Photosynthesis Research **77:** 21–34, 2003.

Minireview

Amino acid sequences and solution structures of manganese stabilizing protein that affect reconstitution of Photosystem II activity

Hana Popelkova[1], Aaron Wyman[1] & Charles Yocum[1,2,*]
[1]*Department of Molecular, Cellular, and Developmental Biology,* [2]*Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1048, USA;* [*]*Author for correspondence (e-mail: cyocum@umich.edu; fax: +1-734-647-0884)*

Received 24 January 2003; accepted in revised form 28 March 2003

Key words: CD spectra, gel filtration, heating, manganese stabilizing protein, natively unfolded protein, Photosystem II, site-directed mutagenesis

Abstract

This minireview presents a summary of information available on the secondary and tertiary structure of manganese stabilizing protein (MSP) in solution, and on the identity of amino acid residues that affect binding and functional assembly of this protein into Photosystem II. New data on the secondary structure of C-terminal mutants and 90 °C-heated manganese stabilizing protein, along with earlier data on the secondary structure of N-terminal mutants and the tertiary structure of all modified MSP species, allow for an evaluation of models for spinach MSP secondary and tertiary structure. This summary of previous and new information better documents the natively unfolded behavior of the protein in solution. A two-step mechanism for binding of manganese stabilizing protein to Photosystem II is discussed and possible solution three-dimensional conformations of the wild-type protein and some of its unfolded mutants, are proposed.

Abbreviations: CD – circular dichroism; EDC – 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide; FT-IR – Fourier transform infrared; MSP – manganese stabilizing protein; PS – photosystem; psbO – gene encoding precursor MSP; *Syn – Synechococcus elongatus*; wt – wild type

Introduction

Photosystem II is unusual among membrane-associated redox enzymes in that it consists of modular components that are fairly easily dissociated from one another. The core module consists of a set of intrinsic membrane proteins that are encoded by chloroplast DNA in eukaryotes. The major proteins of this module are the reaction center polypeptides D1 and D2, the larger accessory chlorophyll binding proteins CP47 and CP43, and the α and β subunits of cytochrome b559 (Debus 2000). The current view of the function of these polypeptides is that they bind the components required for primary electron transfer (P680, Pheo a, Q_A and Q_B, and the redox active tyrosines Y_Z and Y_D, all associated with the D1 and D2 polypeptides), and for energy transfer to the reaction center (CP47, CP43). This group of polypeptides provides the binding sites for the other modules of the enzyme. The second module, made up of a collection of inorganic ions (4 Mn, 1 Ca^{2+}, 1 Cl^-), acts as the catalytic center for H_2O oxidation (Yocum 1991; Debus 1992); current models for PS II structure propose that amino acid residues of the D1 polypeptide ligate the Mn atoms (Svensson et al. 1996). This view is supported by site-directed mutants in D1 of *Synechocystis* (Debus 2001), and by the 3.7–3.8 Å crystal structures of cyanobacterial PS II (Zouni et al. 2001; Kamiya and Shen 2003).

The last module is a set of extrinsic proteins of 33, 23, and 17 kDa molecular masses in eukaryotes (Seidler 1996b). The 33 kDa protein, known as manganese stabilizing protein or MSP, is also found in

cyanobacteria, but the 23 and 17 kDa proteins are absent, and instead, a cytochrome (c550) and a 12 kDa polypeptide with slightly different functions are present in these organisms (Shen et al. 1998). The function of these extrinsic subunits appears to be to form a structure, in concert with the intrinsic subunits, which provides a stable environment for Mn ligation and retention of Ca^{2+} and Cl^- (Ghanotakis and Yocum 1990). All of these extrinsic subunits are encoded by nuclear DNA in eukaryotes and imported into chloroplasts as precursors that are processed in the course of PS II assembly (Keegstra et al. 1989; Hashimoto et al. 1997).

Kuwabara and Murata (1979) first isolated manganese-stabilizing protein from chloroplast extracts. Work by Murata's group, using isolated PS II preparations, established the locale of the protein in the chloroplast. In the course of systematic studies on the conditions for, and consequences of, extraction of extrinsic proteins from PS II, this group also showed that loss of MSP from PS II led to disruption of the Mn cluster (slow dissociation of 2 Mn per reaction center), and that addition of high concentrations of Cl^- (200 mM) would prevent loss of the bound metals (Kuwabara and Murata 1982, 1983; Miyao and Murata 1984, 1989). The former phenomenon is the basis of the name given to MSP. Applications of genetic approaches to PS II research produced mutations that deleted MSP from *Chlamydomonas* and *Synechocystis* 6803. In the former case, the mutation seriously impaired PS II assembly, and the mutants were incapable of photoautotrophic growth (Mayfield et al. 1987). The *Synechocystis* ΔpsbO mutant, on the other hand, was capable of slow growth, although O_2 evolution activity in this mutant was unstable and very sensitive to high light intensities (Burnap and Sherman 1991).

As a result of numerous gene-sequencing initiatives, 20 *psbO* sequences are now available. The sequence of spinach MSP is presented in Figure 1, along with a consensus sequence deduced by de las Rivas et al. (1999). A comparison of the spinach sequence with the consensus sequence illustrates the high degree of conservation of sequence similarity in this group of proteins. Additional alignments are available in the papers by Seidler (1996b), Tucker et al. (2001), and Motoki et al. (2002), where the reader can compare individual sequences with one another. Perhaps the most pronounced sequence differences occur at the N-terminus of MSP. In contrast to the sequence from higher plants, where two highly conserved motifs, T(Y/F)DE and TY, are present after the first 6–7 amino acids, the sequence in green algae starts at the first conserved motif, T(Y/F)DE, and lacks the first 6–7 residues found in the higher plant sequences. In cyanobacteria, a more substantial deviation is observed, in which the N-terminal sequences possess only a conserved TY motif (Popelkova et al. 2002b). Because of the major differences in the MSP N-terminal sequences, it is necessary to introduce a gap in the cyanobacterial sequence to permit optimal alignments with the eukaryotic protein sequences. A gap must also be introduced in the eukaryotic sequence to accommodate additional amino acids present in the cyanobacterial sequence (Seidler 1996b; Tucker et al. 2001; Motoki et al. 2002). This raises an interesting point, namely that in spite of their relatively large differences in sequence at the N-terminus, cyanobacterial and higher plant MSP's contain about the same number of amino acids overall. In addition, if one ignores the sequence differences at the N-termini, then there is a high degree of sequence similarity among all MSP species, including the cysteines present at residues 28 and 51 (spinach numbering system), which form a disulfide bridge (Tanaka and Wada 1988; Tanaka et al. 1989). Work in a number of laboratories over the past decade has provided a comprehensive view of the properties of MSP in solution, and has revealed the identities of amino acid residues that are essential for binding and functional assembly of the protein into PS II. In the following sections we review the available information on MSP structure and on some critical amino acid residues that affect MSP solution structure and PS II binding. We then evaluate models for the protein's secondary and tertiary structure.

Solution structure of MSP

Far-UV CD spectroscopy is a useful probe of protein secondary structure, although it must be recognized that the method is empirical (spectra of proteins of unknown secondary structure content are compared against basis sets containing the spectra of proteins of known structure in order to derive estimates of secondary structure content of the unknown proteins). Several groups have used this method to analyze the structure of MSP in solution. The original investigation by Xu et al. (1994) found that spinach MSP was predicted to contain $< 10\%$ α-helix and about 38% β-sheet. Shutova et al. (1997) arrived at essentially the same conclusion, as have other groups (Sonoyama et al. 1996; Lydakis-Simantiris et al. 1999a, b;

Figure 1. Amino acid sequence of spinach MSP (upper row) and the consensus sequence reported by de las Rivas et al. (1999) (lower row). *Red* amino acid residues, residues identified by Dunker et al. (2001) as promoting structural disorder; *green* residues, order promoting, and *black* residues, neutral. β, α, regions predicted to contain β-sheet or α-helix. Predicted α-helix, β-sheet structure based on the spinach MSP secondary structure reported in Bricker and Frankel (1998); Predicted α-helix, β-sheet content of the consensus MSP sequence (de las Rivas et. al. 1999). See text for a further discussion.

Popelkova et al. 2002a, b). Fourier transform IR spectroscopy (FT-IR) has also been used to probe MSP structure, both in solution and when bound to PS II. Results from this technique yield a wider range of values for α-helix and β-sheet. An isotope editing FT-IR study yielded data that would suggest that upon binding to PS II, random secondary structure in the spinach protein was replaced by additional β-sheet (Hutchison et al. 1998). Results of secondary structure analyses by far-UV CD (Xu et al. 1994; Shutova et al. 1997; Lydakis-Simantiris et al. 1999a), and by FT-IR spectroscopy (Ahmed et al. 1995; Zhang et al. 1996; Hutchison et al. 1998; Lydakis-Simantiris et al. 1999b), are given in Table 1. These data show a range of values, with the consensus being that β-sheet is the dominant form of secondary structure in spinach MSP, and that a substantial amount of the protein's secondary structure consists of turns, coils, and other unordered structures. Substantial β-sheet structure and a large amount of turns plus unordered structures are also predicted for MSP from *Synochococcus elongatus* (Sonoyama et al. 1996; Motoki et al. 2002). The amount of α-helix predicted in *Synochococcus* (Table 1) deviates markedly from the α-helix content predicted by the CD technique in the eukaryotic protein. However, using FT-IR, a more variable α-helix content was found in the eukaryotic protein than was found in *Synochococcus* MSP. In solution, different types of MSP preparations can also lead to variations in secondary structure content. It has been found that calcium- or urea-extracted MSP, and the recombinant protein as well, differ in the estimated amounts of α-helix and β-sheet. These differences are likely to arise from different conformational states of the protein that are generated by differences in hydrogen bonding within the peptide backbone (Hutchison et al. 1998; Lydakis-Simantiris et al. 1999b).

Bricker and Frankel (1998) and de las Rivas et al. (1999) have used computational methods for prediction of protein secondary structure to analyze MSP.

Table 1. Secondary structure estimates for MSP in solution: results from far-UV CD and from FT-IR are given as ranges of values obtained by several groups

	CD		FTIR	
Structure	Spinach	*S. elongatus*	Spinach	*S. elongatus*
α-helix	4–9%	7–17%	2–27%	19%
β-sheet	33–38%	23–39%	36–50%	24%
Turn + other	52–59%	55–60%	37–43%	57%

The results are in very good agreement with those obtained by far-UV CD spectroscopy. Figure 1 shows the locations, in the primary amino acid sequence of MSP, of the predicted secondary structure elements reported by these investigators. The agreement between modeling results and CD data may be fortuitous, however. Both methods utilize data banks of proteins of known structure as the basis sets from which structural predictions are derived, and it is possible that some of the same proteins were used for both methods of secondary structure prediction. At a minimum, however, these investigations are uniform in predicting that the predominant secondary structure in MSP is β-sheet, and that the protein appears to contain large amounts of unordered structure.

The molecular mass of spinach MSP (26 530 Da), computed from the DNA-derived amino acid sequence, is confirmed by MALDI-TOF mass spectrometry, which indicates that there are no posttranslational modifications to the protein (Zubrzycki et al. 1998; Svensson et al. 2002). The name '33 kDa extrinsic protein' was derived from MSP's mobility on SDS–PAGE; given the actual molecular mass, the electrophoretic value is anomalous. Likewise, estimates of molecular mass obtained using gel filtration (37–40 kDa) (Lydakis-Simantiris et al. 1999a, b; Popelkova et al. 2002a, b) reveal a large disagreement with the known molecular mass. One explanation for MSP's behavior on size exclusion columns would be that the protein possesses an oblong (prolate ellipsoid) shape, rather than a globular solution conformation. This possibility is born out by experiments using dynamic light scattering, ultracentrifugation, and low angle X-ray scattering, which yield axial ratio values of 4.2–4.8, consistent with a prolate ellipsoid shape (Zubrzycki et al. 1998; Svensson et al. 2002).

The solubility of MSP is impaired by reduction of the disulfide bridge; the reduced protein is reported to be susceptible to aggregation and is ineffective in reconstitution of PS II activity (Tanaka and Wada 1988; Tanaka et al. 1989). An analysis of the positions of internal crosslinks within spinach MSP (generated with EDC, a H_2O-soluble carbodiimide that forms covalent linkages between $-NH_2$ and $-COO^-$ groups that are within van der Waal's contact distance) showed that in solution, the C-terminus of MSP (Glu246) would crosslink to Lys48, while Glu10 would crosslink to Lys14 within the N-terminus. When MSP was bound to PS II, the same crosslinker generated a bond between residues Glu246 and Lys190 (Enami et al. 1998).

Lastly, Tohri et al. (2002) conducted an analysis of the peptide fragments generated by α-chymotrypsin and *Staphylococcus aureus* V8 protease to examine protein conformations in solution and the accessibility of different MSP species to protease digestion. This work was founded on the experiments of Eaton-Rye and Murata (1989), who had earlier shown for the spinach protein that limited digestion cleaved the first 16 and 18 amino acids, respectively, from the MSP N-terminus. The more recent work examined the cleavage patterns from *Cyanidium caldarium, Chlamydomonas reinhardtii, Euglena gracilis*, *Spinacea oleracea,* and *Oryza sativa*, as well as from the thermophilic prokaryote *Synechococcus elongatus* (Tohri et al. 2002). Cleavage sites of manganese stabilizing protein from various organisms were located in different regions of the primary sequence, in spite of the fact that amino acid residues Tyr, Glu, and Phe are all conserved in these regions. According to Tohri et al. (2001), this result indicates different accessibilities of corresponding regions to proteases, because proteolytic cleavage sites should, in theory, be exposed to the protein's surface. Interpretation of these results also assumes that there are no interferences from surrounding amino acid sequences that would compromise protease specificity or activity. In higher plants, the N-terminal region is exposed to the protein surface since MSP was cleaved only at Y^{16} of spinach by α-chymotrypsin, and at E^{18} and E^{19} of spinach and rice, respectively, by V8 protease. In euglena and green algae, two regions are exposed to the surface of the protein; the N-terminus, and a position around amino acid residues 156–195. Somewhat different results were obtained with cyanobacterial and red algal MSPs, which are accessible to chymotrypsin and V8 protease in solution only in the region around residues 156–195. Based on these results, Tohri et al. (2002) suggest that MSP has solution structures that differ among the various species probed in their experiments.

Near-UV CD spectroscopy provides information on the protein environment of aromatic amino acid residues (Trp, Tyr, Phe). The intensity of the rotational signal from these residues provides an estimate of the hydrophobicity of their protein environment (Schmid 1997). For spinach MSP, such spectra indicate that the lone Trp at position 241 is shielded from solvent, and that the same is true of Tyr residues, although in this case, the number of such residues in MSP (7) leaves open the possibility that not all tyrosines are in hydrophobic environments (Lydakis-Simantiris et al. 1999a). Similar information about MSP hydrophobic domains comes from fluorescence emission spectra of the protein (Shutova et al. 2001; Ruan et al. 2002).

Pazos et al. (2001) used the technique of 'threading' to produce a theoretical model for the tertiary structure of MSP. The protein is predicted to consist of an elongated structure comprised of two β-sheet-rich domains separated by a 'hinge' region. This model is in accord with the secondary structure predictions (high β-sheet content), but does not predict a close interaction between the N- and C-termini of the protein, such as that which was revealed by the crosslinking results discussed above. Selection of model proteins for threading routines is of primary importance in this technique. Ideally, one would prefer to use the crystal structure of a protein that is very closely related to the protein of interest; however, this option is not available in the case of MSP. Pazos et al. (2001) selected proteins such as a human T-lymphocyte adhesion glycoprotein, on the basis that this protein's β-sheet content was comparable to the β-sheet content predicted for MSP, to carry out their structural predictions. The authors caution that a large deviation is likely to exist between their model and the actual solution structure of MSP. It is possible that with a further expansion of the structural information in the data banks, and with more experimental data on MSP's tertiary structure, threading methods will be able to produce a more refined prediction of MSP's tertiary structure in solution.

In addition to its abnormal migration behavior on SDS-PAGE and its unusual elution behavior on gel filtration columns, spinach MSP is also unique in that it is thermostable [preheating MSP at 90 °C, prior to use in *in vitro* reconstitution assays, has little effect on its ability to reconstitute oxygen evolution activity (Lydakis-Simantiris et al. 1999b)], and it is quite acidic, with a pI of 5.2 (Kuwabara and Murata 1979). These properties, taken together, have been cited as evidence that in solution, MSP belongs to a family of proteins whose solution structures are said to be 'natively unfolded' or 'intrinsically disordered' (Lydakis-Simantiris et al. 1999b). It has also been proposed that in solution, MSP is a 'molten globule', i.e., in a structural conformation midway between fully folded and completely unfolded (Shutova et al. 2000). In this latter interpretation, the remarkable thermostability of MSP is ascribed to the presence of the disulfide bond. However, data on 90°C-preheated double mutant C28AC51A, lacking disulfide bridge, indicate that this protein can rebind to PS II and reconstitute activity (A. Wyman, and C.F. Yocum, unpublished data).

In either case, the solution behavior of MSP does not correlate with that of a protein exhibiting a highly ordered, compact structure. Extensive databases are now available that catalog the properties of water-soluble, natively disordered proteins (Dunker et al. 2001;
Uversky 2002). The hallmarks of such proteins are an extremely acidic or basic pI, substantial amounts of unordered secondary structure, thermostability, and almost always participation as a partner in protein-protein, protein-nucleic acid, or other protein-ligand interactions (Dunker et al. 2001; Uversky 2002). It is proposed that the rationale for a flexible, or unordered, solution structure is that this enables such a protein to undergo the structural rearrangements that may be necessary to accommodate its interaction with another biopolymer (Weinreb et al. 1996). A systematic analysis of the amino acid composition of 'intrinsically disordered' proteins has shown that amino acids may be classified as contributing to order or disorder within a protein's structure (Dunker et al. 2001). The results of these theoretical investigations are applied to the amino acid composition of MSP and to other water-soluble proteins in Table 2. As can be seen from the data, the relative amounts of order and disorder-promoting amino acid residues in MSP correlates well with the intrinsically disordered behavior of the protein in solution. The same is true of the non-Aβ precursor of amyloid plaque, NACP, which is included as an example of a thermostable, natively unfolded protein. Results for some well-known water soluble proteins with ordered structures are also shown. As can be seen from the data in the table, these 'normally folded' proteins are characterized by a lower content of disorder promoting amino acid residues.

Table 2. Amino acid composition of MSP and other water-soluble proteins, organized according to the capacity of amino acids to promote order or disorder in the protein's structure

Amino acid residues	% Composition of:				
	Spinach MSP	NACP[a]	BSA[b]	Carbonic anhydase[c]	Trypsin[d]
Order promoting					
W, C, F, I, Y, V, L, N	30.3	24.3	36.5	34.7	42.4
Disorder promoting					
A, R, G, Q, S, P, E, K	55.5	60.8	47.5	48.3	46.5
Neutral					
H, T, M, D	14.2	14.9	16.0	17.0	11.1

[a]NACP–non-Aβ component of Alzheimer's disease amyloid plaque, precursor. [b]Bovine serum albumin. [c]Human carbonic anhydrase. [d]Bovine pancreas trypsin.

Functional consequences of chemical, enzymatic, or genetic modifications of MSP

Chemical and enzymatic modification

The amino acid composition of MSP (Figure 1) presents unusually rich opportunities for application of chemical modifications to research the protein's function, given the numerous Glu, Asp, Lys, and Arg residues in MSP. In the earliest experiments with intact PS II, it was shown that the zero length crosslinker EDC would generate covalent links between MSP and the large extrinsic 'E' loop of CP47 (Enami et al. 1987; Bricker et al. 1988; Odom and Bricker 1992). Glu and Asp residues have been modified by reaction with EDC, glycine methyl ester, and with sulfo-(N-hydroxy)-succinimide (Seidler 1996a; Frankel et al. 1999); the resulting modified MSP species are unable to bind to PSII and reconstitute O_2 evolution activity, implicating carboxy amino acid side groups in the MSP-PS II interaction. Modification of Lys and Arg residues (Miura et al. 1997) also interferes with rebinding to PS II, indicating that both negatively and positively charged residues are clearly involved in MSP binding to PS II. Bricker and Frankel (1998) have provided a thorough discussion of these results and the reader is referred to their review. Modification of Trp241 of spinach MSP by N-bromosuccinimide (NBS) has been reported by Yu et al. (2001). NBS-modified MSP showed low to moderate levels of PS II rebinding, but was unable to reconstitute O_2 evolution activity. Exposure of MSP to chymotrypsin or to V8 protease (Eaton-Rye and Murata 1989) resulted in the proteolytic removal of 16 or 18 N-terminal amino acids. The truncated protein was incapable of rebinding to PS II, even though far-UV CD spectroscopy indicated that proteolysis had not affected MSP's secondary structure.

In vivo *mutagenesis*

The ability to transform *Synechocystis* 6803 has been exploited in some investigations of the consequences of MSP mutations. The most drastic of these is ΔpsbO, whose properties have already been described. The mutant C20S in *Synechocystis* removes the disulfide bridge in the protein, and this mutant exhibits growth and O_2 evolution activity phenotypes that are almost identical to that of the Δ*psbO* mutant (Burnap et al. 1994). This is likely due to proteolytic destruction of the protein since eukaryotic C $\rightarrow$ A mutations are able to reactivate MSP-depleted PS II (see below). On the other hand, replacement of the Asp residue at position 9 by lysine (mutant D9K) does not affect MSP accumulation and has a minimal impact on O_2 evolution activity. Mutation of the strictly conserved Asp159 (D159N mutant) residue produces a mutant whose characteristics are intermediate between the wild-type and Δ*psbO* strains (Burnap et al. 1994).

Mutagenesis of recombinant MSP

Seidler and Michel (1990) demonstrated the feasibility of overexpressing MSP in *E. coli*. The technique has subsequently been used to overexpress MSP from spinach, *Arabidopsis*, and *Synechococcus* (Seidler and Michel 1990; Betts et al. 1994; Motoki et al. 2002), and to generate a number of site-directed mutants. In what follows, we briefly summarize the results of mutagenesis experiments directed at the N-terminus, the intervening sequence, and the C-terminus of the protein.

N-terminal mutations

Effects of deleting partial sequences of the N-terminal 18 amino acids of spinach MSP has been reported (Popelkova et al. 2002a, b). These experiments were undertaken to determine which domains of the MSP N-terminus are required for PS II binding. Deletions of several amino acids simultaneously was used as the experimental strategy, owing to the report by Seidler et al. (1992) that mutation of a single residue, in this case a highly conserved amino acid (Asp9) to Asn, produced little effect on MSP function. In contrast, larger scale deletion mutations had an impact on MSP function. Removal of the first 10 amino acids eliminated binding of one of two copies of MSP to PS II, while deletion of a further eight amino acids reduced binding ability and O_2 evolution activity reconstitution by the second MSP to low levels (about 20% of the control). The sequences ^{4}KRLTYD^{10}E and ^{15}TYL^{18}E have been identified as the essential sequences for MSP binding, and work now completed shows that the conserved Thr residues in these sequence domains are required for MSP binding (Popelkova et al. submitted). Extensions to the MSP N-terminus are without major effect on the protein's activity. Seidler (1994) showed that extending the N-terminus by 6 His residues produced little, if any, effect on activity reconstitution. Popelkova et al. (2002a) showed that unprocessed recombinant MSP, which retains the 82 amino acid transit sequence used to import the protein into the chloroplast, can rebind to PS II and reconstitute activity. As no profound effects are found when using MSP species carrying N-terminal extensions in reconstitutions with MSP-depleted PS II samples, this would suggest that this particular protein domain is likely to be surface-exposed when bound to PS II. It is difficult to imagine how the additional mass of the transit peptide (about 8.2 kDa) could be accommodated in a buried hydrophobic domain of PS II. This assumption is also consistent with the proteolytic cleavage results obtained with spinach MSP, as reported by Tohri et al. (2002).

The disulfide bridge elimination

Betts et al. (1996a) reported that total, or partial, eliminations of the disulfide bridge (mutants C28AC51A, C28A, and C51A) produced recombinant proteins that differed from wild-type MSP in their solubilities. It was necessary to utilize urea during purification, and in some reconstitution studies, to prevent MSP aggregation. Nevertheless, these mutants retained the capability to rebind to PS II and reconstitute O_2 evolution activity. The solubility properties of the C → A mutations parallel the behavior of MSP in which the disulfide bond was reduced (Tanaka and Wada 1988; Tanaka et al. 1989). The reduced protein was insoluble and had a pronounced tendency to aggregate in solution. However, it failed to rebind to PS II, and, as a consequence, was ineffective in reconstituting activity (Tanaka and Wada 1988).

Intervening amino acid sequences between the disulfide bridge and the C-terminus

Seidler et al. (1992) produced a large number of mutations to silence negative charges in spinach MSP (D → N or E → Q), or to reverse the charge (D or E → K). These mutations, distributed throughout the MSP sequence from D9 to D234, were without major effect on the protein's ability to reconstitute PS II activity. Motoki et al. (2002) singled out a highly conserved region in the sequence of *S. elongatus* MSP, ^{148}VPSYRTANFLDPKGRGLASG ^{168}Y, and prepared several mutants. Of these, D158N, K160Q, K160R, R162Q, R162K, R152Q, R152K**,** and F156L dramatically reduced the ability of the protein to reconstitute O_2 evolution activity. The importance of this sequence domain of MSP was further emphasized by Motoki et al. (2002) through a series of insertion mutants in which a Gly, Ala, or Val was inserted into MSP between positions 156 and 157, or when L157 was deleted. In all cases, these insertions or deletions inactivated the ability of the mutagenized MSP to restore activity to MSP-depleted PS II. At the same time, where it was examined, these mutants showed secondary structure content comparable to that found in the wild-type control.

C-terminal mutations

Seidler (1994), in his characterization of His-tagged MSP mutants, prepared a C-terminal spinach mutation with a 6-His extension. The resulting protein would reconstitute activity, but exhibited sigmoidal rebinding to PS II. Betts et al. (1996b) discovered a mutation near the C-terminus of MSP (V235A) that conferred temperature sensitivity on MSP rebinding to PSII. The mutant showed normal O_2 evolution activity, but weakened binding behavior was evident in experiments in which wild-type MSP was able to displace V235A from PSII binding sites (Betts et al. 1997). Betts et al. (1998) and Lydakis-Simantiris et al. (1999a) characterized a series of mutations in which C-terminal residues were systematically elim-

inated from MSP by replacing amino acid codons with stop codons (@), or where Glu246 was changed to Lys and Leu245 to Glu. *Arabidopsis* MSP lacks Q247 and exhibits normal activity with MSP-depleted PSII from spinach. The mutation E246@ showed temperature sensitive binding behavior, but was otherwise normal. Truncation of MSP's C-terminus by three or four residues (L245@, Q244@) had profound affects on MSP function, however. In both cases, only limited binding and reconstitution of activity could be obtained with these recombinant mutants, showing that both Q244 and L245 were essential for MSP binding to PS II and recovery of O_2 evolution activity. The L245E mutant exhibited low reconstitution activity, but surprisingly, the mutant E246K at the adjacent position in the amino acid sequence exhibited an activity that was about 75% that of the control.

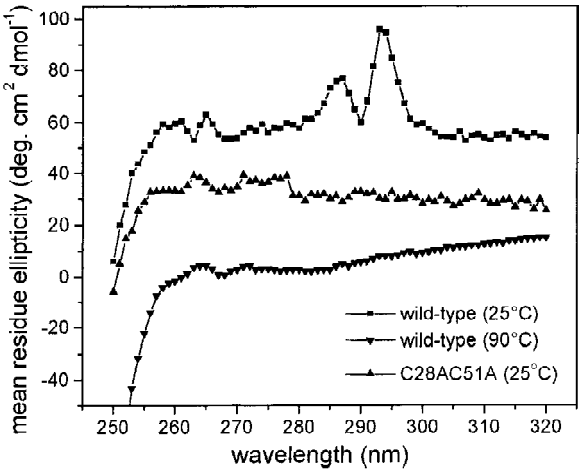

Figure 2. Near-UV CD spectra of wild-type and C28AC51A MSP in 10 mM KH_2PO_4, pH 6. The prominent features at 285 and 293 nm are due to Tyr and Trp, respectively, in hydrophobic environments. Heating at 90 °C, or elimination of the disulfide bridge, greatly reduces these peaks, which is interpreted to indicate that a structural transition has occurred with altered hydrophobicity of the environment around these aromatic amino acid residues.

Structural consequences of chemical or genetic modifications of amino acids in MSP

An advantage of MSP overexpression in *E. coli* is that one can produce sufficient amounts of protein for use in methods such as CD, FT-IR, fluorescence, or gel filtration, which provide information on the protein's secondary structure or solution conformation. As was mentioned above, near-UV CD can provide information on the protein environment surrounding aromatic amino acid residues (Phe, Trp, Tyr) by monitoring the intensity of the ellipticity from these residues at their characteristic absorption wavelengths (Schmid 1997). Likewise, fluorescence emission from these residues has been used to monitor the status of proteins during folding-unfolding. Table 3 collects data for those MSP species (heated or mutated) where information on their secondary or tertiary structures is available. There are several noteworthy points that can be derived from this table.

Firstly, although a number of mutations have a pronounced effect on the activity of MSP with respect to reconstitution of O_2 evolution activity, many of these mutations do not produce a large-scale effect on secondary structure content, as monitored by far-UV CD. The greatest changes occur in the β-sheet content of C-terminal mutants (L245@, Q244@, and L245E). The loss of β-sheet structure is less extensive in wild-type MSP heated at 90 °C. Second, MSP is able to retain some residual secondary structure even upon heating at 90 °C (see Table 3). This phenomenon is probably a reflection of the natively unfolded behavior of the protein.

Thirdly, where measurements have been made, near-UV CD appears to yield results that are consistent with the data from gel filtration experiments. When results from these techniques are combined, it can be seen that a number of N-terminal mutations generate an increase in hydrophobicity (↑) around Tyr and Trp residues and a somewhat diminished size of the resulting proteins, as monitored by gel filtration. However, these mutants' secondary structure remains unchanged. One N-terminal truncation, however, ΔE18M reverses this effect. This mutation shows a modest loss of hydrophobicity (↓), along with a small loss of secondary structure, even though the protein's size is not substantially altered. Significantly, this mutant exhibits dramatically weakened binding to PS II, and only one copy of the protein can rebind (Popelkova et al. 2002b). This is part of the stepwise loss of ability to reconstitute activity that correlates with the loss of binding of first one (ΔT7M), and then the second (ΔT15M), copy of MSP required for full activity of the enzyme system. The absence of large changes in secondary structure and structural compactness upon truncation of MSP's N-terminus, would suggest that this domain functions principally as a recognition sequence that guides MSP to the appropriate docking site, most likely on CP47. At the C-terminus, an opposite effect is observed. Here, truncation or

Table 3. The effect of mutation and heating on the structure and function of manganese stabilizing protein from spinach and *Synechococcus elongatus*

Protein	Secondary structure			Tertiary structure [a]	NSB[b]	size kDa	O_2 evolution activity[c]	Reference[d]
	α-helix	β-sheet	Turn + other					
Wild type	4	41	56	–	no	37	100%	A
PreMSP	5	37	57	↓	yes	49	114%	A
ΔG3M	4	37	58	↑	yes	34	130%	A
ΔR5M	4	37	57	↑	yes	34	100%	S
ΔL6M	4	37	58	↑	yes	35	95%	S
ΔT7M	5	38	58	No	no	34	60%	S
ΔE10M	4	37	59	↑	yes	33	70%	A
ΔS13M	3	38	57	↑	yes	33	70%	B
ΔK14M	3	41	56	↑	yes	33	70%	B
ΔT15M	3	37	59	↑	yes	33	20%	S
ΔE18M	4	34	61	↓	yes	33	20%	B
C28AC51A	4	34	63	↓	yes	51	60%	C
E246K	6	39	55	No	no	41	100%	D
E246@	7	33	59	↓	no	42	88%	D
L245@	8	24	67	↓	yes	52	10%	D
L245E	4	19	75	↓	yes	62	45%	D
Q244@	6	17	78	↓	no	58	0%	D
Rwt-90 °C [e]	6	29	63	↓	ND[g]	ND	ND	E
Nwt-90 °C	4	30	65	↓	ND	ND	ND	E
Syn-wt[f]	7	39	54	–	no	ND	100%	F
Syn-R152Q	8	38	54	↑	yes	ND	16%	F
Syn-D158N	9	38	54	ND	yes	ND	18%	F
Syn-K160Q	8	38	54	ND	yes	ND	11%	F
Syn-R162Q	7	39	55	↓	yes	ND	18%	F

[a]Arrows indicate an increase (↑) or decrease (↓) in the hydrophobic environment around aromatic amino acid residues Trp241 and Tyr242 compared to wild type.
[b]NSB, nonspecific binding of MSP to PS II, compared to wild-type binding.
[c]Oxygen evolution activity is presented as a percentage of the wild-type value.
[d]A: Popelkova et al. 2002a; B: Popelkova et al. 2002b; C: Wyman and Yocum, unpublished data; D: Lydakis-Symantiris et al. 1999a; E: Lydakis-Symantiris et al. 1999b; F: Motoki et al. 2002; S: Popelkova et al., submitted.
[e]Rwt – recombinant wild-type MSP from spinach; Nwt – native wild-type MSP from spinach.
[f]*Syn* – wild type and mutant MSPs from *Synechococcus elongatus*.
[g]ND – not determined.

mutagenesis beyond E246 causes a loss of some β-sheet structure and a rather large increase in size, and also decreases the intensity of the relevant near-UV CD peaks from Trp and Tyr. In this case, the largest changes are seen in the C-terminal mutations L245@, Q244@, and L245E. On the other hand, no change in the near-UV features of Tyr and Trp is observed in the mutant E246K. Moreover, this mutant does not exhibit a higher apparent mass on gel filtration and retains secondary structure. The structural properties of the E246@ mutant are intermediate between E246K and other C-terminal mutants (Lydakis-Simantiris et al. 1999a).

Elimination of the disulfide bridge by mutagenesis gives a result that provides information on the structural changes caused by C-terminal mutations. Figure 2 presents data on the near-UV CD spectra of wild-type MSP, wild-type MSP at 90 °C, and the C28AC51A double mutant at 25 °C. For the heated protein, as well as for the mutant, the CD spectra give evidence for a complete loss of the hydrophobic environments of Tyr residues and Trp241. Data in Table 3 show that some changes occur in the secondary structure elements of these proteins and, in the case of C28AC51A, an increase in apparent mass is also observed. However, the C28AC51A mutant is capable of rebinding to PS II and restoring activity to near-

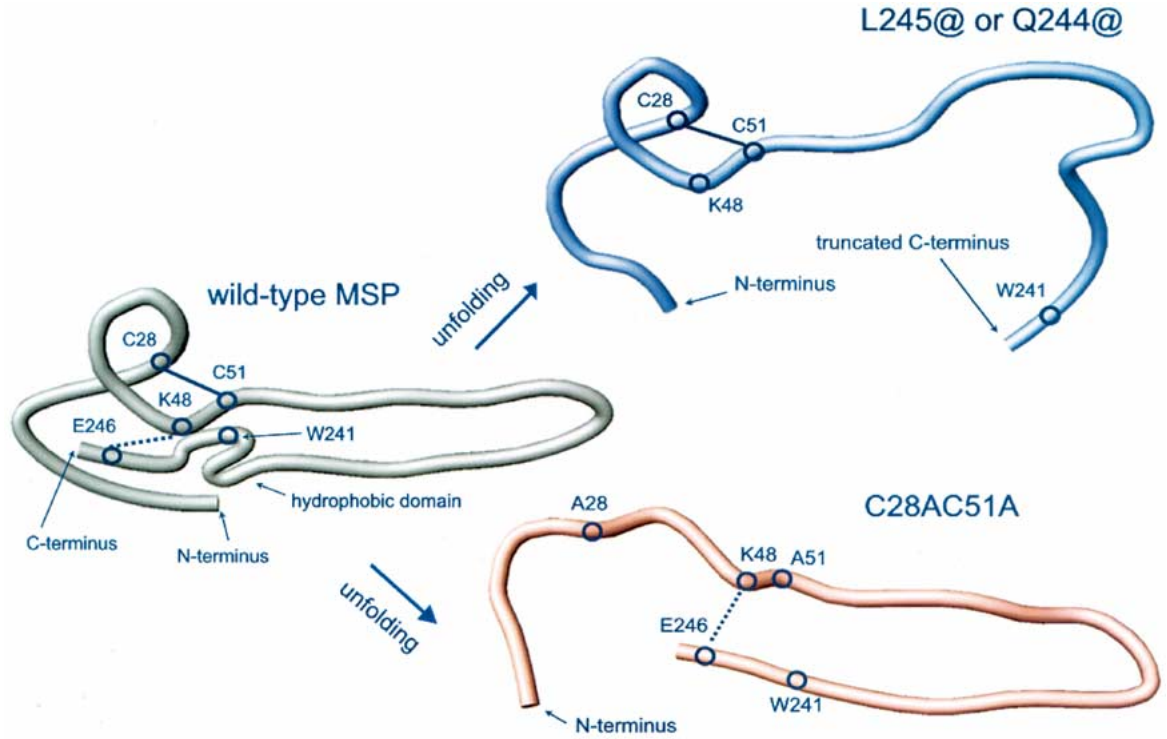

Figure 3. Schematic models of the solution tertiary structure of the wild-type and mutant MSPs from spinach. The dashed line represents the interaction between Glu246 and Lys48, and the solid line represents the covalent bond between Cys28 and Cys51; for other details, see text.

wild-type levels (Betts et al. 1996a). Thus, loss of the hydrophobic domain at the C-terminus, along with some β-sheet structure, as monitored by near- and far-UV CD, is not necessarily diagnostic for loss of MSP function. This would indicate that the elimination of key amino acids at the C-terminus (Leu245, Gln244), which also cause a loss of the hydrophobic character of this domain (Lydakis-Simantiris et al. 1999a), creates the lesion that prevents activation of PS II by MSP lacking critical C-terminal amino acid residues. Thus, two domains of MSP can be clearly identified with the binding and assembly of MSP into PS II. The first of these, the N-terminus, is essential for the preliminary docking of the protein with sites that have been identified as being associated with the extrinsic E loop of CP47. This contact alone is insufficient to permit completion of the steps that lead to tightly bound MSP, however. Key residues at the C-terminus must be present in order for MSP to achieve a functionally bound state. At a minimum, these residues must include Leu245 and Gln244. In accord with the observation that MSP assembly into PS II involves a gain of β-sheet secondary structure (Hutchison et al. 1998), it seems likely that MSP binds to PS II in a process that contains at least two ordered steps, initial contact followed by folding into a new structure that is presumably more compact than the solution structure:

$$\begin{aligned} \text{PS II} + \text{MSP}_{\text{UNFOLDED}} &\rightarrow \text{PS II} - \text{MSP}_{\text{UNFOLDED}} \\ &\rightarrow \text{PS II} - \text{MSP}_{\text{FOLDED}} \end{aligned}$$

Step one can proceed without key C-terminal amino acid residues (Betts et al. 1998), but recovery of function, as well as high affinity binding, requires an intact C-terminus. Part of this rebinding-refolding mechanism may require a substantial rearrangement of MSP structure, such as that revealed by the crosslinking studies of Enami et al. (1998). These investigators found that when MSP was in solution, Glu246 crosslinked to Lys48, as mentioned above. The modified protein, when rebound to PS II, was capable of protecting against Mn loss, but not of restoring O_2 evolution. When PS II-bound MSP was probed, a crosslink between Glu246 and Lys190 was detected, which the authors interpreted as evidence for a major shift in MSP structure upon binding to PS II. Although the hydrophobic environment around Tyr and Trp residues is not important for functional assembly of MSP into PS II, it does appear to play relevant role in the pro-

tein's tertiary structure in solution. An increase or decrease in hydrophobicity in this C-terminal domain correlates, in most cases, with nonspecific binding of MSP to PS II (see Table 3). Thus, the hydrophobic domain at the C-terminus of the wild-type protein may be necessary to prevent nonspecific binding interactions between MSP and PS II.

A model for MSP solution structure

The structure of MSP bound to PS II has been reported as part of the crystal structures from cyanobacterial preparations (Zouni et al. 2001; Kamiya and Shen 2003). In these structures, the protein is bound to the lumenal side of PS II as a prolate ellipsoid shape containing extensive amounts of β-sheet. At the present levels of resolution (3.7–3.8 Å), it is not possible to identify individual amino acids. In addition, there appears to be only one copy of MSP associated with the cyanobacterial preparations, whereas biochemical evidence favors the presence of two copies in higher plant preparations (Xu and Bricker 1992; Seidler 1994; Leuschner and Bricker 1996; Betts et al. 1996b, 1997; Frankel et al. 1999). The shape of the bound subunit agrees with predictions for the prolate ellipsoid shape of MSP in solution, as discussed above. From the effects of mutations and chemical modifications on the solution structure of MSP, it is possible to propose a model for the conformation in solution of the wild-type protein that is consistent with the data that are now available.

Two lines of evidence, the crosslinking results of Enami et al. (1998), and the results from MSP N-terminus truncations by mutagenesis (Popelkova et al. 2002a, b), both suggest that the C-terminal domain of wild-type MSP interacts with the N-terminal domain. If correct, these data predict that wild-type MSP adopts a solution conformation in which the primary sequence and secondary structure elements are folded back upon themselves (see Figure 3–wild type). According to the results of Enami et al. (1998), an interaction between Lys48 and Glu246 exists in this structure, so it is possible that the N-terminus folds back towards this contact point and assists in stabilizing it. This interaction requires the presence of the disulfide bridge and, minimally, the presence of a C-terminal sequence -WYAQLE-. The results of Yu et al. (2001) on NBS-modification of Trp241, where the modified MSP shows a large increase in the amount of random coil and a loss of α-helix and β-sheet structures, would suggest that this residue is also essential for maintaining the protein in an active conformation, and the hydrophobic domain around this residue prevents nonspecific binding of the protein to PS II. Elimination of the stabilizing disulfide bridge disrupts the hydrophobic environment around Trp241, partially unfolding the protein to the more 'open' conformation, but preserving a functional interaction (E246 – K48) between the C- and N-termini and also allowing nonspecific binding of the protein to PS II (see Figure 3 – C28AC51A).

Disruption of the N $\rightarrow$ C-terminus interaction appears to have two consequences. First, in most cases, MSP increases in apparent molecular mass owing to changes in its hydrodynamic properties, and second, for those mutants where C-terminal residues have been removed, high affinity MSP binding and O_2 evolution activity reconstitution are lost, but the protein can bind nonspecifically to PS II. The models for solution structures of the L245@ and Q244@ mutants in Figure 3 represent a schematic of this altered structural state, in which MSP loses the hydrophobic region around Trp241, and adopts an even more 'open' conformation than C28AC51A. This conformational flexibility is likely to be facilitated by the natively unfolded behavior of MSP in solution. The large amount of unassigned secondary structure in the MSP sequence (Figure 1) represents regions where turns and unordered structure could come into play both in disruption of the interactions between the N- and C-termini, and also in regions that might undergo folding when MSP assembles into PS II.

Conclusion

Our knowledge of the solution structure of MSP, and the identity of critical amino acid residues that are needed for its binding and assembly into PS II, has advanced considerably in the last two years. The disorder in MSP's tertiary structure, and its lack of secondary structure as well, are common features of a number of proteins that are involved in protein–protein or protein–nucleic acid interactions. While the disulfide bridge in MSP is not essential to produce a solution structure capable of assembly into PS II, *in vivo* mutagenesis results clearly indicate that this structural feature of the protein is essential to produce a stable species capable of activating water oxidation in intact cyanobacterial cells. Both the N- and C-terminal domains of MSP can now be recognized as essential

contributors to the O_2 evolution activity of the protein, and an interaction between these domains can be identified as a marker for the solution conformation of the wild-type protein. The solution structure models we propose here are consistent with the existing data. Testing such models is a daunting enterprise, however. The size of MSP is at the upper limits where NMR is useful. It is possible that soluble MSP may one day be crystallized. An initial promising report has already appeared (Anati and Adir 2000).

Acknowledgements

This research was supported by a grant to CFY from the Molecular Biochemistry Program of the National Science foundation. We thank David Popelka for valuable assistance with the graphics used in this paper.

References

Ahmed A, Tajmirriahi HA and Carpentier R (1995) A quantitative secondary structure analysis of the 33-kDa extrinsic polypeptide of Photosystem II by FTIR spectroscopy. FEBS Lett 363: 65–68

Anati R and Adir N (2000) Crystalization of dimers of the manganese-stabilizing protein of Photosystem II. Photosynth Res 64: 167–177

Betts SD, Hachigian TM, Pichersky E and Yocum CF (1994) Reconstitution of the spinach oxygen-evolving complex with recombinant *Arabidopsis* manganese-stabilizing protein. Plant Mol Biol 26: 117–130

Betts SD, Ross JR, Hall KU, Pichersky E and Yocum CF (1996a) Functional reconstitution of Photosystem II with recombinant manganese-stabilizing proteins containing mutations that remove the disulfide bridge. Biochim Biophys Acta 1274: 135–142

Betts SD, Ross JR, Pichersky E and Yocum CF (1996b) Cold-sensitive assembly of a mutant manganese-stabilizing protein caused by a Val to Ala replacement. Biochemistry 35: 6302–6307

Betts SD, Ross JR, Pichersky E and Yocum CF (1997) Mutation Val235Ala weakens binding of the 33-kDa manganese stabilizing protein of Photosystem II to one of two sites. Biochemistry 36: 4047–4053

Betts SD, Lydakis-Simantiris N, Ross JR and Yocum CF (1998) The carboxyl-terminal tripeptide of the manganese-stabilizing protein is required for quantitative assembly into Photosystem II and for high rates of oxygen evolution activity. Biochemistry 37: 14230–14236

Bricker TM and Frankel LK (1998) The structure and function of the 33 kDa extrinsic protein of Photosystem II: a critical assessment. Photosynth Res 56: 157–173

Bricker TM, Odom WR and Queirolo CB (1988) Close association of the 33-kDa extrinsic protein with the apoprotein of cpa1 in photosystem II. FEBS Lett 231: 111–117

Burnap RL and Sherman LA (1991) Deletion mutagenesis in Synechocystis Sp PCC6803 indicates that the Mn-stabilizing protein of Photosystem II is not essential for O2 evolution. Biochemistry 30: 440–446

Burnap RL, Qian M, Shen JR, Inoue Y and Sherman LA (1994) Role of disulfide linkage and putative intermolecular binding residues in the stability and binding of the extrinsic manganese stabilizing protein to the Photosystem II reaction center. Biochemistry 33: 13712–13718

Debus RJ (1992) The manganese and calcium ions of photosynthetic oxygen evolution. Biochim Biophys Acta 1102: 269–352

Debus RJ (2000) The polypeptides of Photosystem II and their influence on manganotryosyl-based oxygen evolution. In: Sigel A and Sigel H (eds) Manganese and its Role in Biological Processes, Vol 37, pp 657–711. Marcel Dekker, New York

Debus RJ (2001) Amino acid residues that modulate the properties of tyrosine Y(Z) and the manganese cluster in the water oxidizing complex of Photosystem II. Biochim Biophys Acta 1503: 164–186

de las Rivas J and Heredia P (1999) Structural predictions on the 33 kDa extrinsic protein associated to the oxygen evolving complex of photosynthetic organisms. Photosynth Res 61: 11–21

Dunker AK, Lawson JD, Brown CJ, Williams RM, Romero P, Oh JS, Oldfield CJ, Campen AM, Ratliff CR, Hipps KW, Ausio J, Nissen MS, Reeves R, Kang CH, Kissinger CR, Bailey RW, Griswold MD, Chiu M, Garner EC and Obradovic Z (2001) Intrinsically disordered protein. J Mol Graph 19: 26–59

Eaton-Rye JJ and Murata N (1989) Evidence that the amino-terminus of the 33-kDa extrinsic protein is required for binding to the Photosystem II complex. Biochim Biophys Acta 977: 219–226

Enami I, Satoh K and Katoh S (1987) Crosslinking between the 33 kDa extrinsic protein and the 47 kDa chlorophyll-carrying protein of the PS II reaction center core complex. FEBS Lett 226: 161–165

Enami I, Kamo M, Ohta H, Takahashi S, Miura T, Kusayanagi M, Tanabe S, Kamei A, Motoki A, Hirano M, Tomo T and Satoh K (1998) Intramolecular cross-linking of the extrinsic 33-kDa protein leads to loss of oxygen evolution but not its ability of binding to Photosystem II and stabilization of the manganese cluster. J Biol Chem 273: 4629–4634

Frankel LK, Cruz JA and Bricker TM (1999) Carboxylate groups on the manganese-stabilizing protein are required for its efficient binding to Photosystem II. Biochemistry 38: 14271–14278

Ghanotakis DF and Yocum CF (1990) Photosystem II and the oxygen-evolving complex. Annu Rev Plant Physiol Plant Mol Biol 41: 255–276

Hashimoto A, Ettinger WF, Yamamoto Y and Theg SM (1997) Assembly of newly imported oxygen-evolving complex subunits in isolated chloroplasts: sites of assembly and mechanism of binding. Plant Cell 9: 441–452

Hutchison RS, Betts SD, Yocum CF and Barry BA (1998) Conformational changes in the extrinsic manganese stabilizing protein can occur upon binding to the Photosystem II reaction center: an isotope editing and FT-IR study. Biochemistry 37: 5643–5653

Kamiya N and Shen J-R (2003) Crystal structure of oxygen-evolving Photosystem II from Thermosynechococcus vulcanus at 3.7 Å resolution. Proc Natl Acad Sci USA 100: 98–103

Keegstra K, Olsen LJ and Theg SM (1989) Chloroplastic precursors and their transport across the envelope membranes. Annu Rev Plant Physiol Plant Mol Biol 40: 471–501

Kuwabara T and Murata N (1979) Purification and characterization of 33 kilodalton protein of spinach chloroplasts. Biochim Biophys Acta 581: 228–236

Kuwabara T and Murata N (1982) Inactivation of photosynthetic oxygen evolution and concomitant release of 3 polypeptides in the Photosystem II particles of spinach chloroplasts. Plant Cell Physiol 23: 533–539

Kuwabara T and Murata N (1983) Quantitative analysis of the inactivation of photosynthetic oxygen evolution and the release of polypeptides and manganese in the Photosystem II particles of spinach chloroplasts. Plant Cell Physiol 24: 741–747

Leuschner C and Bricker TM (1996) Interaction of the 33 kDa protein with Photosystem II: rebinding of the 33 kDa extrinsic protein to Photosystem II membranes which contain four, two, or zero manganese per Photosystem II reaction center. Biochemistry 35: 4551–4557

Lydakis-Simantiris N, Betts SD and Yocum CF (1999a) Leucine 245 is a critical residue for folding and function of the manganese stabilizing protein of Photosystem II. Biochemistry 38: 15528–15535

Lydakis-Simantiris N, Hutchison RS, Betts SD, Barry BA and Yocum CF (1999b) Manganese stabilizing protein of Photosystem II is a thermostable, natively unfolded polypeptide. Biochemistry 38: 404–414

Mayfield SP, Bennoun P and Rochaix JD (1987) Expression of the nuclear encoded oee1 protein is required for oxygen evolution and stability of Photosystem II particles in *Chlamydomonas reinhardtii*. EMBO J 6: 313–318

Miura T, Shen JR, Takahashi S, Kamo M, Nakamura E, Ohta H, Kamei A, Inoue Y, Domae N, Takio R, Nakazato K and Enami I (1997) Identification of domains on the extrinsic 33-kDa protein possibly involved in electrostatic interaction with Photosystem II complex by means of chemical modification. J Biol Chem 272: 3788–3798

Miyao M and Murata N (1984) Role of the 33-kDa polypeptide in preserving Mn in the photosynthetic oxygen-evolution system and its replacement by chloride-ions. FEBS Lett 170: 350–354

Miyao M and Murata N (1989) The mode of binding of 3 extrinsic proteins of 33-kDa, 23-kDa and 18-kDa in the Photosystem II complex of spinach. Biochim Biophys Acta 977: 315–321

Motoki A, Usui M, Shimazu T, Hirano M and Katoh S (2002) A domain of the manganese-stabilizing protein from *Synechococcus elongatus* involved in functional binding to Photosystem II. J Biol Chem 277: 14747–14756

Odom WR and Bricker TM (1992) Interaction of cpa-1 with the manganese-stabilizing protein of Photosystem II - identification of domains cross-linked by 1-ethyl-3-[3-(dimethylamino)propyl]carbo diimide. Biochemistry 31: 5616–5620

Pazos F, Heredia P, Valencia A and de las Rivas J (2001) Threading structural model of the manganese-stabilizing protein PsbO reveals presence of two possible beta-sandwich domains. Proteins 45: 372–381

Popelkova H, Im Mm, D'Auria J, Betts SD, Lydakis-Simantiris N and Yocum CF (2002a) N-terminus of the Photosystem II manganese stabilizing protein: effects of sequence elongation and truncation. Biochemistry 41: 2702–2711

Popelkova H, Im Mm and Yocum CF (2002b) N-terminal truncations of manganese stabilizing protein identify two amino acid sequences required for binding of the eukaryotic protein to Photosystem II and reveal the absence of one binding-related sequence in cyanobacteria. Biochemistry 41: 10038–10045

Ruan K, Li J, Liang R, Xu Ch, Yu Y, Lange R and Balny C (2002) A rare fluorescence behavior where the emission is dominated by tyrosine: case of the 33 kDa protein from spinach Photosystem II. Biochem Biophys Res Commun 293: 593–597

Schmid FX (1997) Optical spectroscopy to characterize protein conformation and conformational changes. In: Creighton TE (ed) Protein Structure: a Practical Approach, pp 261–297. IRL Press, New York

Seidler A (1994) Introduction of a histidine tail at the N-terminus of a secretory protein expressed in *Escherichia coli*. Protein Eng 7: 1277–1280

Seidler A (1996a) Intermolecular and intramolecular interactions of the 33-kDa protein in Photosystem II. Eur J Biochem 242: 485–490

Seidler A (1996b) The extrinsic polypeptides of Photosystem II. Biochim Biophys Acta 1277: 35–60

Seidler A and Michel H (1990) Expression in Escherichia coli of the psbO gene encoding the 33 kDa protein of the oxygen-evolving complex from spinach. EMBO J 9: 1743–1748

Seidler A, Roll K and Michel H (1992) Characterization of the 33 kD protein of the oxygen evolving complex of higher plants by site-directed mutagenesis. In: Murata N (ed) Research in Photosynthesis, Vol II, pp 409–412. Kluwer Academic Publishers, Dordrecht, The Netherlands

Shen JR, Qian M, Inoue Y and Burnap RL (1998) Functional characterization of *Synechocystis* sp. PCC 6803 Delta psbU and Delta psbV mutants reveals important roles of cytochrome c-550 in cyanobacterial oxygen evolution. Biochemistry 37: 1551–1558

Shutova T, Irrgang KD, Shubin V, Klimov VV and Renger G (1997) Analysis of pH-induced structural changes of the isolated extrinsic 33 kilodalton protein of Photosystem II. Biochemistry 36: 6350–6358

Shutova T, Irrgang KD, Klimov VV and Renger G (2000) Is the manganese stabilizing 33 kDa protein of Photosystem II attaining a 'natively unfolded' or 'molten globule' structure in solution? FEBS Lett 467: 137–140

Shutova T, Deikus G, Irrgang KD, Klimov VV and Renger G (2001) Origin and properties of fluorescence emission from the extrinsic 33 kDa manganese stabilizing protein of higher plant water oxidizing complex. Biochim Biophys Acta 1504: 371–378

Sonoyama M, Motoki A, Okamoto G, Hirano M, Ishida H and Katoh S (1996) Secondary structure and thermostability of the Photosystem II manganese-stabilizing protein of the thermophilic cyanobacterium *Synechococcus elongatus*. Biochim Biophys Acta 1297: 167–170

Svensson B, Etchebest C, Tuffery P, vanKan P, Smith J and Styring S (1996) A model for the Photosystem II reaction center core including the structure of the primary donor P-680. Biochemistry 35: 14486–14502

Svensson B, Tiede DM and Barry BA (2002) Small-angle X-ray scattering studies of the manganese stabilizing subunit in Photosystem II. J Phys Chem B 106: 8485–8488

Tanaka S and Wada K (1988) The status of cysteine residues in the extrinsic 33 kDa protein of spinach Photosystem II complexes. Photosynth Res 17: 255–266

Tanaka S, Kawata Y, Wada K and Hamaguchi K (1989) Extrinsic 33-kilodalton protein of spinach oxygen-evolving complexes: kinetic studies of folding and disulfide reduction. Biochemistry 28: 7188–7193

Tohri A, Suzuki T, Okuyama S, Kamino K, Motoki A, Hirano M, Ohta H, Shen JR, Yamamoto Y and Enami I (2002) Comparison of the structure of the extrinsic 33 kDa protein from different organisms. Plant Cell Physiol 43: 429–439

Tucker DL, Hirsh K, Li H, Boardman B and Sherman LA (2001) The manganese stabilizing protein (MSP) and the control of O-2 evolution in the unicellular, diazotrophic cyanobacterium, *Cyanothece* sp ATCC 51142. Biochim Biophys Acta 1504: 409–422

Uversky VN (2002) Natively unfolded proteins: a point where biology waits for physics. Protein Sci 11: 739–756

Weinreb PH, Zhen WG, Poon AW, Conway KA and Lansbury PT (1996) NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. Biochemistry 35: 13709–13715

Xu Q and Bricker TM (1992) Structural organization of proteins on the oxidizing side of Photosystem II. J Biol Chem 267: 25816–25821

Xu Q, Nelson J and Bricker TM (1994) Secondary structure of the 33 kDa, extrinsic protein of Photosystem II: a far-UV circular dichroism study. Biochim Biophys Acta 1188: 427–431

Yocum CF (1991) Calcium activation of photosynthetic water oxidation. Biochim Biophys Acta 1059: 1–15

Yu Y, Li R, Xu C, Ruan K, Shen Y and Govindjee (2001) N-bromosuccinimide modification of W241 at the C-terminus of the manganese stabilizing protein of plant Photosystem II influences its structure and function. Phys Plant 111: 108–115

Zhang L-X, Liang H-G, Wang J, Li WR and Yu TZ (1996) Fluorescence and Fourier-transform infrared spectroscopic studies on the role on disulfide bond in the calcium binding in the 33 kDa protein of Photosystem II. Photosynth Res 48: 379–384

Zouni A, Witt HT, Kern J, Fromme P, Krauss N, Saenger W and Orth P (2001) Crystal structure of Photosystem II from *Synechococcus elongatus* at 3.8 angstrom resolution. Nature 409: 739–743

Zubrzycki IZ, Frankel LK, Russo PS and Bricker TM (1998) Hydrodynamic studies on the manganese-stabilizing protein of Photosystem II. Biochemistry 37: 13553–13558