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, 62 first year students are working towards master's degree, and 36 students toward Ph.D. including 13 international students. In undergraduate program, 35 freshmen, 33 sophomores, 35 juniors, and 40 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.)
Assistant Professor : Takita, Teisuke, Dr. Agric. Sci. (Kyoto Univ.)

Students and research fellows
Doctor's program : (6)
Master's program : (11)
Undergraduate : (4)
Research fellow : (1)
Research student : (1)

A. Research Activities (2005.4-2006.3)

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 activesite and charged groups on the surface of the enzyme in the enhancement of the enzyne activity has been suggested. Site-directed mutagenesis and chemical modification have been applied to reveal the roles of tyrosy1, tryptophyl, lysyl, aspartic, and glutamiac residues in the halophilicity of thermolysin.

(ii) Enzymatic properties of MMP-7. The metalloproteinase MMP-7, which contains a zincion 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 *Aeropyru pernix* Kl 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.

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 D_3 hydroxylases. we have succeeded in the expression of human vitamin D_3 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 1α, 25-dihydroxyvitamin D_3. It was noted that a clear difference was-observed between human and rat CYP24 on the metabolism of 1α, 25-dihydroxyvitamin D_3 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 monoclona1

antibodies. Liposome assay and fluorometric assay might be examined for development of homogeneous enzyme immunoassays. In order to increase a sensitivity of the enzyme immunoassays, we have developed an assay system using synchronization of multiple enzymes containing an alkaline phosphatase conjugated with a second antibody. We have also studied an enzyme immunoassay for histamine, which is important in food analysis and a test for allergy.

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

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 staffs are examined. We have developed a method of deodorization of soybean proteins by physicochemical processing with hydrophobic resins. In addition, we have developed a new method to make a bean curd with proteinases.

A-2. Publications and presentations

a) Publications

Books

Inouye, K.: Introduction to Enzyme & Coenzyme Chemistry (Translator: Inouye, K) Springer-Verlag Tokyo, Tokyo, 2005 (Japanese)

Inouye, K. (Editor): Biotechnology Annual Review, Vol. 11, Elsevier, Amsterdam, the Netherlands, 2005

Inouye, K. (Editor): Functional Glyco-Materials: Their Development and Application to Foods (Supervised Editor: Inouye, K.) p.1-447, CMC, Tokyo, 2005 (Japanese)

Inouye, K.: General review on carbohydrate functions In: Functional Glyco-Materials: Their Development and Application to Foods (Editor: Inouye, K.) p.3-23, CMC, Tokyo, 2005 (Japanese)

Inouye, K.: Production of glucosyl ascorbic acid and anti-oxidation effects of food In: Functional Glyco-Materials: Their Development and Application to Foods (Editor: Inouye, K.) p.289-294, CMC, Tokyo, 2005 (Japanese)

Original papers

Kasai, N., Sakaki, T., Shinkyo, R., Ikushiro, S., Iyanagi, T., Ohta, M., and Inouye, K.: Metabolism of 26, 26, 26, 27, 27, 27-F_6-1α, 23S, 25-trihydroxyvitamin D3 by human UDP-glucuronosyltransferase 1A3. Drug Metabol. Dispos. 33; 102-107, 2005

Muta, Y., Oneda, H., and Inouye, K.: Anomalous pH-dependence of the activity of human matrilysin (matrix metalloproteinase-7) as revealed by nitration and amination of its tyrosine residues. Biochem. J. 386; 263-270, 2005

Tsuzuki, S., Murai, N., Miyake, Y., Inouye, K., Hirayasu, H., Iwanaga, T., and Fushiki, T.: Evidence for the occurrence of membrane-type serine protease 1/matriptase on the basolateral sides of enterocytes. Biochem. J. 388; 679-687, 2005

Abe, D., Sakaki, T., Kusudo, T., Kittaka, A., Saito, N., Suhara, Y., Fujishima, T., Takayama, H., Hamamoto, H., Kamakura, M., Ohta, M., and Inouye, K.: Metabolism of 2α-propexy-1α,25-dihydroxyvitamin D3 by human CYP27A1 and CYP24A1. Drug Metab. Dispos. 33; 778-784, 2005

Ito, M. and Inouye, K.: Catalytic properties of an organic solvent resistant tyrosinase from

Streptomyces sp. REN-21 and its high-level production in *Escherichia coli*. J. Biochem. 138; 355-362, 2005

Okumura, S., Saitoh, H., Ishikawa, T., Wasano, N., Yamashita, S., Kusumoto, K., Akao, T., Mizuki, E., Ohba, M., and Inouye, K.: Identification of a novel cytotoxic proteins, Cry45Aa, from *Bacillus thuringiensis* A1470 strain and its selective cytotoxic activity against various mammalian cell lines. J. Agric. Food Chem. 53; 6313-6318, 2005

Yamamoto, K., Uchida, E., Urushino, N., Sakaki, T., Kagawa, N., Sawada, N., Kamakura, M., Kato, S., Inouye, K., and Yamada, S.: Identification of amino acid residues of CYP27B1 responsible for binding of 25-hydroxyvitamin D3 whose mutation causes vitamin D3-dependent rickets type I. J. Biol. Chem. 280; 30511-30516, 2005

Kamo, M., Inouye, K., Nagata, K., Tanokura, M.: Preliminary X-ray crystallographic analysis of thermolysin in the presence of 4 M NaCl. Acta Crystallogr. D Biol. Crystallogr. 61 (Pt 6); 710-712, 2005

Matsumiya, Y., Nishikawa, K., Inouye, K., and Kubo, M.: Mutational effect for stability in a conserved region of thermolysin. Lett. Appl. Microbiol. 40; 329-334, 2005

Orihara, K., Yamazaki, T., Shinkyo, R., Sakaki, T., Inouye, K., Tsukamoto, A., Sugiura, J., and Shishido, K. Rat cytochrome P450-mediated transformation of dichlorodibenzo-*p*-dioxins by recombinant white-rot basidiomycete *Coriolus hirsutus*. Appl. Microbiol. Biotechnol. 69; 22-28, 2005

Tsumura, K., Saito, T., Tsuge, K., Ashida, H., Kugimiya, W., and Inouye, K.: Functional properties of soy protein hydrolysates obtained by selective proteolysis. Food Sci. Technol./LWT 38; 255-261, 2005

Tsumura, K., Kugimiya, W., and Inouye, K.: Emulsifying properties of a peptide from peptic hydrolysates of soy glycinin. Food Sci. Technol. Res. 11; 46-51, 2005

Sakaki, T., Kagawa, N., Yamamoto, K., and Inouye, K.: Metabolism of vitamin D3 by cytochromes P450. Frontiers in Bioscience 10; 119-134, 2005

Kamao, M., Hatakeyama, S., Sakaki, T., Sawada, N., Inouye, K., Kubodera, N., Reddy, G. S., and Okano, T.: Measurement and characterization of C-3 epimerization activity toward vitamin D3. Arch. Biochem. Biophys. 436; 196-205, 2005

Okumura, S., Akao, T., Yamashita, S., Ichimatsu, T., and Inouye, K.: Determination of biotinylated proteins as an index for purification of plasma membrane using surface plasmon resonance-based optical biosensor. Cytotechnology 47; 59-67, 2005

Lee, H.S., Kim, J.S., Shim, K., Kim, J.W., Inouye, K., Oneda, H., Kim, Y.W., Cheong, K.A., Cha, H., Woo, E.J., Auh, J.H., Lee, S.J., Kim, J.W., and Park, K.H. Dissociation/association properties of a dodecameric cyclomaltodextrinase. Effects of pH and salt concentration on the oligomeric state. FEBS J. 273; 109-121, 2006

b) Conference and seminar papers presented

Annual Meeting of the Japan Society (2005) for Bioscience, Biotechnology, and Agrochemistry: 19 papers

78th Annual meeting of the Japanese Biochemical Society: 2 papers

Annual Meetings of Kansai Branch and Nishi-Nippon Branch of the Japan Society for Bioscience, Biotechnology, and Agrochemistry: 11 papers

Annual Meeting of the Japanese Society for Biotechnology: 1 paper

Annual Meeting of the Food Enzyme Chemistry Forum: 1 paper

Mini Symposium on Food Enzyme Chemistry: 1 paper

Mini Symposium on Enzyme Chemistry: 1 paper

Annual Meeting of the Japanese Society of Nutrition and Food Science

A-3. Off-campus activities

Membership in academic societies (roles)

Inouye, K.: The Japanese Biochemical Society (Councilor, Councilor of the Kinki Branch), Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor of the Kansai Branch), The Japanese Society for Food and Technology (Councilor of the Kansai Branch), Japanese Association of Animal Cell Technology (Councilor), Japanese Association of Food Analysis (Councilor)

Research grants

Monbu-Kagaku-sho Research Grant, Research (B): "Protein engineering and reaction control engineering of thermolysin aiming at highly efficient production of an aspartame" (Inouye, 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)

A-4. International cooperations and overseas activities

International meetings (roles)

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

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

Inouye, K.: International Meeting on Proteolysis, Quebec, Canada

Inouye, K.: International Conference on Biotechnology 2004, Tromso, Norway.

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); Research on reaction mechanisms of enzymes (UK) 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 (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Food Basic Biology I (Inouye, Yasukawa), Food Molecular Biology (Inouye, Yasukawa), Enzyme chemistry (Inouye, Yasukawa), Introduction to research I (Inouye, Yasukawa), Seminar on food science and biotechnology (Inouye, Yasukawa), Laboratory

course in enzyme chemistry and biochemistry (Yasukawa), Introduction to foreign literature in food science and biotechnology II (Yasukawa)

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

B-2. Off-campus teaching, etc.

Part-time lecturer

Inouye, K.: Nihon University, Graduate School of Industrial Technology (Life Technology)

B-3. Overseas teaching

Students and research fellows from abroad

Korea (2 graduate students)

C. Other remarks

Committees

Inouye, K.: Member of the Committee for Development of Industry-University Government Cooperation in Kyoto University; Member of the Committee for the Building of the Graduate School of Agriculture; 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

Yasukawa, K.: Member of the Committee for the Eddition of Annual Report of the Graduate School of Agriculture

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 : (2)
Master's program : (8)
Undergraduate : (3)
Research fellow : (1)

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) Functional properties of food proteins: The major component of milk whey protein, ß-lactoglobulin, interacts with casein and both components are linked through disulfide bond. It has been found that ias 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 soybean protein: Soybean 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. Thaumatin and egg white lysozyme are such sweet-tasting proteins. Their taste-eliciting mechanism has been analyzed using chemical modification and site-directed mutagenesis method.

d) Digestion and physiological properties of resistant starch: Degradation and digestibility of starch including resistant 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:

Dendritic cells (DCs), macrophages, NK cells, and epithelial cells are the essential components of the innate immune system and sentinels to sense danger signals of invaders in the mucosal system. In order to elucidate the physiological functions of gut-associated lymphoid tissues, we are studying the immunological role on DCs and macrophages of heat shock protein 70 (hsp70) that is the major intracellular component of all kinds of living cells and functions as an immunoregulatory molecule. Using hsp70 derived from mouse, spinach plant, Lactobacillus acidophilus and Escherichia coli, we found that the mechanism for recognition of hsp70 differently works among various types of antigen-presenting cells, depending on the sequence diversity at the C-terminal region of hsp70. Recently, DCs in peyer's patch tissues were found to have the different binding mode for hsp70 from splenic DCs.

A-2. Publications and presentations

a) Publications

Book

Kitabatake N.:Science of egg constituents (structure and function) (ed. Saito, Nishimura, Matsuda) p160-169, Asakura, Tokyo, 2005

Original papers

Masuda T., Ide N., and Kitabatake N.; Structure-sweetness relationship in egg white lysozyme: role of lysine and arginine residues on the elicitation of lysozyme sweetness. Chem. Senses. **30**, 667-681 (2005).

Tao Y., Nishikawa S., Nomura M., Kitabatake N. and Tani F.; Biotinylation of heat shock protein 70 induces RANTES production in HEK293 cells in a CD40-independent pathway. Biochem. Biophys. Res. Commun. **338,** 700-709 (2005).

Hirose J. Murakami-Yamaguchi, Ikeda, Kitabatake N., and Narita H.: Oligoclonal enzyme-immunosorbent assay capable of determining the major food allergen, ovomucoid, irrespective of the degree of heat denaturation. Cytotechnol. **47**, 145-149 (2005).

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).

Wada R., Fujita Y., and Kitabatake N.; Effects of heating at neutral and acid pH on the structure of ß-lactoglobulin A revealed by differential scanning calorimetry and circular dichroism spectroscopy. Biochim. Biophys. Acta **1760**, 841-847 (2006).

Reports

Kitabatake N. and Tani F.: Promising utilization of beneficial components in the leaves of Japanese cedar and cypress. The Kyoto City Research Foundation ANNUAL REPORT, 2005

b) Conference and seminar papers presented

The annual joint meeting of Kansai and West-Japan branches of the Japan Society for Bioscience, Biotechnology and Agrochemistry: 3 paper

The annual meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry 2006: 10 papers

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, Internaional Food Science and Technology Research Editorial board

Tani, F: Japan Society for Bioscience, Biotechnology, and Agrochemistry (Board in Kansai Branch)

Membership in Science Council of Japan, etc.

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

Research grants

Grant-in-Aid for Scientific Research (B)(2) “Studies on taste-eliciting activity of proteins.” (Kitabatake)

Grant-in-Aid for Scientific Research (B)(2) “Studies on taste-eliciting activity of proteins.”

(Masuda)

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 Scientific Research (Germ) “Studies on the intreaction of salivary proteins with food components and its physiological significance.” (Kitabatake)

Grant-in-Aid for Scientific Research (Germ) “Studies on the intreaction of salivary proteins with food components and its physiological significance.” (Tani)

Grant-in-Aid for Scientific Research (Germ) “Studies on the intreaction of salivary proteins with food components and its physiological significance.” (Masuda)

A-4. International cooperations and overseas activities

b) Other remarks (Co-operative studies)

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

B. Educational Activities (2005.4-2006.3)

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), Food and Environmental Studies (Kitabatake), Laboratory Course in Chemical Engineering (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, 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. Agic. Sci.*
Associate professor : Irie, Kazuhiro, Dr. Agric. Sci.
Assistant professor : Murakami, Akira, Dr. Agric. Sci.
Students and research fellows
Doctor's Program: (5) *Master's Program: (10)* *Undergraduate: (4)*
Research Fellow : (1)
Collaborative Laboratory (IIC) *Professor: Hirai, Nobuhiro, Dr. Agr. Sci.*

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

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

Matrix metalloproteinase (MMP)-7 (matrilysin-1) plays significant roles in the growth, invasion, and metastasis of colorectal tumors, while (-)-epigallocatechin-3-gallate (EGCG), a green tea polyphenol with chemopreventive properties, has been shown to be an inhibitor of MMP-2 and MMP-9. In the present study, HT-29 human colorectal cancer cells were treated with EGCG to examine its effects on pro-MMP-7 induction and production using RT-PCR and western blot analyses. Surprisingly, EGCG (10-100 microM) treatment increased both intracellular and extracellular pro-MMP-7 protein levels in dose- and time-dependent manner, with a significant upregulation of its mRNA expression. EGCG also activated ERK1/2, JNK1/2 and p38 MAPK, as previously reported. In addition, the polyphenol triggered the phosphorylation of c-JUN and induced c-JUN/c-FOS, thereby increasing the DNA binding activity of AP-1. Pharmacological blockade of MAPK activities suggested that pro-MMP-7 expression was induced via JNK1/2 activation, but not in the case of ERK1/2 or p38 MAPK. N-Acetyl-L-cysteine, superoxide dismutase and catalase attenuated the EGCG-induced pro-MMP-7 production, suggesting an involvement of oxidative stress in these events. Conversely, EGCG spontaneously generated superoxide in a cell-free system. Our results suggest that some green tea catechins induce pro-MMP-7 production via superoxide production and the activation of JNK1/2, c-JUN, c-FOS and AP-1 in HT-29 cells.

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

Tumor promoters activate protein kinase C (PKC) isozymes through binding to their C1 domains (C1A and C1B). Since each PKC isozyme is involved in diverse biological events other than tumor promotion, the isozymes serve as promising therapeutic targets. We established a synthetic C1 peptide library of all PKC isozymes to develop agents with binding selectivity for individual PKC C1 domains. Using this peptide library, we identified indolactam-V (IL-V) as a promising lead compound. We suggested that the indole ring of IL-V could be involved in the CH/π interaction with Pro-11 of the C1B domain of PKCδ. The CH/π interaction between the indole ring of IL-V and the hydrogen atom at position 4 of Pro-11 was evaluated using the mutant peptide of the PKCδ C1B domain, in which the CH/π interaction was inhibited by substitution of the hydrogen atom with a fluorine atom. IL-V showed about a 10 times lower binding affinity to the mutant peptide compared to the wild-type peptide, indicating that the CH/π interaction plays a pivotal role on the binding of IL-V to the PKCδ C1B domain. On the other hand,

benzolactam-V8 (BL-V8), with the benzene ring instead of the indole ring of IL-V, lacked the CH/π interaction. The low binding affinity of BL-V8 could be enhanced by the effective formation of the CH/π interaction as exemplified by the design and synthesis of naphtholactam-V8, with the naphthalene ring instead of the benzene ring of BL-V8.

c) Chemistry of Alzheimer's β peptide

The aggregation of 42-mer amyloid β (Aβ42) plays a central role in the pathogenesis of Alzheimer's disease. Our recent research on the systematic replacement with proline in Aβ42 suggested that the formation of a turn structure at positions 22 and 23 could be indispensable to the aggregative ability and neurotoxicity. Since E22K-Aβ42 (Italian mutation) aggregated more rapidly and with more potent neurotoxicity than wild-type Aβ42, the tertiary structure at positions 21-24 of E22K-Aβ42 fibrils was analyzed by solid-state NMR using dipolar assisted rotational resonance (DARR) to identify the 'malignant' conformation of Aβ42.

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 Lys-22 and Asp-23 in the minor conformer could be located on the same side. The data support the presence of a turn structure at positions 22 and 23 in E22K-Aβ42 fibrils. The ionic interaction between Lys-22 and Asp-23 might be a reason why E22K-Aβ42 is more aggregative and neurotoxic than wild-type Aβ42, whose residues at positions 22 and 23 are both anionic. Similar analysis of wild-type Aβ42 fibrils using solid-state NMR did not suggest that the side chains of Glu-22 and Asp-23 could be on the same side.

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. There are two strains of plant-herbivory mite, *Tetranychus kanzawai*, one strain, the red strain, causes change in the color of leaf tissues of lima bean to red after herbivore damage, and the other, the white strain, shows white tissues after herbivore damage. Expression of acidic chitinase in the red tissues is higher than that in the white tissues. This suggests that the red strain produces an elictitor inducing acidic chitinase. The extracts from the red strain did not show activitiy inducing acidic chitinase in lima bean leaves. However, comparison of the extracts from the red strain and the white strain showed that amount of some compounds in the extract from the red strain was higher than that from the white strain. Spectral analyses showed that those compounds were chlorophyll-related compounds, suggesting that the chlorophyll-related compounds might be elicitor of the red strain.

Chimpanzees in the Mahale Mountain National Park in Tanzania were 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 schrimp assay, we isolated and identified 2 compounds, linoleic acid and ß-sitosterol. Linoleic acid was proven to possess antischstosomal activity. The activity of ß-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. However, effects of ABA does not last since ABA is quickly inactivated by the degradading enzyme, ABA 8'-hydroxylase. We investigated the

substrate specificity of the enzyme with the researchers at Shizuoka University , and found that the 4'-carbonyl group is important for recoginition of ABA.

A-2. Publications and presentations

a) Publications

Books

Murakami, A.: The knack of semi-quantification of mRNA with RT-PCR, In: The knack of detection and quantification in biochemical experiments (Ed., Moriyama, T.), Yohdo-Sha, pp.113-119, 2005 (in Japanese).

Ohigashi, H.: Present situation and perspective of thecancer chemoprevention. In: The New Development of Cancer Preventing Foods－The assessment of the biomarker in preventive medicine－(Osawa, T. et al. eds.), CMC (Tokyo), pp. 13-17, 2005 (in Japanese)

Murakami, A., Ohigashi, H. and Koshimizu, K.: Antitumor promoting activity of glyceroglycolipids. In: Food Materials and Physiological Function, CMC (Tokyo), pp. 119-127, 2005 (in Japanese)

Original papers

Takada, Y., Murakami, A. and Aggarwal, B. B.: Zerumbone abolishes NF-kappaB and IkappaBalpha kinase activation leading to suppression of antiapoptotic and metastatic gene expression, upregulation of apoptosis, and downregulation of invasion. Oncogene., 24(46); 6957-6969, 2005

Ichikawa, H., Takada, Y., Murakami, A. and Aggarwal, B. B.: Identification of a novel blocker of I kappa B alpha kinase that enhances cellular apoptosis and inhibits cellular invasion through suppression of NF-kappa B-regulated gene products. J. Immunol., 174(11): 7383-7392, 2005

Murakami, A., Nishizawa, T., Egawa, K., Kawada, T., Nishikawa, Y., Uenakai, K. and Ohigashi, H.: New class of linoleic acid metabolites biosynthesized by corn and rice lipoxygenases: suppression of proinflammatory mediator expression via attenuation of MAPK- and Akt-, but not PPARgamma-, dependent pathways in stimulated macrophages. Biochem. Pharmacol., 70(9): 1330-1342, 2005

Ito, K., Nakazato, T., Xian, M. J., Yamada, T., Hozumi, N., Murakami, A., Ohigashi, H., Ikeda, Y. and Kizaki, M.: 1'-acetoxychavicol acetate is a novel nuclear factor kappaB inhibitor with significant activity against multiple myeloma in vitro and in vivo. Cancer Res., 65(10): 4417-4424, 2005

Kim, M., Murakami, A., Kawabata, K. and Ohigashi, H.: (-)-Epigallocatechin-3-gallate promotes pro-matrix metalloproteinase-7 production via activation of the JNK1/2 pathway in HT-29 human colorectal cancer cells. Carcinogenesis., 26(9): 1553-1562, 2005

Kwon, K. H., Murakami, A., Hayashi, R. and Ohigashi, H.: Interleukin-1beta targets interleukin-6 in progressing dextran sulfate sodium-induced experimental colitis. Biochem. Biophys. Res. Commun., 337(2): 647-654, 2005

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

Ikeda, Y., Murakami, A. and Ohigashi, H.: Ursolic acid promotes the release of macrophage migration inhibitory factor via ERK2 activation in resting mouse macrophages. Biochem.

Pharmacol., 70(10): 1497-1505, 2005

Kaneshiro, T., Suzui, M., Takamatsu, R., Murakami, A., Ohigashi, H., Fujino, T. and Yoshimi, N.: Growth inhibitory activities of crude extracts obtained from herbal plants in the Ryukyu Islands on several human colon carcinoma cell lines. Asian Pac. J. Cancer Prev., 6(3): 353-358, 2005

Murakami, A., Ishida, H., Kobo, K., Furukawa, I., Ikeda, Y., Yonaha, M., Aniya, Y. and Ohigashi, H.: Suppressive effects of Okinawan food items on free radical generation from stimulated leukocytes and identification of some active constituents: implications for the prevention of inflammation-associated carcinogenesis. Asian Pac. J. Cancer Prev., 6(4): 437-448, 2005

Kim, H. W., Murakami, A., Abe, M., Ozawa, Y., Morimitsu, Y., Williams, M. V. and Ohigashi, H.: Suppressive effects of mioga ginger and ginger constituents on reactive oxygen and nitrogen species generation, and the expression of inducible pro-inflammatory genes in macrophages. Antioxid. Redox Signal., 7(11-12): 1621-1629, 2005

Murakami, A., Shigemori, T. and Ohigashi, H.: Zingiberaceous and citrus constituents, 1'-acetoxychavicol acetate, zerumbone, auraptene, and nobiletin, suppress lipopolysaccharide-induced cyclooxygenase-2 expression in RAW264.7 murine macrophages through different modes of action. J. Nutr., 135(12 Suppl): 2987S-2992S, 2005

Eguchi, A., Murakami, A. and Ohigashi, H. Novel bioassay system for evaluating anti-oxidative activities of food items: use of basolateral media from differentiated Caco-2 cells. Free Radic. Res., 39(12): 1367-1375, 2005

Ito, K., Nakazato, T., Murakami, A., Ohigashi, H., Ikeda, Y. and Kizaki, M.: 1'-Acetoxychavicol acetate induces apoptosis of myeloma cells via induction of TRAIL. Biochem. Biophys. Res. Commun., 338(4): 1702-1710, 2005

Nakagawa, Y., Irie, K., Yanagita, R. C., Ohigashi, H. and Tsuda, K.-i.: Indolactam-V is involved in the CH/π interaction with Pro-11 of the PKCδ C1B domain: application for the structural optimization of the PKCδ ligand. J. Am. Chem. Soc., 127(16): 5746-5747, 2005

Murakami, K., Irie, K., Ohigashi, H., Hara, H., Nagao, M., Shimizu, T. and Shirasawa, T.: Formation and stabilization model of the 42-mer Aβ radical: implications for long-lasting oxidative stress in Alzheimer's disease. J. Am. Chem. Soc., 127(43): 15168-15174, 2005

Masuda, Y., Irie, K., Murakami, K., Ohigashi, H., Ohashi, R., Takegoshi, K., Shimizu, T. and Shirasawa, T.: Verification of the turn at positions 22 and 23 of the β-amyloid fibrils with Italian mutation using solid-state NMR. Bioorg. Med. Chem., 13(24): 6803-6809, 2005

Nagao, M., Wen, T.-C., Okamoto, M., Irie, K., Takaku, T. and Sakanaka, M.: *In vivo* neuroprotective activity of Epopeptide AB against ischemic damage. Cytotechnology, 47(1-3): 139-144, 2005

Kitahata, N., Saito, S., Umezawa, T., Miyazawa, Y., Shimada, Y., Min, Y. K., Mizutani, M., Hirai, N., Shinozaki, K., Yoshida S. and Asami, T.: Chemical regulation of abscisic acid catabolism in plants by cytochrome P450 inhibitors. Bioorg. Med.Chem., 13: 4491-4498, 2005

Setha, S., Kondo, S., Hirai, N. and Ohigashi, H.: Quantification of ABA and its metabolites in sweet cherries using deuterium-labeled internal standards. Plant Growth Reguln., 45: 183-188, 2005

Inomata, M., Hirai, N., Takeda, N. and Ohigashi, H.: Mechanism of the formation of a dehydrated ion by an unusual loss of oxygen at the 4'-carbonyl group of abscisic acid methyl ester in electron ionization mass spectrometry. J. Mass Spectrom., 40 (8): 1035-1043, 2005

Ueno, K., Araki, Y., Hirai, N., Saito, S., Mizutani, M., Sakata, K. and Todoroki, Y.: Differences between the structural requirements for ABA 8'-hydroxylase inhibition and for ABA activity. Bioorg. Med. Chem., 13: 3359-3370, 2005

Reviews

Irie, K., Nakagawa, Y. and Ohigashi, H.: Toward the development of new medicinal leads with selectivity for protein kinase C isozymes. Chem. Rec., 5(4): 185-195, 2005

Irie, K., Murakami, K., Masuda, Y., Morimoto, A., Ohigashi, H., Ohashi, R., Takegoshi, K., Nagao, M., Shimizu, T. and Shirasawa, T.: Structure of β-amyloid fibrils and its relevance to their neurotoxicity: implications for the pathogenesis of Alzheimer's disease. J. Biosci. Bioeng., 99(5): 437-447, 2005

Ohigashi, H.: Diet and Health: Exploring new functions. Environment and Health, 18(5), 372-385, 2005 (in Japanese)

Ohigashi, H.: Exploration of useful plants in the subtropics－from the field works－. FOOD Style 21, 9(12), 33-37, 2005 (in Japanese)

Murakami, A. and Ohigashi, H.: Plant constituents produced in the stress enviroment: biological and physiological significance (in Japanese). Shokuhin-Kogyo, 22-277, 2005 (in Japanese)

Reports

Ohigashi, H. and Irie, K.: Development of new agents that regulate cellular signal transduction. Report (2005) for Monbu-kagakusho Research Grant [Scientific Research (A) (2)]

Ohigashi, H.: Elucidation of the suppressive effects of Okinawan citrus, Shikuwasha, on the development of atherosclerosis. Report (2005) Japan Science and Technology Agency.

Ohigashi, H.: Elucidation of physiological functions of food factors and their applications. Report (2005) for funds from the ministry of Agriculture, Forestry, and Fisheries of Japan

Ohigashi, H.: Analysis on the physiological functions of traditional fermented food, pickeled vegetables, in Kyoto. Report for fund from Kyoto Wellness Project (Kyoto Prefecture)

Irie, K.: Structural analysis of β-amyloid fibrils using solid-state NMR. Report (2004-2005) for Monbu-kagakusho Research Grant [Scientific Research (B) (1)]

Irie, K.: Molecular design of agents with protein kinase Cδ selectivity based on the CH/π interaction. Report (2005) for Monbu-kagakusho Research Grant [Priority Areas]

Shirasawa, T., Shimizu, T. and Irie K.: Development of vaccination therapy based on the turn structure of the aggregates of amyloid β 42. Report (2005) for Monbu-kagakusho Research Grant [Priority Areas]

Murakami, A.: Elucidation of physiological functions of food factors and their applications. Report (2005) for funds from the ministry of Agriculture, Forestry, and Fisheries of Japan

Murakami, A.: The role of inflammation in carcinogenesis. Report (2005) for funds from the ministry of Health, Labor and Welfare

Murakami, A.: Effects of lauric acid on the production of pro-inflammatory mediators and its risk assessment [Scientific Research (C) (2)]

Hirai, N.: Regulation mechanism of the resistance induced by insects in plants. Reports (2005) for Japan Science and Technology Corporation, Core Research for Evolutionary Science and Technology

Hirai, N.: Study on elicitor produced by plants afte herbivore damage. Reports (2005) for Japan Science and Technology Corporation, Core Research for Evolutionary Science and Technology

b) Conference and seminar papers presented

The 2005 Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kyoto): general papers (11) and a symposium paper

40th Summer School on Natural Products Chemistry (Atagawa): general papers (2)

47th Symposium on the Chemistry of Natural Products (Tokushima): 1 general paper

24th Meeding of the Japan Society for Dementia: 1 general paper

107th Meeting of the Japanese Pharmacological Society in Kinki branch: 1 general paper

443th Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry in Kansai branch: general papers (3)

79th Meeting of the Japanese Pharmacological Society: 1 invited presentation

The 5th Meeting of AOB research: 1 general paper

The 12th Conference of Japanese Society for Cancer Prevention: 2 general papers

The 64th Annual Meeting of the Japanese Cancer Association: 1 general paper

The 10th Forum of Japanese Society for Food Factors: 4 general papers

The 20th Annual Meeting of Spice Research: 1 general paper

The 5th Annual Meeting of the Japanese Society for Nitric Oxide: 1 invited presentation

The 2005 Annual Meeting of Japanese Society of Plant Physiology: 1 general paper.

The 40th Annual Meeting of Japanese Society of Chemical Regulation of Plants: 2 general papers

A-3. Off-campus activities

Membership in academic societies (roles)

Ohigashi, H.: Japan Society for Bioscience, Biotechnology, and Agrochemistry (chairperson of 2006 annual meeting, councilor, advisory board, councilor of the Kansai Branch), The Japan Society of Analytical Chemistry (councilor of Kinki Branch), The Japanese Society of Food Science and Technology (councilor of Kansai Branch), Japanese Society for Food Factors (councilor), Spice Research Forum of Japan (councilor), Japan Society for Preventive Medicine (councilor), The Japanese Society of Lipid Peroxide and Free Radical Research (councilor), Japanese Association for Animal Cell Technology (councilor), Japanese Association for Cancer Prevention (councilor)

Irie, K.: The Japan Society for Bioscience, Biotechnology, and Agrochemistry in Kansai Branch (general secretary), Organizing committee of Symposium on the Chemistry of Natural Products (member), Organizing committee of Summer School on Natural Products Chemistry (member)

Hirai, N.: Japanese Society for Chemical Regulation of Plants (general secretary), Japan Society for Bioscience, Biotechnology, and Agrochemistry (councilor of the Kansai Branch),

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

Murakami, A.: Food Science Forum (councilor)

Research grant

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)

Scientific Research in Priority Areas: Molecular design of agents with protein kinase Cδ selectivity based on the CH/π interaction (Irie, K., Head)

Scientific Research in Priority Areas: Development of vaccination therapy based on the turn structure of the aggregates of amyloid β 42 (Shirasawa, T., Head, Irie, K., Cooperator)

Scientific Research (C) (2) Effects of lauric acid on the production of pro-inflammatory mediators and its risk assessment (Murakami, A., Head)

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)

Funds from Japan Science and Technology Agency: Elucidation of the suppressive effects of Okinawan citrus, Shikuwasha, on the development of atherosclerosis (Ohigashi, H., Head)

Funds from Japan Science and Technology Agency (Prefecture Collaboration of Regional Entities for Advancement of Technological Excellence): Utilizing Technology on Physiological Functions of the Plants Originated from the Ancient Prefecture, Nara (Ohigashi, H., Murakami, A., Cooperators)

Funds from Kyoto Wellness Project, Kyoto Prefecture (Ohigashi, H., Head)

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)

A-4. International cooperations and overseas activities

International meetings (roles)

Ohigashi, H. , Murakami, A.: Symposium in ACS Annual Meeting (San Diego, 1 invited presentation)

Ohigashi, H., Murakami, A.: International Research Conference on Food, Nutrition and Cancer (Washington DC, 1 invited presentation)

Murakami, A., Ohigashi, H.: The 63rd Annual Meeting of Cancer Research (Anaheim, 2 genral papers)

Murakami, A., Ohigashi, H.: International Meeting of Cancer Prevention (Kyoto, 1 invited presentation, 1 genral paper)

Murakami, A: International Meeting of Chronic Oxidative Stress and Cancer (Heidelberg, 1 invited presentation)

Murakami, A., Ohigashi, H.: The 2005 International Chemical Congress of Pacific Basin Societies (Pacifichem) (Honolulu, 1 invited presentation, 1 genral paper)

Irie, K., Ohigashi, H.: 35th Annual Meeting of Society for Neuroscience (Washington, DC, USA, 3 general papers)

Irie, K., Ohigashi, H.: The 2005 International Chemical Congress of Pacific Basin Societies (Honolulu, USA, 1 general paper)

Irie, K.: Local organizing committee of IUPAC 5th International Conference on Biodiversity and

25th International Symposium on Natural Products (ICOB-5 & ISCNP-25), Kyoto 2006 (member)

Hirai, N.: The 2005 International Chemical Congress of Pacific Basin Societies (Honolulu, USA, 1 invited paper)

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), Journal of Food Science (editor)

Murakami, A.: Journal of Clinical Biochemistry and Nutrition (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 (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: 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., Murakami, A. and Hirai, N.), Introduction and Practice in the Department of Food Science and Biotechnology I, II (Ohigashi, H., Irie, K., Murakami, A. and Hirai, N)

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

B-2. Off-campus teaching, etc.

Part-time lecturer

Ohigashi: H.: Graduate School of Hokkaido Tokai University (Advanced Course of Biotechnogy), Kyoto Women's University (Food Science)

Murakami, A.: Graduate School of Bioagricultural Sciences, Nagoya University (Special Lecture)

Hirai, N.: Faculty of Textile Science, Kyoto Institute of Technology (Phytochemistry), Graduate School of Hiroshima Prefectural University (Special Lecture)

Open seminars

Ohigashi, H.: Seminar for Human Life Science (Health Research Foundation) (lecture), Food Factor Forum in Kyoto 2004 (organizer), 2005 Symposium of the Plant Research Foundation (organizer), 2nd Symposium on Functional Foods (the Ministry of Agriculture, Forestry, and Fisheries of Japan) 'Frontier of Functional Food Research' (special lecture), 6th Forum of the Functional Food Research (the Council fo Osaka Commerce and Industry) (lecture), Technical Seminor of Japan Society of Biotechnology 'New Approach to the Functional Foods' (lecture), Biosymposium for Exploration of Physiological Effects of Functional Food' (lecture)

Irie, K.: The COE seminar in Nara Institute of Science and Technology, 'Development of agents

with selectivity for protein kinase C isozymes' (lecture)

Irie, K.: Seminar in the Department of Chemistry, Stanford University (CA, USA), 'Formation and stabilization model of the 42-mer Aβ radical: implications for the long-lasting oxidative stress in Alzheimer's disease' (lecture)

Irie, K.: First open symposium on Scientific Research in Priority Areas, 'Molecular design of agents with protein kinase Cδ selectivity based on the CH/π interaction' (lecture)

Murakami, A.: The 6th Meeting of Priority Research in Graduate School of Osaka City University (lecture)

B-3. Overseas teaching

Students and research fellows from abroad

Doctors Program: 1 (Korea), Masters Program: 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), Food Safety Council of Kyoto City (chairperson), Plant Research Foundation (councilor), Executive Committee of the Health Research Foundation (member), Nomination Committee for the Financial Support of the Tea Science Foundation of Kyoto Prefecture (member), Research-promoting Committee for the Prefecture Collaboration of Regional Entities for Advancement of Technological Excellence (Nara Prefecture) (member)

Irie, K.: Safeguard Committee of Faculty of Agriculture for Radioisotopes and Radiation (member), Committee of general curriculum in chemistry in Kyoto University (member), Plant Research Foundation (councilor)

Hirai, N.: Dowa and Human Right Problems Committee of Kyoto University (member), Plant Research Foundation (councilor), Coordination of the beer project of Kyoto University and Waseda Univeristy, Coordination of researchg 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: Inoue, Kazuo, Dr. Agric. Sci.
Assistant Professor : Tsuzuki, Satoshi, Dr. Agric. Sci.
Students and research fellows
Doctor's program: (8) *Master's program* *: (11)*
Undergraduate *: (4)*

A. Research Activities (2005.4-2006.3)

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 form 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 the brain elicited the manifestation of central fatigue and depression in willingness to move.

In addition, we showed the administration of TGF-β into the 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 the 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

Books

Fushiki,T: Taste and preference for lipids: Lipid Nutrition and Health (Miyazawa, Yanagida, and Fujimoto eds), pp163-179, Kenpaku-sha (Tokyo), 2005

Fushiki, T: Secrets of Koku and Umami, Shincho-sha shinsho (Tokyo), 2005

Fushiki, T: Human eats by the brain, Chikuma shinsho (Tokyo), 2005

Original papers

Nakano M, Hamada T, Hayashi T, Yonemitsu S, Miyamoto L, Toyoda T, Tanaka S, Masuzaki H, Ebihara K, Ogawa Y, Hosoda K, Inoue G, Yoshimasa Y, Otaka A, Fushiki T, Nakao K. alpha2 Isoform-specific activation of 5'adenosine monophosphate-activated protein kinase by 5-aminoimidazole-4-carboxamide-1-beta-d-ribonucleoside at a physiological level activates glucose transport and increases glucose transporter 4 in mouse skeletal muscle. Metabolism. 2006 Mar; 55(3): 300-8.

Toyoda T, Tanaka S, Ebihara K, Masuzaki H, Hosoda K, Sato K, Fushiki T, Nakao K, Hayashi T. Low-intensity contraction activates the {alpha}1 isoform of 5'AMP-activated protein kinase in rat skeletal muscle. Am J Physiol Endocrinol Metab. 2005 Oct 25; [Epub ahead of print]

Goto T, Takahashi N, Kato S, Egawa K, Ebisu S, Moriyama T, Fushiki T, Kawada T. Phytol directly activates peroxisome proliferator-activated receptor alpha (PPARalpha) and

regulates gene expression involved in lipid metabolism in PPARalpha-expressing HepG2 hepatocytes. Biochem Biophys Res Commun. 2005 Nov 18; 337(2): 440-5. Epub 2005 Sep 21.

Jisaka M, Iwanaga C, Takahashi N, Goto T, Kawada T, Yamamoto T, Ikeda I, Nishimura K, Nagaya T, Fushiki T, Yokota K. Double dioxygenation by mouse 8S-lipoxygenase: specific formation of a potent peroxisome proliferator-activated receptor alpha agonist. Biochem Biophys Res Commun. 2005 Dec 9;338(1): 136-43. Epub 2005 Aug 15.

Hirayasu H, Yoshikawa Y, Tsuzuki S, Fushiki T. Sulfated polysaccharides derived from dietary seaweeds increase the esterase activity of a lymphocyte tryptase, granzyme A. J Nutr Sci Vitaminol (Tokyo). 2005 Dec;51(6): 475-7.

Zhou H, Yamada Y, Tsukiyama K, Miyawaki K, Hosokawa M, Nagashima K, Toyoda K, Naitoh R, Mizunoya W, Fushiki T, Kadowaki T, Seino Y. Gastric inhibitory polypeptide modulates adiposity and fat oxidation under diminished insulin action. Biochem Biophys Res Commun. 2005 Sep 30; 335(3): 937-42.

Hibuse T, Maeda N, Funahashi T, Yamamoto K, Nagasawa A, Mizunoya W, Kishida K, Inoue K, Kuriyama H, Nakamura T, Fushiki T, Kihara S, Shimomura I. Aquaporin 7 deficiency is associated with development of obesity through activation of adipose glycerol kinase. Proc Natl Acad Sci U S A. 2005 Aug 2; 102(31): 10993-8.

Kuroda K, Inoue N, Ito Y, Kubota K, Sugimoto A, Kakuda T, Fushiki T. Sedative effects of the jasmine tea odor and (R)-(-)-linalool, one of its major odor components, on autonomic nerve activity and mood states. Eur J Appl Physiol. 2005 Oct; 95(2-3): 107-14.

Rolls ET, Browning AS, Inoue K, and Hernadi I. Novel visual stimuli activate a population of neurons in the primate orbitofrontal cortex. Neurobiol Learn Mem. 2005 Sep; 84 (2): 111-123.

Rolls ET, Critchley HD, Browning AS, and Inoue K. Face-selective and auditory neurons in the primate orbitofrontal cortex. Exp Brain Res: 2005 Nov. 170 (1), 1-14.

Tsuzuki S, Murai N, Miyake Y, Inouye K, Hirayasu, H, Iwanaga T, and Fushiki T (2005) Evidence for the occurrence of membrane-type serine protease 1/matriptase on the basolateral sides of enterocytes. Biochem. J. 388 (2), 679-687

reviews

b) Conference and seminar papers presented

Annual meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (8 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 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)

Research grant

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).

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-Oriented Technology research Program for Promotion of Basic research Activities for Innovative Biosciences (Fushiki, representative).

A-4. International cooperation and overseas activities

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)

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Nutrition Chemistry (Fushiki), Laboratory course in food and nutrition chemistry (Inoue)

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

B-2. Off-campus teaching etc.

Part-time lecture

Fushiki: Graduate School of Agricultural and Life Sciences, The University of Tokyo, Graduate School of Medicine, Hokkaido University, Faculty of Engineering, Kansai University

Enlightenment lecture

Fushiki, T: 24th Food life culture special lecture, "Food and Exercise, Relax". (Tokyo), Wakayama Prefecture, Gobo City, Parenting support lecture (Wakayama), Lecture at Heisei 17 nenndo Annual Meeting of the Japanese Association for the study of Taste and Smell, Symposium on "the Search of Scientific base of Palatability" (Iwate), High school – University cooperative project on education enhancement program, lectures at the Zeze Shiga prefectural high school (Otsu), Heisei 17 nenndo the Statistic Day anniversary special lecture (Otsu), Heisei 17 nenndo the study group of vegetable and tea science, lecture "Study of preference using experimental animals" (Tsu), The Forum of Food Culture, Summative lecture, "Taste and Preference" (Obama), Nippon Food Education Fair, Keynote lecture, (Tokyo)

Inoue K: Japanese Society of Food Chemistry and FFI Journal Editorial Board, "Fatigue and Recovery" Symposium, lecture (Osaka), University of Shizuoka, School of Food and Nutritional Science monthly seminar, lecture (Shizuoka), Joint Symposium of Research Core and User Science Institute of Kyushu University, lecture (Fukuoka)

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).

Inoue: Advisory Board for the Committee of Animal Experiment in Kyoto University Radioisotope Research Center (member), Advisory Board for the Committee of Animal Experiment in Graduate School of Agriculture, Kyoto University (member), Advisory Board for the Committee of Agricultural Library of Kyoto University (member)

2.7.5 Laboratory of Molecular Function of Food

Staff *Professor* *: Kawada, Teruo, Dr. Agric. Sci.*
Associate Professor: Urade, Reiko, Dr. Agric. Sci.
Students and research fellows
Doctor's Program : (4)
Master's Program : (7)
Undergraduate : (4)
Postdoctoral fellow : (2)
Research fellow : (1)

A. Research Activities (2005.4-2006.3)

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 energy and lipid metabolism aimed at prevention of the life-style related disease and metabolic syndrome

Initiation and progression of the life-style related disease are involved in the lipid metabolism in the various organs including gastrointestinal tract, liver and adipose tissue. For

understanding and prevention of these diseases, we are performing the basic studies about lipid metabolism and adipocyte function, and applied studies about food factors regulating lipid metabolism properly. Our current research topics are 1) the screening of natural products for normalizing lipid metabolism. 2) basic and applied studies about lipoproteins in liver and small intestine. 3) analysis of molecular mechanisms that monitor hypertrophy of adipocytes.

c) Molecular food function on the regulation of obesity-related inflammatory pathologies

Arteriosclerosis and the allergy are the familiar living body inflammations caused by food and the genetic background. The importance of the cytokines and chemokines such as tumor necrosis factor (TNF)α, adiponectin, and monocyte chemoattractant protein-1 (MCP-1) in the lifestyle disease is clarified inside and outside the country in recent years. The chemokine is a super-family of the cytokine of cell migration (chemoatratant), and it is known as inflammatory mediator. In addition, the function has been found receiving the modification by various nutrients and the food factors. This research theme clarifies the realities of a chemical factor that it develops metabolic syndrome from the aspect of the cytokine and chemokine.

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 secretary 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 unfoled 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 affecing 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 srorage 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 plays a primary role on the signal transduction for activation of platelets by using a methods 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

Kobayashi M, Ohno T, Kawada T, Ikegami H, Nishimura M, Horio F: Serum Adiponectin

Concentration: Its Correlation with Diabetes-Related Traits and Quantitative Trait Loci Analysis in Mouse SMXA Recombinant Inbred Strains. Biosci. Biotechnol. Biochem. 70; 677-683, 2006

Shimizu K, Sakai M, Ando M, Chiji H, Kawada T, Mineo H, Taira T: Newly developed primary culture of rat visceral adipocytes and their in vitro characteristics. Cell Biol. Int. 30; 381-388, 2006

Kang JH, Sung MK, Kawada T, Yoo H, Kim YK, Kim JS, Yu R: Soybean saponins suppress the release of proinflammatory mediators by LPS-stimulated peritoneal macrophages. Cancer Lett. 230; 219-227, 2006

Ikeda I, Konno R, Shimizu T, Ide T, Takahashi N, Kawada T, Nagao K, Inoue N, Yanagita T, Hamada T, Morinaga Y, Tomoyori H, Imaizumi K, and Suzuki K: Campest-5-en-3-one, an oxidized derivative of campesterol, activates PPAR α, promotes energy consumption and reduces visceral fat deposition in rats 2. Biochim. Biophys. Acta 1760; 800-807, 2006

Nishiyama T, Mae T, Kishida H, Tsukagawa M, Mimaki Y, Kuroda M, Sashida Y, Takahashi K, Kawada T, Nakagawa K, Kitahara M: Curcuminoids and sesquiterpenoids in turmeric (Curcuma longa L.) suppress an increase in bloodglucose level in type 2 diabetic KK-Ay mice. J. Agric. Food Chem. 53; 959-963, 2005

Kuroda M, Mimaki Y, Nishiyama T, Mae T, Kishida H, Tsukagawa M, Takahashi K, Kawada T, Nakagawa K, Kitahara M: Hypoglycemic Effects of Turmeric (Curcuma longa L. Rhizomes) on Genetically Diabetic KK-A(y) Mice. Biol. Pharm. Bull. 28; 937-939, 2005

Weangsripanaval T, Moriyama T, Kameyama H, Ogawa T, Kawada T: Gastrointestinal Absorption of Major Soybean Allergen, Gly m Bd 30K, in Mice: Effect of Dietary Lipid and Exogenous Emulsifier. J. Nutr. 135: 1738-44, 2005

Goto T, Takahashi N, Kato S, Egawa K, Ebisu S, Moriyama T, Fushiki T, Kawada T: Phytol directly activates peroxisome proliferetor-activated receptor a (PPARa) and regulates gene expression involved in lipid metabolism in PPARa-expressing HepG2 hepatocytes. Biochem. Biophys. Res. Commun. 337; 440-445, 2005

Murakami A, Nishizawa T, Egawa K, Kawada T, Nishikawa Y, Uenakai K, Ohigashi H: New class of linoleic acid metabolites biosynthesized by corn and rice lipoxygenases: Suppression of proinflammatory mediator expression via attenuation of MAPK- and Akt-, but not PPARgamma-, dependent pathways in stimulated macrophages. Biochem. Pharmacol. 70; 1330-1342, 2005

Jisaka M, Iwanaga C, Takahashi N, Goto T, Kawada T, Yamamoto T, Ikeda I, Nishimura K, Nagaya T, Fushiki T, Yokota K: Double dioxygenation by mouse 8S-lipoxygenase: Specific formation of a potent peroxisome proliferator-activated receptor alpha agonist. Biochem. Biophys. Res. Commun. 336; 1-8, 2005

Kamauchi S, Nakatani H, Nakano C, and Urade R: Gene expression in response to endoplasmic reticulum stress in Arabidopsis thaliana. FEBS J. 272; 3461-3476, 2005

Okudo H, Arakaki Y, Urade R.: Cooperation of ER-60 and BiP in the oxidative refolding of denatured proteins in vitro. J. Biochem. 138; 773-780, 2005

Kimura T, Imaishi K, Hagiwara Y., Horibe, T, Hayano, T, Takahashi N, Urade R, Kato K, Kikuchi, M: ERp57 binds competitively to protein disulfide isomerase and calreticulin. Biochem. Biophys. Res. Commun. 331; 224-230, 2005

Reviews

Takahashi N, Kusudo T, Moriyama T, Kawada T,: Structure and functio of PPAR. Nippon Rinsho. 63: 557-564. 2005

Moriyama T, Kawada T: Molecular mechanism of obesity and it regulation. Kagaku to Kogyo 79: 153-162, 2005

Kawada T, Moriyama T: Adipose tissue and nutrition. Kenko Eiyougaku p107-115, p162-170, 2005

Kawada T, Kan M, Goto T: Obesity and food in metabolic syndrome Japan Food Science 44(12): 22-28, 2005

Kusudo T, Moriyama T,, Kawada T: adipocyte proliferation and differentiation Molecular Medicine 42: supplement 33-37, 2005

Yu R, Kwon BS, Kawada T: Chemokines as a novel target for obesity-related inflammatory pathologies. J Japan Society for the Study of Obesity, 11(3). 27-34, 2005.

b) Conference and seminar (paper presented)

The 2006 Annual Meeting of Japan Society for Bioscience, Biotechnology and Biochemistry (8)

The 59th Annual Meeting of Japan Society for Nutrition and Food Science (5)

The 2005 Annual Meeting of Japan Society for Trace Elements Science (1)

The 2005 Annual Meeting of Japan Society for Bio-Quinone (1)

The 75th Annual Meeting of the Japanese Biochmical 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), Study Group on Adipscience (General affair)

Research grants

Monbusho Research Grant: Scientfic Research on Priority Areas "Adipomics; Analysis of molecular mechanism that hypertrophy of adiocytes" (Kawada, representative), Scientific Research (B) "The signal transduction of Dietary fat and energy metabolism via nuclear recptors" (Kawada, representative).

Research and Development Program for New Bio-industry Initiatives "Development of functional food for the prevention hay fever and life-style related disease" (Kawada, representative).

Research and Development Program for New Bio-industry Initiatives "Production of food materials to prevent life style-related diseases based on genomic information. (Urade, collaborator).

A-4. International cooperation and overseas activities

International Joint Research

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

Urade: Studies on roles on interaction of RAP and ER-60 (Washington University of Medicine; United Stats)

Urade: 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 (2005.4-2006.3)

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 (Kawada and Urade), Introduction and practice in the department of food science and biotechnology II (Kawada and Urade), Food biochemistry II (Kawada), Food biochemistry I (Urade), Food Safety II (Kawada), Laboratory Course in Enzymme Chemistry and Biochemistry (Urade)

Graduate level: Advanced Course in Molecular Function of Food (Kawada and Urade), Advanced Course in Food and Health Science (Kawada), 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, T: Dosisha women's University (Enzyme Science), Nara women's University (Nutritional gene regulation)

2.7.6 Laboratory of Physiological Function of Food

Staff *Professor: Yoshikawa, Masaaki, D. Agric. Sci.*
Lecturer : Ohinata, Kousaku, D. Agric. Sci.
Assistant Professor : Yamada, Yuko, M. Agric. Sci.

Students and research fellows

Doctor's Program : (1)	*Postdoctoral Fellow : (2)*
Master's Program : (5)	*Visiting Researcher : (1)*
Undergraduate : (2)	*Research Assistant : (3)*
Research Student : (1)	

A. Research Activities (2005.4-2006.3)

A-1. Main Subjects

a) Anxiolytic activity of opioid peptides derived from plant proteins

In our previous study, rubiscolin-6 (Tyr-Pro-Leu-Asp-Leu-Phe), a δ-selective opioid peptide derived from a large subunit of spinach ribulose bisphosphate carboxylase/oxygenase (Rubisco), inhibited pain perception and stimulated memory consolidation after oral administration in mice. We found that rubiscolin-6 had an anxiolytic activity in elevated plus maze experiment after intracerebroventricular or oral administration at doses of 10 nmol/mouse or 100 mg/kg,

respectively. The anxiolytic activity was inhibited by naltrindole, an antagonist selective for δ receptor, as expected. The anxiolytic activity was also inhibited by BMY14802 and SCH23390, antagonists for σ_1 and dopamine D_1 receptors, respectively. These suggest that an endogenous ligand for σ_1-receptor is released following the binding of rubiscolin-6 to σ_1-receeotor, and dopamine released at the downstream of σ_1-receeotor mediates anxiolytic activity via D_1 receptor.

We found the Tyr-Pro-Phe-Val sequence, which is common to an opioid peptide human β-casomorphin-4, in soy β-conglycinin β subunit, and investigated enzymatic conditions to release the opioid peptides from the protein. Tyr-Pro-Phe-Val-Val-Asn was released by digesting soy 7S globulin with pepsin and pancreatic elastase. On the other hand, Tyr-Pro-Phe-Val-Val and Tyr-Pro-Phe-Val were released by digestion with pancreatic elastase and leucine aminopeptidase (LAP). These were named soymorphins-6, -5, and -4, respectively. Their opioid activities were more potent in the guinea pig ileum assay than mouse vas deferens assay suggesting that they are selective for μ receptor. The IC_{50} values of soymorphins-6, -5, and -4 in the guinea pig ileum assay were 9.2, 6.0, and 20 μM, respectively. Soymorphins-6, and -5 showed anxiolytic activity after oral administration in mice at doses of 10 mg/kg, and 30 mg/kg, respectively. The anxiolytic mechanism of μ-selective soymorphins are different from that of δ-selective rubiscplin-6 since they were blocked by neither BMY14802 nor SCH23390.

b) Antialopecia and hypocholesterolemic activities of a new immunostimulating peptide Met-Ile-Ile-Ile derived from soy glycinin.

Soymetide-4 (Met-Ile-Thr-Leu), a phagocytosis-stimulating peptide derived from β-conglycinin α' subunit, prevented alopecia induced by an anticancer agent etoposide in neonatal rats after oral administration at a dose of 300 mg/kg. We found that a homologous peptide Met-Ile-Ile-Ile was released from soy glycinin A5-A4 subunit after digestion with subtilisin and trypsin. Phagocytosis-stimulating activity of soymetide-4 and Met-Ile-Ile-Ile were blocked by Boc-Met-Leu-Phe, an antagonist for fMLP receptor. The receptor affinity and phagocytosis-stimulating activity of Met-Ile-Ile-Ile were larger than those of soymetide-4. The antialopecia activity of Met-Ile-Ile-Ile was larger than that of soymetide-4 after ip. administration. However, their potencies were almost the same after oral administration. In mice fed hypercholestrol diet, Met-Ile-Ile-Ile showed hypocholesterolemic effect after oral admin istration at a dose of 100 mg/kg while soymetide-4 didn't. The antialopecia activity of Met-Ile-Il-Ile was blocked by indomethacin, a COX inhibitor, while its hypocholestrolemic effect wasn't suggesting that signal transduction systems for both activities are different each other.

c) Suppression of food intake by short peptides activating central prostaglandin E_2 receptor

Prostaglandin (PG) E_2, a bioactive lipid produced in the central nervous system (CNS), has a variety of central effects on wakefulness, fever, pain response and food intake. We examined which of four distinct subtypes of PGE_2 receptors (EP_1-EP_4) mediated the anorexigenic action of PGE_2 using highly selective ligands. A centrally administered EP_4 agonist mimicked the anorexigenic action by PGE_2. The anorexigenic action of PGE_2 or EP_4 agonist was ameliorated by EP_4 antagonist. Thus, activation of PGE_2-EP_4 signaling in the central nervous system suppresses food intake. Furthermore, we explored short peptides suppressing food intake via activation of PGE_2-EP_4 pathway.

Novokinin (RPLKPW), an agonist for angiotensin AT_2 receptor, is a potent antihypertensive peptide designed by replacing 4 amino acids in a vasorelaxing ovokinin(2-7) (RADHPF) derived form ovalbumin. Orally administered novokinin suppressed food intake. The anorexigenic action

of novokinin was blocked by an AT_2 receptor antagonist. This anorexia was also inhibited by a cyclooxygenase (COX) inhibitor or an EP_4 antagonist. Thus, novokinin decreases food intake via AT_2 receptor followed by activation of EP_4 receptor after PGE_2 release.

WPLPR, an agonist for complement C3a receptor, was designed based on an ileum-contracting peptide oryzatensin (GYPMYPLPR) derived form rice albumin. WPLPR decreased food intake. This anorexigenic effect was also ameliorated by a COX inhibitor or an EP4 antagonist, suggesting that WPLPR suppresses food intake through PGE2 release followed by EP4 receptor activation at the downstream of complement C3a receptor.

d) Elongation of antihypertensive activities of tea catechin by complex formation with milk proteins.

Tea catechin reduced blood pressure rapidly in spontaneously hypertensive rats (SHR) 2 hrs after oral administration at a dose of 160 mg/kg. However, the hypotensive activity was not observed 4 hrs after the administration suggesting that its gastrointestinal absorption, renal excretion or metabolism might be rapid. When given as a complex with milk proteins (300 mg/kg body weight), the antihypertensive effect of catechin was observed from 4 to 8 hrs after the administration. When given as a complex with soy protein, catechin never reduced blood pressure. Gastrointestinal absorption of catechin might be inhibited by complex formation with proteins. Catechin might be released gradually from the complex with milk proteins to give long-lasting antihypertensive effect since their digestibility is fairly good. However, release of catechin from the complex with soy protein might be too slow. Among milk proteins, mainly whey proteins contribute to the elongation of antihypertensive effect of tea catechin.

A-2. Publications and Presentations

a) Publications

Books

Agui, S., K. Ohinata, and M.Yoshikawa: Isolation and characterization of a new opioid peptide soymorphin derived from soy β-conglycinin. "Peptide Science 2005" (Ed. by T. Wakamiya), p.196-198, The Japanese Peptide Society, Osaka, 2006

Yoshikawa, M., and T. Tsuruki: Suppression of cancer chemotherapy-induced alopecia by soymetide-4, an fMLP agonist peptide derived from soy protein. "White Hair, Alopecia, and Hair Growth" (*in Japanese*) pp.130-145, NTS, 2005

Ohinata, K., and M. Yoshikawa: Analyses and quantitation of Peptides, "Practical Analyses and quantitation in Bioscience" (*in Japanese*) (Ed. T. Moriyama) pp.83-87, Yodo-sha, 2005

Yoshikawa, M., and T. Tsuruki : Bioactive peptides derived from soy protein. "Soy in Health and Disease Prevention" (Ed. M. Sugano). pp.225-233, Taylor & Francis, 2005

Yoshikawa, M.: Novokinin, anti-hypertensive and hair growth-stimulating peptide designed according to the structure of ovokinin derived from ovalbumin. NIAS Agri-bioscience Series No.2, Bioindustry, Animals, Plants, and Insecta as factories to produce useful substances. (Ed. Y. Izaike, N. Oka, and H. Machii) pp.10-11, 2006

Original papers

Turuki, T., A. Ito, K. Takahata and M. Yoshikawa : Orally administered FPRL1 receptor agonist peptide MMK-1 inhibits etoposide-induced alopecia by a mechanism different from intraperitoneally administered MMK-1. Peptides, 27, p.820-825, 2006

Turuki, T., K. Takahata and M. Yoshikawa : Anti-alopecia mechanism of soymetide-4, an

immunostimulating peptide derived from soy b-conglycinin. Peptides, 26, p.707-711, 2005

Turuki, T., and M. Yoshikawa : Anti-alopecia effect of Gly-Leu-Phe an immunostimulating peptide derived from a-lactalbumin. Biosci. Biotechnol. Biochem., 69, p.1633-1635, 2005

Patents

Patent pending/applied for Patent application no. 2005-284602 "Nobel opioid peptides, nobel anxiolytic peptides", inventors: Yoshikawa, M., Agui, S., and Ohinata, K., applicant: Oike, K., the President of Kyoto Univ., application date: Sept. 29, 2005

Reviews

Yoshikawa, M,: Analyses and exploitation of physiological function of oligopeptides derived from food proteins. Bioscience and Industry (*in Japanese*), 63, p.573-578, 2005

Yoshikawa, M., and K. Ohinata: Design of new food to improve health by combining skim milk and leaf proteins. Animal Industry Information, Agricultural and Livestock Indutries Corporation (*in Japanese*), 3, p.16-33, 2006

b) Conference and seminar papers presented

The Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry: 10 papers

The Annual Meeting of The Society for Biotechnology, Japanen: 1 paper

The 42nd Symposium on Plant Chemistry: 1 paper

Kansai Regional Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry: 2 papers

The Japanese Dairy Science Annual Symposium: 1 paper

The 42nd Japanese Peptide Symposium: 1 paper

The 52nd Annual Meeting of the Japanese Society for Food Science and Technology: 1 papar

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), Japanese Peptide Society (Councilor)

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 reglation in zinc deficiency (Ohinata, representative)

Grant from Ministry of Agriculture, Forestry, and Fisheries: 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 Dairy Science Association: Studies to promote utilization of powdered skim milk. (Yoshikawa, representative)

Grant from The Food Science Institute Foundation: Studies on physiological function of food

proteins. (Yoshikawa, representative)

A-4. International cooperation and overseas activities

International joint researches, overseas research surveys

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

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Introduction to the molecular cell biology (Yoshikawa), Physiological functions of foods (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: Advanced course in health science of foods. Advanced course in physiological functions of foods (Yoshikawa, Ohinata), Seminar in Physiological functions of foods (Yoshikawa, Ohinata), Experimental couse in Physiological functions of foods (Yoshikawa, Ohinata)

B-2. Off-campus teaching

Open seminar, etc

Yoshikawa: JST Seminar on New Technology, lecturer "Nobel anti-amnesic peptide based on new mechanism", 2006, 10, 27, Tokyo,

Yoshikawa: Agri-hi-tech-symposium, Foods in 21st Century, "Prevention of life style-related diseases by bioactive peptides derived from food proteins", 2007, 2, 17, Kyoto

B-3. Overseas teaching

Students and research fellows from abroad

Visiting scientists: 1 (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 (2005)

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

Students and research fellows
Doctor's program: (5) *Research student: (1)*
Master's program: (9)
Undergraduate : (4)

A. Research Activities (2005.4-2006.3)

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 intended 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) Utilization and analysis of characterization of food materials dispersed in nano- or micro-size:

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, focusing on mass transport through interface between oil and water. We also investigate development of food materials, which is W/O/W type emulsion containing functional substances in its inner water phase.

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. Especially, we focus on condensation reaction between hydrophobic and hydrophilic substances via reverse reaction of hydrolase. The following enzymatic reactions are investigated: production of edible surfactants and modification of polyunsaturated fatty acids to retard their autoxidation.

d) Kinetics of deterioration of food materials and its supression:

Deterioration of food materials can not be avoided. The rate of deterioration depends on atmosphere in which the materials were stored and interaction with components in food materials, which generally are composed of multiple components. Analysis of kinetics of deterioration can make us to develop a method to repress the deterioration in a reasonable way. And we also investigate an applicable study, which is suppression of lipid oxidation by

encapsulation of fine emulsion with food polymers.

A-2. Publications and presentations

a) Publications

Books

Adachi, S.: Microencapsulation. In Drying Technology in Food Industry (in Thai), ed. by W. Tanthapanichakoon, pp. 94-113. TPA Book Center, Bangkok, 2005.

Adachi S.: Chapter 3 Chromatography in a batch operation. Separation Engineering, Chromatography (in Japanese), (ed. by K. Hashimoto). p. 53-78, Baifukan, 2005.

Adachi S.: Section 6.1 Introduction in Chapter 6 Emulsifing and dispersion, pp. 144-149, Suppression of oxidation of encapsulated liquid lipid in Section 9.6.2 Quality preservation and stability of Chapter 9 Drying, pp. 276-278, Section 10.7.2 penetration of seasoning in Chapter 10 Cooking with heat, pp. 313, Handbook of Food Engineering (in Japanese), (ed. by Japan Society of Food Engineering). Asakura shoten, 2005.

Original papers

Haghighat Khajavi, S., Kimura, Y., Oomori, T., Matsuno, R., and Adachi, S.: Degradation kinetics of monosaccharides in subcritical water. J. Food Eng., **68**, 309-313 (2005).

Zhang, X.-M., Adachi, S., Watanabe, Y., and Matsuno, R.: Lipase-catalyzed synthesis of *O*-lauroyl L-serinamide and *O*-lauroyl L-threoninamide. Food Res. Intl., **38**(3), 297-300 (2005).

Watanabe, Y., Ishido, E., Fang, X., Adachi, S., and Matsuno, R.: Oxidation kinetics of linoleic acid in the presence of saturated acyl L-ascorbate. J. Am. Oil Chem. Soc., **82**(5), 389-392 (2005).

Asano, T., Maeda, A., Kimura, Y., Takahashi, T., Nakamura, A., Maeda, H., and Adachi, S.: Condensation reaction between angiotensin II and dicarboxylic acid in water at high temperature without any catalytic agent additive. Biotechnol. Progr., **21**, 1169-1174 (2005).

Chen, J., Kimura, Y., and Adachi, S.: Synthesis of linoleoyl disaccharides through lipase-catalyzed condensation and their surface activities. J. Biosci. Bioeng., **100**, 274-279 (2005).

Hung, L.-H., Kimura, Y., and Adachi, S.: Kinetics of moisture-induced-discoloration of L-ascorbic acid powders. Japan J. Food Eng., **6**(4), 289-295 (2005).

Fang, X., Shima, M., and Adachi, S.: Effects of drying conditions on the oxidation of linoleic acid encapsulated with gum arabic by spray-drying. Food Sci. Technol. Res., **11**(4), 380-384 (2005).

Minemoto, Y., Kometani, T., Piao, J., and Adachi, S.: Oxidation of oleoyl residue of its esters with ethylene glycol, glycerol and erythritol. Lebensm.- Wiss. u-. Technol., **39**, 1-5 (2006).

Fujii, T., Khuwijitjaru, P., Kimura, Y., and Adachi, S.: Decomposition kinetics of monoacyl glycerol and fatty acid in subcritical water under temperature-programmed heating conditions. Food Chem., **94**, 341-347 (2006).

Koreishi, M., Zhang, D., Adachi, S., Matsuno, R., and Nakanishi, K.: A novel acylase from Streptomyces mobaraensis that efficiently catalyzes hydrolysis/synthesis of capsaicins as well as *N*-acyl-L-amino acids and *N*-acyl-peptides. J. Agric. Food Chem., **54**, 72-78 (2006).

Shima, M., Tanaka, M., Fujii, T., Egawa, K., Kimura, Y., Adachi, S., and Matsuno R.: Oral administration of insulin included in fine W/O/W emulsions to rats. Food Hydrocolloids, **20**, 523-531 (2006).

Piao, J., Kawahara-Aoyama, Y., Inoue, T., and Adachi, S.: Bacteriostatic abilities of monoacyl sugar alcohols against thermophilic sporeformers. Biosci. Biotechnol. Biochem., **70**(1), 263-265 (2006).

Fang, X., Shima, M., Kadata, M., Tsuno, T., and Adachi, S.: Suppressive effect of alkyl ferulate on the oxidation of linoleic acid. Biosci. Biotechnol. Biochem., **70**(2), 457-461 (2006).

Fang, X., Kikuchi, S., Shima, M., Kadata, M., Tsuno, T., and Adachi, S.: Suppressive effect of alkyl ferulate on the oxidation of microencapsulated linoleic acid. Eur. J. Lipid Sci. Technol., **108**, 97-102 (2006).

Piao, J., Kishi, S., and Adachi, S.: Surface tensions of aqueous solutions of 1-*O*-monoacyl sugar alcohols. Colloids Surf. A: Physicochem. Eng. Aspects, **277**, 15-19 (2006).

Reports

Adachi, S.: Nanoemulsion –What happens in nanoemulsion?– (in Japanese). Food Plant Manage **April**; 36-37, 2005

Adachi, S.: Subcritical water –Its unique property and availavility– (in Japanese). Fooma, 22-4 (88), 22-25 (2006).

b) Conference and seminar papers presented

96th AOCS Annual Meeting (1 paper)

Annual meeting of MICS, 2005 (2 papers)

Asia-Pacific Biochemical Engineering Conference '05 (1 paper)

Annual Meeting of Japan Society for Food Engineering, 2005 (6 papers)

The 37th Autumn Meeting of the Society of Chemical Engineers, Japan (3 papers)

Meeting of Kansai, Chushikoku, and Nishinihon Branches of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 2005 (2 papers)

Enzyme Engineering XVIII (1 paper)

International Symposium on Biocatalysis and Biotechnology (1 paper)

The 54th Meeting of the Japan Society of Enzyme Engineering (1 paper)

PACIFICCHEM2005 (1 paper)

9th Symposium on Biocatalysis Chemistry Japan (1 paper)

Meeting of Kansai Branch of Japan Society for Bioscience, Biotechnology, and Agrochemistry (1 paper)

Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 2006 (3 papers)

A-3. Off-campus activities

Membership in academic societies

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

Research Grant

Adachi, S.: 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.: 96th AOCS Annual Meeting, Salt Lake City, USA (oral presentation), Enzyme Engineering XVIII, Gyeong-ju, Korea, (poster presentation), International Symposium on Biocatalysis and Biotechnology, Taichung, Taiwan (oral presentation) PACIFICCHEM2005, Honollulu, USA (oral presentation).

Kimura Y.: Asia-Pacific Biochemical Engineering Conference '05, Jeju Island, Korea (oral presentation).

Editorial work for international journals

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

Kimura, Y.: Japan Journal of Food Engineering (editor).

B. Educational Activities (2005.4-2006.3)

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.: Graduate School of Bioresources, Mie University (Sustainable Bioscience and Bioengineering (Advanced Course)).

Open seminar, etc.

Kimura, Y.: Academic Plaza in 2005 International Exhibition on Food Machinery, Ariake, Tokyo (lecturer).

B-3. Overseas teaching

Lectures

Adachi, S.: Utilization of Subcritical Water in Food Processing –Basic studies– in Southern Yangtze University in R.P. China

Students and research fellows from abroad

Doctor's program: 5 (China 2, Iran, Chinese Taipei, Thailand)

Research students: 1 (China)

C. Other remarks

Adachi, S.: Division Charperson, 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), Committee for Education Programe in the Graduate School of Agriculture (member).

Kimura, Y.: 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-doctral fellow: (4)
Doctor's program : (3)
Master's program : (4)
Undergraduate : (3)
Research student : (1)

A. Research Activities (2005.4-2006.3)

A-1. Main subjects

a) Expression/function/structure proteomics of pit-forming bacterium

In the presence of polysaccharides, *Sphingomonas* sp. A1 forms a huge pit on the cell surface through rearrangement and/or reconstitution of pleat molecules. The pit functions as a concentrator for extracellular polysaccharides. Through expression proteomics focusing on cell surface proteins of strain A1, eight outer membrane proteins (p1-p8) have been found to be responsible for the formation and/or function of the pit. In this study, function/structure proteomics regarding these proteins were carried out. Transporters p1-p4 for iron-alginate complex were suggested to constitute a tunnel-like β-barrel structure spanning the outer membrane by homology modeling. Flagellin-homologous p5 and p6 show a significant ability to bind alginate at acidic pH and also regulate formation of the pit, suggesting that both function as an alginate sensor on the cell surface. Based on the mutational analysis, p5 was found to form an alginate-binding module consisting of N- and C-terminal domains. p7 showed an affinity to alginate at neutral pH.

b) Molecular breeding of super-bacteria through transplantation of pit

Sphingomonas sp. A1 has a cluster consisting of genes responsible for the transport and degradation of alginate. Strain A1 cells with a mutation in the genetic cluster cannot form the pit. Molecular breeding of pit-forming super-bacteria with a improvement of xenobiotics-degrading ability was carried out through transformation of other sphingomonads, such as dioxin-degrading *Sphingomonas wittichii* and polypropylene glycol-degrading *Sphingomonas subarctica*, with the strain A1 genetic cluster. As a result, both of *S. wittichii* and *S. subarctica* having the genetic cluster formed the pit constitutively on their cell surface and showed a significant

xenobiotics-degrading ability. Thus, a new biotechnology, i.e. molecular transplantation of the pit to other bacteria, has been established. This technology is superior to the old-fashioned metabolic engineering and expected to open a new area for practical use of microbes in bioremedhiation.

c) Interaction between bacteria and hosts: Structural proteomics on bacterial system for degradation of host cell surface polysaccharide

Interactions between bacteria and organisms such as microbes, plants, and animals were analyzed. Degradation of host cell surface polysaccharides by bacteria is one of the first responses when bacteria interact with hosts.

Microbial polysaccharide: *Bacillus* sp. GL1 xanthan lyase degrades cell surface polysaccharide xanthan produced by a bacterium, *Xanthomonas campestris*. A novel catalytic reaction mechanism of the enzyme by using a single tyrosine residue as base and acid catalysts was clarified by X-ray crystallography.

Plant polysaccharide: Degradation of plant cell wall polysaccharide [rhamnogalacturonan (RG)-I] by *Bacillus subtilis* 168 was analyzed. A novel genetic cluster responsible for degrading RG-I was found in the bacterial genome through DNA micro array analysis. YesW encoded in the cluster was identified as a novel RG lyase in that the enzyme depolymerized RG-I to disaccharide. In addition to YesW, YteR was found to be a novel unsaturated galacturonyl hydrolase acting on unsaturated RG produced through YesW reaction. X-ray crystallographic analysis of YteR was carried out. YteR consists of a α6/α6-barrel structure and has a deep cleft in the center of the enzyme molecule. The substrate binds to the cleft, indicating that the cleft is responsible for the enzyme catalysis.

Animal polysaccharide: *Bacillus* sp. GL1 unsaturated glucuronyl hydrolase (UGL) degrades mammalian polysaccharide, glycosaminoglycan, of an extracellular matrix. A novel catalytic reaction mechanism of the enzyme was clarified by X-ray crystallography. This mechanism is distinct from those of ordinary glycoside hydrolases in that UGL triggers hydration of vinyl ether group of the substrate, but not of glycoside bond.

d) Structural biology of NAD kinase

NAD kinase phosphorylates NAD^+ to form $NADP^+$ and is strictly specific to NAD^+, whereas NADH kinase phosphorylates both NAD^+ and NADH, showing relaxed substrate specificity. Based on their primary and tertiary structures, the difference in the substrate specificities between NAD- and NADH-kinases was proposed to be caused by one aligned residue: Gly or polar amino acid (Gln or Thr) in five NADH kinases, and a charged amino acid (Arg) in two NAD kinases. The substitution of Arg with Gly in the two NAD kinases relaxed the substrate specificity, i.e., converted the NAD kinases to NADH kinases. The substitution of Arg in one NAD kinase with polar amino acids also relaxed the substrate specificity, whereas substitution with charged and hydrophobic amino acids did not show a similar result. In contrast, the substitution of Gly with Arg in one NADH kinase failed to convert it to NAD kinase. These results suggest that a charged or hydrophobic amino acid residue in the position of interest is crucial for strict specificity of NAD kinases to NAD^+, whereas Gly or polar amino acid residue is not the sole determinant for the relaxed substrate specificity of NADH kinases.

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

In transformation of *Saccharomyces cerevisiae* cells, DNA has been proposed to enter into cells via membrane-invagination (1). In order to examine further the transformation process, DNA complexed with YOYO-1 (DNA/YOYO-1) was used and successfully visualized by

fluorescence microscope. After incubation of cells with YOYO-1/DNA and PEG, the region around cell surface gave fluorescent light, indicating that DNA was bound around cell surface. Further incubation of the cells in medium, nucleus showed light, being also confirmed by DAPI-staining. Frequency of this nucleus-staining was in good agreement in the transformation frequency, indicating that YOYO-1/DNA enters into cells and reaches to nucleus.

A-2. Publications and presentations

a) Publications

Original papers

Aso, Y., Y. Miyamoto, K. M. Harada, K. Momma, S. Kawai, W. Hashimoto, B. Mikami and K. Murata: Engineered membrane superchannel improves bioremediation potential of dioxin-degrading bacteria. Nat. Biotechnol. 24 (2); 188-189, 2006

Yamasaki, M., K. Ogura, W. Hashimoto, B. Mikami and K. Murata: A structural basis for depolymerization of alginate by polysaccharide lyase family-7. J. Mol. Biol., 352(1); 11-21, 2005

Maruyama, Y., W. Hashimoto, B. Mikami and K. Murata: Crystal structure of *Bacillus* sp. GL1 xanthan lyase complexed with a substrate: insights into the enzyme reaction mechanism. J. Mol. Biol., 350(5);974-986, 2005

Harada, K. M., K. Tanaka, Y. Fukuda, W. Hashimoto and K. Murata: Degradation of rice bran hemicellulose by *Paenibacillus* sp. strain HC1: gene cloning, characterization and function of β-D-glucosidase as an enzyme involved in degradation. Arch. Microbiol.,184(4); 215-224, 2005

Momma, K., Y. Mishima, W. Hashimoto, B. Mikami and K. Murata: Direct evidence for *Sphingomonas* sp. A1 periplasmic proteins as macromolecule-binding proteins associated with the ABC transporter: molecular insights into alginate transport in the periplasm. Biochemistry, 44(13); 5053-5064, 2005

Yamasaki, M., K. Ogura, S. Moriwaki, W. Hashimoto, K. Murata and B. Mikami: Crystallization and preliminary X-ray analysis of alginate lyases A1-II and A1-II' from *Sphingomonas* sp. A1. Acta Cryst., F61; 288-290, 2005

Hashimoto, W., O. Miyake, A. Ochiai and K. Murata: Molecular identification of *Sphingomonas* sp. A1 alginate lyase (A1-IV') as a member of novel polysaccharide lyase family 15 and implications in alginate lyase evolution. J. Biosci. Bioeng., 99(1); 48-54, 2005

Hashimoto, W., J. He, Y. Wada, H. Nankai, B. Mikami and K. Murata: Proteomics-based identification of outer-membrane proteins responsible for import of macromolecules in *Sphingomonas* sp. A1: alginate-binding flagellin on the cell surface. Biochemistry, 44(42); 13783-13794, 2005

Mori, S., M. Yamasaki, Y. Maruyama, M. Momma, S. Kawai, W. Hashimoto, B. Mikami and K. Murata: NAD-binding mode and the significance of intersubunit contact revealed by the crystal structure of *Mycobacterium tuberculosis* NAD kinase-NAD complex. Biochem. Biophys. Res. Commun., 327(2); 500-508, 2005

Kawai, S., C. Fukuda T. Mukai and K. Murata: MJ0917 in archaeon *Methanococcus jannaschii* is a novel NADP phosphatase/NAD kinase. J. Biol. Chem., 280 (47); 39200-39207, 2005

Shi, F., S. Kawai, S. Mori, E. Kono and K. Murata: Identification of ATP-NADH kinase isozymes and their contribution to supply of NADP(H) in *Saccharomyces cerevisiae*. FEBS J., 272

(13); 3337-3349, 2005

Mori, S., S. Kawai, F. Shi, B. Mikami and K. Murata: Molecular conversion of NAD kinase to NADH kinase through single amino acid residue substitution. J. Biol. Chem., 280 (25); 24104-24112, 2005

Reviews

Kawai, S., T. Mukai, S. Mori, B. Mikami and K. Murata: Hypothesis: structures, evolution, and ancestor of glucose kinases in the hexokinase family. *J. Biosci. Bioeng.*, 99 (4); 320-330, 2005

Hashimoto, W., K. Momma, Y. Maruyama, M. Yamasaki, B. Mikami, and K. Murata: Structure and function of bacterial super-biosystem responsible for import and depolymerization of macromolecules. *Biosci. Biotechnol. Biochem.,* 69(4); 673-692, 2005

Reports

Ochiai, A., M. Yamasaki, W. Hashimoto, B. Mikami and K. Murata: Preliminary X-ray study of an alginate lyase Atu3025 from *Agrobacterium tumefaciens* C58, a member of polysaccharide lyase family 15. *SPring-8 User Experiment Report,* 15 (2005A); 298, 2005

Maruyama, Y., B. Mikami, W. Hashimoto and K. Murata: Polysaccharide lyase: crystal structure of *Bacillus* sp. GL1 xanthan lyase complexed with pyruvate mannose. *SPring-8 User Experiment Report,* 14 (2004B); 295, 2005

Maruyama, Y., M. Yamasaki, T. Itoh, A. Ochiai, K. Ogura, B. Mikami, W. Hashimoto and K. Murata: Polysaccharide lyase: crystal structure of *Bacillus* sp. GL1 xanthan lyase complexed with substrate. *SPring-8 User Experiment Report,* 14 (2004B); 173, 2005

b) Conference and seminar papers presented

The Annual Meeting (2005) of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 6 cases

The Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch): 6 cases

The Regular Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch): 2 cases

The Annual Meeting (2005) of The Society for Biotechnology, Japan: 9 cases

The Annual Meeting (2005) of The Japanese Biochemical Society: 2 cases

The Annual Meeting (2005) of The Protein Gakkai, Japan: 1 case

Meeting of C-P Compound Meeting: 1 case

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" (Japanese), Member of Science Consolidating Committee); The Society for Biotechnology, Japan (Director, Editor-in-Chief of "Journal of Bioscience and Bioengineering"); The Society for Biochemistry, Japan (Councilor); The Japan Society for Nutrition and Food (Director), 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 2005, Architectural principles of bacterial cell surface structure and

evolution of flagella through analysis of flagellin molecule; Monbukagakusho Research Grant-in-Aid for Exploratory Research, Principal Investigator, Entry 2004, Molecular mechanism of transformation (DNA transportation) in yeast; Nourinsuisannsho (Bio-oriented Technology Research Advancement Institution) Research Grant-in-Aid, Principal Investigator, Entry 2001, Structural biology of bacterial "Super-channel" and creation of "Super-bacteria" for the protection and regeneration of environment (bioremediation)

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 binding and degrading 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 Hokuto Institute for Biological Scientific Research; Entry in 2005, Establishment of "highly efficient" transformation method of *Saccharomyces cerevisiae* based on cAMP control. Amano Enzyme Inc. Practical Symposium; Entry in 2005, Tertiary structural analysis enabling to applying polyphosphate to enzymatic productions of phosphorylated compounds.

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)

Overseas activities

Hashimoto, Wataru: Sabbatical fellowship from the Ministry of Education, Culture, Sports, Science and Technology of Japan

B. Educational Activities (2005.4-2006.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Food Microbiology (Murata); Basic and Applied Molecular Biotechnology (Murata, Hashimoto); Laboratory Course in Microbiology (Hashimoto, allotment)

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)