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Arginine 116 stabilizes the entrance to the metal ion-binding site of the MntC protein

The cyanobacterium *Synechocystis* sp. PCC 6803 imports Mn^{2+} ions via MntCAB, an ABC transport system that is expressed at submicromolar Mn^{2+} concentrations. The structures of the wild type (WT) and a site-directed mutant of the MntC solute-binding protein have been determined at 2.7 and 3.5 Å resolution, respectively. The WT structure is significantly improved over the previously determined structure (PDB entry 1xvl), showing improved Mn^{2+} binding site parameters, disulfide bonds in all three monomers and ions bound to the protein surface, revealing the role of Zn^{2+} ions in the crystallization liquor. The structure of MntC reveals that the active site is surrounded by neutral-to-positive electrostatic potential and is dominated by a network of polar interactions centred around Arg116. The mutation of this residue to alanine was shown to destabilize loops in the entrance to the metal-ion binding site and suggests a possible role in MntC function.

1. Introduction

Divalent metal ions such as Mn^{2+} , Zn^{2+} and Fe^{2+} are vital microelements in every living cell owing to their ability to provide the electronic functionalities required by many enzymes. One of the most important roles of Mn^{2+} in nature is as a major component of the oxygen-evolving complex in photosystem II, which catalyses the oxidation of water to oxygen, enabling the initiation of linear electron transfer and releasing protons for use in ATP synthesis (Umena *et al.*, 2011; Barber, 2008). In spite of the importance of Mn^{2+} homeostasis, many fundamental details regarding the import and regulation of cellular Mn^{2+} remain unclear (Salomon *et al.*, 2011).

Mn^{2+} uptake in the cyanobacterium *Synechocystis* PCC sp. 6803 (*Syn*) has been investigated over the past 15 years. Transport of Mn^{2+} at different concentrations of extracellular Mn^{2+} was found to be carried out by two systems with different affinities (Keren *et al.*, 2002; Bartsevich & Pakrasi, 1995; Bhattacharyya-Pakrasi *et al.*, 2002), the expression of which is tightly regulated (Ogawa *et al.*, 2002; Chandler *et al.*, 2003). The uptake of Mn^{2+} under deficient conditions ($<1 \mu M$) is carried out by a high-affinity ATP-binding cassette (ABC) transporter (Jones *et al.*, 2009) with high affinity for Mn^{2+} (MnCAB; Bartsevich & Pakrasi, 1995). Deletion of any one of the *mntCAB* genes encoding the three MnCAB transporter subunits leads to diminished oxygen-evolution rates.

The *Syn* MnCAB importer is proposed to be composed of two membrane-spanning permease domains (MntB) associated with two cytoplasmic NBDs (MntA), which supply energy to transport the substrate across the membrane by ATP hydrolysis. The Mn^{2+} substrate is first identified and bound by the solute-binding protein (SBP) MntC, which then associates with the permease, releasing the Mn ion (Bhattacharyya-Pakrasi *et al.*, 2002). MntC is a member of the cluster IX subfamily of ABC-type metal permeases (Tam & Saier, 1993; Lawrence *et al.*, 1998). Proteins of this group are components of transporters involved in the uptake of cations such as Mn^{2+} , Zn^{2+} and Fe^{2+} (Dintilhac *et al.*, 1997). The structure of the MntC protein was first determined to a resolution of 2.9 Å by X-ray crystallography (Rukhman *et al.*, 2005) using heterologously expressed protein. The MntC metal ion (MI) binding site was shown to be bind Mn^{2+} tetravalently with coordination by two histidine $N^{\delta 2}$ atoms (His89 and His154), one aspartic acid $O^{\delta 2}$ atom (Asp295) and one glutamic acid

Table 1

Data-collection and refinement statistics.

Values in parentheses are for the last shell.

Structure (PDB code)	MnWT (3ujp)	MnR116A (4irm)
X-ray data collection		
Space group	<i>P</i> 3 ₁ 21	<i>P</i> 3 ₁ 21
Unit-cell parameters		
<i>a</i> (Å)	127.52	128.40
<i>b</i> (Å)	127.52	128.40
<i>c</i> (Å)	89.73	90.51
α (°)	90.00	90.00
β (°)	90.00	90.00
γ (°)	120.00	120.00
Resolution range (Å)	36.8–2.7 (2.85–2.70)	24.5–3.5 (3.69–3.50)
Observed reflections	70773	58713
Unique reflections	21730 (1569)	11028 (1585)
$\langle I/\sigma(I) \rangle$	7.0 (0.7)	15.0 (6.2)
$R_{\text{merge}}^{\dagger}$	0.095 (1.1)	0.078 (0.324)
Completeness (%)	92.5 (57.0)	99.0 (99.8)
Multiplicity	3.3 (3.2)	5.3 (4.9)
Refinement		
$R/R_{\text{free}}^{\ddagger}$ (%)	23.1/28.4 (35.4/35.3)	29.38/30.48 (51.0/53.5)
Amino acids	921	758
Total No. of non-H atoms	6419	5881
No. of water molecules	12	0
No. of Mn ions	3	3
No. of zinc ions	6	0
No. of cacodylate ions	4	0
Average <i>B</i> factor (Å ²), protein atoms	88.5	97.5
R.m.s.d.		
Bond lengths (Å)	0.026	0.05
Bond angles (°)	1.733	2.95
Ramachandran plot		
Favoured regions (%)	86.6	77.5
Allowed regions (%)	9.7	18.7
Outliers (%)	3.7	3.8

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $\langle I(hkl) \rangle$ is the mean intensity of the symmetry-related reflections $I_i(hkl)$. $\ddagger R/R_{\text{free}} = \sum_{hkl} ||F_{\text{obs}}| - |F_{\text{calc}}|| / \sum_{hkl} |F_{\text{obs}}|$, where R and R_{free} are calculated using the working and test reflections, respectively. The test reflections (10%) were held aside and were not used during the entire refinement process.

O⁶¹ atom (Glu220). The structures of four further metal-transporter SBPs from the cluster IX subfamily have also been determined: PsaA from *Streptococcus pneumoniae* (Lawrence *et al.*, 1998; McDevitt *et al.*, 2011), TroA from *Treponema pallidum* (Lee *et al.*, 1999), ZnuA from *Syn* (Banerjee *et al.*, 2003) and *Escherichia coli* (Li & Jøgl, 2007; Yatsunyk *et al.*, 2008) and the iron-transport protein MtsA from *S. pyogenes* (Sun *et al.*, 2009). MntC, PsaA, TroA and ZnuA have significant differences in terms of amino-acid sequence (Rukhman *et al.*, 2005); however, their overall structures and metal-binding sites are quite similar. The most important difference between MntC and the other SBPs is the presence of a disulfide bond between cysteine residues (Cys219 and Cys268) near the metal-binding site (Rukhman *et al.*, 2005). It has been suggested that the special requirement for manganese in photosynthetic organisms is achieved by structural or molecular characteristics provided by this disulfide bond (Rukhman *et al.*, 2005). Disulfide-bond reduction *in vitro* lowers the affinity of the MntC protein towards Mn²⁺, suggesting that the redox potential of the cell may affect Mn²⁺ import at low Mn²⁺ concentrations (Rukhman *et al.*, 2005). The heterologously expressed MntC protein was found to aggregate into inclusion bodies and thus the protein required unfolding in 8 M urea and subsequent refolding prior to its crystallization (Adir *et al.*, 2002). Crystallization absolutely required the presence of 10–100 mM Zn²⁺; however, the positions of the Zn ions could not be resolved in the structure and there was uncertainty as to whether these ions might compete with the bound Mn ions (McDevitt *et al.*, 2011) or whether they only had a role in crystal lattice formation. The structure deposited as PDB entry 1xvl (Rukhman *et al.*, 2005) had two oxidized disulfide bonds and one

reduced disulfide bond, and the monomer with the reduced disulfide bond had significant disorder. In this work, we present a significantly improved structure of the wild-type MntC protein and present a structure of a site-specific R116A mutant that reveals details of how Arg116 is involved in stabilizing the structure of the MntC protein.

2. Materials and methods

2.1. Isolation, purification and crystallization

The cloning and expression of the *Synechocystis* sp. PCC 6803 *mntC* gene has been described previously (Adir *et al.*, 2002; Rukhman *et al.*, 2005). This clone resulted in insoluble proteins and thus the gene was subcloned into a modified pET-28b vector (a kind gift from Gadi Schuster, Technion) which includes a TEV protease cleavage site enabling His-tag removal by two rounds of chelation chromatography (Ni-NTA Agarose, Qiagen) from *E. coli* BL-21 cells. Expression was initiated by the addition of 1 mM IPTG at 310 K for 4 h. The cells were treated twice with a pressure cell (French Press, Spectronic Instruments Inc., Rochester, New York, USA) and cell debris was removed by centrifugation (15 000 rev min^{−1} for 20 min at 288 K). The supernatant was applied onto a Ni²⁺-bound affinity column and eluted with an appropriate buffer (50 mM Tris-HCl pH 8, 300 mM NaCl, 250 mM imidazole). The elution fractions containing the MntC protein were pooled together and dialyzed against 50 mM Tris-HCl pH 8). The His tag was removed by incubating the protein with TEV protease overnight and the protein was then loaded onto a Ni²⁺ column. The MntC protein without His tag was found in the flowthrough fraction. Further purification was performed by anion-exchange HPLC (PL-SAX 1000 Å, Polymer Laboratories). MntC was eluted with a linear gradient of 0–500 mM NaCl in 50 mM Tris-HCl pH 8.0. MntC-containing fractions were collected and analysed by SDS-PAGE. The main peak fractions were combined, dialyzed against 50 mM Tris pH 8 and concentrated to 10 mg ml^{−1} by ultrafiltration on Centricon-10 concentrators (Amicon).

Preparation of the R116A mutant DNA plasmid was performed according to the Stratagene QuikChange Site-Directed Mutagenesis protocol. The gene was amplified using the PCR reaction with forward and reverse primers containing the desired mutation (5'-AACGGCATGAACCTAGAGGCTTGGTTCGAGCAATTTTTC-3' and 5'-CAAAAATTGCTCGAACCAAGCCTCTAGGTTCATGCCGTT-3', respectively; the sequence that was changed by site-directed mutagenesis is underlined). Sequence determination was performed to verify the presence of the mutations (Macrogen Korea; <http://www.macrogen.com>). Recombinant mutant protein was expressed and purified as described above.

Prior to crystallization, protein samples were incubated overnight with 1 mM MnCl₂. Crystallization was performed by hanging-drop vapour diffusion in 24-well plates (Hampton Research) at 293 K. Extensive screening for alternative crystallization conditions was unsuccessful and thus the crystallization conditions that were used for the structure deposited as PDB entry 1xvl (Rukhman *et al.*, 2005) including 50 mM zinc acetate were used.

2.2. Data collection and structure determination

X-ray diffraction data were collected on beamline ID23-1 at the European Synchrotron Radiation Facility (ESRF), Grenoble, France. The data were indexed and integrated using *MOSFLM* (Leslie, 1992) or *XDS* (Kabsch, 2010) and scaled and merged using *SCALA* (Winn *et al.*, 2011). The structures were solved by molecular replacement using *Phaser* (McCoy *et al.*, 2007) with the coordinates of PDB entry 1xvl as a model. Refinement was performed using *PHENIX* (Adams

et al., 2011) and *REFMAC* (Murshudov *et al.*, 2011). Manual model building, real-space refinement and validation were performed using *Coot* (Emsley & Cowtan, 2004) and *CCP4* (Winn *et al.*, 2011). Data-collection, phasing and refinement statistics are presented in Table 1.

2.3. Determination of metal-ion concentration

The amount of bound Mn^{2+} was measured by ICP-AES (ICP Spectrometer, iCap 6000 Series, Thermo Scientific). MnCl_2 was added to each protein sample and, after an incubation period of 24 h, each sample (up to 10 ml) was dialyzed three times against 2 l 50 mM Tris-HCl pH 8 in order to remove nonspecifically bound MIs.

2.4. Accession codes

The coordinates and structure factors for WT and R116A MntC have been deposited in the RSCB Protein Data Bank under accession codes 3ujp and 3v63, respectively.

3. Results and discussion

3.1. Determination of an improved crystal structure of the MntC protein

Both crystal and X-ray diffraction quality can in many cases be traced back to protein purity and homogeneity. For the previously determined MntC structure (PDB entry 1xvl; Rukhman *et al.*, 2005), the recombinant protein was found to be insoluble, requiring the

resulting inclusion bodies to be unfolded in 8 M urea followed by refolding by removal of urea (Adir *et al.*, 2002; Rukhman *et al.*, 2005). In order to obtain an improved crystal structure of the MntC protein, the *mntC* gene was first recloned into a pQE-60 vector, with which we transformed BL-21 cells. The resulting expressed protein had improved solubility and the addition of a C-terminal 6×His tag enabled improved purification. The expressed protein was truncated by 49 residues at the N-terminus, as it has previously been proposed that this stretch of residues was not part of the manganese-binding functionality of the structure (Rukhman *et al.*, 2005); rather, it is involved in anchoring the protein to the periplasmic membrane. While the change in the expression host and removal of non-essential residues at the N-terminus of MntC enhanced the solubility of this protein, it appeared that addition of the affinity tag actually disturbed the crystal quality, as the diffraction from dozens of crystals proved to be very disordered. Since alternative crystallization conditions could not be identified, we screened alternative expression methods. We finally obtained improved crystals by transferring the *mntC* gene into a modified pET-28b vector that provided soluble protein that could be affinity-purified followed by proteolytic removal of the affinity tag (see §2 for additional details).

3.2. The overall structure of the wild-type MntC protein

We collected a full data set from a WT MntC (MnWT) crystal to 2.7 Å resolution (Table 1). The overall structure of MnWT (PDB entry 3ujp) is similar to the previous determined structure of MntC (PDB entry 1xvl). However, in contrast to the 1xvl structure (Rukhman *et al.*, 2005), all three monomers in the 3ujp structure contained an oxidized form of the disulfide bond. Since the protein was cytoplasmically expressed in both the 1xvl and 3ujp structures, we assume that the disulfide bonds are all reduced upon cell disruption. Oxidation of the disulfides occurs owing to the presence of oxygen in the solvents used during protein isolation and purification. We believe that since the improved protocol provided us with correctly folded MntC in solution, Cys219 and Cys268 are properly aligned and

Figure 1

Crystallographic characteristics of the MnWT structure. The structure of the trimer in the asymmetric unit is represented in cartoon representation and coloured by *B* factor (lowest *B* factors in blue and highest *B* factors in orange-red). The C-terminal domain of each monomer is indicated by a bracket. Cacodylate ions are represented in stick representation and Zn^{2+} ions are represented by grey spheres. Two groups of residues (1 and 2) involved in Zn^{2+} coordination are shown as magenta sticks; group 1 indicates the interactions between Zn^{2+} and three monomers in the asymmetric unit and group 2 those between residues from neighbouring asymmetric units. All molecular figures presented in this work were generated using *PyMOL* (DeLano, 2002).

Figure 2

The structure of MnWT shows that the Arg116 residue forms a polar bonding network at the entrance to the Mn^{2+} -binding site. The structure of the monomer shows the N-terminal, C-terminal and backbone domains (cyan, magenta and yellow cartoons, respectively). The Mn^{2+} ion is represented by a black sphere. Residues involved in the polar interaction cage are shown as sticks (see the enlargement on the left for details, which is rotated 180° with respect to the model on the right). Arg116 is located at the entrance to the Mn^{2+} -binding site of the protein, locking the N-terminal and C-terminal domains by forming polar contacts (black dashed lines) with the side-chain functional groups of Tyr147 and Glu243 and the backbone carbonyl of Asn241. An additional contact with the backbone of His89 is not shown for clarity. The closest distances (Å) between the above residues (monomer A) are shown.

thus oxidation to the disulfide occurred more efficiently. In the 1xvl structure a subpopulation of refolded MntC could not properly form the disulfide bond. In the trimeric asymmetric unit, these monomers were always found at the position with the fewest number of crystal contacts. This was likely to be necessary for crystallization to occur, since monomers lacking disulfide bonds are more structurally heterogeneous and could be more easily accommodated at this position.

The MnWT protein only crystallized well in the presence of 50 mM ZnCl_2 with a trimer in the asymmetric unit. In the 1xvl structure the zinc ions in the mother liquor could not be identified in the electron-density maps and thus there was a possibility that the role of Zn during crystallization was to ensure full occupancy of the metal ion binding site, although the protein was previously incubated with Mn^{2+} and the presence of this ion had been verified crystallographically (Rukhman *et al.*, 2005). In the MnWT structure presented here six zinc ions and four cacodylate ions could be clearly identified in the electron-density maps as being bound in the interfaces between the subunits of the protein, ligated by Glu, Gln and Asn residues (Fig. 1). The concentration of observable zinc ions is thus about 45 mM, accounting for almost all of the available ions in the crystallization solution. We are thus able to propose that the major role of zinc ions in the crystallization liquor is to serve as bridging charges within the crystal lattice.

The monomer of the MntC protein (Fig. 2) can be subdivided into three domains: the N-terminal globular domain (residues 49–137; cyan), the α -helical backbone (residues 158–210 and 306–324; yellow) and the C-terminal globular domain (residues 138–157 and 211–305; magenta). In the MnWT structure the *B* factors of the C-terminal domain are consistently higher than those of the other two domains and vary between the different monomers in the asymmetric unit (Fig. 1). Closer examination of the crystal lattice revealed that the C-terminal domain of monomer *A* is stabilized by polar interactions with the neighbouring asymmetric unit and thus has the lowest *B*

factors. The C-terminal domain of monomer *B* is somewhat less stabilized by the surrounding molecules, while in monomer *C* this domain does not have any stabilization contacts and has the highest *B* factors in the structure. As will be described below, the flexibility of the C-terminal domain appears to be critical for MntC function, and the variation in the position of this domain affords us a more diverse view. It should be noted that no such flexibility of the C-terminal domains has been reported in other SBPs for which structures have been determined.

3.3. An extended view of the Mn^{2+} -binding site

The MntCAB system is only expressed at very low levels of extracellular Mn^{2+} (Bartsevich & Pakrasi, 1995) and also binds Mn^{2+} tightly *in vitro* (Rukhman *et al.*, 2005). On the other hand, the actual MI-binding site is rather similar to those of SBPs that bind other MIs such as Zn^{2+} (Lee *et al.*, 1999; Lawrence *et al.*, 1998; Banerjee *et al.*, 2003; McDevitt *et al.*, 2011). It would thus seem likely that MntC must have structural elements that either selectively bind Mn^{2+} or selectively prevent the dissociation of Mn^{2+} (as opposed to other ions) once it has bound to the site. Both characteristics are most likely to be found in the second and third shells of residues surrounding the binding site: residues within a radius of 6–10 Å of the MI. In comparison with other SBPs of known structure, we can state that what is unique about the third shell of the MntC protein (near the surface of the protein) is that these residues impart a relatively neutral to positive electrostatic surface potential around the binding site (Fig. 3). We analyzed the outer shell residues around the binding site of the MntC protein and revealed one critical residue, Arg116, which appeared to be central to determining the outer shell characteristics. This residue is located close to the putative entrance to the MI-binding site and strongly contributes to the positive electrostatic potential of the shell. In the case of MntC, Arg116 is a central residue in a network of potential hydrogen bonds between the N-terminal

Figure 3 Calculated electrostatic potentials of SBPs. The surface electrostatic potential of three SBPs, (a) PsA (PDB entry 1psz; Lawrence *et al.*, 1998), (b) TroA (PDB entry 1toa; Lee *et al.*, 1999) and (c) MntC (PDB entry 3ujp; this work), were calculated using APBS (Baker *et al.*, 2001) with the AMBER force field option and were visualized in PyMOL (DeLano, 2002). The calculated scale between the strongest blue (positive) and red (negative) electrostatic potentials is 15kT. Black arrows indicate the entrance to the MI binding site. The name and PDB code (in parentheses) of each protein are indicated.

Figure 4

Quality of the electron-density map of the MnR116A structure. A $2F_o - F_c$ electron-density map contoured at 1.5σ (blue) is overlaid onto the MnR116A (subunit B) structure, which is represented by red ribbons. Two missing loops between residues Ala143 and Asp149 and between Gln244 and Asp254 are represented by black dashed lines. The electron-density maps also shows that despite the low resolution the rest of the protein backbone and many of the side-chain configurations were easily traced.

and C-terminal domains of the protein. The network includes the side chains of Glu243, Tyr147, the backbone carbonyl of Asn241 (Fig. 2) and His89 (not shown), all of which are situated prominently in the loops that form the putative entrance to the MI-binding site. The Asn241 side chain juts into the binding site and may affect the Mn^{2+} -binding residues directly, while His89 is one of the four residues that directly bind the Mn^{2+} ion. The Arg116 hydrogen-bonding network thus has the effect of creating a stable cage around the bound Mn^{2+} ion and may help in positioning both the binding-site and first-shell residues correctly in order to enable differentiation between Mn^{2+} and Zn^{2+} (or other MIs). The conformation of the side chain of Glu243 in the three monomers found in the asymmetric unit is variable and can point either towards Arg116 or away towards Glu244; however, when stabilized by Arg116 the residue and indeed the entire loop is stabilized and has lower *B* factors. Since the Glu243 and Arg116 loops both face outwards from the MI-binding site entrance in a fashion that has been suggested to form the interface between the SBP and permeases in ABC transporters (Hvorup *et al.*, 2007), we suggest that the movement of Glu243 between the stabilized bound form (to Arg116) and the looser unbound form is most likely to play a role in the formation of the MntC–MntB complex. In order to further investigate the importance of Arg116, we mutated this residue.

3.4. The crystal structure of the MntC R116A mutant protein

The MntC R116A mutant protein (MnR116A) was expressed, purified and crystallized in the same fashion as the MnWT protein. We expected that growing diffraction-quality crystals of the mutated protein would be difficult, since our analysis revealed that Arg116 serves as the central residue of the hydrogen-bond network in the active-site entrance. In most screens, the mutated protein indeed failed to crystallize. A single diffracting crystal was obtained under conditions similar to those used for the MnWT protein. This crystal enabled determination of the structure of the MnR116A protein to

3.5 Å resolution. Compared with the MnWT structure, the replacement of the Arg116 side chain by the Ala methyl group results in movement of the above residues as well as of entire loops (Supplementary Fig. S1¹). However, the domains remain in similar positions, *i.e.* the protein does not ‘open up’ owing to loss of the hydrogen-bonding network. The crystal-packing forces in the MnR116A structure are similar to those in the MnWT structure and the formation of correct interactions between adjacent asymmetric units is likely to be critical for crystal order and diffraction quality. The lower resolution could be explained by loop movements in both the N-terminal and C-terminal domains which result in stretches of disorder in the electron-density maps (Fig. 4). This observation adds an additional characteristic that differentiates MntC from the PsaA and TroA homologues (which bind either Zn^{2+} or Mn^{2+}), since no homologous network between the N-terminal and C-terminal domains appears to be required by these two latter proteins to stabilize their structure. The calculated electron density obtained from the MnR116A mutant diffraction data is remarkably good for many other segments of the protein, including the Mn^{2+} -binding sites and the disulfide bridge of monomer A (the other two potential disulfides are reduced).

3.5. The suggested role of Arg116 in the MntC protein

The program *CASTp* (Dundas *et al.*, 2006), which can identify cavities within proteins, indicated that mutation of Arg116 increased the size of the cavity between the MI-binding site and the surface by nearly twofold. However, since the resolutions of the two structures presented here are significantly different and the R116A structure lacks surface loops, direct quantitative comparison between the cavity sizes may be inaccurate. In order to visualize the opening of the active site owing to the R116A mutation, we deleted the missing loops from the MnWT structure and then visualized all atoms with a 9 Å radius from the Mn^{2+} ion (Supplementary Fig. S2, red). Exposure of the active site is mainly owing to a shift in the exterior loop containing residues 242–246 (Supplementary Fig. S2, yellow), which moves significantly owing to the loss of the interaction between Arg116 and Asn241. It might be expected that this major change in the binding site might lead to the release of Mn^{2+} ; however, ICP-AES analysis of the MnWT and MnR116A structures showed only a small decrease in the binding affinity towards Mn^{2+} (Supplementary Table S1), which was less than that found for the reduction of the disulfide bond (Rukhman *et al.*, 2005). We suggest that the hydrogen-bonding network between the C-terminal and N-terminal domains in the MntC protein is formed during Mn^{2+} binding, stabilizing the flexible C-terminal domain and bringing specific surface residues into the correct positions required for binding to the MntB permease. The interactions between the MntC and the MntB protein are most likely to open the active site by overcoming the hydrogen-bonding network between His89, Asn241, Glu243, Tyr147 and Arg116, and facilitating the release of Mn^{2+} from the active site of the protein.

The results presented here, coupled with our previous observations on the role of the disulfide bond in Mn^{2+} binding (Rukhman *et al.*, 2005), strongly indicate that the MntC protein is under tighter and more varied control (compared with the other SBP proteins described above) with respect to metal binding and release. This is most likely owing to the absolute requirement of Mn^{2+} import for photosystem II activity coupled with the need to prevent alternative

¹ Supplementary material has been deposited in the IUCr electronic archive (Reference: TT5034).

ion binding (especially Zn^{2+}) when Mn^{2+} concentrations are extremely low.

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