A functional site of Ca^{2+} in the oxygen-evolving photosystem II preparation from *Synechococcus* sp.

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Treatment of the oxygen-evolving photosystem II preparation from the thermophilic cyanobacterium *Synechococcus* sp. with EDTA inhibited electron flow from Z to P680 and consequently induced a back electron flow from Q_A^- to $P680^+$. The inhibition was reversed fully by Ca^{2+} and partially by Mn^{2+} and Mg^{2+} when EDTA-treated preparations had been incubated with respective divalent metal cations for several minutes, whereas diphenylcarbazide had no effect on the recombination between Q_A^- and $P680^+$ in EDTA-treated preparations. It is concluded that Ca^{2+} is essential for electron transport from Z to P680.

Oxygen evolution *Electron transport* *Photosystem II* *Ca^{2+}* *Thermophilic cyanobacterium*

1. INTRODUCTION

There are several lines of evidence suggesting the involvement of Ca^{2+} in photosynthetic oxygen evolution of chloroplasts [1–10] and cyanobacteria [11–16]. We have recently shown that the oxygen-evolving activity of the PS II preparation isolated from the thermophilic cyanobacterium, *Synechococcus* sp., is inhibited by brief treatment with 1 mM EDTA and the addition of Ca^{2+} and Mn^{2+} to the EDTA-treated preparation partially reversed the inhibition [16]. The observations that the activity increases to a maximum level almost immediately after the addition of Mn^{2+}, whereas the activation gradually proceeds in the presence of Ca^{2+} and that the addition of Mn^{2+} to the Ca^{2+}-restored preparation increases the activity to a level which is significantly higher than the sum of the Mn^{2+}- and Ca^{2+}-induced increase in the activity led to the conclusion that the 2 divalent cations reactivate oxygen evolution by different mechanisms [16]. Thus, the *Synechococcus* PS II preparation provides a simple and unique system for the investigation of specific roles of Ca^{2+} and Mn^{2+} in photosynthetic oxygen evolution.

Here, we have examined the functional site of Ca^{2+} in PS II electron transport by measuring flash-induced absorbance changes in EDTA-treated *Synechococcus* oxygen-evolving preparations. The results demonstrate that Ca^{2+} is essential for electron transport from the secondary electron donor Z to the primary electron donor P680.

Abbreviations: PS II, photosystem II; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DPC, diphenylcarbazide

2. MATERIALS AND METHODS

Oxygen-evolving PS II preparations were obtained from *Synechococcus* thylakoid membranes after solubilization with β-octylglucoside as described [16]. For the spectrophotometric assay, the preparations were suspended in a medium containing 1.0 M sucrose, 50 mM Hepes-NaOH (pH 7.5), 10 mM NaCl. EDTA treatment was carried out by incubating the preparations in a medium containing 1 mM EDTA, 0.1 M sucrose, 5 mM Hepes-NaOH (pH 7.5), 1 mM NaCl and 0.5 mM $MgCl_2$ at 25°C for 1 min [16].

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Absorbance changes were measured with a single-beam spectrophotometer with a time constant of 1 μs as in [17]. Xenon flashes with a half peak height duration of 5 μs passed through a Toshiba VR-65 filter and the photomultiplier was protected by 2 Corning 4-96 filters or by a Corning 4-96 and a Toshiba VV-42 filter. When absorbance changes in the red region were measured, the samples were illuminated with blue flashes which passed through 2 Corning 4-96 filters and a monochromator was placed between the cuvette and the photomultiplier. All measurements were carried out at 23–25°C.

3. RESULTS

Figs 1 and 2 show the time courses and difference spectra of flash-induced absorbance changes in the oxygen-evolving preparation, respectively. An initial rapid absorption increase at 410 nm represents photoreduction of Q_A (fig.1a) as judged from the difference spectrum with peaks at 415 and 440 nm and bleachings at 430 and 550 nm (fig.2) which agrees with that for the Q_A reduction [18]. A subsequent decay of the 410 nm signal, which is biphasic but has an overall half-time of about 3 ms, is ascribed to reoxidation of Q_A^- by Q_B because it is sensitive to DCMU (not shown). Details of PS II electron transport in the oxygen-evolving preparation will be described elsewhere [19]. A small bleaching at 680 nm in the difference spectrum indicates that P680 was partially oxidized by flashes (fig.2). The P680 signal has a half-life of about 60 μs (not shown). Schlodder et al. [20] have recently shown that, although flash-oxidized P680 largely undergoes submicrosecond reduction, a small fraction of P680 is reduced with half-times of 40–200 μs in another-oxygen evolving preparation from *Synechococcus*.

The EDTA treatment, which completely inhibits oxygen evolution of the *Synechococcus* PS II preparation [16], markedly enhanced P680 oxidation. Fig.2 shows that the magnitude of the 680 nm bleaching was increased several-fold by the treatment and, correspondingly, subtraction of the spectrum of the untreated preparation in the blue region from that of the EDTA-treated preparation yielded a negative peak centering at 435 nm. The EDTA treatment also caused a marked acceleration of the Q_A oxidation (fig.1b). It should be

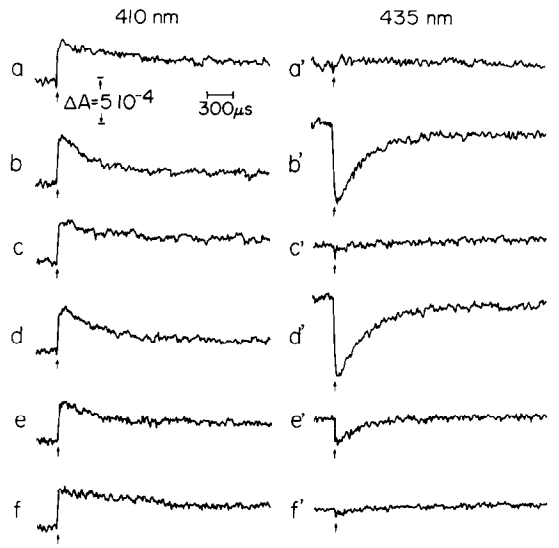

Fig.1. Flash-induced absorbance changes in oxygen-evolving preparations. Chlorophyll concentration, 3.7 μg/ml. Flashes were fired at 1 Hz and 40 signals were averaged. (a,a') Untreated preparations. (b,b') EDTA-treated preparations. (c,c') EDTA-treated preparations incubated with 5 mM $CaCl_2$ for 7 min. (d,d') (b) + 50 μM DPC. (e,e') Untreated preparations + 1 M Tris-HCl (pH 7.5). (f,f') (e) + 50 μM DPC.

noted that the decay of the P680 signal at 435 nm (fig.1b') paralleled that of the Q_A signal at 410 nm (fig.1b), both showing a half-time of 200 μs. We conclude, therefore, that the EDTA treatment inhibits electron transfer from water to P680 and consequently induces a back electron flow from Q_A^- to $P680^+$.

A charge recombination between Q_A^- and $P680^+$ with a 200 μs half-time has been reported for spinach chloroplasts with electron transport from water to Z inhibited by Tris treatment [21,22]. Traces e–f' of fig.1 show experiments in which absorption changes were determined in the presence of 1 M Tris-HCl (pH 7.5). A part of Q_A was reoxidized with the 200 μs half-time and a small P680 signal with similar decay kinetics appeared (fig.1e,e'), indicating a partial inhibition of electron flow from water to P680. Note that the back reaction between Q_A^- and $P680^+$ was totally suppressed on further addition of DPC which serves as an electron donor to Z (fig.1f,f'). The results are consistent with the view that electron transport

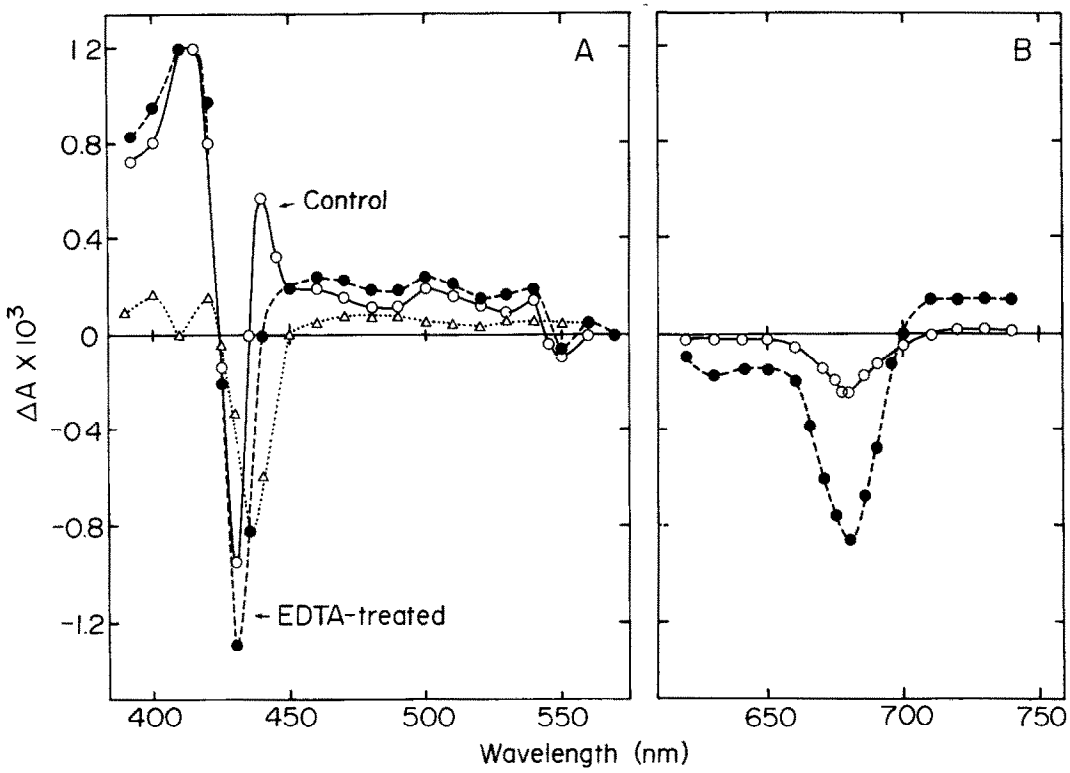

Fig.2. Difference spectra for flash-induced absorption changes. Chlorophyll concentrations were 7.5 and 4.3 μg/ml for A and B, respectively. (○) Untreated preparation, (●) EDTA-treated preparation, (△) (untreated) minus (EDTA-treated).

from water to Z is blocked by Tris. The recombination between Q_A^- and $P680^+$ takes place because Z is kept in the oxidized state during repetitive flash excitation, but is suppressed by DPC which will reduce Z^+ during the flash intervals. In contrast, the addition of DPC to the EDTA-treated preparation had no significant effect on the recombination between Q_A^- and $P680^+$ (fig.1d,d'). Evidently, Z cannot reduce P680 in EDTA-treated preparations. The absence of a functional Z from EDTA-treated preparations is corroborated by an observation that EDTA-treated and dark-adapted preparations showed recombination kinetics even after a single flash excitation (not shown). We conclude, therefore, that the EDTA treatment specifically inactivates electron transfer from Z to P680.

Significantly, the effect of the EDTA treatment was fully reversed by Ca^{2+}. The addition of 5 mM $CaCl_2$ to EDTA-treated preparations diminished the P680 signal and eliminated the 200 μs phase of Q_A oxidation (fig.1c,c'). This is evidence that Ca^{2+} restores rapid electron transport from Z to P680 which effectively competes with the back electron transport from Q_A^-. The Ca^{2+} effect cannot be ascribed to the restoration of the entire electron transport from water to the PS II reaction center because EDTA treatment inhibits the water oxidation by extracting Mn^{2+} and hence Ca^{2+} reactivates oxygen evolution only partially [16]. The almost complete inhibition of the recombination by Ca^{2+} implies, therefore, that Z^+ is slowly but fully re-reduced during the flash intervals with electrons from water or unknown endogenous reductants in the EDTA-treated preparation.

A characteristic of the Ca^{2+}-dependent reactivation of oxygen evolution is that preincubation with the EDTA-treated preparation for several minutes is necessary for the divalent cation to exert its maximal effect [16]. Similarly, the charge recombination between Q_A^- and $P680^+$ was gradually diminished during the incubation of the EDTA-

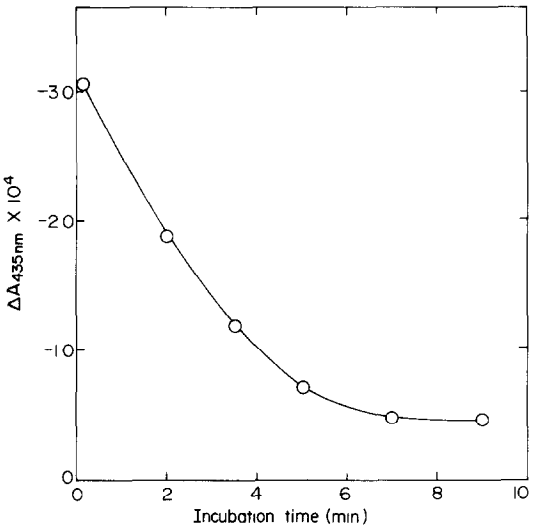

Fig.3. Time-dependent development of the Ca^{2+} effect on the recombination between Q_A^- and $P680^+$ in EDTA-treated preparations. EDTA-treated preparations were incubated with 5 mM $CaCl_2$ for the indicated periods of time, then magnitudes of the P680 signal with 200 μs half-time were determined at 435 nm.

treated sample with Ca^{2+} to reach a minimal level after 7 min (fig.3). Thus, the Ca^{2+}-dependent reactivation of oxygen evolution is ascribed to the restoration of rapid electron flow from Z to P680.

$MnCl_2$ and $MgCl_2$ also suppressed the back electron flow from Q_A^- to $P680^+$ but to a smaller extent (table 1). Again, the preincubation of EDTA-treated preparations with $MnCl_2$ or $MgCl_2$ for several minutes was necessary for maximal effects

Table 1

Effects of $CaCl_2$, $MnCl_2$ and $MgCl_2$ on the recombination between Q_A^- and $P680^+$

	Relative units
(a) Control	1.0
(b) EDTA-treated	11.5
(c) (b) + $CaCl_2$	2.0
(d) (b) + $MnCl_2$	4.0
(e) (b) + $MgCl_2$	7.0

The magnitude of the 200 μs component of Q_A oxidation was determined at 415 nm after incubation of EDTA-treated preparations with the respective divalent metal cations for 7 min

to be observed. The order of the effectiveness of $Ca^{2+} > Mn^{2+} > Mg^{2+}$ is similar to that observed in the reactivation of oxygen evolution when EDTA-treated samples had been incubated with respective divalent cations for 5 min, then the activity was assayed after 5-fold dilution of the cation concentrations [16]. The effect of Mn^{2+} observed here should be distinguished from the fast-developing Mn^{2+} effect on oxygen evolution, which most probably is attributed to the reactivation of the water-oxidation reaction [16].

4. DISCUSSION

The present results show that Ca^{2+} is an essential cofactor in electron transport from Z to P680. We suggest a structural role for Ca^{2+} because the reversal of the EDTA-induced inhibition is not strictly specific to Ca^{2+} (table 1). The slow development of the Ca^{2+} effect during incubation with EDTA-treated preparations is consistent with this view (see [16]). Presumably, the binding of Ca^{2+} to a specific site of the PS II complex would be important for Z to function as an efficient electron carrier.

NH_2OH has been shown to inhibit electron transport from Z to P680 [23]. This cannot be ascribed to the extraction of Ca^{2+} by NH_2OH because electron transport is restored on removal of NH_2OH [23].

Our conclusion that Ca^{2+} functions between Z and P680 is consistent with the earlier experiments with cyanobacteria. Brand [12] has shown that Ca^{2+}-depleted cells of *Anacystis nidulans* show no variable fluorescence in the presence of DCMU or at liquid nitrogen temperature. England and Evans [14] have also reported that Ca^{2+} is required for electron transport from DPC to dichlorophenolindophenol in *A. nidulans*. These observations are well explained in terms of the disconnection of Z from P680 in the absence of the functional Ca^{2+}.

Experiments with higher plant PS II preparations suggest another functional site for Ca^{2+} in PS II electron transport. Salt washing of spinach oxygen-evolving membrane preparations, which releases a 23 kDa and a 17 kDa protein and partly inactivates oxygen evolution, generates a large EPR signal II_f with a slow decay time and the addition of Ca^{2+} to the salt-washed samples markedly accelerates the decay of signal II_f [7,9]. Because

signal II_f arises from Z, it is suggested that Ca^{2+} plays a role in electron transport from water to Z. The function of Ca^{2+} in this span of electron transport has not been studied here because water oxidation in the *Synechococcus* PS II preparation is also inhibited by EDTA. On the other hand, there is no evidence suggesting the involvement of Ca^{2+} between Z and P680 in chloroplasts or PS II preparations from higher plants. Presumably, Ca^{2+} binds more tightly to the PS II complexes in chloroplasts than in cyanobacteria, where the Ca^{2+} deficiency can be readily induced by EDTA treatment [15,16], or even by simply breaking cells [11,12]. Experiments to examine this hypothesis are in progress.

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REFERENCES

[1] Yamashita, T. and Tomita, G. (1974) Plant Cell Physiol. 15, 69–82.
[2] Oku, T., Kukidome, H. and Yamamoto, Y. (1983) Biochem. Biophys. Res. Commun. 116, 803–808.
[3] Ono, T. and Inoue, Y. (1983) Biochim. Biophys. Acta 723, 191–201.
[4] Akerlund, H.E., Jasson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 681, 1–10.
[5] Pan, R., Troxel, K.S. and Crane, F.L. (1983) Plant Physiol. 173, 309–315.
[6] Miyao, M. and Murata, N. (1984) FEBS Lett. 168, 118–120.
[7] Ghanotakis, D.F., Topper, J.N., Babcock, G.T. and Yocum, C.F. (1984) FEBS Lett. 170, 169–173.
[8] Packham, V.K. and Barber, J. (1984) Biochim. Biophys. Acta 764, 17–23.
[9] Dekker, J.P., Ghanotakis, D.F., Plijiter, J.J., Van Gorkom, H.J. and Babcock, G.T. (1984) Biochim. Biophys. Acta 767, 515–523.
[10] Preston, C. and Critchley, C. (1985) FEBS Lett. 184, 318–322.
[11] Picconi, R.G. and Mauzerall, D.C. (1978) Biochim. Biophys. Acta 504, 384–397.
[12] Brand, J.J. (1979) FEBS Lett. 103, 114–117.
[13] Brand, J.J., Mohanty, P. and Fork, D.C. (1983) Carnegie Inst. Year Book, 82, 72–75.
[14] England, R.R. and Evans, E.H. (1983) Biochem. J. 210, 473–476.
[15] Pistorius, E.K. and Schmid, G.H. (1984) FEBS Lett. 171, 173–178.
[16] Satoh, K. and Katoh, S. (1985) Biochim. Biophys. Acta 806, 221–229.
[17] Hirano, M., Satoh, K. and Katoh, S. (1980) Photosynth. Res. 1, 149–162.
[18] Van Gorkom, H.J., Pulles, M.P.J. and Wessels, J.S. (1975) Biochim. Biophys. Acta 408, 331–339.
[19] Takahashi, U. and Katoh, S. (1985) Biochim. Biophys. Acta, submitted.
[20] Schlodder, E., Brettel, K., Schatz, G.H. and Witt, H.T. (1984) Biochim. Biophys. Acta 765, 178–185.
[21] Renger, G. and Wolff, C. (1976) Biochim. Biophys. Acta 423, 610–614.
[22] Haneman, J. and Mathis, P. (1976) Biochim. Biophys. Acta 440, 346–355.
[23] Ghanotakis, D.F. and Babcock, G.T. (1983) FEBS Lett. 153, 231–234.