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Assembly and function of the Photosystem II manganese stabilizing protein: lessons from its natively unfolded behavior

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Abstract

The Photosystem II (PS II) manganese stabilizing protein (MSP) possesses characteristics, including thermostability, ascribed to the natively unfolded class of proteins (Lydakis-Simantiris et al. (1999) Biochemistry 38: 404–414). A site-directed mutant of MSP, C28A, C51A, which lacks the -S—S- bridge, also binds to PS II at wild-type levels and reconstitutes oxygen evolution activity [Betts et al. (1996) Biochim Biophys Acta 1274: 135–142], although the mutant protein is even more disordered in solution. Both WT and C28A, C51A MSP aggregate upon heating, but an examination of the effects of protein concentration and pH on heat-induced aggregation showed that each MSP species exhibited greater resistance to aggregation at a pH near their p*I* (5.2) than do either bovine serum albumin (BSA) or carbonic anhydrase, which were used as model water soluble proteins. Increases in pH above the p*I* of the MSPs and BSA enhanced their aggregation resistance, a behavior which can be predicted from their charge (MSP) or a combination of charge and stabilization by -S—S- bonds (BSA). In the case of aggregation resistance by MSP, this is likely to be an important factor in its ability to avoid unproductive self-association reactions in favor of formation of the protein–protein interactions that lead to formation of the functional oxygen evolving complex.

Abbreviations: BSA – bovine serum albumin; CD – circular dichroism; IPTG – isopropyl-β-D-thiogalactopyranoside; MSP – manganese stabilizing protein; OEC – O_2-evolving complex; PAGE – polyacrylamide gel electrophoresis; PS – Photosystem; *psbO* – gene encoding precursor MSP; WT – wild-type

Introduction

Photosystem II (PS II) is unique in its polypeptide composition, consisting of several intrinsic and extrinsic proteins, along with a tetranuclear Mn cluster, and inorganic cofactors (Ca^{+2} and Cl^{-}) (Debus 1992). The membrane-bound intrinsic proteins of PS II form a core structure that provides binding sites for both the inorganic cofactors and water-soluble extrinsic proteins (MSP, 23, and 17 kDa in eukaryotes) (Debus 1992; Seidler 1996). In solution, MSP has properties similar to natively unfolded proteins (Lydakis-Simantiris et al. 1999), which have unique physical and chemical characteristics, including thermostability, extreme p*I* values, extended solution structures, and anomalous mobility on SDS-PAGE. These proteins are found as components of multisubunit complexes that have diverse activities (protein phosphorylation, ribosomal translation, synapse formation,

cytoskeletal structure) (Weinreb et al. 1996). MSP possesses a low p*I* (5.2), a relatively high content of random coil and turn (50–55%) (Xu et al. 1994; Ahmed et al. 1995; Shutova et al. 1997; Lydakis-Simantiris et al. 1999), and exhibits deviations in estimated molecular mass that range from 26.5 kDa (DNA sequencing, MALDI-TOF mass spectrometry (Betts et al. 1994; Zubrzycki et al. 1998; Svensson et al. 2002)), to 33 kDa (SDS-PAGE) and 34–41 kDa (gel filtration) (Kuwabara and Murata 1979; Popelkova et al. 2002a). These properties, combined with MSP's thermostability (Lydakis-Simantiris et al. 1999) are similar to those possessed by proteins that are said to be natively denatured or intrinsically disordered. A mutant of MSP (C28A, C51A) lacking the single -S—S- bridge of the WT protein (Betts et al. 1996) is also thermostable, although it has a larger apparent mass (51 kDa; data not shown). Here, we show that the thermostability of these MSP species is directly related to their ability to resist protein aggregation upon heating. This behavior may, in turn, be an essential feature of MSP's solution structure that enables it to bind selectively to PS II, rather than forming unproductive protein aggregates.

Materials and methods

Recombinant MSPs were overexpressed, isolated, and purified as described previously (Betts et al. 1994,1996; Popelkova et al. 2002b, 2003a), except that the LB growth medium contained only ampicillin (50 μg ml^{-1}), and 25–32.5 μM IPTG was used to induce protein overexpression. Purified proteins were stored in the solubilization buffer used for protein isolation (3 M urea, 20 mM Bis-Tris (pH 6.4), 5 mM NaCl), or in SMN buffer (0.4 M sucrose, 50 mM Mes (pH 6.0), 10 mM NaCl); urea was removed by dialysis against SMN buffer. MSP concentrations were determined spectrophotometrically (Xu and Bricker 1992). BSA and carbonic anhydrase were purchased from Sigma–Aldrich, and their concentrations were determined spectrophotometrically (Lindskog 1960; Wetlaufer 1962). For CD spectroscopy, MSPs in 10 mM KH_2PO_4 (pH 6.0) were analyzed as previously described (Popelkova et al. 2002a). The time dependence of protein aggregation was assayed by incubation of proteins at 90 °C for up to 5 min in SMN (pH 5.3); after cooling, aggregates were removed by centrifugation, and the protein concentration of the supernatant was determined spectrophotometrically. The concentration dependence of heat-induced protein aggregation was characterized in a similar manner, but proteins were incubated at 90 °C for 20 min in buffers containing 0.4 M sucrose, 10 mM NaCl, and either 50 mM MES (pH 5.3), Tris (pH 6), or KH_2PO_4 (pH 5.7 or 7.7). The pH values are those of the buffers at 90 °C, as determined from their temperature coefficients. The soluble protein concentration was determined as described above.

Results and discussion

Effect of heating on the near-UV CD spectra of wild-type and C28A, C51A MSP

The near-UV CD spectrum of WT MSP has well defined peaks that are assigned to Tyr (at 285 nm) and the lone Trp (W241) at 291 nm (Figure 1a). Additional minor peaks at 258 and 264 nm are assigned to Phe. Near-UV CD signals from these aromatic residues are taken as indicators of the integrity of a protein's tertiary structure; hydrophobic environments enhance the signals, while increased hydrophilicity leads to decreased signal intensities (Kelly and Price 1997). These CD features are absent in C28A, C51A MSP (Figure 1b); this observation, when combined with the mutant protein's estimated mass from gel filtration (51 kDa; data not shown), indicates that loss of the -S—S- bridge has created additional disorder in its solution structure. Heated WT MSP (Figure 1a) has no near-UV CD features and possesses a spectrum similar to that of C28A, C51A MSP at 25 °C (Figure 1b), indicating that the mutant's room temperature disordered state can be achieved by heating WT MSP to 90 °C. WT MSP regains its initial near-UV CD signals upon cooling whereas neither heating nor subsequent cooling affect the spectrum of the mutant (Figure 1b).

Time course of heat-induced aggregation of MSP and model water-soluble proteins

Two reconstitutively active MSP species that differ in their extent of structural disorder provided an opportunity to further examine the intrinsic

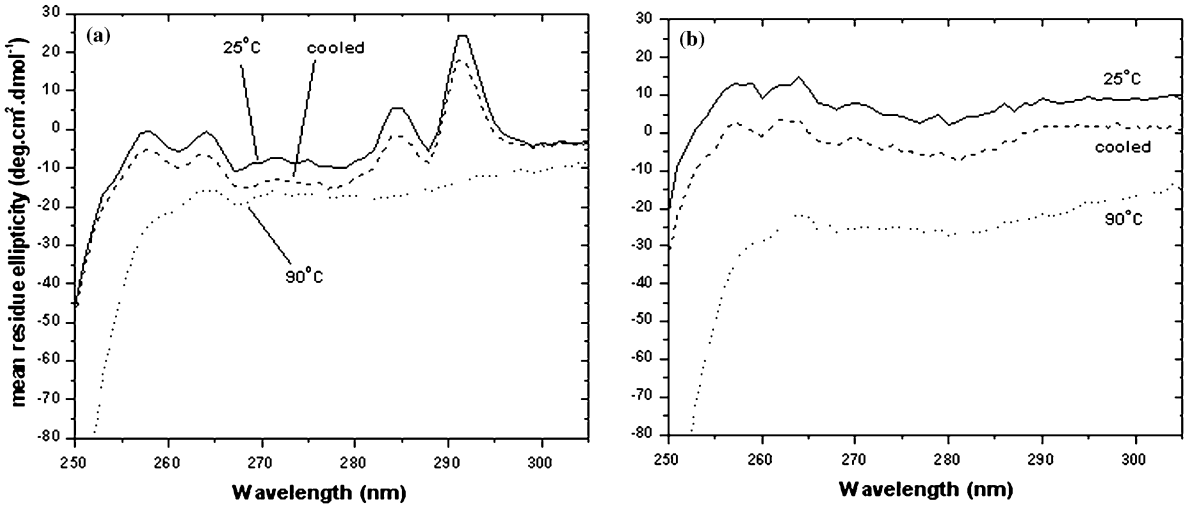

Figure 1. Near-UV CD spectra of WT and C28A, C51A MSP. The spectra are averages of 20 scans each. Experimental conditions: scan width, 320–250 nm; time constant, 1 s; bandwidth, 1 nm; path length, 1 cm.

thermostability of MSP; BSA and carbonic anhydrase were utilized as standards of comparison. BSA (66 kDa) is stabilized by 17 -S—S- bridges, and has a p*I* of 5.0, close to that of MSP, while carbonic anhydrase (29 kDa) has a mass comparable to that of MSP but contains no -S–S- bonds and has a p*I* of 6.6. The heat-induced aggregation behavior of each model protein has been characterized (Alexander and Hamilton 1960; Wetzel et al. 1980; Peters Jr. 1996; Rajaraman et al. 1996; Kundu and Guptasarma 1999). The time dependence of heat-induced aggregation (Figure 2) shows that after 1 min of heating, 45–50% of the MSP species remained soluble, while only 35% of carbonic anhydrase and 10% of BSA resisted aggregation. Prolonged heating further decreased the concentration of soluble carbonic anhydrase (10% by 5 min), while the amount of soluble BSA remained constant at about 10%. Heating beyond 1 min had little effect on the solubilities of unaggregated WT and C28A, C51A MSP (Figure 2).

Effect of pH and protein concentration on resistance to heat-induced protein aggregation

A protein's net charge and concentration should affect the extent to which it self-aggregates. Figure 3a presents the heat-induced aggregation behaviors of WT MSP in buffers over a pH range from 5.3 to 7.7, and at concentrations up to 50 μM. The pH values given in the figures are adjusted to 90 °C using the buffers' temperature coefficients. At pH 5.3, WT MSP exhibits resistance to aggregation at low concentrations, whereas above 30 μM about 50% of the protein remained in solution after heating. Increasing the pH to values above the p*I* of WT MSP produces a marked increase in the amount of aggregation-resistant protein (90–95%) for all concentrations tested. This is likely to be due predominantly to an

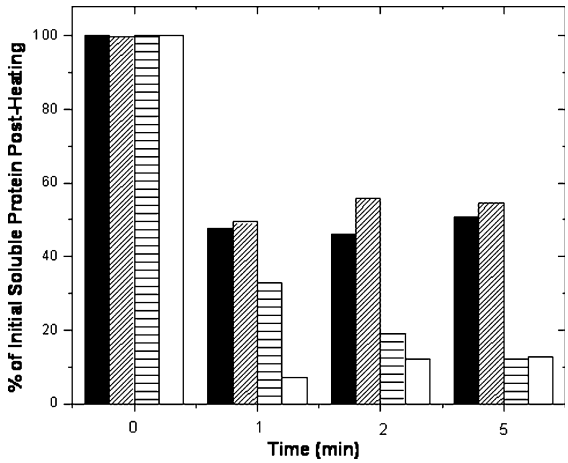

Figure 2. Time course of heat-induced aggregation of MSPs, BSA and carbonic anhydrase. Aggregation was determined as described in Materials and methods. Symbols are: WT MSP (31.5 μM), solid; C28A, C51A MSP (11.9 μM), diagonal stripes; carbonic anhydrase (30.4 μM), horizontal stripes; BSA (34.5 μM), clear.

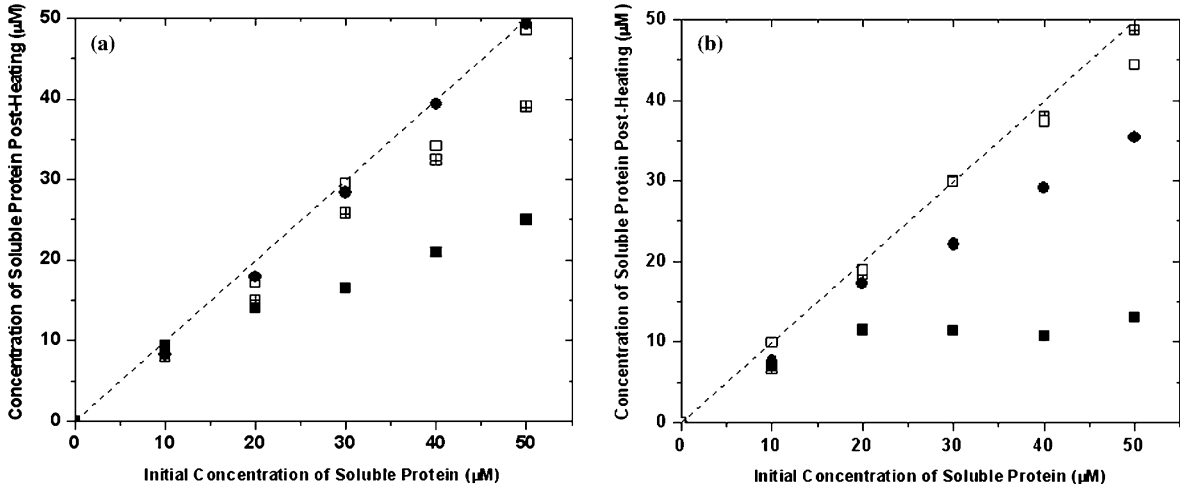

Figure 3. Effect of pH on the aggregation of WT MSP (a) and C28A, C51A MSP (b). The symbols are: MES, pH 5.3, (■); KH_2PO_4, pH 5.7, (●); TRIS, pH 6.0, (⊞); KH_2PO_4, pH 7.7, (□). The dashed line is the result that would be obtained if no protein aggregation were to occur.

increased negative net charge on MSP, which would increase the repulsion between individual protein molecules in solution and in turn limit opportunities for protein–protein interactions that might initiate the formation of complexes that result in insoluble aggregates. A similar experiment with C28A, C51A MSP (Figure 3b) showed that it also resists aggregation at low protein concentrations, but at pH 5.3, only 12–15 μM protein remains soluble, regardless of the initial concentration present in solution. With increasing pH, however, C28A, C51A MSP also exhibits increased resistance to aggregation, attaining 95–100% solubility at pH 7.7 for all concentrations tested at 90 °C.

Figure 4 compares the aggregation resistance of both MSP species to a pair of model water soluble proteins at two pH values. At pH 5.3 (Figure 4a), BSA and carbonic anhydrase exhibit limited solubility after heating, even at the lowest

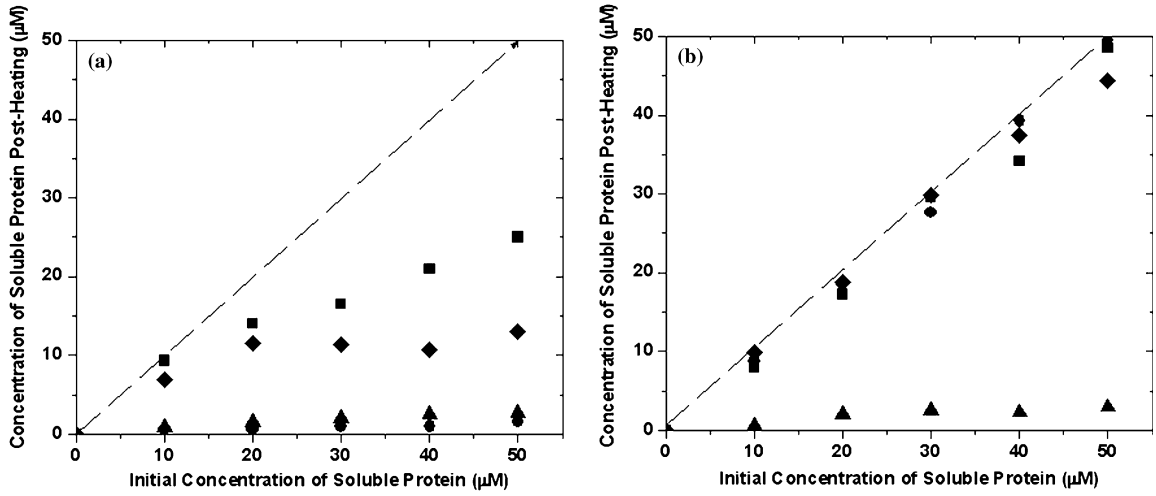

Figure 4. A comparison of aggregation properties of MSPs, BSA, and carbonic anhydrase at pH 5.3 (a) or 7.7 (b). The symbols are: WT MSP, (■); C28A, C51A MSP, (♦); carbonic anhydrase, (▲); BSA, (●). The dashed line is the result that would be obtained if no protein aggregation were to occur.

concentrations used. Little, if any, additional protein remains in solution as their concentrations are increased. When the pH was increased to 7.7 (Figure 4b), carbonic anhydrase showed no change in its aggregation behavior (5–10% remained soluble after heating). However, BSA showed an increased resistance to aggregation comparable to that of both of the MSP species. This can be explained by the increased charge present on BSA at pH 7.7, which normally leads to an increase in its solubility in aqueous buffers, while also acting to prevent interactions between BSA molecules (Hughes 1954; Peters Jr. 1996). However, BSA is not thermostable at a pH near its p*I*, in spite of the presence of 17 -S—S- bridges to impart structural stability to the protein. In agreement with other data on BSA (Militello et al. 2004), the results in Figure 4b shows that some 'normally folded' proteins become more heat-stable as they are shifted away from their p*I* values. In contrast, MSP proved to be innately thermostable, even at a pH near its p*I* value.

Extensive cataloging of intrinsically disordered proteins (Dunker et al. 2001) shows that these polypeptides enter into protein–protein, protein–nucleic acid or protein–small ligand interactions. Their thermostability is probably a byproduct of the high ratio of charged to hydrophobic amino acids in these proteins, which imparts disorder to their solution structures (Uversky 2000; Dunker et al. 2001; Popelkova et al. 2003b). The high charge on these structures, reflected in the extreme p*I* values of such proteins, is also a consequence of this distribution of amino acid residues. The resulting disordered structures have highly flexible conformations in solution, and it is proposed that this feature of natively unfolded proteins is essential to permit them to form initial complexes with their docking partners and undergo the subsequent folding maneuvers that form the more organized mature protein complex (Weinreb et al. 1996; Denning et al. 2002; Permyakov et al. 2003).

Here we have shown that MSP's thermostability is due to its ability to resist aggregation, which may be an emergent property of natively unfolded proteins that can be linked to their high content of charged amino acids, thus allowing them to resist aggregation by charge repulsion. This property would permit high concentrations of a protein like MSP to be maintained in solution in an organelle compartment like the thylakoid lumen, because it can avoid formation of aggregates due to self association. High concentrations of soluble MSP enable rapid targeting of binding sites on PS II, permitting MSP to dock, fold, and assist in the binding of other extrinsic proteins and inorganic cofactors to form a highly active OEC.

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