Molecular Biofunction
- Molecular Microbial Science -

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Visitors

Dr PILON, Marinus — Colorado State University, USA, 1 November 2003 - 6 November 2003
Dr PILON-SMITS, Elizabeth — Colorado State University, USA, 1 November 2003 - 6 November 2003

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 2003)

Presentations

Structure and function of a novel enzyme catalyzing asymmetric reduction of a carbon-carbon double bond, Kurata A, Kurihara T, Kamachi H, Esaki N, 2003 Annual Meeting, Jpn. Soc. Biosci. Biotech. Agrochem., 1 April.

Regulation of the activity of eukaryotic serine racemase by ATP and Mg^{2+}, Yoshimura T, Okubo F, Wu H, Esaki N. 2003 Annual Meeting, Jpn. Soc. Biosci. Biotech. Agrochem., 1 April.

Construction of protein overproduction system using a cold-adapted microorganisms as a host, Kurihara T, Wei Y, Hayashi M, Esaki N. 2003 Annual Meeting, Soc. Biotech. Jpn., 16 September.

Identification of iron-sulfur proteins by differential screening using an IscS-deficient strain, Yamane M, Mihara H, Kurihara T, Takahashi Y, Yoshimura T, Esaki N, 2003 Annual Meeting, Jpn. Biochem. Soc., 16 October.

Grants

Esaki N, Construction and functional analysis of composite biocatalysts, Grant-in-Aid for Scientific Research on Priority Areas (B), 1 April 2001 - 31 March 2004.

Esaki N, Elucidation of the mechanisms of activation of an essential trace element, selenium, and its co-translational incorporation into polypeptide chains, Grant-in-Aid for Scientific Research (B), 1 April 2003 - 31 March 2005.

Esaki N, Isolation of novel cold-adapted microorganisms and exploitation of useful gene resources, Grant-

* Associate professor Tohru Yoshimura transferred to Nagoya University on August 1, 2003.

Protein Overproduction at Low Temperatures with a Cold-Adapted Microorganism

We have constructed a novel host-vector system for the production of proteins at low temperatures using a cold-adapted microorganism as a host. This system is particularly useful for the production of thermolabile proteins.

A cold-adapted bacterium, *Acinetobacter* sp. strain no. 6, was isolated from Siberian tundra soil. The bacterium grew well at low temperatures as low as 4°C. We have constructed an *Escherichia coli* - *Acinetobacter* sp. strain no. 6 shuttle vector by using a replicon from a plasmid isolated from a mesophilic *Acinetobacter calcoaceticus* strain and established an efficient transformation method by electroporation. A powerful promoter that functions in this cold-adapted bacterium was isolated by screening of the genomic DNA library with a promoter probe vector harboring a β-lactamase gene as a reporter. When a thermolabile α-amylase gene from a cold-adapted bacterium, *Shewanella* sp. Ac10, was expressed under the control of the newly isolated promoter, the recombinant *Acinetobacter* sp. strain no. 6 secreted the α-amylase, which amounted to about 35% of the total extracellular proteins. This makes a striking contrast to an *E. coli* expression system, which did not produce a detectable amount of the thermolabile α -amylase.

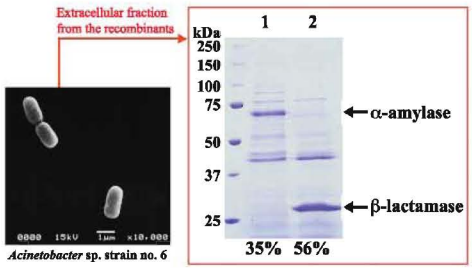

Figure 1. Production of extracellular α-amylase (lane 1) and β-lactamase (lane 2) at 7°C by recombinant *Acinetobacter* sp. strain no. 6.

Thiolation of tRNA Catalyzed by IscS and MnmA

Escherichia coli tRNA contains nucleosides modified with sulfur. A sulfur-containing nucleoside, 5-methylaminomethyl-2-thiouridine (mnm^5s^2U), is found at the wobble position 34 of $tRNA^{Lys}$, $tRNA^{Glu}$, and $tRNA^{Gln}$. The 2-thio group of 2-thiouridine stabilizes the anticodon structure, confers ribosome-binding ability to tRNA and improves reading frame maintenance. Recent studies showed that the biosynthesis of 2-thiouridine requires both MnmA and cysteine desulfurase IscS. However, the mechanism of the thiolation reaction has not been clarified. We cloned the *mnmA* gene from *E. coli* and overproduced and purified the gene product. The purified protein exists as monomers and dimers when determined by gel filtration chromatography. The protein catalyzed the incorporation of ^{35}S from [^{35}S]-L-cysteine into $tRNA^{Lys}$ in the presence of IscS and ATP. We also found that MnmA had the ATP hydrolysis activity and that ATP protected MnmA from trypsinolysis, suggesting the presence of an ATP-binding site in MnmA. These results indicated that MnmA and IscS catalyze the synthesis of s^2U in tRNA by using ATP and L-cysteine.

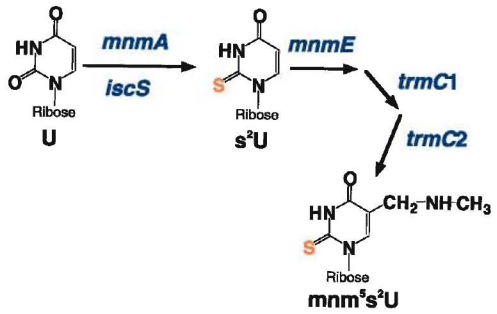

Figure 2. Formation of mnm^5s^2U

in-Aid for Scientific Research (B), 1 April 2003 - 31 March 2005.

Yoshimura T, Physiological role of D-amino acids in eukaryote, Grant-in-Aid for Scientific Research (C), 1 April 2002 - 31 March 2004.

Kurihara T, Bioconversion of fluorinated organic compounds: catalytic mechanisms of elimination and incorporation of fluorine and their application, Grant-in-Aid for Young Scientists (A), 1 April 2002 - 31 March 2005.

Mihara H, Mechanisms of incorporation of sulfur and selenium into the anticodon wobble bases of tRNAs, Grant-in-Aid for Young Scientists (B), 1 April 2003 - 31 March 2006.

Kurihara T, Production of useful compounds and bioremediation of environments by cryobiotechnology using cold-adapted microorganisms, (NEDO), 1 April 2001 - 31 March 2004.

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 2004.