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Crystal Structure of D-Ornithine/D-Lysine Decarboxylase, a Stereoinverting Decarboxylase: Implications for Substrate Specificity and Stereospecificity of Fold III Decarboxylases

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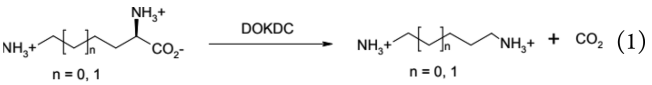
Supporting Information

ABSTRACT: A newly discovered Fold III pyridoxal 5′-phosphate (PLP)-dependent decarboxylase, D-ornithine/lysine decarboxylase (DOKDC), catalyzes decarboxylation of D-lysine and D-ornithine with inversion of stereochemistry. The X-ray crystal structure of DOKDC has been determined to 1.72 Å. DOKDC has a low level of sequence identity (<30%) with *meso*-diaminopimelate decarboxylase (DAPDC) and L-lysine/ornithine decarboxylase (LODC), but its three-dimensional structure is very similar. The distal binding site of DAPDC contains a conserved arginine that forms an ion pair with the L-carboxylate end of DAP. In both LODC and DOKDC, this distal site is modified by replacement of the arginine with aspartate, changing the substrate specificity. L-Ornithine decarboxylase (ODC) and LODC have a conserved phenylalanine on the *re*-face of the PLP complex that has been found to play a key role in the decarboxylation mechanism. We have found that both DAPDC and DOKDC have tyrosine instead of phenylalanine at this position, which precludes the binding of L-amino acids. Because the PLP-binding lysine in ODC, LODC, DAPDC, and DOKDC is located on the *re*-face of the PLP, we propose that this is the acid group responsible for protonation of the product, thus resulting in the observed retention of configuration for decarboxylation of L-amino acids and inversion for decarboxylation of D-amino acids. The reactions of DAPDC and DOKDC are likely accelerated by positive electrostatics on the *re*-face by the lysine ε-ammonium ion and on the *si*-face by closure of the lid over the active site, resulting in desolvation and destabilization of the D-amino acid carboxylate.

Amino acid decarboxylases are critically involved in the biosynthesis of a wide range of biogenic amines and alkaloids. There are two major classes of amino acid decarboxylases: those dependent on pyridoxal 5′-phosphate (PLP) and those that use a pyruvate cofactor. The PLP-dependent decarboxylases are found in two different structural folds, Fold I and Fold III.[1] The Fold I decarboxylases include eukaryotic glutamate, histidine, and aromatic amino acid decarboxylases. All of the Fold I PLP-dependent and the pyruvate-dependent decarboxylases have been found to proceed with retention of configuration at the site of decarboxylation.[2,3] The Fold III decarboxylases include eukaryotic L-ornithine decarboxylase (ODC) and prokaryotic L-arginine and L-lysine/ornithine decarboxylase (LODC), which also react with retention of configuration,[4−9] and diaminopimelate decarboxylase (DAPDC), the only example known to date of a stereoinverting decarboxylase.[10,11] Recently, we discovered a new Fold III amino acid decarboxylase from *Salmonella enterica* serovar typhimurium that decarboxylates D-ornithine and D-lysine (eq 1, DOKDC).[12]



(1)

Decarboxylation of D-ornithine and D-lysine by DOKDC was performed in D_2O, and the progress of the reaction was followed by ^1H nuclear magnetic resonance (NMR). When the decarboxylation reactions were complete, the diamine reaction products were converted to the biscamphanamides with camphanyl chloride.[6] The mass spectra of the biscamphanamides showed the incorporation of ∼0.9 mol of deuterium into the amine products (Table 1 and Figure S1). It has been shown that the diastereotopic α-hydrogens of biscamphanamides can be distinguished by NMR with $Eu(FOD)_3$.[6] The ^1H NMR spectra of these products in $CDCl_3$ in the presence of $Eu(FOD)_3$ showed that the intensity of the upfield peak of the

Table 1. Properties of Biscamphanamides of Putrescine and Cadaverine from DOKDC

substrate	*pro*-S (integration, H)	*pro*-R (integration, H)	M^+ (peak height, %)	$(M + 1)^+$ (peak height, %)
D-ornithine	2.0	1.04	11	89
D-lysine	2.0	1.18	11	89

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Biochemistry XXXX, XXX, XXX−XXX

α-hydrogens is reduced by ~50% due to replacement of one of the diastereotopic *pro*-R hydrogens of the α-carbon with deuterium (Figure 1 and Table 1). The (*R*)-1-[^{2}H]putrescine

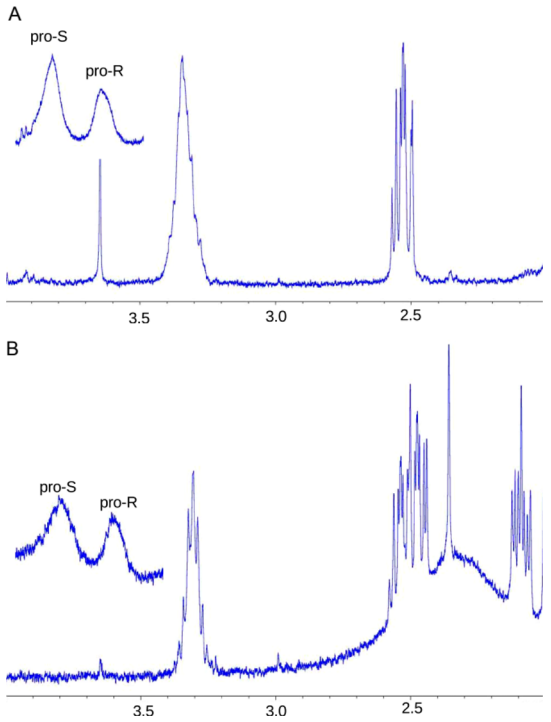

Figure 1. (A) 400 MHz ^{1}H NMR spectrum of putrescine biscamphanamide obtained by decarboxylation of D-ornithine by DOKDC. The inset shows the spectrum of the α-hydrogens in the presence of Eu(FOD)$_3$. (B) 400 MHz ^{1}H NMR spectrum of cadaverine biscamphanamide obtained by decarboxylation of D-lysine by DOKDC. The inset shows the spectrum of the α-hydrogens in the presence of Eu(FOD)$_3$.

product of D-ornithine decarboxylation by DOKDC (Figure 1A) is thus identical to that reported previously for decarboxylation of L-ornithine by ODC, which occurs with retention of configuration.[6] Hence, the decarboxylation of D-ornithine catalyzed by DOKDC occurs with inversion of configuration at the α-carbon. A similar result was also observed with DOKDC decarboxylation of D-lysine (Figure 1B). Thus, the reaction of DOKDC is now the second documented example of a decarboxylase reaction that proceeds with inversion of stereochemistry.

Crystals of DOKDC were grown in hanging drops over wells containing 28−30% dioxane. The diffraction data, collected at the SER-CAT BM22 beamline at APS, were phased by molecular replacement with a search model derived by truncation from DAPDC of *Helicobacter pylori*[13] [Protein Data Bank (PDB) entry 2QGH, 24% identical]. The asymmetric unit is the monomer, crystallizing in space group *C*121. The statistics for the final structure (PDB entry 6N2H) are given in Table S1. The resulting final model after refinement shows the typical PLP-dependent Fold III (β/α)$_8$-barrel structure for the large domain and a β-sandwich for the small domain (Figure 2). As expected for Fold III enzymes, the biologically active assembly of DOKDC is the dimer (Figure S2), with the PLP-binding site formed at the monomer−monomer interface. Good density was observed for all 465 residues (Figure S3), except for a loop comprising

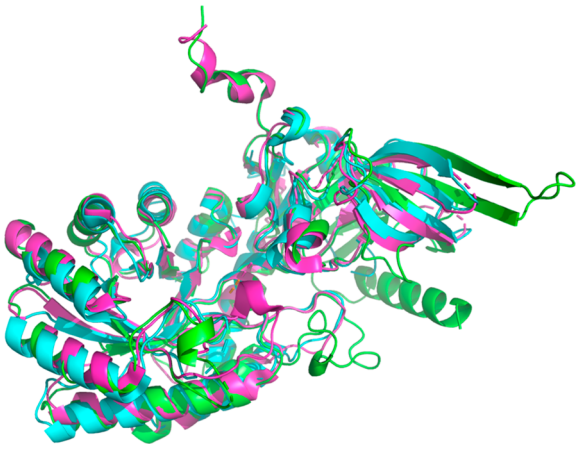

Figure 2. Structure of the DOKDC monomer (green) overlaid with those of the monomer of *H. pylori* DAPDC (magenta) and *Vibrio vulnificus* LODC (cyan).

residues 176−183 that was disordered and did not show enough density for a model to be built. This loop forms a lid over the active site and becomes ordered when substrates are bound to DAPDC[13] and arginine decarboxylase.[14] DOKDC has an N-terminal helix, followed by two β-turns, containing 35 residues, that is not seen in DAPDC or LODC and a β-hairpin extended by 10 residues (334−343) in the small domain (Figure 2 and Figure S1). The secondary structure of DOKDC is shown in Figure S4.

Despite sharing the same fold, DOKDC shows a low level of sequence homology (<30%) with DAPDC and LODC (Figure S5). There are only 31 residues of 465 that are strictly conserved in all three enzymes, the majority of which are glycines and prolines that are critical for the (β/α)$_8$-barrel. The remainder of the conserved residues are located in the active site. Several of the conserved residues are used for PLP binding. These include Lys-80, which forms the Schiff's base; His-218, which π-stacks with the cofactor pyridine ring; Glu-307, which hydrogen bonds to the PLP NH; and, Arg-310 and Tyr-422, which form hydrogen bonds with the phosphate of PLP (Figure 3). DAPDC has two amino acid-binding sites for *meso*-DAP, one that binds the D-end, proximal to the PLP, allowing for Schiff base formation and decarboxylation, and the other that preferentially binds the L-end, distal from the PLP-binding site.[15] An overlay of the structure of DOKDC with the structure of *Escherichia coli* DAPDC with D-lysine bound[16] (PDB entry 1KO0) shows that the changes in the distal amino acid-binding pocket (the "specificity helix") may be responsible for the change in substrate specificity (Figure 3). Hence, Arg-307 of DAPDC forms a salt bridge with the α-carboxylate of the product, L-lysine, and the ε-amino group forms a Schiff base with the PLP. However, D-lysine binds to DAPDC with the α-amino group bound to PLP, and the ε-amino group is in the distal binding site (Figure 3). This would result in an unfavorable electrostatic interaction of the cationic ε-amino group with Arg-307. In DOKDC, this is avoided, because there is no arginine in the distal pocket; rather, there is an aspartate, Asp-356, present to form an ion pair with the terminal ε-ammonium group of D-lysine or D-ornithine (Figure 3).

Comparison of the structures of DOKDC and LODC with putrescine bound[17] shows that LODC also has an aspartate residue on the specificity helix in the distal pocket to bind the ammonium end of L-lysine/L-ornithine (Figure 4). Paradoxi-

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Biochemistry XXXX, XXX, XXX−XXX

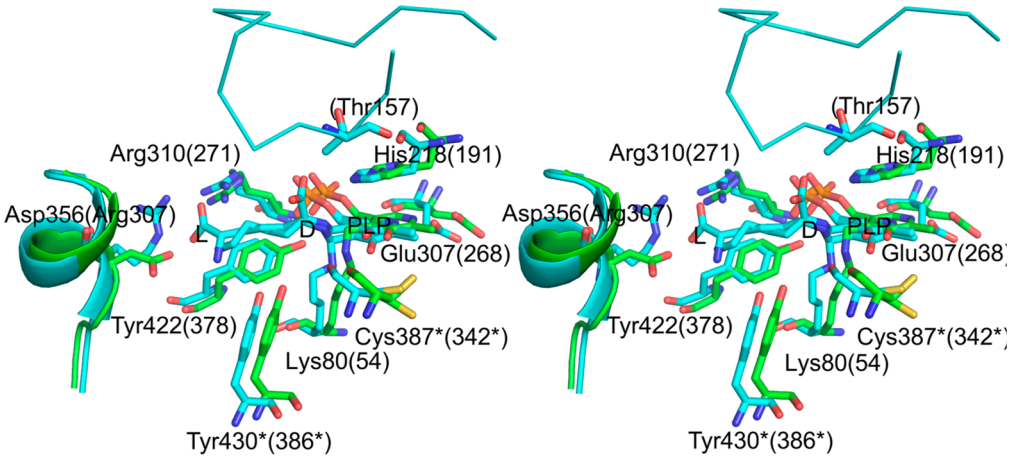

Figure 3. Crossed eye stereoview of the active site of *E. coli* DAPDC (PDB entry 1KO0) with D-lysine and L-lysine bound (cyan) overlaid with the structure of DOKDC (green). The numbers in parentheses are those of *E. coli* DAPDC. The specificity helix is on the left, and the lid of DAPDC can be seen as the cyan ribbon on top.

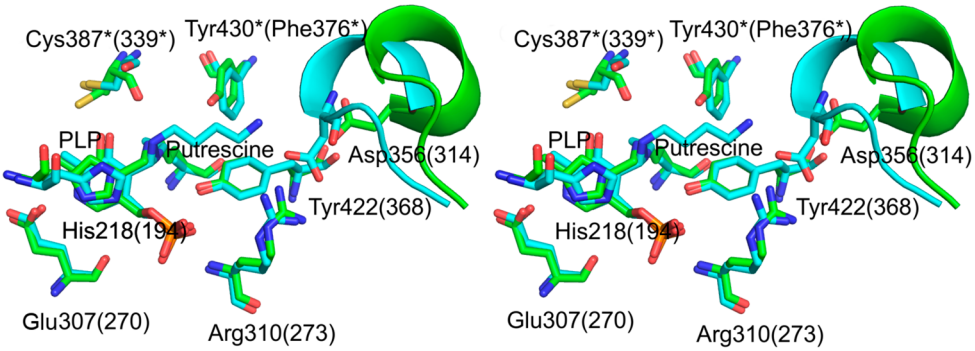

Figure 4. Crossed eye stereoview of the active site of *V. vulnificus* LODC (PDB entry 2PLJ) with putrescine bound (cyan) overlaid with the structure of DOKDC (green). The numbers in parentheses are those of *V. vulnificus* LODC. The specificity helix is on the right. The PLP-binding lysine can be seen behind the putrescine.

cally, DOKDC and LODC catalyze the decarboxylation of enantiomeric substrates within a virtually identical active site. The general acid catalyst in DAPDC and ODC that protonates the amine product has not been determined previously. There is a conserved cysteine in the active site of all of these decarboxylases (Figures 3 and 4 and Figure S4). However, mutagenesis of this cysteine to alanine or serine does not abolish activity,[18,19] so it is not the proton donor. In the structures in Figures 3 and 4, the PLP-binding lysines of DOKDC, DAPDC, and LODC are in identical locations. It should be noted again that the putrescine product of D-ornithine decarboxylation by DOKDC and L-ornithine decarboxylation by ODC in D_2O is identical (Figure 1A). Hence, it is most likely that the PLP-binding lysine ϵ-ammonium is the proton donor for DAPDC, DOKDC, LODC, and ODC reactions, resulting in the observed inversion and retention of configuration.

The remaining question is what controls substrate stereospecificity. In DAPDC and DOKDC, the D-amino acid will orient the reactive α-carboxylate upward toward the solvent, on the *si*-face of the PLP, opposite from the lysine (Figure 3). In contrast, in ODC and LODC, the L-amino acid carboxylate will be oriented downward, on the *re*-face of the PLP, the same face as that of the PLP-binding lysine. The other residues in the active sites of these enzymes that are different are not consistently related to the reaction stereochemistry, with the exception of Tyr-430 in DOKDC, which is also conserved in DAPDC (Figure 3) but is replaced by phenylalanine in LODC and ODC (Figure 4). The phenolic O of Tyr-430 would be only 2 Å from the carboxylate oxygens of bound L-ornithine modeled into DOKDC in the position of the putrescine in Figure 4. This would preclude the productive binding of the L-amino acid to the PLP of DOKDC and DAPDC, due to unfavorable steric clashes. The phenylalanine in ODC and LODC provides space for the α-carboxylate in a hydrophobic pocket, facilitating the decarboxylation by electrostatic destabilization.[20] In support of this hypothesis, mutation of Phe-397 in ODC to alanine decreases the rate of decarboxylation by 2100-fold.[20] Tyr-430 is also a potential proton donor for the decarboxylation reactions of DAPDC and DOKDC. In contrast, there is no significant difference in the environment on the *si*-face of the PLP among DOKDC, DAPDC, ODC, and LODC. Although the active site lid is disordered when the enzyme is unoccupied, the *si*-face is covered by the lid when the substrate is bound (Figure 3). There is no homology in the sequences of the lid for these decarboxylases, although the loop is notably five or six residues longer for DAPDC and DOKDC (Figure S5, blue residues). For DAPDC and DOKDC, there is a conserved threonine that could donate a hydrogen bond to the carboxylate of the D-amino acids (Figure 3 and Figure S5). It has been suggested previously that the mechanism of DAPDC involves concerted decarboxylation and back side protonation, to explain the observed inversion of configuration of the product.[21] We have

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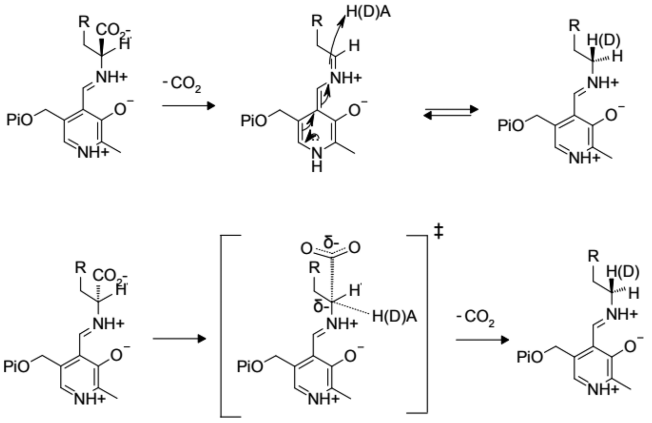
Biochemistry XXXX, XXX, XXX−XXX

found inversion of stereochemistry in the reaction of DOKDC (Figure 1), so it is likely to be concerted, as well. The concerted mechanism is supported by the lack of detectable exchange of the α-hydrogens of putrescine upon incubation with DOKDC in D_2O for 72 h (Figure S6).

Decarboxylation with retention of configuration must occur in a stepwise fashion (Scheme 1, top). The reactive carboxylate

Scheme 1. (Top) Stepwise Mechanism for Decarboxylation of an L-Amino Acid with Retention of Configuration and (Bottom) Concerted Mechanism for Decarboxylation of a D-Amino Acid with Inversion of Configuration



bond must be positioned perpendicular to the plane of the PLP, according to Dunathan's hypothesis.[22] By the principle of microscopic reversibility, the product of the stepwise mechanism must be capable of exchange of protons of the product with solvent. For L-ornithine decarboxylase, this reversal of proton transfer has been exploited in the design of potent and selective mechanism-based inactivators.[23−26] In contrast, in the concerted mechanism for decarboxylation of a D-amino acid (Scheme 1, bottom), there is no pathway for product proton exchange with solvent. The decarboxylation of the D-amino acids in these enzymes may be driven by the combination of a positive electrostatic environment on the *re*-face of the substrate complex by the lysine ϵ-ammonium, together with desolvation and electrostatic destabilization of the leaving α-carboxylate upon lid closure.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.biochem.8b01319.

Experimental methods, mass spectra, topology, sequence alignments, and electron density map (PDF)

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Author Contributions

R.S.P. wrote the paper and determined the structure of DOKDC. T.R.H. and K.A.M. cloned DOKDC, constructed the expression system, and purified the enzyme. P.P. prepared the crystals of DOKDC. D.K. performed the stereochemistry experiments.

Notes

The authors declare no competing financial interest.

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Biochemistry XXXX, XXX, XXX−XXX