（様式５）(Style5)

学位論文全文に代わる要約
Extended Summary in Lieu of Dissertation

氏名：　大志田　達也
Name

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）

学位論文要約：

Dissertation Summary

Introduction

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, because distinctive metabolic systems unknown in general organisms may exist in extremophiles. Moreover, since the enzyme 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.

2-Deoxy-D-ribose-5-phosphate aldolase (DERA) from extreme halophilic archaeon, *Haloarcula japonica*

A gene encoding a DERA was identified in the halophilic archaeon *H. japonica*.

The gene was overexpressed in *Escherichia coli*, and its product was purified and characterized. In preliminary studies, the recombinant *E. coli* cells were disrupted in 2 M NaCl. The crude extract exhibited a high level of DERA activity. In the following Ni-chelating chromatography step, however, a large amount of the absorbed enzyme was eluted as an inactive protein by a linear gradient of imidazole, even though the purification buffers contained 2 M NaCl. The elution profile of the next gel filtration chromatography step indicated that a peak of the inactive protein was present besides the normal peak of the active enzyme. Although the inactive protein was supposed to have a larger molecular mass than the active enzyme, the two proteins were indistinguishable in size as determined with SDS-PAGE analysis. Therefore, the cell disruption and Ni-chelating chromatography steps were carried out without NaCl and the resulting eluate was dialyzed against a high concentration of NaCl. Dialysis against the buffers containing different concentrations of NaCl (ranging from 0.5 to 3 M) showed that the most suitable concentration was 2 M. When the resulting dialysate was subjected to gel filtration chromatography, the enzyme was eluted as a single peak with DERA activity. The purified enzyme showed a single band on SDS-PAGE. *H. japonica* DERA showed typical Michaelis-Menten kinetics at 37°C; the K_m value for DRP was 1.12 ± 0.18 mM and the V_{max} value was 20.7 ± 2.6 μmol/min/mg. At 25°C, these values were calculated to be 1.02 ± 0.22 mM and 8.92 ± 1.4 μmol/min/mg, respectively. Evaluation of the catalytic activity at different pH values revealed the enzyme to be maximally active at around pH 6.4. The optimal temperature of the enzyme reaction was about 60°C. When we examined the thermostability of the enzyme, we found that *H. japonica* DERA retained more than 90% of its activity after heating at 70°C for 10 min. When *H. japonica* DERA was heated for 30 min at 50°C, the enzyme showed no loss of activity at pH ranging from 6.3 to 12.3. 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.

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. AK-HseDH showed typical Michaelis-Menten kinetics at 55°C; the K_m values for NADP and Hse ware 0.064 ± 0.0040 mM and 1.81 ± 0.017 mM, respectively, and those for L-aspartate and ATP were 4.66 ± 0.16 mM and 3.54 ± 0.31 mM, respectively. The V_{max} values for HseDH and AK activities were calculated to be 66.5 ± 0.49 μmol/min/mg and 103 ± 0.52 μmol/min/mg, respectively. 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). Furthermore, in contrast to *A. thaliana* AK-HseDH, Hse oxidation of the *T. maritima* enzyme was almost impervious to inhibition by L-threonine. In the regulatory domain of *T. maritima* AK-HseDH, 32 residues of the C-terminal part of the corresponding domains of *A. thaliana* and *E. coli* AK-HseDHs are not present (Fig. 1 (A)). Both the residues Gln443 and Gln524 in *A. thaliana* AK-HseDH, which are proposed to interact with L-threonine, are completely conserved as Gln351 and Gln432, respectively, in *E. coli* AK-HseDH. However, Gln524 in *A. thaliana* AK-HseDH is replaced by Ala709 in *T. maritima* AK-HseDH, although Gln443 was conserved as Gln631. To obtain structural information about the sites where L-threonine potentially binds, we predicted the secondary structure of the regulatory domains in *A. thaliana* and *T. maritima* AK-HseDHs using SWISS-MODEL. As shown in Fig. 1 (B), the two ACT subdomains (two loop-α-loop-β-loop-β motifs) were predicted for the residues 606-645 and 685-718 of *T. maritima* AK-HseDH, as well as

for the residues 416-453 and 500-534 of *A. thaliana* AK-HseDH. These observations suggest that, although the two enzymes share the structurally similar ACT subdomains, the L-threonine-binding mode in *T. maritima* AK-HseDH is markedly different from that proposed for *A. thaliana* AK-HseDH, which contributes to the distinct manner of the L-threonine-dependent inhibition observed for *T. maritima* AK-HseDH. This study is the first to describe the presence of AK-HseDH in hyperthermophiles.

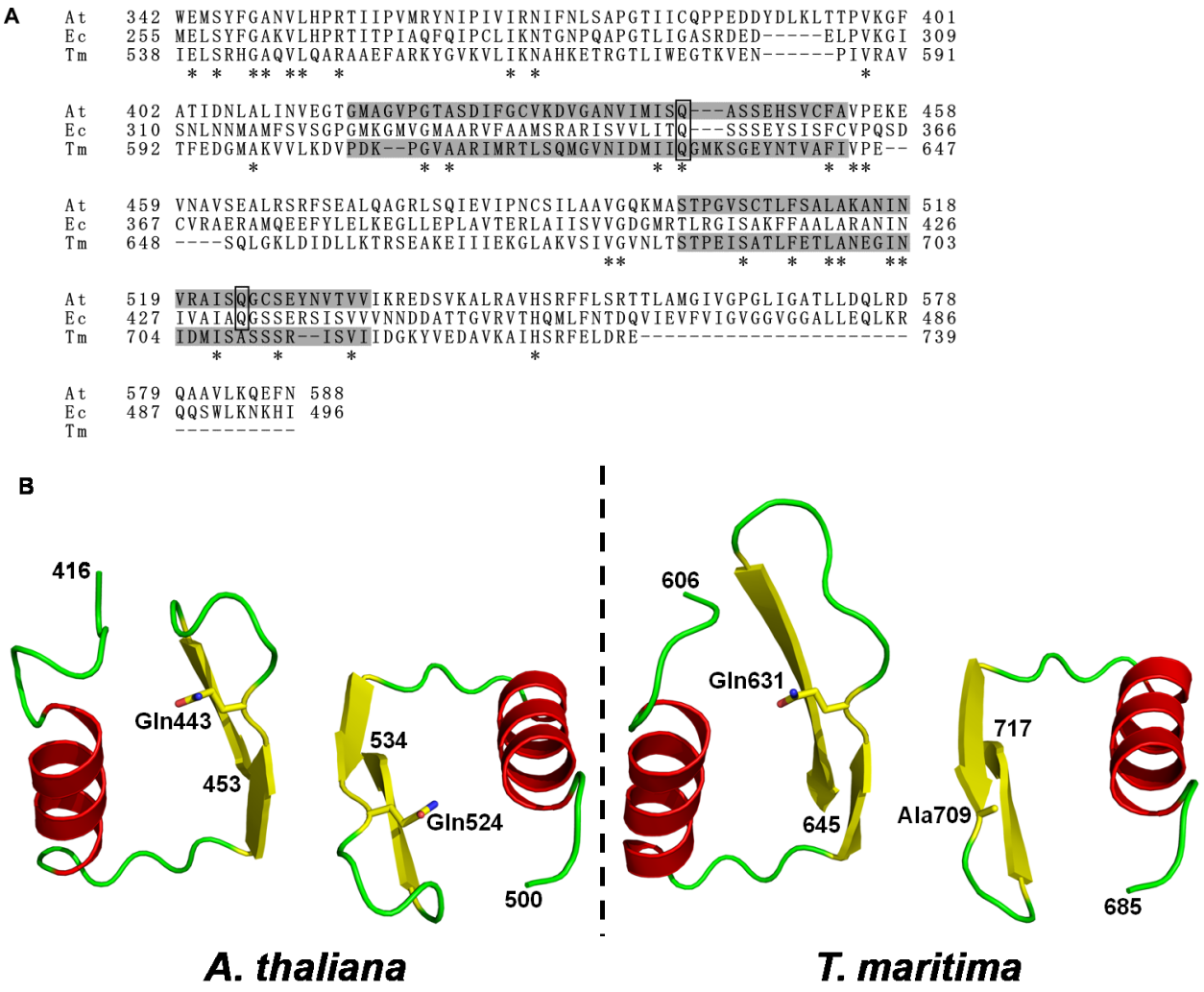

Fig. 1. Comparison of regulatory domains. (A) Amino acid sequence alignment of the regulatory domains of *A. thaliana*, *E. coli*, and *T. maritima* AK-HseDHs. At, *A. thaliana*; Ec, *E. coli*; and Tm, *T. maritima*. Asterisks indicate conserved residues. Gln residues proposed to interact with L-threonine are in boxes. The residues corresponding to the two ACT subdomains of *A. thaliana* (416-453 and 500-534) and *T. maritima* (606-645 and 685-717) enzymes are shaded. Sequences were aligned using ClustalW. (B) Predicted structures of the two ACT subdomains of *A. thaliana* (left panel) and *T. maritima* (right panel) enzymes. Amino acid residues (Gln443 and Gln524 for *A. thaliana*, Gln631 and Ala709 for *T. maritima*) are shown as stick models.