Partial reconstitution of the photosynthetic oxygen evolution system by rebinding of the 33-kDa polypeptide

Mitsue Miyao and Norio Murata

Department of Biology, University of Tokyo, Komaba, Meguro-ku, Tokyo 153, Japan

Received 13 October 1983

Treatment with 2.6 M urea of the Photosystem II particles depleted of two polypeptides of 24 kDa and 18 kDa completely released a polypeptide of 33 kDa and eliminated the oxygen-evolution activity. The 33-kDa polypeptide rebound to the urea-treated particles and partially reactivated the oxygen evolution. A quantitative analysis of the rebinding suggests that there is a specific binding site for the 33-kDa polypeptide on the membrane surface.

33-kDa polypeptide *Oxygen evolution* *Photosystem II* *Photosynthesis* *(Spinach chloroplast)*

1. INTRODUCTION

A large amount of evidence suggests that three membrane-bound polypeptides of 33, 24 and 18 kDa participate in the photosynthetic oxygen evolution [1–8]. In a study on the dissociation and reassociation of the 24-kDa and 18-kDa polypeptides in the PS II particles, we found specific binding sites for the 24-kDa polypeptide on the membrane surface and for the 18-kDa polypeptide on the 24-kDa polypeptide [3]. A quantitative analysis of the oxygen-evolution activity and the binding of the 24-kDa polypeptide indicated that this polypeptide plays an important role in the oxygen evolution system, although oxygen can be evolved at a reduced rate without this polypeptide. Here, we studied dissociation and reassociation of the 33-kDa polypeptide, and found a specific binding site for this polypeptide on the surface of PS II particles, and restoration of oxygen-evolution activity upon binding.

Abbreviations: Chl, chlorophyll; DCIP, 2,6-dichlorophenolindophenol; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PS II, photosystem II; PAGE, polyacrylamide gel electrophoresis

2. MATERIALS AND METHODS

PS II particles were prepared from spinach chloroplasts with Triton X-100 and stored in liquid nitrogen [3]. The PS II particles were washed with 1.0 M NaCl to remove all the 24-kDa and 18-kDa polypeptides as in [3]. The resultant NaCl-treated particles were suspended in 300 mM sucrose, 10 mM NaCl and 25 mM Mes–NaOH (pH 6.5) (hereinafter, medium A) and kept in the dark.

For urea treatment, 5 vols. of 3.1 M urea containing 10 mM NaCl and 25 mM Mes–NaOH (pH 6.5) were added to the suspension of NaCl-treated particles (3 mg Chl/ml), to give final concentrations of 2.6 M for urea and 0.5 mg/ml for chlorophyll. After standing for 30 min in the dark, the particles were collected by centrifugation at 35000 × *g* for 20 min and washed once with medium A by resuspension and recentrifugation.

To prepare the 33-kDa polypeptide, NaCl-treated PS II particles, depleted of both 24-kDa and 18-kDa polypeptides, were treated with 2.6 M urea in the dark as described above. The aqueous phase obtained by centrifugation at 35000 × *g* for 20 min was diluted with 5 vols. of medium A, and then concentrated by ultrafiltration with an Amicon PM 10 Diaflo membrane. After passage

Published by Elsevier Science Publishers B.V.
00145793/83/$3.00

through a membrane filter (1.2 μm, Gelman), the protein solution was dialyzed against medium A for 10 h. The resultant protein solution was used as a 33-kDa polypeptide preparation. The mixture of the 24-kDa and 18-kDa polypeptides with 1:1 molar ratio was prepared by treating the untreated PS II particles with 1.0 M NaCl [3].

In order to investigate the possible rebinding of the 33-kDa polypeptide to the PS II particles, the 33-kDa polypeptide was added to urea-treated particles in medium A at a chlorophyll concentration of 0.4 mg/ml. After standing for 30 min in the dark, the PS II particles were collected and washed with medium A by centrifugation at 35000 × g for 20 min and resuspension.

All the above procedures were performed at 0–4°C. After the NaCl treatment, all operations were done under dim light at an intensity less than 1 lx.

The oxygen-evolution activity was measured with phenyl-*p*-benzoquinone as an electron acceptor at 25°C with a Clark-type oxygen electrode and the reduction of DCIP in the presence and absence of diphenylcarbazide was measured photometrically as in [1]. The polypeptides were analyzed by SDS–urea PAGE, and the gel was stained with Coomassie brilliant blue as in [2]. The relative contents of the 33-kDa polypeptide were estimated according to the peak height of the stained bands in the densitogram (Shimadzu, CS 910). Chlorophyll and protein concentrations were determined as in [3].

3. RESULTS AND DISCUSSION

Treatment of the PS II particles with 1.0 M NaCl released almost all the 24-kDa and 18-kDa polypeptides, while the 33-kDa polypeptide remained bound to the particles (fig.1, lane B). Further treatment of the NaCl-treated particles with 2.6 M urea specifically removed all of the 33-kDa polypeptide (fig.1, lane C) and completely inactivated oxygen evolution (table 1). This treatment also inactivated reduction of DCIP with no addition, but not with diphenylcarbazide as an electron donor (not shown). This suggests that urea attacks the oxygen evolution system specifically.

When the 33-kDa polypeptide was added back to the urea-treated particles, it rebound to the particles (fig.1, lane E) and partially restored the

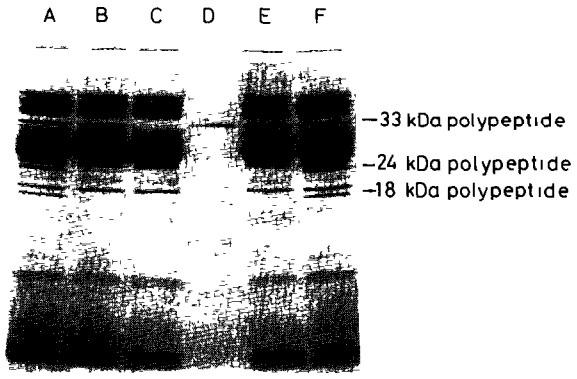

Fig.1. SDS–urea PAGE of the polypeptides in the PS II particles and of the 33-kDa polypeptide fraction. (A,F) Untreated particles; (B) NaCl-treated particles; (C) urea-treated particles; (D) 33-kDa polypeptide preparation; (E) urea-treated particles supplemented with the 33 kDa polypeptide at a protein-to-chlorophyll ratio of 0.9:1 (w/w).

oxygen-evolution activity (table 1). These observations suggest that the 33-kDa polypeptide is an essential component of the oxygen evolution system. Addition of the 24-kDa polypeptide to the urea-treated particles did not restore the oxygen-evolution activity at all (table 1), in contrast to a previous observation [3] that the rebinding of this polypeptide to the NaCl-treated PS II particles fully restored the oxygen-evolution activity.

Rebinding of the 33-kDa polypeptide and restoration of the oxygen-evolution activity were saturated at 60 and 15%, respectively, of the original levels in the NaCl-treated particles (fig.2). The 33-kDa polypeptide could not bind to the NaCl-treated particles nor stimulate oxygen evolution. The mode of the binding of the 33-kDa polypeptide suggests that there is a specific binding site for this polypeptide on the PS II particles. The incomplete rebinding of the polypeptide to the particles may be due to possible damage to the integrity of the PS II particles and/or the 33-kDa polypeptide through the urea treatment. The reason for the discordance between the levels of the 33-kDa polypeptide rebinding and restoration of oxygen-evolution activity is still in question.

Divalent cations did not affect the rebinding of the 33-kDa polypeptide (table 2). The 24-kDa and 18-kDa polypeptides, on the other hand, enhanced the rebinding of the 33-kDa polypeptide. This observation, together with the fact that the 33-kDa

Table 1

Restoration of oxygen-evolution activity with the 33-kDa polypeptide in PS II particles

Type of particles	Addition	O_2 evolution (μmol·mg Chl^{-1}·h^{-1})
NaCl-treated	None	155 (100)
	33-kDa polypeptide	148 (95)
Urea-treated	None	0 (0)
	24-kDa and 18-kDa polypeptides	0 (0)
	33-kDa polypeptide	20 (13)
	33-kDa polypeptide plus 24-kDa and 18-kDa polypeptides	28 (18)

The 33-kDa polypeptide and/or the 24-kDa and 18-kDa polypeptide mixture were added to the NaCl-treated particles or the urea-treated particles. Values in parentheses are percentages of the activity of the NaCl-treated particles with no addition. Amounts of added polypeptides were 1.0 mg/ml Chl for the 33-kDa polypeptide and 0.3 mg/mg Chl for the 24-kDa and 18-kDa polypeptide mixture

polypeptide increases the rebinding of the 24-kDa polypeptide [5], suggests that the 33-kDa and 24-kDa polypeptides attractively interact with each other when they are bound to the PS II particles.

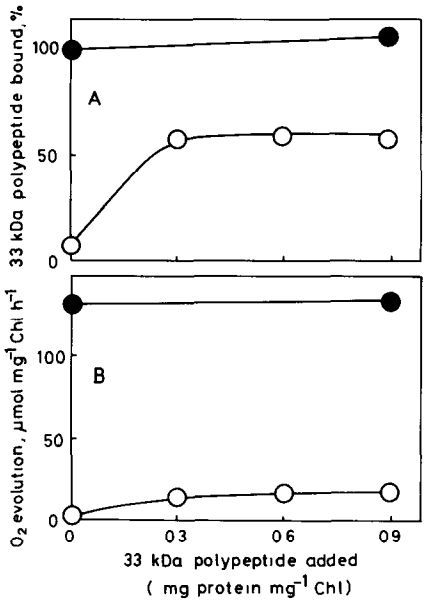

Fig.2. Rebinding of the 33-kDa polypeptide to the urea-treated PS II particles and restoration of oxygen-evolution activity. (A) Amount of bound 33-kDa polypeptide; 100% of binding corresponding to the original level in the NaCl-treated particles. (B) Activity of oxygen evolution. (○—○) Urea-treated particles; (●—●) NaCl-treated particles.

ACKNOWLEDGEMENTS

This work is supported by Grants-in-Aid for Energy Research (56040054) and for Cooperative Research (58340037) to N.M. from the Japanese Ministry of Education, Science and Culture.

Table 2

Effect of divalent cations and the 24-kDa and 18-kDa polypeptide mixture on rebinding of the 33-kDa polypeptide to urea-treated PS II particles

Addition	33-kDa polypeptide rebound (%)
None	56
50 μM $MnCl_2$	51
50 μM $MgCl_2$	55
5 mM $CaCl_2$	54
50 μM $MnCl_2$ and 5 mM $CaCl_2$	52
24-kDa and 18-kDa polypeptides	64

The 33-kDa polypeptide was added to the urea-treated particles in the presence of the cations and the 24-kDa and 18-kDa polypeptide mixture. Rebinding of 100% corresponds to the original level of the 33-kDa polypeptides in the NaCl-treated particles. Amounts of added polypeptides were 1.0 mg/mg Chl for the 33-kDa polypeptide and 0.5 mg/mg Chl for the 24-kDa and 18-kDa polypeptide mixture

REFERENCES

[1] Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 23, 533–539.
[2] Kuwabara, T. and Murata, N. (1983) Plant Cell Physiol. 24, 741–747.
[3] Miyao, M. and Murata, N. (1983) Biochim. Biophys. Acta, in press.
[4] Kuwabara, T. and Murata, N. (1983) in: The Oxygen Evolving System of Photosynthesis (Inoue, Y. et al. eds) pp. 217–222, Academic Press, Tokyo.
[5] Murata, N., Miyao, M. and Kuwabara, T. (1983) in: The Oxygen Evolving System of Photosynthesis (Inoue, Y. et al. eds) pp. 207–216, Academic Press, Tokyo.
[6] Yamamoto, Y., Doi, M., Tamura, N. and Nishimura, M. (1981) FEBS Lett. 133, 265–268.
[7] Åkerlund, H.-E. and Jansson, C. (1981) FEBS Lett. 124, 229–232.
[8] Fukutaka, E., Imaoka, A., Akabori, K. and Toyoshima, Y. (1983) FEBS Lett. 158, 217–221.