BL-5A/2021G143

Crystal structure of an NADP$^+$-dependent L-arginine dehydrogenase belonging to the μ-crystallin family

Ryushi KAWAKAMI[1], Naoki TAKAMI[2], Junji HAYASHI[1], Kazunari YONEDA[3], Taketo OHMORI[4], Toshihisa OHSHIMA[4], and Haruhiko SAKURABA[2, *]

[1] Division of Bioscience and Bioindustry, Graduate school of Technology, Industrial and Social Sciences, Tokushima University, 2-1, Minamijosanjima-cho, Tokushima, Tokushima 770-8513, Tokushima, Japan

[2] Department of Applied Biological Science, Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki-cho, Kita-gun, Kagawa, 761-0795, Japan

[3] Department of Food and Life Sciences, School of Agriculture, Tokai University, 871-12 Sugido, Mashiki-machi, Kamimashiki-gun, Kumamoto 861-2205, Japan

[4] Department of Biomedical Engineering, Faculty of Engineering, Osaka Institute of Technology, 5-16-1 Ohmiya, Asahi-ku, Osaka, 535-8585, Japan.

1 Introduction

NAD(P)$^+$-dependent L-arginine dehydrogenase (EC 1.4.1.25, L-ArgDH) was recently identified as a novel amino acid dehydrogenase belonging to the μ-crystallin/ornithine cyclodeaminase (OCD) family. L-ArgDH was first observed in *Pseudomonas aeruginosa* PAO1, an opportunistic human pathogen, and was found to function together with FAD-dependent D-arginine dehydrogenase to convert D-arginine to L-arginine through its oxo-analog, 5-guanidino-2-oxopentanoate. When the *P. aeruginosa* gene *DauB*, encoding L-ArgDH, was expressed in *Escherichia coli*, the product catalyzed the NAD$^+$-dependent deamination of L-arginine to 5-guanidino-2-oxopentanoate. However, the molecular and catalytic properties of *P. aeruginosa* L-ArgDH have yet to be reported owing to the enzyme's instability. Recently, a gene homolog (*PverR02_12350*) of *DauB* was identified in genomic data from the nonpathogenic bacterium *P. veronii* JCM 11942. Its product possesses 67.8% amino acid sequence homology with *P. aeruginosa* L-ArgDH and exhibits strong NADP$^+$-dependent L-ArgDH activity. Because this enzyme is stable in the presence of 10% (v/v) glycerol, its enzymatic properties have been analyzed in detail [1]. The most notable characteristic of this enzyme is its high substrate specificity for L-arginine. D-Arginine, L-lysine, L-ornithine, L-citrulline, L-leucine, L-phenylalanine, L-histidine, L-glutamate, glycine, and L-alanine are all inert as electron donors. The *Archaeoglobus fulgidus* L-AlaDH, which belongs to the same family, is not substrate-specific; L-valine, L-serine, L-threonine, L-aspartate, and L-isoleucine can also serve as substrates but at rates 12% or less of that for L-alanine. The crystal structure of *A. fulgidus* L-AlaDH complexed with NAD$^+$ has been determined, and modeling of substrate L-alanine into the active site has been reported [2]. Structural analysis of *P. veronii* L-ArgDH may shed light on the substrate-recognition mechanism of this enzyme, which specifically acts on L-arginine.

In the present study, therefore, we determined the molecular structure of the abortive *P. veronii* L-ArgDH complex with its natural substrate, L-arginine, and NADPH bound. We then compared the architecture of the active site with that of *A. fulgidus* L-AlaDH. This is the first description of the structure of an L-ArgDH belonging to the μ-crystallin/OCD family [3].

2 Experiment

Data were collected under cryo conditions at the Beamline BL-5A at Photon Factory in Japan. The program MOLREP in the CCP4 was used for molecular replacement phase determination. The structure of the monomer predicted using AlphaFold2 served as the search model. Model building was performed using the program Coot, and refinement was carried out using REFMAC5.

3 Results and Discussion

Superposition of the *A. fulgidus* L-AlaDH structure with NAD$^+$ bound onto that of *P. veronii* L-ArgDH with L-arginine/NADPH bound showed that the NADPH molecule in the latter was positioned/configured nearly identically to the NAD$^+$ molecule in the former. This suggests *P. veronii* L-ArgDH has *Pro*-R specificity similar to *P. veronii* L-AlaDH for the hydride transfer to NADP$^+$. As proposed for the L-alanine molecule modeled into the *A. fulgidus* L-AlaDH structure, the carboxylate group of L-arginine interacts with the side chain of Arg111 (Fig. 1), which corresponds to Arg108 in *A. fulgidus* L-AlaDH but not in a bidentate manner. The carboxylate group is also recognized by the side chain of Lys71, corresponding to Lys65 in the *A. fulgidus* enzyme. The α-amino group of L-arginine forms hydrogen bonds with the side chains of Thr224 and Asn225. The amino acid residues corresponding to these two residues are not present in *A. fulgidus* L-AlaDH. The guanidino group of the substrate L-arginine hydrogen bonded to the side chain of Asp54 and the main chain O atom of Ser73. To assess the role of Asp54, we constructed a D54A mutant and observed that the specific activity of the mutant was only about 1.8% of the wild-type enzyme activity, which suggests recognition

of the L-arginine guanidino group by the Asp54 side chain is essential for maintaining high reactivity for L-arginine.

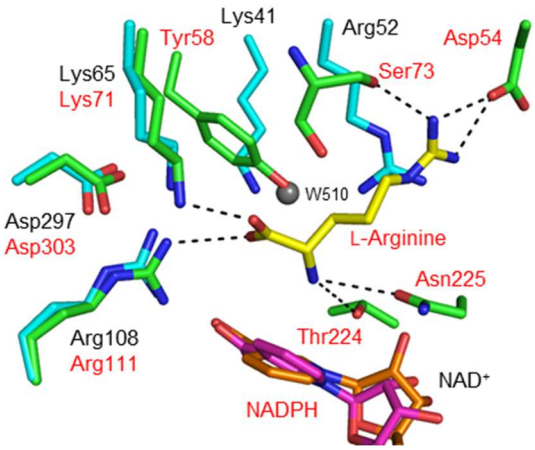

Fig. 1: Comparison of the active site structures in *P. veronii* L-ArgDH with L-arginine/NADPH bound (green and red labels) and *A. fulgidus* L-AlaDH with NAD^+ bound (cyan and black labels). NADPH and L-arginine in *P. veronii* L-ArgDH are shown in magenta and yellow, respectively. NAD^+ and water (W510) in *A. fulgidus* L-AlaDH are shown in orange and gray, respectively. The networks of hydrogen bonds are shown as dashed lines.

A preliminary outline of the reaction mechanism based on the model of *A. fulgidus* L-AlaDH with L-alanine bound was previously reported [2]. The α-amino group of L-alanine modeled into the *A. fulgidus* enzyme formed a hydrogen bond with a water molecule (W510), which was also hydrogen bonded with the side chains of Lys41 and Arg52 (Fig. 1). Consequently, W510 was thought to mediate proton transfer for catalysis. However, the amino acid residues and a water molecule corresponding to those two residues and W510 were not observed in *P. veronii* L-ArgDH.

In the reaction of amino acid dehydrogenases, which catalyze $NAD(P)^+$-dependent dehydrogenation at the Cα position of amino acids accompanied by deamination, the α-amino group (α-keto group in the reverse reaction) of the substrate requires an acid/base capable of catalytically transferring protons. This role is played by His in *Phormidium* L-AlaDH and by Lys in *Rhodococcus* L-PheDH. In *P. veronii* L-ArgDH, no His residue is observed within 5 Å of the substrate Cα, but Lys71 is located within 5 Å. In the above L-AlaDH and L-PheDH, the catalytic acid/base functional group, whether His or Lys, has an Asp or Glu residue as a partner. This appears to be Asp303 in *P. veronii* L-ArgDH, which is within 3 Å of Lys 71 (Fig. 1). The residues corresponding to Lys71 and Asp303 are conserved among the homologs of *P. veronii* L-ArgDH, suggesting that Lys71 acts as the catalytic acid/base in L-ArgDHs as predicted for *A. fulgidus* L-AlaDH.

As shown in Fig. 1, on the other hand, the side chain of Tyr58 (OH) is located nearby the substrate Cα (about 4 Å) in *P. veronii* L-ArgDH and it forms a triad with the side chains of Lys71 (NZ) and Ser73 (OG). In short-chain dehydrogenase/reductase family enzymes, the Ser-Tyr-Lys catalytic triad is essential for catalysis. The side-chain oxygen of the Tyr residue functions as an acid/base catalyst for proton transfer, and the Ser residue plays a secondary role in the stabilization of substrate binding. The Lys residue has two critical roles: it interacts with the OH groups of the nicotinamide ribose (cofactor binding) and lowers the p*Ka* value of the OH group in the side chain of the Tyr residue. In L-ArgDHs, therefore, Tyr58 may serve as the acid/base catalytic residue instead of Lys71. To examine the role of Tyr58 and Lys71, we constructed Y58F and K71A mutants and observed that these substitutions completely abolished enzyme activity. This indicates that Tyr58 and Lys71 play critical roles in enzyme catalysis. The residues corresponding to Tyr58, Lys71, and Ser73 in *P. veronii* L-ArgDH are completely conserved among the L-ArgDH homologs, though Tyr58 and Ser73 are respectively replaced by Met and Val in *A. fulgidus* L-AlaDH. In particular, the presence of Tyr58 in the active site of *P. veronii* L-ArgDH may be a benchmark that distinguishes L-ArgDHs from L-AlaDHs in the μ-crystallin/OCD family, although further experimental verification should be necessary.

Acknowledgement

We are grateful to the staff of the Photon Factory for their assistance with data collection, which was approved by the Photon Factory Program Advisory Committee.

References

[1] T. Ohshima *et al*., *Prot. Exp. Purif.* **199**, 106135 (2022).
[2] D.T. Gallagher *et al*., *J. Mol. Biol.* 342, 119-130 (2004).
[3] R. Kawakami *et al*., *Int. J. Biol. Macromol.* **249**, 126070 (2023).

* sakuraba.haruhiko@kagawa-u.ac.jp