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Crystallization and Preliminary X-Ray Studies of Ornithine Decarboxylase From *Trypanosoma brucei*

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ABSTRACT ***Trypanosoma brucei*** **ornithine decarboxylase, expressed and purified from *E. coli*, has been crystallized by the vapor diffusion method using PEG 3350 as a precipitant. The crystals belong to the monoclinic space group $P2_1$ and have cell constants of a = 66.3 Å, b = 151.8 Å, c = 83.7 Å, and β = 101.2°. While larger crystals are twinned, smaller crystals (0.4 × 0.3 × 0.05 mm^3) are single.**

Key words: polyamines, pyridoxal phosphate, drug design

INTRODUCTION

The first committed step in polyamine biosynthesis is the decarboxylation of ornithine to produce putrescine; this reaction is catalyzed by a pyridoxal phosphate (PLP)-dependent enzyme, ornithine decarboxylase (ODC).[1] Because polyamines are required for cell growth and differentiation, ODC has generated interest as a drug target.[2] α-Difluoromethylornithine (DFMO), an inhibitor of ODC, has been used for the treatment of sleeping sickness caused by African trypanosomes, *Pneumocystis carinii* pneumonia in AIDS patients, and, in combination with other drugs, as an anti-tumor agent.[3,4] We have undertaken structural studies of *Trypanosoma brucei* ODC with the prospect of improving on DFMO as an anti-trypanosomal agent.

T. brucei ODC is a dimer composed of two identical 47 kd monomers; the gene for the enzyme has been cloned and expressed in *E. coli*.[5,6] Mutational analysis suggests that the active site is shared between the two subunits.[6–8] Each subunit is likely to be composed of two domains.[9] The larger N-terminal domain contains residues that interact with PLP; Lys-69 was identified as the residue that forms a Schiff base with PLP[10] and Glu-274 as the residue that stabilizes the pyridine nitrogen of PLP.[11] The C-terminal domain contains residues that may be involved in substrate interactions.[10,11] Additionally, site-directed mutagenesis has identified a number of other residues that are required for full enzyme activity.[8,12,13] Sequence comparison, secondary structure prediction, and hydrophobicity profiles were used to model the structure of the ODC N-terminal domain, which is not homologous to any of the PLP binding enzymes of reported structure. The analysis predicts that the N-terminal domain of ODC folds into a β/α-barrel.[14] In this paper we describe improvements in protein purification and the crystallization conditions that have produced crystals suitable for structure determination by X-ray crystallography.

PROTEIN EXPRESSION AND PURIFICATION

ODC was expressed in *E. coli* as a fusion protein to an N-terminal 6xHis-tag followed by the tobacco etch virus (TEV)-protease cleavage site and was purified from the soluble fraction of the lysed bacteria by Ni^{2+}-NTA agarose column chromatography as described.[6] The 6xHis-tag was removed from ODC by overnight treatment at room temperature with glutathione-S-transferase (GST)-TEV-protease fusion protein,[15] which was immobilized on glutathione-agarose beads. The supernatant from this incubation was loaded onto a Ni^{2+}-NTA agarose column and ODC with the 6xHis-tag removed (processed form) was recovered in the flow-through. The yield of the processed ODC was about 60–70%. To eliminate aggregated forms of the enzyme the sample was concentrated by ultrafiltration in the presence of dithiothreitol (DTT) (10 mM) and PLP (1 mM) and was loaded onto a High-Load 16/60 Superdex 200 gel-filtration column in 10 mM HEPES-NaOH, pH 7.2, 2 mM DTT, 50 mM NaCl, 0.5 mM EDTA, 0.015% Brij35. The major fraction of ODC was eluted with a retention time corresponding to a dimer (90 kd). The protein was homogeneous by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and was concentrated to 20–25 mg/ml for crystallization experiments.

Abbreviations: ODC-Trypanosoma brucei ornithine decarboxylase, PLP—pyridoxal phosphate, PEG—polyethylene glycol, DFMO—α-difluoromethylornithine, GST—glutathione-S-transferase.

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TABLE I. Crystallization of *Trypanosoma brucei* Ornithine Decarboxylase

	Conditions	Crystal form
1.	Hampton screen solution #22: 30% PEG 4000, 0.1 M Tris-HCl, pH 8.5, 0.2 M sodium acetate, 20 mg/ml ODC 16°C	Clusters of microcrystals
2.	20% PEG 3350, 0.1 M Tris-HCl, pH 8.0, 0.2 M ammonium acetate, 10 mM DTT, 20 mg/ml ODC, 16°C	Clusters of plates; the largest plates were $0.7 \times 0.4 \times 0.03$ mm^3
3.	Microseeding from condition 2 using the same conditions	Individual crystals, plates $0.7 \times 0.4 \times 0.1$ mm^3

CRYSTALLIZATION AND DATA ANALYSIS

A Hampton screen[16] was used to identify approximate crystallization conditions in hanging drops. ODC (2 μl of 20 mg/ml) was combined with an equal volume of a reservoir solution. Crystallization trials were carried out for both the His-tagged and processed forms of ODC. The His-tagged protein did not yield crystals under any of the tested conditions, while the processed ODC produced crystals from the Hampton screen solution #22 (Table I, row 1). The conditions were optimized by varying the identity of the salt (included at 0.2 M were sodium chloride, sodium acetate, sodium sulfite, ammonium chloride, ammonium acetate, nickel sulfate, sodium phosphate, or magnesium sulfate), the polyethylene glycol (PEG) type (PEG 400, 1500, 3350, 4000, 6000, and 8000; all obtained from Fluka, Chemika) and concentration (5–30%), the protein concentration (10–35 mg/ml), the buffer (Na-HEPES, Tris-HCl, sodium cacodylate, 0.1 M), the pH (6–9), and the temperature (4°, 16°, 22°C). The best crystals were clusters of thin yellow plates (Table I, row 2). To obtain single crystals, the clustered crystals were crushed and used for microseeding (Table I, row 3). The largest crystals were about $0.7 \times 0.4 \times 0.1$ mm^3 and grew in sitting drops (5–50 μl).

Diffraction measurements were recorded with the rotating anode X-ray source and the Rigaku image plate detection system at −185°C. Crystals were frozen for X-ray analysis in liquid nitrogen after soaking in PEG 400 solutions by 5% stepwise increases in PEG concentration ranging from 15 to 35%. The diffraction limit was 2.6 Å, the space group $P2_1$, and the cell constants a = 66.3 Å, b = 151.8 Å, c = 83.7 Å, and β = 101.2°.

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REFERENCES

1. Tabor, C.W., Tabor H. Polyamines. Annu. Rev. Biochem. 53:749–790, 1984.
2. McCann, P.P., Pegg, A.E. Ornithine decarboxylase as an enzyme target for therapy. Pharm. Ther. 54:195–215, 1992.
3. Marton, L.J., Pegg, A.E. Polyamines as targets for therapeutic invervention. Annu. Rev. Pharmacol. Toxicol. 35: 55–91, 1995.
4. Wang, C.C. Molecular mechanisms and therapeutic approaches to the treatment of African trypanosomiasis. Annu. Rev. Pharmacol. Toxicol. 35:93–127, 1995.
5. Phillips, M.A., Coffino, P., Wang, C.C. Cloning and sequencing of the ornithine decarboxylase gene from *Trypanosoma brucei*. Implications for enzyme turnover and selective α-difluoromethylornithine inhibition. J. Biol. Chem. 262:8721–8727, 1987.
6. Osterman, A., Grishin, N.V., Kinch, L.N., Phillips, M.A. Formation of functional cross-species heterodimers of ornithine decarboxylase. Biochemistry 33:13662–13667, 1994.
7. Tobias, K.E., Kahana, C. Intersubunit location of the active site of mammalian orinithine decarboxylase as determined by hybridization of site-directed mutants. Biochemistry 32:5842–5847, 1993.
8. Coleman, C.S., Stanley, B.A., Viswanath, R., Pegg, A.E. Rapid exchange of subunits of mammalian ornithine decarboxylase. J. Biol. Chem. 269:3155–3158, 1994.
9. Osterman, A.L., Lueder, D.V., Quick, M., Myers, D., Canagarajah, B.J., Phillips, M.A. Domain organization and a protease-sensitive loop in eukaryotic ornithine decarboxylase. Biochemistry 34:13431–13436, 1995.
10. Poulin, R., Lu, L., Ackerman, B., Bey, P., Pegg, A.E. Mechanism of the irreversible inactivation of mouse ornithine decarboxylase by α-difluoromethylornithine: Characterization of sequences at the inhibitor and coenzyme binding sites. J. Biol. Chem. 267:150–158, 1992.
11. Osterman, A., Kinch, L.N., Grishin, N.V., Phillips, M.A. Acidic residues important for substrate binding and cofactor reactivity in eukaryotic ornithine decarboxylase identified by alanine scanning mutagenesis. J. Biol. Chem. 270:11797–11802, 1995.
12. Lu, L., Stanley, B.A., Pegg, A.E. Identification of residues in ornithine decarboxylase essential for enzymic activity and for rapid protein turnover. Biochem. J. 277:671–675, 1991.
13. Tsirka, S., Coffino, P. Dominant negative mutants of ornithine decarboxylase. J. Biol. Chem. 267:23057–23062, 1992.
14. Grishin, N.V., Phillips, M.A., Goldsmith, E.J. Modeling of the spatial structure of eukaryotic ornithine decarboxylases. Protein Sci. 4:1291–1304, 1995.
15. Parks, P.D., Leuther, K.K., Howard, E.D., Johnston, S.A., Dougherty, W.G. Release of proteins and peptides from fusion proteins using a recombinant plant virus proteinase. Anal. Biochem. 216:413–417, 1994.
16. Jancarik, J., Kim, S.H. Sparse matrix sampling: A screening method for crystallization of proteins. J. Appl. Crystallogr. 24:409–411, 1991.