

E021

### Functional Complementation of *E. coli* *glnA* Mutation by Mammalian Glutamine Synthetase

Minsuk Kwon\*, Kyung-min Kim, and Chankyu Park  
*Korea Advanced Institute of Science and Technology*

Exact cellular role of mammalian glutamine synthetase (GS) has not been clearly elucidated due to its biased occurrences in various cell types as well as its presence as various isoforms. Previously, we characterized alternative splicing of the canine glutamine synthetase, leading to an identification of alternative translated enzyme with an extension of N-terminal 40 amino acids. The two forms of GS are different in its affinities to substrate and competitive inhibitor MSOX. In order to further understand their physiological roles *in vivo*, we expressed the corresponding genes of their enzymes in GS-deficient mouse myeloma cell line (NS0) and *E. coli* *glnA* mutant and observed their functional complementation. The results imply that the enzymatic functions of mammalian GS are essentially the same as that of prokaryote, although their regulatory properties are slightly different

[Supported by the BK21 program]

E023

### Screening and Purification of Haloperoxidase from Marine Actinomycetes

Ki Woong Cho  
*Department of Marine Biotechnology, Anyang University*

In search of microbial source of novel enzymes, a marine actinomycetes producing haloperoxidase was isolated from unidentified sponge extract from Guam and identified to be a *Streptomyces* genus through physiological and biochemical studies and designated to be *Streptomyces* sp.4183. The haloperoxidation reaction was followed by the bromination of phenol red in the presence of hydrogen peroxide and potassium bromide with increasing absorbance at 575 nm. The haloperoxidase was purified from the cell extract with 35-75% ammonium sulfate precipitation, High-Q anion exchange chromatography, Hydroxyapatite chromatography, and gel filtration chromatography to a yield of 12% and purification fold 84. This enzyme shows exceptionally high heat stability for a marine originated enzyme without losing activity after 1 hr incubation at 60 °C. The molecular weight of this enzyme, which seems to be a single polypeptide form, is about 65 kD from gel filtration chromatography and SDS-PAGE analysis.

E022

### Deficiency in Tetrahydropteridines Affects Spore Viability in *Dictyostelium discoideum* Ax2

Yong Kee Choi\* and Young Shik Park  
*School of Biotechnology and Biomedical Science, Inje University*

*Dictyostelium discoideum* is one of the simplest eukaryotic organisms. It grows vegetatively in nutrients and upon starvation undergoes development, finally forming fruiting bodies. The organism is notorious for its synthesis of tetrahydrodictyopterin (DH4) together with a much lower amount of L-erythro isomer (BH4). Although both were known to interfere with GTP binding to G protein, the detailed mechanism and physiological implication were not. In order to investigate the putative physiological function of BH4/DH4 in the organism, a mutant was created disrupted in the gene encoding septipenn reductase (SR), which catalyzes the last step of BH4 synthesis. The mutant cells, being completely devoid of SR protein, looked normal in both vegetative and developmental growths. However, the spores formed at the final stage of development showed only 20% of the viability in wild type. As it was presumed that the defect was resulted from malfunctions in nitric oxide synthase and mitochondria, we analyzed the mutant cells by using fluorescence dyes specific to nitric oxide or mitochondria and report here the results. [Supported by a research grant from KOSEF, R05-2003-000-11206-0]