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Crystallization of a Mammalian Ornithine Decarboxylase

Andrew Kern,[1] Marcos A. Oliveira,[1] Ning-Leh Chang,[1] Stephen R. Ernst,[1] Donald W. Carroll,[1] Cory Momany,[1] Karyl Minard,[2] Philip Coffino,[2] and Marvin L. Hackert[1]

[1]Department of Chemistry and Biochemistry, University of Texas at Austin, Austin Texas 78712; and [2]Department of Microbiology and Immunology and Department of Medicine, University of California, San Francisco, California 94143

ABSTRACT **Crystals of truncated (Δ425–461) pyridoxal-5′-phosphate (PLP)-dependent mouse ornithine decarboxylase (mOrnDC′) have been obtained that diffract to 2.2 Å resolution ($P2_12_12$, a = 119.5 Å, b = 74.3 Å, c = 46.1 Å). OrnDC produces putrescine, which is the precursor for the synthesis of polyamines in eukaryotes. Regulation of activity and understanding of the mechanism of action of this enzyme may aid in the development of compounds against cancer. mOrnDC is a member of group IV PLP-dependent decarboxylases, for which there are no known representative structures.**

Key words: polyamines, group IV decarboxylases, pyridoxal phosphate

INTRODUCTION

OrnDC is the enzyme implicated in the first and rate-limiting step of polyamine biosynthesis.[1] It catalyzes the PLP-dependent decarboxylation of ornithine to putrescine, the diamine precursor of polyamines. OrnDC activity and the resulting polyamines have been demonstrated to be essential for the process of cellular proliferation and transformation.[2] The extremely short intracellular half-life of the OrnDC protein[3] is a key characteristic that permits a ready response to the cellular regulatory mechanisms. An antizyme targets the enzyme for degradation in an ATP-dependent, but ubiquitin-independent mechanism by a 26S proteolytic complex known as proteasome.[4]

In its active form the mammalian enzyme is a homodimer of 55 kD subunits. OrnDC from both rat brain[5] and *Lactobacillus* (Carroll and Hackert, unpublished results) are activated by GTP even though their structures differ in the size of subunit, oligomeric state, and their classification based on N-1 sequence analysis.[6] The active site of mOrnDC has now been probed by site-directed mutagenesis and active site-directed irreversible inhibitors. The results provide evidence that the active site is composed of side chains from both monomers with two active sites/dimer.[7,8] In addition, Lys 69 has been identified as forming the Schiff base with PLP.[9,10]

We report here the crystallization of two forms of mouse OrnDC decarboxylase and the initial analysis of crystallographic data from the truncated form of this enzyme.

MATERIALS AND METHODS

The truncated form of mouse ornithine decarboxylase (OrnDC′, Δ425–461), a deletion of the last 37 amino acids involved in degradation of the enzyme,[11] was used for crystallization after initial difficulty in obtaining crystals of the full-length mOrnDC. Six liters of 2xYT media containing carbenicillin were inoculated with bacteria carrying the plasmid with the mOrnDC′ gene. The cells grew at 30°C for 3 hours after induction by 1mM IPTG. The cells were pelleted by centrifugation and frozen at −20°C until the beginning of purification. Frozen cells were resuspended in 25 mM Tris, pH 7.5, with 2.5 mM DTT, 1 mM EDTA, and 0.02% Brij-35. The proteolytic inhibitor PMSF was also added when cells were being broken using a French Press at 16,000 psi. The broken cells were centrifuged and both pellet and supernatant assayed for OrnDC activity using a Gilson differential respirometer. The active soluble fraction was then loaded onto a PLP AFI-Gel-10 column at a flow rate of 3 ml/hr. The protein was allowed to remain on the column for at least 24 hours, and then flushed with buffer lacking PLP until the baseline of the detector was back to near zero. The mOrnDC′ was then eluted from the

Abbreviations: OrnDC, ornithine decarboxylase; mOrnDC, mouse ornithine decarboxylase; mOrnDC′ Δ425–461, mOrnDC truncated by removal of 37 amino acid residues from the C-terminus; OrnDC130a, ornithine decarboxylase from *Lactobacillus* strain 30a; AAT, aspartate aminotransferase; ATP, adenosine triphosphate; GTP, guanosine triphosphate; DTT, dithiothreitol; PLP, pyridoxal-5′-phosphate; TSβ, tryptophan synthase β-subunit; IPTG, isopropyl-β-D-thiogalactoside; MFMO, monofluoromethylornithine; SSRL, Stanford Synchrotron Radiation Laboratory; CCP4, Collaborative Computing Project no. 4; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; PEG, polyethylene glycol.

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Cory Momany's present address is Department of Biological Sciences, Purdue University, West Lafayette, IN 47907.

Address reprint requests to Marvin L. Hackert, Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712.

TABLE I. Statistics for Data Collected at SSRL

Bragg spacing	Unique no.	Total no. measured	No. of multiple*	% Data†	R_{merge}‡
30.0–9.37	231	713	176	76.1	0.042
9.37–6.79	431	1,397	332	89.5	0.039
6.79–5.60	559	1,765	423	92.3	0.042
5.60–4.87	666	2,044	489	95.5	0.043
4.87–4.36	737	2,280	534	94.3	0.043
4.36–3.99	828	2,539	593	96.5	0.048
3.99–3.70	907	2,801	653	97.5	0.059
3.70–3.46	962	2,924	674	97.0	0.067
3.46–3.27	1,013	3,106	744	96.7	0.079
3.27–3.10	1,087	3,409	808	98.4	0.097
3.10–2.96	1,151	3,575	833	98.9	0.112
2.96–2.84	1,201	3,755	888	99.0	0.147
2.84–2.72	1,231	3,777	902	98.1	0.163
2.72–2.63	1,316	4,145	976	99.9	0.219
2.63–2.54	1,347	4,205	1,016	99.8	0.248
2.54–2.46	1,385	3,998	949	99.4	0.254
2.46–2.39	1,407	3,681	900	97.9	0.242
2.39–2.32	1,444	3,780	928	98.1	0.252
2.32–2.26	1,507	3,927	989	99.6	0.320
2.26–2.20	1,518	3,918	957	97.9	0.349
30.0–2.20	20,928	61,739	14,764	97.6	0.084

*Number of reflections for which multiple measurements exist.
†Fraction of theoretically possible data measured.
‡$R_{merge} = (\Sigma|I - \langle I\rangle|)/(\Sigma\langle I\rangle)$.

column using a 50 mM PLP solution at a flow rate of 10 ml/hr. The fractions were assayed to identify the peak containing mOrnDC′ activity. The quality of the purification was verified by both SDS-PAGE and isoelectric focusing and in each experiment a major band was observed (results not shown). The purified mOrnDC′ sample was then screened for crystallization conditions using the approach of Jancarick and Kim.[12] This resulted in crystals that appeared within a week at room temperature. The initial conditions of crystallization were further refined to the use of a combination of 20% PEG 3350 and 30% 2-propanol. We were also able to co-crystallize mOrnDC′ with the inhibitor MFMO. The purified, untruncated samples of mouse OrnDC were also screened for crystallization conditions and produce crystals that have the same morphology as that of the truncated form.

RESULTS AND DISCUSSION

Crystals of mOrnDC′ (0.3 mm in size) were tested for diffraction quality. Using a rotating anode X-ray source (RIGAKU RU200, 50 KV and 110 mA) the limit of diffraction was 3.7 Å. The crystals suffered rapid radiation decay resulting in loss of diffraction within a period of 72 hours. Fortunately, using the synchrotron radiation source at Stanford (SSRL), a 97.6% complete data set was obtained to a maximum resolution of 2.2 Å. The data were collected using a MAR detector at beam line 7.1. The oscillation angle used was 1.2°, with a crystal-to-image plate distance of 130 mm and 20 seconds for each exposure. These data were processed using MOSFLM,[13] and then scaled and merged using the ROTAVATA and AGROVATA programs, available through the CCP4 suite.[14] The statistics for a native data set are indicated in Table I. The refined cell dimensions are a = 119.52 Å, b = 74.32 Å, c = 46.06 Å, space group $P2_12_12$. Assuming a monomer per asymmetric unit, the calculated V_m value is 2.16 Å^3/Da. Additionally, since the active form of the enzyme is a dimer, the crystallographic twofold axis is also the molecular twofold axis that relates two monomers.

Momany et al.[15] reported a sequence motif (based on sequence alignments and the structure of OrnDC130a[16]) that is able to identify a common PLP binding domain in aminotransferases and decarboxylases of groups I, II, and III but not group IV. This finding indicates that the structures of group IV decarboxylases belong to another structural class. Sequence comparisons of group IV decarboxylases with the PLP binding TSβ[17] shows very low homology, although the spatial arrangement of two conserved sequence blocks suggests structural similarity so that TSβ might serve as a model for mOrnDC. One of the conserved blocks is localized near the N-terminus and contains the lysine that binds to the PLP cofactor. The second block is a glycine-rich region found roughly 145 residues away from the lysine in the direction of the C-terminus.

mOrnDC ^{84}AHKTNQV^{90} ^{226}IACVGGGSNA^{235}
TSβ ^{67}AVKCNDS^{73} ^{221}LLDIGGGFPG^{230}

It is known that these two sequence blocks are spatially close to one another in the structure of

TSβ, form part of the active site, and anchor the PLP to the structure. The glycines are found in a loop that leads to the N-terminal end of an α-helix that interacts with the phosphate of the PLP cofactor. In TSβ the PLP binds at the interface of two domains. This also occurs in OrnDCl30a[16] and AAT,[18] where a distinct PLP binding domain is shared between these two structures.[15] Alternatively, an α/β barrel structural model has been recently proposed for the N-terminal domain of eukaryotic OrnDCs.[19]

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