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Crystallization and preliminary X-ray analysis of human uridine-cytidine kinase 2

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Uridine-cytidine kinase (UCK), which converts uridine and cytidine to their corresponding monophosphates, is a rate-limiting enzyme involved in the salvage pathway of pyrimidine-nucleotide biosynthesis. Two members of human UCK, named UCK1 and UCK2, were cloned recently and UCK2 was reported to play a crucial role in activating anti-tumour pro-drugs in human cancer cells. Human UCK2 was expressed, purified and crystallized alone or in complex with various ligands. Free UCK and UCK complexes were crystallized in six crystal forms. Form I (ligand-free) belongs to space group $P2_12_12$, with unit-cell parameters $a = 83.1$, $b = 93.7$, $c = 157.1$ Å. Forms IIa (with CTP), IIb (with UTP) and IIc (with cytidine) belong to space group $F222$, with unit-cell parameters $a = 133.3$, $b = 247.3$, $c = 91.6$ Å (IIa), $a = 132.1$, $b = 247.0$, $c = 91.5$ Å (IIb) and $a = 136.7$, $b = 246.3$, $c = 90.4$ Å (IIc), respectively. Form III (with ATPγS) belongs to space group $C222_1$, with unit-cell parameters $a = 70.3$, $b = 149.9$, $c = 117.2$ Å. Form IV (with cytidine and ATP) belongs to space group $C2$, with unit-cell parameters $a = 89.0$, $b = 109.7$, $c = 64.8$ Å, $\beta = 95.3^\circ$. Diffraction data were collected from these crystals; form IV diffracted to 1.8 Å resolution.

1. Introduction

Uridine-cytidine kinase (UCK; EC 2.7.1.48; uridine kinase is a synonym) catalyzes the phosphorylation of uridine and cytidine to UMP and CMP. In the pyrimidine nucleotide-salvage pathway, UMP and CMP are further phosphorylated by UMP-CMP kinase (Van Rompay *et al.*, 1999) and nucleoside diphosphate kinases (Parks & Agarwal, 1973) to UTP and CTP, respectively. The first phosphorylation step catalyzed by UCK is thought to be rate-limiting (Anderson, 1973). Besides uridine and cytidine, UCK also catalyzes the phosphorylation of several cytotoxic ribonucleoside analogues such as 5-fluorouridine (Greenberg *et al.*, 1977), cyclopentenyl-cytosine (Kang *et al.*, 1989), 3′-*C*-ethynylcytidine and 3′-*C*-ethynyluridine (Tabata *et al.*, 1997; Takatori *et al.*, 1999). In order to exert their pharmacological activities, such as anti-tumour activity, these nucleoside analogues need to be metabolized to their triphosphate forms, which then act as inhibitors of cell growth *via* inhibiting DNA and/or RNA synthesis (Cihak & Rada, 1976; Matsuda *et al.*, 1999). Therefore, for pharmacological activity these nucleoside analogues need to be efficiently phosphorylated to their monophosphate forms by UCK. As with other enzymes involved in pyrimidine metabolism, UCK has also been shown to be regulated through feedback-inhibition by UTP and CTP and to be activated by ATP (Cheng *et al.*, 1986; Ropp & Traut, 1998). This regulation of UCK is thought to be achieved by a mechanism that involves the dissociation of the active native tetramer into the inactive monomer (Traut, 1994).

Recently, two members of human UCK were reported: UCK1, composed of 277 amino acids, and UCK2, composed of 261 amino acids (Van Rompay *et al.*, 2001; Koizumi *et al.*, 2001). While their functional differences remain elusive, it has been reported that expression of UCK2 but not UCK1 was enhanced in human cancer cells and that UCK2 might contribute to the tumour-selective cytotoxicity of 3′-*C*-ethynylcytidine (Hattori *et al.*, 1996; Shimamoto *et al.*, 2002). In this report, we have expressed, purified and crystallized human UCK2 alone and in complex with ligands. By determining the three-dimensional structures of UCK and its complexes with several ligands, the molecular mechanisms of the substrate-specificity, catalytic reaction and regulation of UCK can be elucidated.

2. Experimental

2.1. Expression and purification

As the C-terminal residues (251–261) of human UCK2 were susceptible to proteolysis, these residues were excluded from the construct. The region encoding residues 1–250 was inserted into the pGEX-6p vector (Amersham-Pharmacia) using *Bam*HI–*Eco*RI restriction sites. UCK was expressed in

Table 1
Diffraction data statistics of UCK crystals.

Values in parentheses refer to the highest resolution shell, which is 3.42–3.30 Å for form I, 3.31–3.20 Å for forms IIa, IIb and III, 3.73–3.60 Å for form IIc and 1.86–1.80 Å for form IV.

Crystal form	I	IIa	IIb	IIc	III	IV
Ligands	None	CTP	UTP	Cytidine	ATPγS	Cytidine, ATP
Space group	$P2_12_12$	$F222$	$F222$	$F222$	$C222_1$	$C2$
Unit-cell parameters						
a (Å)	83.1	133.3	132.1	136.7	70.3	89.0
b (Å)	93.7	247.3	247.0	246.3	149.9	109.7
c (Å)	157.1	91.6	91.5	90.4	117.2	64.8
β (°)						95.3
Resolution range (Å)	100–3.3	100–3.2	100–3.2	100–3.6	100–3.2	100–1.8
Observed reflections	59256	42462	66836	28741	15504	238019
Unique reflections	18094	12140	11842	8753	6995	56389
Completeness (%)	96.5 (91.1)	99.5 (99.6)	98.5 (94.6)	95.0 (87.2)	66.5 (66.8)	98.3 (94.3)
$R_{\rm merge}(I)$† (%)	8.8 (28.3)	8.2 (27.3)	11.4 (29.4)	16.7 (36.9)	8.6 (28.8)	4.6 (31.3)
$\langle I/\sigma(I)\rangle$	19.6 (2.2)	15.6 (2.1)	20.7 (2.2)	6.4 (1.7)	11.8 (1.8)	32.8 (1.6)

† $R_{\rm merge}(I) = \sum_{hkl}\sum_i |I(hkl)_i - \langle I(hkl)\rangle| / \sum_{hkl} I(hkl)_i$, where $I(hkl)_i$ is the intensity of the ith observation and $\langle I(hkl)\rangle$ is the mean intensity.

Escherichia coli BL21 DE3 with glutathione-*S*-transferase (GST) fused at the N-terminus. After cell lysis, the GST-fusion protein was first purified by affinity chromatography using a glutathione-Sepharose 4B column. After the GST protein was excised from UCK with PreScission protease (Amersham-Pharmacia), sequential column chromatography (all columns from Amersham-Pharmacia) was performed using HiLoad Superdex200, Mono P and HiLoad Superdex200 again on an ÄKTA system (Amersham-Pharmacia). The purified protein was concentrated to 8–12 mg ml^{-1} in 0.15 *M* NaCl containing 20 m*M* Tris–HCl pH 7.4.

2.2. Crystallization

All crystallization trials were performed with the sitting-drop vapour-diffusion method at 293 K. Initial screening was performed using Crystal Screens I and II provided by Hampton Research and Wizard I, II, Cryo I and II provided by Emerald Biostructures. Typically, 0.5 µl drops of protein solution were mixed with equal amounts of the reservoir solution and ligand solution. Ligand-free and complexed UCK crystallized in six forms: I, IIa, IIb, IIc, III and IV. Form I (ligand-free) was obtained with a reservoir solution consisting of 24–26% PEG 400, 5% PEG 3350, 10% glycerol, 0.1 *M* HEPES pH 6.9. Forms IIa and IIb were obtained with a reservoir solution consisting of 0.8–1.0 *M* sodium tartrate, 0.1 *M* HEPES pH 6–8 and with a ligand solution consisting of 20 m*M* CTP (IIa) or 100 m*M* UTP (IIb). Form IIc was obtained with a reservoir solution consisting of 0.8–1.0 *M* sodium citrate, 0.1 *M* HEPES pH 7 and with a ligand solution consisting of 20 m*M* cytidine. Form III was obtained with a reservoir solution consisting of 10% PEG 3000, 0.2 *M* NaCl, 0.1 *M* sodium acetate pH 5.4 and with a ligand solution consisting of 2 m*M* ATPγS. Form IV was obtained with a reservoir solution consisting of 20% PEG 8000, 0.2 *M* calcium acetate, 0.1 *M* Tris–HCl pH 7.0 and with a ligand solution consisting of 20 m*M* cytidine, 5 m*M* ATP, 10 m*M* $MgCl_2$. All crystals were obtained within a week.

2.3. Preliminary X-ray analysis

All diffraction data were collected at 100 K on a Rigaku R-AXIS IV imaging-plate detector using Cu $K\alpha$ radiation from an in-house Rigaku rotating-anode X-ray generator operating at 50 kV and 100 mA. Crystals were immersed in reservoir solution supplemented with 10–25% glycerol as a cryoprotectant for several seconds and then flash-cooled and kept in a stream of nitrogen gas at 100 K during data collection. Diffraction data were indexed, integrated and scaled with the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). X-ray diffraction data statistics are summarized in Table 1. The acceptable range of the volume-to-mass ratio ($V_{\rm M}$) values (Matthews, 1968) indicates that the form I crystal contains three, four or five molecules and the other crystals contain two or three molecules per asymmetric unit. From the facts that UCK functions as a tetramer in solution and that all these crystals are fragile and seem to have high solvent contents, we consider form I to contain one tetramer (four UCK molecules) per asymmetric unit ($V_{\rm M}$ = 2.7 Å^3 Da^{-1}) and the other crystals to contain two UCK molecules per asymmetric unit ($V_{\rm M}$ = 3.4 Å^3 Da^{-1} for IIa and IIc, 3.3 Å^3 Da^{-1} for IIb and 2.8 Å^3 Da^{-1} for III and IV). Molecular replacement was tried using the crystal structure of phosphoribulokinase from *Rhodobacter spheroides* (Harrison *et al.*, 1998) as a search model, but has so far failed. Phasing experiments using the multiple isomorphous replacement (MIR) method are now in progress.

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