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Structure of human collapsin response mediator protein 1: a possible role of its C-terminal tail

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Collapsin response mediator protein 1 (CRMP-1) is the first identified member of the CRMP family and is crucial for both the mediation of neuronal differentiation and in suppressing the invasion of lung cancer. The crystal structure of full-length human CRMP-1 was determined at a resolution of 3 Å. Human CRMP-1 comprises a tetrameric assembly; its overall structure is similar to that of mouse CRMP-1, but the measured electron density of the C-terminal residues 488–496 show a randomly coiled link that connects the protomers to each other, within which residues 497–572 are proteolytically susceptible *in vivo*. Deletion of residues 472–572 by thrombin *in vitro* not only releases a randomly coiled tail but also transduces observable structural changes of CRMP-1, as revealed by analytical size-exclusive chromatography and circular dichroism spectra. These results indicate a possible alternative role in CRMP dynamics and function.

1. Introduction

Collapsin response mediator proteins (CRMPs) are a family of cytosolic phosphoproteins that were originally investigated as signalling mediators of semaphorin 3A (also called collapsin 1, a secreted protein that acts as a chemorepellent for neuronal axon growth); for a review, see Schmidt & Strittmatter (2007). Among vertebrates, the family currently includes five isoforms (CRMP-1 to CRMP-5) which share a significant similarity in sequence with liver dihydropyrimidinase (DHPase) and with each other. Based on sequence alignment of amino acids, CRMP-1–CRMP-4 are ~75% identical, while CRMP-5 shares ~50% sequence identity with the other CRMPs and DHPase (Wang & Strittmatter, 1996; Fukada *et al.*, 2000; Inatome *et al.*, 2000). Expression of CRMP occurs almost exclusively during the neurogenic period of brain development (Goshima *et al.*, 1995; Minturn *et al.*, 1995; Byk *et al.*, 1996; Wang & Strittmatter, 1996). Significant clinical implications of CRMP have previously been shown; for example, phosphorylated CRMP-2 has been identified as a component of neurofibrillary tangles in the brains of patients with Alzheimer's disease (Yoshida *et al.*, 1998; Gu *et al.*, 2000; Good *et al.*, 2004). Auto-antibodies to CRMP-3 and CRMP-5 have been found in cancer patients suffering from paraneoplastic neurological syndrome (PNS), an inflammatory disorder depending on an autoimmune process and not directly caused by the tumour (Honnorat *et al.*, 1996, 1999; Yu *et al.*, 2001; Cross *et al.*, 2003). CRMP-1 has been found to be both an invasion suppressor and a molecular marker of human lung cancer. A novel long isoform of CRMP-1 with an N-terminus extended by about 100 amino

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acids, named LCRMP-1, has been reported to have functions that are the reverse of those of CRMP-1, promoting non-small-cell lung cancer (NSCLC) invasion and metastasis (Pan *et al.*, 2010). As both CRMP-1 and LCRMP-1 share the same core sequence and structure, CRMP-1 might hetero-oligomerize with LCRMP-1 and antagonize the functions of LCRMP-1 in NSCLC cell invasion (Pan *et al.*, 2011). Moreover, CRMP-1 and CRMP-5 have also been shown to have antagonistic effects, with CRMP-5 negatively regulating and CRMP-1 stimulating the proliferation of neural progenitors and transit-amplifying neuroblasts (Veyrac *et al.*, 2011). One hypothesis is that CRMP-1 forms hetero-oligomers with, and modulates the function of, other CRMP family members (Fukada *et al.*, 2000).

CRMPs are generally accepted to occur preferably as heterotetramers *in vivo* but to form homotetramers when purified from both bovine brain and heterogeneously recombinant hosts (Wang & Strittmatter, 1997). All CRMP monomers possess a conserved core structure (Ponnusamy & Lohkamp, 2013) that excludes approximately 80 residues at the C-terminus of CRMP and is sufficient for hetero- and homo-oligomerization in varied combinations between isoforms (Wang & Strittmatter, 1997; Stenmark *et al.*, 2007; Majava *et al.*, 2008). Compared with the sequence of DHPase, CRMP is extended with a positively charged C-terminal region that is highly susceptible to proteolysis (Deo *et al.*, 2004). The calcium-activated protease calpain specifically cleaves CRMP *in vivo* within the C-terminal region to produce products (55–58 kDa) in response to neuronal cell injury and neurite damage (Jiang *et al.*, 2007; Zhang *et al.*, 2009; Taghian *et al.*, 2012). The effect of the C-terminal truncation correlates with the presence of an inhibiting factor for post-injury neurite regeneration, but little is known about the direct structure and function of CRMP. Knowledge of its structural properties is thus required in order to understand its unique role in these behaviours.

In this work, we determined the crystal structure of human CRMP-1 at a resolution of 3 Å. CRMP-1 is a tetramer in the crystal; the conformations of the four protomers are essentially identical. Each protomer consists of two domains: an N-terminal small domain and an α/β -barrel domain. The crystal structure excludes the proteolytically susceptible C-terminal tail (residues 497–572), circular dichroism measurements of which indicate a randomly coiled structure. Analysis of the oligomerization and structural states after the deletion of residues 472–572 by thrombin *in vitro* revealed discernible differences which, in addition to protein phosphorylation or dephosphorylation events, might be crucial to regulate the function of CRMP and its interaction with other signalling proteins.

2. Materials and methods

2.1. Plasmid construction

The full-length (residues 1–572) coding sequence carrying the human CRMP-1 gene (kindly supplied by Professor Pan-

Chyr Yang, College of Medicine, National Taiwan University, Taipei, Taiwan) was amplified by PCR with Herculanase II fusion DNA polymerase (Merck). The forward primer that contains a factor Xa protease cleavage site (represented in bold) is 5'-**ATCGAAGGTCGTATGTCGTACCAGGGCA-AGAAGAG**-3'; the reverse primer is 5'-TCAACCGAGGCTGGTGATGTTG-3'. The PCR-amplified DNA fragment was ligated into the expression vector pET-SUMO (Invitrogen), resulting in a construct with an N-terminal 6×His tag, a small ubiquitin-related modifier (SUMO) fused to enhance the solubility of expressed proteins and an integrated protease cleavage site for factor Xa between the SUMO fusion and the full-length CRMP-1 gene.

2.2. Protein overexpression, purification and crystallization

The pET-SUMO-CRMP-1 construct was transformed into *Escherichia coli* strain BL21(DE3) (Invitrogen). For expression, the cells were grown in 1.5 l LB medium containing 50 mg l⁻¹ kanamycin at 310 K. When the absorbance at 600 nm reached 0.6, the temperature was decreased to 291 K and the culture was induced with 1 mM IPTG. The cells were harvested by centrifugation (10 000g, 30 min) 20 h after induction and then frozen at 193 K until use.

The cells from the LB medium were resuspended in 100 ml 20 mM Na₂HPO₄-HCl pH 7.5 containing 200 mM NaCl and 1 mM PMSF. The lysate was sonicated on ice and centrifuged (28 500g, 30 min, 277 K). The resulting supernatant was loaded onto a nickel-affinity column (5 ml Ni-NTA resin, Qiagen) followed by wash buffer (ten column volumes; 20 mM Na₂HPO₄-HCl pH 7.5 containing 500 mM NaCl and 100 mM imidazole). The bound His-tagged fusion protein was eluted with elution buffer (three column volumes; 20 mM Na₂HPO₄-HCl pH 7.5 containing 500 mM NaCl and 200 mM imidazole); the buffer was changed to 20 mM Na₂HPO₄-HCl pH 7.5 containing 150 mM NaCl and 35 mM imidazole using 50 kDa cutoff Amicon Centricons (Millipore). The yield of the SUMO-CRMP-1 protein was estimated to be 10–15 mg per litre of culture.

After purification on a nickel-affinity column, the SUMO-CRMP-1 protein was cleaved with factor Xa at the IEGR cleavage site between the SUMO fusion and CRMP-1. SUMO-CRMP-1 fusion protein (~20 mg) was cleaved by incubation with 30 µg factor Xa (BioLabs; 20 h at 277 K) in cleavage buffer (total volume 10 ml; 20 mM Na₂HPO₄-HCl pH 7.5 containing 150 mM NaCl and 35 mM imidazole). The protein mixture was then centrifuged (2000g, 10 min) and the supernatant was loaded onto a nickel-affinity column (3 ml Ni-NTA resin, Qiagen). The CRMP-1 protein lacking the N-terminal 6×His tag was collected in the flowthrough, while the 6×His tag and undigested SUMO-CRMP-1 protein remained bound to the column. The buffer of the purified CRMP-1 was changed to 20 mM Tris-HCl pH 7.5 containing 150 mM NaCl. CRMP-1 was then concentrated to 10 mg ml⁻¹ using 50 kDa cutoff Amicon Centricons (Millipore). The purity of CRMP-1 was greater than 99% as estimated by SDS-PAGE; the yield was determined to be 6–9 mg per litre of

culture. To assay the thrombin protease cleavage of full-length CRMP-1, CRMP-1 (2.5 mg) and thrombin (100 µl; Sigma catalogue No. T6884; 1 kU) were mixed in a final reaction buffer (550 µl; 20 mM Tris–HCl pH 7.4, 150 mM NaCl) and incubated at 277 K overnight. The resulting mixture was then characterized and purified *via* analytical gel filtration as described below. All protein concentrations were determined with a BCA protein-assay kit (Thermo Scientific) using bovine serum albumin as a standard reference.

Crystallization experiments were performed at 293 K using the hanging-drop vapour-diffusion method. Drops were made by mixing protein solution (10 mg ml⁻¹ in 20 mM Tris–HCl with 150 mM NaCl pH 7.5) and well solution (50 mM ammonium sulfate, 50 mM sodium citrate, 12–15% PEG 8000) in a 1:1 ratio. Crystals began to grow within 1 or 2 d. High-quality crystals appeared after incubation for 14 d. Before cooling in liquid nitrogen, the crystals were dehydrated in sequential solutions composed of 50 mM ammonium sulfate, 50 mM sodium citrate and various concentrations of PEG 8000 (17, 19, 21, 23 and 25%), allowing about 5 min in each solution.

2.3. Data collection and structure determination

The protein crystals were screened on beamline 13B1 at the National Synchrotron Radiation Research Center in Taiwan. Crystals were mounted in loops (Hampton Research) and flash-cooled to 100 K. Complete data sets were collected using X-rays of wavelength 1.5 Å over 180° of rotation with 0.5° oscillation. The data were indexed, integrated and scaled with *HKL-2000* (Otwinowski & Minor, 1997). The resolution was truncated at 3 Å to give an R_{merge} of <40%. The structure of CRMP-1 was solved by molecular replacement using the phase from CRMP homologues (PDB entries 1kcx and 2vm8; Deo *et al.*, 2004; Majava *et al.*, 2008). The data were modelled and refined with *PHENIX* (Adams *et al.*, 2010). Table 1 presents a summary of the data-collection and refinement statistics. The figures were created with *PyMOL* (DeLano, 2002). Coordinates and structure factors have been deposited in the PDB as entry 4b3z.

2.4. Analytical size-exclusion chromatography

Oligomerization analyses were performed with full-length and thrombin-cleaved CRMP-1 (using an analytical Superdex 200 10/300 column from GE Healthcare connected to an ÄKTA purifier system). All analyzed proteins were diluted in running buffer (20 mM Tris–HCl with 150 mM NaCl pH 7.5) to a final concentration of 0.5 mg ml⁻¹ before sample injection. All analyses were performed at a flow rate of 0.5 ml min⁻¹ and at least twice in independent experiments. The molecular mass of the proteins was estimated using the marker proteins horse spleen ferritin (450 kDa), bovine liver catalase (240 kDa), bovine albumin (67 kDa), egg albumin (45 kDa) and chymotrypsinogen (25 kDa) (protein molecular-mass standards kit MS II, SERVA).

Table 1

Data-collection and refinement statistics.

Resolution (Å)	30–3.00 (3.11–3.00)
Space group	$P2_1$
Unit-cell parameters (Å, °)	$a = 84.81$, $b = 147.84$, $c = 86.02$, $\beta = 94.612$
Total No. of reflections	308334
Unique reflections	42183
Completeness (%)	99.6 (77.0)
Mosaicity (°)	1.1
Wilson B factor (Å ²)	50.95
R_{merge} (%)	8.5 (43.5)
$\langle I/\sigma(I) \rangle$	18.37 (3.32)
Protein residues	15–496
R_{cryst} (%)	17.83
R_{free} (%)	24.13
R.m.s.d., bonds (Å)	1.106
R.m.s.d., angles (°)	0.008
Ramachandran favoured (%)	96.3
Ramachandran outliers (%)	0.2
Clashscore	12.38
Average B factor (Å ²)	60.6

Figure 1

Crystallization and SDS-PAGE analysis of purified human CRMP-1. (a) Morphology of CRMP-1 crystals. Left, the crystals after crystallization (2 d, 293 K). Right, the crystals after incubation (20 d). (b) Analysis (SDS-PAGE) of CRMP-1 proteins that had not (lane 1) or had (lane 2) been incubated with thrombin protease and a sample taken from a crystallization well containing high-quality CRMP-1 crystals after incubation (20 d at 293 K; lane 3). Samples (3 µg) were characterized using a Tris–glycine gel (12%) and stained with Coomassie Blue. (c) SDS-PAGE analysis of the purified C-terminal tail (residues 472–572) of CRMP-1. Samples were separated with a Tris–tricine (20%) peptide-separation gel and were detected with silver staining.

2.5. Far-ultraviolet circular dichroism (CD) spectra

For far-UV CD spectra, the protein samples were directly collected from FPLC fractionation (estimated protein concentration of 0.04 mg ml^{-1}) and spectra were recorded using a Jasco J-815 CD spectrometer in combination with a temperature controller (Jasco PTC-423S/15; Jasco International, Tokyo, Japan). CD spectra were scanned from 260 to 200 nm at a scanning speed of 100 nm min^{-1} with a response time of 1 s, a bandwidth of 1 nm and an accumulation of five scans in quartz cuvettes (Hellma GmbH, Muehlheim, Germany) with path length 1 mm at 293 K. Using the *Spectra Analysis* software (v.2.09.00; Jasco), the solvent spectrum was background-subtracted from the spectra. Synchrotron-based CD experiments were conducted on beamline BL04B1 as described previously (Liu *et al.*, 2010); CD spectra were collected from 260 to 180 nm.

3. Results and discussion

3.1. The C-terminal tail affects the crystal quality

CRMP-1 protein crystals appeared within 1–2 d after setup (Fig. 1*a*, left), but none of these quickly grown crystals gave satisfactory X-ray diffraction when screened at the synchrotron. Crystals appearing after two weeks had satisfactory diffraction properties and grew to dimensions of approximately $0.4 \times 0.2 \times 0.2 \text{ mm}$ after 20 d (Fig. 1*a*, right). SDS-PAGE analysis showed that the protein sample taken from the crystallization well of these high-quality crystals had a smaller apparent molecular mass (Fig. 1*b*, lane 3) than a fresh CRMP-1 sample (Fig. 1*b*, lane 1) before crystallization trials were undertaken. From matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectra (Microflex MALDI-TOF, Bruker Daltonics), the precise molecular masses of the CRMP-1 protein taken from the crystallization well of these high-quality crystals and of a fresh sample were determined to be 54 681 and 61 343 Da, respectively. Together with the final electron density, these results indicated that the C-terminal tail (residues 497–572) was proteolytically susceptible; the high-quality crystals were grown from molecules from which the C-terminal tails had been hydrolyzed.

3.2. Overall structure of human CRMP-1

Human CRMP-1 crystallizes in space group $P2_1$ with four subunits in the asymmetric unit. The unit-cell parameters are $a = 84.815$, $b = 147.846$, $c = 86.023 \text{ Å}$, $\beta = 94.61^\circ$; the data-processing statistics are reported in Table 1. Electron density was visible for residues 15–496. As no electron density was detectable for residues 1–14 and 497–572 they were excluded from the final model. An example of the quality of the electron-density map is shown in Fig. 2 for residues Arg467–Pro475. The CRMP-1 folds into a structure containing 19 α -helices and 21 β -strands (Fig. 3*a*). The N-terminal small domain comprises seven antiparallel β -sheets ($\beta 1$ – $\beta 7$, residues 15–68). The large portion of the protein is an α/β -barrel domain (residues 69–460); the core scaffold within this domain is a triosephosphate isomerase (TIM)-like structure (which

comprises eight α -helices and seven β -sheets in the order $\alpha 1$, $\beta 11$, $\alpha 2$, $\beta 12$, $\alpha 3$, $\beta 13$, $\alpha 4$, $\beta 14$, $\alpha 7$, $\beta 15$, $\alpha 8$, $\beta 16$, $\alpha 12$, $\beta 17$, $\alpha 15$). Residues 461–496 form a long random coil linking $\beta 21$ to $\alpha 19$ and extending to the C-terminus. The C-terminus of the protein (residues 497–572) was severed during crystallization and was excluded from the structure. CRMP-1 forms tetramers in the crystal (Fig. 3*b*). Four protomers are stabilized by two separate contacts. Interface I is located between chains *A* and *B* and chains *C* and *D*. Residues in $\beta 2$ (Arg23), $\alpha 15$ – $\alpha 16$ (Asp376, Glu377, Asn378 and Gln379) and $\alpha 19$ (Gln480 and Arg481) contribute significantly to the interface. Interface II is located between chains *A* and *C* and chains *B* and *D*. Residues in $\alpha 5$ (Leu204 and Glu208), $\alpha 7$ (Leu231, Phe237, Thr241 and Arg245) and $\alpha 8$ (Ile263) are major contributors to the interface. Besides homotetramerization, CRMP-1 protomers are known to form heterotetramers with other CRMP isoforms. The residues in interface I are conserved among CRMP-1 to CRMP-4 (Fig. 4); this interface is thus likely to serve as a site for hetero-oligomerization. According to the model proposed by Stenmark *et al.* (2007), the tetramerization of CRMP begins with two protomers arranging into homodimers *via* interface II, which then form a homotetramer or heterotetramer *via* interface I. The precise manner in which CRMP heterotetramers are formed, based on either a comparative analysis of the available homotetramer structures (Ponnusamy & Lohkamp, 2013; Ponnusamy *et al.*, 2014) or biochemical experiments (Wang & Strittmatter, 1996; Fukada *et al.*, 2000; Ponnusamy & Lohkamp, 2013), remains inconclusive. Without the structural detail of hetero-oligomers, it is still difficult to precisely identify the residues that might contribute to the preferential formation of such hetero-oligomers.

Human CRMP-1 and the mouse counterpart differ at only 20 amino acids of 572. The main sequence variation is located in the region from β -strand 21 to α -helix 19 (residues 459–493). Seven of 35 amino acids ($\sim 20\%$) differ between mouse and human CRMP-1, indicating some functional disparity related to this region. The crystal structure of CRMP-1 from mouse has been determined (Deo *et al.*, 2004). Compared with the resulting human CRMP-1 structure, the overall backbones of these two closely related proteins are highly similar (pair-

Figure 2

A representative view of the final $2F_o - F_c$ electron-density map (blue, resolution 3 Å, contoured at the 1.0σ level), highlighting the fit of residues Arg467–Pro475 to the maps. The nonconsensus amino acid (depicted in red) in this region is Ala473 of human CRMP-1 (phenylalanine in mouse CRMP-1).

wise C^α r.m.s.d. of 0.338 Å for human CRMP-1 *versus* mouse CRMP-1 comparing the chain *A* monomer). Most nonconserved residues between human CRMP-1 and mouse CRMP-1 are located on the outer surface of the tetramer complex; these residues affect neither the backbone folding nor the oligomerization interfaces.

3.3. A possible functional randomly coiled C-terminal tail

We found that residues 488–496 of CRMP-1, corresponding to the C-terminal end of the electron density, form a randomly coiled link and connect the four protomers to each other (Fig. 5*a*). This connection is located between chains *A* and *C* and chains *B* and *D*, and is stabilized by hydrogen bonds between residues in this segment (Ser495 and Arg496) of one monomer and α -helix 6 (Glu221) and α -helices 10–11 (Tyr290, Ser292 and Lys293) of the other (Fig. 5*b*). A similar inter-

action of C-terminal stretches involving an Arg–Glu pair has been reported in human CRMP-5 (Ponnusamy & Lohkamp, 2013), in which the interactions besides the salt bridge of an Arg–Glu pair are difficult to model unambiguously because of a lack of electron density. Here, we provide a clearer map of the electron density of residues contributing to the cross-linking of CRMP-1. The link is likely to serve as an enhancement of interface II and to contribute to oligomerization, but only one link is built in the four protomers in our model because of the poor quality of electron density for the other three protomers, indicating that this link might be flexible in the crystals.

As mentioned earlier, crystals of high quality were only obtained after the C-terminal tails were auto-cleaved. It is thus of interest to determine whether these links agree with the hypothetical model (Schmidt & Strittmatter, 2007) that a link structure of some kind is located at the CRMP-1 surface and is

Figure 3

Crystal structure of human CRMP-1. (*a*) Ribbon diagram of a monomer. The N-terminal small domain is shown in blue and the α/β -barrel domain is shown in green/yellow. The long link and α -helix 19 are shown in violet. The view in the right panel has been rotated -90° with respect to that in the left panel. (*b*) A CRMP-1 tetramer. *A* chain, green; *B* chain, yellow; *C* chain, cyan; *D* chain, pink. Side chains significantly contributing to interfaces I and II are depicted as blue and red sticks, respectively. The view in the right panel has been rotated -90° with respect to that in the left panel.

critical for tetramerization. Full-length CRMP-1 (amino acids 1–572) was then cleaved at Arg471 using thrombin protease (as predicted using the ExPASy *PeptideCutter* program; <http://www.expasy.org/>; Gasteiger *et al.*, 2005) to release a fragment of 100 amino acids that comprises the auto-cleaved 76 amino acids at the C-terminal tail. As expected, the main proteolytic product has a smaller apparent molecular mass than untreated CRMP-1 protein (Fig. 1*b*, lane 2 *versus* lane 1); the purified C-terminal tail of CRMP-1 (472–572 with theoretical molecular mass 11 014 Da) from size-exclusion chromatography also revealed a sufficiently high purity (Fig. 1*c*). We subsequently replicated the analytical gel-filtration experiments for CRMP-1 but found no pronounced destabilization of homotetramer formation after thrombin cleavage (Fig. 6*a*). For the thrombin-cleaved proteins, the peak profile shows a slight shift towards the tetrameric form, indicating a possible conformational change of its structure. The circular dichroism spectrum of truncated CRMP-1 also shows an observable difference from the spectrum of full-length CRMP-1, which exhibits a decreased depth and broadening of the far-UV CD spectrum

at about 208 nm (Fig. 6*b*). We therefore postulate that the link is likely to extend the interaction with the corresponding protomer. We also identified that the CD spectrum of the purified C-terminal tail (concentration 0.13 mg ml⁻¹) over the wavelength range 180–260 nm shows properties that are significantly different from the spectra of the α -helix-rich protein myoglobin and the β -strand-rich protein concanavalin A (Fig. 6*b*, inset), agreeing with the secondary structure predicted from the protein sequence (Prilusky *et al.*, 2005): the C-terminal tail is intrinsically in a random-coil conformation.

Figure 4

Residues that are potentially important in determining interfaces in CRMP. The crystal structures of mouse CRMP-1 (yellow; PDB entry 1kcx; Deo *et al.*, 2004), CRMP-2 (green; PDB entry 2gse; Stenmark *et al.*, 2007), CRMP-4 (cyan; PDB entry 4bkn; Structural Genomics Consortium, unpublished work) and CRMP-5 (blue; PDB entry 4b91; Ponnusamy & Lohkamp, 2013) are superimposed onto human CRMP-1 (orange; PDB entry 4b3z). Both interfaces I (*a*) and II (*b*) contain conserved and nonconserved residues. The interacting residues are labelled for human CRMP-1; the corresponding residues of mouse CRMP-1, CRMP-2, CRMP-4 and CRMP-5 are given in parentheses.

Figure 5

Links located on the surface of the CRMP-1 tetramers. (*a*) A CRMP-1 tetramer shown as a surface diagram. In the tetramer structure, individual monomers are coloured green (*A* chain), yellow (*B* chain), cyan (*C* chain) and pink (*D* chain). The residues of the linkers (from Val488 to Arg496) are highlighted in dark pink (*D* chain to *B* chain), dark green (*A* chain to *C* chain), dark blue (*C* chain to *A* chain) and orange (*B* chain to *D* chain). The view in the right panel has been rotated 180° with respect to that in the left panel. (*b*) Detailed diagram of a portion of the link from the *D* chain to the *B* chain. Residues contributing to the cross-linking (Val488–Arg496 of the *D* chain and Glu221, Tyr290, Ser292 and Lys293 of the *B* chain) are depicted in stick representation and fitted to the $2F_o - F_c$ electron-density map (blue, resolution 3 Å, contoured at the 0.7σ level). Hydrogen bonds are shown as dashed green lines.

4. Conclusions

Using X-ray crystallography and circular dichroism, we determined the structure of CRMP-1 from residues 14 to 496 and demonstrated that the C-terminal residues 472–572 form random coil. We found also a new structure of links located at the surface of the CRMP-1 tetramers which might contribute

to the structure-induced modulation of the CRMP-1 function instead of regulation of the oligomeric state.

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Figure 6

Oligomerization and characterization of the secondary structure of full-length and thrombin-cleaved CRMP-1. (a) Analytical gel-filtration chromatography of full-length (black line) and thrombin-cleaved (red line) CRMP-1 was performed as described in the *Materials and methods*; the profiles are superimposed based on the 'inject' signal. The elution volumes corresponding to the molecular masses of the protein markers is marked with blue arrows for comparison. V_0 and V_t denote the void and total volumes of the column, respectively. Under these operating conditions, the apparent predominant species of full-length and thrombin-cleaved CRMP-1 are tetramers. (b) CD spectra of CRMP-1 (black line) and thrombin-cleaved CRMP-1 (red line) were recorded in the 260–200 nm wavelength region. In the inset in (b), CD spectra of the C-terminal tail of CRMP-1 (amino acids 472–572; grey line), the α -helix-rich protein myoglobin (blue line) and the β -strand-rich protein concanavalin A (green line) (each protein was dissolved in 10 mM Tris-HCl pH 7.4, 150 mM NaF) are shown in mean residue ellipticity units; the buffer baseline was subtracted.

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