Division of Environmental Chemistry
– Molecular Microbial Science –

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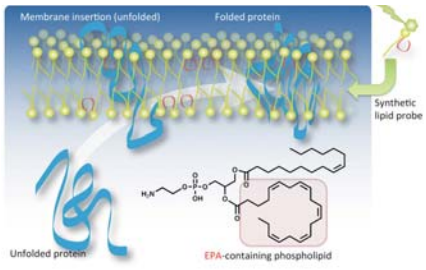
Scope of Research

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

Microbiology
Extremophiles
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Membrane Proteins
Microbial Metal Respiration

Selected Publications

Fukuyama, S.; Mihara, H.; Miyake, R.; Ueda, M.; Esaki, N.; Kurihara, T., Characterization of a Thermostable 2,4-diaminopentanoate Dehydrogenase from *Fervidobacterium nodosum* Rt17-B1, *Journal of Bioscience and Bioengineering*, **117,** 551-556 (2014).
Park, J.; Yamaura, T.; Kawamoto, J.; Kurihara, T.; Sato, S. B., Reciprocal Modulation of Surface Expression of Annexin A2 in a Human Umbilical Vein Endothelial Cell-derived Cell Line by Eicosapentaenoic Acid and Docosahexaenoic Acid, *PloS one*, **9,** e85045 (2014).
Imai, T.; Kurihara, T.; Esaki, N.; Mihara, H., Glutathione Contributes to the Efflux of Selenium from Hepatoma Cells, *Bioscience, Biotechnology, and Biochemistry*, **78,** 1376-1380 (2014).
Kawamoto, J.; Kurihara, T., Proteins and Lipids of Cold-Adapted Microorganisms, *CSJ Current Review*, **17,** 55-61 (2014).
Kurihara, T.; Kawamoto, J., Chemical Approach to Analyze the Physiological Function of Phospholipids with Polyunsaturated Fatty Acyl Chain, *Yakugaku Zasshi : Journal of the Pharmaceutical Society of Japan*, **134,** 507-513 (2014).

Studies of the Microdomian Formation in Biological Membranes of an Antarctic Bacterium

A cold-adapted microorganism, *Shewanella livingstonensis* Ac10, was isolated from Antarctic seawater. This bacterium produces a kind of long-chain polyunsaturated fatty acid, eicosapentaenoic acid (EPA), which is introduced to *sn*-2 position of membrane phospholipids. By using a fluorescence-labeled phospholipid containing eicosapentaenyl group, we found that this strain forms membrane microdomain structure composed of EPA-containing phospholipids, which might regulate the function of cell division proteins at low temperatures. To analyze the mechanism of the microdomain formation, we developed monoclonal antibodies raised against EPA-biosynthesis enzymes of *S. livingstonensis* Ac10 through hybridoma technology and analyzed their subcellular localization at low temperatures by immunofluorescence staining. Orf5, a scaffold protein for EPA synthesis, was localized at the cell division site and cell pole of *S. livingstonensis* Ac10 grown at 4 ˚C. When Orf8, a putative enoyl reductase, was analyzed, we observed the same localization as Orf5. These results suggest that Orf5 and Orf8 colocalize at the cell division site and act as protein complex to contribute to the formation of EPA-containing microdomain at cell division site.

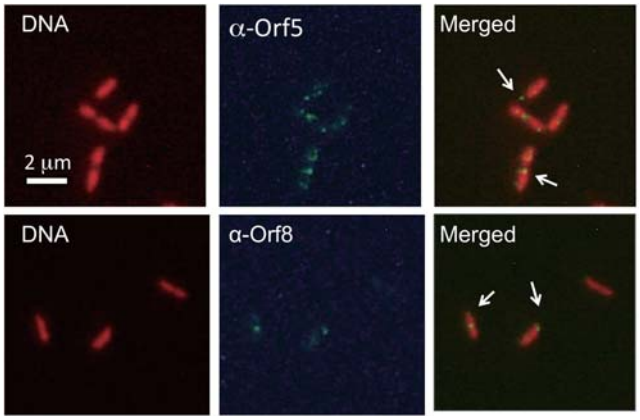

Figure 1. Subcellular localization of EPA-biosynthesis enzymes, Orf5 and Orf8. Fluorescence microscopic images of the cells stained with Hoechst 33342 (false-colored red) (left), immunostained with anti-Orf5 and Orf8 monoclonal antibody as a primary antibody and visualized with a secondary Alexa-488 conjugated anti-mouse antibody (center), and merged images (right). Arrows indicate the position of Orf5 and Orf8, respectively.

Development of a Novel Lipophilic Probe for Functional Analysis of Bioactive Fatty Acids in Human Vein Endothelial Cells

Oleic acid, a kind of monounsaturated fatty acids, is commonly found in olive oil and fish oil and is known to be a bioactive compound that decreases risk of hypertension and various vascular disease of human. However, it remains unclear how oleic acid exerts its physiological function at molecular level in biological membrane. In this study, we synthesized a novel fatty acid probe, oleic acid containing an ω-ethynyl group (click18:1), which is applicable to *in vivo* postlabeling by click chemistry with azide compounds containing fluorescent group and crosslinking reagent. Here, we used human umbilical vein endothelial cells (HUVEC) as a model and attempted to identify the proteins covalently modified with oleic acid in human vascular cells. The crude extracts from HUVEC cultivated with click18:1 were applied to click chemistry with azide biotin. Biotinylated proteins via click18:1 were purified with streptavidin-conjugated beads. Purified proteins were identified by MALDI-TOF MS and following PMF analysis. As the result, three proteins were identified as novel proteins modified with oleic acid.

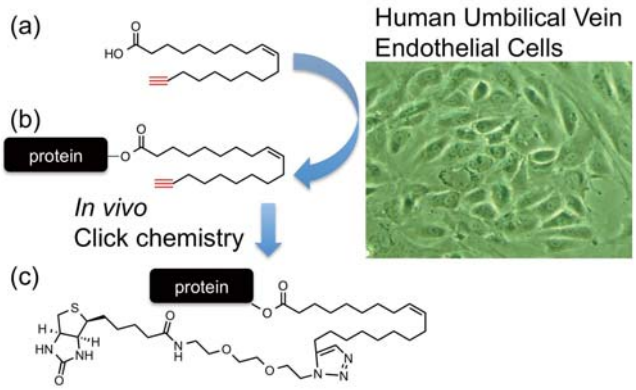

Figure 2. Exploration of oleic acid-modified proteins in human vascular endothelial cells by click chemistry. Oleic acid containing an ω-ethynyl group (click18:1 (a)) was incorporated into HUVEC and recruited to protein lipidation (b). By click chemistry with azide biotin, lipidated proteins were biotinylated and applied to affinity purification with streptavidin-conjugated beads (c).