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Crystal structure of a novel type of ornithine δ-aminotransferase

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1 Introduction

Ornithine δ-aminotransferase (Orn-AT, EC 2.6.1.13) is one of the ω-aminotransferases and a fold type I pyridoxal 5'-phosphate (PLP)-dependent enzyme. This enzyme catalyzes the transfer of the δ-amino group from L-ornithine (L-Orn) to 2-oxoglutarate, yielding L-glutamate (L-Glu) and L-glutamate 5-semialdehyde, which spontaneously cyclizes to form L-1-pyrroline-5-carboxylate (P5C). In the first half reaction of Orn-AT (PLP-form), L-Orn is converted to L-glutamate 5-semialdehyde and the PMP-form intermediate is produced. In the second half-reaction, 2-oxoglutarate binds to the intermediate and after transamination, L-Glu and PLP-form enzyme are produced as the final product (Fig. 1). This means that the enzyme can selectively recognize the ω-amino group of L-Orn and the α-amino group of L-glutamate for the two half reactions. However, the mechanism in the dual substrate specificity of Orn-AT remains to be fully elucidated.

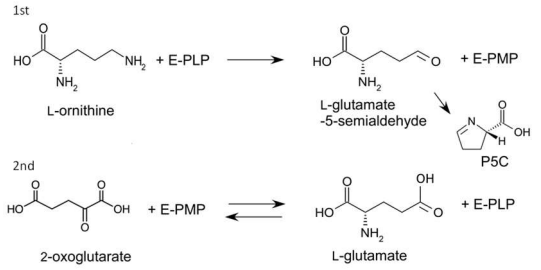

Fig. 1. The two half-transamination reactions catalyzed by Orn-AT. E-PLP: pyridoxal form of the enzyme, E-PMP: pyridoxamine form of the enzyme.

Recently, an Orn-AT has been detected in the hyperthermophilic archaeon *Thermococcus kodakarensis* and reported to be required for its growth in the absence of exogenous proline [1]. That enzyme is the only archaeal Orn-AT so far reported, and genetic analysis confirmed its physiological function as the enzyme involved in the biosynthesis of L-proline. Interestingly, the amino acid sequence of the *T. kodakarensis* Orn-AT (TK2101) is not similar to the previously reported sequences of bacterial and eukaryotic Orn-ATs. The enzyme was originally annotated as γ-aminobutyrate aminotransferase (GABA-AT) in the genome database, and phylogenetic analysis revealed that the clade containing *T. kodakarensis* Orn-AT is clearly distinct from that of previously characterized Orn-ATs [1].

We have been focusing on GABA-AT homologs from *Pyrococcus* and *Thermococcus* strains and found that the PH1423 gene product from *Pyrococcus horikoshii* shares nearly 60% sequence identity with *T. kodakarensis* Orn-AT and exhibits a high level of Orn-AT activity. A structural analysis of the PH1423 gene product may shed light on the substrate-recognition mechanism of a novel group of archaeal Orn-ATs, which are phylogenetically distant from conventional Orn-ATs. In the present study, crystal structures were determined for the enzyme in complex with PLP and L-Orn, and in complex with *N*-(5'-phosphopyridoxyl)- L-glutamate (PLP- L-Glu).

2 Experiment

Data were collected under cryo conditions at the Beamline AR-NW12A at Photon Factory in Japan. The program Phaser-MR in the PHENIX was used for molecular replacement phase determination. The crystal structure of putative GABA-AT from *Sulfolobus tokodaii* (PDB ID: 2eo5) was served as the search model. Model building was performed using the program Coot, and refinement was carried out using REFMAC5.

3 Results and Discussion

The structure of the L-Orn/PLP-bound enzyme was refined to a resolution of 1.92 Å.

The crystal structure of human Orn-AT (PDB ID: 2oat) in complex with the inhibitor 5-fluoromethylornitine (FMO) has been reported previously. Based on the structure of inhibitor-bound human Orn-AT, the natural substrate L-Orn has been modeled into the active site as an external aldimine intermediate. In that model, the main

residues responsible for the specific binding of L-Orn were thought to be Tyr55 and Arg180; the former is likely involved in a hydrogen bond with the α-amino group and the latter forms a salt bridge with the α-carboxylate (Fig. 2A, the human Orn-AT active site with inhibitor bound is indicated). In addition, the two aromatic residues, Tyr85 and Phe177, were observed to form a hydrophobic sandwich that accommodates the side chain of L-Orn. On the other hand, mutation analysis of human Orn-AT suggests that Tyr85 is a major determinant of specificity toward L-Orn; Y85I mutation greatly decreased the reaction rate of the enzyme with L-Orn (1/1000). Because the α-amino group of L-Orn is held between the OH groups of Tyr55 and Tyr85, both of those residues are likely responsible for the suitable binding of L-Orn.

Within *P. horikoshii* Orn-AT, Arg180 and Phe177 in human Orn-AT were respectively conserved as Arg154 and Phe151, whereas the residues corresponding to Tyr55 and Tyr85 were replaced by Thr30 and Ile63, respectively (Fig. 2B). Although the side chain of Ile63 forms hydrophobic interactions with the side chain of L-Orn, no interaction was observed between Thr30 and the substrate. Instead, the side chains of Thr92* and Asp93*, which arise from a loop (residues 90-94) in the neighboring subunit (subunit B), form hydrogen bonds with the α-amino group of L-Orn (Fig. 2B). The residues involved in the corresponding loop in human Orn-AT have no interactions with the substrate. These observations mean that the manner by which the α-amino group of L-Orn is recognized totally differs between the two enzymes.

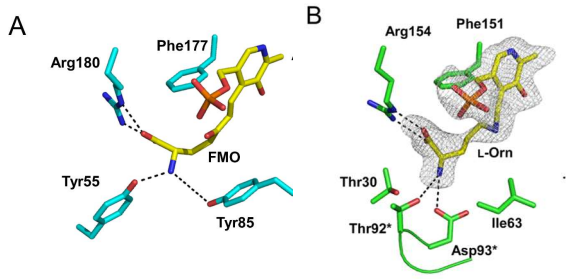

Fig. 2. Close-up of FMO bound to human Orn-AT (A) and L-Orn bound to *P. horikoshii* Orn-AT (B).

Within the active site of the L-Orn-bound model of human Orn-AT, the side chain of Arg413 is covered by the side chain of Glu235, and the α-carboxylate of L-Orn binds to the side chain of Arg180 (Fig. 3A; the active site of inhibitor-bound human Orn-AT is indicated). Therefore, a "Glu235 switch" mechanism that prevents L-Orn from binding in an orientation that would lead to transamination of the α-amino group has been proposed. In that mechanism, the function of Glu235 is thought to be to neutralize the positive charge of Arg413 when L-Orn binds to the active site. In the second half-reaction, the side chain of Glu235 is supposed to switch its position, uncovering Arg413, thereby enabling the Arg413 side-chain to interact with the α-carboxylate of 2-oxoglutarate or L-Glu. Within the *P. horikoshii* Orn-AT structure, Glu235 and Arg413 in human Orn-AT are conserved as Glu236 and Arg419, respectively. In the L-Orn-bound *P. horikoshii* Orn-AT, the side-chain of Glu236 forms a salt bridge with that of Arg419, closing the "Glu switch" and thus preventing unsuitable L-Orn binding (Fig. 3B).

To test the "Glu switch" hypothesis, we performed a crystal structure analysis of the PLP- L-Glu-bound enzyme. The structure was refined to a resolution of 2.99 Å. Although the electron density for the covalent bond connecting the Cα atom of L-Glu and the pyridine ring of PLP was discontinuous, the significant electron density at the L-Glu and PLP moieties enabled us to deduce a plausible structure for PLP-L-Glu (Fig. 3C). In this model, we found that the side-chain of Glu236 rotates clockwise by about 56° around the Cβ atom of Glu236 relative to the L-Orn-bound *P. horikoshii* Orn-AT structure. As a result, the side-chain carboxylate of Glu236 faces in the direction opposite to the side-chain of Arg419. Instead, the α-carboxylate of the L-Glu moiety in PLP-L-Glu interacts with the side-chain of Arg419. These observations clearly indicate that the "Glu switch" functions in this archaeal Orn-AT. Moreover, the *P. horikoshii* Orn-AT structure in complex with the external aldimine PLP-L-Glu adduct provides the first structural evidence for a "Glu switch" mechanism [2].

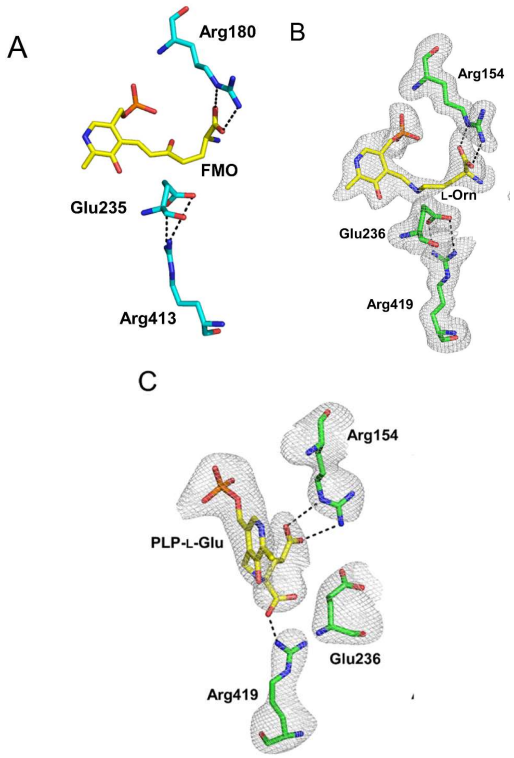

Fig. 3. Close-up of FMO bound to human Orn-AT (A), L-Orn bound to *P. horikoshii* Orn-AT (B), and PLP-L-Glu bound to *P. horikoshii* Orn-AT (C).

The present study describes the first structure of a novel type of Orn-AT, and our results may provide critical information that will facilitate better understanding of the

structure-function relationships within archaeal GABA-AT homologues.

Acknowledgement

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References

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