Title	<Division of Environmental Chemistry> Molecular Microbial Science
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Citation	ICR annual report (2007), 13: 34-35
Issue Date	2007-03
URL	http://hdl.handle.net/2433/65532
Right	
Type	Article
Textversion	publisher

Division of Environmental Chemistry
- Molecular Microbial Science -

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

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Prof LIU, Hung-wen University of Texas at Austin, USA, 2 June–25 July 2006

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 2006)

Presentations

Psychrotrophic Bacteria: Cold-Adaptation Mechanism and Applications, Esaki N, Kurihara T, Kawamoto J, Miyake R, Kitagawa M (Takara Bio Inc.), Kato I (Takara Bio Inc.), International Conference on Alpine and Polar Microbiology, 27 March.

A Comparative Study between Selenocysteine Lyase and Cysteine Desulfurase, Mihara H, Kurokawa S, Omi R, Kurihara T, Miyahara I (Osaka City Univ.), Hirotsu K (Osaka City Univ.), Esaki N, 8th International Sympo. Selenium in Biology and Medicine, 26 July.

Cold-adaptation Mechanism of a Psychrotrophic Bacterium, *Shewanella livingstonensis* Ac10, and Its Applications, Kurihara T, Kawamoto J, Miyake R, Nagayasu M, Tani Y, Inomoto Y, Yamamoto K, Kitagawa M (Takara Bio Inc.), Kato I (Takara Bio Inc.), Esaki N, Extremophiles 2006, 20 September.

Grants

Esaki N, Dynamics of an Essential Trace Element, Selenium, in Mammals and the Molecular Basis of Selenoprotein Biosynthesis, Grant-in-Aid for Scientific Research (B), 1 April 2005–31 March 2007.

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.

Kurihara T, Conversion of Organofluorine Compounds with Microbial Enzymes: Mechanistic Analysis of the Enzyme Reactions and Their Application to Production of

The Cellular Function of Selenocysteine Lyase in Selenoprotein Synthesis

Enzymatic discrimination between selenium compounds and the corresponding sulfur compounds is important for cells to metabolize selenium compounds without interference by sulfur metabolism. Mammalian selenocysteine lyase (SCL) is a pyridoxal 5'-phosphate (PLP)-dependent enzyme that specifically acts on L-selenocysteine to yield L-alanine and selenium. The physiological relevance of the selenium-specific action of the enzyme, however, has remained unclear. To address the role of SCL in mammalian cells, we have used RNA-interference (RNAi) to deplete SCL and thereby assess its function in cell lines. We found that decreasing the level of SCL in HeLa cells results in significant reduction in protein levels of cytosolic glutathione peroxidase (cGPx) and activities of cGPx and thioredoxin reductase (TrxR). We also found that RNAi-mediated reduction of SCL induces cell growth inhibition even in the presence of selenomethionine, selenocysteine, selenite, or FBS in a serum-free medium. This result provides new insights into possible pathways for selenite metabolism. Overexpression of mouse SCL in HeLa cells elevated the activity of cGPx, suggesting that SCL is a rate-limiting enzyme in the synthesis of selenoproteins. These results demonstrate for the first time an essential role of SCL in selenoprotein biosynthesis in mammalian cells.

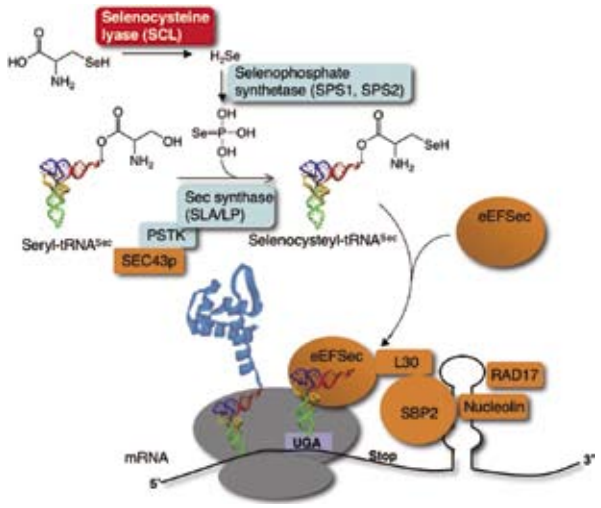

Figure 1. A proposed function of SCL in selenoprotein biosynthesis.

Construction of a Protein Expression System Operating at Low Temperatures by Using a Cold-Adapted Bacterium as the Host

Recombinant protein expression system working at low temperatures is expected to be useful for the production of thermolabile proteins as well as toxic enzymes whose activity can be suppressed by decreasing the temperature. We constructed a low-temperature expression system by using an Antarctic cold-adapted bacterium, *Shewanella livingstonensis* Ac10, as the host. We identified proteins abundantly produced at 4°C in this bacterium by two-dimensional gel electrophoresis and evaluated the promoters for these proteins to express foreign proteins. We used 27 promoters and a broad-host-range vector, pJRD215, to produce β-lactamase in *S. livingstonensis* Ac10. Maximum yield was obtained when the promoter for putative alkyl hydroperoxide reductase, AhpC, was employed and the recombinant cells were grown to the late stationary phase. The yield was 91 mg/L-culture at 4˚C and 139 mg/L-culture at 18˚C. We used this system to produce putative peptidases, PepF, LAP, and PepQ, and a putative glucosidase, BglA, from a psychrophilic bacterium, *Desulfotalea psychrophila* DSM12343. We obtained 48, 7.1, 28, and 5.4 mg/L-culture of these proteins, respectively, in a soluble fraction. The amount of PepF and PepQ produced by this system was higher than that produced by the *Escherichia coli* T7 promoter system, which is regarded as one of the most powerful protein expression systems currently available. This system would greatly contribute to fundamental and application studies of a number of proteins that can not be overproduced by conventional protein expression systems.

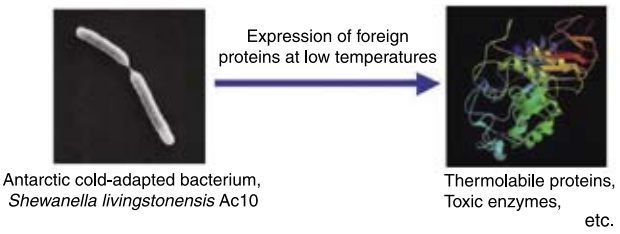

Figure 2. Protein expression at low temperatures by using an Antarctic bacterium, *S. livingstonensis* Ac10 as the host.

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 that Inhabit the Polar Regions and Investigation of Their Useful Enzymes, Grant-in-Aid for Scientific Research (B), 1 April 2005–31 March 2007.

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.