Molecular Biofunction -Molecular Microbial Science-

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Dr Marinus Pilon	Colorad State University, USA, 20 May 2001 - 15 Jun 2001
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Mr Valdez Michael Corpus	University of Santo Tomas, Phillippines, 7 November 2001 - 30 November 2001

Scope of Research

Structure and function 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, molecular structures and functions of psychrophilic enzymes and their application are under investigation.

Research Activities (Year 2001)

Presentations

Purification and characterization of glycine dehydrogenase from *Bacillus megaterium* MN223, Nakano M, Kurihara T, Esaki N, 2001 Annual Meeting, Jpn. Soc. Biosci, Biotech, and Agrochem., 24 March.

D-Amino acid metabolism of Yeasts, Yow GY, Ohkubo F, Yoshimura T, Esaki N. Annual Meeting, Jpn. Soc. Biosci, Biotech, and Agrochem., 24 March. 2001 Annual Meeting, Jpn. Biochem. Soc., 27 October.

Structural analysis of fluoroacetate dehalogenase from *Burkholderia* sp., Takahata M, Kurihara T, Esaki N, Annual Meeting, Jpn. Soc. Biosci, Biotech, and Agrochem., 24 March.

Enzymes involved in the formation of active sulfur and selenium, Mihara H, Kato S, Kurihara T, HataY, Esaki N, 2001 Annual Meeting, Jpn. Biochem.Soc., 25 and 27 October.

Grants

Esaki N, Development and application of new psychrophilic enzymes, Grant-in-Aid for Scientific Research on Priority Areas (B), 1 April 2000 - 31 March 2001.

Kurihara T, Structures, functions and physiological roles of enzymes catalyzing formation of sulfur- and selenium-containing biofactors, Grant-in-Aid for Encouragement of Young Scientists, 1 April 2000 - 31 March 2002.

Yoshimura T, Development of new methods for recovery and elimination of trace amount of substances in water by phage display system, Grant-in-Aid for Exploratory Research, 1 April 2000 - 31 March 2002.

Esaki N, Structural biology and biosynthesis of selenium-containing proteins, Grant-in-Aid for Scientific Research (B), 1 April 2001 - 31 March 2002.

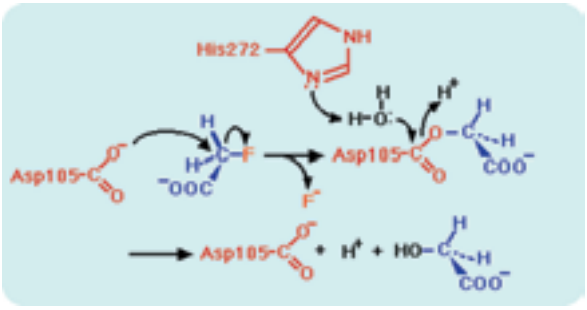
Esaki N, Construction and functional analysis of composite biocatalysts, Grant-in-Aid for Scientific Reseach on Priority Areas (B), 1 April 2001 - 31 March 2003.

Esaki N, Determination of whole genome sequence of psychrophilic bacteria, analysis of genes involved in their adaptation to cold environments, and exploitation of cold-active enzymes, Grant-in-Aid for Scientific Re-

Topics

Probing Active-site Environment of Fluoroacetate Dehalogenase by Catalysis-Linked Inactivation with Ammonia

Fluoroacetate dehalogenase(FAc-DEX; EC 3.8.1.3) from *Moraxella* sp. B catalyzes the hydrolytic dehalogenation of fluoroacetate and other haloacetates to produce glycolate. FAc-DEX is the only enzyme that catalyzes the degradation of an aliphatic fluorinated compound by cleaving the carbon-fluorine bond, whose dissociation energy is among the highest in natural compounds. Asp105 of the enzyme acts as a nucleophile to attack the α-carbon of haloacetate to form an ester intermediate, which is subsequently hydrolyzed by a water molecule activated by His272 (Scheme 1). We found that FAc-DEX is inactivated concomitantly with defluorination of fluoroacetate by incubation with ammonia. Mass spectrometric analyses revealed that the inactivation of FAc-DEX was caused by nucleophilic attack of ammonia on the ester intermediate to convert the catalytic residue, Asp105, into an asparagine residue. The results indicates that ammonia reacted the active site of FAc-DEX without losing its nucleophilicity.

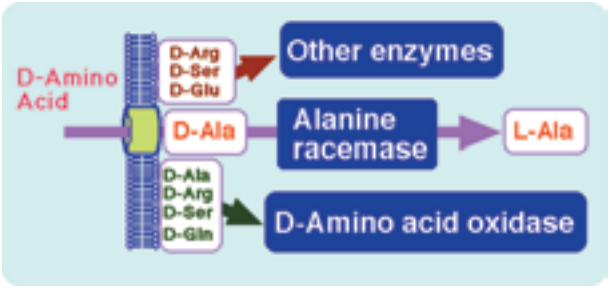


Scheme 1

Analysis of the three-dimensional structure of the enzyme by homology modeling showed that the active site of the enzyme is mainly composed of hydrophobic (Phe35, Tyr148, Trp151, Tyr213, and Phe273) and basic (His104, Arg106, Arg109, and His272) residues, which are considered to be essential for an ammonia molecule to retain its nucleophilicity. In a normal enzyme reaction, the hydrophobic environment is supposed to prevent hydration of the highly electronegative florine atom of the substrate and contribute to fluorine recognition by the enzyme. Basic residues probably play a role in counterbalancing the electronegativity of the substrate.

Functional characterization of Alanine racemase from *Schizosaccharomyces pombe*

Recent discovery of the physiological importance of D-amino acids has attracted much interest in the study of metabolism of D-amino acids in eucaryotic cells. We found that fission yeast, *Schizosaccharomyces pombe*, has several enzymes acting on D-amino acids (Scheme 2). We here describe one of these enzymes, alanine racemase, encoded by the *alr1*$^+$ gene. We cloned the *alr1*$^+$ gene in *E. coli* and purified the gene product (Alr1p) with an *Mr* of 41,590 to homogeneity. Arl1p contains pyridoxal 5'-phosphate as a coenzyme and catalyzes racemization of alanine. The enzyme is almost specific to alanine, but L-serine are racemized slowly at rate of 3.7 % of that of L-alanine. *S. pombe* uses D-alanine as a sole nitrogen source, but deletion of the *alr1*$^+$ gene resulted in retarded growth on the same medium. *Saccharomyces cerevisiae* differes markedly from *S. pombe*: *S. cerevisiae*



Scheme 2. **D**-Amino acid metabolism in *S.pombe*

uses L-alanine but not D-alanine as a sole nitrogen source.

Moreover, D-alanine is toxic to *S. cerevisiae*. However, heterologous expression of the *alr1*$^+$ gene enabled *S. cerevisiae* to grow efficiently on D-alanine as a sole nitrogen source. The recombinant yeast was relieved from the toxicity of D-alanine.

search (B)1 April 2001 - 31 March 2002.

Mihara H, Analyses of *suf* gene cluster and Suf proteins involved in iron transport, Grant-in-Aid for Encouragement of Young Scientists, 1 April 2001 - 31 March2002.

Kurihara T, Production of useful compounds and bioremediation of environments by cryobiotechnology using cold-adapted microorganisms, (NEDO), 1 April 2001 - 31 March 2003.

Kurihara T, *In vivo* and *in vitro* analysis of selenium metabolism – a multidisciplinary approach, Cooperative Research under the Japan-U.S. Cooperative Science Program(JSPS) 1 April 2001 - 31 March 2003.