The Role of Ca^{2+} in Electron Transport of Spinach Chloroplasts

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Abbreviations used: DCMU—3-(3,4-dichlorophenyl)-1,1-dimethylurea; EGTA—ethyleneglycol bis (α-aminoethyl ether)-N,N-tetracetic acid; MV—methylviologen; PS I—Photosystem I; PS II—Photosystem II; TMPD-N,N,N^1, N^1-tetramethyl-p-phenylene diamine.

Introduction

The effect of Ca^{2+} on chloroplasts has frequently been studied from the point of view of its role in the cation-regulated excitation energy transfer between the two photosystems, termed "spillover" (4, 5, 6, 7). Gross and Hess (5) found 2 Ca^{2+} binding sites in chloroplasts: site 1 bound 0.65 μmole/mg chl and had a K_d of 8; site II bound 0.5 μmole/mg chl and had a K_d of 51. Gross, Zimmerman, and Hormats (7) described how low concentrations of monovalent or divalent cations (1-10 mM), including Ca^{2+} ions, decreased the quantum yield of PS II and increased that of PS I. No attempt was made by these authors to remove ions from chloroplasts prior to the addition of mono- or divalent cations.

In the present study we try to demonstrate how removal of Ca^{2+} from chloroplasts by a chelator is correlated with loss of electron transport activity, and how the addition of Ca^{2+} to EGTA-treated chloroplasts can partially restore PS I activity.

Materials and Methods

Sucrose-NaCl chloroplasts (0.4 M sucrose, 0.05 M NaCl) were prepared from market spinach as previously described (2). Chlorophyll was determined according to Arnon (1). PS I and II activities were assayed with a Clark-type O_2 electrode as previously described (2). Reaction components are given in figure legends. Reaction mixtures were irradiated with white light (2.6×10^3 ergs/cm^{-2}•sec^{-1}) from a specially built lamp through a 250 ml round bottom flask containing 1% $CuSO_4$ solution as a heat shield. Reaction rates were recorded with a Sargent-Welch SRG recorder.

The EGTA treatment of chloroplasts was done as follows: 40 ml of unbuffered 200 μM EGTA solution was added to chloroplasts (2.5 mg chlorophyll) suspended in 2.5 ml SN. After stirring, this slurry was kept on ice for 15 min. The treated chloroplasts were pelleted by centrifugation at 600 xg for 10 min. The chloroplasts were drained of residual EGTA solution by resting upside-down on paper towels for 1 min., or by being rewashed with fresh SN solution and recentrifuged. The final chlorophyll concentration was readjusted to 1 mg/ml before assays. Control chloroplasts were osmotically shocked (termed "shocked" in Fig. 1) by suspension in distilled water during the 15 min. EGTA treatment.

EGTA [ethyleneglycol bis (α-aminoethyl ether)-N,N-tetracetic acid] was purchased from the Sigma Chemical Co. and recrystallized from 50% isopropanol before use. Plastocyanin was the gift of Dr. E. Ullrich, Chemistry Department, Purdue University.

Results and Discussion

Ca^{2+} effects on chloroplasts have hitherto been considered as a general effect on the "spillover" of excitation energy along with other mono- or divalent cations by Gross and associates (4, 5, 6, 7), or as an effect on water oxidation in algae (3, 8, 9, 10). We have found a more specific Ca^{2+} effect in PS I of spinach chloroplasts, associated with the plastocyanin region. The evidence for this is 3-fold: (1) EGTA is known to be more selective toward Ca^{2+} ions even in presence of Mg^{2+}, as shown by Schmid and Reilley (11). Therefore, treatment of chloroplasts with EGTA should, presumably, remove Ca^{2+} ions in preference to other ions. Inactivation of chloroplast electron transport reactions by EGTA treatment is shown in Fig. 1. It can be seen that the $H_2O \rightarrow$ MV pathway, which covers both photosystems, is inhibited >75%, as is indophenol reduction by PS II. Since the ascorbate plus TMPD → MV reaction, which donates electrons close to plastocyanin, is inhibited >50% by the EGTA treatment, the implication is that 2 Ca^{2+} sites, one associated with PS II, the other with the plastocyanin region in PS I, exist in the electron transport chain of spinach chloroplasts. No effect of the EGTA treatment on the ascorbate + DCIP → MV shows that Ca^{2+} site I is located before P700. (2) Partial restoration of electron transport activities with Ca^{2+} ions in the ascorbate + TMPD → MV reaction is added evidence that Ca^{2+} ions are implicated. Based on stimulation of the rate seen in EGTA-treated chloroplasts (Fig. 2), 48% stimulation can be obtained, although based on untreated chloroplasts, the restoration by Ca^{2+} ions amounts to 16% (Table 1). However, Table 1 also shows that Ca^{2+} ions gave better restoration of activity than other ions tested. (3) Exogenous plastocyanin with 20 mM $CaCl_2$ can jointly restore activity of the ascorbate + TMPD $\rightarrow$ MV pathway to control levels (Fig. 3).

This, again, is evidence for a functional role of Ca^{2+} ions in the electron transport chain of spinach chloroplasts. Studies are in progress to determine in what manner the plastocyanin region reacts with Ca^{2+} ions.

Acknowledgments

This study was supported by N.S.F. Grant PCM-7820458. The authors wish to thank Dr. R. A. Dilley for providing spinach and Dr. E. Ullrich for the gift of purified plastocyanin.

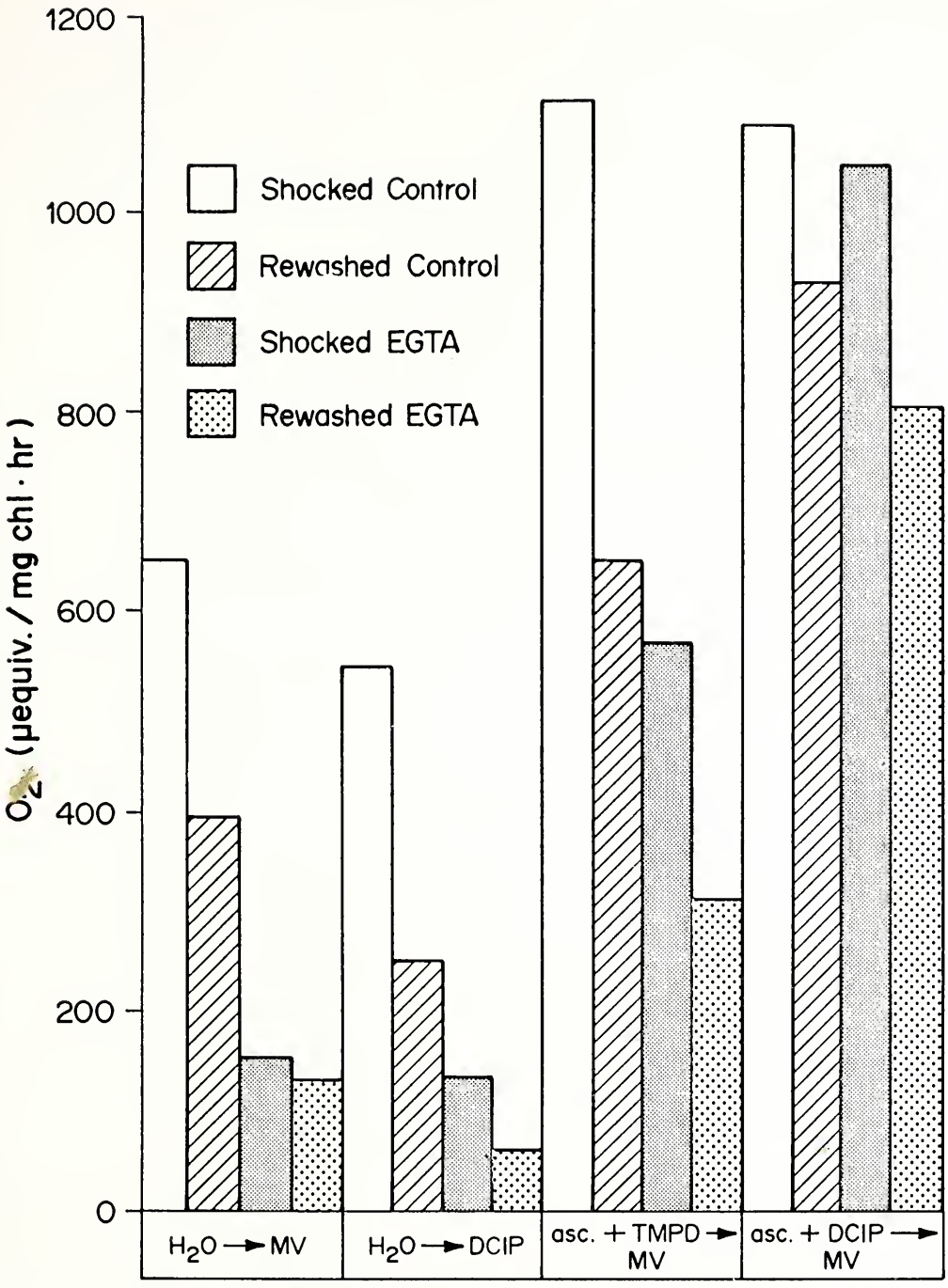

FIGURE 1. *The Effect of EGTA Treatment on Electron Transport Rates in Spinach Chloroplasts. Reaction conditions for O_2 evolution or uptake as described in Materials and Methods. The reaction mixture for the reaction $H_2O \rightarrow MV$ contained chloroplasts (50 μg chlorophyll), 25 mM Tris-Mes buffer, pH 8, 5 mM NH_4Cl, 0.5 mM Na azide, and 0.5 mM MV; for $H_2O \rightarrow$ DCIP-chloroplasts, buffer and NH_4Cl as above, and 0.5 mM DCIP, for asc. + TMPD → MV-chloroplasts, 25 mM Tris-Mes, pH 8, 5 μM DCMU, 0.5 mM Na azide, 0.5 mM MV, 0.05 mM TMPD and 1 mM Na ascorbate; for asc. + DCIP → MV - as for the asc. + TMPD → MV reaction, except 0.5 mM DCIP in place of TMPD.*

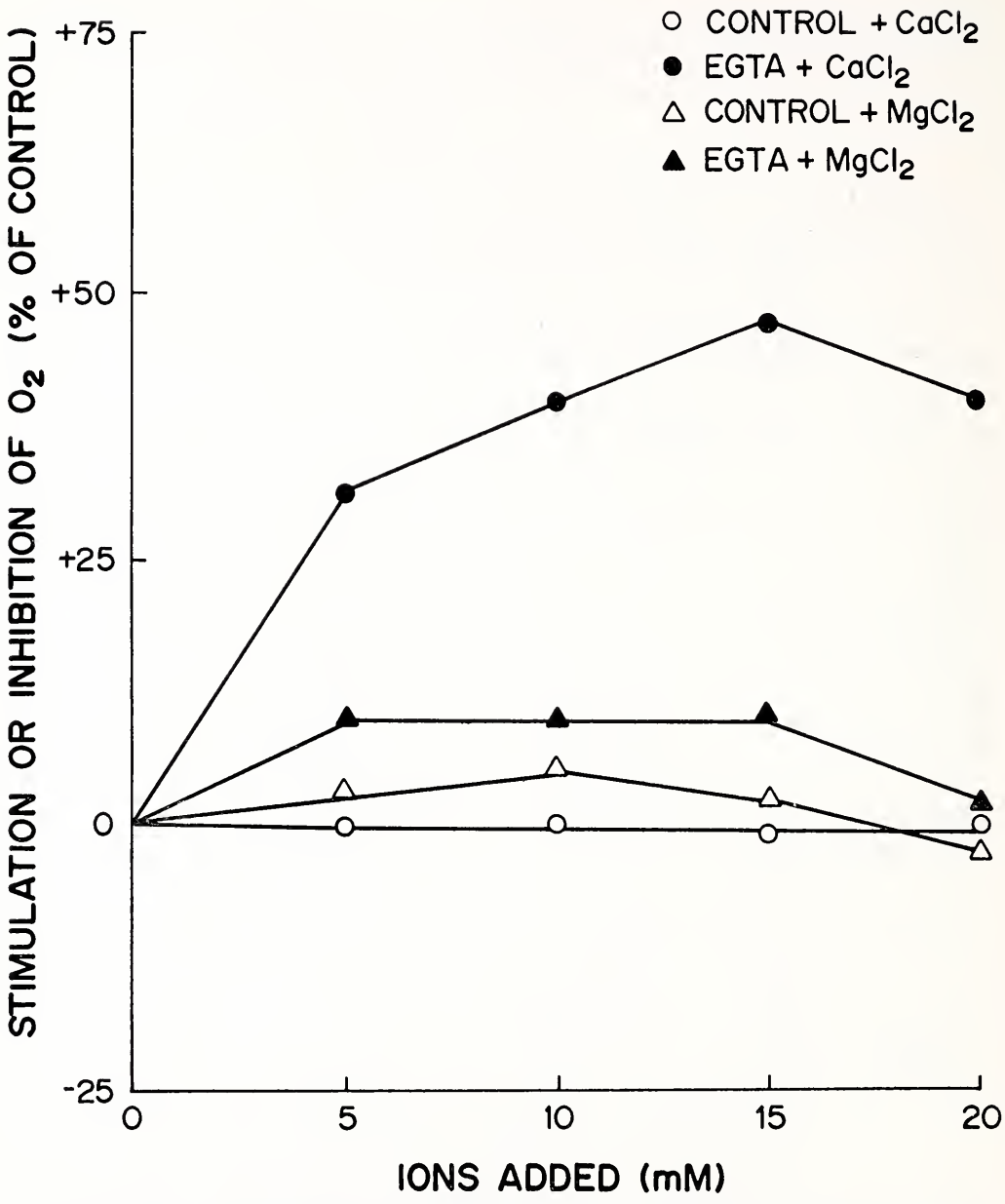

FIGURE 2. *The Effect of Ca^{2+} and Mg^{2+} Ions on The Restoration of Electron Transport in EGTA-Treated Chloroplasts in Photosystem I. The reaction studied was ascorbate + TMPD → MV. The reaction mixture contained chloroplasts, buffer and other ingredients as described in Fig. 1. The control rate without ions was 1137 μmoles O_2/mg chl•hr; the rate of EGTA-treated chloroplasts was 658 μmoles O_2/mg chl•hr; + indicates stimulation, -inhibition of rate in relation to control.*

TABLE 1. *The Effect of Ca^{2+} and Other Ions on Photosystem I Electron Transport in Spinach Chloroplasts.*

Ion Added	Conc. (mM)	Electron transport rate[1] Control O_2	% [2]	EGTA-treated O_2	%
None	-	1137	100	379	-67
KCl	40	1626	+43	462	-59
$CaCl_2$	15	1114	-2	561	-51
$CuCl_2$	0.002	1160	+2	474	-58
$FeCl_3$	0.15	1217	+7	493	-57
$MgCl_2$	15	1160	+2	417	-63
$MnCl_2$	15	1023	-10	349	-69
$ZnCl_2$	15	1023	-10	447	-61

[1] μmole O_2/mg chl•hr in the reaction ascorbate + TMPD → MV. Reaction conditions as in Fig. 1.
[2] + indicates stimulation, - inhibition of rate.

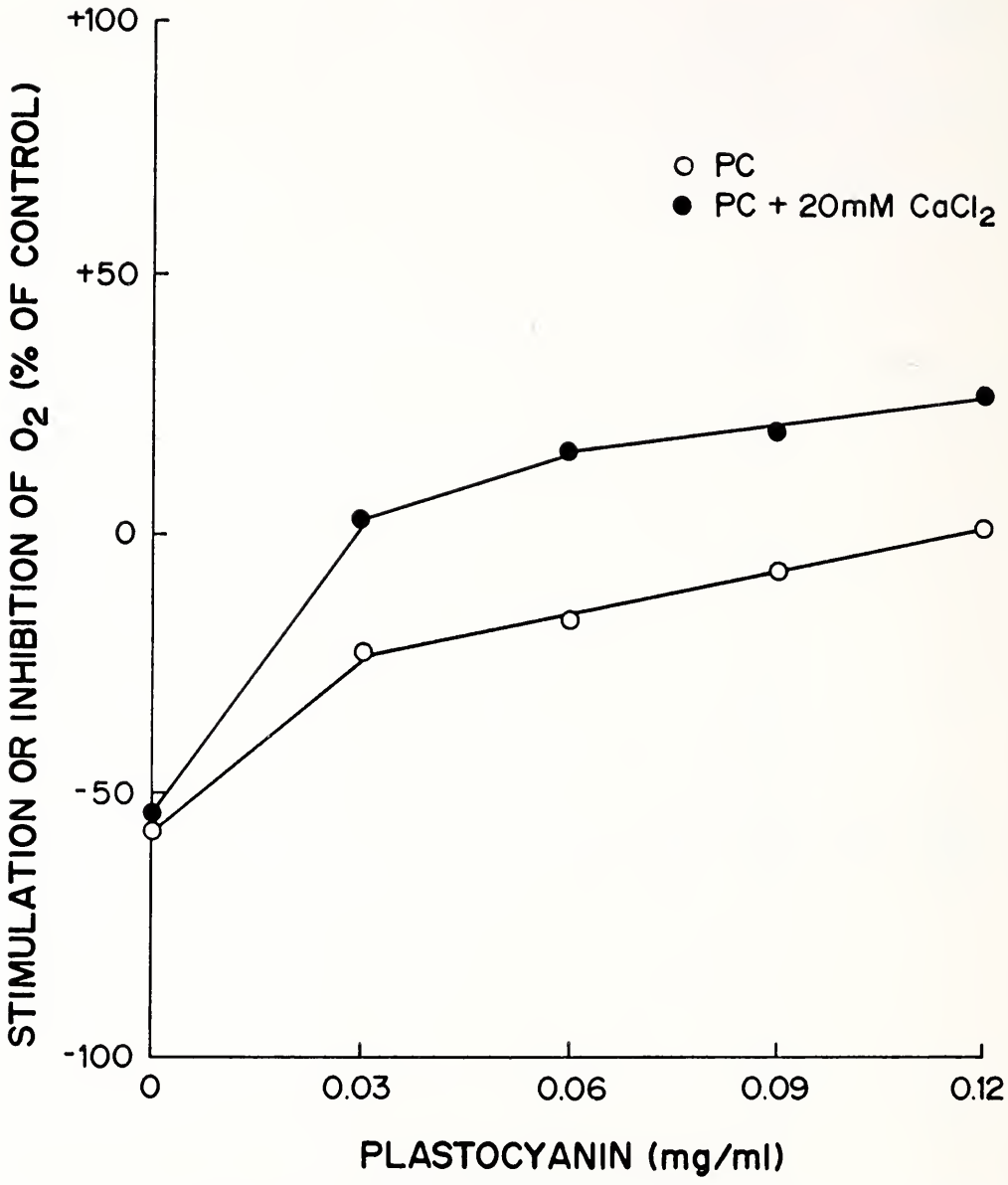

FIGURE 3. *The Effect of Ca^{2+} Ions and Plastocyanin on the Restoration of Electron Transport in EGTA-Treated Cloroplasts. The recation studied was ascorbate + TMPD → MV. The electron transport rate of EGA-treated chloroplasts was 612 μmoles O_2/mg chl•hr. Reaction conditions as in Fig. 2; + indicates stimulation, - inhibition of rate in relation to control.*

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