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Crystallization and Preliminary X-Ray Crystallographic Studies of *Trypanosoma brucei* Prostaglandin $F_{2\alpha}$ Synthase[1]

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Prostaglandin $F_{2\alpha}$ is a potent mediator of various physiological and pathological processes. *Trypanosoma brucei* prostaglandin $F_{2\alpha}$ synthase (TbPGFS) catalyzes the NADPH-dependent reduction of 9,11-endoperoxide PGH_2 to $PGF_{2\alpha}$, and could thus be involved in the elevation of the $PGF_{2\alpha}$ concentration during African trypanosomasis. In the present report, the purification and crystallization of recombinant TbPGFS are described. The active recombinant enzyme was crystallized by the hanging-drop vapor-diffusion method using ammonium sulfate as a precipitant. The crystal belonged to a tetragonal space group, $P4_12_12$ or $P4_32_12$, with unit-cell parameters of $a = b = 112.3$ Å, and $c = 140.0$ Å. Native data up to 2.6 Å resolution were collected from the crystal using our home facility.

Key words: aldo-keto reductase, crystallization, prostaglandin $F_{2\alpha}$ synthase, *Trypanosoma brucei*, X-ray crystallography.

Trypanosoma brucei causes African sleeping sickness in humans and nagana in animals. *T. brucei* $PGF_{2\alpha}$ synthase (EC 1.1.1.188), also termed TbPGFS, is an aldo-keto reductase (AKR) that converts 9,11-endoperoxide PGH_2 (the cyclooxygenase metabolite of arachidonic acid that is the common substrate for prostanoid biosynthesis) to $PGF_{2\alpha}$ in the presence of NADPH through catalytic mechanisms that have not been elucidated yet (*1*). In mammals, $PGF_{2\alpha}$ regulates vascular tone, abortion, ovarian dysfunction, and constriction of uterine muscle and pulmonary arteries (*2–7*), and also acts as a luteolytic hormone, causing luteolysis during the estrous cycle and prior to parturition (*8*). Whereas $PGF_{2\alpha}$ may play a signal-coupling role during phagocytosis, since it elicits vacuole formation in the protozoan parasite *Amoeba proteus* (*9*). In addition, TbPGFS is also known to catalyze the reduction of 9,10-phenanthrenequinone (9,10-PQ), a common non-physiological substrate for most AKRs.

Aldo-keto reductases are monomeric oxidoreductases that are widely distributed in mammals, amphibians, plants, yeasts, bacteria, and protozoa, and bind NAD(P)(H) without a canonical Rossmann-fold motif (*1–4*). AKRs exhibit broad and sometimes overlapping substrate specificities, and catalyze the oxidation/reduction of substrates such as PGs, steroid hormones, monosaccharides, isoflavinoids, aliphatic and aromatic aldehydes, and polycyclic aromatic hydrocarbons (*6–8*).

We have isolated TbPGFS, cloned its cDNA, and overexpressed the recombinant TbPGFS (*1*). Here we report the purification and crystallization of the recombinant TbPGFS in the presence of NADPH.

EXPERIMENTAL

Expression and Purification of TbPGFS—The coding region of TbPGFS cDNA with an *EcoR*I or *Sal*I restriction site at its 5′-end was cloned into the corresponding sites of the pGEX-4T-1 expression vector (Amersham Pharmacia Biotech). The resultant expression vectors were used for transformation of *Escherichia coli* BL21(DE3). Transformed cells were cultured for 8–10 h in the presence of 0.5 mM isopropyl-β-D-thiogalactopyranoside (IPTG) at 30°C. The *E. coli* BL21(DE3) transformants were harvested by centrifugation, washed with a phosphate-buffered saline (PBS) solution containing a cocktail of reversible and irreversible inhibitors (1 tablet in 25 ml) of pancreas-extract, pronase, thermolysin, chemotrypsin, trypsin, and papain (Complete™; Roche Diagnostics, Mannheim, Germany), suspended in the same buffer, and then disrupted by sonication. After removal of debris by centrifugation, the recombinant proteins in the supernatant were purified by affinity chromatography on GSH-Sepharose 4B resin (New England Biolabs) according the manufacturer's protocol. Recombinant TbPGFS bound to GSH-Sepharose 4B gel was cleaved from Glutathione-S transferase with thrombin and eluted with PBS. The resulting recombinant protein was dialyzed against 20 mM Tris/HCl, pH 8.0, buffer and then applied to a DEAE anion exchange column that had been equilibrated with the same buffer. The proteins were eluted

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Fig. 1. **A crystal of $PGF_{2\alpha}$ synthase from *Trypanosoma brucei*.** Its approximate dimensions are 0.1 × 0.1 × 0.1 mm.

TABLE I. **Data collection.** Values in parentheses refer to outermost shells: 2.69–2.6 Å resolution.

Detector system	R-AXIS IV
X-ray source	Rigaku UltraX18
Wavelength (Å)	1.5418
Space group	$P4_12_12$ or $P4_32_12$
Cell constants (Å)	$a = b = 112.3$, $c = 140.0$
Resolution range (Å)	40.0–2.6 (2.69–2.6)
No. of measured/unique reflections	74,276/22,477
Completeness (%)	79.4 (84.5)
R_{sym}[a] (%)	6.4 (21.8)

[a] $R_{sym} = \Sigma |I_h - \langle I_h \rangle| / \Sigma I_h$.

with an increasing linear gradient, 0–500 mM, of NaCl in the same buffer.

Protein concentrations were determined by use of bicinchinonic acid reagent (Pierce, Rockford, Il) with BSA as a standard following the manufacturer's protocol. The purity of the protein was assessed by SDS-PAGE on 14% (w/v) gels, and the gels were stained with Sypro Orange (Bio-Rad Laboratories, Hercules, CA) or Coomassie Brilliant Blue (Daiichi Pure Chemicals).

Enzyme Assays—PGH_2 reductase activity was measured by incubating at 37°C for 2 min a reaction mixture containing an appropriate amount of enzyme, 5 μM [1-^{14}C]PGH_2 (final concentration), 100 μl of 100 mM sodium phosphate (pH 7.0), and 100 μM NADPH generating system (*1*). After stopping the reaction, aliquots were subjected to thin-layer chromatography as described previously (*1*). 9,10-PQ reductase activity was determined with a reaction mixtures consisting of 100 mM sodium phosphate (pH 7.0), the purified recombinant enzyme, 100 μM NADPH, and 40 μM 9,10-PQ, in a total volume of 500 μl. The reaction was initiated by addition of the substrate, and the decrease in absorbance at 340 nm was monitored with a spectrophotometer at 37°C.

Crystallization of the Recombinant TbPGFS and X-ray Diffraction Analysis—Crystals were grown at 20°C by the hanging-drop vapor-diffusion method. The purified recombinant *T. brucei* PGFS was concentrated to 10 mg/ml. The drops consisted of 2 μl of the enzyme solution and an equal volume of well liquor [100 mM Hepes/NaOH, pH 7.5; 2.0 M ammonium sulfate; 5% (w/v) PEG400, and 500 mM NADPH]. Typical crystals with maximum dimensions of 0.15 × 0.15 × 0.1 mm were obtained after 3 weeks (Fig. 1).

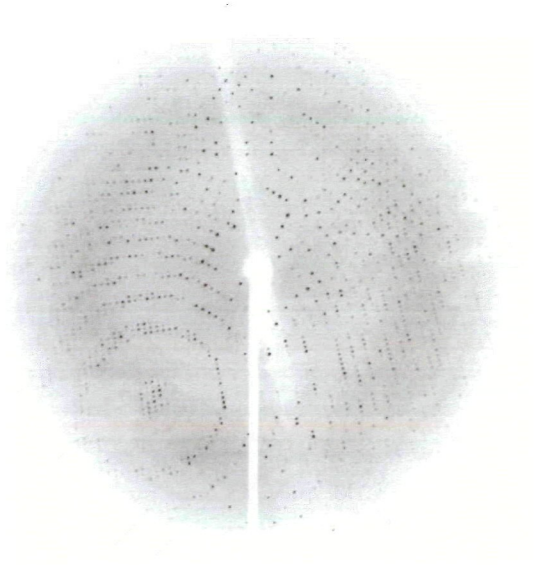

Fig. 2. **X-ray diffraction pattern of a crystal of $PGF_{2\alpha}$ synthase from *Trypanosoma brucei*.** The crystal diffracted beyond 2.6 Å resolution.

X-ray diffraction data were measured at room temperature using an R-AXIS IV imaging-plate detector, with a crystal to detector distance of 150 mm, and Cu $K\alpha$ radiation (λ = 1.5418 Å) produced by a Rigaku ultraX18 rotating-anode generator operated at 45 kV and 100 mA (Fig. 2). Diffraction data were processed with programs *DENZO/SCALEPACK* (*10*). The crystals belonged to a tetragonal space group ($P4_12_12$ or $P4_32_12$) with cell constants of $a = b =$ 112.3 Å, and c = 140.0 Å.

Using an oscillation angle of 1° per frame, a data set up to 2.6 Å resolution was collected. The asymmetric unit in the crystal contains two, three, or four protein molecules with V_m values (*11*) of 3.45, 2.30, and 1.73 Å^3 Da^{-1}, respectively. The mosacity of this crystal is estimated to be about 0.3° after integration and scaling of the data. The final statistics for the data collection are shown in Table I.

RESULTS

Comparison with the amino acid sequences of aldo-keto reductases revealed that the aldehyde reductase from *Sus scrofa* exhibits the highest sequence identity (41%) with TbPGFS. Preliminary molecular-replacement using the structures belonging to the aldo-keto reductase superfamily as a search model was performed with *AMoRe* (*12*). Assuming that the space group was $P4_12_12$, the Patterson correlation coefficient/R factor is 44.1%/49.4% (two molecules per asymmetric unit), 52.1%/46.4% (three molecules), and 47.1%/50.5% (four molecules), respectively. On the other hand, in case of $P4_32_12$, the correlation coefficient/R factor is 35.0%/52.6% (two molecules per asymmetric unit), 44.1%/49.4% (three molecules), and 38.9%/53.1% (four mol-

ecules), respectively. Inspection of the crystal packing and evaluation of the electron density obtained from the initial phase are currently in progress.

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