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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目： Functional analyses of extremophile specific enzymes
Title of Dissertation （極限環境微生物特異的酵素の機能解析）

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Extremophiles are microorganisms that can grow in place where general organisms are not able to live. These environments include, for example, extremely high temperature or high salt concentration. The specific metabolic pathway and related enzymes of these organisms are interested in the viewpoint of biological evolution. Moreover, since the enzymes from extremophiles generally show high stability against various stress such as temperature, pH, salt and organic solvent, these enzymes are expected to have much potential application to industrial processes. In recent years, genome analysis of extremophiles has advanced and entire base sequence of the genomes from various species has been determined. Based on these genome information, various enzymes having unique characteristics have been identified in extremophiles. In this thesis, the two novel enzymes from extremely halophilic archaeon and hyperthermophilic bacterium, were identified and their specific enzymatic properties have been elucidated.

(1) 2-Deoxy-D-ribose-5-phosphate aldolase(DERA) from extreme halophilic archaeon, *Haloarcula japonica*: DERA catalyzes the aldol reaction between two aldehydes and is thought to be a potential biocatalyst for the production of a variety of stereo-specific materials. A gene encoding DERA from the extreme halophilic archaeon, *H. japonica*, was overexpressed in *Escherichia coli*. The gene product was successfully purified, using procedures based on the protein's halophilicity, and characterized. The expressed enzyme was stable in a buffer containing 2 M NaCl and exhibited high thermostability, retaining more than 90% of its activity after heating at 70°C for 10 min. The enzyme was also tolerant to high concentrations of organic solvents, such as acetonitrile and dimethylsulfoxide. Moreover, *H. japonica* DERA was highly resistant to

a high concentration of acetaldehyde and retained about 35% of its initial activity after 5-h' exposure to 300 mM acetaldehyde at 25°C, the conditions under which *E. coli* DERA is completely inactivated. The enzyme exhibited much higher activity at 25°C than the previously characterized hyperthermophilic DERAs. Our results suggest that the extremely halophilic DERA has high potential to serve as a biocatalyst in organic syntheses. This is the first description of the biochemical characterization of a halophilic DERA.

(2) Aspartate kinase-homoserine dehydrogenase(AK-HseDH) from the hyperthermophilic bacterium, *Thermotoga maritima*: The orientation of the three domains in the bifunctional AK-HseDH homologue found in *T. maritima* totally differs from those observed in previously known AK-HseDHs; the domains line up in the order HseDH, AK, and regulatory domain. In the present study, the enzyme produced in *E. coli* was characterized. The enzyme exhibited substantial activities of both AK and HseDH. L-Threonine inhibits AK activity in a cooperative manner, similar to that of *Arabidopsis thaliana* AK-HseDH. However, the concentration required to inhibit the activity was much lower ($K_{0.5}$ = 37 μM) than that needed to inhibit the *A. thaliana* enzyme ($K_{0.5}$ = 500 μM). In contrast to *A. thaliana* AK-HseDH, Hse oxidation of the *T. maritima* enzyme was almost impervious to inhibition by L-threonine. Amino acid sequence comparison indicates that the distinctive sequence of the regulatory domain in *T. maritima* AK-HseDH is likely responsible for the unique sensitivity to L-threonine.