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Crystallization and crystal data on tyrosine phenol-lyase

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Crystals of the apoenzyme of tyrosine phenol-lyase (EC 4.1.99.2), a pyridoxal 5'-phosphate-dependent enzyme from *Citrobacter intermedius*, have been grown by vapor diffusion of an ammonium sulfate solution to a protein solution. The crystals belong to space group $P2_12_12$, with dimensions of $a=75.5$ Å, $b=138.4$ Å and $c=94.1$ Å and diffract up to 2.7 Å resolution. The asymmetric unit contains one half of the enzyme tetrameric molecule. Two heavy-atom derivatives of the crystals have been obtained.

Tyrosine phenol-lyase; Crystallization; Heavy-atom derivative; X-ray analysis; (*Citrobacter intermedius*)

1. INTRODUCTION

Tyrosine phenol-lyase from *Citrobacter intermedius* is a pyridoxal 5'-phosphate-dependent enzyme catalyzing both β-elimination and β-substitution reactions [1]. The enzyme also catalyzes the side transamination reaction [2]. Tyrosine phenol-lyase possesses broad substrate specificity, L-tyrosine and analogs, and non-aromatic acids such as DL-serine and DL-cysteine, as well as their derivatives, are substrates of the enzyme [1]. The broad substrate specificity and multifunctional activity of tyrosine phenol-lyase make it an interesting object for X-ray analysis. At the present time X-ray analysis is being carried out on different classes of pyridoxal 5'-phosphate-dependent enzymes. These investigations enable one to compare the structure and mechanism of action for these enzymes on the basis of data obtained from X-ray analysis. A simple procedure has been developed previously for isolation of the extensively purified enzyme in high yield [3]. One molecule of this enzyme consists of four chemically and catalytically identical subunits of molecular mass 45 kDa [4].

Here, we report a procedure for growing large crystals of tyrosine phenol-lyase that are suitable for X-ray analysis and the preparation of two heavy-atom derivatives.

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Abbreviation: pCMBS, *p*-chloromercuribenzenesulfonic acid

2. MATERIALS AND METHODS

Tyrosine phenol-lyase was purified as in [3]. Crystallization was performed at 4°C in 50 mM potassium phosphate buffer at different pH values. Ammonium sulfate, 2-methyl-2,4-pentanediol and polyethylene glycol (M_r 6000, 4000) were used as precipitants. Crystallization was carried out using the hanging-drop and sitting-drop diffusion technique. The enzyme concentration was varied from 10 to 30 mg/ml. Heavy-atom derivatives were obtained by soaking crystals in solutions of heavy-atom derivatives. The stabilization solution for soaking comprised 1 M $MgSO_4$ in 50 mM Hepes-KOH buffer, pH 7.0.

3. RESULTS AND DISCUSSION

We were not able to obtain enzyme crystals using 2-methyl-2,4-pentanediol and polyethylene glycol as precipitants (hanging-drop technique). Thin, plate-like crystals were formed in the presence of polyethylene glycol (M_r 4000, 6000) as observed with the method of free diffusion. Larger crystals were obtained with ammonium sulfate as precipitant. The largest crystals were grown from

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ammonium sulfate solution in sitting-drop experiments. The enzyme was resolved into an apoenzyme and a coenzyme in concentrated ammonium sulfate solutions. We therefore obtained crystals of the apoenzyme. The results obtained on crystallization did not depend on the pH of the enzyme solution within the range pH 6.5–8.0. The best crystals were obtained when 30 ml of the protein solution (25–30 mg/ml) in 50 mM potassium phosphate buffer, pH 7.0, at 0.2 saturation of ammonium sulfate and 0.2 mM dithiothreitol was equilibrated with ammonium sulfate saturation of 0.3. Prismatic crystals of the enzyme appeared after 1 week and attained dimensions of 0.35 × 0.35 × 0.5 mm^3 within 3 weeks. These crystals belong to space group $P2_12_12$ with unit cell dimensions of a = 75.5 Å, b = 138.4 Å and c = 94.1 Å and contain two subunits in an asymmetric unit. This means that one of the molecular axes coincides with the crystallographic C-axis. The crystals obtained diffract up to 2.7 Å (fig.1).

NH_4^+ can serve as a ligand for some heavy atoms and therefore competes with the protein in a preparation of heavy-atom derivatives of crystals [5]. For this reason, when soaking tyrosine phenol-lyase crystals in solutions of heavy-atom compounds, the crystals must be transferred to a stabilization solution free of ammonium sulfate. To obtain isomorphous heavy-atom derivatives, various heavy-atom compounds were tested. In most cases, soaking of the crystals was accompanied by changes in the X-ray diffraction pattern in terms of both intensities of reflections and unit cell dimensions. In the case of K_2PtCl_4 (0.5 mM solution), soaking for 2–4 days and pCMBS (0.5 mM solution), soaking for 2 h changes in unit cell dimensions allowed us to perform X-ray analysis at about 3.0 Å resolution.

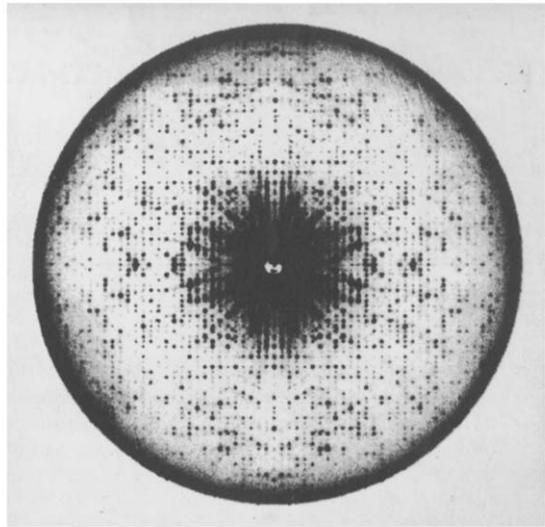

Fig.1. A 15° precession photograph along the a-axis of an apoenzyme crystal taken at a film-to-crystal distance of 7.5 cm using a GX6 rotation anode apparatus. Exposure time was 20 h.

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