The Cl^- effect on photosynthetic oxygen evolution: interaction of Cl^- with 18-kDa, 24-kDa and 33-kDa proteins

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The interaction of Cl^- with the extrinsic proteins of 18 kDa, 24 kDa and 33 kDa in the photosynthetic oxygen-evolution complex was studied by comparing spinach photosystem II particles of different protein compositions. The 33-kDa protein decreased the Cl^- concentration optimum for oxygen evolution from 150 to 30 mM, and the 24-kDa protein decreased it from 30 to 10 mM. The 18-kDa protein did not change the optimum Cl^- concentration, but sustained oxygen evolution at Cl^- concentrations lower than 3 mM. The presence of the 24-kDa and 18-kDa proteins, but not each protein alone, markedly suppressed inactivation of oxygen evolution at a very low Cl^- concentration and its restoration by readdition of Cl^-.

Cl^- effect *Oxygen evolution* *Photosystem II* *Photosynthesis* *(Spinach chloroplast)*

1. INTRODUCTION

Recent biochemical studies on photosynthetic oxygen evolution have revealed that 3 extrinsic membrane proteins of 33 kDa, 24 kDa and 18 kDa [1–3] are components of the oxygen-evolution complex. PS II particles from spinach chloroplasts contain one molecule each of the 3 proteins and 4 Mn atoms per oxygen-evolution complex [4,5]. The 33-kDa protein is necessary for preserving the Mn and full oxygen-evolution activity, and can be partially substituted for 200 mM Cl^- [6,7]. The 24-kDa protein is necessary for oxygen-evolution activity at very low concentrations of Ca^{2+} [8,9], and can be replaced by 5 mM Ca^{2+} [10,11]. This protein seems to change the Cl^- requirement for oxygen evolution [12]. The 18-kDa protein is necessary for oxygen evolution, when Cl^- is depleted from the reaction medium [13].

Although the 3 extrinsic proteins appear to have some influence on the Cl^- requirement for oxygen evolution, the mode of their interaction with Cl^- has not been well clarified. In this study, we studied the effect of Cl^- on oxygen evolution over a wide range of Cl^- concentrations using PS II particles having different protein compositions. We found that the 33-kDa and 24-kDa, but not 18-kDa, proteins decrease the optimum Cl^- concentration, and that the 18-kDa protein greatly stimulates oxygen evolution at Cl^- concentrations lower than 3 mM.

Abbreviations: Chl, chlorophyll; Mes, 4-morpholineethanesulphonic acid; PS, photosystem

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 [14]. Before use, the particles were collected by centrifugation at $35\,000 \times g$ for 10 min and washed 3 times with a medium composed of 300 mM sucrose, 10 mM NaCl and 25 mM Mes-NaOH (pH 6.5). PS II particles depleted of the 24-kDa and 18-kDa proteins were prepared by treating the particles with 1.0 M NaCl, 300 mM

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sucrose and 25 mM Mes-NaOH (pH 6.5) for 30 min under room light [14], and were collected by centrifugation at 35 000 × *g* for 30 min. They were resuspended in 300 mM sucrose, 10 mM NaCl and 25 mM Mes-NaOH (pH 6.5), collected by centrifugation as above, and finally suspended in the same medium. PS II particles depleted of all the 33-kDa, 24-kDa and 18-kDa proteins were prepared by treating the particles with 2.6 M urea, 200 mM NaCl and 25 mM Mes-NaOH (pH 6.5) for 30 min in the dark [6], and were collected by centrifugation at 35 000 × *g* for 20 min. They were resuspended in 300 mM sucrose, 200 mM NaCl and 25 mM Mes-NaOH (pH 6.5), recentrifuged as above, and finally suspended in the same medium.

The 24-kDa and 18-kDa proteins were prepared as in [14]; they were extracted from PS II particles with 1.0 M NaCl and 25 mM Mes-NaOH (pH 6.5) and purified by column chromatography with DEAE-Toyopearl 650M (Toyosoda). With 20 mM sodium phosphate buffer (pH 7.0), the 18-kDa protein was not adsorbed and eluted with the same buffer, and the 24-kDa protein which was adsorbed in 20 mM sodium phosphate buffer (pH 7.0) was eluted with 50 mM NaCl and 20 mM sodium phosphate buffer (pH 7.0). Impurities of the preparations of 24-kDa and 18-kDa proteins, examined by SDS-urea polyacrylamide gel electrophoresis [15], amounted to 9 and 3%, respectively, and were found to be degradation products of the corresponding proteins formed during preparation.

Rebinding of the 24-kDa and/or 18-kDa proteins to the oxygen-evolution complex was performed by incubation at a Chl concentration of 0.4 mg/ml for 30 min of NaCl-treated particles in 300 mM sucrose, 10 mM NaCl and 25 mM Mes-NaOH (pH 6.5) with the purified proteins at a protein to Chl ratio of 0.4:1 (w/w) which corresponded to a doubled amount of stoichiometric rebinding [14]. After dilution with 5 vol of 300 mM sucrose, 10 mM NaCl and 25 mM Mes-NaOH (pH 6.5), the particles were collected by centrifugation at 35 000 × *g* for 15 min, then washed once with, and suspended in, the same medium by resuspension and centrifugation as above. All of these procedures were performed at 0–4°C.

To study the dependence of oxygen-evolution activity on Cl^- concentration, the various types of particles were suspended at 10 μg Chl/ml in 300 mM sucrose and 25 mM Mes-NaOH (pH 6.5) containing various concentrations of NaCl and incubated at 25°C for 5 min. Then oxygen-evolution activity was measured at 25°C with a Clark-type oxygen electrode in the presence of 0.3 mM phenyl-*p*-benzoquinone and 0.05% bovine serum albumin [1]. To study time-dependent inactivation of oxygen evolution at very low Cl^- concentrations, the particle suspension was diluted 250-fold with 300 mM sucrose and 25 mM Mes-NaOH (pH 6.5) to give a final Chl concentration of 10 μg/ml, and incubated at 25°C under room light with stirring. A portion of the suspension was withdrawn and its oxygen-evolution activity was measured at a designated time.

Since no attempt was made to remove Cl^- from the reagents used, the Cl^- concentration in a medium composed of 300 mM sucrose and 25 mM Mes-NaOH (pH 6.5) was 0.1 mM when determined potentiometrically with a Cl^- electrode (Toko Chemical Lab, 5102, MR501DS). Chl was determined as in [17].

3. RESULTS

Treatment of PS II particles with 1.0 M NaCl almost totally removed the 24-kDa and 18-kDa proteins but left all the Mn and the 33-kDa protein bound to the oxygen-evolution complex as reported in [6,15]. Treatment with 2.6 M urea plus 200 M NaCl removed all the 3 proteins but left almost all the Mn bound [6]. Fig.1 shows that these treatments changed the dependence of oxygen evolution on Cl^- concentration. The untreated particles showed their maximum oxygen-evolution activity in 10 mM NaCl. The removal of the 24-kDa and 18-kDa proteins by NaCl treatment reduced the maximum activity to about 40%, and increased the optimum NaCl concentration to 30 mM. It also markedly suppressed the oxygen-evolution activity at Cl^- concentrations lower than 3 mM and eliminated the activity in 0.1 mM NaCl. The untreated particles, on the other hand, retained more than 70% of their maximum activity in 0.1 mM NaCl (fig.1). The removal of all the 33-kDa, 24-kDa and 18-kDa proteins by the urea-plus-NaCl treatment reduced the maximum activity to about one-tenth and increased the optimum NaCl concentration to 150 mM as reported in [6].

The effects of 24-kDa and 18-kDa protein sup-

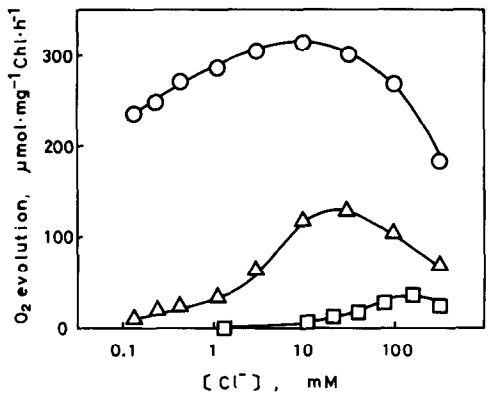

Fig.1. Effects of Cl^- concentration on oxygen-evolution activity in untreated, NaCl-treated and (urea + NaCl)-treated PS II particles. After 5-min incubation at 25°C at various concentrations of NaCl, oxygen evolution was measured. (○—○) Untreated particles, (△—△) NaCl-treated particles, (□—□) (urea + NaCl)-treated particles.

plements to the NaCl-treated particles on the Cl^- dependence of oxygen evolution are presented in fig.2. Our previous study [14] indicates that the 24-kDa protein stoichiometrically rebinds to NaCl-treated particles, thus restoring oxygen-evolution activity, and that the 18-kDa protein also stoichiometrically rebinds to NaCl-treated particles supplemented with the 24-kDa protein without affecting the oxygen-evolution activity measured in 10 mM NaCl. As seen in fig.2, the 24-kDa protein reactivated the oxygen-evolution activity at all Cl^- concentrations from 0.1 to 300 mM, and decreased the optimum concentration of Cl^- from 30 to 10 mM. However, oxygen-evolution activity dropped with a decrease in Cl^- concentration to below 3 mM and reached 30% of the maximum level in 0.1 mM NaCl. Further addition of the 18-kDa protein did not shift the optimum Cl^- concentration but restored the oxygen-evolution activity at the low Cl^- concentrations. Addition of the 18-kDa protein alone did not support the oxygen-evolution activity of the NaCl-treated particles at all the Cl^- concentrations (fig.2).

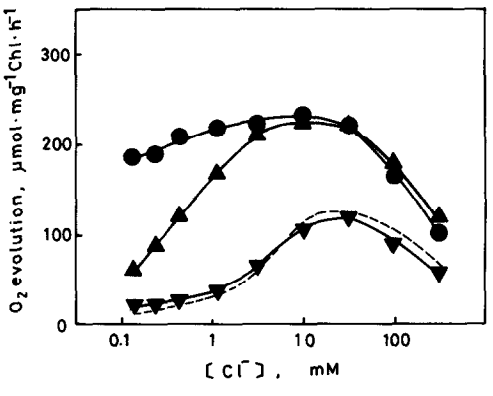

Fig.2. Effect of Cl^- concentration on oxygen-evolution activity in NaCl-treated in PS II particles supplemented with 24-kDa and/or 18-kDa proteins. Experimental conditions were as given in fig.1. (●—●) NaCl-treated particles supplemented with both 24-kDa and 18-kDa proteins, (▲—▲) NaCl-treated particles with the 24-kDa protein, (▼—▼) NaCl-treated particles with the 18-kDa protein, (– – –) NaCl-treated particles.

Another specific feature of the effect of the 18-kDa and 24-kDa protein was found in time-dependent inactivation of oxygen evolution at low Cl^- concentrations. Fig.3 shows that oxygen-evolution activity of the untreated particles in 0.13 mM Cl^- dropped to 70% for 5 min and then gradually decreased, but that those of NaCl-treated particles in 0.13 mM Cl^- and (urea + NaCl)-treated particles in 0.83 mM Cl^- were almost completely lost within 5 min. In the control

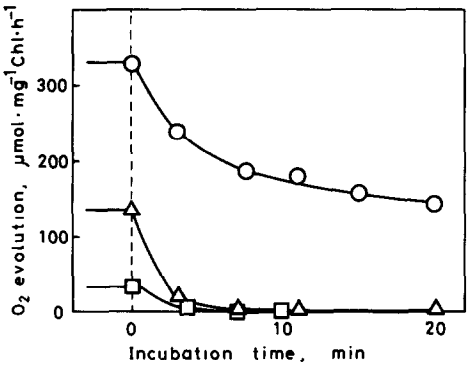

Fig.3. Inactivation of oxygen evolution at low Cl^- concentrations in untreated, NaCl-treated and (urea + NaCl)-treated PS II particles. At zero time of incubation, the Cl^- concentration of the particle suspension was reduced by dilution from 10 to 0.13 mM in untreated and NaCl-treated particles and from 200 to 0.83 mM in (urea + NaCl)-treated particles. Incubation was performed at 25°C. Oxygen-evolution activity at zero time was measured at the Cl^- concentrations before dilution. (○—○) Untreated particles, (△—△) NaCl-treated particles, (□—□) (urea + NaCl)-treated particles.

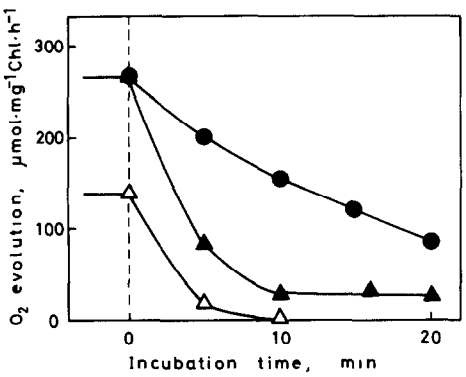

Fig.4. Inactivation of oxygen evolution in 0.13 mM Cl^- in NaCl-treated particles and the particles supplemented with the 24-kDa and/or 18-kDa proteins. At zero time, the Cl^- concentration was reduced from 10 mM to 0.13 mM. Other experimental conditions were as given in fig.3. (△—△) NaCl-treated particles, (●—●) NaCl-treated particles supplemented with both 24-kDa and 18-kDa proteins, (▲—▲) NaCl-treated particles with the 24-kDa protein.

experiment, both the untreated and NaCl-treated particles retained 80–90% of their original activities after 20 min incubation in 200 mM NaCl (not shown). NaCl-treated particles supplemented with 24-kDa and/or 18-kDa proteins were examined for the time-dependent inactivation of oxygen evolution in 0.13 mM Cl^- as seen in fig.4. The 24-kDa protein did not significantly affect the inactivation rate, whereas the 18-kDa protein in addition to the 24-kDa protein markedly retarded it. Here also, the 18-kDa protein alone had no effect on the inactivation rate (not shown). These observations suggest that the 18-kDa and 24-kDa proteins cooperate in suppressing the inactivation of oxygen evolution at the low Cl^- concentration.

Table 1 shows that the oxygen-evolution activity of PS II particles, which had diminished after incubation at the low Cl^- concentration, was markedly restored on re-addition of Cl^-. This effect was the most pronounced in particles lacking the 24-kDa and 18-kDa proteins, i.e., NaCl-treated and (urea + NaCl)-treated particles, in which the activity, which had once dropped almost to zero after 5 min incubation in the low-Cl^- media, returned to about two-thirds of its original level. In contrast, the inactivation and restoration of oxygen evolution were much less significant in the untreated particles and the NaCl-treated particles supplemented with the 24-kDa and 18-kDa proteins. The NaCl-treated particles supplemented with 24-kDa protein alone showed an intermediate feature in both the inactivation and restoration.

Table 1

Inactivation by Cl^- depletion and reactivation by Cl^- re-addition of oxygen evolution in PS II particles

Type of particles	Supplemented protein	Oxygen evolution ($\mu mol \cdot mg^{-1} \cdot h^{-1}$)				
		Before Cl^- depletion	Cl^- depletion for 5 min		Cl^- depletion for 15 min	
			Before Cl^- re-addition	After Cl^- re-addition	Before Cl^- re-addition	After Cl^- re-addition
Untreated	–	304 (100)	200 (66)	236 (78)	141 (46)	192 (63)
(Urea + NaCl)-treated	–	35 (100)	0 (0)	23 (66)	0 (0)	14 (40)
NaCl-treated	–	100 (100)	6 (6)	66 (66)	6 (6)	31 (31)
NaCl-treated	24-kDa	209 (100)	64 (31)	119 (57)	35 (17)	81 (39)
NaCl-treated	24-kDa + 18-kDa	215 (100)	149 (69)	166 (77)	112 (52)	132 (61)

Suspensions of various types of PS II articles were diluted with 300 mM sucrose and 25 mM Mes-NaOH (pH 6.5) to reduce the Cl^- concentration from 10 mM to 0.13 mM in untreated and NaCl-treated particles and from 200 mM to 0.83 mM in (urea + NaCl)-treated particles. After incubation at 25°C for 5 or 15 min, portions of the suspension were withdrawn and oxygen evolution was measured without addition of Cl^-. To the other portions, appropriate amounts of 1.2 M NaCl containing 300 mM sucrose and 25 mM Mes-NaOH (pH 6.5) were added to increase the Cl^- concentrations to 20 mM for the untreated and NaCl-treated particles and to 200 mM for the (urea + NaCl)-treated particles. After further incubation at 25°C for 5 min, oxygen evolution was measured. Values in parentheses represent percentages of the activity

When the particles were kept deficient of Cl^- for a longer time, the restored level of the oxygen-evolution activity became lower (table 1). This was pronounced in the particles depleted of the 18-kDa protein, i.e., (urea + NaCl)-treated, NaCl-treated, and NaCl-treated and 24-kDa protein-supplemented particles. Thus, the Cl^- depletion seemed to produce both reversible and irreversible inactivation, with the proportion of the latter becoming larger with prolonged incubation in a low-Cl^- medium.

4. DISCUSSION

The dependence of oxygen-evolution activity on Cl^- concentration (fig.1,2) indicates that the effect of the 33-kDa and 24-kDa proteins is different from that of the 18-kDa protein; the 33-kDa and 24-kDa proteins decreased the optimum Cl^- concentration and increased oxygen-evolution activity in all the ranges of Cl^- concentrations tested. The 18-kDa protein did not change the optimum Cl^- concentration, but enhanced oxygen-evolution activity at Cl^- concentrations below 3 mM. The latter effect of the 18-kDa protein appeared only in the presence of the 24-kDa protein, as reported by Akabori et al. [13]. This cooperation of the 18-kDa and 24-kDa proteins can be explained by the fact that the 18-kDa protein has its specific binding site on the 24-kDa protein [14].

It seems reasonable to assume that the inactivation of oxygen evolution by Cl^- depletion corresponds to the removal of Cl^- from its functional site(s) in the oxygen-evolution complex [17]. Based on this assumption, all the 33-kDa, 24-kDa and 18-kDa proteins seem to act as 'Cl^--concentrators,' since these proteins either decrease the optimum Cl^- concentration or sustain oxygen evolution at very low Cl^- concentrations.

This study suggests that the 18-kDa and 24-kDa proteins play another role in the oxygen-evolution complex. As seen in fig.4 and table 1, these proteins cooperate to markedly retard the inactivation of oxygen evolution at low Cl^- concentrations, and suppress its restoration by readdition of Cl^-, suggesting that dissociation of Cl^- from, and its reassociation to, the functional site are greatly disturbed by the proteins. These observations may suggest that the 18-kDa and 24-kDa proteins cooperate to act as a Cl^--barrier. The 24-kDa protein alone has a similar, but much less pronounced, effect (table 1).

We must mention, however, that the above inferences are derived from experiments carried out under non-physiological conditions. The Cl^- concentration in stroma ranges from 30 to 60 mM in spinach chloroplasts [18]. Since the thylakoid membranes are very permeable to Cl^- [19], the Cl^- concentration in the intrathylakoid space is likely to be close to that in stroma and is in the range where the 18-kDa protein is unnecessary for oxygen evolution. Therefore the physiological function of the 18-kDa protein remains still obscure.

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