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Crystal structure of thermostable NADP$^+$-dependent *meso*-diaminopimelate dehydrogenase from *Ureibacillus thermosphaericus*

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1 Introduction

meso-Diaminopimelate dehydrogenase (DAPDH; EC 1.4.1.16) catalyzes the reversible NADP$^+$-dependent oxidative deamination of *meso*-diaminopimelate (*meso*-DAP) to produce L-2-amino-6-oxopimelate within cells. The enzyme is the only known NAD(P)$^+$-dependent dehydrogenase that acts stereoselectively on the D-center of *meso*-DAP, and it may be useful for the one-step production of D-amino acids often utilized as source materials for industrial production of medicines, seasonings and agrochemicals. However, the high substrate specificity for *meso*-DAP has thus far precluded use of DAPDH in the practical application of D-amino acid synthesis. On the other hand, Vedha-Peters *et al.* [1] used error-prone PCR and site-directed mutagenesis to create a novel NADP$^+$-dependent D-amino acid dehydrogenase (DAADH) from *Corynebacterium glutamicum* DAPDH. That enzyme could be used for stereoselective synthesis of D-amino acids from the corresponding 2-oxo acids and ammonia in the presence of NADPH. However, because the parent enzyme from mesophilic *C. glutamicum* is rather unstable, this DAADH is not sufficiently stable for use under the conditions necessary for industrial application.

We recently identified and characterized a highly stable DAPDH from a thermophilic bacterium, *Ureibacillus thermosphaericus*. This enzyme showed no loss of activity after incubation for 30 min at temperatures up to 60°C. Moreover, by introducing five point mutations into amino acid residues located around the active site, as in the case of the *C. glutamicum* enzyme, we have already succeeded in creating a stable DAADH from *U. thermosphaericus* DAPDH [2]. In the present study, the factors underlying the dramatic change in substrate recognition from DAPDH to DAADH caused by introducing five point mutations were analyzed based on the active-site architecture of *U. thermosphaericus* DAPDH.

2 Experiment

Diffraction data were collected (λ=1.0 Å) on the beamline AR-NE3A at the Photon Factory. The initial phases for the structure were determined by molecular replacement; the structure of *C. glutamicum* DAPDH (PDB entry 1dap) served as the search model. The crystal structure of the NADP$^+$/*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) -bound *U. thermosphaericus* DAPDH (PDB entry 3wyc) was determined at a resolution of 2.07 Å [3].

3 Results and Discussion

We have already created a stable DAADH from *U. thermosphaericus* DAPDH [2] by introducing five point mutations, Gln154Leu, Asp158Gly, Thr173Ile, Arg199Met and His249Asn, in a manner similar to that used to create the *C. glutamicum* DAADH. The *U. thermosphaericus* DAADH does not act on *meso*-DAP and differs greatly from the parent DAPDH, which acts exclusively on *meso*-DAP as the specific electron donor. In addition, this DAADH catalyzes the reversible deamination of D-amino acids such as D-cyclohexylalanine (relative activity, 100%), D-isoleucine (73%), D-2-aminooctanoate (61%) and D-lysine (53%).

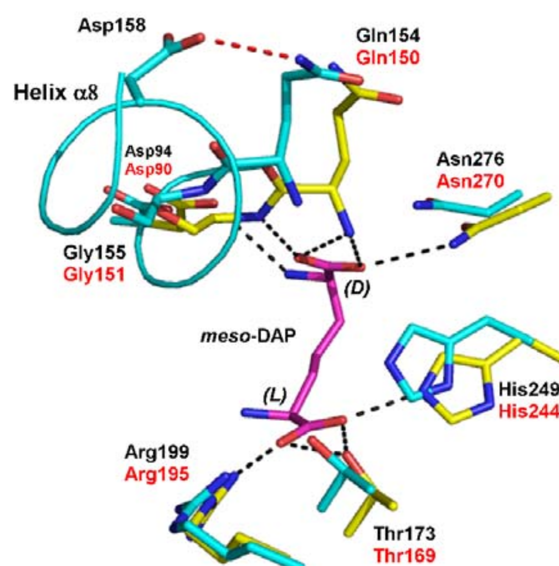

Fig. 1: Substrate binding site in *U. thermosphaericus* DAPDH. The structure of *C. glutamicum* DAPDH (PDB entry 2dap, yellow and red labels) is superimposed on that of *U. thermosphaericus* DAPDH (cyan and black labels).

To assess the difference in the substrate recognition mechanism between DAPDH and DAADH, the active site architecture of *U. thermosphaericus* DAPDH was compared to that of *C. glutamicum* DAPDH.

The crystal structure of the *C. glutamicum* DAPDH/*meso*-DAP binary complex has been reported. Superposition of this structure (PDB entry 2dap) onto that of $NADP^+$/TES-bound *U. thermosphaericus* DAPDH enabled comparison of the amino acid residues involved in substrate binding (Fig. 1). Within the structure of the *C. glutamicum* DAPDH/*meso*-DAP complex, the α-carboxylate of the D-amino acid center of *meso*-DAP forms four hydrogen bonds with the main chain nitrogens of Gln150 and Gly151 and the side-chain of Asn270, while the α-amino group of the D-amino acid center forms one hydrogen bond with the side-chain of Asp90. On the other hand, the α-carboxylate of the L-amino acid center of *meso*-DAP forms four hydrogen bonds with the side-chains of Arg195, Thr169 and His244, and no hydrogen-bonding interactions are observed between the α-amino group of the L-amino acid center and the enzyme. The residues that interact with *meso*-DAP in *C. glutamicum* DAPDH (Asp90, Gln150, Gly151, Thr169, Arg195, His244 and Asn270) are completely conserved in *U. thermosphaericus* DAPDH as Asp94, Gln154, Gly155, Thr173, Arg199, His249 and Asn276, respectively (Fig. 1). Among these residues, Gln154, Thr173, Arg199 and His249 are respectively replaced by Leu, Ile, Met and Asn in DAADH. Although Gln154 is replaced by Leu, the hydrogen-bonding interactions around the D-amino acid center of *meso*-DAP would be conserved in DAADH because the backbone amide of the Leu is thought to be situated at a position where it can interact with the α-carboxylate of the D-amino acid center, as Gln154 does. By contrast, most of the hydrogen-bonding interactions around the L-amino acid center might be lost in DAADH because Thr173 and Arg199 in DAPDH, whose side-chains make hydrogen bonds with the α-carboxylate of the L-amino acid center, are replaced by Ile and Met, respectively. These observations suggest that the loss of interactions between the *meso*-DAP L-amino acid center and the enzyme is likely responsible for the lack of reactivity of DAADH toward *meso*-DAP. Conversely, the Thr173Ile and Arg199Met substitutions may enhance the hydrophobicity of the pocket around the L-amino acid center, and this appears to be related to the high reactivity of DAADH for the hydrophobic D-amino acids, including D-cyclohexylalanine, D-isoleucine and D-2-amino-octanoate.

In *U. thermosphaericus* DAPDH, on the other hand, the side-chain of Asp158, which belongs to helix α8, forms a hydrogen bond with the side-chain of Gln154, the N-terminal residue of helix α8 (Fig. 1). The Gln154Leu and Asp158Gly mutations abolish this interaction and appear to enhance the flexibility of the α8 N-terminal residues that interact with the D-amino acid center of the substrate. This may explain the enlarged substrate range in *U. thermosphaericus* DAADH, because substitution of Asp154Gly in *C. glutamicum* DAADH, which corresponds to the Asp158Gly in the *U. thermosphaericus* enzyme, reportedly also enhances the reactivity toward a broad range of substrates [1]. We anticipate that this structural analysis will provide useful information for the creation of a DAADH mutant exhibiting different substrate specificities. At the present time, however, the details of the substrate-recognition mechanism of DAADH are still unclear. The structure of the *U. thermosphaericus* DAADH/cofactor/substrate ternary complex should be a useful focus for further investigation.

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References

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