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Studies on the molecular mechanism of PSII oxygen-evolving complex using amino acid modification and high hydrostatic pressure

Y Yu[1], KC Ruan[2], J Weng[1], SP Zhang[1], YL Zou[1], J Li[2], CH Xu[1], Govindjee[3]

[1]*Institute of Plant Physiology, Shanghai Institutes of biological sciences, CAS, 300 Fenglin Road, Shanghai 200032, fax: 86-21-64042385, email: xch@iris.sipp.ac.cn;*

[2]*Institute of Biochemistry, Shanghai Institutes of biological sciences, CAS, 320 Yueyang Road, Shanghai 200031;* [3]*Department of Plant Biology, UIUC, Urbana IL 61801-3707 USA*

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Introduction

In oxygenic photosynthesis, oxidization of water to molecular oxygen is catalyzed by photosystem II (PSII). In addition to the reaction center core, PSII includes a manganese stabilizing protein (MSP, 33 kDa protein) that plays an important regulatory role in the process in plants and algae, although it is not directly involved in the electron transfer. MSP is one of the three extrinsic proteins in PSII, located towards the lumen of the thylakoid membrane. The removal of MSP from PSII particles, by incubation with concentrated Tris/HCl buffer or high pH, results in loss of both Mn cluster and the oxygen-evolving activity. MSP can also be released from the particles by washing with $CaCl_2$ or urea plus NaCl without loosing the Mn cluster. The PSII particles can regain the oxygen-evolving activity by rebinding of the protein. MSP was isolated first from spinach by Kuwabara and Murata (1979). Since then, its structure and function have been extensively investigated. It is composed of 247 amino acid residues with only one tryptophan residue (Trp241) and eight tyrosine residues distributed in different protein-domains. It is possible to explore the environment of the protein by studying changes in the fourth derivative UV absorbance spectra. By using NBS modification, high hydrostatic pressure, and Chl a fluorescence transients, molecular properties of MSP were investigated in this study.

Material and methods

PSII membranes were isolated from market spinach leaves as described by Berthold et al. (1981), with minor modifications (Xu et al., 1995). MSP was purified according to Xu and Bricker (1992). The MSP reconstitution was made following the method of Eaton-Rye and Murata (1989). For high-pressure incubation of the PSII membranes, samples were adjusted to 0.5 mg Chl/ml with SCM buffer (0.4 M Sucrose, 60 mM $CaCl_2$, 50 mM MES-NaOH, pH 6.2). High pressure was produced by homemade equipment according to Paladini and Weber (1981). Pressure was increased at a rate of 60 MPa/min and then kept constant for a desired time at 20°C. Then it was decreased with the same rate. Samples were immediately centrifuged and resuspended with SCM buffer. Protein contents were analyzed with SDS-PAGE in the Laemmli system containing 6 M urea. The abundance of manganese was measured with a Shimadzu atomic absorption spectrometer (AA-6501F). For the high

pressure treatment of MSP, the fluorescence measurements were carried out using either an Aminco Bowman Series 2 (AB2) fluorospectrophotometer (SLM Co.) or a SLM 48000 fluorospectrophotometer (SLM Co.) in which the sample housing was modified to measure protein fluorescence under pressure from 0.1 MPa to 600 Mpa. ΔG were estimated from the determination of the ΔV and $P_{1/2}$ (which is the pressure at which the degree of transition α is 0.5), according to the following expression: $\Delta G = \Delta V \bullet P_{1/2}$. Spin trapping EPR spectroscopy was performed in the presence of 10 mM 5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide (DEPMPO). X-band EPR spectra were recorded at room temperature on a Varian E-112 spectrometer at 9.17 GHz and 100KHz field modulation.

Results and discussion

Reconstitution of modified MSP

Trp241 of the MSP was modified with N-bromosuccinimide (NBS), a frequently used chemical in the modification of tryptophan residues in proteins for its special reaction with tryptophan at acidic pH. The affinity of the MSP to PSII decreased greatly after the modification of Trp241, and no oxygen-

Table 1. Activity of oxygen evolution, measured after the reconstitution of the urea/NaCl-washed PSII membranes with the MSPs. Measured at 25°C. Chlorophyll concentration, 10 μg ml^{-1}. 0.8 mM 2,6-dimethyl-*p*-benzoquinone was used as the artificial electron acceptor. (Reproduced from Yu et al., 2001 a)

Samples	Retained 33kDa protein (%)	Oxygen-evolving activity ($\mu mol\ mg^{-1}$ Chl h^{-1})	Oxygen-evolving activity (%)
Control PSII membranes	100	234	100
Urea/NaCl-washed PSII	0	45	19
Urea/NaCl-washed PSII + unmodified MSP (MSP: PSII=8 mol:1 mol)	94	116	50
Urea/NaCl-washed PSII + NBS-modified MSP (MSP:PSII=20 mol:1 mol)	59	49	21

evolving activity was recovered after its reconstitution (Table 1; also see Yu et al., 2001-a). The pH-dependence of the modification, the comparison of features of fluorescence spectra and hydropathy plot suggested that Trp241 is buried in the middle of the hydrophobic region at the C-terminus of MSP. It demonstrated that the C-terminus hydrophobic region of MSP is critical for maintaining its structure and function (Lydakis-Simantiris et al., 1999; Yu et al., 2001 a). Our CD spectra results showed that NBS modification of Trp241 dramatically modified the secondary structure of MSP in solution.

Release of extrinsic proteins by high hydrostatic pressure

We found that three extrinsic proteins and manganese cluster were sequentially released from the membrane when PSII membranes were kept under high hydrostatic pressure. The 17 kDa protein was the most sensitive, while the MSP was the most reluctant to the treatment with high pressure. Results also indicated that the release of manganese was not simply correlated with the loss of MSP. The loss of the oxygen-evolving activity of PSII was synchronized with the release of extrinsic proteins and manganese (Yu et al, 2001 b).

Effects of high hydrostatic pressure: folding and unfolding of MSP

Based on a study of the MSP folding-unfolding with guanidine hydrochloride (GdmCl), Tanaka et al. (1989) concluded that the MSP has a very low free energy upon unfolding (-11.7 kJ•mol^{-1} or -18.4 kJ•mol^{-1} obtained from two different calculation methods). This suggested that rather mild pressure could modulate its unfolding transition. With an excitation at 295 nm, the intrinsic fluorescence of the MSP is mainly due to the tryptophan residue emission. As the pressure was increased to 180 MPa, the protein emission spectra, in the region of 300 to 340 nm, showed a gradual decrease in fluorescence intensity and then a red shift. The peak was shifted from ~316 nm to ~350 nm, the latter being of lower intensity than the former (Figure 1, left). Higher pressures up to 600 MPa did not induce any further significant change in the fluorescence spectra, indicating that a stable transition state induced by pressure-unfolding is obtained. The increase of fluorescence intensity is sigmoidal, implying that the pressure-unfolding process can be described as a two-state transition. The

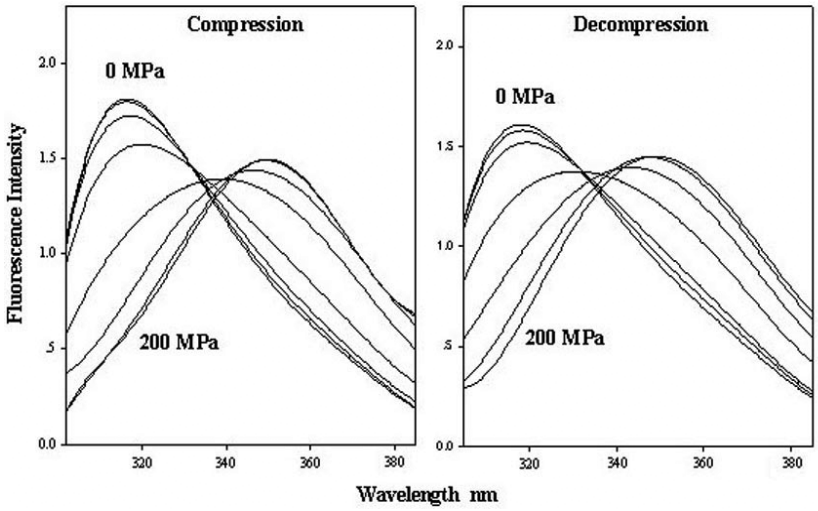

Figure 1. fluorescence emission spectra of MSP upon excitation at 295 nm (exciting only tryptophan) as a function of pressure (compression/decompression). Solution conditions: MSP at 0.1 mg/mL in MES buffer, 0.05 M, pH 6.0, temperature=20˚C (Reproduced from Ruan et al., 2001)

free energy (ΔG) and the standard volume change (ΔV) for the transition at pH 6.0 and 20°C are -14.6 kJ• mol^{-1} and -120 mL• mol^{-1} respectively, in which the ΔG value is consistent with that obtained by chemical denaturation. However, the free energy for the trypsin transition from the native state to the molten globule like state is -146.5 kJ• mol^{-1} (Ruan et al., 1998) with a corresponding pressure-transition at 650 Mpa. The unfolding transition induces, as noted above, a 34 nm red shift of the maximum fluorescence emission (from 316 nm to 350 nm). The observed spectral changes are reversed as the pressure is released (figure 1, right). Further, the oxygen-evolving activity of the reconstituted PSII with the MSP treated with a pressure of 200 MPa is almost the same for that without pressure treatment, indicating also that the unfolding transition of the 33kDa protein by pressure is reversible (Ruan et al., 2001).

Chlorophyll a fluorescence changes and other experiments

The level of maximum chlorophyll a fluorescence remained almost unchanged when all the three extrinsic proteins were released from the PS II membrane. Only when the manganese cluster was removed, chlorophyll a fluorescence decreased dramatically (data not shown). In spinach PSII membranes and three PSII subcomplexes, direct evidence of superoxide formation, in the process of both acceptor- and donor-side-induced photoinhibition of PSII

(under 2000 μE m^{-2} s^{-1}), was obtained by the formation of DEPMPO superoxide adducts (DEPMPO-OOH), using spin trapping EPR spectroscopy.

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