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Crystallization and preliminary crystallographic studies of human coactosin-like protein (CLP)

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The human coactosin-like protein (CLP) belongs to the actin-depolymerizing factor (ADF) family of actin-binding proteins. CLP interacts with 5-lipoxygenase (5LO) and filamentous actin (F-actin) *via* different binding sites. The full-length CLP comprising of 142 amino acids has been overexpressed in *Escherichia coli*. Crystals of CLP were obtained using the hanging-drop vapour-diffusion technique with ammonium sulfate as precipitant at pH 8.5. Diffraction data to 1.9 Å resolution were collected from a crystal belonging to space group $P2_1$, with unit-cell parameters a = 25.6, b = 55.2, c = 37.4 Å, β = 96.0°. There is one molecule per asymmetric unit.

1. Introduction

Human coactosin-like protein (CLP) was initially identified through a yeast two-hybrid screen using 5-lipoxygenase (5LO) as bait. CLP showed high homology with coactosin, a filamentous actin (F-actin) binding protein from *Dictyostelium discoideum*, suggesting that it belongs to the actin-depolymerizing factor (ADF) family of actin-binding proteins and to the coactosin subfamily. CLP is a 16 kDa protein consisting of 142 amino acids. It is widely distributed in tissue and is predominantly expressed in the placenta, lungs, kidneys and peripheral blood leucocytes (Provost *et al.*, 2001). The CLP gene is mapped to human chromosome 17, where it flanks a deletion that characterizes Smith–Magenis syndrome (SMS). This SMS critical region overlaps with a breakpoint cluster region associated with primitive neuroectodermal tumours (Chen *et al.*, 1997). When the molecular mechanism of all-*trans* retinoic acid (ATRA) induced differentiation of acute promyelocytic leukaemia (APL) cells was elucidated by the gene-expression patterns in the APL cell line, it was found that CLP could be modulated by ATRA (Liu *et al.*, 2000). Serological analysis of recombinant cDNA expression libraries (SEREX) identified human tumour antigens, CLP mRNA was overexpressed in pancreatic cancer cell lines and the cellular epitopes encoded at positions 15–24 and 104–113 could be recognized by both cellular and humoral immune systems (Nakatsura *et al.*, 2002)

Leukotrienes play central roles in immune response and tissue homeostasis. 5LO plays a pivotal role in cellular leukotriene synthesis: it converts arachiconic acid to leukotriene A4. The migration of 5LO from the cytosol or nucleus to the membrane is most probably of importance in the regulation of cellular 5LO activity (Kuebler *et al.*, 2000). Regulation of the translocation and activation of 5LO may involve interactions with other proteins. 5LO interacts directly with CLP *in vitro* and *in vivo*. Site-directed mutagenesis has suggested an important role for Lys131 of CLP in mediating 5LO binding (Doucet *et al.*, 2002).

CLP also associates with F-actin *via* a different binding site from 5LO, but does not form a stable complex with globular actin and has no direct effect on actin depolymerization. CLP bound to actin filaments with a 1:2 (CLP:actin subunit) stoichiometry, but could only be cross-linked to one subunit of actin. In transfected mammalian cells, site-directed mutagenesis indicated that CLP could bind F-actin *in vitro* and that *in vivo* CLP was co-localized with actin stress fibres, with Lys75 being essential for this interaction (Provost *et al.*, 2001).

Full-length human CLP has been over-expressed in *Escherichia coli*. Here, we report the crystallization and preliminary X-ray analysis of human coactosin-like protein. Determination of its three-dimensional structure is expected to provide some valuable clues to further understanding the function of CLP binding 5LO/F-actin.

2. Materials and methods

2.1. Protein expression and purification

The coding sequence of CLP from a liver library was cloned between the *Bam*HI and *Xho*I sites of pGEX-6p-1 plasmid DNA (Amersham Biosciences). The recombinant plasmid was transformed into *E. coli* strain BL21 (DE3) and transformed cells were plated onto LB plates containing 100 μg ml^{-1} ampicillin. A single colony was picked and grown overnight at 310 K in 10 ml LB medium

Figure 1
Crystals of CLP (approximate dimensions 0.05 × 0.07 × 0.3 mm).

containing 100 µg ml^{-1} ampicillin. The next day, 10 ml of the overnight culture was added to 1000 ml LB medium. When the culture density reached 0.6–0.7 (A_{600}), induction with 1 mmol l^{-1} isopropyl-β-D-thiogalactopyranoside (IPTG) was performed.

After harvesting by centrifugation (6000*g*, 15 min, 277 K), the cells were resuspended in lysis buffer (140 m*M* NaCl, 2.7 m*M* KCl, 10 m*M* $Na_2HPO_4.12H_2O$, 1.8 m*M* KH_2PO_4) and the cells were sonicated. After centrifugation at 15 000 rev min^{-1} for 30 min at 277 K, the clarified supernatant was passed through a glutathione-Sepharose 4B column (equilibrated with lysis buffer). The GST-fusion protein-bound column was washed with ten column volumes of lysis buffer. The GST-fusion proteins were then cleaved with GST-fusion rhinovirus 3C protease (kindly provided by Dr George F. Gao) at 278 K for 16 h in the cleavage buffer (50 m*M* Tris–HCl pH 7.0, 150 m*M* NaCl, 1 m*M* DTT, 1 m*M* EDTA pH 8.0). The free GST and the GST-3C protease were removed by another passage through the glutathione-Sepharose 4B column (Zhu *et al.*, 2003). The resultant CLP protein was further purified using Superdex 75 (Amersham Pharmacia, USA). The purified and concentrated CLP (20 mg ml^{-1}) was stored in 20 m*M* Tris–HCl pH 8.0.

2.2. Crystallization

The purified protein was concentrated to 20 mg ml^{-1} in 100 m*M* NaCl, 20 m*M* Tris–HCl pH 8.0. Crystallization was performed at 291 K by the hanging-drop vapour-diffusion method. Each drop contained 1 µl protein solution and 1 µl reservoir solution. Initial screening was set up using the sparse-matrix (Jancarik & Kim, 1991) screening kits Hampton Research Crystal Screen Kits I and II (Riverside, CA, USA). The optimized reservoir solution was 30% PEG 4000, 0.1 *M* Tris–HCl pH 8.5, 0.2 *M* sodium acetate. Crystals (0.05 × 0.07 × 0.3 mm; Fig. 1) were obtained within two weeks.

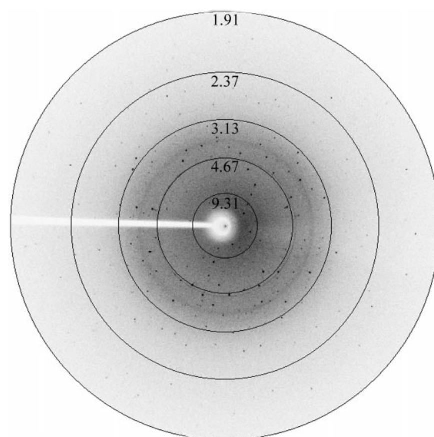

Figure 2
A typical diffraction pattern of CLP crystals to 1.9 Å resolution limit.

2.3. Data collection and processing

Immediately prior to data collection, crystals were soaked in a cryoprotectant solution consisting of 30% PEG 4000, 0.1 *M* Tris–HCl pH 8.5, 0.2 *M* sodium acetate and flash-frozen at 100 K in a stream of nitrogen gas. X-ray diffraction data were collected at 100 K on a MAR 345 image plate using Cu $K\alpha$ radiation from an in-house Rigaku rotating-anode X-ray generator operating at 48 kV and 98 mA (λ = 1.5418 Å). The crystal diffracted to 1.9 Å resolution (Fig. 2). Data processing and scaling were performed with *HKL*2000 and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

Diffraction data were observed to at least 1.9 Å Bragg spacing. Unit-cell parameters were determined to be a = 25.6, b = 55.2, c = 37.4 Å, β = 96.0° in space group $P2_1$. Assuming the presence of one molecule in the asymmetric unit, the solvent content is about 24%. Data-collection statistics are given in Table 1.

Table 1
Data-collection and processing statistics.

Wavelength (Å)	1.5418
Resolution range (Å)	50–1.9
Completeness (%)	96.0 (77.0)
Total reflections	25220
Unique reflections	7780
Redundancy	3.3
R_{merge} (%)	6.9 (26.2)
$I/\sigma(I)$	13.8 (3.0)
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 25.6, b = 55.2, c = 37.4, β = 96.0

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