Division of Environmental Chemistry
- Molecular Microbial Science -

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

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Prof JHEE, Kwang-Hwan Department of Applied Chemistry, Kumoh National Institute of Technology, Korea, 27 February 2008–17 February 2009

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 2008)

Presentations

Sulfur Trafficking in Biosynthesis of Iron-Sulfur Cluster, Mihara H, International Symposium on Chemistry of Reductases, 11 March 2008.

Physiological Role of Eicosapentaenoic acid in a Psychrotroph *Shewanella livingstoensis* Ac10, Esaki N, Kawamoto J, Sato S, Kurihara T, Hosokawa M, Baba T, Sato SB, 3rd International Conference on Polar and Alpine Microbiology, 11 May 2008.

Cold-adaptation Mechanisms and Applications of an Antarctic Psychrotrophic Bacterium, *Shewanella livingstonensis* Ac10, Kurihara T, Esaki N, TBIT's 1st Annual World Congress of ibio-2008, 19 May 2008.

Enzyme Engineering and Microbial Technology for Biocatalysis, Esaki N, 2008 International Symposium and Annual Meeting, 26 June 2008.

A Novel Flavoenzyme Involved in Bacterial Metabolism of 2-Chloroacrylate, Kurihara T, Mowafy A M, Fujita M, Kurata A, Esaki N, 4th Japan-Finland Biotechnology Symposium, 2 October 2008.

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.

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.

Enzymatic Synthesis of (*S*)-2-Chloropropionate by Asymmetric Reduction of 2-Chloroacrylate with 2-Haloacrylate Reductase Coupled with Glucose Dehydrogenase

Asymmetric reduction of carbon-carbon double bonds is one of the most widely used methods for the production of chiral compounds useful as pharmaceuticals, agrochemicals, and so on. Accordingly, enzymes catalyzing this type of reaction have been attracting a great deal of attention from the industrial point of view. We found a novel NADPH-dependent enzyme catalyzing the asymmetric reduction of a carbon-carbon double bond of 2-haloacrylate from 2-chloroacrylate (2-CAA)-assimilating bacterium, *Burkholderia* sp. WS. The enzyme, named 2-haloacrylate reductase, catalyzes the stereospecific conversion of 2-chloroacrylate into (*S*)-2-chloropropionate ((*S*)-2-CPA), which is useful as a chiral synthon for the synthesis of phenoxypropionic acid herbicides. (*S*)-2-CPA is synthesized by optical resolution of a racemic mixture of 2-chloropropionate by a conventional method, in which (*R*)-2-chloropropionate of a racemic mixture is selectively degraded with (*R*)-2-haloacid dehalogenase. However, the theoretical maximum yield of this method is 50%, and a new procedure for the production of (*S*)-2-CPA superior to the conventional method in terms of conversion yield is expected. We constructed a system for asymmetric reduction of 2-CAA to produce (*S*)-2-CPA with recombinant *Escherichia coli* cells producing 2-haloacrylate reductase from *Burkholderia* sp. WS and glucose dehydrogenase from *Bacillus subtilis* for regeneration of NADPH (Figure 1). The system provided 37.4 g/*l* (*S*)-2-chloropropionate in more than 99.9% *e.e.*

The *iscS* Gene Deficiency Affects the Expression of Pyrimidine Metabolism Genes

Inactivation of *iscS* encoding cysteine desulfurase results in a slow growth phenotype associated with the deficiency of iron–sulfur clusters, thiamine, NAD, and tRNA thionucleosides in *Escherichia coli*. By using differential screening strategies, we identified 2 pyrimidine salvage enzymes, namely, uridine phosphorylase and cytidine deaminase, which were down-regulated in the *iscS* mutant (Figure 2). Both enzymes are positively regulated by the cAMP receptor protein. We also identified a novel protein complex, namely, YeiT–YeiA, whose expression level was decreased in the *iscS* mutant. The recombinant YeiT–YeiA complex exhibited NADH-dependent dihydropyrimidine dehydrogenase activity, indicating its role in pyrimidine metabolism. These results provide a clue to the possible role of *iscS* in pyrimidine metabolism by gene regulation.

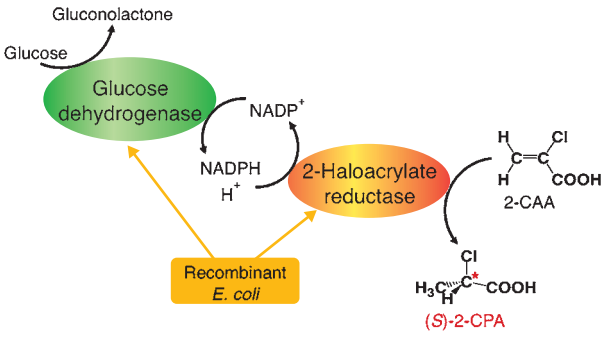

Figure 1. Enzymatic synthesis of (*S*)-2-chloropropionate by asymmetric reduction of 2-chloroacrylate with 2-haloacrylate reductase coupled with glucose dehydrogenase.

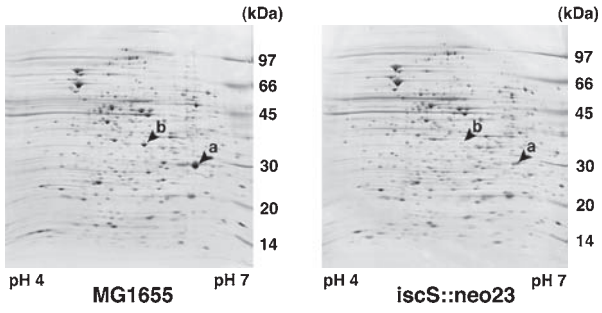

Figure 2. 2-Dimensional electrophoresis of crude extracts of MG1655 (left panel) and iscS::neo23 (right panel).

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.

Kurihara T, Analysis of the Molecular Basis for Cold Adaptation of Psychrotrophic Bacteria, Grant-in-Aid for Scientific Research (B), 1 April 2008–31 March 2011.

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.