Enhancement by Chloride Ions of Photoactivation of Oxygen Evolution in Manganese-Depleted Photosystem II Membranes†

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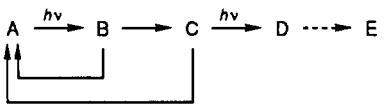
ABSTRACT: The Mn cluster that catalyzes photosynthetic oxygen evolution was removed from the photosystem II (PSII) complex by treating PSII membranes with 1.0 mM NH_2OH with concomitant inactivation of oxygen evolution. The cluster was reconstituted by incubating the treated membranes with 1.0 mM Mn^{2+}, 20 mM Ca^{2+}, 10 μM 2,6-dichlorophenolindophenol, and Cl^- under illumination with continuous or flashing light to restore the oxygen-evolving capacity. This light-dependent activation (photoactivation) of oxygen evolution did not occur to a significant extent at 3 mM Cl^-, but markedly accelerated at higher Cl^- concentrations without showing a saturation phenomenon even at 1 M Cl^-. At 10 mM Cl^- only about 10% of the oxygen-evolving activity before NH_2OH treatment was restored by 5-min illumination with continuous light, whereas at 600 mM Cl^- about 60% of the original activity was recovered. This acceleration resulted from at least two different actions of Cl^-: (1) stabilization of the intermediate state involved in the photoactivation process and (2) increase in the quantum yield of photoactivation. The stabilization of the intermediate was saturated at about 150 mM Cl^-, whereas the increase in yield did not show saturation. The Cl^--induced increase in quantum yield did not involve any changes in the affinity of either Mn^{2+} binding or Ca^{2+} binding for photoactivation, but was rather ascribed to a protective effect of Cl^- against inhibition of photoactivation by high concentrations of Mn^{2+}. We also found that removal of the extrinsic 33-kDa protein from the PSII complex increased the Cl^- requirement for photoactivation.

Photosynthetic oxygen evolution is catalyzed by a Mn cluster which is bound to an intrinsic part of the photosystem II (PSII)[1] complex at the lumenal surface of thylakoid membrane (Miyao & Murata, 1987). The Mn cluster consists of four Mn atoms of valences II and III or III and IV and requires Cl^- and Ca^{2+} as essential cofactors for its proper functioning (Dismukes, 1986; Yocum, 1986; Brudvig et al., 1989). Extrinsic proteins of 33, 23, and 16 kDa are bound to the PSII complex in close vicinity of the Mn cluster and act to decrease the requirements for Cl^- and/or Ca^{2+} (Miyao & Murata, 1987; Homann, 1987).

Photoactivation of oxygen evolution is a process by which an active Mn cluster is formed in the PSII complex under illumination (Radmer & Cheniae, 1977). This process involves at least two successive photoreactions in PSII and a dark reaction in between (Radmer & Cheniae, 1971; Cheniae & Martin, 1973; Inoue et al., 1975), as shown in Scheme I. The PSII complex depleted of Mn cluster (A) is converted to an unstable intermediate (B) by the first photoreaction. This intermediate is insensitive to photoreaction and has to be rearranged in darkness to another unstable intermediate (C) which is sensitive to the second photoreaction. The second photoreaction converts the intermediate C to a stable intermediate (D) which is finally converted to a functional Mn cluster (E) in darkness. Both of the two unstable intermediates (B and C) decay back to A in darkness unless the next steps occur. The two photoreactions (conversions of A to B and of C to D) have been proposed to involve photooxidation of Mn^{2+} by PSII to yield Mn^{3+} (Ono & Inoue, 1987; Tamura & Cheniae, 1987a, 1988; Miller & Brudvig, 1989). At present, only the first photoreaction could be experimentally identified and demonstrated to involve photooxidation of one Mn^{2+} by Y_z^+ (Miller & Brudvig, 1990).

Scheme I



The quantum yield of photoactivation is generally very low: about 200–400 flashes are necessary for full recovery of oxygen evolution in vivo (Cheniae & Martin, 1971; Inoue et al., 1975), whereas many more flashes are required in isolated PSII membranes (Tamura & Cheniae, 1987a). Such a low quantum yield has been ascribed to charge recombination reactions in PSII which cause loss of oxidizing power for Mn^{2+} oxidation, and also to a low quantum yield of the second unstable intermediate (conversion from A to C) (Tamura & Cheniae, 1987a).

Assembly of the Mn cluster can proceed when a Mn-depleted PSII complex is incubated only with Mn^{2+} under illumination (Tamura & Cheniae, 1987a; Tamura et al., 1989). However, the assembled cluster is inactive in oxygen evolution unless supplemented with Ca^{2+} (Miller & Brudvig, 1989; Tamura et al., 1989). Chloride ions are also required for photoactivation of oxygen evolution (Yamashita & Ashizawa, 1985).

It has long been known that Cl^- is essential for oxygen-evolving activity (Izawa et al., 1969; Kelly & Izawa, 1978),

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[1] Abbreviations: Chl, chlorophyll; DCIP, 2,6-dichlorophenolindophenol; EPR, electron paramagnetic resonance; kDa, kilodalton(s); Mes, 4-morpholineethanesulfonic acid; PSII, photosystem II; Tris, 2-amino-2-(hydroxymethyl)propane-1,3-diol; Y_z^+, oxidized form of tyrosine residue in photosystem II which mediates electron transfer from the Mn cluster to the reaction center chlorophyll.

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and its mode of action on intact Mn cluster has been the subject of much research [for reviews, see Critchley (1985), Dismukes (1986), Brudvig et al. (1989), and Coleman (1990)]: Chloride ions are necessary for the proper conformation of the Mn cluster at the S_2 state (Ono et al., 1986b) and for the transition from S_2 to S_3 (Itoh et al., 1984; Theg et al., 1984). Analyses of X-ray absorption of the Mn cluster (Yachandra et al., 1986a) and of the effects of Cl^- substitution on the S_2 multiline EPR signal (Yachandra et al., 1986b) have suggested that Cl^- is not associated with the Mn cluster in the S_1 and S_2 states. As for the function of Cl^- in photoactivation, little is known yet.

In the present study, we analyzed the effects of Cl^- on photoactivation using wheat PSII membranes depleted of the Mn cluster by treatment with NH_2OH. We found that Cl^- has at least two independent actions on photoactivation both of which give rise to an increase in the overall yield of photoactivation on a quantum basis. We also found that removal of the extrinsic 33-kDa protein increases the Cl^- requirement for photoactivation.

Experimental Procedures

Preparation of PSII Membranes and Extrinsic Proteins. Thylakoids were prepared from 8-day-old wheat seedlings grown under continuous light (Ono et al., 1986a). PSII membranes were prepared with Triton X-100 from the thylakoids as previously described (Miyao & Inoue, 1991). The PSII membranes were first treated with 1.5 M NaCl in darkness to remove the extrinsic 23- and 16-kDa proteins from PSII complex (Miyao & Murata, 1983) and then further treated with 1.0 mM NH_2OH for 5 min in darkness to remove Mn atoms from the PSII complex (Miyao & Inoue, 1991). The NH_2OH-treated PSII membranes were collected by centrifugation at 40000*g* for 10 min, washed once with 2.0 mM EDTA, 10 mM NaCl, 0.4 M sucrose, and 25 mM Mes–NaOH (pH 6.5) by resuspension and recentrifugation, washed again with 10 mM NaCl, 0.4 M sucrose, and 25 mM Mes–NaOH (pH 6.5) (designated hereafter as medium A), and finally suspended in medium A. The NH_2OH-treated PSII membranes prepared as above were almost completely devoid of Mn (Miyao & Inoue, 1991). In order to further remove the extrinsic 33-kDa protein, the NH_2OH-treated PSII membranes were treated with 3.4 M urea, 10 mM NaCl, and 25 mM Mes–NaOH (pH 6.5) for 10 min in darkness (Miyao & Murata, 1984a). The treated membranes were washed once with medium A by centrifugation and resuspension and finally suspended in medium A. Handling of the treated membranes was done under dim green light.

The 23- and 16-kDa proteins were extracted with 1.5 M NaCl from untreated PSII membranes, and the 33-kDa protein with 3.4 M urea from NaCl-treated membranes as described above. The extracted proteins were concentrated by ultrafiltration with an Amicon YM 10 Diaflo membrane and dialyzed against 10 mM Mes–NaOH (pH 6.5). SDS–polyacrylamide gel electrophoresis (Laemmli, 1970) revealed that these protein preparations were free of other protein components. The concentration of the 33-kDa protein was determined by using the molar extinction coefficient determined for spinach 33-kDa protein of 16 $mM^{-1}\cdot cm^{-1}$ at 276 nm (Miyao & Murata, 1989). All the procedures were done at 0–4 °C.

Photoactivation of Oxygen Evolution. Conditions for photoactivation of oxygen evolution were essentially the same as those reported by Tamura and Cheniae (1987a) with slight modifications as described in our previous report (Miyao & Inoue, 1991). The concentration of DCIP was fixed at 10 μM. Concentrations of Mn^{2+} and Ca^{2+} were 1.0 and 20 mM, respectively, unless otherwise stated.

The NH_2OH-treated or urea-treated PSII membranes were suspended in 10 μM DCIP, 0.4 M sucrose, and 25–80 mM Mes (pH 6.5) containing the designated concentrations of Mn^{2+}, Ca^{2+}, and Cl^- at 0.25 mg of Chl/mL. For photoactivation with Cl^- at concentrations above 40 mM, Ca^{2+} was supplied as $CaCl_2$, Cl^- was supplied as $CaCl_2$ and NaCl, and Mes concentration was 25 mM. For photoactivation with Cl^- below 40 mM, Ca^{2+} was added as $CaCl_2$ and/or $Ca(OH)_2$, and the Cl^- concentration was varied by changing the ratio between $CaCl_2$ and $Ca(OH)_2$. In this case, $Ca(OH)_2$ was taken from a stock solution consisting of 200 mM $Ca(OH)_2$, 0.4 M sucrose, and 600 mM Mes (pH 6.5). Manganese ion was supplied as $MnCl_2$ in all experiments. The membrane suspension was incubated in darkness for 1–2 min at 25 °C and then illuminated with continuous light from fluorescent lamps (30 $\mu E\cdot m^{-2}\cdot s^{-1}$) or with flashing light from a 4-μs xenon flash lamp at 25 °C. After illumination, the suspension was diluted with 0.4 M sucrose and 25 mM Mes–NaOH (pH 6.5) containing NaCl and the 23- and 16-kDa proteins sufficient to maximize oxygen-evolving activity. For the urea-treated PSII membranes depleted of the 33-kDa protein, purified 33-kDa protein was also included in the dilution medium to give a protein-to-Chl ratio of 0.6 (w/w). The oxygen-evolving activity was measured at 25 °C with 0.45 mM phenyl-1,4-benzoquinone using a Clark-type oxygen electrode. Final Chl concentrations of the assay mixture were 5 and 10 μg/mL when photoactivated by continuous and flashing light, respectively. The NaCl concentration of the dilution medium was designed to give a final Cl^- concentration of 10–45 mM on assay.

Activity recovery was expressed by the oxygen-evolving activity of photoactivated PSII membranes as a percentage of the activity of NaCl-treated PSII membranes measured in medium A containing 10 mM $CaCl_2$. The activities of NaCl-treated membranes measured in the presence of 10 mM $CaCl_2$ were equivalent to those of untreated PSII membranes.

Other Methods. DCIP was freshly dissolved in 10 mM Mes–NaOH (pH 6.5) at about 0.5 mM, and an accurate DCIP concentration was determined by using an extinction coefficient at 600 nm of 18.1 $mM^{-1}\cdot cm^{-1}$ at pH 6.5 (Armstrong, 1964). Chl was determined according to Arnon (1949).

Results

We have previously shown that the oxygen-evolving activity of PSII membranes once completely lost by treatment with 1.0 mM NH_2OH could be restored up to 80% of the original level when the treated membranes were incubated with 1.0 mM Mn^{2+}, 20 mM Ca^{2+}, 10 μM DCIP, and 150 mM Cl^- under continuous illumination (Miyao & Inoue, 1991). When the Cl^- concentration during illumination was reduced to 3 mM, oxygen-evolving activity did not substantially increase even after a 60-min illumination (Figure 1). With 10 mM Cl^-, the activity rapidly increased to about 40% of the original in a 20-min illumination and remained constant. On further raising the Cl^- concentration, the initial rise of activity became more rapid and the final recovery of activity higher (Figure 1). The final recovery reached maximum, about 80% of the original, at Cl^- concentrations above 300 mM, whereas the acceleration of the initial rise was not saturated even at 1200 mM Cl^- (data not shown).

As previously shown (Miyao & Inoue, 1991), photoactivation under continuous illumination is accompanied by two types of irreversible photodamage, one of which deteriorates the Mn-depleted PSII complexes before photoactivation while the other inactivates the photoactivated PSII complexes, and

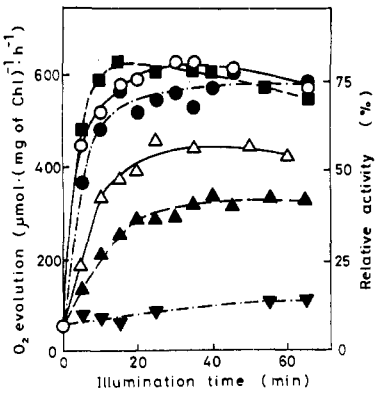

FIGURE 1: Effects of Cl^- concentration of photoactivation by continuous illumination of NH_2OH-treated PSII membranes retaining the extrinsic 33-kDa protein. NH_2OH-treated membranes were illuminated with continuous light in 1.0 mM Mn^{2+}, 20 mM Ca^{2+}, and 10 μM DCIP containing the designated concentrations of Cl^-. After a designated time, a portion of the suspension was diluted with a medium containing the 23- and 16-kDa proteins, and oxygen-evolving activity was measured. Final Cl^- concentration on assay was 10–20 mM. 100% corresponds to the activity of NaCl-treated membranes before NH_2OH treatment measured in medium A containing 10 mM $CaCl_2$. Cl^- concentration: (▼) 3.0 mM; (▲) 10 mM; (△) 30 mM; (●) 150 mM; (○) 300 mM; (■) 600 mM.

the rate of activity recovery depends not only on the photoactivation rate but also on rates of these two inhibitory processes. We first examined whether or not Cl^- affects either of these two inhibitory processes. Figure 2A shows the course of photoinhibition of Mn-depleted PSII complexes at two different Cl^- concentrations. The NH_2OH-treated PSII membranes were illuminated with continuous light in the absence of Mn^{2+} at 3 or 600 mM Cl^- for different time lapses, and then Mn^{2+} and Cl^- were added to initiate photoactivation under the same conditions. Under these conditions, the extent of photoinhibition can be estimated from the decrease in the maximum activity recovery after the Mn^{2+} addition (Miyao & Inoue, 1991). At both Cl^- concentrations, the photoinhibition proceeded in the same course with a half-time of about 30 min. Effects of Cl^- on photoinactivation of once photoactivated PSII complexes are shown in Figure 2B. NH_2OH-treated PSII membranes which had been photoactivated were illuminated with continuous light at different Cl^- concentrations under the photoactivation conditions. At 3 mM Cl^-, oxygen evolution was inactivated rapidly, and about 70% of activity was lost after a 60-min illumination. In contrast, the photoinactivation was much suppressed at 10 mM Cl^-, and almost negligible at higher Cl^- concentrations above 30 mM. The suppression of photoinactivation at high Cl^- concentrations can be ascribed to the protection by Cl^- against inhibition of oxygen evolution by exogenous Mn^{2+} (Muallem & Izawa, 1980).

Data in Figure 2A,B clearly indicate that at Cl^- concentrations above 10 mM rates of both types of photodamage are practically unchanged. It is therefore inferred that the marked acceleration by Cl^- of the initial rate of activity recovery observed in Figure 1 is not due to protection from photodamage but can solely be ascribed to acceleration of photoactivation. At Cl^- concentrations below 10 mM, on the other hand, the slow activity recovery observed in Figure 1 is probably contributed by both factors: the retardation of photoactivation and the acceleration of photoinactivation of photoactivated complexes. The low final recovery levels at low Cl^- concentrations (Figure 1) can be generally explained by the competition between photodamage and photoactivation when the photoactivation proceeds slowly (Miyao & Inoue, 1991).

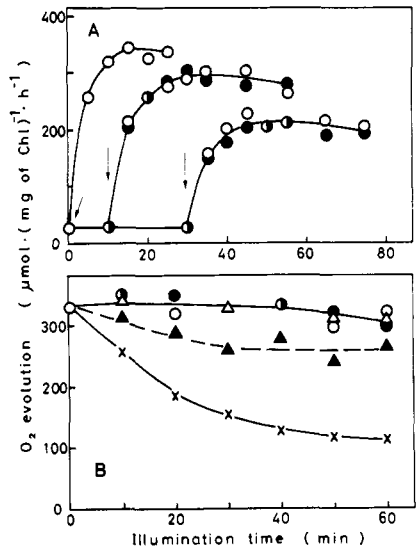

FIGURE 2: Effects of Cl^- on two types of photodamage of PSII complexes. (A) Photoinhibition of NH_2OH-treated PSII membranes. NH_2OH-treated membranes were suspended in 20 mM Ca^{2+}/10 μM DCIP containing either 3 or 600 mM Cl^- at 0.25 mg of Chl/mL and illuminated with continuous light for 0, 10, or 30 min. Then, one-fourth volume of a medium containing Mn^{2+} and Cl^- was added to give final concentrations of Mn^{2+} and Cl^- of 1.0 and 600 mM, respectively, and further illuminated. Arrows indicate the time at which Mn^{2+} and Cl^- were added to the suspension. Cl^- concentration during illumination before Mn^{2+} addition: (●) 3 mM; (○) 600 mM. (B) Photoinactivation of photoactivated PSII complexes. NH_2OH-treated membranes were photoactivated under continuous illumination with 1.0 mM Mn^{2+}/20 mM Ca^{2+}/1.2 M Cl^-/10 μM DCIP for 20 min. The photoactivated membranes were washed once with medium A containing 2.0 mM EDTA, again with medium A by centrifugation and resuspension, and finally suspended in medium A. The washed membranes were suspended in 1.0 mM Mn^{2+}/20 mM Ca^{2+}/10 μM DCIP containing designated concentrations of Cl^- at 0.25 mg of Chl/mL and illuminated with continuous light. Cl^- concentration during illumination: (×) 3 mM; (▲) 10 mM; (△) 30 mM; (●) 300 mM; (○) 600 mM. Oxygen-evolving activity was measured as in Figure 1.

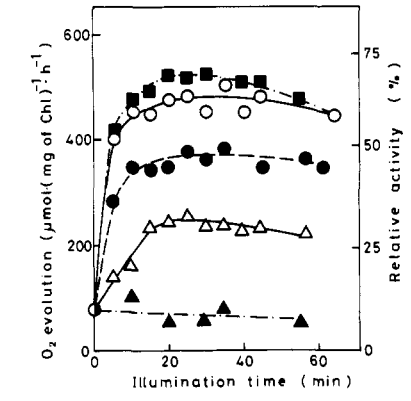

FIGURE 3: Effects of Cl^- concentration on the photoactivation by continuous illumination of urea-treated PSII membranes lacking the extrinsic 33-kDa protein. Urea-treated membranes were illuminated as described in the Figure 1 legend. After illumination, the suspension was diluted with a medium containing all three extrinsic proteins, and oxygen-evolving activity was measured. 100% is the same as in Figure 1. Cl^- concentration: (▲) 50 mM; (△) 100 mM; (●) 250 mM; (○) 750 mM; (■) 1000 mM.

Photoactivation can proceed in the absence of the extrinsic 33-kDa protein (Tamura & Cheniae, 1986, 1987a). When the 33-kDa protein was removed from NH_2OH-treated PSII membranes by urea treatment, Cl^- similarly accelerated the initial rise in activity and also increased the final activity recovery (Figure 3). However, higher Cl^- concentrations were

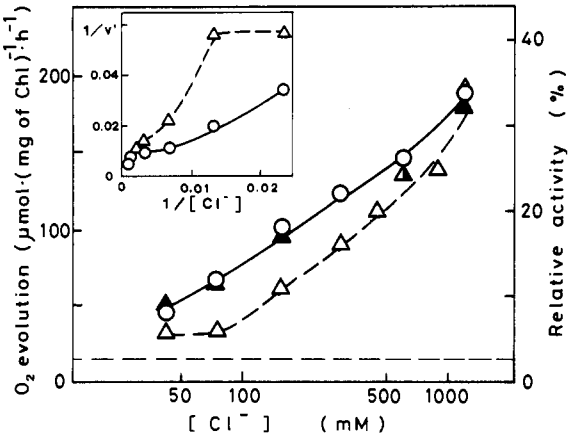

FIGURE 4: Dependence on Cl^- concentration of the photoactivation with flashing light. NH_2OH-treated or urea-treated PSII membranes were suspended in 1.0 mM Mn^{2+}, 20 mM Ca^{2+}, and 10 μM DCIP containing designated concentrations of Cl^- with or without supplement of 33-kDa protein and illuminated with 100 flashes at 1-s intervals, and oxygen-evolving activity was measured in the presence of all three extrinsic proteins at 10–45 mM Cl^-. Averages of three or four measurements are presented. 100% is the same as in Figure 1, and the horizontal dashed line represents the activity before illumination. (○) NH_2OH-treated membranes; (△) urea-treated membranes; (▲) urea-treated membranes supplemented with 33-kDa protein at a protein-to-Chl ratio of 0.6 (w/w). (Inset) Lineweaver–Burk plot of the restored activity against Cl^- concentration. Restored activity (v') represents the difference in oxygen-evolving activity before and after flash illumination. Units of the ordinate and abscissa are micromoles of O_2 per milligram of Chl per hour and mM^{-1}, respectively.

required in the absence of the protein as compared with that in its presence: Cl^- concentration above 750 mM was necessary to maximize the activity recovery. At an optimum Cl^- concentration, 1000 mM, the activity was restored to about 70% of the original in the first 20 min of illumination (Figure 3). The slightly reduced level of maximum recovery did not result from depletion of the 33-kDa protein, since readdition of the protein during illumination did not improve the maximum recovery (data not shown). It may be due to some additional effects of urea treatment employed to remove the 33-kDa protein.

To accurately analyze the Cl^- effect on photoactivation rate, illumination with 100 flashes instead of continuous light was employed (Figure 4). In NH_2OH-treated PSII membranes containing the extrinsic 33-kDa protein, the activity recovery by flash illumination increased with Cl^- concentration without showing a saturation phenomenon even at 1200 mM. In urea-treated PSII membranes depleted of the 33-kDa protein, on the other hand, the activity recovery remained at a very low level at Cl^- concentrations below 100 mM, and then increased with Cl^- concentration, although the recovery was always lower than that in membranes retaining the 33-kDa protein. These Cl^- dependences are the same as those observed for the photoactivation rate under continuous illumination (Figures 1 and 3), indicating that the activity recovery by flashes corresponds to the photoactivation rate under continuous illumination. Readdition of the 33-kDa protein to the urea-treated PSII membranes reversed this Cl^- dependence back to that before protein removal. This indicates that the 33-kDa protein reduces the Cl^- requirement for photoactivation. Either in the presence or in the absence of the 33-kDa protein, the Lineweaver–Burk plot of the restored activity against Cl^- concentration did not give a straight line but a sigmoidal curve (inset of Figure 4), suggesting that the Cl^- effect does not result from a single action of Cl^-.

Table I lists the effects of various salts at 300 mM on activity recovery by flash illumination. Bromide ion restored

Table I: Effects of Various Salts on Photoactivation of Oxygen Evolution in NH_2OH-Treated PSII Membranes[a]

salt added	O_2 evolution [μmol·(mg of Chl)$^{-1}$·h^{-1}]: NaCl-treated, incubated in darkness	O_2 evolution [μmol·(mg of Chl)$^{-1}$·h^{-1}]: NH_2OH-treated, illuminated	activity recovery (%)
NaCl	840	210	25
NaBr	850	210	25
$NaNO_3$	680	70	10
CH_3COONa	670	50	7
HCOONa	460	40	9
tetramethylammonium chloride	850	180	21
choline chloride	870	160	18
KCl	800	130	16

[a] NH_2OH-treated PSII membranes were suspended in 1.0 mM Mn^{2+}, 20 mM Ca^{2+}, and 10 μM DCIP containing a designated salt at 300 mM and illuminated with 100 flashes at 1-s intervals. NaCl-treated PSII membranes were also suspended in the same medium but followed by dark incubation for 5 min. Then, oxygen-evolving activity was measured after dilution with a medium containing the 23- and 16-kDa proteins. Final Cl^- concentration on the assay was 10 mM. Averages of three or four measurements are presented. Since some salts at 300 mM inactivated oxygen evolution by NaCl-treated PSII membranes, activity recovery was expressed as the ratio of oxygen-evolving activities between illuminated NH_2OH-treated membranes and NaCl-treated membranes after dark incubation with a salt. The activity of NH_2OH-treated membranes before illumination was 20 μmol of O_2·(mg of Chl)$^{-1}$·h^{-1}.

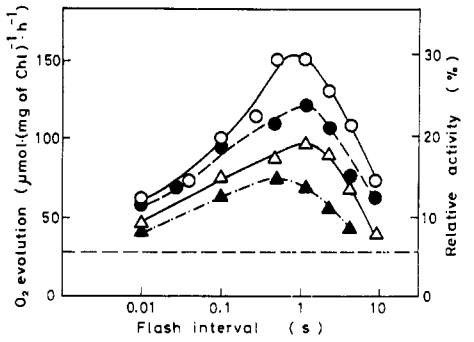

FIGURE 5: Effects of Cl^- concentration on the flash interval dependence of photoactivation. NH_2OH-treated PSII membranes were suspended in 1.0 mM Mn^{2+}, 20 mM Ca^{2+}, and 10 μM DCIP containing the designated concentrations of Cl^- and illuminated with 100 flashes at designated intervals, and oxygen-evolving activity was measured. Averages of three or four measurements are presented. 100% and the horizontal dashed line are the same as in Figure 4. Cl^- concentration: (▲) 60 mM; (△) 150 mM; (●) 300 mM; (○) 600 mM.

the activity as effectively as Cl^-. Nitrate, acetate, and formate ions, on the other hand, did not substantially substitute for Cl^-. This specificity among anion species is consistent with previous results (Yamashita & Ashizawa, 1985; Tamura & Cheniae, 1987a) and resembles that in supporting oxygen evolution by the intact Mn cluster (Hind et al., 1969; Kelly & Izawa, 1978). The effectiveness of Cl^- depended on the countercation, and decreased in the order of Na^+, tetramethylammonium ion, choline ion, and K^+ (Table I). When the salt concentration was reduced to 10 mM (in this case, photoactivation was effected by continuous illumination because of the very low activity recovery by flash illumination), the anion specificity did not change, but the dependence of Cl^- effect on countercation disappeared except for K^+: KCl was appreciably less effective than the others (data not shown). The low effectiveness of KCl may be due to inhibition by K^+ of photooxidation of Mn^{2+} by Y_z^+ (Hoganson et al., 1989). Sodium ion, in contrast, has been reported to be less inhibitory

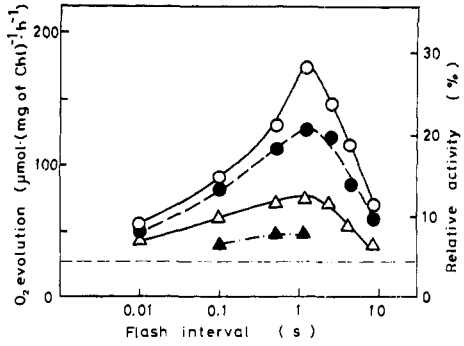

FIGURE 6: Effects of Mn^{2+} concentration on the flash interval dependence of photoactivation. NH_2OH-treated PSII membranes were suspended in 20 mM Ca^{2+}, 150 mM Cl^-, and 10 μM DCIP containing the designated concentrations of Mn^{2+} and illuminated with 100 flashes at designated intervals, and oxygen-evolving activity was measured. Averages of three or four measurements are presented. 100% and the horizontal dashed line are the same as in Figure 4. Mn^{2+} concentration: (▲) 0.1 mM; (△) 0.3 mM; (●) 1.0 mM; (○) 3.0 mM.

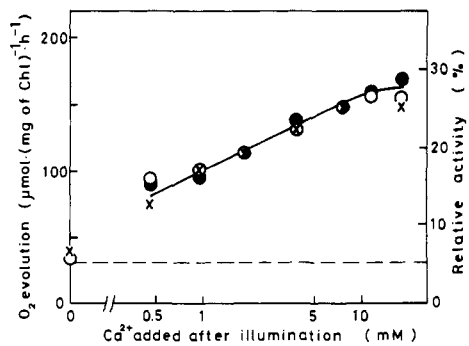

FIGURE 7: Effects of Cl^- on the Ca^{2+} requirement of oxygen evolution by the reconstituted Mn cluster. NH_2OH-treated PSII membranes were suspended at 0.5 mg of Chl/mL in 2.0 mM Mn^{2+}, 600 mM Cl^-, and 10 μM DCIP and illuminated with 100 flashes at 1-s intervals. Then, the suspension was diluted with an equal volume of 0.4 M sucrose and 25 mM Mes–NaOH (pH 6.5) containing $CaCl_2$ and NaCl to give the designated concentrations of Ca^{2+} and Cl^-. After incubation at 25 °C for 15 min in darkness, oxygen-evolving activity was measured at 45 mM Cl^-. Averages of three or four measurements are presented. 100% and the horizontal dashed line are the same as in Figure 4. Cl^- concentrations during dark incubation with Ca^{2+} were 300 mM (×), 600 mM (●), and 1200 mM (○).

to Mn^{2+} photooxidation than K^+ (Hoganson et al., 1989).

Figure 5 shows the effect of Cl^- on the flash interval dependence of activity recovery. At every Cl^- concentration, the flash interval dependence exhibited a bell-shaped curve. According to the model of photoactivation involving a two-quantum process as depicted in Scheme I, rise and decay of the bell-shaped curve correspond to formation of the second unstable intermediate (C) after the first photoreaction and its decay in darkness, respectively. At 60 mM Cl^-, the recovery showed a maximum at an interval of 0.5 s. At 150 mM Cl^-, the recovery increased at every flash interval, and the interval for maximum recovery was shifted to 1 s. This indicates that the intermediate is more stable at 150 mM than at 60 mM Cl^-. We have previously reported that decay of the intermediate is stimulated by raising the DCIP concentration during illumination (Miyao & Inoue, 1991). Thus, the lower stability of the intermediate observed at 60 mM Cl^- would be simply ascribable to a lower yield of the intermediate relative to a fixed amount of DCIP. However, this possibility is ruled out by Figure 6 experiments that clearly indicate that the stability of the intermediate in the presence of 150 mM Cl^- did not change even though the yield of intermediate was much suppressed by decreasing the Mn^{2+} concentration. On raising the Cl^- concentration above 150 mM, the recovery increased further at every flash interval without any change either in the interval for maximum recovery or in the overall shape of the dependence curve (Figure 5). This suggests that Cl^- above 150 mM increases the overall quantum yield of photoactivation without changing the rates of formation and decay of the second unstable intermediate.

Reactivation of oxygen evolution requires not only reassembly of the Mn cluster but also rebinding of Ca^{2+} to its functional site in the PSII complex (Ono & Inoue, 1983; Miller & Brudvig, 1989; Tamura et al., 1989). One may thus assume that Cl^- might facilitate the binding of Mn^{2+} and/or Ca^{2+} and thereby increase the photoactivation yield. We first examined whether or not Cl^- facilitates the binding of Ca^{2+} to its functional site (Figure 7). A certain amount of Mn cluster was reconstituted in the absence of Ca^{2+}, and then the Ca^{2+} requirement for oxygen evolution of the reconstituted cluster was investigated at three different Cl^- concentrations. Obviously, the Ca^{2+} requirement was identical irrespective of the Cl^- concentration, suggesting that Cl^- does not facilitate the binding of Ca^{2+} to its functional site in the PSII complex. This observation, on the other hand, suggests that Na^+, a countercation of Cl^-, does not affect Ca^{2+} binding. This might contradict the previous finding that Na^+ inhibits Ca^{2+} binding in the oxygen-evolving PSII complex (Waggoner et al., 1989). However, it is noted that in the experiments of Figure 7 the Ca^{2+} requirement was examined in the presence of Mn^{2+} which has a stronger affinity to the Ca^{2+} binding site than Ca^{2+} (Ono & Inoue, 1983; Miller & Brudvig, 1989). Probably the Ca^{2+} effect shown in Figure 7 represents the Ca^{2+} demand to exclude Mn^{2+} away from the Ca^{2+} binding site. In contrast, the inhibition by Na^+ observed by Waggoner et al. (1989) might reflect the competition between Na^+ and Ca^{2+} for vacant Ca^{2+} binding sites.

Next we examined the effects of Cl^- on the affinity for Mn^{2+} of the Mn^{2+} binding site. To eliminate the influence of Ca^{2+}, a competitive inhibition for Mn^{2+} binding (Ono & Inoue, 1983; Miller & Brudvig, 1989), Ca^{2+} was excluded from the photoactivation mixture but supplied after flash illumination. Figure 8A shows the dependence of activity recovery on Mn^{2+} concentration at different Cl^- concentrations between 150 and 1200 mM. Within this concentration range of Cl^-, the second unstable intermediate was equally stable irrespective of Cl^- concentration (Figure 5). We can thus assume that the activity recovery reflects the relative quantum yield of photoactivation. As shown in Figure 8A, the dependence on Mn^{2+} concentration exhibited a saturation phenomenon at every Cl^- concentration. On raising the Cl^- concentration, the yield was increased at every Mn^{2+} concentration, and the Mn^{2+} concentration for maximum yield was shifted to a higher concentration. However, the extent of Cl^--induced increase in yield was small at low Mn^{2+} concentrations but enlarged with Mn^{2+} concentration: on raising the Cl^- concentration from 150 to 1200 mM, the yield was almost doubled at 0.6 mM Mn^{2+}, while the increase was only about 20% at 0.15 mM Mn^{2+}. The data in Figure 8A were replotted with the Mn^{2+} concentration divided by the restored activity as the ordinate and with Mn^{2+} concentration as the abscissa (Segel, 1975). Since the reciprocal of the ordinate corresponds to the photoactivation yield per unit Mn^{2+} concentration, this plot is more suitable than the Lineweaver–Burk plot to compare the extent of Cl^- concentration effect at different Mn^{2+} concentrations. As shown in Figure 8B, the plot gave straight lines which intersected at one point on the ordinate. This indicates that the Cl^--induced increase in the photoactivation yield decreases on lowering the Mn^{2+} concentration and diminishes at an indefinitely low Mn^{2+}

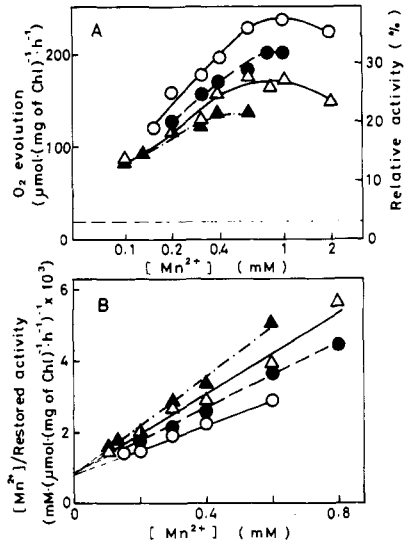

FIGURE 8: Dependence on Mn^{2+} concentration of the flash photoactivation yield at different Cl^- concentrations. NH_2OH-treated PSII membranes were suspended in 10 μM DCIP and the designated concentrations of Mn^{2+} and Cl^-, illuminated with 100 flashes at 1-s intervals, and then supplemented with 20 mM Ca^{2+} by adding 0.4 M $CaCl_2$ (pH 6.5). After incubation at 25 °C for 15 min in darkness, oxygen-evolving activity was measured at 45 mM Cl^-. Averages of three or four measurements are presented. (A) Dependence on Mn^{2+} concentration of the flash photoactivation yield. 100% and the horizontal dashed line are the same as in Figure 4. (B) Plot of Mn^{2+} concentration divided by the restored activity against Mn^{2+} concentration. Data in (A) with Mn^{2+} concentrations between 0.1 and 0.8 mM are replotted. Restored activity represents the difference in oxygen-evolving activity before and after flash illumination. Data were approximated to straight lines by the least-squares method. Cl^- concentrations during illumination were 150 mM (▲), 300 mM (△), 600 mM (●), and 1200 mM (○).

concentration where binding of Mn^{2+} limits the whole process. It is therefore suggested that the affinity of Mn^{2+} binding and the efficiency of proper assembly of the bound Mn^{2+} are not affected by Cl^-.

In enzymatic reactions, the intersection of straight lines on the ordinate as in the plot of Figure 8B [equivalent to parallel straight lines in the Lineweaver–Burk plot (Segel, 1975)] can be explained by acceleration of one of the steps involved in the reaction sequence. However, this cannot be directly applied to photoactivation, if we take into account the unique situation of photoactivation effected by flashing light. In enzymatic reactions, individual reactions proceed sequentially in either forward or reverse direction on every enzyme molecule, and the enzyme molecule repeatedly reacts with the substrate after releasing the product, so that the rate of every step can affect the overall reaction rate. In photoactivation with flashing light, in contrast, the process is driven by the two photoreactions intermittently triggered at a relatively long dark interval. Consequently, a change in the rate of a step which rapidly completes within the interval cannot affect at all the overall yield. In addition, the rate of the last step does not affect the yield either, because a PSII complex once successfully photoactivated will not repeatedly participate in the process. A likely candidate for the step, whose change in rate will give rise to an increase in the overall yield of photoactivation, will be the rearrangement of the first unstable intermediate (conversion of B to C in Scheme I). However, the rearrangement rate roughly estimated from the half rise time of the flash interval dependence curve (Figure 5) did not show any clear increase with Cl^- concentration. Thus, it is unlikely to explain the results in Figure 8B by assuming that Cl^- accelerates some specific step in photoactivation.

Another possible explanation will be that Mn^{2+} itself at high concentrations causes some inhibitory effect on photoactivation and Cl^- suppresses this inhibitory effect. This is not unlikely, since a protective effect of Cl^- against inhibition by Mn^{2+} of oxygen evolution has been reported previously (Muallem & Izawa, 1980). The suppression of the inhibition by Mn^{2+} would enable PSII complexes to efficiently utilize concentrated Mn^{2+} for photoactivation, resulting in a higher yield at higher Mn^{2+} concentrations.

Discussion

Effects of Cl^- on Photoactivation with Continuous Light. Chloride ion apparently exhibited two effects on activity recovery by photoactivation with continuous light: acceleration of the initial rise in activity and increase in the final level of activity recovery (Figure 1). Both can originate in a single effect of Cl^-, that is, acceleration of photoactivation. This acceleration did not saturate even at 1.2 M Cl^-, but the final recovery level reached a maximum at Cl^- above 300 mM (Figure 1). When the extrinsic 33-kDa protein was removed from the PSII complex, the maximum recovery required Cl^- above 750 mM (Figure 3).

The Cl^- effect on photoactivation has been investigated previously, and the apparent Michaelis constant for Cl^- was reported to be 0.3 mM in Tris-treated grana (Yamashita & Ashizawa, 1985) and 3.8 mM in NH_2OH-treated PSII membranes (Tamura & Cheniae, 1987a). Since these values were determined for the maximum recovery after a 40-min illumination with continuous light, they will probably be much lower than the values determined for the photoactivation rate.

Effects of Cl^- on Photoactivation with Flashing Light. Dependences on Cl^- concentration of activity recovery by illumination with 100 flashes at 1-s intervals (Figure 4) were almost the same as those of the photoactivation rate observed under continuous illumination (Figures 1 and 3). This indicates that the recovery by flash illumination preferentially reflects the photoactivation rate under continuous illumination. The activity recovery by flash illumination increased with Cl^- concentration without showing a saturation phenomenon even at 1.2 M (Figure 4). Also in this case, removal of the 33-kDa protein shifted the Cl^- requirement to higher concentrations.

This effect of Cl^- does not appear to result from a single action of Cl^- but a combination of two effects: stabilization of the intermediate state [the second unstable intermediate (C) in Scheme I] and increase of the quantum yield of photoactivation. In the PSII complex retaining the 33-kDa protein, the stabilization was sufficed by Cl^- at about 150 mM (Figure 5). In contrast, the increase in quantum yield of photoactivation, which can be distinguished from the stabilizing effect by comparing the yield at flash intervals shorter than 0.5 s, was observed at all Cl^- concentrations tested (Figure 5). The Cl^--induced increase in yield did not result from changes in the affinity of either Ca^{2+} binding or Mn^{2+} binding (Figures 7 and 8) but could rather be ascribed to a protective effect of Cl^- against some inhibition caused by high concentration of Mn^{2+}. One might consider that at high Cl^- concentrations Cl^- may coordinate to Mn^{2+} to form Mn–Cl complexes such as $MnCl_3^-$ and $MnCl_4^{2-}$, and thereby reduces the concentration of free Mn^{2+}. However, this does not seem to be the case if we refer to the formation constants for these complexes. Since the stability of a Mn–ligand complex is much lower than that of the corresponding Cu^{2+} complex irrespective of the coordinating ligand species or the number of ligands involved (Irving & Williams, 1953), the cumulative formation constants for $MnCl_3^-$ and $MnCl_4^{2-}$ are estimated to be much smaller than those for $CuCl_3^-$ of $10^{-2.4}$ and $CuCl_4^{2-}$

of $10^{-4.2}$ (Bjerrum, 1950). Thus, the Mn–Cl complexes will not be substantially formed even in 1 M Cl^-. We suggest that Cl^- affects the PSII complex itself but not Mn^{2+} and thereby suppresses the Mn^{2+} inhibition.

The requirement of highly concentrated Cl^- implies that concentrated Na^+ is not inhibitory to photoactivation. This is surprising in view of the fact that concentrated Na^+ is known to inhibit binding of divalent cations [e.g., see Waggoner et al. (1989)]. In addition, as demonstrated in Table I, large bulky cations such as tetramethylammonium and choline ions, which are known to be less inhibitory to binding of divalent cations because of their larger size [e.g., see Waggoner and Yocum (1990)], were slightly inhibitory as compared with Na^+. We consider that this might have resulted from the difference in effectiveness of these monovalent cations to exclude Ca^{2+}, a competitive inhibitor for Mn^{2+} binding, away from the Mn^{2+} binding site. This appears possible when we take into account the much higher affinity for Mn^{2+} than Ca^{2+} of the Mn^{2+} binding site (Ono & Inoue, 1983; Miller & Brudvig, 1989) and the relatively high Mn^{2+} concentration in the photoactivation mixture employed in this study. In fact, the dependence of the Cl^- concentration effect on the countercation observed with salts at 300 mM (Table I) disappeared when the salt concentration was reduced to 10 mM (data not shown). This could be the reason why the effectiveness of countercations exhibited an order of the capacity to inhibit the binding of divalent cations. Probably, Na^+ to some extent contributes to the increase in photoactivation yield by preferentially lowering the affinity for Ca^{2+} of the Mn^{2+} binding site when both Mn^{2+} and Ca^{2+} are present. Potassium ion, on the other hand, inhibits not only Ca^{2+} binding but also Mn^{2+} binding to the Mn^{2+} binding site [see Hoganson et al. (1989)].

As discussed above, the multiple action of Cl^- (and Na^+ when Ca^{2+} is present during photoactivation) is suggested. This will probably be the reason why the Lineweaver–Burk plot with respect to Cl^- concentration gave a sigmoidal curve (inset of Figure 4).

Effects of the Extrinsic 33-kDa Protein on Photoactivation. It is known that the 33-kDa protein reduces the Cl^- requirement for oxygen evolution of the intact Mn cluster (Miyao & Murata, 1985). The present study revealed that the protein also reduces the Cl^- requirement for photoactivation (Figures 3 and 4). Since the Mn cluster is unstable in the absence of the 33-kDa protein unless supplemented with Cl^- above 100 mM (Miyao & Murata, 1984b; Kuwabara et al., 1985), the higher Cl^- requirement for photoactivation in the absence of the protein can partly be ascribed to destabilization of the reconstituted Mn cluster in the absence of both the protein and sufficient Cl^-. However, the photoactivation yield in the absence of the protein was still lower than that in its presence even at Cl^- concentrations above 100 mM sufficient to stabilize the Mn cluster (Figure 4). This indicates that the 33-kDa protein not only stabilizes the reconstituted cluster but also acts to reduce the Cl^- requirement for photoactivation.

Mode of Action of Cl^- in Relation to the Mechanism of Photoactivation. Since the functional Mn cluster consists of four Mn atoms of a higher average oxidation state than Mn^{2+}, the two photoreactions (conversions of A to B and of C to D in Scheme I) have been proposed to involve photooxidation of Mn^{2+} by PSII to yield Mn^{3+} (Ono & Inoue, 1987; Tamura & Cheniae, 1987a, 1988; Miller & Brudvig, 1989). Thus, the first unstable intermediate (B) would contain one Mn^{3+} bound. This intermediate is rearranged to the next unstable intermediate in darkness (dark conversion of B to C). This step has been proposed to involve binding of another Mn^{2+} in preparation for the second photoreaction (Tamura & Cheniae, 1987a; Miller & Brudvig, 1989). We found that the rearrangement rate estimated from the half rise time in the flash interval dependence curve (Figure 6) did not increase with Mn^{2+} concentration. This indicates that binding of the second Mn^{2+} is not rate-limiting in the rearrangement. Probably, the rearrangement itself occurs independent of Mn^{2+} binding. Chloride ion also does not affect the rearrangement rate (Figure 5).

The second unstable intermediate (C) was appreciably stabilized by Cl^- (Figure 5). This intermediate is sensitive to reducing reagents and decays back to A, probably through re-reduction of Mn^{3+} by reductants (Cheniae & Martin, 1973; Ono & Inoue, 1987; Tamura & Cheniae, 1987b). Under the photoactivation conditions in this study, the reduced form of DCIP formed during illumination acts as the reductant (Miyao & Inoue, 1991). On the other hand, the first intermediate (B) appears to be less sensitive to reductants than the second intermediate. This view is based on our previous observation and others that the flash photoactivation yield at flash intervals shorter than 0.1 s was not at all affected by reductants which effectively accelerated the decay of the second intermediate (Tamura & Cheniae, 1987b; Miyao & Inoue, 1991). These infer that Mn^{3+} in the first intermediate becomes more susceptible to re-reduction after rearrangement to the second intermediate and that Cl^- makes the susceptible Mn^{3+} more resistant to the attack by reductants.

Chloride ion markedly increased the quantum yield of photoactivation probably by suppressing some inhibitory effect of concentrated Mn^{2+}. It has been generally recognized that the low quantum yield of photoactivation results from the charge recombination reactions in PSII and a low efficiency in yielding the second unstable intermediate [conversion from A to C (Tamura & Cheniae, 1987a)]. Recently, Miller and Brudvig (1990) have demonstrated that oxidation of exogenous Mn^{2+} by Y_z^+ in the first photoreaction occurs at a very low quantum efficiency and proposed that this is the main cause for the low yield of photoactivation. In addition, it has been reported that Mn^{2+} oxidation by Y_z^+ is inhibited when the Mn^{2+} concentration is too high (Hoganson et al., 1989). Taking this and the high concentration of Mn^{2+} in the photoactivation mixture in this study into consideration, it is very likely that Cl^- acts to increase the quantum yield of Mn^{2+} photooxidation in the first photoreaction that limits the overall yield of photoactivation. However, the possibilities that Cl^- affects some other steps (conversions of C to D and of D to E) cannot be excluded at present.

The Cl^- requirements for photoactivation described above are largely similar to that for oxygen evolution by the intact Mn cluster in terms of the anion specificity and the effect of the 33-kDa protein. However, the dependences on Cl^- concentration are different from each other. The stimulation by Cl^- of oxygen evolution exhibits a saturation phenomenon at a Cl^- concentration between 10 and 150 mM depending on the composition of extrinsic proteins (Miyao & Murata, 1985), whereas the Cl^--induced increase in the quantum yield of photoactivation was not saturated even at 1.2 M (Figures 4 and 8). In addition, Cl^- does not appear to have its specific binding site(s) in the PSII complex in the latter case, since the Lineweaver–Burk plot with respect to Cl^- concentration of the data in Figure 8 did not give a straight line (not shown). Thus, it is likely that Cl^- functions in photoactivation in a way different from that in intact Mn clusters. Highly concentrated Cl^- is known to act as a chaotropic reagent which alters the higher structure of proteins or disrupts hydrophobic interac-

tions (von Hippel & Schleich, 1969). Taking into account this and the fact that quite highly concentrated Cl^- is effective in photoactivation, we presume that Cl^- alters the conformation around the Mn^{2+} binding site and thereby increases the quantum yield of photoactivation.

Chloride Requirement for Photoactivation in Vivo. The Cl^- requirement for photoactivation reported here is much above the physiological level of Cl^-: the Cl^- concentration in thylakoid lumen is likely to be close to that in stroma of 30–60 mM (Demmig & Gimmler, 1983), since thylakoid membranes are permeable to Cl^- (Schuldiner & Avron, 1971). However, it is noted that the Cl^- action in increasing the quantum yield of photoactivation is observed only at Mn^{2+} concentrations above 0.1 mM (Figure 8).

The concentration of Mn^{2+} in thylakoid lumen in vivo can be estimated to be around several micromolar, since exogenous Mn^{2+} at 10 μM well compensated the suppression of photoactivation by an ionophore, A23187, in intact chloroplasts from leaves grown under intermittent illumination (Ono & Inoue, 1983). At such a low Mn^{2+} concentration, Cl^- may act to stabilize the second unstable intermediate but not to increase the quantum yield of photoactivation. In addition, the photoinhibition of Mn-depleted PSII complexes, which sometimes limits activity recovery by photoactivation in vitro, appears to be well suppressed in vivo: We have previously observed that the photoinhibition can be minimized by adding an efficient electron acceptor of PSII, phenyl-1,4-benzoquinone, instead of less efficient DCIP (Miyao & Inoue, 1991). This infers that the photoinhibition is suppressed when a negative charge on the acceptor side is efficiently removed. In native thylakoid membranes, electrons on the acceptor side are efficiently removed by electron-transfer reactions through cytochrome *b/f* complex and photosystem I. Therefore, it is likely that Mn-depleted PSII complexes hardly suffer the photoinhibition in vivo, so that oxygen evolution can be fully restored in vivo even though photoactivation proceeds rather slowly with the limited concentration of Mn^{2+}. Thus, Cl^- concentration below 100 mM will be sufficient for photoactivation in vivo.

The low quantum yield of photoactivation is an obstacle to the study of the molecular mechanism of photoactivation and has to be much improved. Higher Mn^{2+} concentration above the physiological level is required to enhance the yield, but it tends to inhibit photoactivation at the same time. As discussed above, concentrated Cl^- enables the PSII complexes to efficiently utilize concentrated Mn^{2+} for photoactivation. Thus, we conclude that highly concentrated Cl^- is a prerequisite to artificially increase the quantum yield of photoactivation.

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Site-Directed Mutagenesis of the *psb*C Gene of Photosystem II: Isolation and Functional Characterization of CP43-less Photosystem II Core Complexes†

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ABSTRACT: Two mutants of *Synechocystis* PCC 6803 lacking the *psb*C gene product CP43 were constructed by site-directed mutagenesis. Analysis of cells and thylakoid membranes of these mutants indicates that PS II reaction centers accumulate to a concentration of about 10% of that of WT cells. PS II core complexes isolated from mutants lacking the CP43 subunit show light-driven electron transfer from the secondary electron donor Z to the primary quinone electron acceptor Q_A with a quantum yield similar to that of wild type, indicating that CP43 is not required for binding or function of Q_A. The use of mutants for the removal of CP43 thus avoids the loss of Q_A function associated with biochemical extraction of CP43 from intact core complexes. Both absorbance and fluorescence emission maxima of the mutant complexes show a blue shift in comparison to the WT PS II core complex, indicating that the absorbance spectrum of CP43 is red-shifted relative to that of the remainder of the core complex. The antenna size of these CP43-less complexes is about 70% of that of WT, indicating that approximately 15 chlorophyll molecules are bound by CP43. The molecular mass of the PS II complex, including the detergent shell, shifts from 310 ± 15 kDa in WT to 285 ± 15 kDa in the CP43-less mutants.

The photosystem II reaction center is the site of one of the two primary photoreactions of the oxygenic electron-transport chain of photosynthesis [see Hansson and Wydrzynski (1990) for a recent review]. The redox components responsible for the primary charge separation are bound by two polypeptides, D1[1] and D2 (Nanba & Satoh, 1987). These form part of a larger ensemble, the oxygen-evolving core complex which may be isolated through detergent extraction techniques [see, for example, Ghanotakis et al. (1987)]. This complex retains the ability to evolve oxygen in the presence of artificial quinone electron acceptors and is composed of some 20 polypeptides (Ikeuchi et al., 1990; Koike et al., 1990).

The two major chlorophyll–protein complexes of the PS II core complex are CP47 and CP43 (Delepelaire & Chua, 1979; Camm & Green, 1980; Bricker, 1990), the apoproteins of which are ~56 and 50 kDa, respectively, as deduced from the known nucleotide sequences of the corresponding genes, *psb*B and *psb*C. Both are thought to contain six transmembrane α-helices and homologous histidine residues near the stromal

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[1] Abbreviations: β-DM, dodecyl β-D-maltoside; bp, basepair(s); Chl, chlorophyll; CP43, one of two major chlorophyll–protein complexes of the PS II core complex, and the *psb*C gene product; CP47, one of two major chlorophyll–protein complexes of the PS II core complex and the *psb*B gene product; cyt *b*559, cytochrome *b*-559; D1, one of two polypeptides that coordinate the primary photoreactants of PS II and the *psb*A gene product; D2, one of two polypeptides that coordinate the primary photoreactants of PS II and the *psb*D gene product; DCMU, Diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DCPIP, dichlorophenolindophenol; kb, kilobase(s); kDa, kilodalton(s); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HPLC, high-performance liquid chromatography; NH_2OH, hydroxylamine; P680, primary electron donor; PCC, Pasteur culture collection; PCR, polymerase chain reaction; PQ, plastoquinone; PQ^-, plastosemiquinone anion; PS I, photosystem I; PS II, photosystem II; Q_A, primary quinone electron acceptor; Q_B, secondary quinone electron acceptor; RC, reaction center; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; WT, wild type; Z, secondary electron donor identified as tyrosine-161 in polypeptide D1.

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