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Thermal unfolding of the manganese stabilizing 33 kD protein photosystem II: implications for a molten globular state

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Introduction

Manganese-stabilizing 33 kDa protein (MSP; encoded by the psbO gene; 247 amino acids of known sequence) is a constituent of PS II in all oxygenic photosynthetic organisms from cyanobacteria to higher plants (Bricker and Frankel,1998). This protein is associated with the lumenal surface of thylakoid membrances and plays an important role in maintaining the stability and efficient turnover of the tetranuclear manganese cluster of the water-oxidizing complex (WOC).

This protein exhibits an elongated shape in solution, with a highly amount of β-sheet in its secondary structure. The solution structure is need for its binding to PS II, upon reduction clearage of the conserved disulfide bridge in the protein results in inability of its to bind to PS II (Tanaka et al.1989). The conformation of MSP would be change upon binding to the photosystem II reaction center, more over the intramolecular cross-linking of the extrinsic 33-kDa protein would leads to loss of oxygen evolution but not its ability of binding to photosystem II and stabilization of the manganese cluster (). Some other properties of the protein, such as high percent of random structure, a larger apparent molecular mass and thermostability, suggest that it may be a native unfolded protein instead of a typical globular protein (Lydakis-Simantiris et al.1999).

The thermodynamic properties of MSP unfolding apper different from that of typical globular proteins. Chemical unfolding of MSP is brought about by 0.8 M guanidine hydrochloride or 2.5 M Urea (Tanaka et al.1989). A previous analysis of the unfolding of MSP led to an estimate of the free energy of stabilization (25 ºC) = 4.4 Kcal/mol, compared to 5-15 Kcal/mol typical for globular proteins. By using adiabatic differential scanning calorimetry, we demonstrate that MSP undergoes a low-cooperativity non-two-state thermal unfolding and displays other properties consistent with the molten globular state, which is defined as a compact folding intermediate with a near-native secondary structure but lax tertiary structure. This is first evidence support the hypothesis recently proposed that the MSP in solution maybe attain a molten globular state. Our results suggest that MSP may perform its major physiological function via the molten globular state in vivo.

Materials and methods

Oxygen-evolving PS II membranes were prepared from spinach leaves according to Kuwabara and Murata (1982). The PS II membranes were suspended in buffer A (50 mM MES-NaOH, pH 6.0, containing 400 mM sucrose, 10 mM NaCl, and 5 mM $MgCl_2$) and stored in liquid nitrogen until use. The 33 kDa protein was extracted from the PS II membranes and purified according to Kuwabara et al., migrated as a single band on Urea-SDS/13.75% polyacrylamide gel. The concentration of the 33 kDa protein was determined using an extinction coefficient of 20 $mM^{-1}cm^{-1}$ at 276 nm.

High-sensitivity differential scanning calorimetry (DSC), The heat capacity Cp (T) of samples was recorded over a temperature range of 5~140 ºC, by using a high-sensitivity differential scanning calorimeter (Dupont 1090). The volume of the calorimetric cell was 0.05 ml and the protein concentrations used were 2.0 mg/ml. The measurements were carried out with a microcalorimeter at the scan rate of 10 K/min.

Results and Discussion

The typical excess heat capacity curves of the spinach MSP at pH 6.5 are shown in Fig. 1. It is seen that the denaturation transition is highly asymmetrical. Tm was defined as the temperature at the peak of the curve. The thermal transition of MSP is resolved as a peak extending from 35 to 124 ºC. The calorimetric enthalpy, van Hoff enthalpy, and the heat capacity change for the denaturation of the MSP was obtained directly from the excess heat capacity curves.

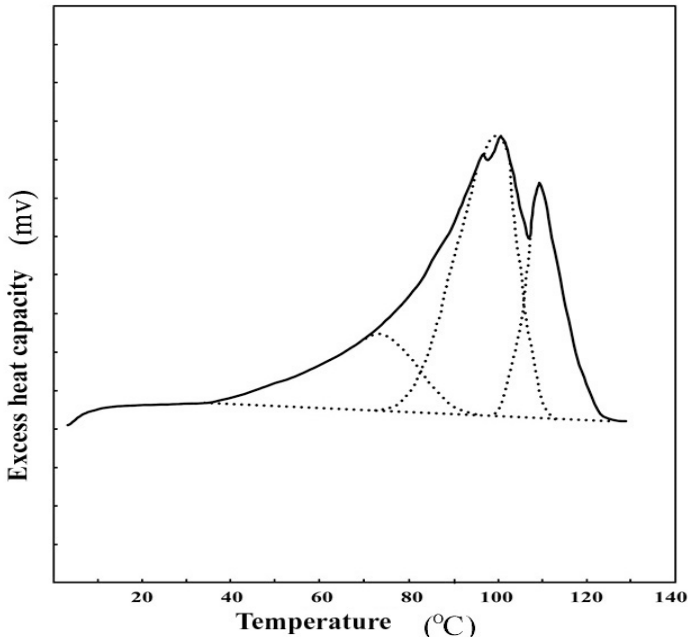

Fig. 1 The typical excess heat capacity curves of the spinach MSP at pH 6.5. Thermal unfolding of MSP is fitted by four consecutive non-two-state transition (in dashed lines) of combined enthalpy, major transition is at Tm = 100.8 ºC. It is preceded by a small reversible transition [Tm = 73 ºC]. The major transition is followed by an irreversible transistion [Tm = 73 ºC] which occurs at a slower rate than the major transition.

MSP was subjected to temperature denaturation/ renaturation experiments to evaluate the reversibility of its thermal transition. The results demonstrate that the thermal transition which MSP undergoes at a Tm of approximately was reversible, If the temperature was not allowed to increase above 100 ºC, a point just above the transition temperature.When the temperature of the initial run was allowed to rise to 120 ºC before thermal cutoff, the second run, after recooling, did not display a significant thermal transition, indicating that the MSP had been irreversible unfolded by the initial heating. Thus, it appears that irreversible thermal denaturation of MSP occurred in the range of 120 ºC.

The Tm of the MSP was 100.8 ºC. This is an unusually high value and probably reflects the role of the MSP as an external structural protein in photosystem. It is well known that small globular proteins can increase their stability by forming multiple disulfide bridges. Since MSP contain only one intrachain disulfide bond between Cys-28 and Cys-51 (Bricker and Frankel, 1998). This type of transition may be a result, at least in part, of MSP containing one disulfide bridge. Since there is a significant change in the Tm for MSP in the presence of DTT which can destoried the disulfide bridge (Fig.2 curve 3).

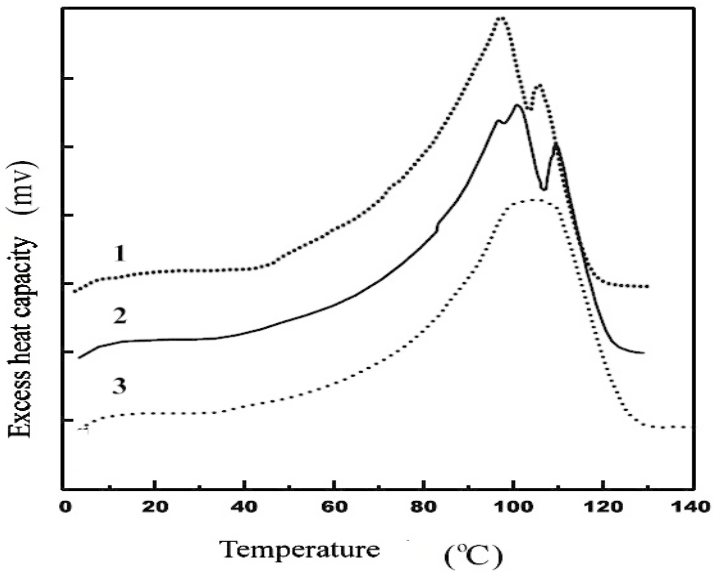

Fig. 2 The typical excess heat capacity curves of the spinach MSP under some condition.
Curve 1 is as that in Fig.1, while other were obtained in the presence of 100mM $CaCl_2$ (curve 2) or 2 mM DTT (curve 3).

MSP was analysis by DSC under some condition in order to determine its thermal denaturation properties. In the case where MSP was examined in low ionic strength buffer at pH 6.5. the deconvolution analysis clearly established the presence of no two-state transition (Fig. 1). This strongly suggests that the molecule is thermally denatured a noncooperative unfolding step with segregation of region within the molecule. Since the shape of the DSC curves could be differential feature for protein characterization in the manner of a fingerprint.

When Ca^{2+} ions were added to the MSP, the DSC results show some difference in the posistion [Fig.2 curves 2]. The Tm of the MSP was 98.9 ºC and 107.7 ºC in the presence of 100mM $CaCl_2$, which is about 2 ºC lower than that of MSP in the absence Ca^{2+}. Recently, the calcium-saturated protein contain large amount of α- helix and β- sheet (Zhang et al. 1996).

The temperature dependence of the far- and near-UV CD spectra indicate that the β- sheet of MSP are only weakly stabilized by tertiary interactions (Lydakis-Simantiris et al.1999). These features suggest a molten globular-like state for MSP in solution. Several experimentally observed properties of MSP are consistent with the molten globular stste: well-defined secondary structure (Shutova 2000), low unfolding cooperativity (this work), loose tertiary contacts (this work), and substantial solvent exposure of hydrophobic group, tendency to aggregate and high susceptibility to denaturants (Tanaka et al.1989).

These results demonstrated that MSP undergoes a low-cooperativity non-two-state thermal unfolding. This is first evidence support the hypothesis recently proposed that the MSP in solution maybe attain a molten globular state (Shutova 2000), which is defined as a compact folding intermediate with a near-native secondary structure but lax tertiary structure. Our results suggest that MSP may perform its major physiological function via the molten globular state in vivo.

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