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The extrinsic 18-kDa protein in photosystem II restores the ion-retention activity of a mutant extrinsic 23-kDa protein lacking 19 amino-acid residues on the amino terminus.

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Introduction

The photosynthetic oxygen-evolving complex (OEC) in photosystem II (PS II) participates in the use of strong oxidants produced in the light to oxidize water, forming molecular oxygen as a by-product. This process is catalyzed with the Mn_4 cluster surrounded by at least three extrinsic subunits bound to the lumenal surface of PS II reaction center. In all types of oxygenic photosynthetic organisms, OEC33 (named for its molecular weight) encoded by the *psbO* gene stabilizes the Mn_4 cluster. Plants and green algae have two other OEC polypeptides: OEC23 (PsbP) and OEC18 (PsbQ). OEC23 is involved in both Ca^{2+} and Cl^- retention, essential cofactors for water-splitting reaction. OEC18 is reported to enhance Cl^- retention in the reaction center at a low Cl^- condition (for review, see Seidler 1996*)*.

Among OEC polypeptides, OEC33 was most intensively analyzed through limited proteolysis, chemical modification or site-directed mutagenesis. Molecular genetics using cyanobacteria also promotes the studies to characterize the function of OEC33. On the other hand, information of the functional domains, binding regions or important amino acid residues for ion retention about OEC23 and OEC18 is so far limited. We have tried to elucidate the molecular mechanism of ion retention by OEC23 and reported that amino-acid sequence of N-terminal putative domain in OEC23 was important for the activity of ions retention (Ifuku & Sato 2001). Here, we further confirm the importance of N-terminal sequence of OEC23 for ion retention by using deletion mutant of OEC23 lacking 19 amino-acid residues on the N-terminus. The possible cooperation of OEC23 and OEC18 in ion retention is also discussed.

Materials and methods

Spinach leaves used to prepare PS II membrane were purchased at the local market. PS II membrane was prepared as reported elsewhere (Ghanotakis et al. 1984). O_2 evolution was measured at 25 ^oC with a Clark-type O_2 electrode (Hansatech, UK) in the presence of 2 mM DMBQ as the electron acceptor for PS II. Red actinic light, at an intensity of 2 mE m^{-2} s^{-1}, was provided by an incandescent lamp that was used in conjunction with HA50 heat-absorbing filter and an R-60 red optical filter (Kenko, Tokyo, Japan). The concentrations of Ca^{2+} and Cl^- in the buffer for measurements were confirmed by the atomic-absorption spectrometer (AA-640-12, Shimadzu, Kyoto, Japan) and the ion-meter with a chloride-selective electrode (ME-20E, TOA, Tokyo, Japan), respectively.

Recombinant OEC23s were expressed in *E. coli* by T7 phage RNA polymerase-driven system (pET) (Ifuku et al. 1998). Reconstitution of the extrinsic polypeptides to the NaCl-washed PS II membrane was previously described (Ifuku & Sato 2001).

Results and Discussion

N-terminal 19 residues of OEC23 are indispensable for ion retention.

OEC23 Δ18 was produced using the methionine at position 19 of OEC23 as the first Met of translation. N-terminal amino acid sequence determination showed that OEC23 Δ18 lost initial Met during the expression and purification process. OEC23 Δ18 used in this study is thus a mutant OEC23 lacking 19 residues on the N-terminus. Reconstitution of OEC23 Δ18 to salt-washed PS II membrane lacking OEC23 and OEC18 showed that OEC23 Δ18 completely lacked the ability to restore PS II activity. SDS-PAGE and immunoblotting of reconstituted PS II membrane confirmed that it bound to PS II membrane. An increase in the amount of protein in the reconstitution experiment did not lead to an increase in PS II activity. Circular dichroism spectrum of OEC23 Δ18 indicated the similar overall structure to OEC23. Since the deletion of N-terminal 9 residues of OEC23 by chymotrypsin digestion didn't prevent the function of OEC23 (Miyao M et al. 1988), the critical amino acid residues for ions retention should exist between G10 and F19 (Fig. 1). Residues in this region that are highly conserved among higher plant OEC23s are G10, K11, K13, and T16. Further analysis of these amino acid residues by site-directed mutagenesis should reveal the critical amino acid(s) indispensable for ion retention.

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                        Q
                   E    L
                   S    VT
                 A TD DYIA
NH2-AYGEAANVFG KPKKNTEFMP------
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Fig.1 The amino-terminal amino acid sequence of spinach OEC23. Varieties of amino acids found in other plant species are also shown. The sequence data are from: spinach (X05511), cucumber (AB03225), tobacco 2AF (X62427), arabidopsis (X98108), pea (X15552), tomato (X63007), wheat (X57407), and rice (D49713).

OEC23 Δ18 could cooperate with OEC18 in the retention of both Cl^- and Ca^{2+}.

Since OEC23 is necessary for the tight binding and functioning of OEC18 in PS II complex (Miyao & Murata 1989), we examined whether bound OEC23 Δ18 cooperated with OEC18. When OEC18 was rebound together with OEC23, the PS II activity was markedly increased, especially when it was measured without ions in the assay medium (Fig 2a). OEC18 bound to PS II without OEC23; however, this binding hardly restored the PS II activity and the amount of bound OEC18 was lower than that with OEC23 (Fig. 2b). Interestingly, OEC23 Δ18 restored PS II activity in the presence of OEC18, although the restored activity was lower than that with OEC23 and OEC18 (Fig.2d). This difference could be due to the amount of bound OEC18, however, we cannot exclude the possibility that the N-terminal 19 residues are necessary for the optimum activity. OEC18 complemented the function of OEC23 Δ18 even in the presence of 20 mM NaCl, which is a saturating concentration of Cl^- for oxygen-evolution. Our data suggests that OEC18 cooperates with OEC23 in the retention of both Ca^{2+} and Cl^-.

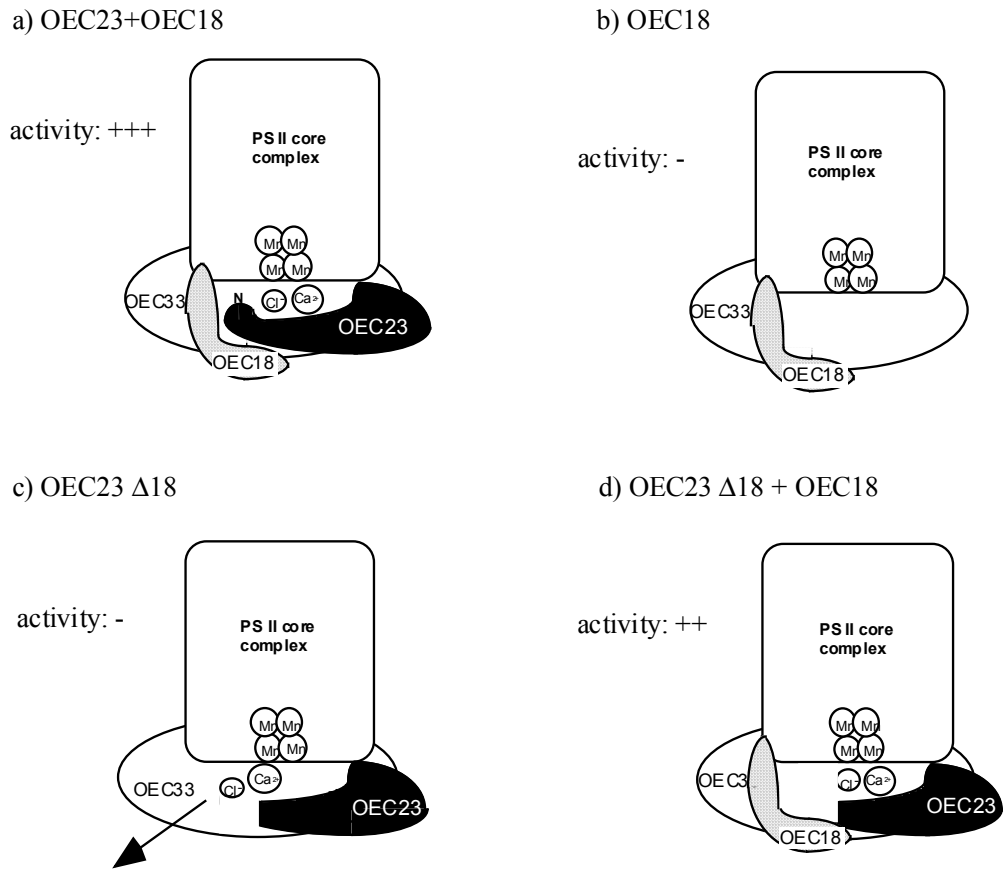

Fig. 2. A model for OEC23 and OEC18 function in ion retention. a) Intact OEC23 and OEC18 cooperate to retain Ca^{2+} and Cl^- near the Mn cluster. OEC23 by itself can retain Ca^{2+} and Cl^-; however, OEC18 will stabilize this function. b) OEC18 alone is not sufficient to retain ions for PS II activity. c) The deletion of N-terminal 18 residues of OEC23 completely eliminates the activity for ion retention and ions are not retained in PS II complex. d) OEC18 compensates the role of N-terminal 19 residues of OEC23 at least in part. The numbers of symbols representing Ca^{2+} and Cl^- do not show the actual number of these ions in the PS II complex.

What is the physiological function of OEC18?

Previous studies concluded that OEC18 was involved in only Cl^- retention because the PS II activity restored by OEC18 binding was small in the presence of saturating amount of Cl^- (Akabori K et al. 1984 and Miyao M et al. 1985). However, the Cl^- concentration in stomata ranges from 30 to 60 mM in spinach chloroplasts and the thylakoid membrane is very permeable to Cl^-, the Cl^- concentration in the intrathylakoid space is likely to be within a range in which OEC18 is unnecessary for oxygen evolution (Miyao M et al. 1985). Thus, the physiological function of OEC18 has been unclear. The fact that OEC18 partially complemented the function of OEC23 Δ18 provides an insight: OEC23 and OEC18 would have a common function in the water-splitting process and thus cooperate to optimize the Ca^{2+} and Cl^- concentrations, although OEC18 absolutely required OEC23 for the tight binding and functioning in PS II complex (Miyao & Murata 1989). Structural information regarding the PS II core complex and OEC proteins is necessary to fully understand our results. X-ray crystallographic studies about OEC23 crystals were currently undergoing for the further 3D structural studies.

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