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THE HIGH AFFINITY BINDING SITE FOR CALCIUM ON THE OXIDIZING SIDE OF PHOTOSYSTEM II

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Preparations of photosystem II complex from spinach chloroplasts with Triton X-100 were treated with 1 M KCl to release 17 KDa and 23 KDa polypeptides. The inhibited oxygen evolution activity could be reactivated by adding high concentration (mM) of Ca^{++} or by reconstituting 17 KDa and 23 KDa polypeptides which were found to promote high affinity binding of Ca^{++} to the reconstituted membranes (Ghanotakis et al. FEBS (1984) 170, 169-173). Inclusion of 50 mM Ca^{++} during KCl treatment did not prevent the release of 17 KDa and 23 KDa polypeptides but protected oxygen evolution from being inactivated. It is explained by preservation of the high affinity binding site for Ca^{++} if Ca^{++} is present during the salt treatment even though depletion of 17 KDa and 23 KDa polypeptides usually results in replacement by a low affinity (mM) binding site for Ca^{++}. It also implies that the high affinity binding site is not located on 17 KDa and 23 KDa polypeptides.

Recent investigations have revealed that three peripheral proteins with molecular masses of 33 KDa, 23 KDa and 17 KDa are closely related to the function of photosynthetic oxygen evolution (1-8). A variety of treatments on oxygen evolving PSII preparations can remove these polypeptides from thylakoids. 23 KDa and 17 KDa polypeptides can be specially liberated by high concentration of monovalent salt washing of PSII preparations and oxygen evolution activity is partially inactivated (2,3,4). Reconstitution of 23 KDa polypeptide to salt washed PSII preparations would restore some activity (2,8). The 23 KDa polypeptide has been suggested to play either regulatory (9) or essential (10) role in oxygen evolution.

On the other hand, studies on cyanobacteria (11,12,13) as well as higher plant chloroplasts (14,15,16) suggest that Ca^{++} is a necessary factor for proper functioning of PSII. High (non-physiological) concentration of Ca^{++} (mM) has been shown to restore oxygen evolution activity

Abbreviations: Chl, chlorophyll; DCIP, 2,6-dichlorophenolindophenol; EGTA, ethylenegycol-bis-(β-aminoethyl ether)N,N-tetra-acetic acid; Mes, 4-morpholineethanesulfonic acid; PSII, photosystem II; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

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in salt washed PSII preparations while 23 KDa and 17 KDa polypeptides have been depleted (9,17). Authors in (18) demonstrated that reconstitution of 23 KDa and 17 KDa polypeptides to PSII created a high affinity binding for Ca^{++} which is responsible for reactivation of oxygen evolution activity.

In this study, we investigated the effect of Ca^{++} on oxygen evolution of salt washed PSII preparations and found that by including Ca^{++} during KCl wash, the high affinity Ca^{++} binding site on PSII seemed to be preserved even after the depletion of 23 KDa and 17 KDa polypeptides. It also implies that the binding site is not located on either 23 KDa or 17 KDa polypeptide.

MATERIALS AND METHODS

Preparation of PSII particles from local market spinach was carried out with Triton X-100 as in (19) and stored in liquid nitrogen. The electron transport activity was about 160 μmole DCIP.$mgChl^{-1}.hr^{-1}$. The PSII particles, when used, were defrosted and washed with 10 mM NaCl and 20 mM Mes-NaOH (pH6.1). For salt treatment, the particles (400 μgChl/ml) were incubated at room temperature for 15 min in a medium containing 1 M KCl, 10 mM NaCl, 20 mM Mes-NaOH (pH6.1) and a designated concentration of $CaCl_2$. The particles were then washed twice with and resuspended in 10 mM NaCl and 20 mM Mes-NaOH (pH6.1). Chlorophyll concentration was estimated according to (20).

The electron transport activity was measured by DCIP reduction at room temperature using a home made spectrophotometer. The reduction was monitored by absorbance change at 590 nm and an extinction coefficient of 20.6 $mM^{-1}.cm^{-1}$ was used. The composition of polypeptides of PSII particles was examined by SDS-PAGE in the buffer system of (21) containing 6 M urea. The acrylaminde concentration of the stacking and separation gels were 6% and 12.5% respectively. The gel was stained with Coomassie brilliant blue R-250.

RESULTS

Treatment of PSII particles with high concentration of NaCl removes two water soluble polypeptides with molecular masses of 23 KDa and 17 KDa and reduces oxygen evolution activity by about half (6). Similar results were obtained by treating PSII particles with 1 M KCl. The removel of the two polypeptides is demonstrated in Fig.1. The activity was lowered to about 10% of control as shown in Fig.2. This activity can be restored to substantial level by adding 10 mM $CaCl_2$ (Fig.2) similar to that of NaCl treated PSII particles. The effect of $CaCl_2$ on restoration of the activity of KCl treated PSII particles is shown in Fig.3. Maximal restored activity could be achieved at a concentration higher than 2 mM.

If $CaCl_2$ were included during KCl treatment, a protective effect against KCl inactivation was observed. In Fig.4 it shows that the maximal protection was achieved with the presence of 50 mM $CaCl_2$ during the

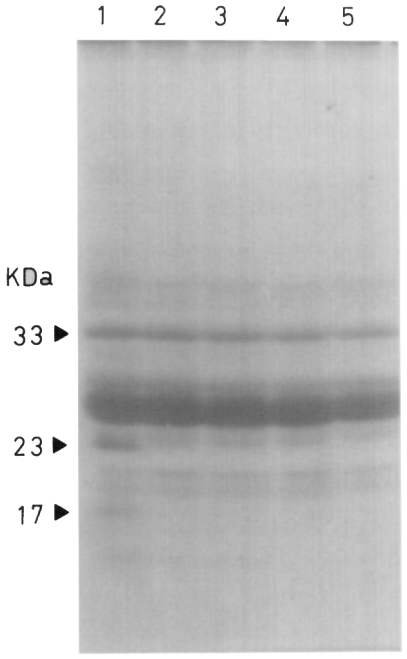
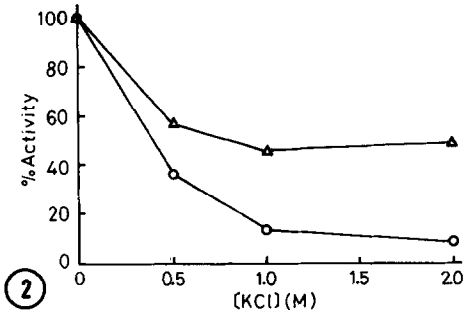


Fig.1 SDS-urea gel electrophoresis of polypeptides in PSII particles:(1) Control, and washed in (2) 1 M KCl, (3) 1 M KCl and 20 mM $CaCl_2$, (4) 1 M KCl and 50 mM $CaCl_2$, (5) 1 M KCl and 100 mM $CaCl_2$.

treatment. Similar activities could be attained either by including 50 mM $CaCl_2$ during KCl treatment or by adding 10 mM $CaCl_2$ to KCl treated particles (without $CaCl_2$ protection) during activity assay. Addition of 10 mM $CaCl_2$ during activity assay to KCl treated particles protected by $CaCl_2$ did not induce much stimulation. $Ca(NO_3)_2$ had the same effect as



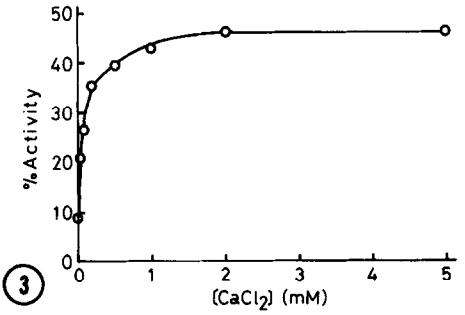


Fig.2 Effect of KCl treatment and $CaCl_2$ on oxygen evolution activity in PSII particles. (○—○) Particles washed with various concentrations of KCl. (△—△) Treated particles with the presence of 10 mM $CaCl_2$ during activity assay. Control (100%) activity: 160 μmole DCIP.mgChl^{-1}.hr^{-1}.

Fig.3 Effect of $CaCl_2$ on oxygen evolution activity in 1 M KCl treated PSII particles. Various concentrations of $CaCl_2$ were added to the reaction medium during activity assay. Control (100%) activity: 160 μmole DCIP.mgChl^{-1}.hr^{-1}.

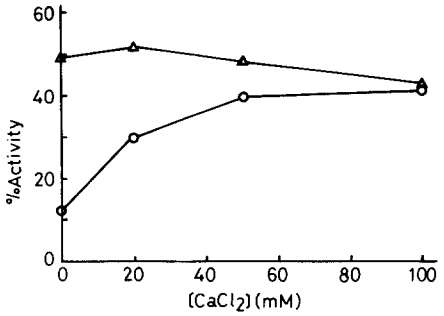


Fig.4 Effect of the presence of $CaCl_2$ during salt treatment on oxygen evolution activity in 1 M KCl treated PSII particles. (O—O) Treated PSII particles. (Δ—Δ) Treated particles with the presence of 10 mM $CaCl_2$ during activity assay. Control (100%) activity: 160 μmole DCIP. $mgChl^{-1}.hr^{-1}$.

$CaCl_2$ (data not shown) suggesting that it is Ca^{++} which plays the protective role during KCl treatment.

Oxygen evolution activity of salt washed PSII particles can be restored by addition of Ca^{++} as well as 23 KDa polypeptide. Ghanotakis et al. (17) suggested that 23 KDa and 17 KDa polypeptides restored oxygen evolution activity by creating a high affinity binding for Ca^{++} on the oxidizing side of PSII. The presence of Ca^{++} during KCl treatment might prevent 23 KDa and 17 KDa polypeptides from being released from thylakoid membranes. However, this is shown in Fig.1 not to be the case. The polypeptide content of KCl treated particles is not very different from that of the particles protected by $CaCl_2$ during KCl wash.

DISCUSSION

Studies on cyanobacteria (11,12,13) and higher plant chloroplasts (14,15,16) suggest that Ca^{++} is required for proper functioning of PSII. However, attempt to remove Ca^{++} from chloroplasts either by prolonged incubation with EGTA or by extensive dialysis against EGTA failed to inhibit oxygen evolution activity. Authors in (18) therefore concluded that if Ca^{++} is required for PSII activity, it is very tightly bound.

Liberation of 23 KDa and 17 KDa polypeptides by salt washing PSII preparations creates a low affinity binding site for Ca^{++}. The inhibited oxygen evolution activity of salt depleted PSII preparation, although can be restored by addition of Ca^{++}, requires 2 mM or higher for full reactivation (Fig.3) (4 mM in (18) under their condition). Reconstitution of 23 KDa and 17 KDa polypeptides is able to replace this low affinity binding site (9,17). No extrinsic Ca^{++} is required for reactivation under this condition. However, if Ca^{++} is removed, before reconstitution, by extensive dialysis of the two polypeptides (23 KDa and 17

KDa) and salt depleted PSII preparations against EGTA separately, no reactivation of oxygen evolution activity is resulted even though the two polypeptides rebind. Neverthless, the reconstituted PSII preparation, under this condition, requires much lower concentration of Ca^{++} for reactivation (18). The results demonstrate that reconstitution of 23 KDa and 17 KDa polypeptides creates a high affinity binding site for Ca^{++} which is responsible for reactivation. It is not known whether the high affinity binding site is located on the two polypeptides or on thylakoid membranes.

In this report, inclusion of 50 mM Ca^{++} (either $CaCl_2$ or $Ca(NO_3)_2$) during KCl wash seems to retain this high affinity binding site. 23 KDa and 17 KDa polypeptides (Fig.1) as well as any loosly bound Ca^{++} were effectively removed by wash twice with Ca^{++}-free medium after salt treatment. The PSII particles so treated still had the oxygen evolution activity as high as that of KCl washed PSII particles if 10 mM $CaCl_2$ was added during activity assay. Not much activation was observed in addition of 10 mM $CaCl_2$ to the PSII particles proctected by Ca^{++} during KCl wash (Fig.4).

It also implies that if the high affinity binding site for Ca^{++} does exist as suggested above, it is certainly not on either 23 KDa or 17 KDa polypeptide, although the affinity for Ca^{++} binding seems to be modulated by the presence or absence of the two polypeptides. Therefore we suggest that one of the possible roles played by 23 KDa and 17 KDa polypeptides in oxygen evolution system is regulatory. They create a special configuration which facilitates the binding of Ca^{++}, an important cofactor for activity of oxygen evolution. The presence of high concentration of Ca^{++} during removal of the two polypeptides seems to be able to retain this special configuration.

Sparrow and England (22) isolated a Ca^{++} binding protein (molecular mass 13-15 KDa) from an oxygen evolving PSII preparation. It was not identical to any of the three polypeptides of 33 KDa, 23 KDa and 17 KDa. Besides, none of the three polypeptides was found to bind Ca^{++}. It is not clear whether the high affinity binding site we discussed above is located on this Ca^{++} binding protein.

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