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Reconstitution of the extrinsic 23 and 17 kDa proteins with spinach PSII which had been exchanged for the 33 kDa protein from different plant species

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Introduction

The 33 kDa protein of photosystem II (PSII) plays an important role in maintaining function of the Mn cluster, and is common to all of the organisms. Cross-reconstitution experiments have shown that the 33 kDa protein is totally exchangeable in binding to PSII from various different organisms (Enami et al., 2000). The amino acid sequence of the 33 kDa protein showed a relatively high homology above 40% from cyanobacteria to higher plants. Thus, the structure of the 33 kDa protein has been considered to be conserved during evolution from cyanobacteria to higher plants. However, it has been reported that the protease-cleavage sites of the 33 kDa protein are different among cyanobacteria, red algae and higher plants (Tohri et al. 2001, and references cited therein). In terms of the protease-cleavage pattern, it has been suggested that the red algal 33 kDa protein resembles the cyanobacterial one but not the higher plant one (Tohri et al. 2001). In addition to the 33 kDa protein, higher plant PSII contains two other extrinsic proteins of 23 kDa and 17 kDa; we have shown previously (Enami et al. 2000) that these two proteins bound to cyanobacterial and red algal PSII only through non-specific interactions. These facts suggest that the functional binding sites for these two proteins have been newly developed in the higher plant PSII. Thus, it would be interesting to study the possible interaction of the 33 kDa protein from different sources with the other two extrinsic proteins, in order to elucidate the structural difference of the 33 kDa protein among different plant species. Here, we report cross-reconstitution experiments of the 23 kDa and 17 kDa proteins with spinach PSII which had been exchanged with the 33 kDa protein from different sources.

Materials and methods

Oxygen-evolving PSII complexes were prepared from spinach, a red alga *Cyanidium caldarium* (Enami et al. 1998) and a thermophilic cyanobacterium *Synechococcus vulcanus* (Shen et al. 1993) as described previously. Various extrinsic proteins from three PSII complexes were purified as described in Shen et al. (1993) and Enami et al. (2000).

Spinach PSII complexes were treated with 2.6 M urea/0.2 M NaCl to remove the three extrinsic proteins. The treated PSII was reconstituted with the 33 kDa protein of various sources first, and then used to reconstitute with the spinach 23 kDa and 17 kDa proteins. Reconstitution experiments were performed according to Enami et al. (2000). For convenience, we name the three extrinsic proteins of spinach as H33, H23 and H17, and the red algal and cyanobacterial 33 kDa protein as R33 and C33, respectively.

Oxygen evolution was measured with a Clark-type oxygen electrode at 25 °C with 0.4 mM phenyl-*p*-benzoquinone as electron acceptor in the absence or presence of 10 mM NaCl or 5 mM $CaCl_2$. SDS-PAGE was performed according to Enami et al. (2000).

Results and discussion

Table 1 shows the binding abilities of the extrinsic 23 kDa (H23) and 17 kDa (H17) proteins with spinach PSII in which the 33 kDa protein (H33) had been replaced with the red algal 33 kDa (R33) or cyanobacterial 33 kDa (C33) protein. Consistent with the previous results (Enami et al. 2000), all of the 33 kDa proteins from three different sources completely bound to spinach PSII which had been depleted of its three extrinsic proteins. Both H23 and H17 bound completely to the spinach PSII which had been reconstituted with H33. H23, however, bound partially to the spinach PSII which had been reconstituted with R33 or C33. Furthermore, H17 did not bind to spinach PSII carrying R33 or C33 at all.

Table 1 Binding of the extrinsic 23 kDa (H23) and 17 kDa (H17) proteins from spinach with the spinach PSII in which the 33 kDa protein (H33) had been replaced with red algal 33 kDa (R33) or cyanobacterial 33 kDa (C33) protein. Relative binding abilities of the extrinsic proteins were shown: "+++" represents effective binding; "-" represents no binding.

	Binding of H23 and H17	
	H23	H17
H33-bound spinach PSII	+++	+++
R33-bound spinach PSII	++	-
C33-bound spinach PSII	++	-

The restoration of oxygen evolution upon reconstitution with various extrinsic proteins was shown in Table 2. Reconstitution of the 33 kDa protein alone, either from higher plant, red alga or cyanobacterium, resulted in no restoration of oxygen evolution in the absence of Cl^- and Ca^{2+} ions. The activity showed a partial recovery when it was measured in the presence of Cl^-; this recovery increased further when it was measured in the presence of both Cl^- and Ca^{2+} ions. The extent of the activity recovery was similar upon reconstitution with either H33, R33 or C33. Reconstitution of H33-bound spinach PSII with H23 and H17 resulted in an appreciable restoration of oxygen evolution in both the absence and presence of Cl^- and Ca^{2+}. However, reconstitution of R33- or C33-bound spinach PSII with H23 and H17 only slightly restored the activity in the absence of Cl^- and Ca^{2+} ions; a remarkable Cl^- and Ca^{2+} requirement for oxygen evolution still remained in these reconstituted PSIIs. This is in support of the above results that H23 partially bound and H17 did not bind to R33- or C33-bound spinach PSII. These results indicate that unlike H33, R33 and C33 are unable to support the effective and functional binding of H23 and H17 to spinach PSII. This suggests that the 33 kDa protein has a different structure between higher plants and red algae or cyanobacteria, at least from the viewpoint of its interaction with H23 and H17.

Table 2 Restoration of oxygen evolution upon reconstitution of the spinach 23 kDa and 17 kDa proteins to spinach PSII which had been exchanged for the 33 kDa protein from different species. Oxygen-evolving activities of control PSII (100) were 447, 464 and 508 μmole O_2/mg chl/h, respectively, in the absence or presence of 10 mM NaCl or 5 mM $CaCl_2$.

	Oxygen evolution (Relative value %)		
	- ion	+ 10mM NaCl	+ 5 mM $CaCl_2$
Spinach PSII (Control)	100	100	100
Urea/NaCl-washed PSII	0	0	1
+ H33	0	16	50
+ H33 + H23 + H17	52	65	68
+ R33	0	16	45
+ R33 + H23 + H17	3	25	53
+ C33	0	15	43
+ C33 + H23 + H17	6	31	54

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