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BRIEF COMMUNICATION

The release of extrinsic polypeptides and manganese cluster from photosystem 2 membranes under high hydrostatic pressure

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Abstract

Three extrinsic polypeptides and manganese cluster were sequentially released from the membrane when photosystem 2 (PS2) membranes were kept under high hydrostatic pressure. The 17 kDa polypeptide was the most sensitive, while the 33 kDa polypeptide was the most reluctant to the treatment with high pressure. The release of manganese was not simply correlated with the loss of 33 kDa polypeptide. The losing of oxygen-evolving activity of PS2 was synchronised with the releasing of extrinsic polypeptides and manganese.

Additional key words: oxygen evolution; spinach; *Spinacia oleracea*.

Photosystem 2 (PS2), a multisubunit pigment-protein complex, catalyses the photon-driven oxidation of water to molecular oxygen and the reduction of plastoquinone to plastoquinol. At the electron donor side of PS2, a cluster of four manganese atoms plays a crucial role in the oxygen evolution. Meanwhile, three extrinsic polypeptides with apparent molecular masses of 17, 23, and 33 kDa at the lumen side of PS2 membrane are also essential for efficient oxygen evolution by PS2 (Seidler 1996). The 17 and 23 kDa polypeptides regulate the requirement of calcium and chloride in PS2. The 33 kDa polypeptide, the manganese-stabilising protein, stabilises binding and maintains functional conformation of the manganese cluster (Bricker and Frankel 1998). Usually, extrinsic polypeptides can be removed from the PS2 membrane with methods using high concentrations of salt (NaCl or $CaCl_2$), urea, Tris (pH 8.0), or heat treatment (Seidler 1996). High concentration of trichloroacetate also may be used to release extrinsic polypeptides (Xu *et al.* 1995). Different treatments caused different alteration on the functional structure of the donor side of PS2.

Currently, high hydrostatic pressure technique is applied actively in the biological research and biotechnology (Mozhaev *et al.* 1994, 1996). It was frequently used as a method to study intramolecular or intermolecular interactions in biosystems, and showed advantages especially in researches of unfolding of oligomeric protein and dissociation of multiprotein (Mozhaev *et al.* 1996). In this study, PS2 membranes were incubated under high hydrostatic pressure. With increasing the pressure, three extrinsic polypeptides and manganese cluster were sequentially released from the PS2 membranes. Consequently, the oxygen-evolving activity of PS2 was gradually lost.

PS2 membranes were isolated from spinach (*Spinacia oleracea* L.) leaves with *Triton X-100* following the method of Berthold *et al.* (1981). The high-pressure equipment was home made following Paladini and Weber (1981). For high-pressure incubation, PS2 membranes were diluted to a concentration of 0.5 kg(Chl) m^{-3} with SCM buffer (0.4 M sucrose, 60 mM $CaCl_2$, 50 mM MES-NaOH, pH 6.2). Then membranes were filled into soft Eppendorf tubes, and placed into a sample cavity. A water bath was linked to control the sample temperature of 20 °C. Pressure was increased with a rate of 1 MPa s^{-1} and then kept constant for a desired time. After measure-

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Abbreviations: Chl, chlorophyll; DMBQ, 2,6-dimethyl-*p*-benzoquinone; PS2, photosystem 2; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.
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ments, it was depressed with the same rate. Samples were immediately centrifuged at 40 000×*g* for 15 min after the depressing, and re-suspended with the SCM buffer to reach a final concentration of 1.0 kg(Chl) m^{-3}. Control samples were treated following the same procedures under air pressure.

For reconstitution, PS2 membranes were incubated under 250 MPa for 2.5 h to remove all the extrinsic polypeptides. 33 kDa polypeptide, purified from freshly prepared PS2 membranes following the method of Xu and Bricker (1992), was then added to meet a protein-to-Chl ratio of 0.4 : 1.0 (m:m). The final concentration of Chl in the reaction system was adjusted to 0.1 kg m^{-3} with the SCM buffer. After a dark incubation at 4 °C for 30 min, it was centrifuged at 40 000×*g* for 20 min. Pellets were washed twice with the SCM buffer to remove the loosely bound 33 kDa polypeptide.

Protein contents were analysed with SDS-PAGE in the Laemmli (1970) system containing 6 M urea. A slab gel containing 5.00 (stacking gel) and 13.75 % (resolving gel) acrylamide was used. The densitogram of the gel stained in Coomassie Brilliant blue R-250 was obtained using a Digital Imaging System (*IS-1000*), and the relative amounts of 17 and 23 kDa polypeptides were determined by integrating the peak areas. Since the quantification of 33 kDa polypeptide is always disturbed by D1 and D2 polypeptides, whose positions are close to that of 33 kDa polypeptide on electrophoresis gel, immunodetection was designed. Polypeptides were electro-transferred to a nitrocellulose paper after electrophoresis, and the Western-blot was done following the conventional method with anti-33 kDa polypeptide antiserum of rabbit. Then the density of blots was scanned for further quantification. The abundance of manganese was measured with a *Shimadzu* atomic absorption spectro-meter (*AA-6501F*). Before measurement, samples (20 mm^3) were dried at 105 °C for 30 s, ashed at 1 000 °C for 20 s, and atomised at 2 500 °C for 3 s.

The oxygen-evolving activity of PS2 membranes was measured with a Clark-type oxygen electrode in the SCM buffer at 25 °C. The Chl concentration in the reaction medium was 10 g m^{-3}. 0.8 mM DMBQ was used as the artificial electron acceptor.

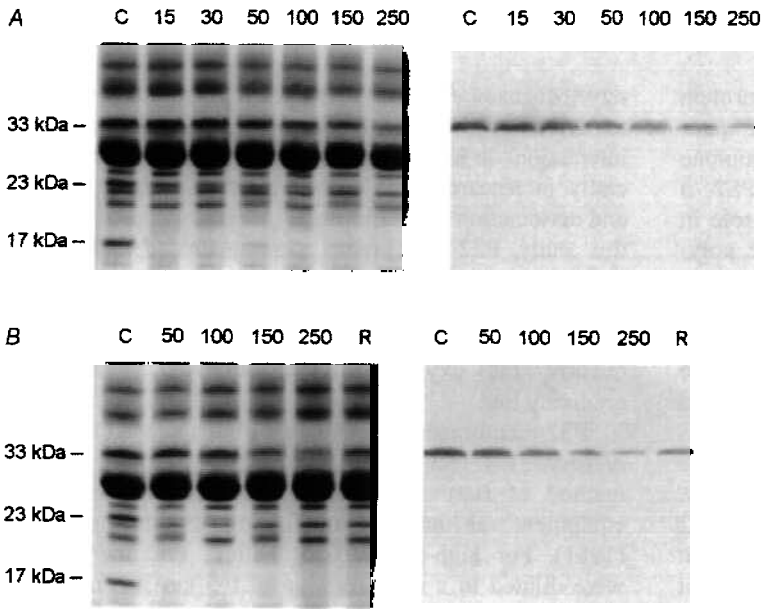

Fig. 1. SDS-PAGE and immunodetection of PS2 membranes that were pre-incubated under high pressure for either 30 (*A*) or 150 (*B*) min. The *left panels* are SDS-PAGE (resolving gel: 13.75 %), and the *right ones* are the Western-blot analysis with anti-33 kDa polypeptide rabbit antiserum. Lines labelled C stand for the control PS2 membranes, while R for 33 kDa polypeptide-reconstituted PS2 membranes. Numbers represent high pressure in MPa.

After PS2 membranes were incubated under high pressure, extrinsic polypeptides were sequentially released from membranes (Fig. 1). The 17 kDa polypeptide was released completely after PS2 membranes were incubated under 15 MPa pressure for 30 min (Fig. 1*A*). 23 and 33 kDa polypeptides were released afterwards with different responses to pressure (Fig. 2*A,B*). Comparison of the remaining contents revealed that the binding of 33 kDa polypeptide with PS2 membrane was more tight than that of 23 kDa polypeptide. More 23 kDa polypeptide was released when incubation time was increased from 30 to 150 min, while the release of 33 kDa polypeptide changed little (cf. Fig. 2*A* with 2*B*). In addition, no free pigment was detected in the suspension after high-pressure treatment.

With the release of extrinsic polypeptides, the oxygen-evolving activity of PS2 membranes changed after high-pressure treatment. Release of 17 and 23 kDa

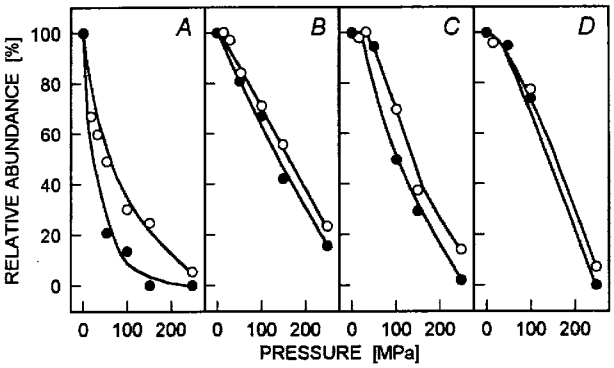

Fig. 2. Relative abundance of 23 kDa (*A*) and 33 kDa (*B*) polypeptides, the oxygen-evolving activity (*C*), and manganese content (*D*) in PS2 membranes that were pre-incubated under high pressure for either 30 (○) or 150 (●) min. A ratio of 3.96 manganese atoms per 260 chlorophyll molecules was obtained for the control PS2 membrane.

polypeptides had little influence on the oxygen-evolving activity, when high concentration of Cl^- was included in the buffer. Oxygen-evolving activity of PS2 decreased correspondingly with the release of 33 kDa polypeptide. When the 33 kDa polypeptide is completely removed, the oxygen-evolving activity of PS2 is about 20 % of the normal value (Bricker 1992). After PS2 membranes were treated under 250 MPa for 150 min, 16 % of 33 kDa polypeptide remained (Fig. 2*B*), but the oxygen-evolving activity of PS2 was only 3 % of the original value (Fig. 2*C*). The activity of oxygen evolution did not recover after 50 % of 33 kDa polypeptide was reconstituted onto the PS2 membranes (Fig. 1*B*). The high-pressure treatment might affect not only binding of extrinsic polypeptides, but also intrinsic structure of PS2.

Also the Mn cluster was released with the incubation under high pressure (Fig. 2*D*). Little manganese remained after incubating PS2 membranes under 250 MPa for 150 min, causing the completely loss of oxygen-evolving activity of PS2. High concentration (>100 mM) of Cl^- can replace the role of 33 kDa polypeptide to preserve the Mn cluster (Miyao and Murata 1984). Since we included 120 mM Cl^- in the buffer throughout the treatment, the release of Mn cluster must be caused by the influence of high pressure rather than the release of 33 kDa polypeptide.

Comparing with other methods, high pressure is a gentle way to remove the extrinsic polypeptides from the PS2 membrane, avoiding the violent influences on the polypeptides and membrane after addition of chemical reagent. Furthermore, controlling the pressure and other experimental conditions is able to regulate the remaining contents of extrinsic polypeptides and manganese.

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