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Crystallization and preliminary X-ray analysis of IND, an enzyme with indole oxygenase activity from *Chromobacterium violaceum*

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Abstract

IND, a redox flavoprotein from *Chromobacterium violaceum* has been crystallized in the presence and absence of NADH. The crystals belong to the space group $P4_12_12$ or its enantiomorph $P4_32_12$ with a = 73.9 and c = 153.6 Å. There is one molecule per asymmetric unit and the crystals diffract beyond 2.1 Å resolution.

1. Introduction

Chromobacterium violaceum is a Gram-negative bacterium common in soil and fresh water in tropical regions. It is an opportunistic pathogen in humans and animals. It derives its name from violacein, a purple pigment which serves as an antibiotic against Gram-positive bacteria and discourages predation by protozoa (Sneath, 1974; Gillis & De Ley, 1992) and trypanosomes (Riveros *et al.*, 1988). Violacein may also protect against ultraviolet radiation (DeMoss, 1967). The gene cluster that codes for the enzymes for violacein biosynthesis has been isolated and partially characterized (Pemberton *et al.*, 1991).

Within the violacein gene cluster, the gene which encodes indole oxygenase is not essential for violacein biosynthesis. *Escherichia coli* colonies transformed with this gene turn blue (Quiggin *et al.*, 1998, in preparation). The pigments responsible for this effect are indigo and indirubin, and they are probably derived from indole *via* the reaction shown on Fig. 1 (Quiggin *et al.*, 1998). The exact biological function of IND remains unclear but it is of commercial interest since its expression in specific plant tissues may result in coloration of these tissues. We have purified the protein so that we can characterize better its activity (Quiggin *et al.*, in preparation). Structure determination of IND would facilitate engineering the active site to increase indigo biosynthesis *via* enzymatic conversion of indole to indoxyl, and its subsequent spontaneous oxidation to indigo.

IND is a 41.6 kDa monomeric protein which binds FAD and consumes NAD(P)H. Although the overall sequence does not resemble that of any other reported protein, the N-terminal sequence does contain the FAD-binding motif that is found in many flavoproteins (Wierenga & Drenth, 1983; Eggink *et al.*, 1990). We report here the crystallization and preliminary X-ray analysis of IND.

2. Crystallization

IND was overexpressed *E. coli* and purified to homogeneity as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis. Crystallization conditions were screened using hanging drops and the sparse-matrix method (Jancarik & Kim, 1991). Several conditions produced IND crystals and some of them have been optimized. The most reliably reproducible crystals are grown from a starting drop (10 µl) which has an equal volume of protein solution (6.6 mg ml^{-1}) and precipitant. This is equilibrated against 1 ml of the precipitant. The precipitant contains 20–28% PEG 1500, 0.2 *M* $(NH_4)_2SO_4$ or Li_2SO_4 and 0.2 *M* cacodylate pH 6.1–6.4. Yellow tetragonal crystals (Fig. 2) appear after a 2 week incubation at 277 K. The crystals are typically 0.4 × 0.4 × 0.13 mm. Crystals grown in the presence of NADH appear using the same precipitants and 1 m*M* NADH in the starting drop.

3. X-ray analysis

The data were collected at 277 K on a R-AXIS II using Cu $K\alpha$ radiation from a Rigaku rotating-anode generator running at 50 kV and 200 mA. The space group was initially determined from stills and using software provided by the manufacturer. This was later confirmed with precession photography. The cell dimensions and space group give a Matthews coefficient, V_m of 2.52 Å^3 Da^{-1} assuming one molecule of 41.6 kDa per asymmetric unit. This is close to the average observed for protein crystals (Matthews, 1968). Diffraction from the crystals has been observed to 2.1 Å resolution and the crystals are sufficiently stable to enable complete data sets to be collected with a single crystal. A complete 3.0 Å native data set has been recorded with an overall $R_{\rm merge}$ of 6.8%. Crystals grown in the presence of NADH belong to the same space group and have similar cell dimensions to the native crystals. The crystals of

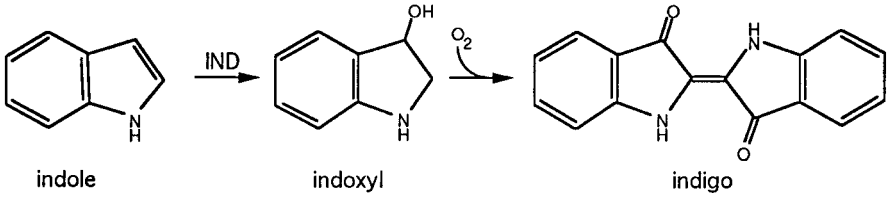

Fig. 1. Indigo production from indole. The hydroxylation of indole gives indoxyl and this spontaneously oxidizes to indigo.

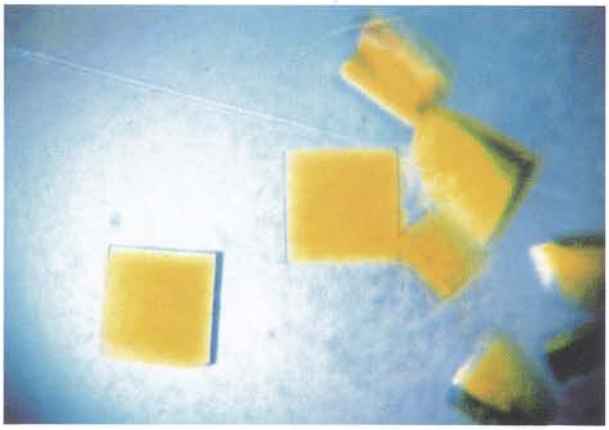

Fig. 2. Crystals of IND from *C. violaceum*.

IND are suitable for high-resolution X-ray analysis and the search for heavy-atom derivatives is currently under way.

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