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Crystallization and preliminary X-ray crystallographic studies of cPOP1

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Cellular pyrin domain-only protein 1 (cPOP1) is a pyrin domain (PYD)-containing protein that can regulate inflammation by preventing the assembly of inflammasome *via* direct interaction with ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain). In this study, cPOP1, corresponding to amino acids 1–87, was overexpressed in *Escherichia coli* using an engineered C-terminal polyhistidine tag. cPOP1 was then purified to homogeneity and crystallized at 293 K. Finally, X-ray diffraction data were collected to a resolution of 3.6 Å from a crystal belonging to the cubic space group $P2_13$ with unit-cell parameters $a = b = c = 94.12$ Å, $\alpha = \beta = \gamma = 90.00^\circ$.

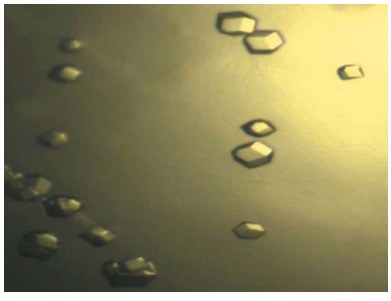
1. Introduction

Pyrin domain (PYD) is a member of the death domain superfamily, which includes the death domain (DD) subfamily, death effector domain (DED) subfamily and caspase recruitment domain (CARD) subfamily (Park, 2012). The death domain superfamily is a protein interaction module that mediates protein interactions for apoptosis and inflammation signalling pathways (Park, 2011). More than 100 proteins are known to contain a member of the death domain superfamily (Park *et al.*, 2007).

Cellular pyrin domain-only protein 1 (cPOP1) is a PYD-containing protein that can regulate inflammation (Stehlik *et al.*, 2003). Around 23 PYD-containing proteins have been identified to date (Bae & Park, 2011). cPOP1 inhibits inflammation by blocking the formation of inflammasome, which is a critical protein complex for activation of caspase-1. Caspase-1 activated by the formation of inflammasome activates inactive inflammatory cytokines including pro-interleukin1β and pro-interleukin18, leading to NF-kB activation and elicitation of inflammation and fever for innate immunity (Dinarello, 2004; Franchi *et al.*, 2009). NOD-like receptors (NLRs) (Martinon & Tschopp, 2005) such as NALP3 (NACHT, LRR and PYD domains containing protein 3), ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and caspase-1 are three protein components that form the inflammasome. PYD-domain-containing NALP3 plays an important role in signal sensing and oligomerization. ASC is a bipartite adaptor protein that contains an N-terminal PYD domain and a C-terminal CARD domain. CARD-domain-containing caspase-1 is a prototypical inflammatory caspase (Thornberry *et al.*, 1992; Ghayur *et al.*, 1997). NALP3 inflammasome is assembled *via* a PYD:PYD interaction between ASC and NALP3 and a CARD:CARD interaction between ASC and caspase-1. cPOP1 can modulate the assembly of inflammasome through direct interaction with ASC PYD instead of NALP3 (Stehlik *et al.*, 2003).

Despite the fundamental importance of inflammasome and regulatory protein containing PYD in innate immunity and many immune disorders, limited structural information is available. Structural studies of the PYD domain and PYD:PYD interaction in the inflammasome have been especially difficult because PYD domains are subject to self-aggregation under physiological conditions (Jang & Park, 2011).

As a first step towards elucidating the molecular structure of cPOP1 and further understanding the homotypic interaction of PYD in the inflammation signalling pathway, we overexpressed, purified



and crystallized cPOP1. Overexpressed cPOP1 was solubilized at pH 8.0 under high salt conditions and then purified by affinity chromatography followed by gel-filtration chromatography. Initial cPOP1 crystals were obtained in a solution containing 3.5 *M* sodium formate and 0.1 *M* Tris–HCl pH 8.0. The crystals belonged to space group $P2_13$ and had the unit-cell parameters $a = b = c = 94.12$ Å, and $\alpha = \beta = \gamma = 90.00^\circ$. The crystals were obtained at 293 K and diffracted to a resolution of 3.6 Å. Details regarding the structure of cPOP1 should enable an understanding of the assembly and inhibition mechanism of the inflammasome *via* the PYD:PYD interaction.

2. Materials and methods

2.1. Expression and purification

The full-length human cPOP1 corresponding to amino acids 1–89 was amplified by PCR and the PCR product was then digested with the restriction enzymes *Nde*I and *Xho*I (New England Biolabs, USA). The digested products were then inserted into the pOKD vector, which had been cut with the same restriction enzymes. The pOKD vector is a home-made vector that is modified from commercially available pET26b (Novagen, USA) (Dzivenu *et al.*, 2004).

The plasmid was transformed into BL21 (DE3) *Escherichia coli* competent cells and its expression was then induced by treating the bacteria with 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) overnight at 293 K. Cells expressing cPOP1 were subsequently pelleted by centrifugation, resuspended and lysed by sonication in 50 ml lysis buffer (20 m*M* Tris pH 8.0, 500 m*M* NaCl, 5 m*M* imidazole). The lysate was then centrifuged at 16 000 rev min^{-1} for 1 h at 277 K, after which the supernatant fractions were applied onto a gravity-flow column (Bio-Rad) packed with Ni–NTA affinity resin (Qiagen). The unbound bacterial proteins were subsequently removed from the column using washing buffer (20 m*M* Tris pH 8.0, 500 m*M* NaCl, 60 m*M* imidazole). The target protein was eluted from the column using elution buffer (20 m*M* Tris buffer pH 8.0, 500 m*M* NaCl, 250 m*M* imidazole). The elution fractions were collected at a 0.8 ml scale to 5 ml. Fractions containing more than 95% homogeneous cPOP1 upon SDS–PAGE analysis were selected, combined and concentrated to 30 mg ml^{-1} using a concentration kit (Millipore). The concentrated proteins were then applied onto a Superdex 200 gel-filtration column (GE Healthcare) that had been pre-equilibrated with a solution of 20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl.

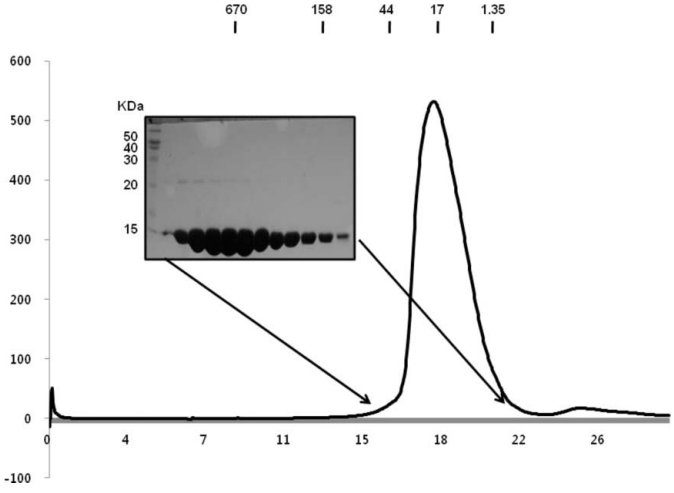


Figure 1
Purification of cPOP1. Gel-filtration chromatography and SDS–PAGE of cPOP1.

cPOP1 (molecular weight 10 450 Da) that eluted at around 18 ml was collected and concentrated to 7–8 mg ml^{-1}. The protein concentration was measured using a protein-assay kit (Bio-Rad) and was determined using the Bradford method (Bradford, 1976). The peak was then confirmed to contain cPOP1 by SDS–PAGE (Fig. 1). Purified cPOP1 contained the C-terminal extra residues LEHHHHHH and was not removed. The selenomethionine-substituted cPOP1 was expressed in methionine auxotrophic strain B834 (Novagen) grown in minimal medium supplemented with seleno-L-methionine (Sigma) and other nutrients. It was then purified and crystallized using the same procedure as that used for the native protein.

2.2. Crystallization

The crystallization conditions were initially screened at 293 K by the hanging-drop vapour-diffusion method using screening kits from Hampton Research (Crystal Screen and Crystal Screen 2, Natrix, MembFac, Index, Cryo, Lite and Salt Rx) and from the deCODE Biostructures Group (Wizard I, II, III and IV). Initial crystals were grown on the plates by equilibrating a mixture containing 1 µl of protein solution (7–8 mg ml^{-1} protein in 20 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl) and 1 µl of a reservoir solution from number 31 of Salt Rx (3.5 *M* sodium formate and 0.1 *M* Tris–HCl pH 8.0) against 0.4 ml of reservoir solution. Crystallization was further optimized by searching over a range of concentrations of protein and sodium formate and the best crystal was grown in the presence of 3.6 *M* sodium formate and Tris–HCl pH 8.5. Crystals appeared within 2 weeks and grew to maximum dimensions of 0.1 × 0.1 × 0.1 mm (Fig. 2). The crystals diffracted to a resolution of 3.6 Å.

2.3. Crystallographic data collection

For data collection, the crystals were transiently soaked in a solution corresponding to the reservoir solution supplemented with 40%(*v*/*v*) glycerol. The soaked crystals were then cooled in liquid nitrogen. A 3.6 Å data set was collected on beamline 5C (SB II) at the Pohang Accelerator Laboratory (PAL), South Korea. The SAD (single-wavelength anomalous diffraction phasing) data set was collected at the selenium peak wavelength (E = 12 669 eV, λ = 0.979 Å). The data sets were indexed and processed using *HKL*-2000 (Otwinowski & Minor, 1997). Selenium-labelled methionine was found and the structure was phased using the program

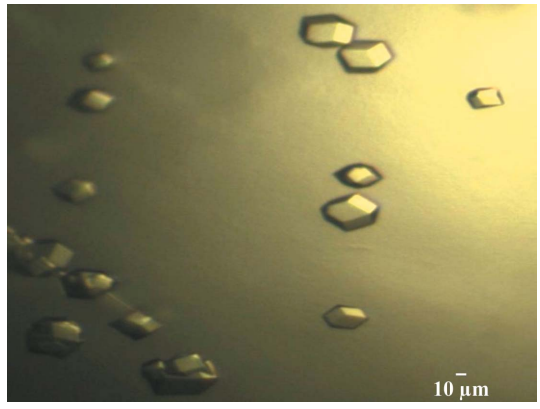


Figure 2
Crystals of cPOP1. Crystals were grown for 2 weeks in the presence of 3.6 *M* sodium formate and 0.1 *M* Tris–HCl pH 8.5. The approximate dimensions of the crystals were 0.1 × 0.1 × 0.1 mm.

Table 1
Diffraction data pertaining to cPOP1 crystals.

Values in parentheses refer to the highest-resolution shell.

	SeMet	Native
X-ray source	5C (SB II) at PAL	5C (SB II) at PAL
Wavelength (Å)	0.979	1.000
Space group	$P2_13$	$P2_13$
Unit-cell parameters (Å, °)	$a = b = c = 94.12$, $\alpha = \gamma = \beta = 90.00$	$a = b = c = 93.38$, $\alpha = \gamma = \beta = 90.00$
Resolution limits (Å)	50–3.6	50–4.0
No. of observations	70 690	36 181
No. of unique reflections	3425	2426
Mean $I/\sigma(I)$	39.6 (12.7)	38.6 (7.1)
Completeness (%)	100 (100)	99.9 (100)
$R_{\rm merge}$† (%)	18.7 (55.0)	11.1 (60.6)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity for all observations i of reflection hkl

SOLVE/*RESOLVE* (Terwilliger, 2004) or *SHELX* (Sheldrick, 2008). The data-collection statistics are summarized in Table 1.

3. Results and discussion

cPOP1 was purified by two chromatography steps, polyhistidine-tag affinity chromatography and gel-filtration chromatography, which produced a 98% pure target protein. The generated protein was subsequently analysed by SDS–PAGE (Fig. 1). No contaminating bands were visible upon SDS–PAGE. The calculated monomeric molecular weight of cPOP1 including the additional C-terminal tag was 10 450 Da and it eluted at approximately 12 kDa, suggesting that it may exist as a monomer in solution.

The success in crystallizing cPOP1 was a result of understanding critical features of cPOP1. cPOP1 was only crystallized and diffracted when purified under high salt conditions. We used 500 m*M* NaCl for the last step of purification.

The crystals belong to space group $P2_13$, with unit-cell parameters of $a = b = c = 94.12$ Å, $\alpha = \gamma = \beta = 90.00^\circ$. Assuming the presence of three monomers in the crystallographic asymmetric unit, the Matthews coefficient ($V_{\rm M}$) was calculated to be 2.22 Å^3 Da^{-1}, which corresponds to a solvent content of 44.5% (Matthews, 1968). Diffraction data statistics are given in Table 1. The data set was indexed and processed using *HKL*-2000 (Otwinowski & Minor, 1997). The SAD method was conducted with *SOLVE*/*RESOLVE* (Terwilliger, 2004).

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References

Bae, J. Y. & Park, H. H. (2011). *J. Biol. Chem.* **286**, 39528–39536.
Bradford, M. M. (1976). *Anal. Biochem.* **72**, 248–254.
Dinarello, C. A. (2004). *J. Endotoxin Res.* **10**, 201–222.
Dzivenu, O. K., Park, H. H. & Wu, H. (2004). *Protein Expr. Purif.* **38**, 1–8.
Franchi, L., Eigenbrod, T., Muñoz-Planillo, R. & Nuñez, G. (2009). *Nature Immunol.* **10**, 241–247.
Ghayur, T., Banerjee, S., Hugunin, M., Butler, D., Herzog, L., Carter, A., Quintal, L., Sekut, L., Talanian, R., Paskind, M., Wong, W., Kamen, R., Tracey, D. & Allen, H. (1997). *Nature (London)*, **386**, 619–623.
Jang, T. H. & Park, H. H. (2011). *J. Biotechnol.* **151**, 335–342.
Martinon, F. & Tschopp, J. (2005). *Trends Immunol.* **26**, 447–454.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Park, H. H. (2011). *Apoptosis*, **16**, 209–220.
Park, H. H. (2012). *Apoptosis*, **17**, 1247–1257.
Park, H. H., Lo, Y., Lin, S., Wang, L., Yang, J. K. & Wu, H. (2007). *Annu. Rev. Immunol.* **25**, 561–586.
Sheldrick, G. M. (2008). *Acta Cryst.* A**64**, 112–122.
Stehlik, C., Krajewska, M., Welsh, K., Krajewski, S., Godzik, A. & Reed, J. C. (2003). *Biochem. J.* **373**, 101–113.
Terwilliger, T. (2004). *J. Synchrotron Rad.* **11**, 49–52.
Thornberry, N. A. *et al.* (1992). *Nature (London)*, **356**, 768–774.