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, 70 first year students are working towards master's degree, and 25 students toward Ph.D. including 6 international students. In undergraduate program, 37 freshmen, 33 sophomores, 34 juniors, and 39 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 : (2)Research fellow : (1)Master's program : (8)Research student : (2)Undergraduate : (4)

A. Research Activities (2007.4-2008.3)

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 enzyme activity has been suggested. Site-directed mutagenesis and chemical modification have been applied to reveal the roles of tyrosyl, tryptophyl, lysyl, aspartic, and glutamiac residues in the halophilicity of thermolysin.

(ii) Enzymatic properties of MMP-7. The metalloproteinase MMP-7, which contains a 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 their inhibitors.

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

e) Studies on application of monoclonal antibodies.

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

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. We are studying the aggregation process of soyproteins induced by various proteinases with physicochemical methods. On the other hand, whey protein especially gluten is also an important food protein but it has unique amino acid composition so that its proteolytic digestion in vitoro and in vivo proceeds hardly. Presently, we are in process to develop an effective method for whey protein degradation to amino acids.

g) Studies on reverse transcriptase

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

A-2. Publications and presentations

a) Publications

Books

- Inouye, K.: Recent development of chromatographic technology and its application to therapy and diagnosis (S26 Biotechnology). 20th IUBMB International Congress of Biochemistry and Molecular Biology Reports (Eds., Congre Co.; Taniguchi, N. and Suzuki, K.) p. 65, 2007
- Inouye, K. and Kamo, M.: Mechanism of the substrate recognition for thermolysin. Structutal Biology - Introduction and Background (Ed. by Kawano, K. and Tanokura, M.), Kyoritsu, 91-98.

Original papers

- Sawada, N., Yamamoto, K., Yamada, S., Ikushiro, S., Kamakura, M., Ohta, M., Inouye, K., and Sakaki, T.: Role of Gln 85 of human CYP27A1 in 25-hydroxyvitamin D3-binding and protein folding. Biochem. Biophys. Res. Commun. 355; 211-216, 2007
- Tatsumi, C., Hashida, Y., Yasukawa, K., and Inouye, K.: Effects of site-directed mutagenesis of the surface residues Gln128 and Gln225 of thermolysin on its catalytic activity. J. Biochem., 141; 835-842, 2007
- Hashida, Y. and Inouye, K.: Kinetic analysis of the activation-and-inhibition dual effects of cobalt ion on thermolysin activity. J. Biochem., 141; 843-853, 2007
- Hashida, Y. and Inouye, K.: Molecular mechanism of the inhibitory effect of cobalt ion on thermolysin activity and the suppressive effect of calcium ion on the cobalt ion-dependent inactivation of thermolysin. J. Biochem., 141; 879-888, 2007
- Inouye, K., Shimada, T., and Yasukawa, K.: Purification to homogeneity of a neutral metalloproteinase from *Streptomyces caespitousus*. Biosci. Biotechnol. Biochem., 71; 1773-1776, 2007
- Inouye, K., Nakamura, K., Kusano, M., and Yasukawa, K.: Improvement in performances of affinity gels containing Gly-D-Phe as a ligand to thermolysin by increasing the spacer chain length. Biosci. Biotechnol. Biochem., 71; 2083-2086, 2007
- Yasukawa, K., Kusano, M., and Inouye, K.: A new method for the extracellular production of recombinant thermolysin by co-expression of the mature sequence and the prosequence in *Escherichia coli*. Protein Eng. Des. Sel., 20; 375-383, 2007
- Inouye, K., Shimada, T., and Yasukawa, K.: Effects of neutral salts and alcohols on the activity of *Streptomyces caespitousus* neutral protease (ScNP). J. Biochem., 142; 317-324, 2007
- Yasukawa, K. and Inouye, K.: Improving the activity and stability of thermolysin by site-directed mutagenesis. Biochim. Biophys. Acta, 1774; 1281-1288, 2007
- Yasukawa, K., Nemoto, D., and Inouye, K.: Compariosn of the thermal stabilities of reverse transcriptases from avian myeloblastosis virus and moloney murine leukemia virus. J. Biochem., 143; 261-268, 2008
- Kojima, K., Tsuzuki, S., Fushiki, T., and Inouye, K.: Roles of functional and structural domains of hepatocyte growth factor inhibitor type 1 in the inhibition of matriptase. J. Biol. Chem., 283; 2478-2487, 2008
- Takita, T., Aono, T., Sakurama, H., Itoh, T., Wada, T., Minoda, M., Yasukawa, K., and Inouye, K.: Effects of introducing negative charges into the molecular surface of thermolysin by site-directed mutagenesis on its activity and stability. Biochim. Biophys. Acta, 1784; 481-488, 2008

Ueda, M., Asano, T., Nakazawa, M., Miyatake, and K., Inouye, K.: Purification and characterization of novel raw-starch-digesting and cold-adapted alpha-amylases from *Eisenia foetida*. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 150, 125-130, 2008

Reviews and others

- Inouye, K., Kusano, M., Hashida, Y., Minoda, M., and Yasukawa, K.: Engineering, expression, purification, and production of recombinant thermolysin. Biotehnol. Annu. Rev. 13; 43-65, 2007
- Inouye, K., Okumura, S., and Mizuki, E.: Parasporin-4, a novel cancer cell-killing protein produced by *Bacillus thuringiensis* (Minireview). Food Sci. Biotechnol. 17; 219-227, 2008
- b) Conference and seminar papers presented
- Annual Meeting of the Japan Society (2007) for Bioscience, Biotechnology, and Agrochemistry: 11 papers
- Annual Meeting of Kansai Branch Chubu Branch of the Japan Society for Bioscience, Biotechnology, and Agrochemistry: 6 papers
- 54th Annual Meeting of Kinki Branch of the Japanese Biochemical Society: 5 papers

59th Annual Meeting of the Japanese Society for Biotechnology: 1 paper

7th Annual Meeting of the Food Enzyme Chemistry Forum: 1 paper

30th Annual Meeting of the Molecular Biology Society of Japan and 80th Annual Meeting of Japanese Biochemical (BMB 2007): 3 papers

Enzyme Chemistry Mini-symposium: 1 paper

60th Annual Meeting of the Japanese Society of Nutrition and Food Science: 1 paper

Annual Meeting of the Japanese Association of Food Analysis: 1 paper

9th Soyprotein Research Conference of the Fuji Foundation: 1 paper

38th Open Seminar of the Research Institute of Humanosphere: 1 paper

5th Symposium of the Graduate School of Agriculture, Kyoto University: 1paper

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, 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), The Society of Proteases and Inhibitors in Pathophysiology and Therapeutics (Councilor).

Research grants

- JSPS 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, Research (C): "Establishment of the expression system of recombinant AMV reverse transcriptase and improvement of its productivity, activity, and thermal stability" (Yasukawa, representative)

A-4. International cooperations and overseas activities

International meetings (roles)

- Inouye, K.: Korea-Japan Symposium on Food Enzyme Chemistry, Seoul National University, Seoul, Korea (Feb., 2007) (Symposium speaker)
- Inouye, K.: International Symposium on the Clinical Aspects of Matrix Metalloproteinases, University of Leiden (April, 2007) (Speaker)
- Inouye, K.: UK-Japan Mini-symposium on the Recent Progress of Enzyme Inhibition Mechanism, University of Warwick (June, 2007) (Speaker)
- Inouye, K.: 5th General Meeting of the International Proteolysis Society, University of Patras, Greece (Oct., 2007) (Speaker)
- Inouye, K.: 3rd International Conference on Polyphenols and Health, Kyoto (Nov., 2007) (Speaker)
- Inouye, K.: International Symposium on Frontier in Plant Proteome Research, Tsukuba (March, 2008) (Speaker)

International joint researches, overseas research surveys

Inouye, K.: Japan-Korea Core Universities Joint Research on the Energy Science between Kyoto University and Seoul National University (Korea); Proteases on bacterial infection, diagnosis, and therapy (University of Michigan, USA), Characterization of soy and wheat proteins (University of Wageningen, the Netherlands); Role of MMPs in digestive diseases (University of Leiden and University of Groningen, the Netherlands); Reaction mechanism of MMPs (Imperial College, London, UK); Biotechnology of enzymes and antibodies (University of Tromso, Norway); Research on structure-function relationship of thermophilic enzyme (Seoul National University, Yonsei University and Gwanju Institute of Technology, Korea); Enzyme reaction mechanism (Warwick, UK); Research on reaction mechanism of amylase and proteinase and their application to food science and technology (Seoul, Korea); Application of amylases and proteinases (University of Guelph, Canada); Collaborative study on the stabilization of proteins (Graz Technical University, BOKU, and Austrian Research Center for Science and Technology, Austria)

Editorial work for international journals (roles)

Inouye, K.: Biotechnology Annual Review (Elsevier) Vols. 13-15 (Editor)

Inouye, K.: Applied Microbiology and Biotechnology (Springer) (Editor)

Inouye, K.: Enzyme and Microbial Technology (Elsevier) (Editor)

Inouye, K.: Food Science and Biotechnology (Editor)

B. Educational Activities (2007.4-2008.3)

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), Laboratory Course in Enzyme Chemistry and Biochemistry (Yasukawa, Takita), Pocket Seminar on Function of Enzymes on Food and Nutrition (Inouye, Yasukawa).
- Graduate level: Advanced Course of Enzyme Chemistry (Inouye, Yasukawa), Enzyme Chemistry Seminar (Inouye, Yasukawa, Takita), Experimental Course of Enzyme Chemistry (Inouye, Yasukawa, Takita).

B-2. Off-campus teaching, etc.

Part-time lecturer

Inouye, K.: Nishi-Kyushu College Graduate School (Enzyme Chemistry)

- Inouye, K.: Iwate University Graduate School (Life Technology)
- Inouye, K.: Tokushima University Graduate School (Enzyme Chemistry)
- Inouye, K.: Toyo University Graduate School
- Inouye, K.: Ritsumeikan University Graduate School

B-3. Overseas teaching

Lecture

Inouye, K.: Warwick University (Warwick, UK) (Enzyme Chemistry)

Inouye, K.: Seoul National University Graduate School of Life Science and Agriculture (Seoul, Korea) (Enzyme Chemistry)

C. Other remarks

Committees

- Inouye, K.: Member of the Committee for Planning of Open-seminar in Kyoto University; Member of the Committee for the Building of the Graduate School of Agriculture; Member of the Committee for Undergraduate Education of the Graduate School of Agriculture; Member of Judging Committee for Industrialization by Minor Enterprises; Member of the Judging Committee for Member of the Ministry of Industry and Trades, Kyoto Municipal Bio-industrial Business Promotion Forum, Cooperative-researcher of the Research Institute of Humanosphere, Kyoto University, Member of the Committee of Protein 3000 Project, Member of the Institute of Science and Technology Policy of the Ministry of Education, Member of the Science Committee of the Iijima Foundation, Visiting Researcher of the Nippon University Biotechnology Research Center
- Yasukawa, K.: Member of the Committee for the Edition of Annual Report of the Graduate School of Agriculture; Member of the Committee for the public relations 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 : (3) Master's program : (11) Undergraduate : (3) Research fellow : (0)

A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Functional properties of food proteins: The major component of milk whey protein, 8-lactoglobulin, was mixed and heated together with the major components of egg white, ovalbumin and lysozyme. Molecular complex was observed by heating at neutral and acidic pH. Binding mechanism and molecular interaction have been analyzed. This hetero-complex of these protein would develop new type of food materials

b) Astringent taste at acidic pH induced by milk whey protein and its reduction by novel technique: Milk whey protein shows an astringent taste at acidic pH. In this study the characteristics of this astringency induced by protein were analyzed.

c) Studies on the taste-eliciting activity of proteins: Protein has usually no taste and flavor. However, we found some kinds of protein elicit sweet and/or astringent taste. The mechanism of taste-eliciting activity by proteins have been studied. Egg white lysozyme is one of sweet-tasting proteins. Its taste-eliciting mechanism has been analyzed using chemical modification and site-directed mutagenesis method.

d) Digestion and physiological properties of food polysaccharides: Degradation and digestibility of starch have been analyzed in vitro and in vivo experiments using mice and humans.

e) Studies on the response of innate immunity to changing environments and its regulation by foodstuff:

B cells, dendritic cells (DCs), and macrophages are the essential sentinels to sense danger signals by invaders in the gastrointestinal mucosal immunity. In order to elucidate the physiological functions of gut-associated lymphoid tissues (GALT), we are studying the immunological role on those sentinels of heat shock protein 70 (hsp70) that is the major intracellular component in all kinds of living cells and functions as an immunoregulatory molecule. Using hsp70 derived from four different organisms, we found that the mechanism for recognition of hsp70 differently works among various types of antigen-presenting cells (APCs), depending on the sequence diversity at the C-terminal region of hsp70. Also, the components of foodstuff were found to contribute the organization of a variety of APCs in GALT.

A-2. Publications and presentations

a) Publications

Original papers

- 1) Ohta K, Masuda T, Ide N, Kitabatake N. Critical molecular regions for elicitation of the sweetness of the sweet-tasting protein, thaumatin I. FEBS J.2008.
- 2) Ide N, Masuda T, Kitabatake N. Effects of pre- and pro-sequence of thaumatin on the secretion by Pichia pastoris. Biochem Biophys Res Commun. 2007 Nov 23; 363(3):708-14.
- 3) Ide N, Kaneko R, Wada R, Mehta A, Tamaki S, Tsuruta T, Fujita Y, Masuda T, Kitabatake N. Cloning of the thaumatin I cDNA and characterization of recombinant thaumatin I secreted by Pichia pastoris. Biotechnol Prog. 2007;23(5):1023-30.
- 4) Ohno M, Kitabatake N, Tani F. Functional region of mouse heat shock protein 72 for its binding to lymphoid neoplastic P388D1 cells. Mol Immunol. 2007, 44(9):2344-2354.
- 5) Tao Y, Nomura M, Kitabatake N, Tani F. Mouse CD40-transfected cell lines cannot exhibit the binding and RANTES-stimulating activity of exogenous heat shock protein 70. Mol Immunol. 2007; 44(6):1262-1273.
- b) Conference and seminar papers presented
- The meeting of Chubu-Kansai Branches in the Japan Society for Bioscience, Biotechnology and Agrochemistry 2007: 5 papers
- The annual meeting of the Japan Society for Bioscience, Biotechnology and Agrochemistry 2008: 3 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)

A-4. International cooperations and overseas activities

- a) International meeting
- $98^{\rm th}\,\rm AOCS\,Annual$ Meeting and Expo, Quebec (2007): 2 papers

B. Educational Activities (2007.4-2008.3)

B-1. On-campus teaching

a) Courses given

- Undergraduate level: Introduction to the Molecular Cell Biology II (Tani), Introduction and Practice in the Department of Food Science and Biotechnology I (Kitabatake), Food Safety I (Kitabatake, Tani), Food and Environmental Studies (Kitabatake), Basic Laboratory Course in Food Science and Biotechnology (Tani), 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,

B-2. Off-campus teaching, etc. *Part-time lecturer* Shiga Prefetural University Nakasaki University Kyoto Women's University

2.7.3 Laboratory of Organic Chemistry in Life Science

StaffProfessor: Irie, Kazuhiro, Dr. Agric. Sci.Assistant professor : Murakami, Akira, Dr. Agric. Sci.Technician: Yamaguchi, KanokoPostdoctoral fellow : Nakagawa, Yu, Dr. Agric. Sci.Students and research fellowsDoctor's Program : (5)Master's Program : (9)Undergraduate: (3)Research student : (1)Collaborative Laboratory (comparative agricultural science):Professor : Hirai, Nobuhiro, Dr. Agr. Sci.

A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Toward the development of new anti-cancer agents like bryostatins

Bryostatins, macrocyclic lactones isolated from marine bryozoa, are promising compounds for the treatement of various types of cancer. Their cellular targets are protein kinase C (PKC) isozymes, but the activation mechanism is quite different from that of tumor promoters like phorbol etsers. Since bryostatins are isolated in extremely low yield from natural sourses, and since their total synthesis requires over 70 steps, their surrogate compounds are urgently needed to clarify the molecular mechanism of action. We focused on aplysiatoxin, a potent tumor promoter with a similar partial structure of bryostatins, and synthesized its new simplified analogue. The compound named Aplog-1 showed PKC activation pattern in HeLa cells partly similar to that by bryostatins.

b) Chemistry of amyloid β peptides

Aggregation of the 42-residue β -amyloid (A β 42) plays a crucial role in the pathogenesis of Alzheimer's disease (AD). Despite numerous structural studies on A β aggregates, the relationship between tertiary structure and toxicity remains unclear. Since the mutation sites related to cerebral amyloid angiopathy are mainly observed at position 22, we marked the central region of A β 42. Our proline scanning and solid-state NMR studies suggested both aggregates of wild-type A β 42 and E22K-A β 42 exist in two conformational states in the central region: a major conformer with a turn at positions 25 and 26, and a minor conformer with a turn at positions 22 and 23. To identify the toxic conformer, $A\beta 42$ -lactam(22K-23E), whose side chains at positions 22 and 23 were covalently linked, was synthesized as a minor conformer surrogate, along with $A\beta 42$ -lactam(25K-26E) as a major conformer surrogate. $A\beta 42$ -lactam(22K-23E) showed stronger aggregation, neurotoxicity, radical-generation, and oligomerization than wild-type $A\beta 42$, while these properties of $A\beta 42$ -lactam(25K-26E) were significantly weaker. These data imply that the transition from the physiological conformation with a turn at positions 25 and 26 to the toxic conformation with a turn at positions 22 and 23 might be a key event in the pathogenesis of AD. c) Studies on the regulation of life style-related disease

IL-1beta has been shown to play a pivotal role in the development of inflammatory disorders. In this study, we examined the effects of UA on the production of several cytokines in resident murine peritoneal macrophages (pMphi). UA increased the protein release of IL-1beta, IL-6, and MIF, but not of TNF-alpha. This triterpene also strikingly induced the activation of p38 MAPK and ERK1/2 together with that of upstream kinases. The release of UA-induced IL-1beta was significantly inhibited by the inhibitors of p38 MAPK, MEK1/2, ABC transporter, and caspase-1. Furthermore, UA induced intracellular ROS generation for IL-1beta production. Pretreatment with an anti-CD36 Ab significantly suppressed IL-1beta release, and surface plasmon resonance assay results showed that UA bound to CD36 on macrophages. In addition, the amount of IL-1beta released from UA-treated pMphi of CD36-deficient mice was markedly lower than that from those of wild-type mice. Taken together, our results indicate that aggregated UA is recognized, in part, by CD36 on macrophages for generating ROS, thereby activating p38 MAPK, ERK1/2, and caspase-1, as well as releasing IL-1beta protein via the ABC transporter.

d) Chemical ecology of plants

Some ectomicorrhiza form a fairy ring which shows the cyclic formation of fruit body. The inside of the fairy ring of *Tricholoma* is a whitish mycelium-soil aggregated zone, and called "shiro". The density of bacteria and fungi in the shiro is significantly lower than that of the outside of the shiro. The researchers of our University demonstrated in 1967 that the shiro had antimicrobial activity against bacteria. However, the antimicrobial compound(s) has been remained unclear. We have isolated an antimicrobial compound from the extract of *P pinidensiflorae* roots forming ectomicorrhiza, and identified it as a diterpene, totarol. Totarol was not contained in the leaves and barks, suggesting that totarol is specifically formed in he ectomicorrhiza to protects it from bacteria.

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, the effect of ABA does not last since ABA is quickly inactivated by the degradation enzyme, ABA 8'-hydroxylase. The inhibitor of the hydroxylase may delay the inactivation of ABA to increase resistance of plants to environmental stress. We have developed a new hydroxylase inhibitor AHI 1 based on the substrate specificity of the hydroxylase with the researchers at Shizuoka University. A test of AHI 1 for water stress tolerence of apple trees was carried out by the researchers at Chiba University, and AHI 1 gave a water stress tolerance to apple trees as well as the known inhibitor uniconazole-P. The endogenous level of ABA was higher than that of control trees. Development of the hydroxylase inhibitors with high specificity are under progress.

A-2. Publications and presentations

a) Publications

Books

- Tsushida, T., Umegaki, K., Inoue, K. and Murakami, A. (Eds): Guidebook for the Safety of Functional Foods., Science Forum, Tokyo, 2007 (in Japanese)
- Murakami, A. and Morimitsu Y.: Health Guidebook for Freshmen, Maruzen, Tokyo, 2007 (in Japanese)
- Murakami, A. and Ohigashi, H.: Polysaccharides. In: Guidebook for the Safety of Functional Foods., Tsushida, T., Umegaki, K., Inoue, K. and Murakami, A. (Eds), pp.351-354, Science Forum, Tokyo, 2007 (in Japanese)
- Murakami, A. and Ohigashi, H.: Diacylglycerols. In: Guidebook for the Safety of Functional Foods., Tsushida, T., Umegaki, K., Inoue, K. and Murakami, A. (Eds), pp.369-372, Science Forum, Tokyo, 2007 (in Japanese)
- Murakami, A. and Ohigashi, H.: Polyphenols Including Catechins. In: Guidebook for the Safety of Functional Foods., Tsushida, T., Umegaki, K., Inoue, K. and Murakami, A. (Eds), pp.380-383, Science Forum, Tokyo, 2007 (in Japanese)
- Murakami, A. and Ohigashi, H.: A Mutagenic Assay by using Animal Cell Cultures. In: Guidebook for the Safety of Functional Foods., Tsushida, T., Umegaki, K., Inoue, K. and Murakami, A. (Eds), pp.476-478, Science Forum, Tokyo, 2007 (in Japanese)
- Murakami, A. and Ohigashi, H.: Pharmacologically Active Compounds. In: Dictionary for Functional Foods., Arai, S., Abe, K., Yoshikawa, T., Kanazawa, K., Watanabe, S. (Eds.), pp.244-253, Asakura Shoten, Tokyo, 2007 (in Japanese)
- Ikeda, Y., Murakami, A. and Ohigashi, H.: Terpenes. In: Dictionary for Functional Foods., Arai, S., Abe, K., Yoshikawa, T., Kanazawa, K., Watanabe, S. (Eds.), pp.118-127, Asakura Shoten, Tokyo, 2007 (in Japanese)
- Miyamaoto, S., Yasui, Y., Murakami, A. and Tanaka, T.: Molecular mechanism of colorectal cancer. In. Cancer: Disease Progression and Chemoprevention, Takuji Tanaka (Ed.), pp. 45-56, Research Singpost, Kerala, India, 2007
- Murakami, A. and Ohigashi, H.: Cancer preventive agents in zingiberaceous plants from Southeast Asian countries. In. Cancer: Disease Progression and Chemoprevention, Takuji Tanaka (Ed.), pp.217-238, Research Singpost, Kerala, India, 2007
- Nishikawa, A., Nakamura, H., Lee, I-S., Furukawa, F., Murakami, A., Ohigashi, H., Umemura, T. and Hirose, M.: Chemoprevention effects of 1'-acetoxychavicol acetate and auraptene on stomach carcinogenesis in rats initiated with N-methyl-N'-nitro-N-nitrosogaunidine. In. Cancer: Disease Progression and Chemoprevention, Takuji Tanaka (Ed.), pp. 267-276, Research Singpost, Kerala, India, 2007

Original papers

- Yanagita, R. C., Nakagawa, Y., Yamanaka, N., Kashiwagi, K., Saito, N. and Irie, K.: Synthesis, conformational analysis, and biological evaluation of 1-hexylindolactam-V10 as a selective activator for novel protein kinase C isozymes. J. Med. Chem., 51(1); 46-56, 2008
- Murakami, K., Uno, M., Masuda, Y., Shimizu, T., Shirasawa, T. and Irie, K.: Isomerization and/or racemization at Asp-23 of Aβ42 do not increase its aggregative ability and neurotoxicity in vitro. Biochem. Biophys. Res. Commun., 366(3); 745-751, 2008

- Sugimoto, T., Itagaki, K. and Irie, K.: Design and physicochemical properties of new fluorescent ligands of protein kinase C isozymes focused on CH/π interaction. Bioorg. Med. Chem., 16(2); 650-657, 2008
- Yanagita, R. C., Torii, K., Nakagawa, Y. and Irie, K.: Binding selectivity of 1- or 12-substituted indolactam derivatives for protein kinase C isozymes. Heterocycles, 73; 289-302, 2007
- Murakami, K., Hara, H., Masuda, Y., Ohigashi, H. and Irie, K.: Distance measurement between Tyr-10 and Met-35 in amyloid β by site-directed spin labeling ESR: implications for the stronger neurotoxicity of Aβ42 than Aβ40. ChemBioChem, 8(18); 2308-2314, 2007
- Miyamoto, S., Yasui, Y., Kim, M., Sugie, S., Murakami, A., Ishigamori-Suzuki, R. and Tanaka, T.: A novel rasH2 mouse carcinogenesis model that is highly susceptible to 4-NQO-induced tongue and esophageal carcinogenesis is useful for preclinical chemoprevention studies. Carcinogenesis, 29(2); 418-426, 2008
- Sandur, S.K., Pandey, M.K., Sung, B., Ahn, K.S., Murakami, A., Sethi, G., Limtrakul, P., Badmaev,
 V. and Aggarwal, B.B.: Curcumin, demethoxycurcumin, bisdemethoxycurcumin,
 tetrahydrocurcumin and turmerones differentially regulate anti-inflammatory and
 anti-proliferative responses through a ROS-independent mechanism. Carcinogenesis,
 28(8); 1765-1773, 2007
- Murakami, A., Song, M. and Ohigashi, H.: Phenethyl isothiocyanate suppresses receptor activator of NF-kappaB ligand (RANKL)-induced osteoclastogenesis by blocking activation of ERK1/2 and p38 MAPK in RAW264.7 macrophages. Biofactors, 30(1); 1-11, 2007
- Kim, M., Murakami, A. and Ohigashi, H. Modifying effects of dietary factors on (-)-epigallocatechin-3-gallate-induced pro-matrix metalloproteinase-7 production in HT-29 human colorectal cancer cells. Biosci. Biotechnol. Biochem., 71(10); 2442-2450, 2007
- Kwon, K.H., Ohigashi, H. and Murakami, A.: Dextran sulfate sodium enhances interleukin-1 beta release via activation of p38 MAPK and ERK1/2 pathways in murine peritoneal macrophages. Life Sci., 81(5); 362-371, 2007
- Eguchi, A., Kaneko, Y., Murakami, A. and Ohigashi, H.: Zerumbone suppresses phorbol ester-induced expression of multiple scavenger receptor genes in THP-1 human monocytic cells. Biosci. Biotechnol. Biochem., 71(4); 935-945, 2007
- Ikeda, Y., Murakami, A., Fujimura, Y., Tachibana, H., Yamada, K., Masuda, D., Hirano, K., Yamashita, S. and Ohigashi, H.: Aggregated ursolic acid, a natural triterpenoid, induces IL-1beta release from murine peritoneal macrophages: role of CD36. J. Immunol., 178(8); 4854-4864, 2007
- Unahara, Y., Kojima-Yuasa, A., Higashida, M., Kennedy, D.O., Murakami, A., Ohigashi, H. and Matsui-Yuasa, I.: Cellular thiol status-dependent inhibition of tumor cell growth via modulation of p27(kip1) translocation and retinoblastoma protein phosphorylation by 1'-acetoxychavicol acetate. Amino Acids, 33(3); 469-476, 2007
- Kamo, T., Endo, M., Sato, M., Kasahara, R., Yamaya, H., Hiradate, S., Fujii, Y., Hirai, N., and Hirota. M.: Limited distribution of natural cyanamide in higher plants: occurrence in *Vicia villosa* subsp. varia, V. cracca, and Robinia pseudo-acacia. Phytochemistry, 69; 1166-1172, 2007
- Hattori, Y., Horikawa, K., Makabe, H., Hirai, N., Hirota, M., and Kamo, T.: A refined method for determining the absolute configuration of the 3-hydroxy-3-methylglutaryl group.

Tetrahedron Asym., 18; 1183-1186, 2007

Shimomura, H., Etoh, H., Mizutani, M., Hirai, N. and Todoroki, Y.: Effect of the minor ABA metabolite 7'-hydroxy-ABA on Arabidopsis ABA 8'-hydroxylase CYP707A3. Bioorg. Med. Chem. Lett., 17; 4977-81, 2007

Patents

Patent pending

- No. 2007-193062 'Scavenger receptor suppressants and medicines and foods including them', Ohigashi, H., Murakami, A. and Eguchi, A., 07/25/07, The Support Center for Small and Medium-sized Enterprises of Nara Prefecture
- No. 2007-224294 'Ulcerative colitis suppressants and medicines and foods including them', Murakami, A., Irie, K., Ohkubo, T. and Washida, K., 08/30/07, The Support Center for Small and Medium-sized Enterprises of Nara Prefecture

Reviews

- Irie, K., Murakami, K., Masuda, Y., Morimoto, A., Ohigashi, H., Hara, H., Ohashi, R., Takegoshi, K., Fukuda, H., Nagao, M., Shimizu, T. and Shirasawa, T.: Malignant conformation of the 42-mer amyloid β peptide. Mini-Rev. Med. Chem., 7(10); 1001–1008, 2007
- Ikeda, Y., Murakami, A. and Ohigashi, H.: Ursolic acid: an anti- and pro-inflammatory triterpenoid. Mol. Nutr. Food Res., 52(1); 26-42, 2008
- Murakami, A. and Ohigashi, H.: Targeting NOX, INOS and COX-2 in inflammatory cells: chemoprevention using food phytochemicals. Int. J. Cancer., 121(11); 2357-2363, 2007
- Murakami, A.: Who is to blame? FOOD Style 21, 11(4); 93-95, 2007 (in Japanese)
- Murakami, A.: A food factor quintet: Part 1. FOOD Style 21, 11(5); 84-86, 2007 (in Japanese)
- Murakami, A.: A food factor quintet: Part 2. FOOD Style 21, 11(6); 84-86, 2007 (in Japanese)
- Murakami, A.: A food factor quintet: Part 3. FOOD Style 21, 11(7); 90-92, 2007 (in Japanese)
- Murakami, A.: A food factor quintet: Part 4. FOOD Style 21, 11(8); 85-87, 2007 (in Japanese)
- Murakami, A.: A plant hormone latent in human. FOOD Style 21, 11(9); 81-83, 2007 (in Japanese)
- Murakami, A.: 'Cancer Prevention II' in ICoFF. FOOD Style 21, 11(10); 106-108, 2007 (in Japanese)
- Murakami, A.: The Front Line of Cancer Prevention with Food Factors. FOOD Style 21, 11(10); 56-58, 2007 (in Japanese)
- Murakami, A. : Miscellaneous thoughts on health foods. FOOD Style 21, 11(11); 92-94, 2007 (in Japanese)
- Murakami, A.:Miscellaneous thoughts on basic experiments. FOOD Style 21, 11(12); 103-105, 2007 (in Japanese)
- Murakami, A.: The know-how of research presentation. FOOD Style 21, 11(1); 78-80, 2007 (in Japanese)
- Murakami, A. : The ABC's of international conference. FOOD Style 21, 11(2); 83-85, 2007 (in Japanese)

Murakami, A. : What COMT tells us. FOOD Style 21, 11(3); 96-98, 2007 (in Japanese)

Reports

Irie, K.: Development of antibodies and aggregation inhibitors for amyloid β peptides based on the new aggregation model. Report (2006) for Monbu-kagakusho Research Grant [Scientific Research (A)]

- Irie, K.: Molecular design of agents with selectivity for protein kinase C isozymes based on the CH/π interaction. Report (2006) for Monbu-kagakusho Research Grant [Priority Areas, Creation of Biologically Functional Molecules]
- Murakami, A.: Molecular mechanisms underlying inflammation-associated carcinogenesis and its prevention. Report (2007) for funds from the Ministry of Health, Labor, and Welfare
- b) Conference and seminar papers presented
- The 2008 Annual Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry (Nagoya): 13 general papers and 1 invited presentation
- The 42th Summer School on Natural Products Chemistry (Sendai): 1 general paper
- The 112th Meeting of the Japanese Pharmacological Society in Kinki branch (Osaka): 1 general paper
- The 49th Symposium on the Chemistry of Natural Products (Sapporo): 1 general paper
- The 30th Annual Meeting of the Molecular Biology Society of Japan, the 80th Annual Meeting of the Japanese Biochemical Society (Yokohama): 1 general paper
- The 46th Annual Meeting of the NMR Society of Japan (Sapporo): 1 general paper
- The 2nd Meeting of the Society for Shampoo Ginger Research (Osaka): 1 invited presentation
- The 14th Conference of Japanese Society for Cancer Prevention (Tokyo): 1 general paper
- The 66th Annual Meeting of the Japanese Cancer Association (Yokohama): 1 general paper
- The 54th Annual Meeting of the Japanese Society for Food Science and Technology (Fukuoka): 1 invited presentation
- The Joint Meeting of Chugoku, Shikoku, and Kinki branches of the Japanese Society for Nutrition and Food Science (Hiroshima): 1 invited presentation
- The 2008 Meeting of Chemistry and Biology (Kyoto): 1 general paper
- The 42th Annual Meeting of Japanese Society for Chemical Regulation of Plants (Shizuoka): 2 general papers

A-3. Off-campus activities

Membership in academic societies (roles)

- Irie, K.: The Japan Society for Bioscience, Biotechnology, and Agrochemistry in Kansai Branch (councilor), Organizing committee of Symposium on the Chemistry of Natural Products (member), Organizing committee of Summer School on Natural Products Chemistry (member), The Japanese Association for the Pursuit of New Bioactive Resources (councilor)
- Murakami, A.: Japanese Society for Food Factors (director), Japanese Society for Oxidative Stress Research (councilor), The Society for Antioxidant Unit (councilor), Food Science Forum (councilor)

Research grants

Monbu-Kagakusho Research Grant

- Scientific Research (A): Development of antibodies and aggregation inhibitors for amyloid β peptides based on the new aggregation model (Irie, K., Head)
- Scientific Research in Priority Areas: Molecular design of agents with selectivity for protein kinase C isozymes based on the CH/π interaction (Irie, K., Head)
- Grant-in-Aid from Japan Science and Technology Agency
- The Prefecture Collaboration of Regional Entities for the Advancement of Technological

Excellence, Evaluation of anti-inflammatory activity of Yamatomana and its applications for new cultivars and foods (Murakami, A., Cooperator)

Grant-in-Aid from the Ministry of Health, Labor, and Welfare

Molecular mechanisms underlying inflammation-associated carcinogenesis and its prevention (Murakami, A., Head)

Foundations

The Uehara Memorial Foundation: Design and synthesis of bryostatin surrogates (Irie, K., Head); Asahi Beer Research Foundation: Functions and safety of food factors binding with scavenger receptors (Murakami, A., Head).

A-4. International cooperations and overseas activities

International meetings (roles)

Irie, K.: The 37th Annual Meeting of Society for Neuroscience (Sandiego, USA, 3 general papers)

- Murakami, A.: The 65th Annual Meeting of the American Association for Cancer Research (Los Angels,, 2 general papers)
- Murakami, A.: The 6th International Meeting of Medicinal Chemistry (Istanbul, 1 invited presentation)
- Murakami, A.: The 3rd International Meeting of Polyphenols and Health (Kyoto, 3 general papers, 1 invited presentation, Organizing Committee Member)
- Murakami, A.: The 4th International Meeting of Food Factors (Kyoto, 3 general papers, Organizing Committee Member, Chief of Programme Committee)
- Murakami, A.: The 2nd International Meeting of Translational Research on Cancer Therapy (Mumbai, India, 1 invited presentation)
- Hirai, N.: The 19th International Congress on Plant Growth Substances (Puerto Vallarta, Mexico, 3 general papers)

Editorial work for International journals

Murakami, A.: Journal of Clinical Biochemistry and Nutrition (editor)

Membership in international academic societies

Irie, K.; American Chemical Society (member), The Society for Neuroscience (member)

B. Educational Activities (2007.4-2008.3)

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 (Irie, K.), Organic Chemistry in Life Science (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 (Irie, K., Murakami, A. and Hirai, N., a partial charge), Outline of Agricultural Science (Irie, K., a partial charge)
- Graduate level: Seminar of Organic Chemistry in Life Science (Irie, K., Murakami, A. and Hirai, N.), Experimental Course of Organic Chemistry in Life Science (Irie, K., Murakami, A. and Hirai, N.)

B-2. Off-campus teaching, etc.

Open seminars

- Irie, K.: Tohoku Pharmaceutical University, The 2nd Symposium of High Technology Research Center (presentation)
- Murakami, A.: The Society for Industrial Creation of Kyoto Wellness, Seminar for Health Business Seeds (presentation)
- Murakami, A.: Joint Symposium with The Drug and Food Efficacy Evaluation Center of Osaka City University (presentation)

B-3. Overseas teaching

Students and research fellows from abroad

Research Student: 1 (Thailand)

C. Other remarks

- 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 (derector & councilor), Program Officer of the Task Force for Research Strategy at Kyoto University
- Murakami, A.: Animal Research Committee, Graduate School of Agrticulture, Kyoto University (member), Hygiene Administrator of Graduate School of Agrticulture (member), The Best Paper Award of *Biosci. Biotechnol. Biochem*.

Chair of Food Bioscience

2.7.4 Laboratory of Nutrition Chemistry

Staff	Professor	: Fushiki, Tohru, Dr. Agric. Sc	i.	
	Associate Professor: Inoue, Kazuo, Dr. Agric. Sci.			
	Assistant Professor : Tsuzuki, Satoshi, Dr. Agric. Sci.			
Students and research fellows				
	Doctor's program: (2	?) Master's program	: (11)	
	Undergraduate :(4)		

A. Research Activities (2007.4-2008.3) 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: Consideration of a global environment through a dining table "Acceptability of foods for human" pp1-23, Showado Shuppan (Kyoto), 2007

Original papers

Tetsuro Shibakusa, Wataru Mizunoya, Yuki Okabe, Shigenobu Matsumura,

- Yoko Iwaki, Alato Okuno,2Katsumi Shibata, Kazuo Inoue, and Tohru Fushiki (2007) Transforming growth factor- in the brain is activated by exercise
- and increases mobilization of fat-related energy substrates in rats (2007)*Am.J.Physiol. Regul* Integr Comp Physiol 292: R1851–R1861
- Shigenobu Matsumura,1 Tetsuro Shibakusa,Teppei Fujikawa,Hiroyuki Yamada,1 Kiyoshi
 Matsumura, Kazuo Inoue, and Tohru Fushiki Intracisternal administration of
 transforming growth factor- evokes fever, through the induction of cyclooxygenase-2 in
 brain endothelial cells(2007) Am J Physiol *Regul Integr Comp Physiol* 294: R266–R275
- Fujikawa T, Matsumura S, Yamada H, Inoue K, Fushiki T. Transforming growth factor-beta in the brain enhances fat oxidation via noradrenergic neurons in the ventromedial and paraventricular hypothalamic nucleus. (2007) Brain Res. Oct 10;1173:92-101
- Miyamoto L, Toyoda T, HayashiT, Yonemitsu S, Nakano M,Tanaka S, Ebihara K, Masuzaki H, Hosoda K, Ogawa Y,Inoue G Fushiki T, Nakao K. Effect of Acute Activation of 5'-AMP-Activated Protein Kinase on Glycogen Regulation in Isolated Rat Skeletal Muscle (2007) J Appl Physiol (Mar)102(3) 1007-1013
- Koji Tajino, Kiyoshi Matsumura, Kaori Kosada,1Tetsuro Shibakusa, Kazuo Inoue, Tohru Fushiki, Hiroshi Hosokawa, and Shigeo Kobayashi Application of menthol to the skin of whole trunk in mice induces autonomicand behavioral heat-gain responses (2007) Am J Physiol *Regul Integr Comp Physiol* 293: R2128–R2135
- Kenji Kojima, Satoshi Tsuzuki Tohru Fushiki, and Kuniyo Inouye Roles of Functional and Structural Domains of Hepatocyte Growth Factor Activator Inhibitor Type 1 in the Inhibition of Matriptase (2007) J. Biol. Chem.283(5)2478-2487
- Ikeda H, Yong Q, Kurose T, Todo T, Mizunoya W, Fushiki T, Seino Y, Yamada Y. Clock gene defect disrupts light-dependency of autonomic nerve activity. (2007) Biochem Biophys Res Commun. Dec 21;364(3):457-63
- Yoneda T, Saitou K, Mizushige T, Matsumura S, Manabe Y, Tsuzuki S, Inoue K, Fushiki T. The palatability of corn oil and linoleic acid to mice as measured by short-term two-bottle choice and licking tests (2007)
- Yoneda T, Taka Y, Okamura M, Mizushige T, Matsumura S, Manabe Y, Tsuzuki S, Inoue K, Fushiki T. Reinforcing effect for corn oil stimulus was concentration dependent in an operant task in mice. (2007) Life Sci. 81, 1585-1592

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 (1 papers) Annual meeting of the Japanese Association for the Study of Taste and Smell (4 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: Grant from Bio-Oriented Technology research Program for Promotion of Basic research Activities for Innovative Biosciences (Fushiki, representative).
- Inoue, K: Monbusho Research Grant: Scientific Research (B) "Establishment of the evaluation system for central fatigue and the development of preparative basis for food which aims the control of 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

A-4. International cooperation and overseas activities

Editorial work for international journals

Fushiki, T.: Journal of Nutritional Science and Vitaminology (Chairman), American Journal of Physiology (nominated as a part time reviewer), registered reviewer, for the Elsevier Editorial System

B. Educational Activities (2007.4-2008.3)

B-1. On-campus teaching

- a) Courses given
- Undergraduate level: Nutrition Chemistry (Fushiki), Taste and Preference (Fushiki) Laboratory course in food and nutrition chemistry (Inoue, Tsuzuki), Introduction to foreign literature in food science and biotechnology II (Inoue, Masuda)
- Graduate level: Nutrition Chemistry (Advanced course) (Fushiki), Laboratory Course in Nutrition Chemistry (Fushiki, Inoue)

B-2. Off-campus teaching etc.

Part-time lecture

Fushiki: Faculty of Life Sciences, Kyoto Wemen's University, Graduate School of Medicine, Hokkaido University

Enlightenment lecture

Fushiki, T: Lecture in Annual meeting in Japan Society of Pharmacology "Mechanisms on the preference of food" (Nagoya), Educational lectule in Annual Meeting for Japan Society for Clinical Nutrition "Food preference and human health" (Kyoto), Forum "A New Century for the Global Science, "Structure of human food preference" (Kyoto), Lecture for Japan Perfumery & Flavoring Association "Mechanisms on the preference of food" (Tokyo, Osaka), Lecture for Japan Association of Soysauce "Mechanisms on the preference of food" (Tokyo)

Lecture for high school student in High school-University Cooperation Project "Taste and preference of food" Lecture for dieticians in north part of Kyoto " Strategy of health and foods" (Kyoto), Lecture for dieticians in Wakayama "Japanese health and traditional foods" (Gobo, Wakayama)

Inoue K: "Why do we feel this fatigue? -exercise, nutrition, and mechanism of manifestation of central fatigue-", Symposium of graduate school of agriculture, Kyoto university (Kyoto)
"The role of the brain during exercise -central fatigue and regulation of energy homeostasis by central nervous system-", Open forum, 07 Japan women's college of physical education (Tokyo)

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), 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), The Board of Dirctors for Japanese Culinary Academy (Director). Writing colum series: in Journal of Association of Communication "A lively tongue", Writing colum series in the evening paper of Sankei newspaper "The way of eating of Japanese" A member of Association of sake spread
- 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. Assistant Professor : Takahashi, Nobuyuki, Dr. Med. Sci Postdoctoral fellow : Hirai, Shizuka, Dr. Agric. Sci. Students and research fellows Doctor's Program : (7) Research fellow : (2) Master's Program : (10) Research student : (1) Undergraduate

A. Research Activities (2007.4-2008.3) A-1. Main subjects

a) Genomic regulatory science on lipid metabolism and obesity

: (3)

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

Books

- Uemura T., Goto T., Kawada T. Anti-obesity food stuffs and their applications: anti-obesity Mechanism CMC Press In press
- Goto T., Uemura T., Murakami Y., Kawada T. Saiwaisyobo Metabolic syndrome and food function aspects on diabetes175-187, 2007
- Hirai S., Kusudo T., Mukai S., Kawada T. Protocol of cultured adipocyte differentiation Current Library on Diabetes 276-279, 2007

Original Papers

- Kasahara Y, Takayanagi Y, Kawada T, Itoi K, Nishimori K.: Impaired thermoregulatory ability of oxytocin-deficient mice during cold-exposure. Biosci Biotechnol Biochem. 71:3122-3126, 2007
- Sakane N, Kotani K, Hioki C, Yoshida T, Kawada T.: Up-regulation of muscle uncoupling protein 3 gene expression by calcium channel blocker, Benidipine Hydrochloride in rats. Endocr. J. 54:771-775, 2007

Hirai S, Kim Y-I, Goto T, Kang M-S, Yoshimura M, Obata A, Yu R, Kawada T.: Inhibitory effect of

naringenin chalcone on inflammatory changes in the interaction between adipocytes and macrophages. Life Sci. 81:1272-1279, 2007

- Kang J-H, Kim C-S, Han I-S, Kawada T, Yu R.: Capsaicin, a spicy component of hot peppers, modulates adipokine gene expression and protein release from obese-mouse adipose tissues and isolated adipocytes, and suppresses the inflammatory responses of adipose tissue macrophages. FEBS Letters 581:4389-4396, 2007
- Yano Y, Matsumura T, Senokuchi T, Ishii N, Motoshima H, Taguchi T, Matsuo T, Sonoda K, Kukidome D, Sakai M, Kawada T, Nishikawa T, Araki E.: Troglitazone inhibits oxidized low-density lipoprotein-induced macrophage proliferation: Impact of the suppression of nuclear translocation of ERK1/2. Atherosclerosis 191:22-32, 2007
- Yano Y, Matsumura T, Senokuchi T, Ishii N, Murata Y, Taketa K, Motoshima H, Taguchi T, Sonoda K, Kukidome D, Takuwa Y, Kawada K, Brownlee M, Nishikawa T, Araki E.: Statins Activate Peroxisome Proliferator-Activated Receptor y Through Extracellular Signal-Regulated Kinase 1/2 and p38 Mitogen-Activated Protein Kinase-Dependet Cyclooxygenase-2 Expression in Macrophages. Circ. Res. 100:1442-1451, 2007
- Oi-Kano Y, Kawada T, Watanabe T, Koyama F, Watanabe K, Senbongi R, Iwai K.: Extra virgin olive oil increases uncoupling protein 1 content in brown adipose tissue, and enhances noradrenaline and adrenaline secretion in rats. J Nutr Biochem. 18:685-692, 2007
- Woo H-M, Kang J-H, Kim C-S, Kawada T, Yoo H, Sung M-K, Yu R.: Spices-derived active components inhibit obesity-induced inflammatory responses by modulating the behavior of adipose tissue macrophages and the release of MCP-1 from adipocytes. Life Sci. 80:926-931, 2007
- Kawachi H, Moriya NH, Korai T, Tanaka S, Watanabe M, Matsui T, Kawada T, Yano H.: Nitric oxide suppresses preadipocyte differentiation in 3T3-L1 culture. Mol. Cell. Biochem. 300:61-67, 2007
- Kondoh Y, Kawada T, Urade R.: Activation of caspase 3 in HepG2 cells by elaidic acid (t18:1) Biochim. Biophys Acta, 1771:500-505, 2007
- Wadahama, H., Kamauchi, S., Nakamoto, Y., Nishizawa, K., Ishimoto, M., Kawada, T. and Urade, R.: A novel plant protein disulfide isomerase family homologous to animal P5: molecular cloning and characterization as a functional protein for folding of soybean seed storage proteins. FEBS J. 275; 399-410, 2008
- Ukai, T., Matsumura, Y. and Urade, R.: Disaggregation and reaggregation of gluten proteins by sodium chloride. J Agric. Food Chem. 102; 225-231, 2008
- b) Conference and seminar paper presented
- The 2008 Annual Meeting of Japan Society for Bioscience, Biotechnology and Biochemistry (4)

The 61th Annual Meeting of Japan Society for Nutrition and Food Science (10)

The 2007 Annual Meeting of Japan Society for the Study of Obesity (5)

The 77th Annual Meeting of the Japanese Biochmical Society (2)

The 10th Asian Congress of Nutrition(3)

The 59th Annual Meeting of The Society for Biotechnology(1)

A-3. Off-campus activities

Membership in academic societies

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: Scientific 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 receptors" (Kawada, representative), Scientific Research (B) "Gene targeting analysis of a folding enzyme ER-60 localized in the endoplasmic reticulum" (Urade, representative), Scientific Research (Germ) "Quality control system of the endoplasmic reticulum regulating the generation of soybean storage proteins" (Urade, representative). Scientific Research on Priority Areas "Adipomics; Analysis of developiong mechanism of insulin resistance through cell voiume regulation inadipocytes" (Takahashi, 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).
- The Salt Science Research Foundation: Research Grant "Functions of salt on the formation of gliadin aggregate in wheat dough" (Urade, representative).

A-4. International cooperation and overseas activities

International joint researches, overseas research surveys

- Kawada: Studies on cytokine and chemokine from adipose tissue and obesity related deseases (South Korea, University of Ulsan)
- 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. Medicinal Food (editorial board), J. Nutritional Science and Vitaminology (expert editor), Obesity (referee), B.B.A. (referee), Life Science (referee), Lipid (referee), J. Agric. Food Chem. (referee), B.B.B. (referee)
- Urade, R.: Journal of Biological Chemistry (referee), BMC Genomics (referee), The Plant and Cell Physiology (refree), Plant Science (refree), Journal Biologia (refree), The Molecular Genetics and Genomics (referee)

B. Educational activities (2007.4-2008.3)

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), 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 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), Saga University (Bioresource chemisrty)

B-3. Overseas teaching

Students and research fellows from abroad

Doctor's Program : (2)	Research fellow	: (2)
	Research student	: (1)

2.7.6 Laboratory of Physiological Function of Food

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

Students and research fellows

Doctor's Program : (1)Postdoctoral Fellow : (1)Master's Program : (8)Visiting Researcher : (1)Undergraduate : (3)

A. Research Activities (2007.4-2008.3)

A-1. Main Subjects

a) Anxiolytic activity of rubimetide via the prostaglandin $D_2 \ system$

We investigated effect of rubimetide (Met-Arg-Trp), which is a vasorelaxing and hypotensive peptide isolated from enzymatic digest of spinach Rubisco, on emotional behavior using the elevated-plus maze test in mice. We found that rubimetide increases ratio of time spent in the open arm and of visit to the open arm after i.p. (0.1 mg/kg) or oral (1 mg/kg) administration, suggesting that rubimetide has anxiolytic activity. We previously reported that the vasorelaxing and hypotensive activities of rubimetide were blocked by an antagonist for DP₁ receptor among two receptor subtypes for prostaglandin (PG) D₂. Then we tested whether rubimetide-induced anxiolytic activity was mediated by PGD₂. Anxiolytic activity of rubimetide was completely blocked by DP₁ antagonist BWA868C. These results suggest that rubimetide-induced anxiolytic activity is mediated via the PGD₂-DP₁ receptor system.

We also investigated whether PGD_2 has anxiolytic activity by itself. Centrally administered PGD_2 showed anxiolytic activity at a dose of 10 pmol/mouse, which was much lower than minimum dose necessary for food intake stimulation and sleep induction, suggesting that PGD_2 plays a critical role in emotional behavior in the central nervous system. Orexigenic effect of PGD_2 was blocked by neuropeptide Y (NPY) Y₁ antagonist, while its anxiolytic activity was not inhibited by antagonists for both NPY Y₁ and Y₅ receptors, which are known to be associated with anxiolytic behavior. Taken together, centrally administered PGD_2 exhibits anxiolytic activity independently of the NPY system to mediate food intake stimulation.

b) Mechanism for memory-enhancement by rubiscolin-6 derived from Rubisco

Rubiscolin-6 (Tyr-Pro-Leu-Asp-Leu-Phe) is a δ opioid peptide derived from Rubisco. It shows anxiolytic and memory-enhancing activities after oral administration in mice. Previously, we have shown that the anxiolytic activity of rubiscolin was mediated by release of endogenous σ ligands, such as neurosteroids, which binds to σ_1 receptor, followed by the release of dopamine. After all, the anxiolytic activity was mediated by dopamine D₁ receptor. In the case of momory-enhancing activity, steps leading to the release of dopamine were essentially the same as the anxiolytic activity. However, dopamine D₂ receptor mediated the memory-enhancing activity of rubimetide-6 downstream of δ and σ_1 receptors. In fact, dopamine released from hippocampus elevated by infusion of rubimetide-6 by in vivo microdialysis experiment.

c) Bioactive peptide suppressing food intake derived from egg protein

We previously reported that complement C3a agonist suppresses food intake. We focused the amino acid sequence (Ile-Gly-Lue-Phe-Arg) necessary for binding C3a receptor in primary structure of egg white albumin, synthesized low molecular weight peptides including this sequence, and investigated effect of these peptides on food intake. We found that Met-Met-Tyr-Glu-Ile-Gly-Leu-Phe-Arg (MMYGIGLFR) persistently suppressed food intake after i.p. administration, and this anorexigenic activity was 30 times more potent than that of IGLFR. Unexpectedly, MMYQIGLFR lost guinea pig ileum (GPI)-contracting activity, indicating this is not C3a agonist peptide. The C-terminal Arg-deleted peptide suppressed food intake; however, N-terminal Met-deleted peptide was inactive. Since MMYQIGLFR shows affinity for fMLP receptor (Ki=35 μ M), this may be a candidate of receptor for this peptide. Furthermore, the anorexigenic effect of MMYQIGLFR was blocked by COX inhibitor and PGF_{2a} antagonist but not PGE₂ antagonist. Taken together, MMYQIGLFR may suppress food intake via PGF_{2a} independently of C3a receptor and the PGE₂ system.

d) Mechanism underlying vasorelaxing and hypotensive activities of Arg-Ile-Tyr derived from rapeseed.

Arg-Ile-Tyr (RIY), which has been isolated as an inhibitor for angiotensin-converting enzyme (ACE) from an enzymatic digest of rapeseed protein. We found that RIY showed vasorelaxing activity in the mesenteric artery from spontaneously hypertensive rats (SHR). The vasorelaxing activity of RIY was blocked by CCK₁ receptor antagonist, lorglumide. However, RIY has no affinity for CCK₁ receptor, suggesting that RIY relaxes mesenteric artery after CCK release and CCK₁ receptor activation. RIY-induced vasorelaxation was also inhibited by PGI₂ receptor (IP) antagonist, CAY10441. Vasorelaxing activity of an IP agonist iloprost was inhibited by CCK₁ antagonist lorglumide, indicating that the CCK-CCK₁ receptor system is activated downstream of the PGI₂-IP receptor in the mesenteric artery. Taken together, RIY exhibits vasorelaxing activity via CCK₁ receptor downstream of IP receptor.

e) Anti-analgesic effect of angiotensin AT₂ receptor agonists.

Novokinin (Arg-Pro-Leu-Lys-Pro-Trp) is a highly potent vasorelaxing and hypotensive peptide designed based on the structure of ovokinin(2-7) (Arg-Ala-Asp-His-Pro-Phe), a vasorelaxing peptide derived from ovalbumin. Its vasorelaxing and hypotensive activities are mediated by angiotensin AT_2 receptor. We used novokinin as a probe to searching for novel functions of AT_2 receptor on pain response. Centrally administrated novokinin inhibited the analgesic effect of μ -opioid agonist morphine in mice using the tail-pinch test. The anti-analgesic effect of novokinin was blocked by PD123319, an antagonist of the AT_2 receptor. Ang II and [p·NH₂-Phe⁶]-Ang II, a synthetic AT₂ receptor agonist, also inhibited the analgesic effect of morphine, and the effects were also blocked by PD123319. The anti-analgesic effects induced by novokinin, Ang II and [p-NH₂-Phe⁶]-Ang II were blocked by ONO-AE3-240, an antagonist of the EP₃ receptor. These results suggest that the anti-analgesic effects of AT₂ agonists were mediated through PGE₂-EP₃ receptor system downstream of the AT₂ receptor. We have reported that the vasorelaxing and hypotensive activities of novokinin were mediated by PGI₂, but not by PGE₂ downstream of the AT₂ receptor. It is interesting that the prostaglandin molecular species acting downstream of the AT₂ receptor differ between central and peripheral actions.

A-2. Publications and Presentations

a) Publications

Books

- Yoshikawa, M.: Science of functional food constituents. Bioactive peptides. in "Encyclopedia of Functional Foods" (Ed. By S. Arai *et al.*) pp.43-57, Asakura Inc. (2007)
- Fujita, H., and Yoshikawa, M.: Marine-derived protein hydrolysates, Their biological activities and potential as functional food ingredients: ACE-inhibitory peptides derived from bonito. in "Marine Neutraceuticals and Functional Foods" pp. 247-257, Tayler & Francis (2007)
- Ohinata, K., and Yoshikawa, M.: Milk proteins and peptides, in "Science of Food Functions", pp.376-382, SGS Inc. (2008)

Original papers

- Ohinata, K., Sonoda, S., Inoue, N., Yamauchi, R., Wada, K., and Yoshikawa, M. β-Lactotensin, a neurotensin agonist peptide derived from bovine β-lactoglobulin, enhances memory consolidation in mice, *Peptides*, 28, 1470-1474 (2007)
- Hirata, H., Sonoda, S., Agui, S., Yoshida, M., Ohinata, K., and Yoshikawa, M. Rubiscolin-6, a δ opioid peptide derived from spinach Rubisco, has anxiolytic effect via activating σ_1 and dopamine D₁ receptors. *Peptides*, **28**, 1998-2003 (2007)
- Ohinata, K., Agui, S., and Yoshikawa, M. Soymorphins, Novel μ opioid peptides derived from soy β-Conglycinin β-subunit have anxiolytic activities. *Biosci. Biotech. Biochem.*, 71, 2618-2621 (2007)
- Yamada, Y., Yamauchi, D., Yokoo, M., Ohinata, K., Usui, H., and Yoshikawa, M., A. potent hypotensive peptide, novokinin, induces relaxation of by AT₂- and IP-receptor dependent mechanism in the mesenteric artery from SHRs. *Biosci. Biotechnol. Biochem.*, 72, 257-259 (2008)
- Zhao, H., Usui, H. Ohinata, K., and Yoshikawa, M.: Met-Arg-Trp derived from Rubisco lower blood pressure via prostaglandin D₂-dependent vasorelaxation in spontaneously hypertensive rats. *Peptides*, **29**, 345-349 (2008)
- Yamada, Y., Yamauchi, D., Usui, H., Zhao, H., Yokoo, M., Ohinata, K., Iwai, M., Horiuchi, M., and Yoshikawa, M.: Hypotensive activity of novokinin, a potent analogue of ovokinin(2-7), is mediated by angiotensin AT₂ receptor and prostaglandin IP-receptor. *Peptides*, 29, 412-418 (2008)
- Yamada, Y., Nishizawa, K., Yokoo, M., Zhao, H., Onishi, K., Teraishi, M., Utsumi. S., Ishimoto, M., and Yoshikawa, M.: Anti-Hypertensive activity of genetically modified soybean seeds accumulating novokinin. *Peptides*, **29**, 331-337 (2008)
- Watanabe-Kamiyama, M., Kamiyama, S., Horiuchi, K., Ohinata, K., Shirakawa, H., Furukawa, Y.,

and Komai, M.: Antihypertensive effect of biotin in stroke-prone spontaneously hypertensive rats. *Br J Nutr*, **99**(4), 756-763 (2008)

- Zhao, H., Ohinata, K., and Yoshikawa, M.: Rubimetide (Met-Arg-Trp) derived from Rubisco exhibits anxiolytic-like activity via DP₁ receptor in male *ddY* mice. *Peptides*, **29**, 629-632 (2008)
- Ohinata, K., Fujiwara, Y., Fukumoto, S., Iwai, M., Horiuchi, M., and Yoshikawa, M.: Angiotensin II and III suppress food intake via angiotensin AT₂ receptor and prostaglandin EP₄ receptor in mice. *FEBS Lett.*, **562**, 773-777 (2008)
- Ohinata, K., Takagi, K., Biyajima, K., Fujiwara, Y., Fukumoto, S., Eguchi, N., Urade, Y., Asakawa, A., Fujimiya, M., Inui, A., and Yoshikawa, M.: Central prostaglandin D₂ stimulates food intake via the neuropeptide Y system in mice. *FEBS Lett.*, **582**, 679-682 (2008)

Patents

- Patent pending/applied for Patent application no. 2007-226614 "Hair growth stimulant, drug stimulating hair growth, and food stimulating hair growth", inventors: Yoshikawa, M., and Tsuruki, T., applicant: Kew-Pie, Inc., application date: Aug. 31, 2007
- Patent pending/applied for Patent application no. 2008-093178 "Anxiolytics and Anxiolytic drugs", inventors: Yoshikawa, M., Ohinata, K., and Zhao, H., applicant: Kagome, Inc., application date: March 31, 2008

Reviews

- Ohinata, K.: Adrenomedullin-related peptides and brain-gut axis –A variety of physiological actions of adrenomedullin in the central nervous and peripheral enteric systems. Journal of clinical and experimental medicine, (*in Japanese*) **233**, 555-558 (2007)
- Yoshikawa, M.: Physiological functions of foods and prevention of life style-related diseases. –How shall will encounter flood of information on physiological functions of foods-, *Eiyou-kyoyu* (*in Japanese*), **2008/Winter**, 58-61 (2008)
- Yoshikawa, M., and Ohinata, K.: Anxiolytic peptides derived from plant proteins. *Food Research* (*in Japanese*) **633**, 33-37 (2008)
- b) Conference and seminar papers presented

Annual Meeting of Japan Society for Bioscience, Biotechnology and Agrochemistry: 8 papers The 54th Annual Meeting of the Japanese Society for Food Science and Technology: 1 paper Annual Meeting of the Japanese Society for Food Science and Technology, Kansai Branch: 1 paper

A-3. Off-campus activities

Roles in academic societies

Yoshikawa: Japan Society for Bioscience, Biotechnology and Agrochemistry Japan (Councilor), 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): Mechanism for low molecular weight peptides stimulating hair growth and protecting alopecia (Yoshikawa, representative), Grant-in-Aid for Young Scientists (B): Studies on short peptide regulating food intake (Ohinata, representative), Grant-in-Aid for Young Scientists (Start-up): Novel function

and mode of action of Novokinin, an agonist for angiotensin AT_2 receptor

- Grant from Core Research for Evolutional Science and Technology (CREST): Biocommunication between mother and child supporting brain development. (Yoshikawa, representative)
- JST Grants for Co-operation between Industry and University (Stage for Developing Innovative Seeds): Exploitation of hair-growth stimulants acting through a new mechanism, based on immunostimulating peptides derived from soy (Yoshikawa, representative)
- Kiei Research Foundation: Studies on egg protein-derived peptides regulating food intake (Ohinata, representative)
- Japan Dairy Association: Studies on anorexigenic peptides derived from milk protein (Ohinata, representative)
- Fuji Foundation for Protein Research: Novel low molecular weight peptides originated from soy protein preventing life style-related disease (Ohinata, representative)

A-4. International cooperation and overseas activities

International joint researches, overseas research surveys International meeting

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

International meeting

Yoshikawa: Polish Peptide Symposium, 2007.9.23-27 Poland (Invited lecture)

Ohinata: 4th International Workshop on Complement Associated Diseases, Animal Models, and Therapeutics 2007.6.10-15 Greece (Oral presentation), ICoFF 2007 International Conference on Food Factor for Health Promotion (Poster presentation) 2007.11.27-12.1 Kyoto, 9th Neuropeptide Y International Meeting 2008.3.16-20 Okinawa (Invited lecture)

Editorial work for international journals

Yoshikawa: Peptides (Editorial board), J. Agr. Food Chem. (Reviewer), J. Food Sci. (Reviewer)

B. Educational Activities (2007.4-2008.3)

B-1. On-campus teaching

a) Courses given

- Undergraduate level: Principles of Biochemistry in Food Science I (Ohinata), Physiological functions of foods (Yoshikawa), Seminar in Food Science and Biotechnology (Yoshikawa), Introduction to experimental food bioscience (Yoshikawa, Ohinata), Basic data processing (Ohinata), Laboratory course in food and nutrition chemistry (Ohinata)
- Graduate level: Seminar in physiological function of foods (Yoshikawa, Ohinata), Experimental course in physiological function of foods (Yoshikawa, Ohinata), Advanced course in health science of foods (Yoshikawa), Advanced course in physiological function of foods (Yoshikawa, Ohinata)

B-2. Off-campus teaching

Open seminar, etc

Yoshikawa: Bioscience Seminar on Frontier in Hair Growth, -Targeting on New Hair-Growth Stimulants-, "Effect of food-derived low-molecular weight peptides on hair-growth system", 2007.7.4 Fukuoka

Yoshikawa: Public Seminar on Milk, "Physiological function of milk and importance as a basic

food", 2008.1.26 Kyoto

Ohinata: Tohoku Pharmaceutical University (Lecture), "Food intake regulation by bioactive peptide", 2007.12.7 Sendai

B-3. Overseas teaching

Students and research fellows from abroad Students from abroad: 2 (China and Taiwan) Invited professor from abroad: 1 (Poland)

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

Ohinata: Safeguard Committee of Faculty of Agriculture for Radioisotopes and Radiation (member), Advisory Board for the Committee of Animal Experiment in Graduate School of Agriculture, Kyoto University (member).

Chair of Food Production Technology

2.7.7 Laboratory of Bioengineering

Staff	Professor	: Adachi, Shuji, Dr. Agric. Sci.
	Associate Professor	: Kimura, Yukitaka, Dr. Agric. Sci.
	Assistant Professor	: Shima, Motohiro, Dr. Agric. Sci.
	Assistant Professor	: Kobayashi, Takashi, Dr. Agric. Sci.
	Part-time Secretary	: Kamiya, Rumiko
Students	and research fellows	
	Doctor's program	: (1)
	Master's program	: (8)
	Undergraduate	: (4)
	Research fellow	: (1)
	Research student	: (1)

Foreign research student: (1)

A. Research Activities (2007.4-2008.3)

A-1. Main subjects

a) Development of novel food processing using subcritical water:

Water which maintains its liquid state at 100 to 374°C under pressurized conditions is called 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. Production of functional substances from less utilized agricultural resources, and hydrolyses of esters, saccharides and amino acids by subcritical water have also been investigated.

b) Properties and utilization of nano- or microemulsions:

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 transfer through interface between oil and water. We also investigate preparation of 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. We intend to elucidate factors affecting the reaction performance and to construct reactor systems reasonably. Enzymatic production of edible and functional substances are investigated.

d) Development and analysis of food process engineering:

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

A-2. Publications and presentations

a) Publications

Reviews

T. Kobayashi: Production of functional eaters by lipase-catalyzed reaction. – Factors affecting the productivity– *Foods & Food Ingredients Journal of Japan*, **221**, 273-280 (2007).

Original papers

- S. Kikuchi, T. Kobayashi, and S. Adachi: Dependence of the distribution coefficients of hydrophobic slutes on porous methyl methacrylate resin on the temperature and methanol content of the eluent. *Food Sci. Technol. Res.*, 14, 144-147 (2008).
- Y. Watanabe, Y. Sawahara, S. Asai, and S. Adachi: Decomposition kinetics of 6-O-monoacyl ascorbate in air. *Food Sci. Technol. Res.*, 14, 139-143 (2008).
- N. Iwamoto, M. Shima, and S. Adachi: Synthesis of xylitoyl fatty acid monoesters by immobilized lipase in subcritical acetone. *Biochem. Eng. J.*, **38**, 16-21 (2008).
- P. Khuwijitjaru, K. Chalooddong, and S. Adachi: Phenolic content and radical scavenging capacity of kaffir lime fruit peel extracts obtained by pressurized hot water extraction. *Food Sci. Technol. Res.*, 14, 1-4 (2008).

- R. Nakazawa, M. Shima, and S. Adachi: Effect of oil-droplet size on the oxidation of microencapsulated methyl linoleate. J. Oleo Sci., 57, 225-232 (2008).
- J. Wiboonsirikul, Y. Kimura, Y. Kanaya, T. Tsuno, and S. Adachi: Production and characterization of functional substances from a by-product of rice bran oil and protein production by a compressed hot water treatment. *Biosci. Biotechnol. Biochem.*, **72**, 384-392 (2008).
- J. Wiboonsirikul, Y. Sakai, A. Hosoda, H. Morita, Y. Kimura, H. Taniguchi, T. Tsuno, and S. Adachi: Mutagenicity of the extract from defatted rice bran by subcritical water treatment. Japan J. Food Eng., 9, 75-78 (2008).
- C. Usuki, Y. Kimura, and S. Adachi: Degradation of pentaoses and hexauronic acids in subcritical water. *Chem. Eng. Technol.*, **31**, 133–137 (2008).
- S. Katagi, Y. Kimura, and S. Adachi: Continious preparation of O/W nano-emulsion by the treatment of a coarse emulsion under subcritical water conditions. LWT-Food Sci. Technol, 40, 1376-1380 (2007).
- J. Wiboonsirikul, S. Hata, Y. Kimura, T. Tsuno, and S. Adachi: Production of functional substances from black rice bran by its treatment in subcritical water. LWT Food Sci. Technol, 40, 1732-1740 (2007).
- J. Piao, K. Takase, and S. Adachi: Enzymatic synthesis of myristoyl disaccharides and their surface activity. J. Sci. Food Agric., 87, 1743-1747 (2007).
- P. Khuwijitjaru, P. Nualchan, and S. Adachi: Foaming and emulsifying properties of rice bran extracts obtained by subcritical water treatment. *Silpakorn U Sci. Technol. J.*, 1, 7-12 (2007).
- L.-H. Hung, Y. Horagai, Y. Kimura, and S. Adachi: Decomposition and discoloration of L-ascorbic acid freeze-dried with saccharides. *Innov. Food Sci. Emerg. Technol.*, **8**, 500-506 (2007).
- C. Usuki, Y. Kimura, and S. Adachi: Isomerization of hexoses in subcritical waters. *Food Sci. Technol. Res.*, **13**, 205-209 (2007).
- J. Wiboonsirikul, Y. Kimura, M. Kadota, H. Morita, T. Tsuno, and S. Adachi: Properties of extracts from defatted rice bran by its subcritical water treatment. J. Agric. Food Chem., 55, 8759-8765 (2007).
- J. Wiboonsirikul, P. Khuwijitjaru, Y. Kimura, H. Morita, T. Tsuno, and S. Adachi: Production optimization of the extract with high phenolic content and radical scavenging activity from defatted rice bran by subcritical water treatment. Japan J. Food Eng., 8, 311-315 (2007).
- T. Kobayashi, T. Nagao, Y. Watanabe, and Y. Shimada: Analysis of equilibrium state for synthesis of oleic acid L-menthyl ester in an oil-aqueous biphasic system with *Candida rugosa* lipase. *Enzyme. Microb. Technol.*, **40**, 1300-1304 (2007).
- Y. Watanabe, P. Pinsirodom, T. Nagao, A. Yamauchi, T. Kobayashi, Y. Nishida, Y. Takagi, and Y. Shimada: Conversion of acid oil by-produced in vegetable oil refining to biodiesel fuel by immobilized *Candida antarctica* lipase. J. Mol. Cat. B: Enzymatic, 44, 99-105 (2007).
- T. Nagao, Y. Watanabe, T. Kobayashi, M. Sumida, N. Kishimoto, T. Fujita, and Y. Shimada: Enzymatic purification of dihomo-γ-linolenic acid from *Mortierella* single-cell oil. J. Mol. Cat. B: Enzymatic, 44, 14-19 (2007).
- b) Conference and seminar papers presented
- 98th AOCS Annual Meeting (1 paper)
- Annual Meeting of Japan Society for Food Engineering, 2007 (3 papers)

The 46th Annual Meeting of Japan Oil Chemists' Society (2 papers) The 39th Autumn Meeting of the Society of Chemical Engineers, Japan (2 papers) The 58th Meeting of the Japan Society of Enzyme Engineering (1 paper) Asia-Pacific Biochemical Engineering Conference 2007 (1 paper) Yamaguchi Meeting of the Society of Chemical Engineers (1 paper) The 6th Meeting of Lipid Engineering Division of the Society for Biotechnology, Japan (2 papers) Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry, 2008 (1 paper)

A-3. Off-campus activities

Membership in academic societies

Adachi, S.: The Japan Society for Food Engineering (director), The Japanese Society on Enzyme Engineering (vice-president), Japan Oil Chemists' Society (secretary of Kansai branch), The Society of Chemical Engineers, Japan (Section-chief of Foodstuffs and Food Processing Subcommittee).

Research Grant

- Adachi, S.: Japan Society of the Promotion of Science, Grant-in-Aid for Exploratory Research: Does nanoemulsion delay autoxidation of lipid in the oil phase? (representative).
- Nanotechnology project, the Ministry of Agriculture, Forestry and Fisheries: Assessment of anti-oxidative and dispersion stabilities of nano- and microparticles in food emulsions and their control (co-operative).
- Cooperation of Innovative Technology and Advanced Research in Evolutional Area (CITY AREA), the Ministry of Education, Culture, Sports, Science and Technology, Japan: Development of functional food materials (co-operative).
- Salt Science Research Foundation: Estimation of binding constant between salt and oligosaccharide by chromatographic method. (representative).
- Kimura Y.: Japan Society of the Promotion of Science, Grant-in-Aid for Scientific Research (C): Effects of pH at high pressure and temperature in subcritical water on hydrolytic kinetics of food componets. (representative).

A-4. International cooperations and overseas activities

International meetings

 $Adachi,\,S.:\,Invited\ lecture\ in\ Southern\ Yangtze\ University,\ Wuxi,\ China.$

Editorial work for international journals

Adachi, S.: Food Science and Technology Research (editor).

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

B. Educational Activities (2007.4-2008.3)

B-1. On-campus teaching

a) Courses given

Undergraduate level: Food Engineering (Adachi, S., 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., Shima, M., Kobayashi, T.), Laboratory Course in Chemical Engineering (Kimura, Y., Shima, M., Kobayashi, T.).

Graduate level: Food Engineering (Advanced Course) (Adachi, S.), Bioengineering Seminar

(Adachi, S., Kimura, Y., Shima, M., Kobayashi, T.), Experimental Course in Bioengineering (Adachi, S., Kimura, Y., Shima, H., Kobayashi, T.).

B-2. Off-campus teaching, etc.

Open seminar, etc.

Adachi, S.: Basic Training Course of the Japan Society for Food Engineering (lecturer).

- Kimura, Y.: Academic Plaza in 2007 International Exhibition on Food Machinery, Ariake, Tokyo (lecturer).
- Kobayashi, T.: Meeting of Research Exchange for Process of Organic Reaction Harmonized with Environment (lecturer).

B-3 Overseas teaching

Students and research fellows from abroad

Doctor's program: 1 (Thailand) Foreign research student: 1 (Netherlands) Research student: 1 (R. P. China)

C. Other remarks

- Adachi, S.: Advisory Board for Utilization of Academic Center for Computing and Media Studies, Kyoto University (member), Department Chairperson of Food Science and Biotechnology, Committee for Assessment of Faculty of Agriculture, Chairperson of Committee for Information Systems in Faculty of Agriculture, Chairperson of Technical Subcommittee for Information Systems in Faculty of Agriculture, Committee for Informational Security in the Graduate School of Agriculture (member), Committee for Safety Control in the Graduate School of Agriculture (member).
- Kimura, Y.: Committee for Informational Security in the Graduate School of Agriculture (member), Representative for Safety Control of Research Activity in Division of Food Science and Biotechnology, 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.
 Postdoctoral fellows: Maruyama Yukie, Dr. Agric. Sci.
 Postdoctoral fellows: Itoh, Takafumi, Dr. Agric. Sci.

Students and research fellows

Post-doctral fellow: (2)Doctor's program: (2)Master's program: (6)Undergraduate: (2)

A. Research Activities (2007.4-2008.3)

A-1. Main subjects

a) Functional 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. A flagellin homologue p5 expressed on the cell surface functions as a receptor for extracellular alginate. In this study, localization mechanism of p5 on the cell surface was investigated. A p5 deletion mutant (p5 Δ NC) with a lack of N- and C-terminal 50 residues responsible for alginate binding was constructed. A strain A1 cell surface protein SPH2681 with a molecular mass of 50 kDa was found to associate with the p5 Δ NC-bound resin. The gene for SPH2681 was identified in the strain A1 genome database through the peptide mass finger printing analysis. To clarify the function of SPH2681, its overexpression and purification systems were constructed in *Escherichia coli*. Surface plasmon resonance biosensor analysis indicated that SPH2681 is able to bind to the p5 Δ NC with a disassociation constant K_d of 1.6 x 10⁻⁴ M. SPH2681 is, therefore, suggested to function as an anchoring protein for p5 localized on the cell surface.

b) Structural proteomics of pit-forming bacterium

Sphingomonas sp. A1 directly incorporates macromolecule polysaccharide from the pit to cytoplasm through periplasmic binding proteins-dependent ABC importer. A structural model of the ABC importer (AlgM1-AlgM2/AlgS-AlgS: 4 subunits) was architected by homology modeling and docking simulation. Both of AlgM1 and AlgM2 are six-spanning membrane proteins and the heterodimer consists of twelve membrane-spanning α -helices. The P loop and LSGGQ motif conserved in ABC proteins are present around the ATP-binding site in AlgS. The ABC importer is constituted through the subunit interaction between the membrane heterodimer (AlgM1-AlgM2) and cytoplasmic homodimer (AlgS-AlgS). A short a-helix of the membrane heterodimer extruded from membrane to cytoplasm and ENI motif/Q loop of the homodimer are involved in the subunit interaction.

c) Gas biology

Nitrogen-fixing *Azotobacter vinelandii* assimilates nitrogen to ammonia through the action of nitrogenase in the nitrogen source-free environment. Nitrogenase is immediately inactivated in

the presence of oxygen. In this study, the bacterial regulation mechanism for maintenance of nitrogen and oxygen in the intracellular fraction was analyzed. Cells of *A. vinelandii* were grown in the nitrogen source-free minimal medium under different compositions of nitrogen, oxygen and/or helium gasses. The bacterial growth was inhibited by high level of oxygen (more than 25%). The comprehensive gene expression in the bacterial cells was analyzed by DNA microarray. In comparison with the cells grown in the air, gene clusters for synthesis of extracellular alginate biofilm and cytoplasmic polyhydroxylbutyrate (PHB) inclusion body were inducibly expressed in the cells grown in the mixed gas consisting of 50% nitrogen and 50% oxygen. Cells of *A. vinelandii* are known to convert to cysts resistant to the dried condition through production of alginate and PHB. The above-described results indicate that, other than dryness, nitrogen/oxygen gas level also becomes the factor for formation of cysts.

d) Interaction between bacteria and hosts: Structural biology of 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.

Rhamnogalacturonan (RG) lyases are responsible for degradation of plant cell wall polysaccharide RG. Saprophytic *Bacillus subtilis* 168 produces two types of RG lyases, endotype YesW and exotype YesX, both of which are mutually similar in the primary structure. To clarify the structural factor for mode of action, i.e. endo/exo type, comparative structural analysis regarding YesW and YesX was conducted. X-ray crystal structure of YesX was determined at 1.65 Å resolution by molecular replacement method using YesW as a search model. Superimposition of crystal structures of YesX and galacturonan disaccharide-bound YesW indicated that a lid loop covering nonreducing end of the disaccharide is situated specific for YesX. The YesX mutant with a lack of the loop showed an endotype-like activity similar to YesW. The loop is, therefore, suggested to inhibit the accommodation of long chain substrate in the active site, and provide YesX with the exolytic activity.

e) Physiological function of NADP(H) biosynthesis in yeast Saccharomyces cerevisiae

In yeast Saccharomyces cerevisiae, there are three NAD kinases (cytosolic Utr1p, cytosolic Yef1p, and mitochondrial Pos5p). The NADH kinase reaction catalyzed by Pos5p, rather than the NAD kinase reaction followed by the NADP+-dependent dehydrogenase reaction, has been regarded to be critical as a source of mitochondrial NADPH, which has vital roles in various mitochondrial functions. In this study, we demonstrated that mitochondrial NADH kinase reaction is dispensable as the source of mitochondrial NADPH. This emphasized an importance of the NAD kinase reaction followed by the mitochondrial NADP⁺-dependent dehydrogenase reaction. Among such dehydrogenases (malic enzyme, Mae1p, isocitrate dehydrogenase, Idp1p, and acetaldehyde dehydrogenases, Ald4/5p), the evidence was obtained that at least Ald4/5p, in particular Ald4p, has a prominent role to supply mitochondrial NADPH in the absence of NADH kinase reaction. Physiological significance of mitochondrial NADH kinase reaction was also demonstrated in the absence of Ald4p. In addition, Pos5p was confirmed to show much higher NADH kinase activity over NAD kinase one. Collectively, we propose that there are two sources of mitochondrial NADPH in yeast; one is the mitochondrial Pos5p-NADH kinase reaction and the other isthe mitochondrial Pos5p-NAD kinase reaction followed by mitochondrial NADP⁺-dependent acetaldehyde dehydrogenase reaction.

f) Structure and function of human NAD kinase

NAD kinase (EC 2.7.1.23) catalyzes the phosphorylation of NAD⁺ to NADP⁺. The NAD kinase from human neutrophiles has been implied to be activated by calcium and calmodulin, although this activation had not been reexamined in the homogeneous system despite of its biological significance. In this study, recombinant human NAD kinase and human calmodulin (CALM1) was purified. Although no effect of purified CALM1 on the activity of purified human NAD kinase was demonstrated, we found that activity of human NAD kinase was inhibited by NADH and NADPH, but not by NADP⁺. Taken together the lower NADH kinase activity (about 1.3 % compared to that of NAD⁺ kinase activity), our data indicated the significance of NADP⁺-dependent dehydrogenase, which produces NADPH from NADP⁺, for the regulation of the synthesis of NADP⁺ by NAD kinase.

A-2. Publications and presentations

a) Publications

Books

- Murata, K., W. Hashimoto and S. Kawai: Chapter 4 Cell structure of microbes. Genetic aspects Applied microbiology. (Eds. H. Kumagai, N. Kato, K. Murata and Y. Sakai), p. 55-77, Asakura Publishing Co., Tokyo, 2008
- Murata, K., Y. Aso: Chapter 7 Isolation and growth of microbes. ^{Genetic aspects}Applied microbiology. (Eds. H. Kumagai, N. Kato, K. Murata and Y. Sakai), p. 113-128, Asakura Publishing Co., Tokyo, 2008

Original papers

- Maruyama, Y., M. Momma, B. Mikami, W. Hashimoto and K. Murata: Crystal structure of a novel bacterial cell-surface flagellin binding to polysaccharide. Biochemstry, 47 (5); 1393-1402, 2008
- Fukuda, C., S. Kawai and K. Murata: NADP(H) phosphatase activities in archaeal inositol monophosphatase and eubacterial 3'-phosphoadenosine 5'-phosphate phosphatase. Appl. Environ. Microbiol., 73 (17); 5447-5452, 2007
- Chaudhari, A., M. Mahfouz, A. M. Fialho, T. Yamada, A. T. Granja, Y. Zhu, W. Hashimoto, B. Schlarb-Ridley, W. Cho, T. K. D. Gupta and A. M. Chakrabarty: Cupredoxin-cancer interrelationship: azurin binding with EphB2, interference in EphB2 tyrosine phosphorylation, and inhibition of cancer growth. Biochemistry, 46 (7); 1799-1810, 2007
- Ochiai, A., T. Itoh, A. Kawamata, W. Hashimoto and K. Murata: Plant cell wall degradation by saprophytic *Bacillus subtilis*: gene clusters responsible for rhamnogalacturonan depolymerization. Appl. Environ. Microbiol., 73 (12); 3803-3813, 2007
- Cui, Z., Y. Maruyama, B. Mikami, W. Hashimoto and K. Murata: Crystal structure of glycoside hydrolase family 78 α-L-rhamnosidase from *Bacillus* sp. GL1. J. Mol. Biol., 374 (2); 384-398, 2007
- Ochiai, A., T. Itoh, Y. Maruyama, A. Kawamata, B. Mikami, W. Hashimoto and K. Murata: A novel structural fold in polysaccharide lyases: *Bacillus subtilis* family 11 rhamnogalacturonan lyase YesW with an eight-bladed b-propeller. J. Biol. Chem., 282 (51); 37134-37145, 2007

Reviews

Murata, K., S. Kawai, B. Mikami and W. Hashimoto: Superchannel of bacteria: biological significance and new horizons. Biosci. Biotechnol. Biochem., 72 (2); 265-277, 2008

- Hashimoto, W., T. Itoh, Y. Maruyama, B. Mikami and K. Murata: Hydration of vinyl ether groups by unsaturated glycoside hydrolases and their role in bacterial pathogenesis. Int. Microbiol., 10 (4); 233-243, 2007
- Hashimoto, W., Y. Maruyama, Y. Aso and K. Murata: Molecular transplantation of bacterial organ and bioremediation. Kagaku-to-Seibutsu, 45 (5); 320-329, 2007

Reports

- Maruyama, Y., M. Momma, T. Itoh, K. Ogura, B. Mikami, W. Hashimoto and K. Murata: X-ray crystallographic study on *Sphingomonas* sp. A1 cell surface protein associating with alginate-binding flagellin homologue. SPring-8 User Experiment Report, 2007B1624, On-line publication, 2007
- Ochiai, A., T. Itoh, K. Ogura, B. Mikami, W. Hashimoto and K. Murata: Crystal structure of exotype rhamnogalacturonan lyase YesX from *Bacillus subtilis* strain 168: structural factors responsible for mode of action. SPring-8 User Experiment Report, 2007B1268, On-line publication, 2007
- Itoh, T., Y. Maruyama, A. Ochiai, K. Ogura, B. Mikami, W. Hashimoto and K. Murata: Structural identification of bacterial *yihS*-encoded proteins to an aldose-ketose isomerase. SPring-8 User Experiment Report, 2007B1176, On-line publication, 2007
- Itoh, T., Y. Maruyama, A. Ochiai, K. Ogura, B. Mikami, W. Hashimoto and K. Murata: Structural analysis of N-acyl-D-glucosamine 2-epimerase superfamily. SPring-8 User Experiment Report, 2007A1154, On-line publication, 2007
- Maruyama, Y., M. Momma, B. Mikami, W. Hashimoto and K. Murata: Alginate recognition by cell surface flagellin homologous proteins of *Sphingomonas* sp. A1. SPring-8 User Experiment Report, 2007A1330, On-line publication, 2007
- Maruyama, Y., T. Itoh, A. Ochiai, K. Ogura, B. Mikami, W. Hashimoto and K. Murata: Crystal structure of unsaturated glucuronyl hydrolase from *Streptococcus agalactiae*. SPring-8 User Experiment Report, 2007A1407, On-line publication, 2007
- b) Conference and seminar papers presented
- The Annual Meeting (2007) of Japan Society for Bioscience, Biotechnology, and Agrochemistry: 11 cases
- The Annual Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch): 2 cases
- The Regular Meeting of Japan Society for Bioscience, Biotechnology, and Agrochemistry (Kansai Branch): 4 cases
- The Annual Meeting (2007) of The Society for Biotechnology, Japan: 2 cases
- Japan-Korea Joint Seminar on Microbial and Plant Biotechnology: 2 cases

The Meeting on phosphorous compounds (The 28th Meeting on C-P compounds): 2 cases

A-3. Off-campus activities

Membership in academic societies (roles)

Murata, Kousaku: Japan Society for Bioscience, Biotechnology, and Agrochemistry (Councilor of Nation-Wide, Editor-in-Chief of "Chemistry and Biology (Japanese)"; The Society for Biotechnology, Japan (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); Others. Hashimoto, Wataru: Yeast Research Society of Japan (Operator).

Research grants

- Murata, Kousaku: Monbukagakusho Research Grant-in-Aid for Scientific Research (B), Principal Investigator, Entry 2005, Molecular bases for regulation of bacterial cell surface structure and evolution of flagella; Monbukagakusho Research Grant-in-Aid for Exploratory Research, Principal Investigator, Entry 2006, Membrane engineering through molecular transplantation of bacterial pit-organ and its application; Monbukagakusho Research Grant-in-Aid for Scientific Research on Priority Areas (A), Principal Investigator, Entry 2006, Roles of water in structure and function of proteins
- Hashimoto, Wataru: Monbukagakusho Research Grant-in-Aid for Scientific Research (C), Principal Investigator; Entry in 2006, Molecular and structural biology of streptococcal invasion and infection mechanisms through degradation of glycosaminoglycan; Monbukagakusho Targeted Proteins Research Program, Principal Investigator; Entry in 2007, Structural biology of bacterial super-biosystem for import and degradation of polysaccharides
- Kawai, Shigeyuki: Research Grant-in-Aid for Encouragement of Young Scientists (B), Principal Investigator; Entry in 2007, Molecular mechanism and physiological function of the regulation of NAD(H) and NADP(H) corresponding to the extracellular environment

B. Educational Activities (2007.4-2008.3)

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)

C. Other remarks

Murata, Kousaku: The Japan Bioscience, Biotechnology and Agrochemistry Society Award Kawai, Shigeyuki: The Japan Bioscience, Biotechnology and Agrochemistry Society Award for the Encouragement of Young Scientists