Course for Biotechnology

Department of Chemistry and Biotechnology
Graduate School of Engineering
The Department of Chemistry and Biotechnology has been founded since 2007 by reorganization of the Faculty of Engineering. The department is composed of two courses, Applied Chemistry and Biotechnology.

Chemistry and Biotechnology are bases for the modern industry. It is now well recognized that academic and technological breakthroughs in science can be realized most readily when two divergent principles in different fields are combined. Due to the diverse field of chemistry, the combination of varied scientific principles is becoming particularly important. Many phenomena found in nature are now explained in terms of the recognition of different molecules via intermolecular interactions, and the integration of the so-called biological and chemical fields is proceeding rapidly.

It is generally regarded that living things represent an extremely complex phenomenon. It is possible, however, to understand a phenomenon as a system composed of large organic molecules, each with a unique structure and capable of mediating many chemical reactions necessary for life. In this course, a broad study of many types of bacteria will be performed in order to find novel genes and biological activities which have industrial applications. These useful biological phenomena will then be studied at the molecular level to elucidate various new applications which are not found in living cells. Also, by isolating, modifying, expanding or mimicking the processes found in living cells, these useful biological processes will be adapted to industrial uses. The course will provide basic and applied research opportunities and education to each candidate which will allow him/her to take part in the adaptation of biological phenomena in industrial and environmental control processes.
RESEARCH SUBJECTS

1. Microbial desulfurization of organic sulfur compounds

Regulations to reduce the sulfur content of fuels for motor vehicles will be increasingly stringent. However, it is difficult to meet the environmental regulations in a cost-effective manner by the conventional hydrodesulfurization. Therefore, the microbial desulfurization has attracted much attention as an alternative method. Dibenzothiophene (DBT) is regarded as a model sulfur heterocycle, and some bacteria have been found to metabolize DBT to 2-hydroxybiphenyl (2-HBP) without DBT’s carbon skeleton. In such bacteria, DBT is desulfurized by consecutive three enzyme reactions catalyzed by DszC (DBT monooxygenase) oxidizing DBT to DBT sulfone, DszA (DBT sulfone monooxygenase) catalyzing the transformation of DBT sulfone to 2’-hydroxybiphenyl 2-sulfinate (HBPSi), and DszB (HBPSi desulfinate) converting HBPSi to 2-HBP and sulfite. The first two enzymes (DszC and A) require a flavin reductase for activities.

We have purified to homogeneity and characterized all the enzymes. Among them, DszB catalyzes the release of sulfite, and is considered to be responsible for the actual desulfurization. DszB is thought to be a novel enzyme in that it can specifically cleave the carbon-sulfur bond of the substrate without the aid of any other proteinic components and coenzymes. Recently, we elucidated the 3-D structure of DszB, which was the first X-ray crystallographic study of the enzymes involved in DBT desulfurization. The catalytic activity and thermostability of DszB were enhanced by the two amino acid substitutions based upon the 3-D structure of the enzyme protein.

2. Microbial biotin biosynthesis

Biotin (vitamin H) is an essential cofactor required for a number of carboxylation, transcarboxylation, and decarboxylation reactions in all living organisms. All studies of the biosynthetic pathway of biotin and the microbial biotin production have so far been performed exclusively using mesophilic organisms. However, the biotin biosynthetic pathways in thermophiles have not yet been characterized at all. Enzymes from thermophiles are attractive in terms of higher resistance to physical/chemical denaturation.
than mesophiles. Also, the production of useful compounds at higher temperatures by organisms having thermophilic enzymes should have advantages in terms of low risk of contamination and reactor cooling requirements. Thus, with regard to both fundamental and applied aspects, we have tried to classify various thermophilic enzymes based on the known genome databases of thermophilic organisms. As a result, we found that the TTHA1582 gene codes putative α-oxoamine synthase family enzymes, 8-amino-7-oxononanoate (KAPA) synthase (BioF, EC 2.3.1.47) and 2-amino-3-ketobutyrate CoA ligase (KBL, EC 2.3.1.29). The TTHA1582 gene product was overexpressed in *E. coli* and found to have the activity of BioF, which catalyzes the condensation of pimeloyl-CoA and L-alanine to produce KAPA, CoASH, and CO2, with the participation of pyridoxal 5’-phosphate.

### 3. Halogenating enzymes from macro-algae

Haloperoxidases catalyze the halogenation of organic substrates in the presence of hydrogen peroxide. A variety of halogenated compounds are found in many kinds of marine macro-algae, and activities of haloperoxidase were detected in these organisms. The enzymes were thought to function in the biosynthesis of halogenated natural products with biological activities. Among marine macro-algae, bromoperoxidase (BPO) from the red alga, *Corallina pilulifera*, has been extensively characterized, and was found to require a vanadium as an essential cofactor for the enzyme activity. The cDNA of BPO has been cloned from *C. pilulifera*, expressed in *Saccharomyces cerevisiae*, and the X-ray structure of the enzyme has been determined. The analysis of the three-dimensional structure suggests that one calcium ion per subunit is bound in a loop at the top of the active site cleft, and we have confirmed the importance of calcium and other metal ions for protein stability. Moreover, the specificity for halide of the enzyme was altered by the single amino acid substitution based upon the structural data.

### PUBLICATIONS


GROUP OF BIOORGANIC CHEMISTRY

Kise Naoki, Professor (Dr. of Engineering)
Sakurai Toshihiko, Associate Professor (Dr. of Engineering)

RESEARCH SUBJECTS
1. Synthesis of Physiologically Active Compounds utilizing Electrochemical Reaction
Electrochemical method generally has the advantages of simple operation, mild condition, and non-polluting. The aim of this study is to develop useful electrochemical reactions applicable to the synthesis of physiologically active compounds. For example, the reductive intramolecular coupling of aromatic $\alpha$-, $\beta$-, and $\gamma$-imino esters was realized by electrochemical reduction. This reaction provides a new method for the stereoselective synthesis of cyclic amines, such as azetidines, pyrrolidines, and piperidines. Among them, one of the obtained pyrrolidines is a precursor of neurokinin NK1 receptor antagonist L-733,060.

![Chemical structure of pyrrolidine](image1)

2. Amyloid-like structural properties of the alternating seq-polypeptide and their assembly kinetics
The term “amyloid” meant the starch-like which was accumulated to intercellular cement originally, but now it was used to describe the proteinaceous fibrillar aggregates. The fibril formation denominated “amyloid fibril” is rich in $\beta$-structure that have a high tendency to form aggregates, and it has recently been shown that several proteins can aggregate into amyloid fibrils. Negative-stain electron microscopy (EM) studies show that these fibril structure have a several nanometers width, and is straight, rigid, and unbranched formation. All the amyloid fibrils contain $\beta$-sheet structure in which the peptide strand aligned perpendicular to the long axis of the fibril. This “cross-$\beta$” motif is a typical formation in amyloid fibrils and shows very similar morphologies. We report the amyloid fibril formation by a synthetic model peptide having an alternating sequence analogue and containing a pyrene group at the N-terminal in order to analyze the amyloid fibril formation and formation process. This model peptide has three key characteristics: (1) the sequence is alternating with hydrophobic and hydrophilic amino acids; (2) the degree of polymerization is monodispersed. If $\beta$-structure is formed in an aqueous solution, it is expected that the monodispersity may promote one-dimensional aggregation by amphiphilic property; (3) the terminal fluorescent chromophore enables us to evaluate the molecular orientation spectrophotometrically.

![Transmission electron micrograph images](image2)
This kind of research using model peptide gives us specific information for not only the fundamental information of the amyloidogenesis, but also the development to the luminescence probe which utilized amyloid fiber.

**PUBLICATIONS**

Group of Protein Engineering  
Yasushi Kawata, Professor (D. Sc.)  
Tomohiro Mizobata, Associate Professor (Ph. D.)  
Kunihiro Hongo, Research Associate (M. Eng.)

Research Interests

1. Elucidation of the structural and functional characteristics of molecular chaperones

Molecular chaperones are a group of proteins that assist in protecting the integrity of the cell under stressful conditions. Many molecular chaperones are specifically involved in the maintenance and recovery of protein structures, in assisting the translocation of proteins through biological membranes, and in assisted protein degradation and recycling. Our laboratory is performing studies to elucidate the molecular mechanisms by which these molecular chaperones fulfill their respective roles in vivo. Our research focuses specifically on the chaperonin proteins found in all types of cell. Our studies have succeeded in clarifying a number of aspects of the mechanism of chaperonin function, and we have begun to probe some methods of harnessing the unique activity of chaperonins in various applications.

<i>Recent findings</i>

i) Elucidation of the importance of hydrophilic amino acid side chains in the bacterial chaperonin GroEL from <i>E. coli</i> \(^2\).

ii) Structural stability of the cochaperonin GroES from <i>E. coli</i>, using novel experimental methods such as atomic force microscopy and covalent subunit linkage \(^4,10,12\).

iii) Detailed kinetic studies on the dynamic subunit movements of the chaperonin GroEL using mutant proteins, functional analyses, and stopped-flow fluorescence analyses \(^5, 9, 13, 14\).

iv) Characterization of a chaperonin protein from a hyperthermophilic organism, and a comparison with <i>E. coli</i> GroEL \(^3, 6\).

2. Characterization of thermostable enzymes, elucidation of the principles of structural stabilization in proteins, and development of methods to confer structural stability to useful biological activities

Proteins evolve in numerous ways according to the environment of the host organism. An interesting discovery in this context is the existence of thermophilic and hyperthermophilic organisms, whose constituent enzymes are, on average, markedly more stable compared to related enzymes isolated from mesophilic sources. Our group studies various enzymes isolated from thermophilic and hyperthermophilic organisms in order to elucidate the underlying mechanisms of enzyme stability. We also attempt to apply this knowledge to produce stable applications of biological activity, specific stabilization of some interesting biological activities, and the invention of novel enzymes that are capable of functioning in various non-native conditions.

<i>Recent findings</i>

i) Cloning, overproduction, and characterization of members of the Class II fumarase superfamily from various thermophilic and archaean sources \(^16\). Attempts to improve the stability of these proteins using chimeric gene recombination and covalent modification (ongoing research).

ii) Adaptation of superoxide dismutase activities to function under non-aqueous conditions (ongoing research).

iii) Characterization of an extremely thermostable manganese catalase activity from thermophilic bacteria \(^15\).

3. Studies regarding the mechanisms of protein aggregation, protein fibrillation, and the diseases caused by fibrillar protein deposits

Recent novel findings have found that under specific conditions and after a prolonged interval, certain cellular
proteins have a tendency to form filamentous insoluble particles that are deposited within or around a cell. These insoluble proteins are implicated in the pathology of various neurodegenerative disorders that are propagated solely by protein-derived particles (prions or protein fibrils). As part of our effort to understand all aspects of protein structure, our group is involved in probing the molecular mechanisms of protein fibril formation, mainly under in vitro conditions. We have found a number of surprising insights regarding the protein fibrillation phenomenon, and are also working on discovering various factors that either enhance or suppress protein fibril formation, with an interest toward utilizing our findings in medical and industrial applications.

**Recent discoveries**

i) Determination of “core amino acid sequence regions” that are responsible for the association and fibrillation of multiple homologous proteins in vitro. The proteins in question are unrelated to specific neurological diseases.

ii) Characterization of the fibril forming behavior of the cochaperonin GroES from *E. coli*, a protein that is completely unrelated to any disease or disorder. The fibrils formed were capable of triggering the formation of a different protein linked to Parkinson’s disease.

iii) Suppression of amyloid fibril formation of certain proteins using the biological activities of molecular chaperones.


GROUP OF FOOD AND BIOENGINEERING

Takeshi Furuta, Professor (Dr. of Engineering)
Hidefumi Yoshii, Associate Professor (Dr. of Engineering)

RESEARCH SUBJECTS

1. Microencapsulation of food and pharmaceutical related ingredients by spray drying and the storage stability

The incorporation of hydrophobic flavors into powders by encapsulation is great importance in the food and flavors industries. Microencapsulation of flavors is a technology of enclosing flavor compounds in a carrier matrix to provide dry and free-flowing powders, which are easy to handle. Furthermore, it also provides protection against the degradative reaction and prevents the loss of flavors during food processing and storage. Among various microencapsulation methods, spray drying is the most common technique to produce flavor powders, since it has many merits such as low process cost, wide choice of carrier solids, good retention of flavors, and good stability of the finished flavors. In this research, the main emphasis of the microencapsulation of flavors has concentrated on preventing the flavor losses during spray drying and extending the shelf life of the products. The focus is on the effect of emulsion droplet size, powder size as well as the type of the model flavors. More specifically the aim is to understand the mechanism loss and oxidation of encapsulated flavor from the droplet which directly relate to the shelf life of product. The work is focused on the effect of water activity as well as the change of the capsule structure on the stability of encapsulated flavor. Furthermore, in order to understand the morphology of spray dried powder and encapsulated flavor powder, CLSM was used to view the cross sectional of the spray dried powder and the arrangement of encapsulated flavor in powder without the destruction of the powders.

2. Encapsulation of flavors by molecular inclusion in cyclodextrins

Cyclodextrin (CD) is a family of cyclic oligosaccharides with truncated molecular structure. The relatively hydrophobic cavity of CD provides a less polar microenvironment in CD solution for the appropriately-sized hydrophobic molecules to reside in. Their applications are mainly intended for the entrapment of smaller molecules, stabilization of reactive intermediates and drug delivery device as potential molecular transport. In food related applications, flavor compounds are being encapsulated into CDs for better retention and protection from various possible means of deterioration, as well as for controlled delivery. However, the inclusion and release characteristics of the guest molecules have not been fully understood. The aim of this study was to examine the effects of inclusion methods on the properties of inclusion complex powders especially with respect to flavor retention and release of the guest flavor compounds under various temperatures and relative humidities.

3. Crystal transformation of sugars by ethanol dehydration technique

α,α-Trehalose(α-D-glucopyranosyl α-D-glucopyranoside) is a non-reducing disaccharide found in many organisms as an energy source and protection of living cells from the damage caused by dryness. Since trehalose is also present at low concentration in various food we continually ingest this sugar as part of a
The main purposes for using trehalose are lowering of sweetness, moisture retention and prevention of starch retrogradation. Trehalose crystal exists in the dihydrate and polymorphic anhydrous crystalline forms, which exhibit complicated polymorphism depending on the given thermodynamic conditions. In this study, an innovative process to produce anhydrous crystal of trehalose with fine porosity using ethanol as the dehydration medium was explored. Using this method, a new type of porous crystal particle with a three-dimensional fine network structure could be obtained. The specific surface area of the anhydrous crystal transformed at 50 °C was 3.3 m²/g, with a median pore diameter of 0.21 μm, and void volume of 0.22 mL/g. The crystal transformation was monitored by measuring the crystal moisture content. The crystal transformation rates could be correlated with the Avrami equation, using the mechanism parameter n = 11.5, suggesting that the change of surface area occurred during crystal transformation from dehydrate to anhydrous trehalose. The activation energy of the crystal transformation rate was 132 kJ/mol.

**PUBLICATIONS**

Our study is focused on the microbial biotechnology for environmental and pharmaceutical industries. To improve useful functions of biocatalysts, microbial cells and enzymes, we use the techniques of metabolic engineering and protein engineering.

1) Biofuel production from lignocellulosic waste materials

We focus on the production of fuel ethanol from agricultural wastes and municipal solid waste by using unique alcohol-producing bacteria, *Zymomonas mobilis* and *Zymobacter palmae*. To breed the organisms that can convert cellulose to ethanol directly, the cellulolytic enzyme genes were introduced into the strains by metabolic engineering.

*NEPO Project: Development of Preparatory Basic Bioenergy Technology
“Genetic Engineering of a Novel Ethanologenic Bacterium for SSCF of Lignocellulosic Biomass”

2) Microbial deodorization of VOC (BTEX and HCHO)

BTEX (Benzene, Toluene, Ethylbenzene, and xylene) and formaldehyde are air pollutants and are harmful. We focus on the abilities to degrade various toxic VOC
(Volatile Organic Compounds) of microorganisms, and design a bioreactor filled with VOC-degrading strains and a biofilter immobilized with VOC-degrading enzymes.

3) **Microbial degradation of cyanide compounds and its application for bioremediation**

Large quantities of cyanide compounds are released into the environment as waste products of various industries related to metal plating, aluminum electrolysis, coal gasification, coal coking, and ore leaching. Because of its extreme toxicity, environmental cyanide pollution causes great damage to ecosystems. We isolate various microorganisms with the ability to degrade cyanide compounds, and characterize the enzymes catalyzing hydrolysis or oxidation of cyanide compounds.

4) **Screening of useful microbial enzymes for pharmaceutical industry**

The bacterium *Helicobacter pylori* associated with gastric diseases (gastritis and gastric cancer) is living in human gastric mucosa. Adherence to the gastric epithelial cells is mediated by fucosylated sugar chain of mucosa as a receptor. To remove *H. pylori* on the gastric epithelial cells, we are isolating enzymes destroying its receptor of bacterial adhesion.

**PUBLICATIONS**


