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Crystallization and preliminary crystallographic studies of human indoleamine 2,3-dioxygenase

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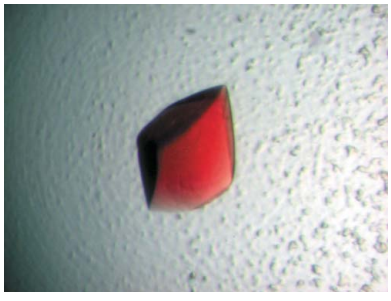

Indoleamine 2,3-dioxygenase (IDO) is a haem-containing dioxygenase that catalyzes the oxidative cleavage of the pyrrole ring of indoleamines by the insertion of molecular oxygen. This reaction is the first and the rate-limiting step in the kynurenine pathway, the major Trp catabolic pathway in mammals. Recombinant human IDO was crystallized by the vapour-diffusion technique. The addition of 4-phenylimidazole as a haem ligand was essential for crystallization. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters a = 86.1, b = 98.0, c = 131.0 Å. Diffraction data were collected to 2.3 Å resolution.

1. Introduction

Indoleamine 2,3-dioxygenase (IDO) catalyzes the oxidative cleavage of the pyrrole ring of indoleamines by the insertion of molecular oxygen (Higuchi & Hayaishi, 1967; Yamamoto & Hayaishi, 1967). This reaction is the first and the rate-limiting step of the kynurenine pathway, the major Trp catabolic pathway in mammals. IDO is a 45 kDa monomeric cytosolic protein containing haem (protoporphyrin IX) as the prosthetic group , which is essential for enzymatic activity. In addition to the major substrate D-Trp, a variety of molecules such as D-Trp, L-Trp, serotonin, 5-hydroxy Trp and tryptamine can also be the substrate of IDO (Hirata & Hayaishi, 1972). *N*-Formylkynurenine is the primary product for the main substrate L-Trp. The amino-acid sequences of IDOs show homology to the 40 kDa haem proteins known as myoglobins from archaeogastropod molluscs (Suzuki *et al.*, 1998). IDO-expressing cells are found in many tissues and IDO expression is inducible by endotoxin, interferon-γ or other proinflammatory cytokines (Yoshida *et al.*, 1979; Babcock & Carlin, 2000). Early literature documented the ability of IDO to inhibit the proliferation of pathogens and tumour cells *in vitro* through consumption of the essential amino acid Trp (Taylor & Feng, 1991). An important role for Trp catabolism in the control of immune responses was demonstrated by Munn and Mellor by showing that inhibition of IDO induces foetal allograft rejection in mice (Munn *et al.*, 1998). Considerable evidence supports the hypothesis that dendritic cells expressing IDO contribute to the regulation of T-cell-mediated immune responses (Munn *et al.*, 1999, 2002; Mellor *et al.*, 2002; Platten *et al.*, 2005).

Despite its physiological importance, the chemistry of the reaction catalyzed by IDO is poorly understood, primarily because of a lack of structural information. In contrast, structure–function correlations and the nature of intermediates for the haem-containing monooxygenases such as cytochrome P450 and peroxidase have been extensively studied on the basis of their crystal structures and model systems (Baek & Van Wart, 1989; Schlichting *et al.*, 2000; Davydov *et al.*, 2001). Determination of the tertiary structure of IDO will provide the first example of a haem-dependent dioxygenase structure and would help to understand how the substrate or dioxygen is activated in the enzyme. In the present study, we describe the purification, crystallization and preliminary X-ray study of human IDO.

Table 1
Crystallographic data for IDO.

Values in parentheses are for the highest resolution shell.

Beamline	BL26B1, SPring-8
Wavelength (Å)	0.98
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 86.1, b = 98.0, c = 131.0
Resolution range (Å)	20.0–2.30 (2.38–2.30)
No. of observed reflections	225581
No. of unique reflections	45130
R_{sym}†	0.052 (0.244)
Completeness (%)	90.9 (72.0)
$I/\sigma(I)$	34.8 (4.9)
Redundancy	5.0 (3.0)

† $R_{sym} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i |I_i(hkl)|$, where the outer summation is over all unique reflections with multiple observations and the inner summation is over all observations of each reflection.

2. Experimental procedures

2.1. Protein expression and purification

The coding region for human IDO was cloned into the pET-15b vector (Novagen) using the *Nde*I and *Bgl*II restriction sites. The plasmid encodes a 6×His tag with a thrombin cleavage site in the N-terminus of the full-length protein. The transformed *Escherichia coli* BL21(DE3) cells were grown in a rotary shaker at 300 K using LB medium containing 100 µg ml^{-1} ampicillin and 0.5 m*M* δ-aminolaevulinic acid. The expression of His-tagged IDO fusion protein was induced by the addition of 0.5 m*M* isopropyl β-D-thiogalactoside (IPTG) at an OD_{600} of 1.0. Cell growth was continued for a further 12 h at 303 K. The harvested cells were resuspended in phosphate buffer *A* (100 m*M* potassium phosphate pH 8.0, 200 m*M* NaCl, 5 m*M* 2-mercaptoethanol, 20 m*M* imidazole and 0.5 m*M* PMSF) and one tablet of Complete (EDTA-free) protease-inhibitor cocktail (Roche) was added per 50 ml. The suspension was homogenized with an ultrasonic processor after treatment with 0.4 mg ml^{-1} lysozyme for 30 min. The solution was centrifuged at 125 000*g* for 1 h and cell debris was discarded. The supernatant was then loaded onto 30 ml Ni–NTA agarose resin. The column was washed with 300 ml buffer *A*. His-tagged IDO was then eluted with 100 ml buffer *A* containing 150 m*M* imidazole. The eluted fraction was dialyzed against phosphate buffer *B* (50 m*M* potassium phosphate pH 8.0, 100 m*M* NaCl and 5 m*M* 2-mercaptoethanol). The N-terminal His tag was cleaved using the Thrombin Cleavage Kit (Novagen) and any uncleaved His-tagged IDO was removed by passing the solution through a column with Ni–NTA agarose. The cleaved protein was dialyzed against buffer *C* (16 m*M* Tris–HCl pH 8.0 and 5 m*M* 2-mercaptoethanol) and further purified using a MonoQ column (Amersham). The protein was eluted with a linear gradient of 0–0.3 *M* NaCl in buffer *C*. Fractions with a high absorbance at 423 and 280 nm were pooled. Prior to crystallization, the sample buffer was exchanged to buffer *D* (25 m*M* MES pH 6.5, 25 m*M* NaCl, 2 m*M* 4-phenylimidazole; Sono & Cady, 1989) using a Fast Desalting Column HR (Amersham) and concentrated by centrifugation in a Centriprep filter (Amicon Inc.) to 30–40 mg ml^{-1}.

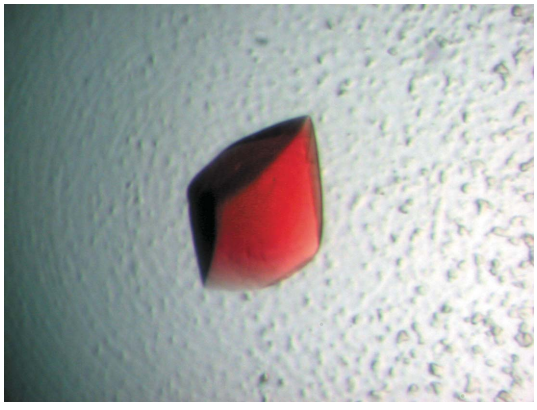

Figure 1
A crystal of human IDO grown using PEG 8000 as a precipitant.

2.2. Crystallization

Initial crystallization conditions were screened by the sitting-drop vapour-diffusion technique using commercially available crystallization screening kits: Crystal Screen, Crystal Screen II (Hampton Research), Wizard I and Wizard II (Emerald Biostructures). Several crystals were obtained from Crystal Screen condition No. 36 (8% PEG 8000, Tris–HCl pH 8.5). This condition was optimized using hanging-drop vapour-diffusion experiments. The best crystals were obtained using reservoir solution containing 10% PEG 8000 and 0.1 *M* CHES buffer pH 9.0. Droplets containing 4 µl protein solution and 4 µl mother liquor were equilibrated against 200 µl reservoir solution at 293 K. Typically, the crystals appear within two weeks and grow to dimensions of 0.3 × 0.3 × 0.1 mm.

2.3. X-ray diffraction study

X-ray diffraction data were collected at beamline BL26B1 at SPring-8 using a Rigaku/MSC Jupiter 210 CCD detector. The crystals were transferred into a solution of mother liquor containing 30%(*w*/*v*) xylitol as a cryoprotectant and placed directly into a nitrogen stream at 90 K using a nylon loop (Hampton Research). The data were integrated and scaled using the *HKL*2000 program suite (Otwinowski & Minor, 1997).

3. Results

The crystallization conditions for the recombinant human IDO were obtained by the sparse-matrix method and optimized to produce a single crystal suitable for X-ray analysis (Fig. 1). The addition of 4-phenylimidazole as a haem ligand in the final stage of the purification was essential for crystallization. The crystals diffracted X-rays to 2.3 Å resolution on beamline BL26B1 at SPring-8. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 86.1, b = 98.0, c = 131.0 Å. A summary of the data-collection statistics is given in Table 1. The calculated Matthews coefficient (V_M) for two monomers of 45 kDa protein in the asymmetric unit is 3.0 Å^3 Da^{-1}, with a solvent content of 59%. The structure determination of the crystal of IDO is currently under way.

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