S9-013

Distribution of extrinsic proteins among various organisms as an index of evolution of oxygen-evolving PSII

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Keywords: evolution, oxygen-evolving PSII, extrinsic proteins, antibody

Introduction

Oxygen-evolving PSII complex contains different extrinsic proteins functioning to maintain its activity and stability among cyanobacteria, red algae and higher plants. Cyanobacterial and red algal PSIIs contain 33 kDa, 12 kDa proteins and cytochrome c550 (cyt c550) as their extrinsic proteins (Shen et al. 1993, Enami et al. 1995), whereas PSIIs of green algae and higher plants contain the 33 kDa, 23 kDa and 17 kDa proteins (for review, see Seidler 1996). In addition, red algal PSII contains a unique 20 kDa protein (Enami et al. 1998). This implies that cyt c550 and the 12 kDa protein were replaced by the 23 kDa and 17 kDa proteins during evolution from cyanobacteria and red algae to green algae and higher plants, and the 20 kDa protein appeared during evolution from cyanobacteria to red algae and disappeared from red algae to green algae. The distribution of these extrinsic proteins among various organisms, therefore, provides a clue to elucidate the evolutionary process of the oxygen-evolving complex. In this study, we prepared the antibodies raised against the 20 kDa, 12 kDa proteins and cyt c550 from red algal PSII complex and examined their cross-reactivity with PSIIs and/or thylakoid membranes from cyanobacteria, red algae, diatom, brown algae, green algae and higher plants.

Materials and methods

Red algal PSII was prepared from an acidophilic red alga, *Cyanidium caldarium* (Enami et al. 1995). The extrinsic 20 kDa, 12 kDa proteins and cyt c550 were purified from the red algal PSII with a DEAE-Toyopearl 650M column (Enami et al. 1998). The genes encoding the 20 kDa, 12 kDa proteins and cyt c550 were cloned and sequenced by means of PCR and a rapid amplification of cDNA ends (RACE) procedure. Their cloned genes were expressed in *Escherichia coli* and the resulting proteins were purified with His-bind

resin and calmodulin-affinity column. The recombinant protein of cyt c550 was an apoprotein to bind no heme c. These recombinant proteins were used for preparation of the antibodies against the extrinsic 20 kDa, 12 kDa proteins and cyt c550 of red algal PSII. The resultant antibodies specifically cross-reacted with each of the corresponding proteins. The antibodies raised against the extrinsic 23 kDa and 17 kDa proteins from spinach PSII were generously provided by Prof. T. Horio and Dr. T.

Kakuno. PSII complexes from a cyanobacterium, *Synechococcus vulcanus*, were prepared according to Shen et al. (1993). Thylakoid membranes from brown algae (*Laminaria japonica* and *Undaria pinnatifida*) or a diatom (*Chaetoceros gracilis*) and a green alga (*Chlamidomonas reinhardtii*) were prepared by centrifuged fractionation after homogenization of their sporophyte with blender or after disruption of the cells by agitation with glass beads, respectively. PSII complexes from a higher plant (spinach) and a cyanobacterium (*Synechococcus vulcanus*) were prepared according to Enami et al. (2000) and Shen et al.(1993), respectively. The thylakoid membranes and PSII complexes from various organisms were solubilized 5% lithium lauryl sulfate and 75 mM dithiothreitol. The solubilized samples were applied into SDS-polyacrylamide gel electrophoresis (SDS PAGE) with a gradient gel of 16~22% containing 7.5 M urea. For Western blotting, proteins on the gel were transferred onto a PVDF membrane, reacted with respective antibodies, and visualized with biotinylated anti-rabbit IgG.

Results and Discussion

Table 1 summarized the results of immunoblot analysis of the extrinsic proteins in PSII complexes and thylakoid membranes from various species. Thylakoid membranes from a green alga (*Chlamidomonas reinhadti*) cross-reacted with the antibodies raised against the extrinsic 23 kDa protein from spinach PSII (anti-H23) but not with the antibody against spinach 17 kDa protein (anti-H17). This may be due to a high specificity of anti-H17 and also a low homology of the 17 kDa protein between higher plants and green algae. The green algal thylakoid membranes did not cross-react with any antibodies against the extrinsic proteins from red algal PSII (anti-R20, anti-Rc550 and anti-R12). In contrast, thylakoid membranes from brown algae (*Laminaria japonica* and *Undaria pinnatifida*) and from a diatom (*Chaetoceros gracilis*) cross-reacted with anti-R20 and anti-Rc550 but not with anti-R12, anti-H23 and anti-H17. PSII complex from a cyanobacterium, *Synechococcus vulcanus*, cross-reacted with anti-Rc550 but not with anti-R20 and anti-R12 as well as anti-H23 and anti-H17. Therefore, we examined the cross-reactivity of cyanobacterial PSII complex with the antibody (anti-C12) raised against the extrinsic 12 kDa protein from the cyanobactrium and confirmed that the PSII complex cross-reacted with anti-C12. PSII complex from a red alga, *Cyanidium caldarium*, cross-reacted with anti-R20, anti-Rc550 and anti-R12

Table 1. Immunoblot analysis of extrinsic proteins in PSII complexes and thylakoid membranes from various plant species.

	Antibodies against extrinsic proteins					
	H23	H17	R20	Rc550	R12	C12
Higher plant						
Spinach (PSII)	+	+	-	-	-	-
Green alga						
Chlamidomonas reinhadtii (Thy.)	+	-	-	-	-	-
Brown algae						
Laminaria japonica (Thyl.)	-	-	+	+	-	-
Undaria pinnatifida (Thyl.)	-	-	+	+	-	-
Diatom						
Chaetoceros gracilis (Thyl.)	-	-	+	+	-	-
Red algae						
Cyanidium caldarium (PSII)	-	-	+	+	+	-
Cyanobacteria						
Synechococcus vulcanus (PSII)	-	-	-	+	-	+

but not anti-C12. These indicate that the antibodies raised against cyanobacterial or red algal 12 kDa protein did not cross-react with red algal or cyanobacterial 12 kDa protein, respectively, showing the high specificity of the antibody of the 12 kDa protein. The fact that thylakoid membranes from *brown algae* and a diatom did not cross-react with anti-R12 and anti-C12 may be related to the specificity of the antibody of the 12 kDa protein.

In summary, cyanobacterial PSII complex contains the 12 kDa protein and cyt c550 but not the 20 kDa protein; PSII complexes of red alga, diatom and brown algae have the 20 kDa protein and cyt c550 (and the 12 kDa protein); higher plant and green algal PSII contain the 23 kDa and 17 kDa proteins. These results indicate that the oxygen-evolving PSII complex can be divided into two major groups from the viewpoint of distribution of the extrinsic proteins; chla/c group involving diatom and brown algae and chla/b group involving green algae and higher plant. These imply that the 20 kDa protein was appeared during evolution from cyanobacteria to red algae and conserved in chla/c plants together with cyt c550 and the 12 kDa protein, while the 20 kDa, 12 kDa proteins and cyt c550 had been replaced by the 23 kDa and 17 kDa proteins during evolution from red algae to chla/b plants.

Acknowledgements

We thank Dr. Y. Kashino for generously providing us with a diatom, *Chaetoceros gracilis*.

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