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Purification, crystallization and preliminary X-ray studies of GMP reductase 2 from human

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GMP reductase 2 from human has been expressed in *Escherichia coli*, purified and crystallized. The crystals belong to space group $P3_221$, with unit-cell parameters $a = b = 110.6$, $c = 209.8$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$. Diffraction data were collected to 3.0 Å with a completeness of 100% (100% for the last shell), an $R_{\rm merge}$ value of 0.089 (0.189) and an $I/\sigma(I)$ value of 7.3 (3.2).

1. Introduction

Purines and their nucleosides and nucleotides have been found to control numerous biochemical and developmental reactions (Endo *et al.*, 1983). Guanosine-5′-monophosphate (GMP) reductase (NADPH:GMP oxidoreductase; EC 1.6.6.8) catalyzes the only known metabolic step by which guanine nucleotides can be converted to the pivotal precursor of both adenine and guanine nucleotides and plays an essential role in salvage pathways of purine ribonucleotide biosynthesis (Benson *et al.*, 1971). It catalyzes the irreversible NADPH-dependent conversion of GMP to IMP: GMP + NADPH + $2H^+$ $\rightarrow$ IMP + NH_4^+ + $NADP^-$. The enzyme exists in various organisms, *e.g. Artemia salina* (Renart & Sillero, 1974), *Escherichia coli* (Andrews & Guest, 1988), *Salmonella typhimurium* (Benson & Gots, 1975), *Leishmania donovani* (Spector & Jones, 1982) and calf thymus (Stephens & Whittaker, 1973) as well as human erythrocytes (Spector *et al.*, 1979; Kondoh *et al.*, 1991).

The human GMP reductase with NADPH-linked activity was first confirmed by purifying it from erythrocytes and was regulated in a negatively cooperative manner as a tetramer (Mackenzie & Sorensen, 1973). A cDNA for human GMP reductase (named GMPR1 compared with the GMPR2 of this paper) was discovered in 1991, which was assigned to human chromosome 6 (Kondoh *et al.*, 1991). We cloned a distinct cDNA (GenBank accession No. AF419346) for a human GMP reductase isoenzyme (named GMPR2) isolated from a human foetal brain library, which was assigned to human chromosome 14. The deduced protein is composed of 348 amino-acid residues and shows 90% identity with human GMPR1 and 69% identity with *E. coli* GMP reductase (the results will be published elsewhere). No structure of GMP reductase has been reported to date. The structure of human GMPR2 will help in understanding the function and reaction mechanism of both the enzyme itself and the enzyme family. As a preliminary step in the study of the GMPR2 structure, we report here the purification, crystallization and preliminary X-ray diffraction analysis of GMPR2.

2. Material and methods

2.1. Protein purification

The gene encoding GMPR2 was cloned into pQE31 between endonuclease *Kpn*I and *Hin*dIII sites after PCR amplification. The gene had no errors by sequencing and was overexpressed in *E. coli* M15 with IPTG induction. Approximately 10 g (wet weight) of cells were disrupted by sonication in 100 ml buffer containing 10 m*M* Na_2HPO_4–NaH_2PO_4 pH 7.4, 0.5 *M* NaCl. The cell debris was removed by centrifugation and the supernatant was applied to pre-equilibrated Ni–NTA Superflow (Qiagen). After washing with buffer consisting of 10 m*M* Na_2HPO_4–NaH_2PO_4 pH 7.4, 0.5 *M* NaCl, 50 m*M* imidazole pH 7.4, the column was eluted with an imidazole-concentration gradient from 10 m*M* Na_2HPO_4–NaH_2PO_4 pH 7.4, 0.5 *M* NaCl, 50 m*M* imidazole pH 7.4 to 10 m*M* Na_2HPO_4–NaH_2PO_4 pH 7.4, 0.5 *M* NaCl, 300 m*M* imidazole pH 7.4. GMPR2 protein eluted at about 100 m*M* imidazole. It showed a single band on SDS–PAGE and was concentrated to 20 mg ml^{-1} in 10 m*M* Tris–HCl buffer pH 8.0 at 298 K.

2.2. Crystallization and preliminary X-ray analysis

Crystallization was performed at 277 and 293 K using the hanging-drop vapour-diffusion method by combining 5 µl GMPR2 and 5 µl precipitant solution. A total of 200 conditions were used for crystallization screening. The diffraction data were collected at 100 K [cryoconditions; 100 m*M* sodium citrate pH 5.6 at 298 K, 1 *M* Li_2SO_4, 2.0 *M* $(NH_4)_2SO_4$] using

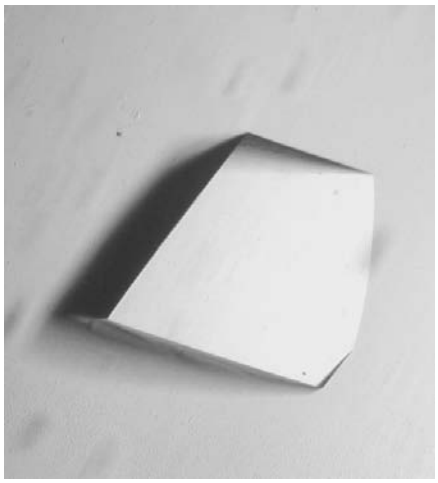

Figure 1
Crystal of GMPR2 from human. The dimensions of the largest crystal were 0.2 × 0.2 × 0.04 mm.

an ADSC Quantum 4R detector on beamline 18B at the Photon Factory in Japan and were processed with *DPS* (Rossmann & van Beek, 1999) and *MOSFLM* (Leslie, 1994).

3. Conclusions

Small crystals were observed in several drops. The initial conditions were optimized and the crystals used for data collection were grown at 293 K by combining 3 µl of 20 mg ml^{-1} protein solution and 3 µl of 100 m*M* sodium citrate pH 5.6 at 298 K, 1 *M* Li_2SO_4, 1.2 *M* $(NH_4)_2SO_4$. The dimensions of the largest crystals were 0.2 × 0.2 × 0.04 mm (Fig. 1).

Table 1
Crystal parameters and data-collection statistics for GMPR2.

Values in parentheses are for the highest resolution shell.

Space group	$P3_221$
Unit-cell parameters (Å, °)	$a = b = 110.6$, $c = 209.8$, $\alpha = \beta = 90$, $\gamma = 120$
V_M (Å^3 Da^{-1})	2.32
Resolution (Å)	20–3 (3.16–3)
No. of observations	463679
No. of unique reflections	30157
R_{merge} (%)	0.089 (0.189)
Multiplicity	8.4 (8.6)
Completeness (%)	100 (100)
$I/\sigma(I)$	7.3 (3.2)

GMPR2 crystals belong to the trigonal space group $P3_221$, with unit-cell parameters $a = b = 110.6, c = 209.8$ Å, $\alpha = \beta = 90, \gamma = 120°$. A calculation assuming six 159.6 kDa tetramers per unit cell yields a V_M of 2.32 Å^3 Da^{-1} and a solvent content of 47% (Matthews, 1968). The data statistics are given in Table 1. Structure determination by molecular replacement (using inosine 5′-monophosphate dehydrogenase, PDB code 1eep, as a model) is currently under way.

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