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Crystal structure of L-tryptophan dehydrogenase

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1 Introduction

NAD(P)-dependent L-amino acid dehydrogenases (EC 1.4.1.x) catalyze reversible oxidative deamination of L-amino acids to their corresponding 2-oxo acids and ammonia in the presence of NAD(P). So far, more than fifteen types of L-amino acid dehydrogenases have been identified from various organisms and characterized extensively. In particular, detailed structure and function analyses of L-Glu/L-Leu/L-Phe dehydrogenases have led to the elucidation of their catalytic mechanisms. Consequently, several L-amino acid dehydrogenases have been successfully used for the syntheses for chiral amino acids and their analogs, for developing biosensors for L-amino acids.

Among all the amino acid dehydrogenases, biochemical information and application on the L-Trp dehydrogenase (TrpDH, EC 1.4.1.19) have been limited so far, mainly due to its extremely limited distribution and less stability. The enzyme is known to catalyze reversible oxidative deamination of L-Trp to 3-indolepyruvate (IPyA) in the presence of NAD(P). IPyA is known to show pharmacological and antioxidant effects. TrpDH has high potentiality for applications such as synthesis of IPyA and biosensor for L-Trp/IPyA. The enzyme was first identified in several higher plants, including *Pisum sativum*, in the middle of 1980s, and was partially characterized at that time. Since then, no studies were reported for this enzyme until Npun_R1275 from a cyanobacterium *Nostoc punctiforme* ATCC 29133 (29133TrpDH) was identified to be the first NAD-dependent TrpDH of microbial origin.

We recently characterized TrpDH from *N. punctiforme* NIES-2108 (NpTrpDH), which is a 29133TrpDH homologous protein and found that the enzyme catalyzed reversible oxidative deamination of L-Trp to IPyA in the presence of NAD and exhibited B-type stereospecificity for transferring hydrogen from NADH, just like L-Glu/L-Leu/L-Phe/L-Val dehydrogenases. The most striking feature was that NpTrpDH uses L-Trp as the preferred electron donor for oxidative deamination and exhibits much less activity when L-Phe was used as the electron donor. Other proteinogenic L-amino acids and D-Trp, the stereoisomer of L-Trp, were all inert as electron donors for NpTrpDH. For reductive amination, IPyA was the most preferred substrate, whereas phenylpyruvate, the 2-oxo analog of L-Phe, was inert as a substrate. Despite these interesting observations, there exists no structural information on TrpDH. Thus, we employed X-ray crystallography to solve the apo-structure of NpTrpDH,

2 Experiment

Data were collected under cryo conditions at the Beamline AR-NE3A at Photon Factory in Japan. The crystal structure of LeuDH from *Sporosarcina psychrophila* (SpLeuDH, PDB ID: 3VPX, amino acid sequence identity: 49%) was applied as a search model, and the program PHENIX was used for molecular replacement phase determination and initial model building. Model building was performed using the program Coot, and refinement was carried out using Refmac5.

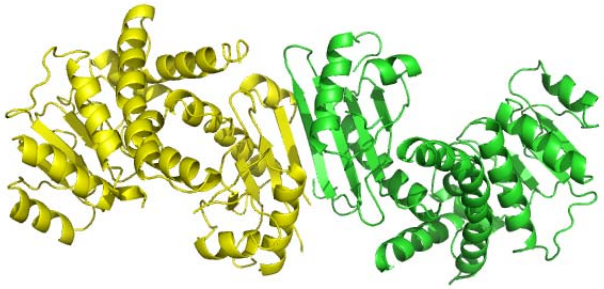

Fig. 1: Overall structure of NpTrpDH.

3 Results and Discussion

The structure of NpTrpDH monomer, which exhibited high similarity to the structures of L-Glu/L-Leu/L-Phe dehydrogenases, consisted of a substrate-binding domain (domain I: residues: 2–133 and 328–343) and a Rossmann-fold domain (domain II, residues: 142–327) separated by a deep cleft. The apo-NpTrpDH existed in an open conformation, where the domains I and II move apart from each other. The subunits dimerized themselves mainly due to interactions between amino acid residues around the β-1 strand of each subunit (Fig. 1), as was observed in the case of L-Phe dehydrogenase. Several hydrophobic

residues, which were located in the active site of NpTrpDH and possibly interacted with the side-chain of the substrate L-Trp, were arranged similar to those found in L-Leu/L-Phe dehydrogenases.

To predict the possible L-Trp binding site, we initially performed a molecular docking using the MOE software tool. The calculated binding energy was -14 kcal/mol, which suggested that this binding model was reasonable significantly. In contrast, the calculated binding energy with L-Phe was 0.18 kcal/mol, which indicated that L-Phe is unsuitable as a substrate compared with L-Trp. In this model, the side-chain of L-Trp was hydrophobically interacted with those of Ala-42 (4.1Å), Met-65 (3.3Å), Lys-68 (4.2Å), Gly-112 (4.0Å), Leu-288 (3.7Å), and Val-291 (3.7Å) (Fig. 2). Based on these results and active sites comparison, we identified several residues including other hydrophobic residues around the active site as the candidate residues which might be involved in recognizing the side chain of L-Trp.

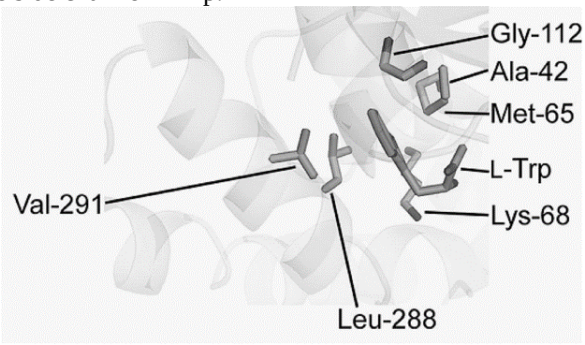

Fig. 2: Predicted L-Trp-binding site in the NpTrpDH. The docking simulation was performed using the MOE software tool.

The site-directed substitution mutants at residues Met-40, Met-65, Ala-69, Ile-74, Ile-110, Val-132, and Val-133 in domain I and Leu-288, Ile-289, Val-291, Tyr-292, Met-295, and Ile-296 in domain II of NpTrpDH revealed that Met-40, Ala-69, Ile-74, Ile-110, Leu-288, Ile-289, and Tyr-292 formed a hydrophobic cluster, which may play a critical role in both protein folding and L-Trp recognition.

To our knowledge, there is no previous report demonstrating that a hydrophobic cluster in the active site of any L-amino acid dehydrogenase may have a critical impact on protein folding. Furthermore, our results suggest that this hydrophobic cluster could strictly and stereoselectively accommodate L-Trp as compared to other L-amino acid dehydrogenases such as L-Glu/L-Leu/L-Phe dehydrogenases. These studies not only show the first structural characteristics of L-Trp dehydrogenase, but may also facilitate the novel applications of L-Trp dehydrogenase.

Acknowledgement

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Reference

[1] T. Wakamatsu *et al.*, *Appl. Env. Microbiol.* e02710-16 (2017).

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