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EFFECT OF UREA ON PHOTOSYSTEM II PARTICLES

EVIDENCE FOR AN ESSENTIAL ROLE OF THE 33 KILODALTON POLYPEPTIDE IN PHOTOSYNTHETIC OXYGEN EVOLUTION

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Treatment with concentrated urea of Photosystem II particles, which were depleted of two polypeptides of 24 and 18 kDa by a prior washing with 1.0 M NaCl, released a polypeptide of 33 kDa and reduced photosynthetic oxygen-evolution activity. Light stimulated the polypeptide release and inactivation. The degree of inactivation was proportional to the release of the 33 kDa polypeptide from the particles, both in the light and in the dark. These observations suggest that the 33 kDa polypeptide plays an essential role in the oxygen-evolution system. The urea treatment also released Mn from the particles. A quantitative analysis suggests that one molecule of 33 kDa polypeptide interacts with two Mn atoms in the oxygen-evolution system.

Introduction

A large amount of evidence has suggested that three membrane-bound polypeptides of 33, 24 and 18 kDa are components of the photosynthetic oxygen-evolution system of PS II membrane preparations [1–9] and cholate-treated thylakoid membranes [10]. Treatment of the PS II preparations with concentrated NaCl released the 24 and 18 kDa polypeptides, and partially inactivated oxygen evolution [2]. The 24 kDa polypeptide can rebind to the membrane and reactivate oxygen evolution [3,7,9]. The 18 kDa polypeptide also can rebind, but has no effect on activity [3,7,9]. A quantitative analysis of oxygen-evolution activity and the binding of the 24 kDa polypeptide indicated that this polypeptide is regulatory rather than essential for oxygen evolution [3].

In the present study, we used PS II particles depleted of the 24 and 18 kDa polypeptides to analyze quantitatively the dissociation of the 33 kDa polypeptide and Mn from the PS II particles in relation to the inactivation of oxygen evolution.

Materials and Methods

PS II particles were prepared from spinach chloroplasts with Triton X-100 according to the method described previously [1] and stored in liquid nitrogen in the presence of 30% (v/v) ethylene glycol [3]. Before use, the particles were collected and washed three times with 300 mM sucrose/10 mM NaCl/25 mM Mes-NaOH (pH 6.5) (designated hereinafter as medium A) by centrifugation at 35 000 × *g* for 10 min and resuspension. For NaCl treatment of the PS II particles to remove all the 24 and 18 kDa polypeptides, the particles were suspended in 1.0 M NaCl contain-

Abbreviations: Chl, chlorophyll; DCIP, 2,6-dichlorophenolindophenol; Mes, 4-morpholineethanesulfonic acid; PS, Photosystem; Tris, 2-amino-2-hydroxymethylpropane-1,3-diol.

ing 300 mM sucrose and 25 mM Mes-NaOH (pH 6.5), at a final chlorophyll concentration of 0.5 mg/ml. After standing for 30 min under room light, the suspension was centrifuged at $35\,000 \times g$ for 30 min. The pellet was washed once with medium A by resuspension and recentrifugation. The resultant pellet, consisting of the NaCl-treated PS II particles, was suspended in medium A and kept in the dark.

Effects of urea on oxygen-evolution activity and the release of polypeptides and Mn were studied by adding an urea solution containing 10 mM NaCl and 25 mM Mes-NaOH (pH 6.5) to 1/6 vol. of the suspension of the NaCl-treated particles to give a final chlorophyll concentration of 0.5 mg/ml. After standing for 30 min under white fluorescent light of 1000 lx or in the dark, the PS II particles were collected by centrifugation at $35\,000 \times g$ for 20 min and washed once with medium A by resuspension and recentrifugation. The final pellet of the urea-treated PS II particles was suspended in medium A, and then used for measurement of oxygen-evolution activity, SDS-urea gel electrophoresis and Mn determination. To investigate the time-course of the inactivation of oxygen evolution by urea, the NaCl-treated particles were kept in the dark for 2 h and then incubated in the light or dark with 2.3 M urea containing 10 mM NaCl/25 mM Mes-NaOH (pH 6.5) at a chlorophyll concentration of 0.5 mg/ml. After a designated period of incubation, part of the particle suspension was withdrawn and diluted with 40 vol. medium A, and immediately assayed for oxygen evolution. A stock solution of 5.0 M urea was deionized by passage through a column of AG 501-X8D mixed bed ion exchange resin (Bio-Rad Lab.) just before use. All the above procedures were performed at 0–4°C.

Photosynthetic electron transport reactions were measured at 25°C in medium A supplemented with 0.05% bovine serum albumin as described previously [1]: the oxygen-evolution activity was measured using phenyl-*p*-benzoquinone as an electron acceptor with a Clark-type oxygen electrode, and the reduction of DCIP in the presence or absence of an electron donor, diphenylcarbazide, was measured photometrically [1]. The concentrations of the artificial electron donor and acceptors were 0.3 mM for phenyl-*p*-benzoquinone, 0.06 mM for DCIP, and 1 mM for diphenylcarbazide.

SDS-urea gel electrophoresis was performed with a slab gel plate containing 6.0 M urea, using the buffer system of Chua and Bennoun [11] except that the electrode buffer was 0.1% SDS and 25 mM Tris-glycine (pH 8.3) [2]. The polyacrylamide concentrations of the stacking and the separation gel were 5% and 12%, respectively. The gel was stained with Coomassie brilliant blue R-250 [12] and the electrophoretic pattern was photographed or recorded with a dual-wavelength TLC scanner (Shimadzu, CS-910). The relative amounts of the 33 kDa polypeptide were estimated within 5% accuracy according to the peak heights of the stained bands in the densitogram.

Mn was determined by atomic absorption at 279.5 nm with an automated flameless atomic absorption spectrometer (Shimadzu, AA-640-12, GFA-3). The sample solutions were made up to 1% HNO_3, dried at 150°C for 40 s, ashed at 800°C for 30 s and atomized at 2500°C for 6 s. Three measurements were made for the same sample, and deviation of the obtained values did not exceed 5%. Chlorophyll was determined according to Arnon [13].

Results and Discussion

Treatment of the PS II particles with 1.0 M NaCl removed almost all the 24 and 18 kDa polypeptides (Fig. 1) and reduced oxygen-evolution activity to about half (Table I). Further treatment of the NaCl-treated particles with 2.3 M urea removed the 33 kDa polypeptide (Fig. 1), and almost completely eliminated oxygen-evolution activity (Table I). Treatment with 2.3 M urea also removed the 33 kDa polypeptide and parts of 24 and 18 kDa polypeptides from the untreated particles which contained all the three polypeptides and completely inactivated oxygen evolution [5]. Electron transport activity from water to DCIP, measured by reduction of DCIP, was also suppressed by the urea treatment (Table I). The DCIP reduction in the urea-treated particles was restored when an electron donor, diphenylcarbazide, was added. This observation suggests that urea specifically attacks the oxygen evolution system.

Fig. 2 shows the time-course of the inactivation of oxygen evolution by incubation of the NaCl-

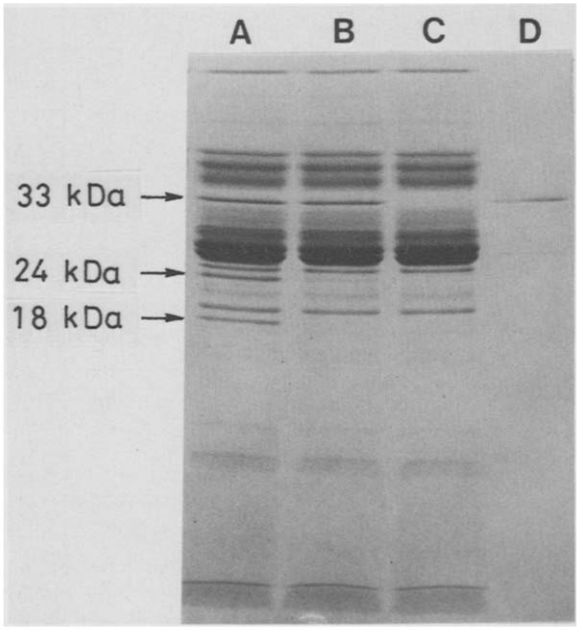

Fig. 1. SDS-urea gel electrophoresis of polypeptides in PS II particles. (A) Untreated PS II particles; (B) NaCl (1.0 M)-treated particles; (C) urea (2.3 M)-treated particles; (D) polypeptides released from the NaCl-treated particles by treatment with 2.3 M urea.

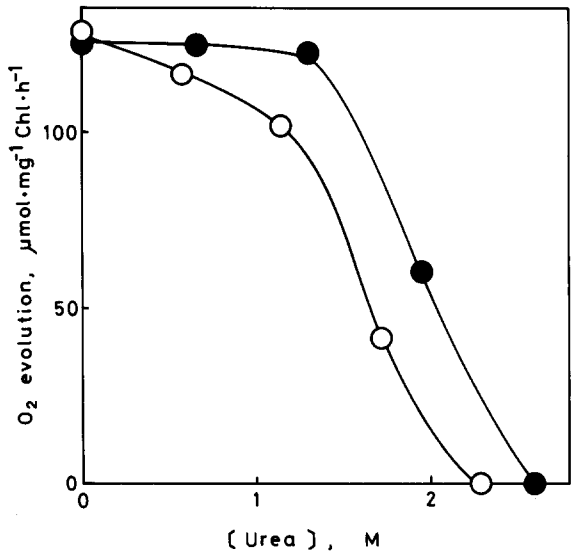

Fig. 3. Inactivation of oxygen evolution upon treatment with various concentrations of urea. The NaCl-treated PS II particles were treated with urea for 30 min in the light (○——○) or in the dark (●——●).

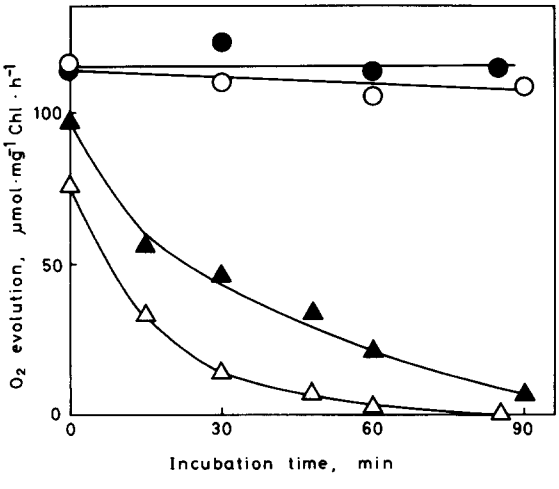

Fig. 2. Time course of inactivation of photosynthetic oxygen evolution with urea. NaCl-treated PS II particles were incubated with 2.3 M urea in the light (△——△) or in the dark (▲——▲), and with medium A in the light (○——○) or in the dark (●——●).

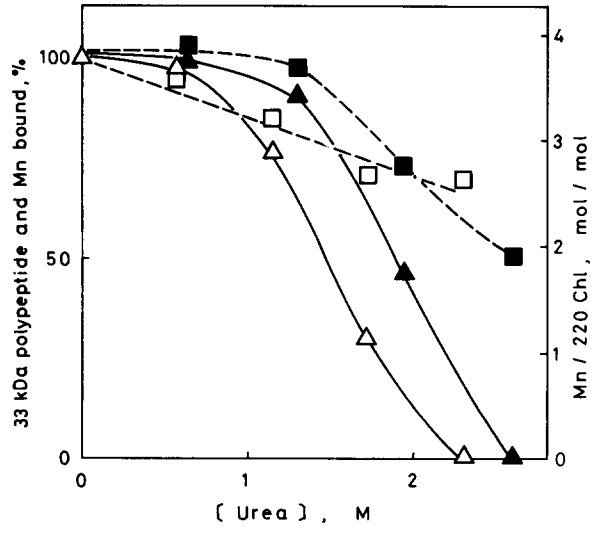

Fig. 4. Changes with urea treatment in the amounts of the particle-bound 33 kDa polypeptide and Mn. The relative contents of the polypeptide and Mn were determined for the same samples as in Fig. 3. The binding of 100% corresponds to that in the NaCl-treated PS II particles. The 33 kDa polypeptide bound to the particles after treatment in the light (△——△) or in the dark (▲——▲). The Mn bound to the particles after treatment in the light (□-----□) or in the dark (■-----■).

treated PS II particles with 2.3 M urea. The oxygen-evolution activity declined immediately after exposure of the particles to urea, and decreased further with incubation time. Illumination accelerated the inactivation: an incubation for 60 min almost completely eliminated the activity under illumination with white fluorescent light of 1000 lx, whereas it reduced the activity to 20% of the original in the dark.

The dependence of the inactivation of oxygen evolution on the urea concentration is shown in Fig. 3. The activity stayed at a relatively constant level at concentrations lower than 1.0 M, but fell steeply in a concentration range between 1.3 and 2.3 M. The oxygen evolution was completely inactivated at 2.3 M in the light, and at 2.6 M in the dark.

The urea treatment specifically released the 33 kDa polypeptide from the NaCl-treated particles (Fig. 1). The dependence of this polypeptide release on the urea concentration was similar to that of the inactivation of oxygen evolution (Fig. 4). A complete removal was achieved at 2.3 and 2.6 M in the light and dark, respectively. In a previous study [3], illumination enhanced the effectiveness of NaCl treatment in inactivating the oxygen evolution and releasing the 24 and 18 kDa polypeptides. In the urea treatment in the present study, illumination also enhanced the release of 33 kDa polypeptide as well as the inactivation of oxygen evolution.

From the data in Figs. 3 and 4, the quantitative relationship between the oxygen evolution and the bound 33 kDa polypeotide can be obtained as shown in Fig. 5A. Oxygen-evolution activity was proportional to the amount of 33 kDa polypeptide bound to the particles. Total removal of the polypeptide completely inactivated oxygen evolution. These findings indicate that the inactivation by urea treatment is due to the release of the 33 kDa polypeptide.

In previous studies [2,5], we showed that the PS II particles contain four Mn atoms and 220 Chl molecules per one reaction center II, and that this metal can be released by concentrated Tris buffer and alkaline pH but not by concentrated NaCl. In the present study, urea was found to release Mn (Fig. 4). The effect of illumination on the release of Mn was rather complicated. In the dark, Mn

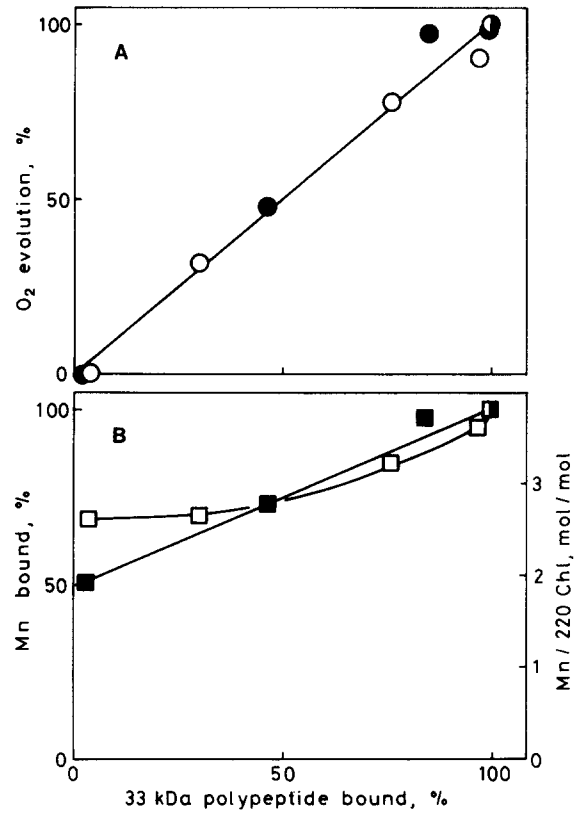

Fig. 5. Effect of the bound 33 kDa polypeptide on oxygen-evolution activity and binding of Mn in the urea-treated PS II particles. Oxygen-evolution activity and the amounts of bound Mn and bound 33 kDa polypeptide were changed by treatment with various concentrations of urea. The data were taken from Figs. 3 and 4. The urea treatment was performed in the light (open symbols) or in the dark (closed symbols). (A) Relationship between oxygen-evolution activity and the amount of the bound 33 kDa polypeptide. (B) Relationship between the amounts of the bound Mn and the bound 33 kDa polypeptide.

was released at urea concentrations higher than 1.0 M, similar to the release of 33 kDa polypeptide in the dark, although two Mn atoms per 220 Chl molecules still remained bound to the particles at 2.6 M urea. In the light, the release of Mn was apparently proportional to the urea concentration. The relationship between the amount of bound Mn and 33 kDa polypeptide after treatment with various concentrations of urea is presented in Fig. 5B. When the NaCl-treated PS II particles were treated with urea in the dark, Mn was released in a linear relation to the 33 kDa polypeptide release.

TABLE I

CHANGES IN THE PHOTOSYNTHETIC ELECTRON TRANSPORT ACTIVITIES OF PS II PARTICLES UPON TREATMENT WITH SODIUM CHLORIDE AND UREA

PS II particles were treated with 1.0 M NaCl and then 2.3 M urea for 30 min under room light. Electron acceptors were 0.3 mM phenyl-*p*-benzoquinone for measurement of O_2 evolution, and 0.06 mM DCIP for measurement of DCIP reduction. The values in parentheses are percentages of the activity of untreated particles.

Type of particles	O_2 evolution (μmol·mg^{-1} Chl·h^{-1})	DCIP reduction (μmol·mg^{-1}Chl·h^{-1})	
		−DPC	+DPC
Untreated	290 (100)	130 (100)	130 (100)
NaCl-treated	130 (45)	100 (77)	130 (100)
Urea-treated	20 (7)	30 (25)	230 (180)

Since the PS II particles contain one molecule of 33 kDa polypeptide and four atoms of Mn per one reaction center II [5], the release of a molecule of 33 kDa polypeptide corresponds to the release of two Mn atoms, When the PS II particles were treated in the light, on the other hand, no linear relationship was found between the release of Mn and the 33 kDa polypeptide. At the maximum release of the 33 kDa polypeptide, 70% of the Mn (corresponding to about three atoms per 220 Chl molecules) still remained bound. These observations seem to suggest that the 33 kDa polypeptide interacts with two of the four Mn atoms in the particles, although this interaction is affected by illumination.

It is concluded that the 33 kDa polypeptide plays an essential role in the oxygen-evolution system. This polypeptide probably influences the binding of two Mn atoms to the oxygen-evolution system. Recently, Mn-binding polypeptides of 32–36 kDa were purified by Dismukes et al. [14] and Yamamoto and Nishimura [15], although it is not confirmed that the Mn-binding polypeptides correspond to the 33 kDa polypeptide in the present study. We tried to detect Mn in the 33 kDa polypeptide released from the PS II particles upon urea treatment but were unsuccessful. The possible binding of Mn to the 33 kDa polypeptide is still in question.

Acknowledgements

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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 725, 87–93
4 Kuwabara, T. and Murata, N. (1983) in The Oxygen-Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 223–228, Academic Press Japan, Tokyo
5 Murata, N., Miyao, M. and Kuwabara, T. (1983) in The Oxygen-Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 213–222, Academic Press Japan, Tokyo
6 Yamamoto, Y., Doi, M., Tamura, N. and Nishimura, M. (1981) FEBS Lett. 133, 265–268
7 Ljungberg, U., Jansson, C., Andersson, B. and Åkerlund, H.-E. (1983) Biochem. Biophys. Res. Commun. 113, 738–744
8 Åkerlund, H.-E. and Jansson, C. (1981) FEBS Lett. 124, 229–232
9 Åkerlund, H.-E., Jansson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 681, 1–10
10 Fukutaka, E., Imaoka, A., Akabori, K. and Toyoshima, Y. (1983) FEBS Lett. 158, 217–221
11 Chua, N.-H. and Bennoun, P. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 2175–2179
12 Kuwabara, T. and Murata, N. (1979) Biochim. Biophys. Acta 581, 228–236
13 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
14 Dismukes, G.C., Abramowicz, D.A., Ferries, K.F., Mathur, P., Siderer, Y., Upadrashta, B. and Watnick, P. (1983) in The Oxygen-Evolving System of Photosynthesis (Inoue, Y., Crofts, A.R., Govindjee, Murata, N., Renger, G. and Satoh, K., eds.), pp. 145–158, Academic Press Japan, Tokyo
15 Yamamoto, Y. and Nishimura, M. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. 1, pp. 333–336, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague