Role of the 33-kDa polypeptide in preserving Mn in the photosynthetic oxygen-evolution system and its replacement by chloride ions

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Treatment of Photosystem II particles from spinach chloroplasts with Triton X-100 with 2.6 M urea in the presence of 200 mM NaCl removed 3 polypeptides of 33 kDa, 24 kDa and 18 kDa, but left Mn bound to the particles. The (urea + NaCl)-treated particles could evolve oxygen in 200 mM, but not in 10 mM NaCl. Mn was gradually released with concomitant loss of oxygen-evolution activity in 10 mM NaCl but not in 200 mM Cl^-. The NaCl-treated particles, which contained Mn and the 33-kDa polypeptide but not the 24-kDa and 18-kDa polypeptides, did not lose Mn or oxygen-evolution activity in 10 mM NaCl. These observations suggest that the 33-kDa polypeptide maintains the binding of Mn to the oxygen-evolution system and can be functionally replaced by 200 mM Cl^-.

Cl^- *Mn* *33-kDa polypeptide* *Oxygen evolution* *Photosystem II* *Photosynthesis*

1. INTRODUCTION

Biochemical studies on photosynthetic oxygen evolution have recently been concentrated on the 3 membrane-bound polypeptides of 33, 24 and 18 kDa [1–3]. The oxygen-evolution system of PS II particles from spinach chloroplasts contains one molecule each of the 3 polypeptides, one reaction center II and 4 Mn atoms per 220 Chl molecules [4,5]. Treatment of PS II preparations with concentrated NaCl specifically released the 24- and 18-kDa polypeptides and partially inactivated oxygen evolution [6–9]. Treatment with concentrated urea released the 3 polypeptides and about half of the Mn and also inactivated oxygen evolution [4,10,11]. Quantitative analyses of the polypeptide binding and the oxygen-evolution activity [4,6,7,10] suggest that the 24-kDa polypeptide is a regulatory factor and the 33-kDa polypeptide is most essential for oxygen evolution among the 3 polypeptides. The 33-kDa polypeptide seems to interact with two Mn atoms in the oxygen-evolution system [11,12].

Here, we prepared PS II particles which retain Mn but none of the 3 polypeptides by treating particles with concentrated urea and NaCl. We found that the 33-kDa polypeptide is essential for preserving Mn in the oxygen-evolution system and that it can be replaced by concentrated Cl^-.

Abbreviations: Chl, chlorophyll; Mes, 4-morpholineethanesulfonic acid; PS II, Photosystem II

2. MATERIALS AND METHODS

PS II particles were prepared from spinach chloroplasts with Triton X-100 as in [1] and stored in liquid nitrogen in the presence of 30% (v/v) ethylene glycol [7]. Before use, the particles were collected by centrifugation at 35000 × *g* for 10 min and washed 3 times with a medium composed of 10 mM NaCl, 300 mM sucrose and 25 mM Mes–NaOH (pH 6.5, low-salt medium) and kept in the dark for 2 h.

The particles were treated with 1.0 M NaCl containing 300 mM sucrose and 25 mM Mes–NaOH

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(pH 6.5) for 30 min under room light as in [7] and washed once with the low-salt medium by centrifugation and resuspension at 35000 × *g* for 20 min; the resultant particles were designated as NaCl-treated particles. The PS II particles were also treated with 2.6 M urea containing 10 mM NaCl and 25 mM Mes–NaOH (pH 6.5) or 2.6 M urea containing 200 mM NaCl and 25 mM Mes–NaOH (pH 6.5) for 30 min in the dark according to [10]. Then both types of particles were collected by centrifugation at 35000 × *g* for 20 min and washed once with, and suspended in, a medium composed of 200 mM NaCl, 300 mM sucrose and 25 mM Mes–NaOH (pH 6.5, high-salt medium) by resuspension and recentrifugation; the resultant particles were designated as urea-treated and (urea + NaCl)-treated particles, respectively. Control treatment of PS II particles was performed with the low-salt medium under room light and the particles were washed with, and suspended in, the same medium. All the above procedures were performed at 0–4°C.

Oxygen-evolution activity was measured with phenyl-*p*-benzoquinone as an electron acceptor at 25°C with a Clark-type oxygen electrode [1]. Polypeptides of the particles were analyzed by SDS-urea gel electrophoresis and stained with Coomassie brilliant blue as in [6]. The relative amount of each polypeptide was determined according to the peak heights of the stained bands in the densitogram (Shimadzu, CS-910). Mn content of the particles 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. Chlorophyll was determined as in [1].

3. RESULTS AND DISCUSSION

Treatment of the PS II particles with 1.0 M NaCl almost totally removed the 24- and 18-kDa polypeptides, but left Mn and the 33-kDa polypeptide bound to the particles (table 1). Treatment with 2.6 M urea plus 10 mM NaCl removed all the 33-kDa polypeptide, most of the 24- and 18-kDa polypeptides and about 40% of Mn (table 1). Previous reports [4,5,11] showed that the oxygen-evolution system contained 4 Mn atoms and one reaction center II per ~220 Chl molecules and that the urea treatment with 10 mM NaCl released 2 of the 4 Mn atoms. When 200 mM NaCl was present during the urea treatment, only about 10% of the Mn, but all of the 3 polypeptides were lost. This protective effect of NaCl against the Mn release was observed at concentrations above 100 mM (not shown).

Authors in [13] reported that 1.0 M $CaCl_2$ removed the 3 polypeptides from PS II particles but left all the Mn bound. As described above, the treatment with 2.6 M urea plus 200 mM NaCl had a similar effect on the oxygen-evolution system of PS II particles.

Table 1

Changes in oxygen-evolution activity and contents of Mn and polypeptides upon various treatments of PS II particles

Treatment	Polypeptide bound (%)			Mn		O_2 evolution ($\mu mol \cdot mg^{-1}$ Chl $\cdot h^{-1}$)		
	18 kDa	24 kDa	33 kDa	%	Mn/ 220 Chl	10 mM NaCl[e]	200 mM NaCl[e]	200 mM NaCl + 5 mM $CaCl_2$[e]
Control[a]	100	100	100	100	3.8	330 (100)	230 (100)	300 (100)
NaCl[b]	3	6	100	95	3.6	150 (45)	110 (48)	270 (90)
Urea[c]	22	19	0	61	2.3	10 (3)	20 (9)	50 (17)
Urea + NaCl[d]	3	9	0	89	3.4	10 (3)	50 (22)	110 (37)

The treatment medium contained: [a] 10 mM NaCl, 300 mM sucrose, and 25 mM Mes–NaOH (pH 6.5); [b] 1.0 M NaCl, 300 mM sucrose and 25 mM Mes–NaOH (pH 6.5); [c] 2.6 M urea, 10 mM NaCl and 25 mM Mes–NaOH (pH 6.5); [d] (2.6 M urea, 200 mM NaCl and 25 mM Mes–NaOH (pH 6.5). [e] The oxygen-evolution activity was measured in 300 mM sucrose, 25 mM Mes–NaOH (pH 6.5) and designated salt(s). The values in parentheses are percentages of the control

Oxygen-evolution activity of the (urea + NaCl)-treated particles depended on the concentration of NaCl added to the assay medium (table 1); it was 50 μmol $O_2 \cdot mg^{-1}$ Chl $\cdot h^{-1}$ in 200 mM NaCl but decreased to one-fifth of that level in 10 mM NaCl. As shown in [14], 5 mM Ca^{2+} restores oxygen-evolution activity of the NaCl-treated particles and functionally replaces the 24-kDa polypeptide. Also in the (urea + NaCl)-treated particles, 5 mM $CaCl_2$ doubled the oxygen-evolution activity (table 1). However, the activity of the (urea + NaCl)-treated particles was much lower than that of the NaCl-treated particles both in the presence and absence of 5 mM $CaCl_2$ (table 1). This suggests that the 33-kDa polypeptide is necessary for full oxygen-evolution activity.

Fig.1A shows the time courses of release of Mn from the (urea + NaCl)- and NaCl-treated particles. When the (urea + NaCl)-treated particles were incubated in 200 mM NaCl, Mn was not released at all. When they were incubated in 10 mM NaCl, Mn was gradually released and about one-third was lost after 4 h incubation. Oxygen evolution was gradually inactivated in 10 mM NaCl, but not in 200 mM NaCl (fig.1B). When the NaCl-treated particles were suspended in 10 mM NaCl, no Mn release or inactivation of oxygen evolution was observed (fig.1). These findings suggest that the 33-kDa polypeptide acts to preserve Mn in the oxygen-evolution system and Cl^- at concentrations higher than 100 mM can functionally substitute for the 33-kDa polypeptide.

Fig.2 shows the relationship between the amount of bound Mn and the oxygen-evolution activity in the (urea + NaCl)-treated particles, which were incubated in the low-salt medium for various periods. There was a linear relationship between the activity and the amount of bound Mn. Extrapolation of the line to the abscissa suggests that inactivation will be complete when the Mn level reaches two atoms per reaction center II. These findings confirm our previous conclusions [11] that the 33-kDa polypeptide participates in the binding of two of the four Mn atoms to the oxygen-evolution system.

Table 2 shows the effect of various salts on preserving Mn bound to the (urea + NaCl)-treated particles. Mn remained bound to the particles in 200 mM KCl, 200 mM $MgCl_2$ and 100 mM $CaCl_2$, but not in 100 mM Na_2SO_4, suggesting that the effect of 200 mM NaCl can be ascribed to Cl^- and not to Na^+.

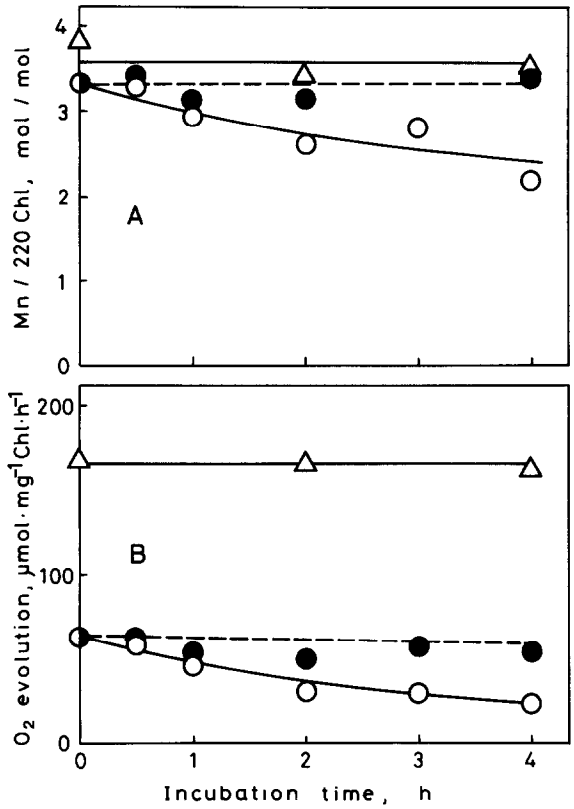

Fig.1. Changes in bound-Mn contents and oxygen-evolution activities in NaCl-treated and (urea + NaCl)-treated PS II particles during incubation in 10 mM and 200 mM NaCl. The particles were suspended at 0.6 mg Chl/ml and incubated in the dark. After a designated period, a portion of suspension was withdrawn, and the particles were collected by centrifugation at 35000 × g for 10 min and suspended in the high-salt medium. (A) Amount of bound Mn. (B) Oxygen-evolution activity measured in the low-salt medium for NaCl-treated particles and in the high-salt medium for (urea + NaCl)-treated particles. (○—○) (Urea + NaCl)-treated particles incubated in the low-salt medium, (●---●) (urea + NaCl)-treated particles incubated in the high-salt medium, (△—△) NaCl-treated particles incubated in the low-salt medium.

In intact thylakoid membranes, Cl^- is essential for photosynthetic oxygen evolution [15] and also protects the oxygen-evolution system against inactivation by hydroxylamine [16] and exogenous Mn [17]. Authors in [16] proposed that Cl^- interacts with the functional Mn of the oxygen-

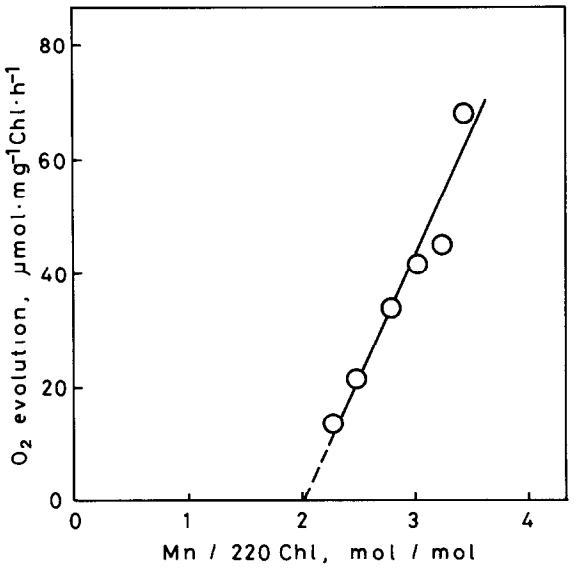

Fig.2. Relationship between the amount of bound Mn and the oxygen-evolution activity measured in the high-salt medium in (urea + NaCl)-treated PS II particles. The activity and the amount of bound Mn were changed by incubating the particles in the low-salt medium for various periods from 0 to 6 h. The experimental procedures were the same as those in fig.1.

Table 2

Effect of salts on the preservation of Mn in (urea + NaCl)-treated PS II particles

Salt	Mn/220 Chl
10 mM NaCl	2.6 (65)
200 mM NaCl	3.9 (98)
200 mM KCl	3.8 (95)
100 mM Na_2SO_4	2.6 (65)
100 mM $MgCl_2$	3.5 (88)
100 mM $CaCl_2$	3.9 (98)

The (urea + NaCl)-treated particles were suspended at 0.4 mg Chl/ml in a designated salt solution containing 300 mM sucrose and 25 mM Mes–NaOH (pH 6.5). After incubation for 4 h in the dark, the particles were collected by centrifugation at 35000 × *g* for 20 min, then washed with and suspended in the high-salt medium by resuspension and recentrifugation. The values in parentheses are percentages of the Mn content of untreated particles, which was 4.0 atoms per 220 chlorophylls

evolution system. This inference is supported by our findings that Cl^- retains Mn in the oxygen-evolution system of the PS II particles depleted of the 33-kDa polypeptide.

Authors in [13] treated PS II particles with 1.0 M $CaCl_2$ to prepare particles containing Mn but lacking the 3 polypeptides and inferred that Ca^{2+} was essential for preserving Mn in the oxygen-evolution system. However, our present results suggest that under their experimental conditions the concentrated Cl^- would be effective for preserving Mn and the concentrated Ca^{2+} for releasing the 3 polypeptides.

4. CONCLUSIONS

The 33-, 24- and 18-kDa polypeptides are released from PS II particles by 2.6 M urea plus 200 mM NaCl, but almost all the Mn remains bound. The 33-kDa polypeptide is necessary for the binding of Mn to the oxygen-evolution system, as well as for full oxygen-evolution activity. Cl^- can replace the 33-kDa polypeptide for preserving Mn bound to the oxygen-evolution system.

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