Effect of Magnesium and Calcium Ions on the Photoelectron Transport Activity of Low-Salt Suspended *Hydrilla verticillata* Thylakoids: Possible Sites of Cation Interaction

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Z. Naturforsch. **53c,** 849–856 (1998); received March 16/May 12, 1998

Aquatic Angiosperm, Divalent Cation, Electron Transport, Fluorescence, *Hydrilla verticillata*

Stimulatory effect of divalent cations like calcium (Ca^{2+}) and magnesium (Mg^{2+}) was investigated on electron transport activity of divalent cation deficient low-salt suspended (LS) thylakoid preparation from a submerged aquatic angiosperm, *Hydrilla verticillata.* Both the cations stimulated electron transport activity of LS-suspended thylakoids having an intact water oxidation complex. But in hydroxylamine (NH_2OH) – or alkaline Tris – washed thylakoid preparations (with the water oxidation enzyme impaired), only Ca^{2+} dependent stimulation of electron transport activity was found. The apparent K_m of Ca^{2+} dependent stimulation of electron flow from H_2O (endogenous) or from artificial electron donor (exogenous) to dichlorophenol indophenol (acceptor) was found to be identical. Calcium supported stimulation of electron transport activity in NH_2OH – or Tris – washed thylakoids was electron donor selective, i.e., Ca^{2+} ion was only effective in electron flow with diphenylcarbazide but not with NH_2OH as electron donor to photosystem II. A magnesium effect was observed in thylakoids having an intact water oxidation complex and the ion became unacceptable in NH_2OH – or Tris – washed thylakoids. Indirect experimental evidences have been presented to suggest that Mg^{2+} interacts with the water oxidation complex, while the Ca^{2+} interaction is localized between Y_z and reaction center of photosystem II.

Introduction

The role of divalent cations in higher plant thylakoid membrane function have been well studied over years (Butler, 1978; Debus, 1992) Divalent cations like Ca^{2+}, Mg^{2+} and Mn^{2+} have been shown to satisfy different functional role to maximize the electron flow and also the energy distribution process of the thylakoid membrane. Among these divalent cations, Ca^{2+} is considered to the most essential co-factor of photosynthetic O_2 evolution. Calcium dependent reactivation of O_2 evolution activity has been extensively studied both in mesophytic higher plant thylakoids and cyanobacterial photosynthetic membrane systems (Yocum, 1991; Debus, 1992). The ion effect in a cyanobacterial system, unlike the higher plants, is discernible by simple washing the membranes in Ca^{2+} deficient buffer or even growing the cells in Ca^{2+} depleted medium (Brand *et al.*, 1983; Satoh and Katoh, 1985). The ion effect in higher plants requires preparation of PS-II particles followed by high concentration of NaCl (1–2 M) – or low pH (3.0/citrate) – washings (Akerlund *et al.*, 1982; Ono and Inoue, 1988). The high salt-washing have been shown to deplete the 17 and 24 kDa extrinsic polypeptide of water oxidation complex and it is the 24 kDa polypeptide which lowers the Ca^{2+} requirement (Miyao and Murata, 1984; Ghanotakis *et al.*, 1984).

Abbreviations: BSA, bovine serum albumin; Chl, chlorophyll; DCIP, 2, 6 dichlorophenol indophenol; DPC, diphenylcarbazide; EDTA, ethylene diamine tetraacetic acid; LHC II, light harvesting chlorophyll protein of photosystem II; PMSF, phenylenemethene sulfonyl fluoride; PpBQ, phenyl-*para*-benzoquinone; P_{680}, reaction center chlorophyll II; PS, photosystem; SDS-sodium dodecyl sulphate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; Tricine, N-(tris-(hydroxymethyl)-methyl)glycine; Tris, N-tris (hydroxymethyl) amino ethane; Y_Z and Y_D, secondary electron donors functioning between Mn and P_{680}.

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0939–5075/98/0900–0849 $ 06.00

The specific role of Ca^{2+} in O_2 evolution activity is still unclear. However, a large number of experimental findings indicate that the ion functions in close association with Cl^- and Mn to maintain the normal S-state transition (different Mn oxidation-state) of water oxidation (Boussac and Rutherford, 1994; Vliet *et al.*, 1994; Latimer *et al.*, 1995). Other pockets of Ca^{2+} interactions, like Y_z (in cyanobacteria ref. Satoh and Katoh, 1985) or in the LHC-II (Han and Katoh, 1993) have also been recognized. Besides, Ca^{2+} may have a role in structural co-ordination of cytochrome b_{-559} with water oxidation enzyme protein (Hulsebosch *et al.*, 1996).

The functional requirement of Ca^{2+} ion, in the cyanobacterial membrane system, can also be reproduced by substituting Na^+ or Mg^{2+} in place of Ca^{2+}. However, in higher plant thylakoids, the monovalent cations such as Na^+, K^+ or Cs^+ are inhibitory on Ca^{2+} mediated reactivation of O_2 evolution. Magnesium ion is neither an activator nor an inhibitor of O_2 evolution in higher plant thylakoids (Debus, 1992).

Unlike higher plant thylakoids, the divalent cations like Ca^{2+} and Mg^{2+} in divalent cation deficient LS-suspended *Hydrilla verticillata* thylakoid do not support energy transfer ("spill-over", "state-change", stacking and destacking phenomenon). Furthermore, the cations stimulate the electron transport activity in a light intensity independent manner. The maximum stimulation of photoelectron transport activity (measured as O_2 evolution) could be obtained with Ca^{2+} than with other divalent cations like Mg^{2+}, Sr^{2+} or Ba^{2+}. The Ca^{2+} and Mg^{2+} dependent stimulation is also pH dependent; being higher at alkaline that at acidic pH (unpublished observations in authors' laboratory). These observations imply that Ca^{2+} and Mg^{2+} most probably modulate electron flow by interacting with electron transport component(s).

Since the divalent cation effect in *Hydrilla verticillata* thylakoid electron transport activity was readily observed upon washing them in LS-salt medium; experiments were conducted to locate the sites of their interaction in the electron transport chain. In this investigation we have studied the effect of physiologically active divalents like Ca^{2+} and Mg^{2+}.

Materials and Methods

Thylakoid isolation and divalent deficient low salt-suspension

Leafy shoots of *Hydrilla verticillata* were homogenized in ice cold homogenizing medium containing 300 mM sucrose, 5 mM $MgCl_2$, 20 mM $CaCl_2$, 10 mM NaCl, 10 mM ascorbic acid, 0.02% BSA and 20 mM Tricine-NaOH (pH 7.5). The slurry was filtered and the filtrate was centrifuged at 6000 x *g* for 5 min. The pellet was suspended in 100 mM sucrose, 5 mM $MgCl_2$, 20 mM $CaCl_2$, 10 mM NaCl and 0.02% BSA and 20 mM Tricine-NaOH (pH 7.5). The suspension was centrifuged for 1 min at 300 x *g* to pellet the debris. Thylakoids from the supernatant of 300 x *g* centrifugation was collected by 6000 x *g* centrifugation for 5 min and taken up in small volume of suspending buffer as mentioned before. Chlorophyll was estimated following Porra *et al.*, (1989). The divalent cation deficient, LS-suspension of thylakoids was prepared by twice washing the thylakoid membranes in a medium containing 100 mM sucrose, 10 mM NaCl, 20 mM Tricine-NaOH (pH 7.5) and taken up in the same medium.

Inactivation of water oxidation by NH_2OH/alkaline Tris washing

For NH_2OH and Tris washings, thylakoids (Chl 500 μg ml^{-1}) in LS-medium were incubated with 5 mM NH_2OH (Ort and Izawa, 1973) 16 or 0.8 M Tris (pH 8.0) (Yamashita and Butler, 1969), in dark for 20 min. The medium was supplemented with 1 mM EDTA to chelate the extracted Mn. The treated thylakoids were spun down at 6000 x *g* for 5 min, washed twice in LS-medium and finally suspended in the same medium.

Photoinhibitory treatment

The photoinhibitory treatment of NH_2OH-extracted *Hydrilla* thylakoids was done by exposing the membranes (Chl 250 μg ml^{-1}) to light (250 and 500 μmol m^{-2} s^{-1}) for 90 sec at 25 °C. The samples were centrifuged and pelleted membranes were taken up in LS-buffer.

Room temperature (25 °C) fluorescence emission measurement

The fluorescence emission of thylakoid preparations was measured in Hitachi-3010 spectrofluoro-

meter keeping the excitation and emission slit widths 5 and 3 nm respectively. The sample was excited at 437 nm and the emission was collected at 685 nm. For all measurements the Chl concentration was adjusted to 5 μg ml^{-1}. The concentration of exogenous electron donors like DPC and NH_2OH, when used, was 0.5 and 10 mM respectively.

Electron transport measurement

Photosystem (PS) II catalyzed electron transport activity was assayed in terms of O_2 evolution using an O_2 electrode assembly (Hansatech) at 25 °C. Light minus dark rate of DCIP reduction was measured at 590 nm in 1 ml reaction mixture containing thylakoids (40 μg Chl), 50 μM DCIP, 100 mM sucrose, 10 mM NaCl and 20 mM Tricine-NaOH (pH 7.5). The electron transport rates were expressed in terms of μmol O_2 evolved $mgChl^{-1}$ h^{-1}. Other reaction details have been mentioned in respective figure legends.

Thylakoid polypeptide analysis

The thylakoid proteins from *Hydrilla* (control, LS-washed, Tris and NH_2OH washed) were resolved in LDS-PAGE using discontinuous buffer system of Laemmli (1970). The resolving gel was of 10–15% continuous gradient. The acrylamide concentration in the stacking gel was 4%. The thylakoid membranes were solubilized in sample buffer [62.5 mM TRIS-HCI (pH 6.8), 10% (v/v) glycerol, 2% (v/v) LDS, 1 mM PMSF, 5% (v/v) β-mercaptoethanol] at room temperature for nearly 30 to 35 min. The gel was run at 25 °C under constant corrent of 15 mA. The separated proteins on the gel were visualized after coomassie brilliant blue staining and the gel was scanned in Personal Densitometer SI (Molecular Dynamics, USA).

Results and Discussion

Cation concentration dependent stimulation of electron transport rate

Cation (Ca^{2+} and Mg^{2+}) concentration dependent stimulation of O_2 evolution activity has been shown in Fig. 1 (A). The cation titration (used as their chloride salts) was done with 2 mM increment in concentration, ranging from 2 to 20 mM. The O_2 evolution activity showed a concentration dependent stimulation both with Ca^{2+} and Mg^{2+}; stimulation being more with Ca^{2+} than Mg^{2+} (the stimulatory effects were independent of chloride ion, as 10 mM NaCl was found to be sufficient to satisfy the maximum chloride requirement, which was predetermined in these preparations, data not shown). The reciprocal analysis of the data yielded a K_m of nearly 3.0 and 2.7 mM for Ca^{2+} and Mg^{2+} respectively (Fig. 1B). It should be mentioned that in high NaCl washed higher plant PS II particles, the low affinity site of Ca^{2+} (in water oxidation complex) as determined by different workers, has K_m values ranging from 2 to 7 mM (Debus, 1992). However, the value differs with back ground concentration of Na^+ ion (Debus, 1992). The ion dependent stimulation of electron transport rate was

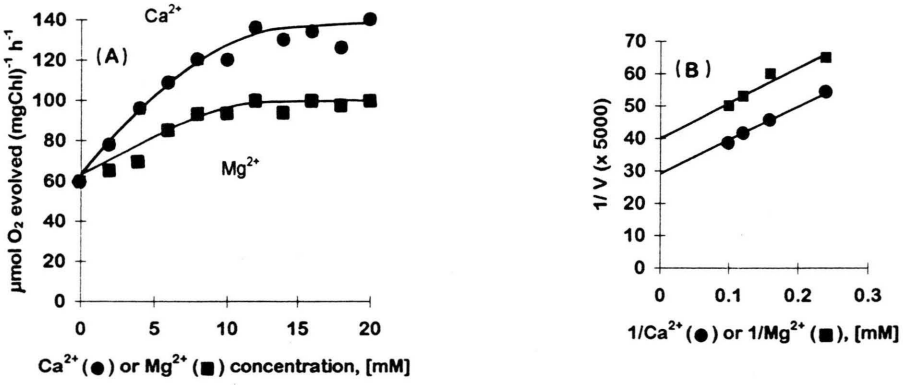

Fig. 1. (A) PpBQ supported PS-II catalyzed O_2 evolution activity as a function of increase in concentration of Ca^{2+} (●), and Mg^{2+} (■) in LS-suspended (●) *Hydrilla* thylakoids. The electron transport activity at pH 7.5 was measured in 1 ml of reaction mixture containing 100 mM sucrose, 10 mM NaCl and 20 mM Tricine-NaOH (pH 7.5). (B) Double reciprocal plot of same data. The data points are the mean of four separate experiments.

also discernible in presence of NH_4Cl or nigercin (data not shown). These compounds have been well characterized to dissipate the *trans*-thylakoid pH gradient (Δ pH, NH_4Cl) and also the cation gradient (electrochemical gradient, nigercin) formed across the thylakoid membrane during electron transport. Therefore, the stimulatory effect of the cations on electron transport is unrelated to any change in cation related changes in protonmotive force (ΔpH and electrochemical potential), built-up during electron transport, rather the effect is very much intrinsic to electron transport activity of *Hydrilla verticillata* thylakoids.

Cation effect on the electron transport activity and fluorescence emission intensity of NH_2OH and Tris washed thylakoids

The Ca^{2+} and Mg^{2+} effect was further examined in *Hydrilla* thylakoid, inactivated in H_2O oxidation enzyme by NH_2OH or Tris treatments. The cation sensitivity, in these thylakoids were examined in presence of exogenous electron donors like NH_2OH and DPC; known to donate electron largely at Y_z (Babcock, 1987) and also at Y_D (Blubaugh and Cheniae, 1990).

No dye (DCIP) reduction was marked in NH_2OH or Tris-washed thylakoids. Also the Chl *a* fluorescence intensity was reduced by about 60–65% as compared to LS-suspended thylakoids (Table I). Addition of NH_2OH or DPC restored the dye reduction and the fluorescence emission intensity as well (Table I). The reason for including these results is to show that the NH_2OH and DPC donor systems were indeed functioning in *Hydrilla* thylakoids as shown for many other thylakoid systems.

The rate of dye reduction in LS-suspended *Hydrilla* thylakoids (Table II) was stimulated in presence of Ca^{2+} (1.90–2.00-fold) or Mg^{2+} (1.40–1.50-fold). The extent of stimulation was comparable to the stimulation of O_2 evolution activity in presence of Ca^{2+} and Mg^{2+} (Table II and Fig. 1). Opposite to the results obtained with intact H_2O oxidation system (Table II, control), Ca^{2+} and Mg^{2+} failed to stimulate the electron transport activity in NH_2OH or Tris washed thylakoids with NH_2OH as electron donor. But on the other hand, DPC supported electron flow to DCIP was stimulated with Ca^{2+} but not with Mg^{2+}. Similar to the intact H_2O oxidation system, the extent of Ca^{2+} dependent stimulation with DPC as electron donor was nearly two-fold (Table II). Electron donation efficiency of DPC can be suppressed by Mn (extracted by Tris or NH_2OH treatment if left untrapped) and Ca^{2+} competes with Mn to release this inhibition (Preston and Seibert, 1991). Since in all washing buffers EDTA was included to chelate the extracted Mn, it is very unlike that the Ca^{2+} dependent stimulation of DPC supported electron flow as shown in *Hydrilla* thylakoids is

Table I. The effect of NH_2OH and Tris washing on the NH_2OH or DPC supported DCIP photoreduction and fluorescence intensity of *Hydrilla* thylakoids. The DCIP photoreduction and fluorescence intensity of treated and donor supported samples are presented as relative to control value taken as 1.00. DCIP reduction in control sample was 47 μmol DCIP reduced $mgChl^{-1}\ h^{-1}$ (mean of four determinations). NH_2OH and DPC donor concentrations were 10 and 0.5 mM respectively. 'ND'-means activity not detected.

Treatment and addition	Dye reduction (Relative unit)	Fluorescence emission intensity (F_{685}) (Relative unit)
None	1.00	1.00
NH_2OH washed (W1)	ND	0.36
Tris washed (W2)	ND	0.32
W1 + NH_2OH	1.36	1.04
W1 + DPC	1.12	1.02
W2 + NH_2OH	0.96	0.99
W2 + DPC	1.44	1.02

Table II. Effect of Ca^{2+} and Mg^{2+} ions on the control (H_2O oxidation functional), NH_2OH and Tris washed (H_2O oxidation inactivated) *Hydrilla* thylakoid electron transport activity measured in terms of DCIP photoreduction. The data shown here for DPC and NH_2OH were 0.5 and 10 mM respectively. The cation effect was, however remained identical altering the DPC and NH_2OH concentrations to 1 and 5 mM respectively. Light intensity was 800 μmol $m^{-2}\ s^{-1}$. Mg^{2+} and Ca^{2+} concentration was 20 mM each. '00' denotes no detectable dye reduction and '—' means measurements were not done. The depicted values are the mean of three independent experiments. The mean difference was noted to be within 5–7 percent deviation.

Addition	Electron transport rate (μmol DCIP reduced $mgChl^{-1}\ h^{-1}$) None	Mg^{2+}	Ca^{2+}
None(control)	25	36	48
NH_2OH washed (W1)	00	—	—
Tris washed (W2)	00	—	—
W1 + DPC	28	26	55
W2 + DPC	36	32	71
W1 + NH_2OH	34	35	32
W2 + NH_2OH	24	25	24

due to the release of Mn inhibition of DPC electron donation by Ca^{2+}.

In NH_2OH extracted PS-II particles of wheat (Blubaugh and Cheniae, 1990), the reductants like DPC, I^- and Mn^{2+} has been shown to be oxidized preferentially by Y_z (under rate – limiting and – saturating light intensities) and presumably also by Y_D (under rate saturating light intensities). It may be possible that the donors (DPC and NH_2OH) used in our investigation for *Hydrilla* thylakoids may have preferential electron donation to either of these two sites. The DPC donation site is largely identified as the Y_z (Babcock, 1987). Hence, in NH_2OH or Tris washed *Hydrilla* thylakoids the reductant NH_2OH whether donate electron to Y_D in a Ca^{2+} insensitive manner remains to be identified. The functional status of Y_D in D_2 reaction center polypeptide in coordinating electron flow between water oxidation complex and P_{680^+} is not yet fully deciphered. However, the sluggish redox active species Y_D has been shown to compete efficiently with Y_z for reduction of oxidized primary electron donor chlorophyll (P_{680^+}) at moderately low temperature and at alkaline pH, Y_D can reduce Y_z in Tris washed chloroplasts (see Blubaugh and Cheniae, 1990 and refs. therein). In water oxidation inactivated PS-II membranes the contribution of Y_D versus Y_z to oxidize various exogenous electron donors although has not been clearly understood the available reports (Babcock, 1987) indicate that the relative contribution of these two sites to oxidize the reductants (electron donors) vary significantly.

An indirect approach was taken to assign the differential electron donation sites of DPC and NH_2OH in *Hydrilla* thylakoids based on the evidence generated by Blubaugh *et al.*, (1991). As shown by these authors, DPC feeds electron at much reduced rate than NH_2OH in NH_2OH-extracted-photoinhibited PS-II (NH_2OH-PS II) wheat particles. Their observations further suggest that Y_z is relatively more susceptible to photodamage compared to Y_D.

DPC and NH_2OH in *Hydrilla* thylakoids, if has selective electron donation to either Y_z or Y_D then it is expected to show a change in the ratio of NH_2OH to DPC supported fluorescence emission intensity (F_{685}) in NH_2OH-PS II samples as compared to control. The F_{685} fluorescence intensity of NH_2OH-extracted and NH_2OH-PS II samples were increased with increasing concentrations (0–300 μM) of the donor (NH_2OH or DPC). Under identical concentration of the donors a higher restoration in fluorescence emission was obtained with DPC than NH_2OH. The electron donation capacity of both the donors was reduced in photoinhibited samples. Comparatively, NH_2OH donation was more affected than DPC. This difference was significant at low concentration of the donor (data not shown).

The extent of photoinhibition is a light intensity dependent phenomenon. Therefore, it is expected that the NH_2OH/DPC F_{685} ratio shall alter depending on the intensity of photoinhibitory light treatment. Under low light photoinhibition (250 μmol m^{-2} s^{-1}), the reduction in ratio was nearly 10–12% while it was about 40% at 500 μmol m^{-2} s^{-1} (Table III). These results indicate that in *Hydrilla* thylakoids the DPC and NH_2OH probably do not prefer to donate electrons at an identical site, and possibly uses different sites for electron donation and NH_2OH site of electron donation is Ca^{2+} insensitive. These results are interesting on the basis that the Y_D redox active species which normally reacts poorly with virtually all exogenous electron donors (Boussac *et al.*, 1992) may have NH_2OH associated

Table III. DPC and NH_2OH dependent restoration of Chl a fluorescence emission (F_{685}) in NH_2OH-extracted (control) and NH_2OH-extracted-photoinhibited (photoinhibited at 250 and 500 μmol m^{-2} s^{-1} light intensities for 90 sec) *Hydrilla* thylakoids suspended in LS-medium. The donor concentration of DPC and NH_2OH were 100 μM each. Thylakoids were dark adapted for 2 min before measurements. The light intensity depicted in the table refers to the intensity used for photoinhibitory treatment. The control samples were not exposed to light. The results were confirmed from three separate batches of thylakoid preparations.

Donor	Photoinhibitory light intensity [μmol m^{-2} s^{-1}]	Fluorescence emission intensity (relative unit, F_{685})
NH_2OH washed (control)		
↓		
DPC		95
NH_2OH		65
NH_2OH washed (Photoinhibited)		
↓		
DPC	250	65
NH_2OH	250	36
DPC	500	40
NH_2OH	500	16

high rate of electron flux in *Hydrilla* thylakoids. This assumption needs further detail study in *Hydrilla* thylakoids.

Since Ca^{2+} stimulates DPC supported photoreduction of DCIP in H_2O oxidation impaired *Hydrilla* thylakoids it may be concluded that the ion has a site of effect on Y_z. The Ca^{2+} effect was donor (site) selective. Furthermore, the Ca^{2+} concentration required to induce half maximal stimulation (i.e. K_m) with H_2O (control) and DPC (H_2O oxidation inactivated) supported electron flow to DCIP was found to be nearly equal (nearly 2.40 mM, see Fig. 2 A, B and C). These results suggest that Ca^{2+} ion in LS-suspended *Hydrilla* thylakoids has no appreciable effect on the H_2O oxidation complex, but effects the electron flow from Y_z to P_{680^+}; similar to a situation reported in cyanobacterial membrane system (Satoh and Katoh, 1985).

Thylakoid polypeptide analysis

In higher plant thylakoids a 24 kDa extrinsic polypeptide of water oxidation complex has been shown to enhance the binding of Ca^{2+} (Miyao and Murata, 1984; Ghanotakis *et al.*, 1984). Removal of this polypeptide attenuates the electron transport activity. Addition of high concentration of Ca^{2+} to 24 kDa polypeptide depleted samples can restore the electron transport function (Miyao and Murata, 1984; Ghanotakis *et al.*, 1984). Removal of weakly bound Ca^{2+} ion by depleting 24 kDa polypeptide from higher plant thylakoid reversibly slows the electron flow from Y_z to P_{680^+} (Ghanotakis *et al.*, 1984) due to disruption of normal Mn cycle through S-state (Yocum, 1991).

To check the participation of 24 kDa polypeptide in inducing the Ca^{2+} effect in *Hydrilla* thylakoids, we studied the presence or absence of this polypeptide through SDS-PAGE in control and LS-washed thylakoids. To ascertain that the 24 kDa polypeptide is the extrinsic polypeptide of PS-II complex in *Hydrilla verticillata* thylakoids, we also studied the protein profile of Tris – and high salt – washed (1.5 M NaCl) thylakoid preparations. These treatments are known to deplete the 24 kDa polypeptide including polypeptides of other molecular weights like 17 and also 33 kDa to a various extent. The 24 kDa polypeptide is discernible as faint band in control and LS-washed thylakoids whereas the band is completely abolished in high salt washed or Tris treated samples (data not shown). These features support that the appearance of Ca^{2+} effect in LS-washed *Hydrilla* thylakoids is not due to the release of 24 kDa extrinsic PS-II polypeptide during preparation of LS-suspended thylakoids.

Possible site of Mg^{2+} effect

Considering the appearance of Mg^{2+} effect in thylakoids with intact water oxidation complex and absence of Mg^{2+} dependent stimulation in

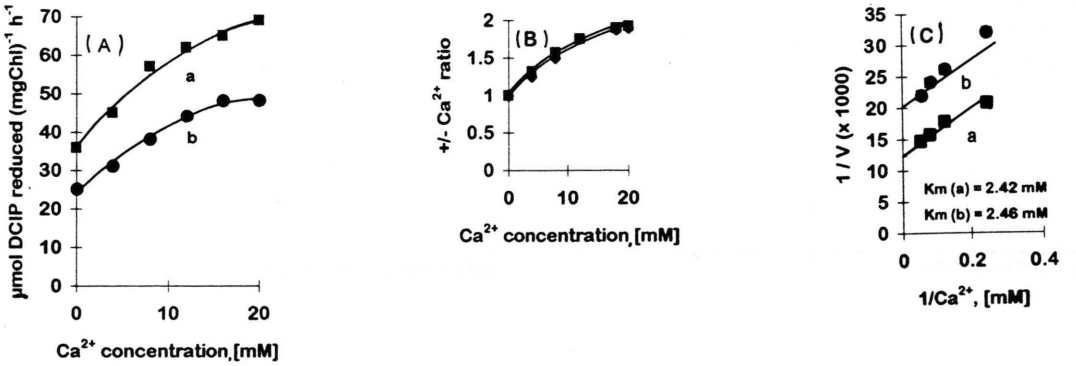

Fig. 2. (A) Graph showing the Ca^{2+} concentration dependent stimulation of DCIP photoreduction in LS-suspended *Hydrilla* thylakoids without (a, DPC → DCIP) and with (b, H_2O → DCIP) functional H_2O oxidation system. The relative extent of stimulation for 'a' and 'b' have been depicted in figure 2 (B). Figure 2 (C) shows the reciprocal plot of observations from figure 2 (A). The observed K_m values for respective reactions (a, b) has been depicted in numbers. DPC concentration was 500 µM. The reaction was done at pH 7.5 under 800 µmol m^{-2} s^{-1} light intensity. The experiment was repeated thrice and the mean value has been shown in the graph. The deviation was found to be between 3–5% of mean.

DPC or NH_2OH supported DCIP reduction (in water oxidation impaired thylakoids) it can be inferred that Mg^{2+} effect is largely restricted to H_2O oxidation complex; an observation so far has not been reported (Debus, 1992). Our observation on the Mg^{2+} activation of O_2 evolution by interacting with H_2O oxidation complex is first of its kind; identified in the thylakoids of a higher submerged aquatic plant, *Hydrilla verticillata.* More detailed work on the effect of Mg^{2+} on PS-II should be obtained.

In this investigation we have presented some new observations on the effect of Ca^{2+} and Mg^{2+} ion on the photo-electron transport activity of a submerged aquatic plant *Hydrilla verticillata.* It is worth mentioning that these group of plants which constitutes a major fraction of photosynthesizing organisms in fresh water aquatic ecosystem has not been taken care for photosynthetic studies at thylakoid level.

Acknowledgements

The work was supported from a Grant (SP/SO/A14/92) of DST Government of India to SCS. Thanks are due to The Director Institute of Life Sciences. The senior author (SRM) is thankful to UGC for a SRF.

Akerlund H.-E., Jansson C. and Andersson B. (1982), Reconstitution of photosynthetic water spilting in inside-out thylakoid vesicles and identification of a participating polypeptide. Biochim. Biophys. Acta **681,** 1–10.

Babcock G. T. (1987), The photosynthetic oxygen-evolving process. In: Photosynthesis. (Amesz, J., ed.). Elsevier Science Publishers, Amsterdam, pp. 125–158.

Blubaugh D. J. and Cheniae G. M. (1990), Kinetics of photoinhibition in hydroxylamine-extracted photosystem II membranes: relevance to photoinactivation and sites of electron donation. Biochemistry **29,** 5109–5118.

Blubaugh D. J., Atamian M., Babcock G. T., Golbeck J. H. and Cheniae G. M. (1991), Photoinhibition of hydroxylamine-extracted photosystem II membranes: identification of the site of photodamage. Biochemistry **30,** 7586–7597.

Boussac A., Setif P. and Rutherford A. W. (1992), Inhibition to tyrosine z photooxidation after formation of S_3 state in Ca^{2+} depleted and Cl^--depleted photosystem II. Biochemistry **32,** 1224–1234.

Boussac A. and Rutherford A. W. (1994), Electron transport events in chloride-depleted photosystem II. J. Biol. Chem. **269,** 12462–12467.

Brand J. J., Mohanty P. and Fork D. C. (1983), Reversible inhibition of photochemistry of photosystem II by Ca^{2+} removal from intact cells of *Anacystis nidulans.* FEBS. Lett. **155,** 120–124.

Butler W. L. (1978), Energy distribution in photochemical apparatus of photosynthesis. Annu. Rev. Plant Physiol. **29,** 345–378.

Debus R. J. (1992), The manganese and calcium ions of photosynthetic oxygen evolution. Biochim. Biophys. Acta. **1102,** 269–352.

Ghanotakis D. F., Topper J. N., Babcock G. T. and Yocum C. F. (1984), Water soluble 17 and 23 kDa polypeptides restore oxygen evolution by creating a high-affinity binding site for Ca^{2+} on the oxidizing side of photosystem II. FEBS Lett. **170,** 169–173.

Ghanotakis D. F., Babcock G. T. and Yocum C. F. (1984), Calcium reconstitutes high rates of oxygen evolution in polypeptide depleted photosystem II preparations. FEBS Lett. **167,** 127–130.

Han K.-C. and Katoh, S. (1993), Different localization of two Ca^{2+} in spinach oxygen-evolving photosystem II membranes. Evidence for involvment of only one Ca^{2+} in oxygen evolution. Plant Cell Physiol. **34,** 585–593.

Hulsebosch R. J., Hoff A. J. and Shuvalo V. A. (1996), Influence of KF, DCMU and removal of Ca^{2+} on the high-spin EPR signal of cytochrome b-559 heme (III) ligated by OH^- in chloroplasts. Biochim. Biophys. Acta **1277,** 103–106.

Laemmli U. K. (1970), Cleavage of structural proteins during the assembly of head of bacteriophage T_4. Nature **277,** 680–685.

Latimer M. J., DeRose V. J., Mukerji I., Yachandra V. K., Sauer K. and Klein M. P. (1995), Evidence for the proximity of calcium to maganese cluster of photosystem II: Determination by x-ray absorption spectroscopy. Biochemistry **34,** 10898–10909.

Miyao M. and Murata N. (1984), Calcium ions can be substituted for the 24-kDa polypeptide in photosynthetic oxygen evolution. FEBS Lett. **168,** 118–120.

Ort D. R. and Izawa S. (1973), Studies on energy coupling sites of phosphorylation II. Treatment of chloroplasts with NH_2OH plus ethylenediaminetetraacetate to inhibit water oxidation while maintaining energy-coupling efficiencies. Plant Physiol. **52,** 595–600.

Ono T. and Inoue Y. (1988), Discrete extraction of the Ca atom functional for O_2 evolution in higher plant photosystem II by simple low pH treatment. FEBS Lett. **227,** 147–152.

Porra R. J., Thompson W. A. and Kriedemann P. E. (1989), Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim. Biophys. Acta **975,** 384–394.

Preston C. and Seibert M. (1991), The carboxyl modifier 1-ethyl-3-[3-(dimethylamino) propyl] carbosiimide (EDC) inhibits half of the high-affinity Mn-binding site in photosystem II membrane fragments. Biochemistry **30,** 9615–9624.

Satoh K. and Katoh S. (1985), A functional site of Ca^{2+} in oxygen-evolving photosystem II preparation from *Synechococcus sp.* FEBS. Lett. **190,** 199–203.

Vliet P. V., Boussac A. and Rutherford A. W. (1994), Chloride depletion effects the calcium-deficient oxygen-evolving complex of photosystem II. Biochemistry **33,** 12998–13004.

Yamashita T. and Butler W. L. (1969), Photoreduction and photophosphorylation with Tris-washed chloroplasts. Plant Physiol. **43,** 1978–1986.

Yocum C. F. (1991), Calcium activation of photosynthetic water oxidation. Biochim. Biophys. Acta **1059,** 1–15.