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Cross-reconstitution of extrinsic cytochrome *c*-550 and 12 kDa protein between cyanobacterial and red algal PSII

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Introduction

Cyanobacterial and red algal PSIIs contain two similar extrinsic proteins, cyt *c*-550 and a 12 kDa protein (Shen et al 1992, Enami et al. 1995). Previous release-reconstitution experiments have shown that the binding patterns of these extrinsic proteins with PSII are different between cyanobacteria and red algae. In cyanobacterial PSII, cyt *c*-550 can bind to PSII in a way essentially independent of the other extrinsic proteins (Shen et al. 1993), whereas effective binding of red algal cyt *c*-550 to the red algal PSII requires the presence of all of the other extrinsic proteins, namely, the 33 kDa, 20 kDa and 12 kDa proteins (Enami et al. 1998). On the other hand, binding of the 12 kDa protein requires the presence of all the other extrinsic proteins in both cyanobacterial (Shen et al. 1993) and red algal PSII (Enami et al. 1998). The functions of cyt *c*-550 and 12 kDa protein in red algal PSII resemble those of the 23 and 17 kDa proteins in higher plant PSII in that their removal created a strong requirement for Ca^{2+} and Cl^- of oxygen evolution (Enami et al. 1998). These effects were less apparent in the case of cyanobacterial PSII (Shen et al. 1993). In this study, cross-reconstitution experiments of cyt *c*-550 and the 12 kDa protein between cyanobacterial and red algal PSII were performed to compare the binding and functional properties of these extrinsic proteins between the two organisms.

Material and methods

Cyanobacterial and red algal PSIIs were prepared from *Synechococcus vulcanus* (Shen et al 1992) and *Cyanidium caldarium* (Enami et al. 1995), respectively. The extrinsic proteins from these PSIIs were purified according to our previous papers (Shen et al. 1993, Enami et al. 1998).

For reconstitution experiments, cyanobacterial and red algal PSIIs were treated with 1 M $CaCl_2$ to remove all of the extrinsic proteins, and suspended in 40 mM Mes (pH 6.5) and 25% glycerol. Reconstitution of the $CaCl_2$-treated PSIIs with various extrinsic proteins either alone or in combination was performed in the above buffer for 30 min on ice in the dark, at 0.1 mg chl/ml and a ratio of each extrinsic protein-to-PSII of 3:1. After reconstitution, an aliquot of concentrated PEG 6000 was added to the reconstitution mixtures to a final concentration of 10% and then the mixtures were centrifuged at 410,000 x g for 30 min. The resulting precipitates were suspended in the above buffer, and used for oxygen evolution measurements (and electrophoretic analysis) (Enami et al. 2000). Oxygen evolution was measured with a Clark-type electrode at 25°C with 0.4 mM phenyl-*p*-benzoquinone as electron acceptor.

For convenience, we name the three extrinsic proteins of cyanobacterium as C33, C*c*-550, C12, and the four extrinsic proteins of red alga as R33, R20, R*c*-550, R12, respectively.

Results and discussion

Table 1 shows oxygen-evolving activities of cyanobacterial PSII reconstituted with red algal cyt *c*-550 and 12 kDa protein. When the extrinsic proteins were reconstituted to $CaCl_2$-treated cyanobacterial PSII in various combinations as shown in Table 1, all of the extrinsic proteins completely bound to cyanobacterial PSII (data not shown, Enami et al. 2000). Oxygen-evolving activities of cyanobacterial PSII reconstituted with C33 + R*c*-550 or C33 + R*c*-550 + R12 recovered up to the same levels as those reconstituted with C33 + C*c*-550 or C33 + C*c*-550 + C12, respectively, either in the absence or presence of NaCl or $CaCl_2$. This indicates that red algal cyt *c*-550 and 12 kDa protein can functionally replace cyanobacterial cyt *c*-550 and 12 kDa protein in the cyanobacterial PSII. When the PSII was reconstituted with C33 + C*c*-550 + R12 or C33 + R*c*-550 + C12, recovery of oxygen evolution was slightly lower than those reconstituted with C33 + C*c*-550 + C12 or C33 + R*c*-550 + R12 in the absence of ions. This may be due to that cyt *c*-550 and 12 kDa have an interaction with each other, and the combination of these two proteins from red algae and cyanobacteria gave rise to a less effective interaction between them.

Table 1. Reconstitution of red algal cyt *c*-550 and 12 kDa protein to cyanobacterial PSII. Oxygen-evolving activities of non-treated PSII (100) were 1577, 1787 and 1839 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	+ 10 mM NaCl	+ 5 mM $CaCl_2$
Synechococcus PSII	100	100	100
$CaCl_2$-treated PSII	1	3	3
+ C33	6	20	34
+ C33 + C*c*-550	18	41	49
+ C33 + C*c*-550 + C12	53	56	56
+ C33 + R*c*-550	18	41	49
+ C33 + R*c*-550 + R12	51	48	50
+ C33 + C*c*-550 + R12	41	47	49
+ C33 + R*c*-550 + C12	40	41	46

In red algal PSII, cyanobacterial cyt *c*-550 could replace the red algal one, while the 12 kDa protein could not. As shown in Table 2, oxygen-evolving activity of red algal PSII reconstituted with C*c*-550 instead of R*c*-550 in the presence of R33, R20 and R12 recovered up to the same level as that with all of the red algal extrinsic proteins. In contrast, red algal PSII reconstituted with C12 instead of R12 in the

Table 2. Reconstitution of cyanobacterial cyt *c*-550 and 12 kDa protein to red algal PSII.

Oxygen-evolving activities of non-treated PSII (100) were 2310, 2330 and 2342 μ mole O_2/mg chl/h in the absence and the presence of 10 mM NaCl or 5 mM $CaCl_2$, respectively.

	Oxygen evolution (Relative value %)		
	-ion	+ 10 mM NaCl	+ 5 mM $CaCl_2$
Cyanidium PSII	100	100	100
$CaCl_2$-treated PSII	0	0	5
+ R33	0	13	33
+ R33 + R20 + R*c*-550	7	26	48
+ R33 + R20 + R*c*-550 + R12	63	63	67
+ R33 + R20 + C*c*-550	4	20	42
+ R33 + R20 + C*c*-550 + C12	4	21	40
+ R33 + R20 + R*c*-550 + C12	4	18	39
+ R33 + R20 + C*c*-550 + R12	59	61	60

presence of R33, R20 and R*c*-550 or C*c*-550 resulted in no recovery of oxygen evolution in the absence of Cl^- and Ca^{2+} ions. This is due to the fact that C12 bound to a very low level to red algal PSII (data not shown, Enami et al. 2000). Furthermore, the antibodies raised against cyanobacterial or red algal 12 kDa protein did not cross-react with red algal or cyanobacterial 12kDa protein, respectively, while the antibodies against cyt *c*-550 cross-reacted each other between cyanobacterial and red algal cyt *c*-550 (data not shown).

These results suggest that the structure and function of cyt *c*-550 were largely conserved, whereas those of the 12 kDa protein have been changed during evolution from cyanobacteria to red algae.

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