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The D1 protein, CP43 and OEC33 are the neighboring polypeptides at the lumenal side of PS II : evidence from a photo-induced cross-linking study

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Introduction

Photosystem (PS) II is the site of photosynthetic water oxidation. The PS II complex is composed of more than 25 polypeptides, which are either integrated in the thylakoid membrane as the intrinsic components of the PS II complex or extrinsically bound to the complex. Recent studies using electron microscopy, electron crystallography, and X-ray crystallography have revealed the spatial organization of the core subunits of PS II (Ree et al. 1998, Zouni et al. 2001). In spite of the progress of the research, however, there is much speculation over the relative location of the PS II core subunits and the extrinsic oxygen-evolving subunit proteins (OEC33, 24 and 18). Here, we studied cross-linking between that the D1 protein and CP43 in donor-side photoinhibition of PS II. The cross-linking was prevented when the OEC33 was reconstituted to the OEC33-depleted membranes before illumination. From these results, we suggest the relative location of D1, CP43 and OEC33 at the lumenal side of PS II.

Materials and Methods

PS II-enriched membranes were prepared from spinach by the treatment of thylakoids with Triton X-100. To impair the water-splitting system of PS II, the samples were washed with 0.8 M Tris (pH 9.3) or 5 mM hydroxylamine (pH 6.5) as described previously (Yamamoto and Akasaka 1995). All the procedures were carried out in darkness at 4˚C under the safe light. For isolation of thylakoid lumen fraction, intact thylakoids were ruptured with a French pressure cell, and were centrifuged at 200,000 g for 20 min. The supernatant obtained was used as a thylakoid lumen fraction. Isolation of the OEC33 and the reconstitution of the OEC33 with Tris-treated PSII-enriched membranes were carried out as described previously (Yamamoto and Akasaka 1995). Anaerobic conditions were obtained with glucose, glucose oxidase and catalase. Photoinhibitory illumination of the PS II samples, SDS/urea-PAGE and Western blot analysis were carried out as described previously (Ishikawa et al. 1999).

Results

Cross-linking of the D1 protein and CP43 in the donor-side photoinhibition

Tris- or hydroxylamine-treated PS II membranes and native PS II membranes were illuminated under either aerobic or anaerobic conditions, and they were subjected to SDS/urea-PAGE and Western blot analysis with the antibody against the D1 protein (Fig.1A). By the illumination of the native PS II membranes under aerobic conditions, cross-linked products D1/D2, D1/theα-subunit of Cytb_{559} and D1/CP43 appeared, depending on the light intensity. The cross-linking was more prominent in the Tris- or hydroxylamine-treated PS II

membranes. In contrast to the illumination under aerobic conditions, no cross-linked products were detected under anaerobic conditions in the native and hydroxylamine-treated PS II membranes. However the cross-linked bands of D1/CP43 were still observed in the Tris-treated membranes under anaerobic conditions (Fig.1B). The major difference between the Tris-washed and hydroxylamine-washed membranes is the absence of OEC subunits in the former samples. These results strongly indicate that the OEC subunits prevent the cross-linking between D1 and CP43. It is noted here that degradation products were not observed under anaerobic conditions, regardless of the samples used in the experiments. These results raise a possibility that oxygen is required for efficient degradation of the D1 protein not only in the acceptor-side photoinhibition, but also in the donor-side photoinhibition.

Reconstitution of the OEC33 to the Tris-treated PSII membranes

Tris-treated PS II membranes were reconstituted with the OEC33 and illuminated with strong light under anaerobic conditions (Fig.2A). With increasing the OEC33, the amount of the protein bound to the PSII membranes increased. The light-induced cross-linking of the D1 protein with CP43 was completely suppressed in the presence of a saturating amount of the OEC33 (Fig.2B).

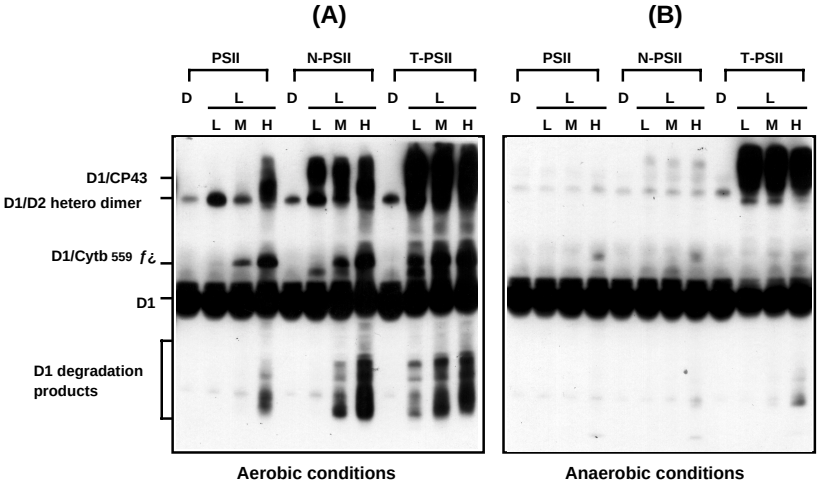

Fig. 1. Cross-linking of the D1 protein and CP43 in the donor-side photoinhibition. Native PS II membranes (PS II) and Tris- or hydroxylamine-treated PS II membranes (T- PS II, N- PS II) were illuminated with high light (H, 4000 μE m^{-2} s^{-1}), medium light (M, 200 μE m^{-2} s^{-1}) and low light (L, 20 μE m^{-2} s^{-1}) for 60 min under either aerobic (A) or anaerobic (B) conditions at 25 ^{o} C The samples were subjected to SDS/urea-PAGE and Western blot analysis with the specific antibody against the C-terminal part of the D1 protein.

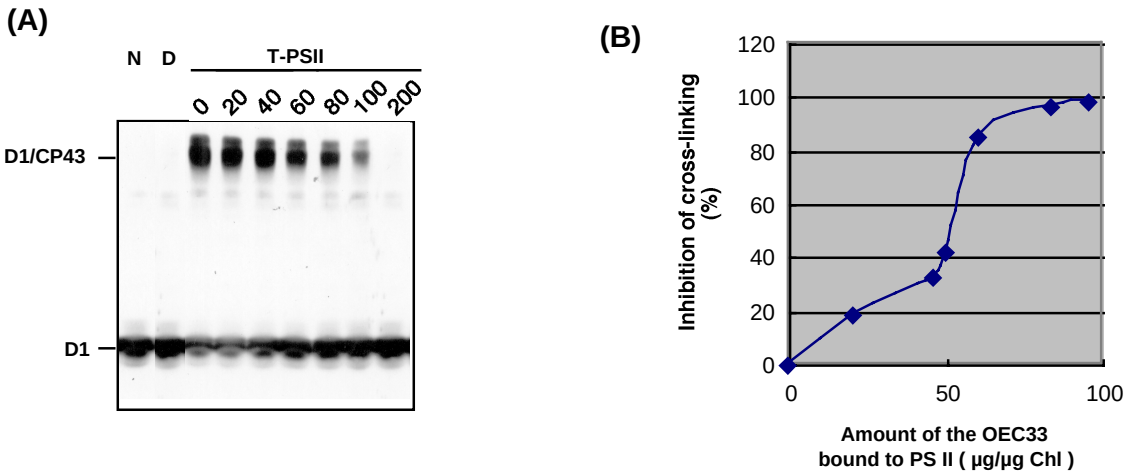

Fig. 2. Reconstitution of the OEC33 to the Tris-treated PS II membranes and the effects of reconstitution on cross-linking of the D1 protein by donor-side photoinhibition. Native PS II membranes (N) and Tris-treated membranes (T- PS II) reconstituted with the OEC33 were illuminated with strong light (2500 µE m^{-2} s^{-1}) for 30 min under anaerobic conditions at 25 ^{o} C. The amount of the OEC33 added to PS II (µg OEC33 added/ml of the reaction mixture) is shown at the top of each lane. D denotes the dark control. The samples were subjected to SDS/urea-PAGE and Western blot analysis with specific antibody against the DE-loop of the D1 protein (A). Relationship between the binding of the OEC33 to PS II and inhibition of the cross-linking is shown in (B).

Discussion

Our present data show that closely located D1 and CP43 cross-link with each other during the donor-side photoinhibition of PS II. The OEC33 prevents the cross-linking almost completely, indicating that the OEC33 is also located in the close neighborhood of these intrinsic proteins. The nearest location of the D1 protein and CP43 was shown previously by the cross-linking study in the acceptor-side photoinhibition where the cross-linking sites are at the stroma-exposed loops of D1 and CP43 (Yamamoto and Akasaka 1995, Ishikawa et al. 1999). In the present study, we showed that the D1 protein, CP43 and the OEC33 are located closely at the lumenal side of PS II. As the OEC33 is a structurally extended protein with enriched β-sheet content, the protein probably shields the surface of the D1 protein and CP43, and should protect the both proteins from photo-induced cross-linking. The present results support the recently published three-dimensional structure of PSII showing the spatial arrangement of these components, which were derived from the x-ray diffraction analysis of the crystal structure of PS II from *Synechococcus elongatus* (Zouni et al. 2001). It is highly probable that the D1 protein, CP43 and OEC33 form a special domain for the catalytic Mn-cluster which is bound to the D1 protein and is responsible for water oxidation in PS II.

References

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