operative);

Kimura Y.: Japan Society of the Promotion Science Grant, Grant-in-Aid for Scientific Research (C): Non-catalytic synthesis of food material in subcritical water (representative);

A-4. International cooperations and overseas activities

International meetings

Adachi, S.: 95th AOCs Annual Meeting, Cincinnati, USA (oral presentation), 10th the Asian Pacific Confederation of Chemical Engineering Congress, Kitakyushu (poster presentation), The 8th China-Japan-Korea joint Symposium on Enzyme Engineering, Hangzhou, R.P. China (oral presentation).

Kimura Y.: 10th the Asian Pacific Confederation of Chemical Engineering Congress, Kitakyushu (oral presentation)

Editorial work for international journals

Adachi, S.: Food Science and Technology Research (editor), Japan Journal of Food Engineering (editor), Journal of Bioscience and Biotechnology (editor).

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Food Engineering (Adachi, S. and Kimura, Y.), Physical Chemistry in Food Science I (Kimura, Y.), Physical Chemistry in Food Science II (Adachi, S.), Basic Laboratory Course in Food Science and Biotechnology (Kimura, Y.), Laboratory Course in Chemical Engineering (Kimura, Y.).

Graduate level: Food Production Technology (Adachi, S.), Food Engineering (Advanced Course) (Adachi, S.), Bioengineering Seminar (Adachi, S. and Kimura, Y.), Experimental Course in Bioengineering (Adachi, S. and Kimura, Y.)

B-2. Off-campus teaching, etc.

Part-time Lectures

Adachi, S.: Faculty of Agriculture, Kagawa University (Food Chemical Engineering), Graduate School of Bioresources, Mie University (Sustainable Bioscience and Bioengineering

(Advanced Course)).

Open seminar, etc.

Adachi, S.: Academic Plaza in 2004 International Exhibition on Food Machinery, Ariake, Tokyo (lecturer), Seminar on Agricultural High Technology (lecturer), Seminar of Industrial Lipid Technology Division, Japan Oil Chemists' Society (lecturer).

B-3. Overseas teaching

Students and research fellows from abroad

Doctor's program: 4 (China 2, Iran, Chinese Taipei)

Research students: 1 (China)

C. Other remarks

Adachi, S.: Division Chairperson, Advisory Board for Basic Education Program, Division of Information Education (member), Advisory Board for Utilization of Academic Center for Computing and Media Studies, Kyoto University (member), Committee for Safety Control in the Graduate School of Agriculture (member), Advisory Board for Open Campus, Kyoto University (member).

Kimura, Y.: Committee for Student Administrative Activity in Faculty of Agriculture (member), Representative for Safety Control of Research Activity in Division of Food Science and Biotechnology, the Graduate School of Agriculture.

Shima, M.: Advisory Board for Information Systems in Faculty of Agriculture (member)

2.7.8 Laboratory of Basic and Applied Molecular Biotechnology

Staff Professor : Murata, Kousaku, Dr. Agric. Sci.

Associate Professor: Hashimoto, Wataru, Dr. Agric. Sci.

Assistant Professor : Kawai, Shigeyuki, Dr. Agric. Sci.

Students and research fellows

Post-doctoral fellow: (2)

Doctor's program : (5)

Master's program : (3)

Undergraduate : (3)

Research student : (1)

A. Research Activities (2004.4-2005.3)

A-1. Main subjects

a) Expressional and functional proteomiscs of "super-channel"-forming bacterium

Sphingomonads are widely distributed in nature and frequently found in various soils and aqueous environments. They contain sphingolipid in their outer membrane in place of lipopolysaccharide (LPS) structure characteristic of conventional Gram-negative heterotrophic aerobes such as *Pseudomonas*. Sphingomonards is also known as the bacterial group having

striking biodegradative capabilities of environmental hazardous compounds. In addition to these, we showed that *Sphingomonas* sp. A1 isolated from soil as a potent alginate producer forms a pit, when they grow on macromolecules (alginate) as a sole source for carbon, on their cell surface through rearrangement and/or reconstitution of pleat, which is also an inherent property of Sphingomonads. To elucidate the molecular route for the creation of pit and the role of pit in transport and assimilation of alginate, proteomics-based identification of the molecules responsible for the formation and function of the pit was conducted. Eight proteins (p1-p8) were found to be specifically expressed in the outer membranes of alginate-grown cells. They were identified to be: p1-p4, siderophore-dependent outer membrane transporters for ferric ions; p5 and p6, flagellin; p7, putative lipoprotein; and p8, granule-binding protein. Two flagellin homologs (p5 and p6) were inducibly produced on the cell surface and tightly bound alginate with a dissociation constant of below nM, thus strongly suggesting that the two flagellin-like proteins function as the receptors for alginate. p7 (putative lipoprotein) and p8 (granule-binding protein) were also confirmed to be alginate-binding proteins. These findings, particularly alginate-binding ability of flagellins, are significant when we think the origin and evolution of flagella of bacterial cells.

b) Molecular breeding of bacteria, “*micromonster*”

Members of the genus *Sphingomonas* can degrade numerous naturally occurring and environmentally hazardous compounds. However, their ability to degrade these compounds is not necessarily sufficient and, except for a few rare cases, strains in Sphingomonads can not be applied to the practical bioremediation processes. Attempts have been made to increase the pollutant-degrading ability by using conventional gene engineering techniques. However, the results don't meet with the practical requirement for bioremediation. Fortunately, *Sphingomonas* sp. A1 exhibits an ability to form a pit on their cell surface, which will permit the permeation of various low- and high-molecular weight compounds, or increase the permeability of them through the cell membrane structures. Then, the genes responsible for the formation of the pit were introduced into various cells of *Sphingomonas* strains, and we found that the pit is successfully transplanted and the cells acquiring the pit-forming ability show extremely high pollutant-degrading capability.

c) Structural proteomics of polysaccharide lyases and other enzymes

Based on their primary structures, polysaccharide lyases are classified into fifteen families (PL-1-15). Polysaccharide lyases recognize uronic acid residues in polysaccharide molecules, and catalyze a β -elimination reaction that accompanies the release of unsaturated saccharides with C=C double bonds at the nonreducing terminal uronate residues. In order to elucidate the common structural properties, the structures and functions of family PL-5, -7, and -8 lyases were analyzed. The molecular evolutionary process of alginate lyase and post-translational modification system of polysaccharide lyase have also been constructed and discussed.

Family PL-5: Alginate lyase A1-III of strain A1 consists of only 12 α -helices, and has a α 6/ α 5-barrel supersecondary structure with a tunnel-like cleft and a mobile loop over the cleft. Through the site-directed mutagenesis, Tyr68 in the loop and Tyr246 in the cleft were found to be essential for the movement of the loop and catalytic reaction.

Family PL-7: Alginate lyase PA1167 from *Pseudomonas aeruginosa* shows a glove-like β -sandwich structure, which is distinct from those of other polysaccharide lyases. Through the catalytic site-targeted mutagenesis, reaction mechanism of Family PL-7 was elucidated

completely.

Family PL-8: Xanthan lyase of *Bacillus* sp. GL1 consists of N-terminal α/α -barrel and C-terminal β -barrel domains. By constructing the mutants of the enzyme and analyzing the three dimensional structure of them, detailed reaction mechanism of the lyase was established.

Unsaturated glucuronyl hydrolase: Three dimensional structure of unsaturated glucuronyl hydrolase (UGL) of *Bacillus* sp. GL1 was determined, and reaction mechanism and its relevance with the infection processes were discussed, since the enzyme can act universally on the products of polysaccharide lyase reactions.

d) Structural biology of inorganic polyphosphate [poly(P)]/ATP-NAD kinase

NAD kinase is a key enzyme in NADP biosynthesis. We solved the crystal structure of polyphosphate/ATP-NAD kinase from *Mycobacterium tuberculosis* (Ppnk) complexed with NAD (Ppnk-NAD) at 2.6 Å resolution using apo-Ppnk structure being also solved in this work. Consequently, we revealed the details of the structure and NAD-binding site of Ppnk. Superimposition of tertiary structures of apo-Ppnk and Ppnk-NAD demonstrated a substantial conformational difference in a loop (Ppnk-flexible loop). As a quaternary structure, these Ppnk structures exhibited tetramer as in solution condition. It should be noted that the Ppnk-flexible loop was involved in the intersubunit contact and probably related to the NAD-binding of the other subunit. Furthermore, the two residues (Asp189, His226) substantially contributed to creating NAD-binding site on the other subunit. The two residues and the residues involved in NAD-binding were conserved. However, residues corresponding to the Ppnk-flexible loop were not conserved, making us to speculate that the Ppnk-flexible loop may be Ppnk-specific.

e) Mechanism for DNA-uptake of yeast *Saccharomyces cerevisiae*

The transformation of intact cells of yeast *Saccharomyces cerevisiae* with exogenous DNA generally requires polyethylene glycol (PEG). We attempted to elucidate the role of PEG in the transformation. PEG showed at least two distinct roles: enhancement of transformability during incubation of cells, even in the absence of DNA, dependently on time and temperature of the incubation and on PEG concentration, and that of attachment of DNA to cell surface in the presence of DNA dependently on PEG concentration, but independently of time and temperature of the incubation. When the attached DNA was removed just before the spread of cells onto selective media, transformability was lowered by 5- to 20-folds than that obtained without removing, indicating that transformation process undergoes during the growth conditions on the selective solid media. To address why PEG can enhance the transformability, microarray analysis was conducted. However, the enhanced transformability could not be attributed to the transcriptional change during incubation with PEG.

A-2. Publications and presentations

a) Publications

Books

Murata, K.: "Future of genetically engineered crops" in "Gene Problems", Ed. Okuno, T. and Human Runessansu Institute, p.163-198, Iwanami publisher, Tokyo, 2004

Original papers

Hashimoto, W., H. Nankai, B. Mikami and K. Murata: Crystal structure of *Bacillus* sp. GL1 xanthan lyase, which acts on the side chains of xanthan. *J. Biol. Chem.*, 278, 7663-7673, 2004

- Miyake, O., A. Ochiai, W. Hashimoto and K. Murata: Origin and diversity of alginate lyases of families PL-5 and -7 in *Sphingomonas* sp. strain A1. *J. Bacteriol.*, 186(9), 2891-2896, 2004
- Miyake, O., E. Kobayashi, H. Nankai, W. Hashimoto, B. Mikami and K. Murata: Post-translational processing of polysaccharide lyase: maturation route for gellan lyase in *Bacillus* sp. GL1. *Arch. Biochem. Biophys.*, 422, 211-220, 2004
- Kawai, S., S. Mori, T. Mukai and K. Murata: Cytosolic NADP phosphatases I and II from *Arthrobacter* sp. strain KM: Implication in regulation of NAD⁺/NADP⁺ balance. *J. Basic Microbiol.*, 44(3), 185-196, 2004
- Kawai, S., T. A. Pham, H. T. Nguyen, H. Nankai, T. Utsumi, Y. Fukuda and K. Murata: Molecular insights on DNA delivery into *Saccharomyces cerevisiae*. *Biochem. Biophys. Res. Commun.* 317, 100-107, 2004
- Jinshan H., H. Nankai, W. Hashimoto and K. Murata: Molecular identification and characterization of an alginate-binding protei on the cell surface of *Sphingomonas* sp. A1. *Biochem. Biophys. Res. Commun.*, 322, 712-717, 2004
- Mukai, T., S. Kawai, S. Mori, B. Mikami and K. Murata: Crystal structure of bacterial inorganic polyphosphate/ATP-glucomannokinase: INSIGHTS INTO KINASE EVOLUTION. *J. Biol. Chem.*, 279(48), 50591-50600, 2004
- Yamasaki, M., S. Moriwaki, O. Miyake, W. Hashimoto, K. Murata and B. Mikami: Structure and function of a hypothetical *Pseudomonas aeruginosa* protein PA1167 classified into family PL-7: A NOVEL ALGINATE LYASE WITH A β -SANDWICH FOLD. *J. Biol. Chem.*, 279(30), 31863-31872, 2004
- Mori, S., M. Yamasaki, Y. Maruyama, K. Momma, S. Kawai, W. Hashimoto, B. Mikami and K. Murata: Crytallographic studies of *Mycobacterium tuberculosis* polyphosphate/ATP-NAD kinase complexed with NAD. *J. Biosci. Bioeng.*, 98(5), 391-393, 2004
- Itoh, T., S. Akao, W. Hashimoto, B. Mikami and K. Murata: Crystal structur of unsaturated gucuronyl hydrolase, responsible for the degradation of glycosaminoglycan, from *Bacillus* sp. GL1 at 1.8 Å resolution. *J. Biol. Chem.*, 279(30), 31804-31812, 2004
- Ochiai, A., S. Mori, S. Kawai and K. Murata: Overexpression, purification, and characterization of ATP-NAD kinase of *Sphingomonas* sp. A1. *Protein Expr. Purifi.*, 36, 124-130, 2004
- Miyake, O., M. Yamasaki, S. Kawai, W. Hashimoto, B. Mikami and K. Murata: Study on the molecular diversity, evolution, and crystal structure of bacterial alginate lyase. *Ouyoubiseibutugakukennkyuu*, 2(2), 103-111, 2004

Reviews

- Hashimoto, W., M. Yamasaki, T. Itoh, K. Momma, B. Mikami and K. Murata: Super-channel in bacteria: Structural and functional aspects of a novel biosystem for the import and depolymerization of macromolecules. *J. Biosci. Bioeng.*, 98(6), 399-413, 2004
- Kawai, S.: How to transport and locate vacuole correctly. *Kagaku to Seibutu*, 42(2); 116-117, 2004
- Kawai, S. and K. Murata: The transformation mechanism of yeast revealed by screening for 5,000 strains. Is an endocytosis a key event? *Kagakuto Seibutu*, 42(8), 503-505, 2004
- Murata, K., W. Hashimoto and S. Kawai: Involvement of bacteria in chronic lung diseases and development of therapy. In "Annual Report of Kouseiroudousho Special disease Investigation: Micribiology group" 55-59, 2004

Reports

- Mori, S., S. Akao, H. H. Yoon, W. Hashimoto, K. Murata, K. Mizutani and B. Mikami: X-Ray crystallographic analysis of a new polysaccharide-degrading enzyme. SPring-8 User Experiment Report, (in press), 2004
- Maruyama, Y., M. Yamasaki, T. Itoh, A. Ochiai, K. Ogura, B. Mikami and K. Murata: Polysaccharide lyase: crystal structure of *Bacillus* sp. GL1 xanthan lyase complexed with substrate. SPring-8 User Experiment Report, BL38B1 , 2004B0315-NL1-np, 2004.
- Itoh, T., S. Akao, S. Mori, W. Hashimoto, M. Yamasaki, K. Murata and B. Mikami: Crystal structure of unsaturated glucuronyl hydrolase, responsible for the degradation of glycosaminoglycan, from *Bacillus* sp. GL1 at 1.8 Å resolution. SPring-8 User Experiment Report, BL38B1 , 2004A0713-np-P3k, 2004
- Yamasaki, M., S. Moriwaki, W. Hashimoto, K. Murata, K. Mizutani and B. Mikami: Crystal structure of family PL-7 alginate lyase PA1167 from *Pseudomonas aeruginosa*. SPring-8 User Experiment Report No. 12 (2003B) p. 211, 2004

b) Conference and seminar papers presented

- The Annual Meeting (2004) of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 8 cases
- The Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branches): 5 cases
- The Annual Meeting (2004) of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Joint meeting of Nishinihon and Hokkaido Branches): 1 cases
- The Regular Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch): 3 cases
- The Annual Meeting (2004) of The Society for Biotechnology, Japan: 9 cases
- The Annual Meeting (2004) of The Japanese Biochemical Society: 4 case
- The Annual Meeting (2004) of The Applied Glycobiology, Japan: 2 case
- Meeting of Ministry of Health, Labour and Welfare: 1 case
- The Annual Yeast Symposium (2004): 1case

A-3. Off-campus activities

Membership in academic societies (roles)

- Murata, Kousaku: Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor of Nation-Wide; Editor of Chemistry and Biology; Member of Science Consolidating Committee), The Society for Biotechnology, Japan (Director, Editor-in-Chief of Journal of Bioscience and Biotechnology), The Vitamin Society of Japan (Councilor of Nation-Wide)

Research grants

- Murata, Kousaku: Monbukagakusho Research Grant-in-Aid for Scientific Research (B), Principal Investigator, Entry 2002, Biological significance of non-general amino acid sequences determining general function of enzyme: lyases Monbukagakusho Research Grant-in-Aid for Exploratory Research, Principal Investigator, Entry 2004, Molecular mechanism of transformation (DNA transportation) in yeast. Monbukagakusho Research Grant-in-Aid for Scientific Research on Priority areas (B), Principal Investigator, Entry 2003, Clarification of genetic network responsible for the formation of “vesicular structure” through fluidization of bacterial cell membrane.

Kouseiroudouosho Grant-in-Aid for Research, Allotment Investigator, Entry 2002, Function of microbes in chronic lung diseases and development of bacterial infection therapy. Nourinsuisansho (Bio-oriented Technology Research Advancement Institution) Research Grant-in-Aid, Principal Investigator, Entry in 2001, Structural biology of bacterial “super-channel” and creation of “super-bacteria” for the protection and regeneration of environment.

Hashimoto, Wataru: Monbukagakusho Research Grant-in-Aid for Encouragement of Young Scientists (B), Principal Investigator; Entry in 2003, Analysis of dynamic structure determining the function of protein/enzyme responsible for the binding and degrading of macromolecule.

Kawai, Shigeyuki: Monbukagakusho Research Grant-in-Aid for Encouragement of Young Scientists (B), Principal Investigator; Entry in 2003, Evolution and structure-function analysis for energy metabolising-enzyme in microbes. Research Grant for Noda Institute for Scientific Research, Principal Investigator; Entry in 2004, Establishment of “highly efficient” transformation method of *Saccharomyces cerevisiae* based on molecular basis for yeast’s competence.

A-4. International cooperations and overseas activities

Membership in international academic societies

Murata, Kousaku: American Society for Microbiology (Member); American Society for Molecular Biology and Biochemistry (Member)

Scholars from abroad

Invited foreign researcher: 1 (Food Industries Research Institute, Viet Nam • Deputy head)

B. Educational Activities (2004.4-2005.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Food Safety I (Murata); Food Microbiology (Murata); Basic and Applied Molecular Biotechnology (Murata, Hashimoto); Laboratory Course in Microbiology (Hashimoto, allotment); Seminar for Basic Information Processing (Hashimoto, allotment)

B-2. Off-campus teaching, etc.

Part-time lecturer

Murata, Kousaku: Invited lecture (Kameoka, Kyoto) on Transgenic crops.

B-3. Overseas teaching

Students and research fellows from abroad

Foreign students: Doctor’s program: 2 (China and Brazil), Master’s program: 1 (Vietnam)

Research fellow: 1 (China)

Research student: 1 (Vietnam)

C. Other remarks

Murata, Kousaku: International Innovation Foundation (Fellow); International Biographical Center (Deputy Director General)