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Crystallization and preliminary X-ray analysis of the C-terminal domain of δ-COP, a medium-sized subunit of the COPI complex involved in membrane trafficking

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Coat protein I (COPI) is a protein complex composed of seven subunits that mediates retrograde transport of proteins and lipids from the *cis*-Golgi network to the endoplasmic reticulum and intra-Golgi membranes. The medium-sized δ subunit of COPI (δ-COP) is a 57 kDa protein with a C-terminal domain (CTD) and an N-terminal longin domain. Here, the δ-COP CTD was successfully cloned, purified and crystallized. Diffraction data were collected from native and selenomethionyl crystals of δ-COP CTD to resolutions of 2.60 and 2.30 Å, respectively. Both crystals belonged to space group $P2_12_12$, with similar unit-cell parameters. The native crystals had unit-cell parameters a = 100.23, b = 136.77, c = 44.39 Å.

1. Introduction

Intracellular trafficking between membrane compartments is mediated by small vesicles. Three main classes of coated vesicles have been identified to mediate particular transport steps within the cell. Specifically, COPII-coated vesicles transport newly synthesized proteins from the endoplasmic reticulum (ER) to the Golgi membrane. Clathrin-coated vesicles mediate mature protein trafficking from the *trans*-Golgi network (TGN) to plasma membranes and endosomes *via* post-Golgi trafficking routes. Lastly, COPI-coated vesicles mediate retrograde transport from the *cis*-Golgi network (CGN) to the ER and intra-Golgi transport (Bonifacino & Glick, 2004).

The heptameric protein complex COPI can be divided into two subcomplexes: the cargo-binding subcomplex comprised of the β, γ, δ and ζ subunits and the cap–cage subcomplex comprised of the remaining three subunits (α, β′ and ε; Kreis *et al.*, 1995). Previous studies have characterized the cap–cage subcomplex (Hsia & Hoelz, 2010; Lee & Goldberg, 2010), γ-COP and ζ-COP crystal structures (Watson *et al.*, 2004; Yu *et al.*, 2012). In contrast, the structure of the cargo-binding subcomplex remains to be elucidated owing to the lack of structural details for the β and δ subunits.

In this study, we focus on the δ subunit, a medium-sized 57 kDa protein in the COPI complex. It contains a putative N-terminal longin domain (NTD) and a 26 kDa C-terminal domain (CTD) (Faulstich *et al.*, 1996). Previous studies have shown that the μ2 CTD, a homologue of the δ-COP CTD in the adaptor protein 2 (AP2) complex, plays an important role in cargo recognition. Upon binding to specific cargo molecules, the closed conformation of the AP2 complex switches to an open conformation in order to initiate vesicle formation (Jackson *et al.*, 2010). Recent research has demonstrated that the δ-COP CTD is an interesting component of COPI; it can sometimes be nonspecifically cleaved off, leaving incomplete COPI, which is still involved in vesicle transport (Sun *et al.*, 2007). Here, we purified and crystallized human δ-COP CTD (residues 274–511). Preliminary X-ray diffraction analysis of this protein should provide further structural information on the COPI complex and reveal the molecular mechanism of COPI-regulated vesicle formation.

2. Materials and methods

2.1. Cloning, expression and purification

Based on secondary-structure prediction of human δ-COP, a series of truncations were designed. Genes encoding human full-length and truncated δ-COP were amplified from a human cDNA library by polymerase chain reaction (PCR) using specific primers which contained *Nhe*I and *Xho*I restriction sites; the NCBI reference sequence is NP_001646.2/gi:11863154. The PCR products were then inserted in frame into the corresponding restriction sites in the pET28a vector. To aid protein purification, the constructs contained a hexahistidine tag at the N-terminus followed by a thrombin protease cleavage site before the protein sequence. The full-length and truncated δ-COPs all expressed as precipitates, with the exception of the C-terminal truncation residues 274–511; therefore, residues 274–511 of human δ-COP (referred to below as δ-COP CTD) were chosen to proceed to subsequent purification and crystallization.

The human δ-COP CTD protein was expressed in *Escherichia coli* strain BL21 (DE3). 800 ml LB culture was grown to an OD_{600} of 0.8 before induction with 1 m*M* IPTG for 3 h at 310 K. Cells were harvested by centrifugation at 4670*g* for 30 min. All subsequent purification steps were carried out at 277 K. The bacterial pellet was resuspended in buffer *A* (20 m*M* Tris pH 8.0, 150 m*M* NaCl) supplemented with protease inhibitor (1 m*M* PMSF) and lysed by sonication. The lysate was clarified by centrifugation in an R20A2 rotor (Hitachi) at 18 000 rev min^{-1} for 60 min. The supernatant was incubated for 60 min with 2 ml Ni–IDA beads. After incubation, the slurry was transferred to an affinity column and washed with 50 ml buffer *A* with 50 m*M* imidazole. The bound protein was eluted from the beads using 250 m*M* imidazole in buffer *A*.

For crystallization trials, eluted protein from the Ni–IDA beads was applied onto a Superdex 200 10/300 GL gel-filtration column pre-equilibrated with buffer *A* at 277 K. The δ-COP CTD protein eluted from the gel-filtration column at a flow rate of 0.5 ml min^{-1} and the purified protein was concentrated for crystallization.

To obtain selenomethionine-labelled protein, the pET28a δ-COP CTD expression construct was transformed into the methionine-auxotrophic *E. coli* strain B834. Cells were grown in M9 minimal medium in the presence of 50 µg ml^{-1} L-selenomethionine (SeMet). 800 ml M9 culture was grown to an OD_{600} of 0.8 and induced with 0.5 m*M* IPTG for 16 h at 289 K. The protein was purified in the same way as the native protein.

The N-terminal hexahistidine tag was not cleaved before crystallization for both native and SeMet δ-COP CTD; the additional residues MGSSHHHHHHSSGLVPRGSHMAS remained before the protein sequence.

2.2. Crystallization and data collection

Native δ-COP CTD was crystallized by the sitting-drop technique at 289 K. Initial crystallization trials were performed using several commercial screening kits: Crystal Screen, Crystal Screen 2, PEG/Ion, Index (Hampton Research) and Wizard I and II (Emerald BioSystems). Drops consisting of 300 nl protein solution in buffer *A* and 300 nl reservoir solution were dispensed using a Mosquito robot and were equilibrated against 30 µl reservoir solution. The crystallization condition was optimized by varying the types and concentrations of precipitant, salts and buffers, the pH and by using additives. The optimized crystallization conditions for SeMet δ-COP CTD were based on those for the native protein with minor modifications.

An X-ray diffraction data set for native δ-COP CTD was collected at 100 K using a MAR CCD area detector on beamline BL17U (beam size 0.1 mm) at Shanghai Synchrotron Radiation Facility (SSRF). A total of 360 frames of 1° oscillation were measured with 1 s exposure per frame. A single-wavelength anomalous diffraction (SAD) data set for SeMet δ-COP CTD was also collected on beamline BL17U at SSRF. A total of 180 frames were collected at the peak wavelength of 0.979 Å with 1° oscillation and 1 s exposure per frame. *HKL*-2000 (Otwinowski & Minor, 1997) and *CCP*4 (v.4.2.2; Winn *et al.*, 2011) were used to process, reduce and scale the diffraction data sets.

3. Results and discussion

The protein eluted from the Superdex 200 10/300 GL gel-filtration column as a large peak at 14.1 ml, corresponding to the predicted molecular weight of dimeric δ-COP CTD (Fig. 1). The native δ-COP CTD protein (fractions 22–24) was collected and concentrated to approximately 12 mg ml^{-1}. Its purity was examined by 12% SDS–PAGE and it was determined to be >95% pure (Fig. 2). The SeMet δ-COP CTD protein was concentrated to 3.5 mg ml^{-1}.

The best crystals of native δ-COP CTD grew in 4 d using Index condition No. 86 consisting of 0.2 *M* potassium sodium tartrate

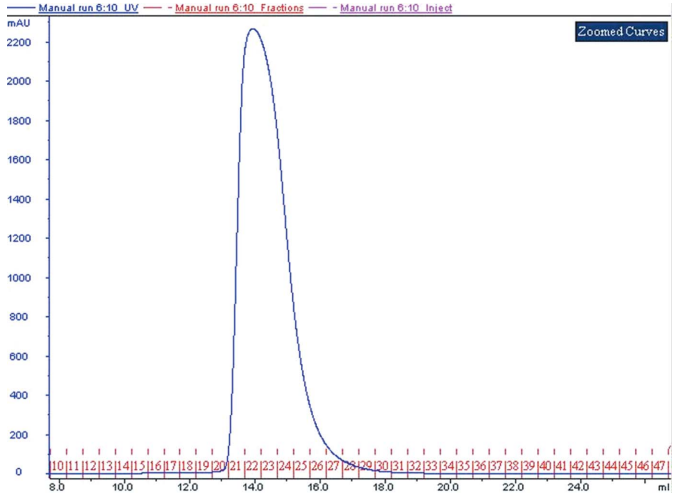

Figure 1
Superdex 200 10/300 GL chromatogram of native δ-COP CTD.

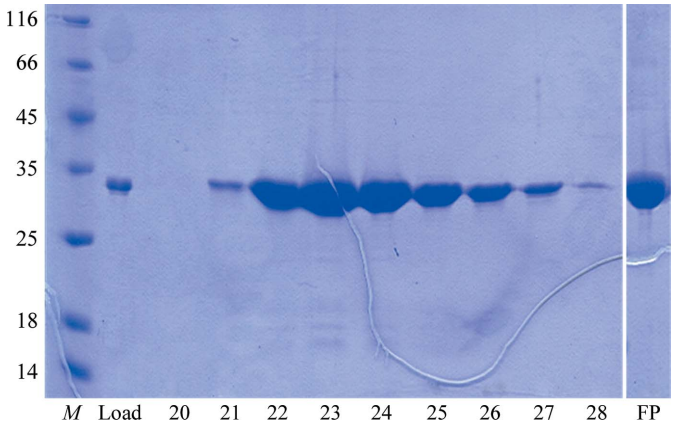

Figure 2
SDS–PAGE analysis of δ-COP CTD after purification on Superdex 200 10/300 GL. The fraction numbers of the gel-filtration column are labelled. Lane FP, the final product at a concentration of 12 mg ml^{-1}. Lane *M*, molecular-mass marker (labelled in kDa).

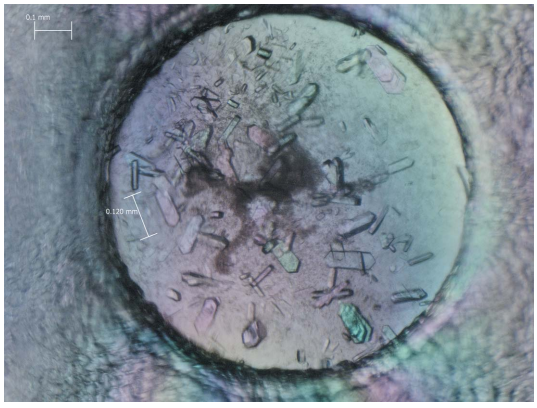

Figure 3
Crystals of native δ-COP CTD. The crystal dimensions are 0.12 × 0.05 × 0.03 mm.

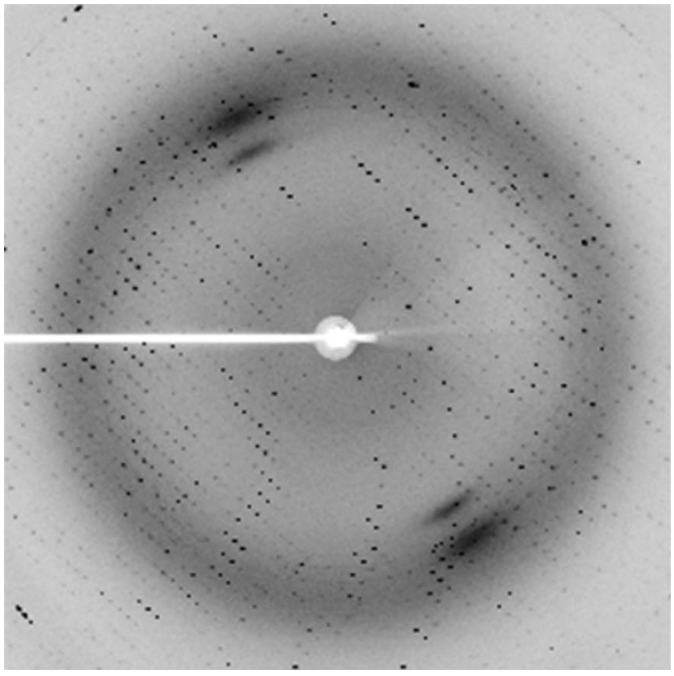

Figure 4
Diffraction image of a native δ-COP CTD crystal.

tetrahydrate, 20%(*w*/*v*) polyethylene glycol 3350 and Index condition No. 87 consisting of 0.2 *M* sodium malonate pH 7.0, 20%(*w*/*v*) polyethylene glycol 3350. The initial crystallization condition was further optimized by varying the pH, the type of salt and the concentration of PEG 3350 and by using additives. The optimal crystallization conditions were obtained at 289 K and consisted of 0.2 *M* lithium sulfate monohydrate, 0.1 *M* sodium cacodylate pH 6.0, 15% PEG 3350, 10 m*M* 1,4-naphthalene dicarbonic acid (Fig. 3). After soaking for 3 s in a cryoprotectant solution consisting of 0.2 *M* lithium sulfate monohydrate, 0.1 *M* sodium cacodylate pH 6.0, 15% PEG 3350, 30%(*v*/*v*) glycerol, the crystal was flash-cooled in liquid nitrogen for data collection.

Table 1
X-ray diffraction data statistics for native and SeMet δ-COP CTD crystals.

Values in parentheses are for the highest resolution shell.

	Native	SeMet
Space group	$P2_12_12$	$P2_12_12$
Resolution (Å)	50–2.60 (2.69–2.60)	30–2.30 (2.38–2.30)
Unit-cell parameters (Å, °)	a = 100.23, b = 136.77, c = 44.39, $\alpha = \beta = \gamma$ = 90	a = 100.02, b = 136.83, c = 44.34, $\alpha = \beta = \gamma$ = 90
Solvent content (%)	51.29	51.38
Matthews coefficient (Å^3 Da^{-1})	2.52	2.53
Total reflections	20037 (1960)	27967 (2741)
Unique reflections	3285 (321)	1828 (177)
Multiplicity	6.1 (6.1)	15.3 (15.5)
Completeness (%)	100 (100)	100 (100)
$R_{\rm merge}$† (%)	8.7 (66.1)	11.1 (46.6)
Average $I/\sigma(I)$	18.4 (2.9)	26.2 (9.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the measured reflection and $\langle I(hkl)\rangle$ is the mean intensity of all symmetry-related reflections.

SeMet δ-COP CTD was crystallized using the sitting-drop technique at 289 K and the best crystals were obtained after 90 d of growth in 0.2 *M* lithium sulfate monohydrate, 0.1 *M* sodium cacodylate pH 6.0, 12.5% PEG 3350, 0.4 *M* 1,4-butanediol.

Data-collection statistics for native and SeMet δ-COP CTD crystals are given in Table 1. The native crystals of human δ-COP CTD diffracted to 2.60 Å resolution and belonged to space group $P2_12_12$, with unit-cell parameters a = 100.23, b = 136.77, c = 44.39 Å (Fig. 4). The crystals of SeMet δ-COP CTD diffracted to 2.30 Å resolution and also belonged to space group $P2_12_12$, with unit-cell parameters a = 100.02, b = 136.83, c = 44.34 Å. The asymmetric unit contained two molecules of δ-COP CTD, with a crystal volume per unit mass ($V_{\rm M}$) of 2.53 Å^3 Da^{-1} and a solvent content of 51.38% (Matthews, 1968).

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References

Bonifacino, J. S. & Glick, B. S. (2004). *Cell*, **116**, 153–166.
Faulstich, D., Auerbach, S., Orci, L., Ravazzola, M., Wegchingel, S., Lottspeich, F., Stenbeck, G., Harter, C., Wieland, F. T. & Tschochner, H. (1996). *J. Cell Biol.* **135**, 53–61.
Hsia, K.-C. & Hoelz, A. (2010). *Proc. Natl Acad. Sci. USA*, **107**, 11271–11276.
Jackson, L. P., Kelly, B. T., McCoy, A. J., Gaffry, T., James, L. C., Collins, B. M., Höning, S., Evans, P. R. & Owen, D. J. (2010). *Cell*, **141**, 1220–1229.
Kreis, T. E., Lowe, M. & Pepperkok, R. (1995). *Annu. Rev. Cell Dev. Biol.* **11**, 677–706.
Lee, C. & Goldberg, J. (2010). *Cell*, **142**, 123–132.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Sun, Z., Anderl, F., Fröhlich, K., Zhao, L., Hanke, S., Brügger, B., Wieland, F. & Béthune, J. (2007). *Traffic*, **8**, 582–593.
Watson, P. J., Frigerio, G., Collins, B. M., Duden, R. & Owen, D. J. (2004). *Traffic*, **5**, 79–88.
Winn, M. D. *et al.* (2011). *Acta Cryst.* D**67**, 235–242.
Yu, X., Breitman, M. & Goldberg, J. (2012). *Cell*, **148**, 530–542.