Calcium reconstitutes high rates of oxygen evolution in polypeptide depleted Photosystem II preparations

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Exposure of highly resolved Photosystem II preparations to 2 M NaCl produces an 80% inhibition of oxygen-evolution activity concomitant with extensive loss of two water-soluble polypeptides (23 and 17 kDa). Addition of Ca^{2+} to salt-washed PS II membranes causes an acceleration in the decay of Z^{+}, the primary donor to P-680^{+}, and we show here that this acceleration is due to reconstitution of oxygen-evolution activity by Ca^{2+}. Other cations (Mg^{2+}, Mn^{2+}, Sr^{2+}) are much less effective in restoring oxygen evolution. On the basis of these observations we propose that Ca^{2+}, perhaps in concert with the 23 kDa polypeptide, is an essential cofactor for electron transfer from the 'S'-states to Z on the oxidizing side of PS II.

Photosystem II *Oxygen evolution* *Polypeptide* *Calcium*

1. INTRODUCTION

Photosynthetic oxygen evolution is significantly inhibited when PS II membrane preparations are treated with 2 M NaCl [1,2]; extensive removal of the 17 and 23 kDa polypeptides is observed but functional manganese is not perturbed by this treatment. Authors in [3] reported that treatment of inside-out spinach thylakoid vesicles with 250 mM NaCl inhibited 75% of oxygen evolution activity; this inhibition was also accompanied by the release of the 17 and 23 kDa polypeptides. Addition of the purified 23 kDa polypeptide to the extracted inside-out vesicles restored about 60% of the activity lost by salt washing. Even though the 23 and 17 kDa polypeptides have been purified and their amino acid content is known [4], it is not clear what role they play in oxygen evolution. All investigations so far have correlated loss of a 33 kDa polypeptide with loss of functional manganese, whereas a structural role has been postulated for the 23 and the 17 kDa species [1,3].

We have examined the behavior of PS II preparations after high-salt extraction and have found that 80% of the control oxygen evolution activity is restored upon addition of $CaCl_2$. A parallel study of the EPR signal arising from the primary donor to P-680^{+}, Z^{+}, strongly suggests a site of Ca^{2+} action on the oxidizing side of PS II.

Abbreviations: DCBQ, 2,5-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; $Fe^{III}CN$, potassium ferricyanide; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; Mes, 4-morpholinoethanesulfonic acid; PS II, Photosystem II

2. MATERIALS AND METHODS

Subchloroplast membranes, free of Photosystem I and having high rates of oxygen evolution, were prepared as in [1,5]. Salt treatment to release polypeptides from the PS II complex was carried out by incubation of the preparation in 2 M NaCl (pH 6.0) on ice for 1 h in the dark. After centrifugation the membranes were washed once with SMN (0.4 M sucrose/50 mM Mes, pH 6.0/15 mM NaCl) and then stored in the same buf-

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fer. Calcium content in the PS II preparations was determined at 4227 Å with a Jarell-Ash atomic absorption/flame emission spectrometer. Samples for the above analysis were prepared as follows: PS II preparations were pelleted by centrifugation and the pellets were resuspended in 0.1 N HCl. The suspensions were heated for 4 min at 80°C and then centrifuged for 5 min at 10000 × *g*; the clear supernatants were combined with the supernatants from a subsequent wash and were analyzed by flame emission spectroscopy. Gel electrophoresis was carried out as in [6] with the modification that 2.5 M urea was present in the gel and in denaturing solutions.

EPR spectroscopy was carried out on a Bruker ER-200D spectrometer operated at X-band and interfaced to a Nicolet 1180 computer. Instrument modifications as well as the xenon flash lamp circuitry and the protocol followed in signal-averaged, flashing-light kinetic experiments are described in [7].

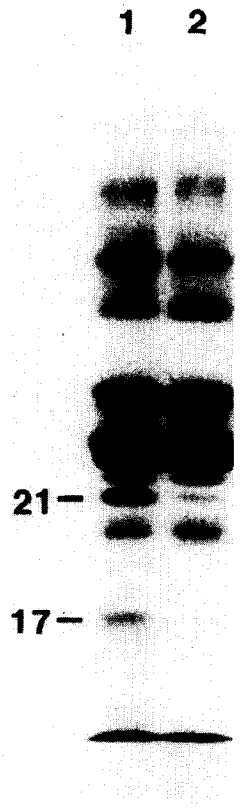

Fig.1. Gel electrophoresis patterns: (1) of the untreated PS II complex; (2) after high-salt treatment. The gel contains 2.5 M urea. Under these conditions the apparent molecular mass of the 23 kDa polypeptide is shifted to 21 kDa as shown on the gel.

3. RESULTS

Fig.1 shows that exposure of PS II membranes to 2 M NaCl for 1 h at pH 6.0 produces an extensive depletion of two polypeptides (17 and 23 kDa). As we reported earlier, this treatment generates Z^+ with an increased decay time [1]. A further analysis of the decay of flash-induced Z^+ in salt-washed PS II membranes produced the results shown in fig.2, where addition of Ca^{2+} induces an accelerated decay of the radical. The effect shown in fig.2 could be due either to the induction of a fast back-reaction from Q^- to Z^+, or to an effect of Ca^{2+} on the oxidizing side of PS II. The latter is shown to be true by the data of fig.3, where added Ca^{2+} reconstitutes high rates of oxygen evolution activity in the salt-washed PS II preparations.

An examination of the Ca^{2+} content of salt-washed PS II preparations revealed that about

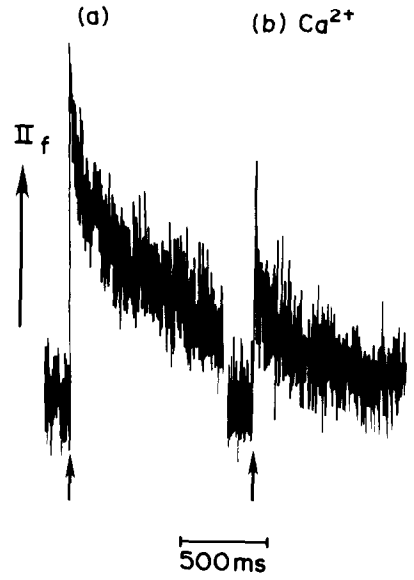

Fig.2. Kinetic transients for Z^+ at room temperature in high-salt-treated PS II complex. An equimolar mixture of ferricyanide and ferrocyanide was used as an acceptor system. (a) No further addition; (b) 10 mM $CaCl_2$. Each kinetic trace is the average of 200 flashes. Time constant = 1 ms and dark time between flashes t_d = 3.5 s.

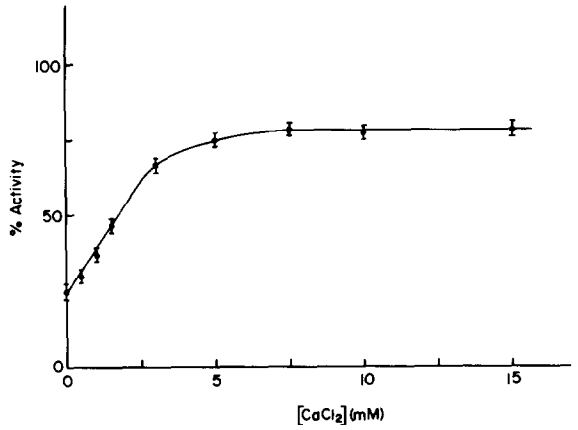

Fig.3. Rates of oxygen evolution as a function of added $CaCl_2$. High-salt-treated PS II complex was illuminated with continuous light in a 0.4 M sucrose/50 mM Mes (pH 6.0) buffer containing $CaCl_2$ as indicated, 6 μg chlorophyll/ml, 250 μM dichlorobenzoquinone and 3.5 mM ferricyanide (control activity ($\pm Ca^{2+}$): 780 μmol $O_2 \cdot$ mg $Chl^{-1} \cdot h^{-1}$).

40% of the Ca^{2+} was removed by exposure to 2 M NaCl. Control PS II membranes contained 0.84 gatom Ca/mol Chl; in salt-washed preparations, 0.52 gatom Ca/mol Chl was found. Since the salt treatment of the PS II complex could remove other cations as well as Ca^{2+}, we examined the ability of several divalent cations to restore oxygen evolution activity. These data are summarized in table 1. At the concentration shown (15 mM), Ca^{2+} is most effective in restoring activity. None of the other cations can produce oxygen-evolution activity equal to that of Ca^{2+}; Mn^{2+} is slightly inhibitory. We

Table 1

Effects of cations on rate of oxygen evolution in high-salt-treated PS II preparations

Additions	Activity (μmol $O_2 \cdot$ mg $Chl^{-1} \cdot h^{-1}$)	% Activity[a]
None	196	25
$CaCl_2$ (15 mM)	616	79
$MgCl_2$ (15 mM)	202	26
$SrCl_2$ (15 mM)	330	42
$MnCl_2$ (15 mM)	160	20
DCMU (80 μM)	28	3
$CaCl_2$ (15 mM) + DCMU (80 μM)	50	6

[a] Control activity ($\pm Ca^{2+}$): 780 μmol $O_2 \cdot$ mg $Chl^{-1} \cdot h^{-1}$ (conditions the same as those in fig.3)

emphasize here that the relatively high concentrations of Ca^{2+} required to reconstitute activity implies that the cation is weakly bound to the membrane; this is borne out by the observations that we cannot incubate PS II membranes at high concentrations with Ca^{2+} and then successfully dilute the suspension for assay of activity, and that EGTA at appropriate concentrations blocks reconstitution (not shown). The data of table 2 show that the ability of Ca^{2+} to reconstitute oxygen evolution activity is independent of the acceptor system used to assay the PS II preparation, although as we reported earlier [1,5], a lipophilic redox mediator such as DCBQ produces the highest rates of oxygen evolution activity.

Table 2

Effect of calcium on rate of oxygen evolution in high-salt-treated PS II preparations in the presence of various acceptor systems

Acceptor system	Additions	Activity[a] (μmol $O_2 \cdot$ mg $Chl^{-1} \cdot h^{-1}$)
DCBQ (250 μM)	None	180
	$CaCl_2$ (15 mM)	610
$Fe^{III}CN$ (3.5 mM)	None	30
	$CaCl_2$ (15 mM)	130
DCBQ (250 μM) + $Fe^{III}CN$ (3.5 mM)	None	185
	$CaCl_2$ (15 mM)	600

[a] Control activity ($\pm Ca^{2+}$): 780 μmol $O_2 \cdot$ mg $Chl^{-1} \cdot h^{-1}$ (conditions the same as those in fig.3)

4. DISCUSSION

Several previous studies on cyanobacteria [8,9] and higher plant chloroplast systems [10,11] have implicated Ca^{2+} as a possible cofactor in PS II-catalyzed electron transfer, although speculations as to its role have not produced a consensus on a specific side of action. Likewise, the role of water-soluble polypeptides (23 and 17 kDa) in reactivating oxygen evolution activity has not been established. Authors in [3] demonstrated a requirement for the 23 kDa species, but this protein contains no appreciable amounts of bound manganese nor does its release from inside-out thylakoid vesicles perturb the membrane-bound manganese required for oxygen evolution activity. As we show here, Ca^{2+} can reconstitute oxygen evolution activity in salt-washed membranes where the 23 and 17 kDa polypeptides are depleted. This effect of Ca^{2+} is located on the oxidizing side of PS II at a site where electron transfer from the S-states to Z^{+} is facilitated.

From the data presented here, we can propose 3 hypotheses concerning the roles of polypeptides and Ca^{2+} in oxygen evolution activity. The first hypothesis would state that Ca^{2+} by itself is an essential cofactor for oxygen evolution. This assertion is not supported by the results presented here, since Ca^{2+} cannot be made to bind to the PS II preparations in such a way as to reconstitute activity under conditions where the cation is present at low concentrations. The second hypothesis would state that the 23 kDa is essential for oxygen evolution activity and that its role can be mimicked by Ca^{2+}. This model is not entirely satisfactory because:

(i) Extensive depletion of the 23 kDa polypeptide does not entirely inhibit oxygen evolution activity (see also [2]);

(ii) Release of the 23 kDa polypeptide results in concomitant removal of Ca^{2+}.

The third hypothesis holds that both Ca^{2+} and the 23 kDa polypeptide are necessary for optimal rates of oxygen evolution activity. We favor this model because:

(i) The absence of the 23 kDa polypeptide creates a situation in which loosely-bound Ca^{2+} reconstitutes activity;

(ii) Addition of EGTA to PS II preparations at pH 6.0 does not inactivate oxygen evolution, but does prevent reconstitution by Ca^{2+} of activity in salt-washed preparations;

(iii) The variety of systems (intact thylakoids, cyanobacterial cells and membrane vesicles) which have been demonstrated to require Ca^{2+} for PS II activity must certainly include preparations where Ca^{2+} and not the 23 kDa polypeptide, has been removed to create the lesion in PS II.

Experiments are now in progress to examine the above hypothesis.

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