Division of Environmental Chemistry
– Molecular Microbial Science –

http://www.scl.kyoto-u.ac.jp/~mmsicr/mmstojp/Top_en.html

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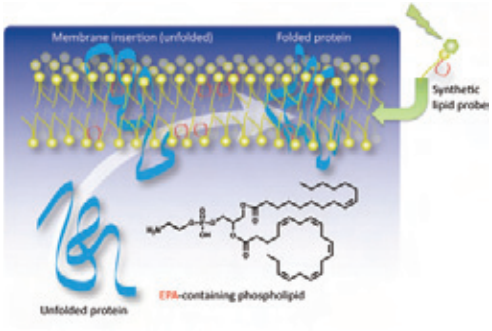
Visitors

Prof KROL, Alain — Centre National de la Recherche Scientifique (CNRS), France, 9 February
Prof ARNER, Elias — Department of Medical Biochemistry and Biophysics, Karolinska Institutet, Sweden, 8 June

Scope of Research

Microorganisms are found almost anywhere on Earth. They have a great diversity of capacities to adapt to various environments including chemically and physically unusual environments. Our main subject is to clarify the molecular basis of environmental adaptation of microorganisms and their application. Specific functions of proteins and lipids that play essential roles in environmental adaptation of extremophilic microorganisms are of our particular interest. Mechanistic analysis of microbial enzymes, in particular those involved in unique metabolic pathways, and their application are also undertaken.



KEYWORDS

Molecular Microbial Science
Biochemistry
Psychrotroph
Polyunsaturated Fatty Acids
Bioengineering

Selected Publications

Zhang W, Urban A, Mihara H, Leimkuhler S, Kurihara T, Esaki N: IscS Functions as a Primary Sulfur-donating Enzyme by Interacting Specifically with MoeB and MoaD in the Biosynthesis of Molybdopterin in *Escherichia coli*, *J Biol Chem*, **285,** 2302-2308 (2010).

Toyoda M, Jitsumori K, Mikami B, Wackett LP, Kurihara T, Esaki N: Crystallization and Preliminary X-ray Analysis of L-Azetidine-2-Carboxylate Hydrolase from *Pseudomonas* sp. Strain A2C, *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.*, **66,** 801-804 (2010).

Omi R, Kurokawa S, Mihara H, Hayashi H, Goto M, Miyahara I, Kurihara T, Hirotsu K, Esaki N: Reaction Mechanism and Molecular Basis for Selenium/sulfur Discrimination of Selenocysteine Lyase, *J. Biol. Chem.*, **285,** 12133-12139 (2010).

Mowafy AM, Kurihara T, Kurata A, Uemura T, Esaki N: 2-Haloacrylate Hydratase, a New Class of Flavoenzyme that Catalyzes the Addition of Water to the Substrate for Dehalogenation, *Appl Environ Microbiol*, **76,** 6032-6037 (2010).

Kawamoto J, Kurihara T, Yamamoto K, Nagayasu M, Tani Y, Mihara H, Hosokawa M, Baba T, Sato SB, Esaki N: Eicosapentaenoic Acid Plays a Beneficial Role in Membrane Organization and Cell Division of a Cold-adapted Bacterium, *Shewanella livingstonensis* Ac10, *J. Bacteriol.*, **191,** 632-640 (2009).

Studies on Dynamics of Eicosapentaenoic-acid-containing Phospholipids by Using Fluorescence-labeled Ether Phospholipids

Shewanella livingstonensis Ac10, a psychrotrophic Gram-negative bacterium isolated from Antarctic seawater, grows at a temperature range from 4˚C to 25˚C. This bacterium produces eicosapentaenoic acid (EPA), a long-chain polyunsaturated fatty acid, as an acyl component of membrane phospholipids at low temperatures close to 0˚C. We identified the genes required for synthesis of EPA and disrupted them to obtain EPA-less mutants. The mutants lacking EPA showed significant growth retardation at 4°C but not at 18°C. Supplementation of a synthetic phosphatidylethanolamine containing EPA at the *sn*-2 position complemented the growth defect. The EPA-less mutant became filamentous, and multiple nucleoids were observed in a single cell at 4°C, indicating that the mutant has a defect in cell division.

To analyze the physiological function of EPA, we synthesized fluorescence-labeled phospholipids shown in Figure 1 as molecular probes to visualize the localization of EPA-containing phospholipids. In AcylalkylEPA and DialkylEPA, the eicosapentaenyl group is bound to the glycerol backbone of the phospholipid by an ether bond to prevent separation of EPA and the fluorescence group by hydrolysis *in vivo*. These fluorescent phospholipids were added to the EPA-less mutant of *S. livingstonensis* Ac10, and the cells were grown at 4–7˚C for fluorescence microscopic analysis. When AcylalkylEPA was used, fluorescence was localized between two nucleoids at the center of the cells during cell division, suggesting that EPA-containing phospholipids are involved in this cellular process (Figure 2).

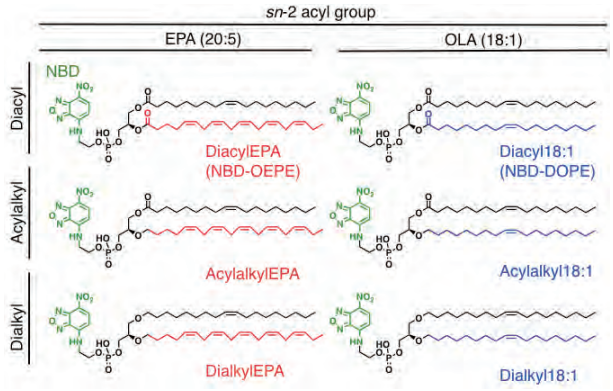


Figure 1. Fluorescent phospholipids synthesized in this study to analyze the physiological role of EPA.

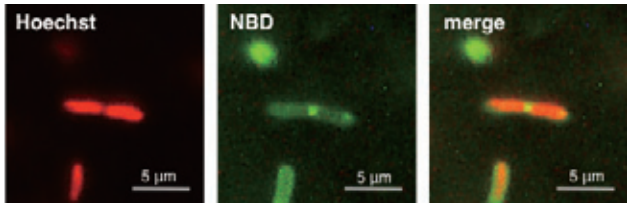


Figure 2. Fluorescence microscopic images of an EPA-less mutant cell that incorporated AcylalkylEPA. Nucleoids were stained with Hoechst.

Physiological Role of Eicosapentaenoic-acid-containing Phospholipids in the Folding of a Cold-inducible Membrane Protein of a Psychrotrophic Bacterium, *Shewanella livingstonensis* Ac10

Polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid, are known to have various beneficial effects on human health. PUFAs existing as acyl components of membrane phospholipids alter the physicochemical properties of lipid bilayers, including permeability, curvature, fluidity, and thickness, and affect the function of membrane proteins. However, molecular mechanisms how PUFAs influence the functions of membrane proteins are not well understood. A cold-adapted microorganism, *Shewanella livingstonensis* Ac10 isolated from Antarctic seawater, produces EPA as an acyl chain of its membrane phospholipids at 4˚C. In order to elucidate the physiological role of EPA at low temperatures, we performed *in vitro* reconstitution of a cold-inducible membrane protein, OmpC74, with the liposomes containing or not containing EPA and analyzed the effect of the presence of EPA on the folding of OmpC74. The larger amounts of folded OmpC74 were observed in the liposomes containing EPA than those without EPA. Circular dichroism analysis indicated that OmpC74 rapidly interacts with the membrane surface and forms ß-sheet structures in the EPA-containing liposome at 4˚C. These results suggest that EPA-containing phospholipids play a role as a molecular chaperone in the membrane insertion and folding of OmpC74 at low temperatures.

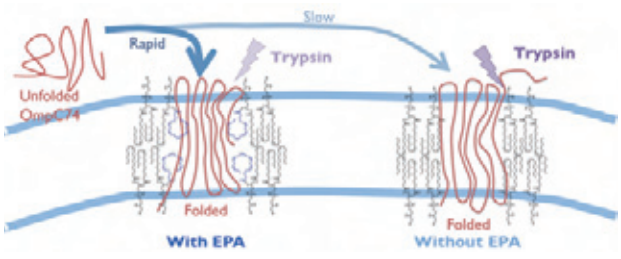


Figure 3. EPA-containing phospholipids accelerate the membrane insertion and folding of a cold-inducible outer membrane protein, OmpC74, at low temperatures.