Hypothesis

Is the manganese stabilizing 33 kDa protein of photosystem II attaining a 'natively unfolded' or 'molten globule' structure in solution?

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Abstract This study compares the properties of the extrinsic 33 kDa subunit acting as 'manganese stabilizing protein' (MSP) of the water oxidizing complex with characteristic features of proteins that are known to attain a 'natively unfolded' or a 'molten globule' structure. The analysis leads to the conclusion that the MSP in solution is most likely a 'molten globule' with well defined compact regions of β structure. The possible role of these structural peculiarities of MSP in solution for its function as important constituent of the WOC is discussed.

Key words: Manganese stabilizing protein; Photosystem II; Water oxidation; 'Molten globule' structure

1. Introduction

The overall pattern of the structural and functional organization of photosynthetic water oxidation into molecular oxygen and four protons is resolved (for reviews see [1–3]) but key questions on the mechanism and ligand-cofactor arrangement of the manganese containing catalytic site of water oxidation are still far from a satisfying answer (for a list see [4]). There is general agreement that the cofactors [redox active chlorophyll-a of PS II (P680), redox active tyrosine of polypeptide D1 (Y_Z), pheophytin (Pheo), primary (Q_A) and secondary (Q_B) quinone acceptor of PS II that assure a stable light induced charge separation] are located within a heterodimeric matrix consisting of polypeptides D1 and D2 [5–7] but the situation is less clear for the manganese cluster of the water oxidizing complex (WOC). Although site directed mutagenesis reveals that several amino acid residues of D1 and D2 are of functional and/or structural relevance in establishing a competent WOC [8–13], other polypeptides cannot be excluded as potential candidates for being essential constituents of the WOC. It was recently shown that the large lumen exposed loop E of the chlorophyll containing 47 kDa protein (CP47) is essential for the dark stability of the WOC [14–16] and the regulation of its chloride demand [17,18].

Apart from the above mentioned membrane bound integral proteins the properties of the WOC also depend on extrinsic subunits that are bound on the lumenal side of photosystem II (PS II) and exert regulatory functions. Among these the 33 kDa protein (encoded by the *psb*O gene) is of special relevance. It is a constituent of PS II in all oxygen evolving photosynthesizing organisms (for recent reviews, see [19,20]). The key role of this subunit is the stabilization of the manganese cluster of the WOC and therefore it is referred to as the 'manganese stabilizing protein' (MSP) [19,20]. This protein exhibits unusual mobility properties in solution: the apparent molecular mass values of 33 and 41 kDa gathered from migration in SDS gels [21] and by gel filtration [22], respectively, are markedly larger than the real mass of 26.54 kDa calculated from the DNA sequence [23] and confirmed by matrix assisted laser desorption ionization mass spectroscopy (MALDI) [54]. Spectral analysis (CD, FTIR) and model calculations revealed that the secondary structure of this protein is characterized by a comparatively high content of antiparallel β sheets and turns together with a low fraction of α-helices ([24–26] and references therein). Recently the MSP was shown to be thermostable [26] and that Leu-245 of the C-terminus is essential of maintaining its solution structure [27]. Based on these features the MSP was inferred to attain a flexible structure in solution that is typical for 'natively unfolded' proteins and a prerequisite for functional competent binding to PS II [26,27]. However, the structural feature of a 'natively unfolded' state is not the only possibility for conformational flexibility of a protein to achieve optimal conditions for interaction with other proteins. An alternative state with a high potential for structural adaptability is that of a 'molten globule'. This state has been predicted to be a folding intermediate [28] that exists as an equilibrium state under mild denaturing conditions [29] as well as a kinetic intermediate in the process of protein folding [30]. This state was shown to occur in several proteins like cytochrome *c* [31], lysozyme [32], apomyoglobin [33] and carbonic anhydrase B [34]. It is not only a kind of transition state in the folding but is expected to exist as a distinct state of proteins in vivo that participates in physiological processes, e.g. protein translocation through membranes or recognition by chaperones [35,36]. When the properties of several proteins with a characteristic 'natively unfolded' structural pattern are compared with those of the MSP its assignment to this group of proteins appears to

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Abbreviations: CP47, chlorophyll containing 47 kDa protein; Q_A amd Q_B, primary and secondary quinone acceptor, respectively, of PS II; MSP, manganese stabilizing protein; NACP, non-Aβ component of Alzheimer's disease amyloid plaque, precursor; P680, redox active chlorophyll-a of PS II; Pheo, pheophytin; PKI, protein kinase inhibitor; PS II, photosystem II; R_S, Stokes radius; S_e, sedimentation coefficient; WOC, water oxidizing complex; Y_Z, redox active tyrosine of polypeptide D1

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be less convincing. The present report provides a detailed discussion of this problem which leads to the conclusion that the structure of MSP in solution is better described as a 'molten globule'.

2. Compilation of typical (diagnostic) properties of 'natively unfolded' and 'molten globule' proteins

In order to permit a comparison of the MSP with structural characteristics of 'natively unfolded' and 'molten globule' proteins, the following section briefly summarizes features that are typical for both conformational states. 'Natively unfolded' proteins are characterized by:

1. high stability to thermal denaturation, e.g. harsh heat treatment (boiling) does not significantly affect the conformation of non-Aβ component of Alzheimer's disease amyloid plaque, precursor (NACP) [37],
2. high percentage of charged amino acid residues (often negatively), e.g. prothymosin α with a sequence of 109 amino acids contains 44 acidic residues [38],
3. maximum negative ellipticity in the far UV-CD spectrum near 200 nm as an indicator of random coil structure, observed in several 'natively unfolded' proteins like NACP, prothymosin α, protein kinase inhibitor (PKI), microtubule-associated protein 2 (MAP2) and τ-protein [37–40],
4. abnormally large Stokes radius (R_S) and sedimentation coefficient (S_e) together with unusual mobility in gel electrophoresis [37–39], and
5. lack of a hydrophobic core as indicated for NACP by the lack of significant ellipticity within the near-UV region [37].

Typical properties of 'molten globule' proteins can be summarized as follows:

1. high content of secondary structure elements (α-helices, β-sheets, turns),
2. considerable molecular compactness compared with the unfolded state as reflected by the R_S values gathered from the diffusion coefficient (in β-lactamase from *Bacillus cereus* this value is 31 Å versus 23 Å and 51 Å of the native and unfolded state, respectively [41]),

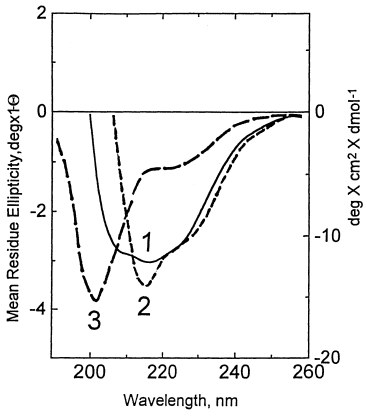

Fig. 1. Far-UV CD spectra of MSP in solution [25] (solid lined curve 1), α-lactalbumin in 'molten globule' state [53] (dashed curve 2) and prothymosin α in 'natively unfolded' state [38] (dashed curve 3).

3. significant flexibility monitored e.g. by NMR in carbonic anhydrase [42],
4. absence of a definite tertiary structure (e.g. markedly reduced near-UV CD in α-lactalbumin [29]), and
5. existence of a hydrophobic core.

3. Structural characteristics of the MSP within the framework of a 'molten globule' versus 'natively unfolded' state

Using the above mentioned characteristics of 'natively unfolded' and 'molten globule' proteins together with typical properties compiled in Table 1 and based on an inspection of the far UV-spectra shown in Fig. 1 it appears likely that the MSP in solution attains a 'molten globule' rather than a 'natively unfolded' structure. We propose that the MSP as a molten globule belongs to β-type proteins and forms a hydrophobic core of β-sheets that is stabilized by the disulfide bridge. Reduction of this bridge by dithiothreitol or addition of guanidinium hydrochloride gives rise to drastic structural changes as reflected by markedly altered spectra of tryptophan fluorescence [25,43] and near-UV circular dichroism [44]. Likewise, the unusual thermostability [26] of the MSP can be explained by the effect of the S–S bridge on the sec-

Table 1
Typical structural features of the MSP in comparison with 'natively unfolded' and 'molten globule' proteins

Proteins	Properties				
	Number of amino acid residues	M_{GF}/M_{SQ}^{a} or $Rs(MG)/Rs(N)$	Charged residues (%)	Presence of near-UV CD	R_S (Å)
A. *Natively unfolded*					
Phosphatase inhibitor [55]	193	3.2	38.4	−	N.A.[b]
Protein kinase [39,54]	208	2.3	47.1	−	31
NACP [37]	140	4.1	25.6	−	34
Prothymosin α (pH ∼ 7) [38]	111	4.9	58.6	−	31
B. *Molten globule*					
β-Lactamase [41]	227	1.1	26.4	+	$26.5_{(MG)}$; $24_{(N)}$, $51_{(U)}$
α-Lactalbumin [42]	123	1.2	29.3	+	$21_{(MG)}$; $18_{(N)}$, $32_{(U)}$
Carbonic anhydrase [42]	260	∼1	26.3	+	$Rs_{(MG)} = Rs_{(N)}$
Cytochrome *c* [41]	104	1.2	35.5	+	$20_{(MG)}$; $17_{(N)}$, $34_{(U)}$
C. *MSP*	247	1.55	23.1	+	31 [22]

[a] M_{GF}/M_{SQ}: ratio of molecular mass gathered from gel filtration to values calculated from amino acid sequence experimental data taken from table 1 of [37] and table 1 of [42] and table 2 of [26].
[b] N.A.: data are not available.

ondary structure. The idea of a classification of MSP as β-type protein is supported by the close similarity of its far-UV CD spectrum with that of carbonic anhydrase B as a prominent example of this class of proteins and to less pronounced extent with carbonic anhydrase A, concanavalin and lysozyme (for detailed discussion see [25]). Recently, the 'molten globule' as the third thermodynamic state of a protein (in addition to unfolded and native) was shown to be characterized by a structured domain that is common to all non-native forms [45]. In MSP a compact β-structure core most likely satisfies this condition.

4. Possible role of the 'molten globule' structure for the function of the MSP in the WOC

The 'molten globule' state as a folding intermediate is assumed to be an important structural feature of the MSP in order to satisfy several regulatory functions. At first the flexibility of the protein permits an optimal binding to the PS II complex in the thylakoid membrane. Taking into account the pronounced heterogeneity in the distribution of PS I and PS II between grana and stroma lamellae (for a review see [46] and references therein) it appears likely that the MSP structure in solution is also of relevance for the targeting of the mature protein inside the lumen to the surface of PS II.

With respect to regulation of the function in the WOC it is well established that in PS II complex deprived of the MSP there is a markedly enhanced Ca^{2+} demand for stabilizing the manganese cluster of the WOC and sustaining some oxygen evolution activity ([1,19] and references therein). Therefore it is attractive to speculate that in the MSP the structurally invariable part of the 'molten globule' state might be of relevance for transient Ca^{2+} binding and/or transport. This idea is supported by the finding that the metal-free apoenzymes of lysozyme, calmodulin and α-lactalbumin [42] are in a 'molten globule' state. Apart from this specific problem of Ca^{2+} effect(s) it has to be emphasized that the process of photosynthetic water oxidation is generally coupled with dynamic structural changes that are necessary for substrate (H_2O, OH^-) transport to the catalytic site and the product (O_2, protons) release pathway [3]. The role of protein dynamics in water oxidation is clearly illustrated by the striking temperature dependencies of the different redox steps in the WOC as reflected by their characteristic values of freezing [47] and thermal activation [48].

One fundamental question of photosynthetic water cleavage is the mode of coupling between electron and proton transfer. Recently, evidence has been presented that a hydrogen bond network affects the functional pattern of $P680^{+\bullet}$-reduction by Y_Z [49–51]. With this respect it is interesting to note that under natural illumination conditions the lumenal space becomes acidified [52]. It is therefore reasonable to assume that the MSP plays a role in steering the local pH in the vicinity of the WOC. The MSP exhibits an unusual hysteresis in pH titration experiments [25] that might be important for the regulation of the WOC under in vivo conditions. It remains to be clarified in future studies as to what extent a 'molten globule' state of the protein in solution is of relevance for structure-function relations in the WOC that are modulated by the membrane bound MSP in response to changes in the thylakoid lumen.

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