# Division of Environmental Chemistry
## - Molecular Microbial Science -

http://www.kuicr.kyoto-u.ac.jp/labos/bm2/Pages/lab.html

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### Visitor

Prof GLADYSHEV, Vadim N  Nebraska Redox Biology Center, University of Nebraska, USA, 30 May–30 June 2007

## Scope of Research

Structures and functions of biocatalysts, in particular, pyridoxal enzymes and enzymes acting on xenobiotic compounds, are studied to elucidate the dynamic aspects of the fine mechanism for their catalysis in the light of recent advances in gene technology, protein engineering and crystallography. In addition, the metabolism and biofunction of sulfur, selenium, and some other trace elements are investigated. Development and application of new biomolecular functions of microorganisms are also studied to open the door to new fields of biotechnology. For example, cold-adaptation mechanism and applications of psychrotrophic bacteria are under investigation.

## Research Activities (Year 2007)

### Presentations

Application of Cold-adapted Microorganisms and Cold-active Enzymes, Esaki N, Kurihara T, Kawamoto J, Miyake R, Yamamoto K, Wei Y, Kitayama K, International Conference on Biotechnology Engineering (ICBioE '07), 9 May 2007.

Strict Discrimination between Sulfur and Selenium by a Selenium-specific Enzyme, Selenocysteine Lyase, Esaki N, The 41st IUPAC World Chemistry Congress, 6 August 2007.

Physiological Role of Phospholipids Containing Eicosapentaenoic Acid in Cold Adaptation of an Antarctic Psychrotrophic Bacterium, *Shewanella livingstonensis* Ac10, Kurihara T, Kawamoto J, Sato S (Grad. Sch. Sci., Kyoto Univ.), Esaki N, Gordon Research Conference: Molecular and Cellular Biology of Lipids, 24 July 2007.

The Cellular Function of Selenocysteine Lyase in Selenoprotein Synthesis, Mihara H, Kurokawa S, Esaki N, TRACE ELEMENTS in DIET, NUTRITION, & HEALTH: Essentiality and Toxicity, 24 Octorber 2007.

Function of Selenium-specific Enzymes in Selenoprotein Biosynthesis, Mihara H, Kurokawa S, Omi R, Kurihara T, Miyahara I, Hirotsu K, Esaki N, International Symposium on Metallomics 2007, 1 December 2007.

### Grants

Esaki N, Investigation of Organisms Carrying a Unique Selenium Metabolism and Its Application to Bioremediation, Grant-in-Aid for Scientific Research (B), 1 April 2006–31 March 2008.

## Biosynthesis of Molybdopterin Requires Cysteine Desulfurase IscS

The persulfide sulfur formed on an active site cysteine residue of pyridoxal 5'-phosphate-dependent cysteine desulfurases is subsequently incorporated into the biosynthetic pathways of a variety of sulfur-containing cofactors and thionucleosides. It has been shown that three cysteine desulfurases IscS, CsdA (CSD) and SufS (CsdB) of *Escherichia coli* can transfer sulfur from L-cysteine to the C-terminal thiocarboxylate of the small subunit of molybdopterin (MPT) synthase in a defined *in vitro* system for the generation of the dithiolene group of MPT from precursor Z. Here we report that an *iscS*-deletion strain of *E. coli* accumulates compound Z, a direct oxidation product of precursor Z, to the same extent as a *ΔmoaD* strain. In contrast, analysis of the compound Z content of *ΔsufS* and *ΔcsdA* strains revealed no such accumulation. In addition, a genetic complementation experiment confirmed that the *in vivo* conversion of precursor Z to MPT requires IscS. These findings suggest that IscS but not SufS or CsdA is the physiological sulfur-donating enzyme for the generation of the thiocarboxylate of MPT synthase for the MPT biosynthesis (Figure 1). Furthermore, bioinformatic analysis shows that most of *moaD*/*moaE*-containing bacteria also carry at least one *iscS*-like group I cysteine desulfurase gene, suggesting that this type of system is widely distributed in bacteria.

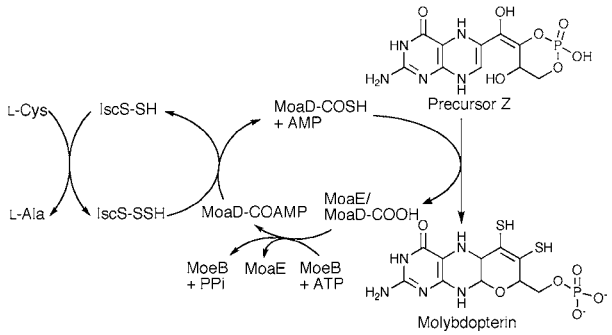


**Figure 1.** Proposed scheme for the biosynthesis of molybdopterin.

## Physiological Role of Eicosapentaenoic Acid in a Cold-adapted Bacterium, *Shewanella livingstonensis* Ac10

*Shewanella livingstonensis* Ac10, a psychrotrophic Gram-negative bacterium isolated from Antarctic seawater, grows at a temperature range of 4˚C to 25˚C. The bacterium produces eicosapentaenoic acid (EPA) as a component of phosphatidylglycerol and phosphatidylethanolamine at low temperatures. EPA constitutes about 5% of the total fatty acids of the cells grown at 4˚C. We found that five genes termed *orf2*, *orf5*, *orf6*, *orf7*, and *orf8* are essential for the production of EPA by targeted disruption of the respective genes. The mutant cells lacking EPA exhibited significant growth retardation at 4˚C, whereas they grew normally at 18˚C. Microscopic observation revealed that the EPA-less strains became filamentous at 4˚C, suggesting that they have a defect in cell division (Figure 2). We analyzed the fluidity of the cell membrane at low temperatures by using pyrene as a fluorescence probe: the diffusion rate of pyrene is regarded as an index of the membrane fluidity. We found that the diffusion rate of pyrene in the membrane from the EPA-less strain was not significantly different from that in the membrane from the parent strain. The results suggest that EPA has a physiological function other than the function to maintain the membrane fluidity. Proteomic analysis of the membrane proteins revealed that the amounts of five proteins were decreased and the amounts of three proteins were increased by the absence of EPA. The cold-sensitive phenotype of the EPA-less strains may be ascribed to a defect in the function of these membrane proteins.


**Figure 2.** Fluorescence microscopic images of the EPA-less mutant of *S. livingstonensis* Ac10. DAPI and FM4-64 were used to stain DNA and the membrane, respectively.

Esaki N, Structure-Function Analysis of Selenium-specific Chemical Conversion System and Co-translational Insertion of Selenium into Protein, Grant-in-Aid for Scientific Research (B), 1 April 2007–31 March 2009.

Kurihara T, Conversion of Organofluorine Compounds with Microbial Enzymes: Mechanistic Analysis of the Enzyme Reactions and Their Application to Production of Useful Compounds and Bioremediation of Environments, Grant-in-Aid for Scientific Research (B), 1 April 2005–31 March 2008.

Kurihara T, Exploration of Novel Cold-adapted Microorganisms to Develop a System for the Production of Useful Compounds at Low Temperatures, Grant-in-Aid for Scientific Research (B), 1 April 2007–31 March 2009.

Mihara H, Studies on Mechanism of Selenium-specific Recognition and Selenoprotein Biosynthetic Machinery, Grant-in-Aid for Young Scientists (B), 1 April 2006–31 March 2008.