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Studies on the structural and functional role of the extrinsic manganese stabilizing protein in higher plants

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Introduction

Photosynthetic water oxidation takes place at a manganese containing unit, the water oxidizing complex (WOC) that is incorporated into the membrane integral protein matrix of PS II (for a review see Renger 2001 and references therein). Extrinsic proteins bound to the lumenal side of PS II are of physiological relevance as stabilizing and regulatory subunits. Among these the PsbO protein with an apparent mol. wt. of 33 kDa is unique because it is the constituent of PS II in all oxygen evolving organisms, in marked contrast to other extrinsic polypeptides that were entirely changed during evolutionary development from cyanobacteria to plants (Enami et al. 2000 and references therein). The PsbO protein is essential for stabilizing the manganese cluster and therefore often referred to as "manganese stabilizing protein" (MSP). The structure of the bound MSP depends on the redox state of the manganese cluster as reflected by the different susceptibilities to digestion by trypsin (Hong et al. 2001). In addition to its stabilizing effect the MSP also regulates the Cl^- and Ca^{2+} demand of the WOC (for a review, see Seidler 1996).

The secondary structure of this protein in solution is characterized by a high content of antiparallel β-sheets and turns together with a low fraction of α-helices (Xu et al. 1994, Shutova et al. 1995, 1997) and probably attains a molten globule structure (Shutova et al. 2000). Striking features are the content of several carboxylic groups with unusually high pK_a values of about 5.7 and the pronounced hysteresis in pH titration of opposite direction (Shutova et al. 1995, 1997). As outlined in Shutova et al. (1997) these properties suggest that the MSP might participate in proton transfer from the WOC into the lumen through a hydrogen bond network and, in addition, exert a regulatory role when the lumen becomes acidified in the light down to pH of about 5 (Siggel 1975) and reequilibrated in the dark. The C-terminus which contains the only Trp residue-W241 of higher plant MSP was shown to be critical for binding to PS II and restoration of O_2 evolution (Betts et al. 1998). Likewise, chemical modifications of amino acid residues in the C-terminus, i. e. in the regions of D_{157} - D_{168} and E_{212} - Q_{247} is only observed for the MSP in solution (Frankel et al., 1999). In a recent study the fluorescence spectrum of MSP was shown to be the composite of two

emitters: tyrosine(s) and W241 with the latter exhibiting an unusual strong quenching (Shutova et al. 2001). The present communication describes comparative studies on the properties of the MSP and a synthetic peptide corresponding to that of the C-terminus of MSP.

Materials and methods

The 33-kDa protein was isolated by a salt-washing as described before (Shutova et al., 2001), dialyzed against 5 mM amino acetate buffer, pH 6.0, and lyophilized. The lyophilized sample was dissolved in distilled water and further purified by chromatography on a DEAE-Sepharose 6-B. The protein homogeneity was checked via SDS/urea/PAGE. The protein concentration was spectrophotometrically determined at 276 nm. Prior to the measurements the protein was additionally dialyzed against phosphate/NaCl buffer, pH=7.2 and concentrated. The synthetic peptide was synthesized by INNOVAGEN, Lund, Sweden. The molecular weight of the peptide was confirmed by Mass Spectral Analysis (MALDI.TOF VOYAGER). The sample concentration was adjusted by using the optical density. Spectroscopic measurements were corrected for spectral sensitivity of the instruments. The bandwidth did not exceed 2 nm. The reactivation of the oxygen evolution in salt washed PS II membranes were performed as in Lydakis-Simantiris et al., 1999.

Results and discussion

Fig. 1 shows the fluorescence spectra of solubilized MSP (full-lined curve 3), of the synthetic C-terminus peptide KDVKIQGVWYAQLES (dash-dot-lined curve 2) and of a 7:1 mixture of N-acetyl-tyrosine-amide and N-acetyl-tryptophan-amide in aqueous solution (dash-lined curve 1), excited either at 275 nm or at 297 nm. For the sake of comparability the concentration of the tryptophan chromophore is nearly the same in the three sample types. An inspection of this data readily shows that the Trp emission is strongly quenched in MSP whereas in the peptide this effect is virtually absent at neutral pH. This finding indicates that at neutral pH the quenching of W241 emission in the native MSP is not caused by the neighbored protonated Y242 but probably arises from interaction with the S-S bridge as outlined in Shutova et al, 2001.

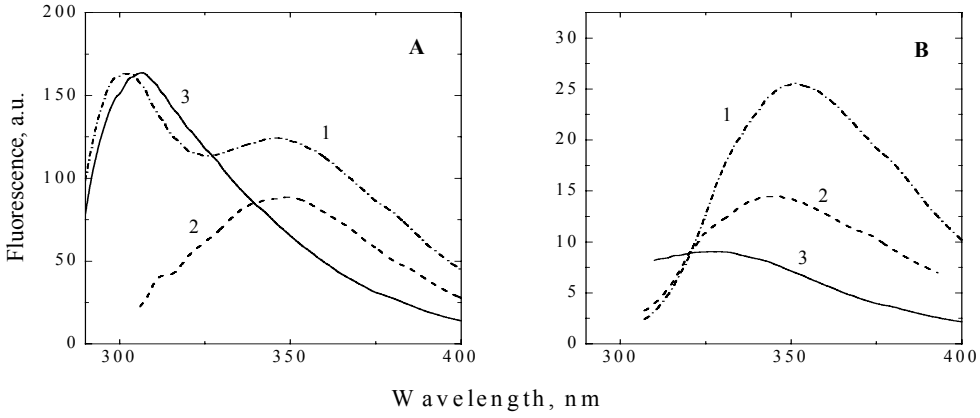

Fig. 1. Fluorescence emission spectra of the mixture of the N-acetyl-Tyr amide and N-acetyl Trp amide in 7:1 molar ratio equal to that in protein sequence (curve 1), C-terminus peptide (curve 2). The spectrum of the native MSP (curve 3) is presented for comparison. The samples were excited at 275 nm (panel A) or at 297 nm (panel B). The concentrations of the all samples were adjusted to an 0.2 D.

On the other hand, the Trp fluorescence of the C-terminus peptide is drastically suppressed in the alkaline region as illustrated in the left panel of Fig. 2. The titration of the quenching effect reveals that the deprotonation of a single residue with a pK value of about 9.7 leads to a drastic decrease of the fluorescence emission from the tryptophan. This pK closely resembles that of 10.1 for tyrosine in solution. It is therefore most likely that the tyrosinate form acts as quencher. In this respect it is interesting to compare the data with the pH dependence of W241 emission in native MSP. In this case the emission ascribed to the W241 fluorescence exhibits a complex pH dependence with a steep decline above pH 11.3 while the quenching of tyrosine emission can be described by the titration curve with a single pK of 11.7. The latter effect was ascribed to the deprotonation of Tyr residues that are buried into an environment with low dielectric constants within the MSP (Shutova et al. 2001).

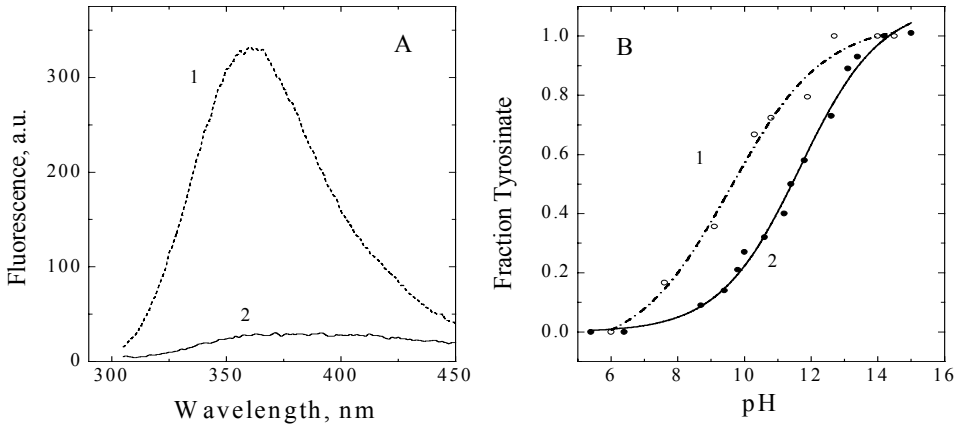

Fig 2: Panel A: Fluorescence spectra of the C-terminus peptide at pH=7.2 (curve 1) and at pH=12.4 (curve 2). Panel B: Ratio [F360 (pH 7.2) - F360 (pH)] / F360 (pH 7.2) in synthetic C-terminus peptide (open circles) and normalized tyrosinate concentration in MSP (filled circles). Curves 1 and 2 are calculated single proton titration curves with pKa = 9.7 and 11.7, respectively. The pH-dependence of fluorescence for the synthetic peptide was the same for both, 275 nm and 297 nm excitation.

Since the spinach MSP contains seven Tyr residues it is most likely that not all of these are in the same dielectric environment and that the titration curve represents an average with slight variations of the pK values of individual groups. Therefore the pK of the neighbor Y242 could be in the range of 11.5 - 12 and therefore perfectly fits with the idea that also in native MSP the deprotonation of this residue is responsible for the strong quenching of the W241 emission above pH 11.3. The mechanism of this effect is not yet clarified but it seems reasonable to assume that the negatively charged pair in its excited state $^{1}W241^{*}\,(YO^{-})_{242}$ forms a ground state biradical pair. The state $W241(YO^{\bullet})_{242}$ of the same charge but with the electron located on W241 undergoes rapid electron transfer back to $W241(YO^{-})_{242}$. This reaction can only occur if Y242 attains its deprotonated tyrosinate state at sufficiently high pH. Further experiments are required to support this mechanism of an additional quenching of W241 in the alkaline region.

In order to analyze the possible functional role of the C-terminus of the MSP, reconstitution experiments of oxygen evolution were performed in the absence and presence of the C-terminus peptide. The results obtained are shown in Fig. 3. Two important features emerge

from this data: i) the C-terminus peptide competes with the native MSP for the binding alone

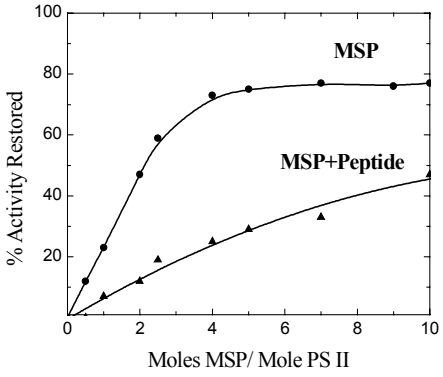

Fig. 3. Reconstitution of the O_2 evolution of the salt-treated BBY particles by the MSP in presence and in absence of the C-terminus peptide.

is not sufficient for restoring the domain of the integral PS II and ii) binding of the C-terminus peptide oxygen evolution. Based on these findings it is concluded that the C-terminus plays a key role for the binding of the MSP and that a larger part of this protein is required for optimum oxygen evolution of PS II. In this respect it is interesting to note that in mildly trypsinized PS II membrane fragments a 15 kDa proteolytic degradation product of the MSP can still retain the oxygen evolution activity (Völker et al. 1985). In forthcoming studies the structural elements of the MSP that are essential for a fully competent oxygen evolution will be analyzed.

Acknowledgments

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