Crystal structure of truncated human coatamer protein complex subunit ζ 1 (Cop ζ 1)

Sergey Lunev,^{a,‡} Marije F. W. Semmelink,^{a,‡} Jia Ling Xian,^a Kai Yu Ma,^a Anna J. A. Leenders,^a Alexander S. S. Dömling,^a Michael Shtutman^b and Matthew R. Groves^{a*}

^aDepartment of Drug Design, Groningen Research Institute of Pharmacy, University of Groningen, Antonius Deusinglaan 1, 9700 AD Groningen, The Netherlands, and ^bDepartment of Drug Discovery and Biomedical Sciences, South Carolina College of Pharmacy, University of South Carolina, 715 Sumter Street, Columbia, SC 29208, USA.
*Correspondence e-mail: m.r.groves@rug.nl

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‡ These authors contributed equally to this study.

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The majority of modern anticancer approaches target DNA/protein targets involved in tumour-cell proliferation. Such approaches have a major drawback, as nonproliferating cancer cells remain unaffected and may cause relapse or remission. Human coatamer protein complex I (COPI) subunit ζ (Cop ζ), a component of the coat protein involved in cell apoptosis and intracellular trafficking, has recently been proposed as a potential anticancer drug target. Previous studies have shown that two different isoforms of the Cop ζ subunit exist in mammalian cells. While normal cells express both Cop ζ 1 and Cop ζ 2 isoforms, various types of tumour cells display a loss of Cop ζ 2 expression and rely solely on Cop ζ 1 for growth and survival. Subsequent knockdown of Cop ζ 1 results in specific inhibition of both proliferating and dormant tumour-cell populations, with no adverse growth effects on normal cells. Therefore, a Cop ζ 1-targeting therapy was proposed to bypass the problem of dormant cancer cells that are resistant to conventional antiproliferative drugs, which is the major cause of tumour relapse. In order to aid in structure-based inhibitor design, a crystal structure is required. In this article, the recombinant expression, purification, crystallization and crystal structure of Cop ζ 1, as well as the expression and purification of Cop ζ 2, are reported.

1. Introduction

The optimal anticancer drug should target a particular feature or a pathway of the tumour that does not exist or is significantly different in normal cells. The majority of anticancer drugs on the market are focused on DNA or protein targets that are involved in the proliferation of the tumour cells. However, even if the proliferation of cancer cells is completely inhibited or eliminated during such treatments, the remaining nonproliferating dormant (Aguirre-Ghiso, 2006), senescent (Roninson, 2003) and resting stem cells (Sell, 2006) can re-enter the cell cycle and cause tumour relapse after initial remission. Therefore, truly effective anticancer therapies should affect both proliferating and nonproliferating populations of tumour cells. However, the pathways targeted are unlikely to be completely unique to tumours, and inhibition of these pathways in nontumour cells may lead to toxicity and adverse effects in patients (Eastman & Perez, 2006). In some cases the growth and survival of tumour cells depends on a pathway that, while being common to both kinds of cell, is not essential for the normal cells, as they possess an additional alternative/redundant pathway. This phenomenon is known as ‘oncogene addiction’ (Eastman & Perez, 2006; Weinstein & Joe, 2008), and it can be exploited for selective and successful

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anticancer drug design. An example of oncogene addiction is the interplay between human coatamer protein complex I (COPI) subunits $\zeta 1$ and $\zeta 2$. Human COPI is a coat protein that is involved in autophagy and intracellular protein trafficking between the endoplasmic reticulum and the Golgi apparatus in the early secretory pathway (Beck *et al.*, 2009; Razi *et al.*, 2009; Béthune *et al.*, 2006; Lee *et al.*, 2004).

The COPI consists of a cytosolic seven-subunit protein complex, which reversibly associates with the non-clathrin-coated vesicles of the Golgi and mediates transport from the endoplasmic reticulum (Waters *et al.*, 1991). The subunits forming COPI are organized into two subcomplexes: α -COP, β' -COP and ε -COP comprise a heterotrimer, while β -COP, γ -COP, δ -COP and ζ -COP form a heterotetramer (Lowe & Kreis, 1995). ζ -COP (Cop ζ) is an ~ 20 kDa subunit of COP that is required for coated vesicle assembly and binding of coatamer to the Golgi membranes (Kuge *et al.*, 1993). Subsequently, ζ -COP and γ -COP were both shown to exist in two distinct isoforms (Futatsumori *et al.*, 2000), and each isoform is present in the coatamer as a single copy. These findings resulted in the suggestion that there are several distinct COPI forms that are present in many or most mammalian cells (Wegmann *et al.*, 2004; Blagitko *et al.*, 1999). These COPI isoforms were later shown to have different distributions and localizations within the Golgi apparatus (Moelleken *et al.*, 2007). The abundance of different COPI isoforms was reassessed and the relative ratio of $\gamma 1/\zeta 1$, $\gamma 1/\zeta 2$ and $\gamma 2/\zeta 1$ was shown to be $\sim 10:3:5$, with the majority of $\zeta 1/\zeta 2$ -COP localized within the early Golgi compartment. The authors have estimated the amount of the $\gamma 2/\zeta 2$ -COPI isoform to be $<5\%$ of the total.

It has recently been shown that the Cop $\zeta 2$ isoform (but not Cop $\zeta 1$) is significantly down-regulated in the majority of tumour-cell lines and clinical samples of different cancer types (Shtutman *et al.*, 2011). This down-regulation makes the tumour cells dependent on Cop $\zeta 1$. Interestingly, no oncogenic activities of overexpressed Cop $\zeta 1/\zeta 2$ have been reported both *in vitro* and *in vivo*. Knockdown of Cop $\zeta 1$ was shown to be lethal to cancer cells, causing collapse of the Golgi apparatus, loss of autophagy and induced apoptosis, whereas normal cells were unaffected. Simultaneous knockdown of both Cop $\zeta 1/\zeta 2$ subunits caused similar effects in normal cells. Furthermore, tumour cells in which Cop $\zeta 1$ was knocked down could be rescued by re-expression of Cop $\zeta 2$, supporting the hypothesis that tumour-associated Cop $\zeta 2$ silencing causes the 'addiction' of tumour cells to Cop $\zeta 1$. These results also suggest that specific inhibition of Cop $\zeta 1$ subunit insertion into the coatamer (ideally with no adverse effect on Cop $\zeta 2$ assembly) represents a promising anticancer drug avenue. A structure of the Cop $\zeta 1$ subunit is required to provide a basis for structure-based rational drug design. In 2009, Yu and coworkers reported a solution NMR structure of the Cop $\zeta 1$ subunit (Yu *et al.*, 2009), but no crystal structure of the Cop $\zeta 1$ or the Cop $\zeta 2$ subunit is yet available in the Protein Data Bank. In this article the cloning, recombinant expression and purification of both Cop $\zeta 1$ and Cop $\zeta 2$ are reported, as well as the high-resolution crystal structure of Cop $\zeta 1$.

2. Materials and methods

2.1. Cloning

A commercially available (Eurofins) plasmid (pEX-A2) containing a synthetic gene encoding N/C-terminally truncated human Cop $\zeta 1$ (residues 7–150; UniProt ID P61923) was purchased and used for further subcloning into the *Escherichia coli* pETM-11 expression vector (Dümmler *et al.*, 2005). The pETM-11 expression system is based on the T7 promoter, allowing the use of high-yield autoinduction medium (Studier, 2005). A synthetic *E. coli* codon-optimized gene was used to increase the probability of successful soluble expression. The sequence encoding residues 7–150 of human Cop $\zeta 1$ contained NcoI and HindIII restriction sites at the 5' and 3' ends, respectively. pEX-A2-Cop $\zeta 1$ plasmid and pETM-11 vector were both digested with NcoI and HindIII restriction enzymes (Thermo Scientific) according to the manufacturer's recommendations and ligated by T4 DNA ligase (Thermo Scientific). The ligation mixture was incubated at 293 K for 10 min followed by heat inactivation at 338 K for 10 min according to the manufacturer's recommendations. The final construct contained a synthetic sequence encoding truncated Cop $\zeta 1$ (7–150) with a His₆ tag, linker sequence and *Tobacco etch virus* (TEV) digestion site fused at the N-terminus (Table 1).

Cloning of truncated human Cop $\zeta 2$ (UniProt ID Q9P299) was performed similarly. The final Cop $\zeta 2$ construct contained a synthetic sequence encoding truncated human Cop $\zeta 2$ (40–183) with a His₆ tag, linker sequence and TEV digestion site fused to the N-terminus (Table 1).

2.2. Expression and purification

E. coli BL21 (DE3) RIL competent cells transformed with the pETM-11-Cop $\zeta 1$ expression plasmid were propagated in 0.5 l selective medium (ZYM5052 in the presence of 100 $\mu\text{g ml}^{-1}$ kanamycin and 35 $\mu\text{g ml}^{-1}$ chloramphenicol) overnight at 310 K according to the autoinduction protocol of Studier (2005). At an OD₆₀₀ of 2.0, the temperature was decreased to 291 K. After overnight expression, the cells were harvested by centrifugation at 4000g for 30 min, resuspended in 30 ml wash buffer [50 mM Tris–HCl pH 8.0, 300 mM NaCl, 5% (v/v) glycerol, 3 mM β -mercaptoethanol (BME), 20 mM imidazole] and lysed by sonication on ice for 6 min at 50% amplitude (Branson W250D). The lysate was clarified by centrifugation at 41 000g for 60 min. All steps were performed at 277 K and no protease-inhibitor cocktails were used. No protein degradation was observed during the purification.

The lysate containing soluble His₆-tagged recombinant Cop $\zeta 1$ was filtered with a 2 μm filter membrane (Whatman) and further incubated with 2 ml Ni–NTA agarose (Macherey Nagel) for 10 min at 277 K. The agarose was washed with 100 ml wash buffer in order to remove contaminations using a gravity-flow column (BioRad). After the washing step, the recombinant His₆-tagged Cop $\zeta 1$ bound to Ni–NTA agarose was mixed with 8 ml digestion buffer [50 mM Tris–HCl pH 8.0, 300 mM NaCl, 5% (v/v) glycerol, 5 mM BME, 2 mg recombinant TEV protease] and incubated at 277 K overnight in order

Table 1

Macromolecule-production information.

A synthetic gene encoding human Cop ζ 1 was subcloned into the pETM-11 expression vector using NcoI (CCATGG) and HindIII (AAGCTT) restriction sites (underlined). Cloning of Cop ζ 2 was performed similarly. The His₆ tag (bold) and TEV protease cleavage site (underlined) are also indicated. The beginning and ending numbers of the amino-acid sequences are shown as subscripts.

Cop ζ 1	
Source organism	<i>Homo sapiens</i>
DNA source	Synthetic codon-optimized gene (Eurofins)
Insert sequence	CCATGGAACCATCGCTCTATACCGTAAAGCGAT- TCTGATTCTGGACAATGACGGAGATCGCCTGT- TTGCGAAGTATTACGATGATACGATACCGTCT- GTTAAAGAGCAGAAAGCCTTTGAGAAGAAT- CTTCAACAAAACCATCGCACAGATTCCGAAA- TTGCCCTGTTAGAAGGTCTGACTGTCGTGTAC- AAATCGAGCATTGACCTGTACTTCTATGTGAT- TGGGTCTAGCTATGAAAACGAAGTATGCTGA- TGGCTGTGCTTAATTGCCTGTTGATAGCTTG- TCCCAATGCTGCGGAAGAATGTGGAGAAACG- TGCAATTGCTGAAAACATGGAAGGCCTCTTTC- TTGCTGTAGACGAAATCGTGGATGGTGGCGTT- ATCCTGGAGTCAGATCCTCAGCAAGTCGTACA- CCGTGTTGCATTACGTGGCTAAGCTT
Cloning vector	pEX-A2
Expression vector	pETM-11
Expression host	<i>Escherichia coli</i>
Complete amino-acid sequence of the construct (including the expression tag and linker sequence)	MKHHHHHPMSDYDIPTTENLYFEGAME ₄₀ PSLYT- VKAILILDNDGRLFAKYDDTYPVKEQKAF- EKNIFNKTHTDSEIALLEGLTVVYKSSIDL- FYVIGSSYENELMLMAVLNCLFDSLQMLRKN- VEKRALLEMEGLFLAVDEIVDGGVILESDPQ- QVVRVALRG ₁₅₀
Cop ζ 2	
Source organism	<i>Homo sapiens</i>
DNA source	Synthetic codon-optimized gene (Eurofins)
Insert sequence	CCATGGAACCATCGCTCTATACCATCAAAGCTGT- GTTCAATCTGGACAATGATGTCGCTGCCTGT- TAGCCAAATACTATGACGATACATTCGCCAGT- ATGAAAGAACAGATGGTCTTTGAGAAGAAGCT- GTTCAACAAAACGAGTCGCACTGAATCGAAA- TTGCGTCTTTGGCGGCATGACCATTTGTGAC- AAGAACAGCATTGACCTTTTCTGTATGTTGT- TGGGTGCTCTTATGAGAATGAAGTATGCTCA- TGAGTGTGCTGACATGCCTTTTCGAGAGCTTG- AACCATATGCTGCGGAAGAATGTTGAGAAACG- CTGGCTCCTTGAACATGGATGGCGCATTTC- TGGTACTCGATGAGATCGTTGATGGTGGCGTT- ATCCTTGAATCCGATCCCAACAGGTGATTCA- GAAAGTCAACTTTCGGGCGTAAGCTT
Cloning vector	pEX-A2
Expression vector	pETM-11
Expression host	<i>Escherichia coli</i>
Complete amino-acid sequence of the construct (including the expression tag and linker sequence)	MKHHHHHPMSDYDIPTTENLYFEGAME ₄₀ PSLY- TIKAVFILDNDGRLRLAKYDDTTPSMKEQMV- FEKNVFNKTSRTESEIAFFGGMTIVYKNSIDL- FLYVVGSSYENELMLSVLTCLFESLNHMLRK- NVEKRWLLENMDGAFLVLDEIVDGGVILESDP- QQVIQKVNFR ₁₈₃

to perform on-column His₆-tag digestion. After overnight digestion the flowthrough fraction containing digested non-His₆-tagged Cop ζ 1 was collected and subsequently concentrated to a volume of 1 ml using a centrifugal concentrator with a 5 kDa cutoff (Sartorius).

The digested Cop ζ 1 was further purified using a Superdex 75 16/60 size-exclusion column (GE Healthcare). The protein was purified in SEC buffer [20 mM 4-(2-hydroxyethyl)-1-

piperazineethanesulfonic acid (HEPES) pH 7.5, 20 mM sodium citrate, 300 mM NaCl, 5% (v/v) glycerol, 3 mM BME] and eluted at a volume of ~80 ml as a single peak. This peak was pooled and concentrated using a centrifugal concentrator with a 5 kDa cutoff (Sartorius) to 10 mg ml⁻¹ for crystallization. The final protein concentration was determined based upon its theoretical absorbance at 280 nm. The protein purity was estimated to be better than 95% (not shown) as assessed by Coomassie Brilliant Blue-stained SDS-PAGE (Laemmli, 1970). The final yield of Cop ζ 1 was in excess of 40 mg per litre of culture.

The expression and purification of human Cop ζ 2 was performed identically apart from the use of *E. coli* BL21 (DE3) Rosetta2 pLysS as the expression cell line. Similarly, the elution volume of Cop ζ 2 during size-exclusion purification was ~80 ml. The final yield of Cop ζ 2 was comparable to that of Cop ζ 1.

2.3. Differential scanning fluorimetry

The buffer composition for the size-exclusion purification steps of Cop ζ 1 and Cop ζ 2 was chosen based upon a differential scanning fluorimetry (DSF; Thermofluor) assay (Ericsson *et al.*, 2006; Nettleship *et al.*, 2008). SYPRO Orange fluorescent dye (Invitrogen) dissolved in 100% DMSO was added to the protein sample (concentrated to 2 mg ml⁻¹) at 1:500 dilution. Each well of the 96-well plate PCR plate contained 5 μ l protein-dye solution and 45 μ l of the buffer component to be screened. A stability assay was performed using a CFX96 Real-Time PCR Detection System (Bio-Rad) at a temperature gradient from 293 to 368 K in steps of 0.5 K. The inflection points in graphs of relative fluorescence units (RFU) against temperature (*i.e.* melting temperature; T_m) were determined manually and used as an indicator of the sample thermal stability in the buffer component screened. Comparisons were made against control samples containing only protein/water. The optimal SEC buffer composition for both Cop ζ 1 and Cop ζ 2 was selected based on the components that showed a positive shift in the melting temperature and consisted of 20 mM HEPES pH 7.5, 20 mM sodium citrate, 300 mM NaCl, 5% (v/v) glycerol, 3 mM BME. The stabilization effect of the optimal SEC buffer on Cop ζ 1 compared with the wash buffer is shown in Fig. 1(a).

In order to select Cop ζ 2/Cop ζ 1 candidate-binding partners from a small-molecule compound library (Professor Alexander Dömling, Department of Drug Design, University of Groningen), further DSF-based experiments were performed. Candidate compounds were selected based on the positive shifts of the inflection point. The assay was performed similarly to the initial stability assay. Experiments were composed of 5 μ l of the candidate compound (100 mM solubilized in DMSO) and 45 μ l of the protein-dye mixture diluted in SEC buffer. Comparisons were made against a control sample containing only protein and DMSO and no compound. Based on these trials, 2-(3-hydroxy-4-methoxyphenyl)acetic acid was selected for further co-crystallization (Figs. 1c and 1e).

2.4. Crystallization

Initial high-throughput crystallization screening (Gryphon robot, ARI) was performed in MRC2 96-well plates using the sitting-drop technique. Screening was performed against the PACT premier, JCSG plus (Molecular Dimensions) and Index (Hampton Research) sparse-matrix screens. Screening for the crystallization conditions for Cop ζ 1 without additives did not yield positive results. Co-crystallization screening was also performed using the compound selected from the small-

molecule library using DSF (Fig. 1c). The sitting-drop experiments were performed using MRC2 96-well plates with 0.1 μ l protein/compound solution (10 mg ml⁻¹/10 mM) mixed with 0.1 μ l screen condition and stored at 289 K. Multiple Cop ζ 1 crystals appeared in various conditions containing 2–3 M sodium malonate, DL-malic acid or sodium citrate at pH 6.0 within a few days. Further optimization of the crystallization buffer was performed by varying the precipitant type and concentration using the hanging-drop technique. Drops of protein sample mixed with crystallization reagent in a 1:1 ratio were equilibrated against 500 μ l reservoir solution at 295 K. The optimal conditions consisted of equal volumes (1.5 μ l) of Cop ζ 1 sample at 14 mg ml⁻¹ and 2.2 M DL-malic acid, 0.2 M sodium malonate pH 6.2 in the presence of 10 mM 2-(3-hydroxy-4-methoxyphenyl)acetic acid and 10% (v/v) DMSO (Table 2). Small rectangular-shaped thin plate-like crystals of Cop ζ 1 (Fig. 2) appeared within a few days and were subsequently flash-cooled in liquid nitrogen for further storage and shipment. No further cryoprotection was necessary owing to the high precipitant concentration in the crystal-growth buffer.

Crystallization trials of Cop ζ 2 were performed identically. However, no Cop ζ 2 crystals have yet been observed.

Figure 1

Examples of DSF melting curves for Cop ζ 1/ ζ 2 samples. (a) Stabilizing effect of the optimum SEC buffer (thick line, $T_m = 342.5$ K) compared with the wash buffer (thin line, $T_m = 336.5$ K) used during the purification stages. (b) Addition of 10% (v/v) DMSO in SEC buffer shows a destabilizing effect ($\Delta T_m = 10.5$ K). The high melting temperature of the DMSO sample (thin line, $T_m = 332$ K), however, suggests that the protein sample can tolerate such a high DMSO concentration. (c) The presence of 10 mM 2-(3-hydroxy-4-methoxyphenyl)acetic acid in SEC buffer (thick line, $T_m = 337.5$ K) shows a stabilizing effect owing to an increased melting temperature ($\Delta T_m = 5.5$ K). (d) Similarly to Cop ζ 1, 10% (v/v) DMSO in SEC buffer (thick line, $T_m = 326.5$ K) destabilizes Cop ζ 2 compared with SEC buffer (thin line, $T_m = 331$ K). (e) 10 mM 2-(3-hydroxy-4-methoxyphenyl)acetic acid in SEC buffer (thick line, $T_m = 329.5$ K) stabilizes Cop ζ 2 ($\Delta T_m = 3$ K).

Table 2

Crystallization of Copζ1.

The buffer composition of the protein solution was chosen based upon the results of the DSF stability assay (Fig. 1)

Method	Hanging drop
Plate type	24-well
Temperature (K)	295
Protein concentration (mg ml ⁻¹)	14
Buffer composition of protein solution	20 mM HEPES pH 7.5, 20 mM sodium citrate, 300 mM sodium chloride, 5% (v/v) glycerol, 3 mM BME, 10 mM 2-(3-hydroxy-4-methoxyphenyl)acetic acid, 10% (v/v) DMSO
Composition of reservoir solution	2.2 M DL-malic acid, 0.2 M sodium malonate pH 6.2
Volume and ratio of drop	3 µl (1:1)
Volume of reservoir (µl)	500

Table 3

Data collection and processing.

Values in parentheses are for the outer shell. No ice rings were observed.

Diffraction source	P11, PETRA III
Wavelength (Å)	1.03
Temperature (K)	100
Detector	PILATUS 6M
Crystal-to-detector distance (mm)	277.74
Rotation range per image (°)	0.1
No. of images collected/No. used	3600/1700
Exposure time per image (s)	0.1
Space group	C121
<i>a</i> , <i>b</i> , <i>c</i> (Å)	109.4, 65.4, 37.3
α , β , γ (°)	90.0, 96.6, 90.0
Resolution range (Å)	56.03–1.60 (1.63–1.60)
Total No. of reflections	101312 (16680)
No. of unique reflections	33495 (5408)
Completeness (%)	97.3 (95.4)
Multiplicity	3.02 (3.0)
$\langle I/\sigma(I) \rangle$	16.0 (2.29)
R_{meas} (%)†	5.6 (42.2)
CC _{1/2} (%)	99.8 (89.1)
Overall <i>B</i> factor from Wilson plot (Å ²)	17.9

† R_{meas} is defined as $\sum_{hkl} \{ [N(hkl)/[N(hkl) - 1]]^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl) \}$, where $I_i(hkl)$ is the *i*th intensity measurement of reflection *hkl* and $\langle I(hkl) \rangle$ is the average intensity from multiple observations. The free *R* factor was calculated using a randomly selected 5% of reflections omitted from the refinement.

2.5. Data collection and processing

Copζ1 crystals were shipped to the PETRA III synchrotron facility, Hamburg, Germany using a dry-shipping container (Taylor–Wharton), and a 1.6 Å resolution data set was collected at 100 K in a nitrogen stream on the P11 beamline. The crystal belonged to space group C121, with unit-cell parameters *a* = 109.4, *b* = 65.4, *c* = 37.3 Å, α = 90.0, β = 96.6, γ = 90.0° and diffracted to below 1.6 Å resolution. A data set consisting of 3600 frames was collected. After initial data processing performed automatically using pipelines based on the XDS software (Kabsch, 2010), the data set was truncated to 1700 frames. No crystal twinning was detected. The data-collection statistics are shown in Table 3.

The structure of human Copζ1 was solved by molecular replacement using MOLREP (Vagin & Teplyakov, 2010) within the CCP4 package (Winn *et al.*, 2011). The solution NMR structure of human Copζ1 (PDB entry 2hf6; Yu *et al.*,

Table 4

Structure solution and refinement.

Values in parentheses are for the highest resolution shell.

Resolution range (Å)	56.03–1.60
Completeness (%)	97.34
No. of reflections, working set/free	33496/1619
Final R_{cryst}	0.21
Final $R_{\text{free}}^{\dagger}$	0.25
Cruickshank DPI	0.11
No. of non-H atoms	
Protein	2224
Ion	0
Water	116
Total	2340
R.m.s. deviations	
Bonds (Å)	0.03
Angles (°)	2.57
Average <i>B</i> factors (Å ²)	
Protein	20.15
Water	24.1
Ramachandran plot	
Most favoured (%)	96.35
Allowed (%)	2.92

† The free *R* factor was calculated using a randomly selected 5% of reflections omitted from the refinement.

2009) was used as a search model, yielding a solution with two monomers in the asymmetric unit. The model was further optimized using manual rebuilding *via* Coot (Emsley & Cowtan, 2004) and was refined with REFMAC (Murshudov *et al.*, 2011). All refinement steps were carried out with automatically generated local NCS restraints and TLS restraints calculated *via* the TLSMD web server (Painter & Merritt, 2006a,b). The final 1.6 Å resolution model of human Copζ1 consisted of two molecules in the asymmetric unit with an *R* factor of 0.21 and a free *R* factor of 0.25 (Table 4), and was deposited in the PDB with accession code 5mc5.

3. Results and discussion

A truncated version of human Copζ1 (residues 7–150) was cloned, recombinantly expressed and purified. The N- and

Figure 2

Small (50–100 µm) diffraction-quality crystals of Copζ1 grown in 2.2 M DL-malic acid, 0.2 M sodium malonate pH 6.2 in the presence of 10 mM 2-(3-hydroxy-4-methoxyphenyl)acetic acid.

C-terminal truncations were based on the results of Yu *et al.* (2009), in which full-length Cop ζ 1 showed no secondary structure for the first ten amino acids of the N-terminus and self-proteolysis near the C-terminus. A shorter construct of Cop ζ 1 (residues 17–150) expressed insolubly (data not shown). Similarly, a truncated version of human Cop ζ 2 (residues 40–183) was cloned, expressed and purified, and the truncations were based on the high degree of homology between Cop ζ 1 and Cop ζ 2 (74.9% identity).

A differential scanning fluorimetry (DSF)-based screening of purified Cop ζ 1 and Cop ζ 2 was performed against a fragment library of small-molecule compounds. A potentially binding fragment [2-(3-hydroxy-4-methoxyphenyl)acetic acid] was selected based on the increased thermal stability of both Cop ζ 1 ($\Delta T_m = 5.5$ K) and Cop ζ 2 ($\Delta T_m = 3$ K) in the presence

of this compound (Figs. 1c and 1e). Co-crystallization trials of both proteins yielded diffraction-quality crystals of Cop ζ 1 in the presence of 2-(3-hydroxy-4-methoxyphenyl)acetic acid. Interestingly, no apo crystals of Cop ζ 1 were observed in the absence of the compound even after an extended time period, suggesting that the presence of the small molecule is essential for crystallization. Extensive crystallization trials of Cop ζ 2 were performed similarly in the presence and absence of the compound, but to date no Cop ζ 2 crystals have been observed.

A 1.6 Å resolution X-ray data set from a Cop ζ 1 crystal grown in the presence of 2-(3-hydroxy-4-methoxyphenyl)acetic acid was collected on the P11 beamline at PETRA III, DESY, Hamburg, and the structure of Cop ζ 1 with two molecules in the asymmetric unit and an R factor of 0.21 and an R_{free} of 0.25 has been deposited in the PDB with accession code 5mc7.

Overall, the Cop ζ 1 monomer consists of five β -sheets surrounded by five α -helices (Fig. 3a). A comparison of our crystal structure of human Cop ζ 1 with the solution NMR structure (Yu *et al.*, 2009) indicates that the crystal structure and solution NMR structures are highly similar (Fig. 3b), strongly suggesting that the crystal structure represents the solution form of free Cop ζ 1. Based on this comparison, we suggest that the N-terminal region of Cop ζ 1 shows no secondary structure. The inability to detect electron density for the first five N-terminal residues of the construct (residues 7–11) supports this hypothesis. A region between α 1 and β 3 (residues 52–58) shows a significant rearrangement (8.3 Å

Figure 3

(a) Overall structure of human Cop ζ 1. (b) Superposition of the crystal structure of human Cop ζ 1 (pale green) and the solution NMR Cop ζ 1 structure (orange; PDB entry 2hf6; Yu *et al.*, 2009) shows no significant differences except for the flexible loop between α 1 and β 3 (residues 52–58) and the region between β 5 and α 2 (residues 81–87). (c) A comparison between two superimposed molecules (pale green and blue) in the asymmetric unit of the crystal structure of human Cop ζ 1 shows significant differences in the flexible-loop region. The coordinates were superimposed using *Coot* (Emsley & Cowtan, 2004; Jomaa *et al.*, 1999).

Figure 4

(a) A superposition of the crystal structure of human Cop ζ 1 (pale green) and the electron-microscopy structure of the ζ/γ -Cop complex (PDB entry 5a1u, pink and grey; Dodonova *et al.*, 2015). (b) A closer look at the superposition of the crystal structure of human Cop ζ 1 (both molecules of the asymmetric unit; pale green and blue) and the electron-microscopy structure of Cop ζ 1 (dark grey) bound to γ -Cop (pink) suggests that the flexible-loop region may show structural rearrangement during assembly into the COPI complex. The coordinates were superimposed using *Coot* (Emsley & Cowtan, 2004; Jomaa *et al.*, 1999).

difference for Thr55 and 9.3 Å for Asp56), suggesting high flexibility of this loop. Comparison between the two molecules within our Cop ζ 1 structure shows similar behaviour of the flexible loop, with shifts of 5.4 Å for Thr55 and 6.9 Å for Asp56. Another loop region between β 5 and α 2 (residues 81–87) shows a 4.9 Å shift between both molecules in our Cop ζ 1 structure and the solution NMR structure of Yu and co-workers.

Further comparison with the electron-microscopy structure of the ζ/γ -Cop complex (PDB entry 5a1u; Dodonova *et al.*, 2015) suggests a rearrangement of the flexible loop (residues 52–58) upon binding to γ -Cop. This shift may indicate that Cop ζ 1 undergoes structural rearrangement during assembly into the COPI complex that can potentially be used in the design of small molecules (Fig. 4). However, the low resolution of the EM structure strongly suggests that this hypothesis needs to be further assessed.

Despite the fact that Cop ζ 1 only crystallized in the presence of the potentially interacting compound 2-(3-hydroxy-4-methoxyphenyl)acetic acid, no unambiguous electron density for this compound was observed in the crystal structure. Further co-crystallization and biophysical experiments are under way in order to identify compounds that interact selectively with Cop ζ 1. The availability of the high-resolution crystal structure of Cop ζ 1 will aid in the search for hit compounds and further structure-based drug design.

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