Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization of native and selenomethionyl yeast orotidine 5′-phosphate decarboxylase

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Received 5 October 1999
Accepted 18 January 2000

Crystals of the *Saccharomyces cerevisiae* pyrimidine biosynthetic enzyme orotidine 5′-phosphate decarboxylase (ODCase) were grown by the hanging-drop vapor-diffusion technique at 277 K using polyethylene glycol 4000 as the precipitant. Crystals of native and selenomethionyl ODCase diffract to less than 2.2 Å and belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 90.1, b = 116.2, c = 117.0 Å. Crystals of ODCase grown in the presence of the postulated transition-state analog inhibitor 6-hydroxyuridine 5′-phosphate (BMP) diffract to less than 2.5 Å and belong to space group $P2_1$, with unit-cell parameters a = 79.9, b = 80.0, c = 98.2 Å, β = 108.6°.

1. Introduction

Orotidine 5′-phosphate decarboxylase (ODCase; E.C. 4.1.1.23) catalyzes the final step in *de novo* pyrimidine biosynthesis yielding uridine 5′-phosphate (UMP), an essential precursor for synthesis of nucleic acids. In eukaryotes, orotate phosphoribosyltransferase (OPRTase; E.C. 2.4.2.10) and ODCase comprise separate catalytic domains of the bifunctional enzyme UMP synthase. However, in fungi and bacteria these enzymes exist as independent monofunctional dimeric proteins (Yablonski *et al.*, 1996). Compounds that are ODCase inhibitors impair biosynthesis of nucleic acids in all cell types and several have been tested as potential anticancer agents without success (Handschumacher *et al.*, 1962; Sweeney *et al.*, 1973). One of the most potent ODCase inhibitors is 6-hydroxyuridine 5′-phosphate (BMP; $K_i = 9 \times 10^{-12}\,M$), a compound which resembles the postulated intermediate generated in the transition state during OMP decarboxylation (Levine *et al.*, 1980).

The spontaneous loss of CO_2 from OMP is an extremely slow reaction that proceeds with a half-time of nearly 7.8×10^7 y in aqueous solutions at 298 K (Radzicka & Wolfenden, 1995). Despite numerous studies involving model decarboxylation reactions (Beak & Siegel, 1976; Silverman & Groziak, 1982; Lee & Houk, 1997), the mechanism employed by ODCase remains a mystery. In contrast to other enzymatic decarboxylations, ODCase is unique in that it appears to function independently of known cofactors and metals (Shostak & Jones, 1992; Miller *et al.*, 1999) while enhancing the spontaneous rate of OMP decarboxylation by nearly 17 orders of magnitude. Thus, structural studies of ODCase provide a source of new information that should enhance understanding of this enzyme's reaction mechanism. Here, we describe crystallization conditions for recombinant yeast ODCase, free and in complex with 6-hydroxyuridine 5′-phosphate, and present initial X-ray diffraction results for both the native and selenomethionine-substituted enzymes.

2. Materials and methods

Native recombinant yeast ODCase was expressed in *Escherichia coli* strain SS6130 and

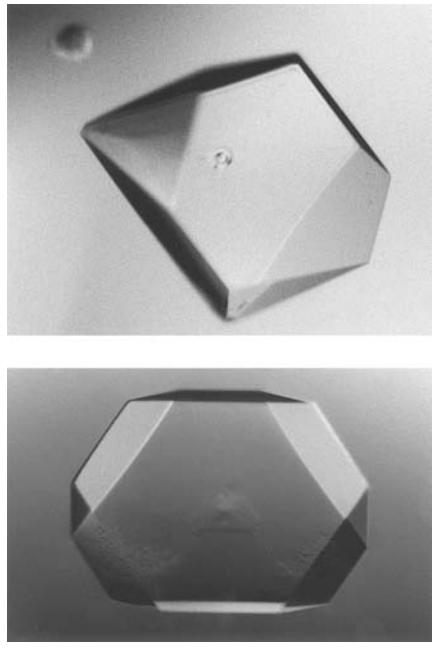

Figure 1
Photograph of native ODCase crystals grown at 277 K.

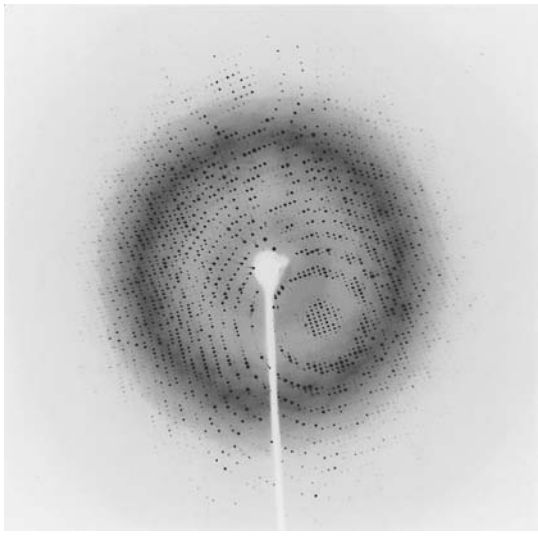

Figure 2
A 1.5° oscillation image of a crystal of native ODCase. The crystals diffract nominally to 2.0 Å.

purified as described previously (Miller *et al.*, 1999). Prior to its expression in *E. coli*, the AGA and AGG arginine codons of the yeast *ura3* gene were changed to CGC or CGU. Stock solutions of purified ODCase (18–25 mg ml^{-1}) were stored at 253 K in 50 m*M* Tris–HCl (pH 7.0) containing 5 m*M* β-mercaptoethanol and 5% glycerol. Protein concentration was estimated from the absorbance at 280 nm using a calculated molar extinction coefficient of 28 830 cm^{-1} (Edelhoch, 1967).

Selenomethionyl ODCase was prepared by growing SS6130 (pBGM88) in a minimal media containing Vogel and Bonner salts (Vogel & Bonner, 1956), 0.002% vitamin B_1, 0.6% glycerol and 150 µg ml^{-1} ampicillin. To this solution, sterile amino acids were added to give the following final concentrations: DL-selenomethionine, 120 mg ml^{-1}; alanine, glycine, serine and threonine, 0.5 mg ml^{-1}; arginine, 0.6 mg ml^{-1}; asparagine, aspartate, glutamine and lysine, 0.4 mg ml^{-1}; cysteine, 0.03 mg ml^{-1}; glutamate, 0.7 mg ml^{-1}; histidine and proline, 0.1 mg ml^{-1}; isoleucine, leucine and valine, 0.25 mg ml^{-1}; phenylalanine, 0.15 mg ml^{-1}; tryptophan and tyrosine, 0.2 mg ml^{-1}. The expression cultures were inoculated to an initial OD_{600} of 0.005 and incubated at 300 K for 19–24 h, after which the OD_{600} was ∼4.5. ODCase from bacteria grown in selenomethionine medium was purified to greater than 95% homogeneity using the same conditions employed for purification of the native protein. Analysis of the selenomethionyl enzyme by electrospray mass spectrometry showed that all six methionine residues per subunit were replaced by selenomethionine.

Crystals of native ODCase were grown at 277 K by the hanging-drop vapor-diffusion method using 0.1 *M* Na HEPES pH 7.0, 10%(*v*/*v*) 2-propanol and 20%(*w*/*v*) polyethylene glycol 4000 as the precipitant. The protein drops were prepared by adding 2 µl of ODCase solution to 3 µl of the reservoir solution. Crystals of native protein typically appeared overnight. Prior to data collection, the crystals were equilibrated in a cryoprotectant composed of 0.085 *M* Na HEPES pH 7.0, 8.5%(*v*/*v*) 2-propanol, 17%(*w*/*v*) polyethylene glycol 4000, 15% glycerol (Hampton Cryo #41). Crystals of selenomethionine-substituted ODCase were grown as described for the native enzyme except that these crystals required incubation for 6–10 d at 277 K. Diffraction data for native crystals were collected at 93 K on a Rigaku RU-200 rotating-anode generator equipped with an MSC R-AXIS 4 area detector.

Crystals of ODCase grown in the presence of BMP were obtained at 295 K in 0.18 *M* NaH_2PO_4, 18%(*w*/*v*) PEG 3350. The hanging drops contained 8 mg ml^{-1} enzyme and BMP added in a 2.7:1 molar ratio. Crystals generally appeared after 7–10 d and were equilibrated in cryoprotectant containing 0.18 *M* NaH_2PO_4, 18%(*w*/*v*) PEG 3350, 25%(*v*/*v*) glycerol prior to data collection.

3. Results and discussion

Screening for optimal crystallization conditions was performed using sparse-matrix sampling (Jancarik & Kim, 1991; Crystal Screens I and II, Hampton Research) at temperatures in the range 277–295 K. Single crystals of native recombinant yeast ODCase (0.74 × 0.66 × 0.37 mm) were obtained from hanging drops incubated overnight at 277 K (Fig. 1). The diffraction pattern shown in Fig. 2 represents a typical 10 min, 1.5° oscillation image obtained with crystals of native enzyme. These crystals diffracted to less than 2.0 Å resolution, were orthorhombic and belonged to the space group $P2_12_12_1$, with unit-cell parameters a = 90.1, b = 116.2, c = 117.0 Å. Assuming a protein density of 2.3 Å Da^{-1} and a solvent content of 40%, there are four molecules per asymmetric unit (Matthews, 1968). In general, ODCase crystals remained stable on storage at 277 K for six months without loss in resolution.

Replacement of all six ODCase methionine residues with selenomethionine did not appear to change the physical properties of this protein. The selenomethionyl enzyme had a specific activity that did not differ noticeably from the activity of the native enzyme. Crystals of the selenomethionyl protein were grown under the same crystallization conditions as defined for the native enzyme. However, these crystals were much slower to develop and were markedly smaller than those of the native enzyme. Attempts to increase crystal size by microseeding or to obtain crystals under different conditions were not successful.

The mechanism of enzymatic OMP decarboxylation has to date eluded definition. Our initial approach to the study of this reaction involves defining those enzyme residues that might participate in catalysis owing to their juxtaposition to the proposed transition-state analog inhibitor BMP (K_i = 9 × 10^{-12} *M*; Brody & Westheimer, 1979). To this end, structural studies were initiated on ODCase complexed with BMP. Crystals of ODCase (270 µ*M* in subunits) grown in the presence of excess BMP (740 µ*M*) belonged to the space group $P2_1$, with unit-cell parameters a = 79.9, b = 78.0, c = 98.2 Å, β = 108.6°, suggesting that these crystals represent the enzyme–ligand complex. These crystals diffracted to less than 2.5 Å and contained four ODCase monomers per asymmetric unit.

Our ability to grow ODCase crystals was aided by the original crystallization studies of yeast ODCase complexed with BMP (Bell *et al.*, 1991); however, the crystallization conditions and crystal space groups differed dramatically between the two studies. This lack of similarity may be because of physical differences between ODCase purified from yeast and the recombinant yeast enzyme expressed in and purified from bacteria (Miller *et al.*, 1999). Diffraction data collected for selenomethionyl ODCase and the native enzyme complexed with BMP are currently being analyzed. The ability to generate diffraction-quality crystals offers hope that the structures of free and liganded ODCase will be determined, thereby providing an understanding of the forces responsible for this enzyme's remarkable catalytic capabilities.

We thank Jennifer Neugebauer for conducting mass-spectrometric analysis of native and selenomethionine-substituted ODCase. This work was supported in part by NIH Training Grant GM-08570.

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