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学 位 論 文 要 旨
Dissertation Abstract

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学位論文題目：
Title of Dissertation

Unique coenzyme binding mode of hyperthermophilic archaeal NAD(P)-dependent dehydrogenases
(超好熱アーキア由来NAD(P)依存性脱水素酵素が示す新規な補酵素結合様式)

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The two NAD(P)-dependent dehydrogenases showing new variations of the cofactor binding were found in hyperthermophilic archaea. One is homoserine dehydrogense (HseDH) involved in the biosynthetic pathway from aspartate to homoserine, which is a common precursor for the synthesis of methionine, threonine, and isoleucine. The other is glycerol-1-phosphate dehydrogenase (G1PDH) involved in synthesis of *sn*-glycerol-1-phosphate, which is an archaea-specific glycerophosphate backbone of phospholipids. In the present study, the detailed characteristics and the crystal structures of these enzymes were determined, and the factors responsible for their unique cofactor-binding modes were analyzed.

(1) HseDH: A gene encoding a HseDH was identified in the hyperthermophilic archaeon *Pyrococcus horikoshii*. The gene was overexpressed in *Escherichia coli*, and its product was purified and characterized. The expressed enzyme is the most thermostable HseDH yet described, retaining 70% of its activity even after incubation for 10 min at 95ºC. The crystal structure of the enzyme was determined at a resolution of 2.30 Å. Refinement of the structure and mass analysis showed the presence of the bound cofactor NADPH, although it has not been added during crystallization. The structure of *Pc. horikoshii* HseDH showed this enzyme to have a strong preference for NADP(H): the C2’ phosphate group of the cofactor adenine ribose is tightly held at the nucleotide-binding site through three direct hydrogen bonding interactions with the side chains of Arg40 and Lys57. Strikingly, however, NADP did not act as a cofactor with this enzyme, but as a strong inhibitor of NAD-dependent homoserine oxidation. Furthermore, K57A mutant acquired high reactivity against NADP. The crystal structure of the K57A mutant with bound NADPH was then determined to a resolution of 2.43 Å. Structural comparison suggests that the large number of interactions between the cofactor and the enzyme are responsible for the lack of reactivity of the enzyme towards NADP.

(2) G1PDH: A gene encoding a G1PDH was identified in the hyperthermophilic archaeon *Pyrobaculum calidifontis*. The gene was overexpressed in *E. coli*, and its product was purified and characterized. In contrast to conventional G1PDHs, the expressed enzyme showed strong preference for NADH: the reaction rate (Vmax) with NADPH was only 2.4% of that with NADH. The crystal structure of the enzyme was determined at a resolution of 2.45 Å. The asymmetric unit consisted of one homohexamer. Refinement of the structure and HPLC analysis showed the presence of the bound cofactor NADPH in subunits D, E, and F, even though it was not added in the crystallization procedure. The phosphate group at C2' of the adenine ribose of NADPH is tightly held through the five biased hydrogen bonds with Ser40 and Thr42. In comparison with the known G1PDH structure, the NADPH molecule was observed to be pushed away from the normal coenzyme binding site. Interestingly, the S40A/T42A double mutant enzyme acquired much higher reactivity than the wild-type enzyme with NADPH, which suggests that the biased interactions around the C2'-phosphate group make NADPH binding insufficient for catalysis.

The molecular basis for the cofactor preference observed in the two enzymes greatly differs from those of the conventional NAD(P)-dependent dehydrogenases. The present study indicates that the molecular details underlying cofactor preference in NAD(P)-dependent dehydrogenases are more complex than expected, and the cofactor specificity cannot be predicted even from the structural information in the absence of biochemical data.