# ABSENCE OF DIVALENT CATION EFFECTS ON PHOTOSYSTEM II REACTIONS AS MONITORED BY OXYGEN EVOLUTION

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Received 26 February 1981

## 1. Introduction

Mono- and divalent cations have extensive effects on the photoreactions in isolated chloroplasts [1,2]. Based on changes in chlorophyll *a* fluorescence intensity at room temperature and spectra at 77 K, and the quantum yields of photosystems (PS) I and II, a number of models for cation action have been suggested. Divalent cation induced increase in PS II fluorescence has been postulated to be due to an inhibition of energy transfer from PS II to PS I [3] or an increased PS I to PS II transfer [4]. However, divalent cations may alter the efficiencies of fluorescence and radiationless de-excitation processes in favor of fluorescence [5,6]. Divalent cations may enhance PS II activity by activating PS II reaction centers [7,8]. In [8], using a rate electrode, increases in oxygen flash-yield were observed upon addition of $MgCl_2$ and it was concluded that $Mg^{2+}$ induced activation of PS II reaction centers. However, in [9], based on several measurements of PS II activity, it was concluded that cations do not activate PS II centers. To resolve this discrepancy, we have re-examined these studies and measured the effect of divalent cations on oxygen evolution, using both concentration and rate electrodes. Here, we demonstrate that the apparent stimulation of oxygen evolution, as observed [8], may only be due to the slower settling of $Mg^{2+}$-depleted chloroplasts on the electrode surface and not due to enhancement of PS II activity. We also present data to show that there is no effect of $Mg^{2+}$ on oxygen flash-yield as measured by a concentration electrode.

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## 2. Material and methods

### 2.1. *Chloroplast preparation*

Broken chloroplasts were isolated by grinding pea leaves in a Waring Blender in 0.1 M Na-tricine (pH 7.8) containing 0.4 M sorbitol as in [8]. The isolated chloroplasts were washed once in 10 mM NaCl and then recentrifuged to obtain a 'low salt' chloroplast preparation. The pellet was resuspended in 0.4 M sorbitol, 10 mM Na-tricine (pH 7.8) and 10 mM NaCl.

### 2.2. *Oxygen flash-yield measurements*

The oxygen evolution rates of chloroplasts were measured with a Yellow Springs instrument 5331 Clark electrode (concentration electrode) and Easterline Angus recorder. Oxygen yield as a function of the number of flash was measured by a Joliot type [10] rate electrode. The platinum electrode was polarized at −0.6 V with respect to Ag/AgCl electrode. Unless stated otherwise, the upper chamber of the rate electrode was filled with 20 mM NaCl and 10 mM Na-tricine (pH 7.8) for measurements of divalent-depleted samples. When samples with divalent salts present were measured, 10 mM $MgCl_2$ was added to the upper chamber. Short saturating flashes were provided by a General Radio 1538-A Xenon strobe lamp, which has a 3 $\mu$s pulse width at one-half peak height. The signals were amplified and recorded on a photographic recorder or transient digitizer [11].

### 2.3. *Fluorescence yield changes*

Time-dependent changes in chlorophyll *a* fluorescence were observed to establish that the chloroplast preparation used in these experiments had been successfully depleted of divalent cations [3]. Fluorescence yield changes were measured with a conven-

tional fluorimeter with the actinic source at right angles to the photomultiplier. The actinic light was an incandescent lamp filtered by Corning CS 4-96 and CS 3-71 glass filters. The photomultiplier was shielded with a Corning CS 2-64 glass filter.

## 3. Results

### 3.1. *Divalent salt effect on $O_2$ evolution using a concentration electrode*

The oxygen evolution activity on a chlorophyll basis can be determined with a concentration electrode illuminated by a series of saturating flashes of a few $\mu$s duration [12,13]. Fig.1 shows oxygen evolution measured in this way for samples with and without divalent cations present. Divalent salts had no significant effect on oxygen evolution activity, which was 0.26 mmol $O_2$/mol chl in this case. The values for oxygen evolution activity were dependent on plant growth conditions, but were always insensitive to the presence of divalent cations.

### 3.2. *Divalent salt effect on $O_2$ evolution using a rate electrode*

The effect of $MgCl_2$ on the flash-yield of oxygen evolution measured with a rate electrode can be seen

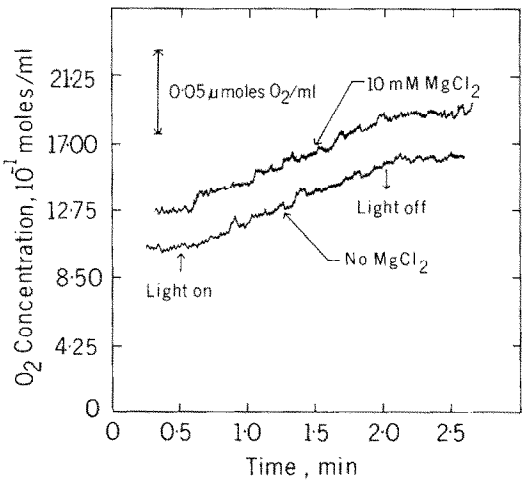


Fig.1. Oxygen evolution measured using a concentration electrode with illumination provided by saturating flashes given at a frequency of 4 Hz. Chloroplast samples were at 50 $\mu$g chl/ml with 1 mM ferricyanide present as an electron acceptor. The oxygen evolution traces were offset for clarity. The addition of $MgCl_2$ to the sample was made 10 min or more prior to the beginning of the measurement.

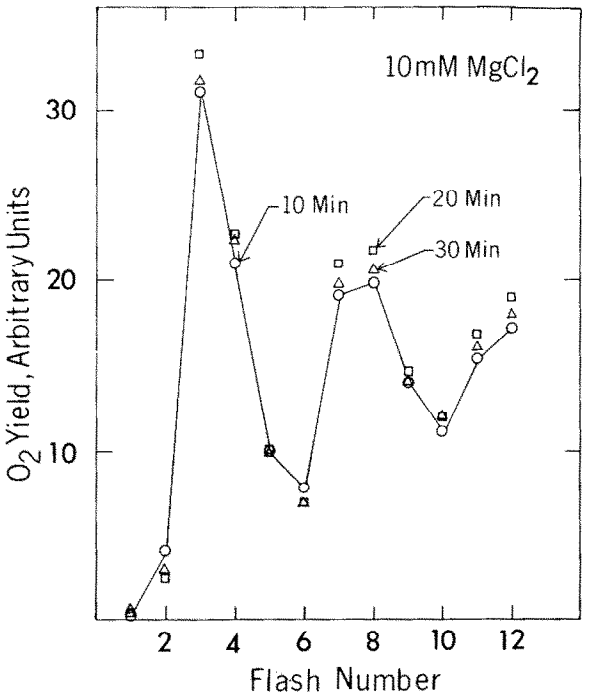


Fig.2. Oxygen evolution flash-yield as a function of flash number given to chloroplasts that had been dark-adapted for at least 5 min. Oxygen yield patterns are shown for samples that had been incubated on the electrode for a total of 10 min (○), 20 min (□) or 30 min (△). These chloroplasts had been depleted of divalent cations, then 10 mM $MgCl_2$ was added ⩾10 min prior to the beginning of the measurement. Chloroplasts were at 500 $\mu$g chl/ml. Flashes were given at a rate of 2 Hz.

by comparing fig.2,3. Measurements were made for samples kept on the electrode for a total time of 10, 20 and 30 min. The sample containing $MgCl_2$ showed only a small change with time (fig.2), whereas the depleted sample showed a marked increase in the oxygen flash-yield as the incubation time increased (fig.3). A plot of the steady-state oxygen yield for these samples is shown in fig.4. For the sample with $MgCl_2$ present, the steady-state oxygen flash-yield remains essentially unchanged except for a decrease that becomes apparent after 30 min on the electrode, which is probably due to the inactivation of the chloroplasts at room temperature. The $Mg^{2+}$-depleted sample undergoes a rapid increase in steady-state yield of >2-fold by 40 min.

Occasionally, samples that had been divalent cation-depleted, as shown by the altered fluoresence rise curves, would show little or no increase of their oxygen

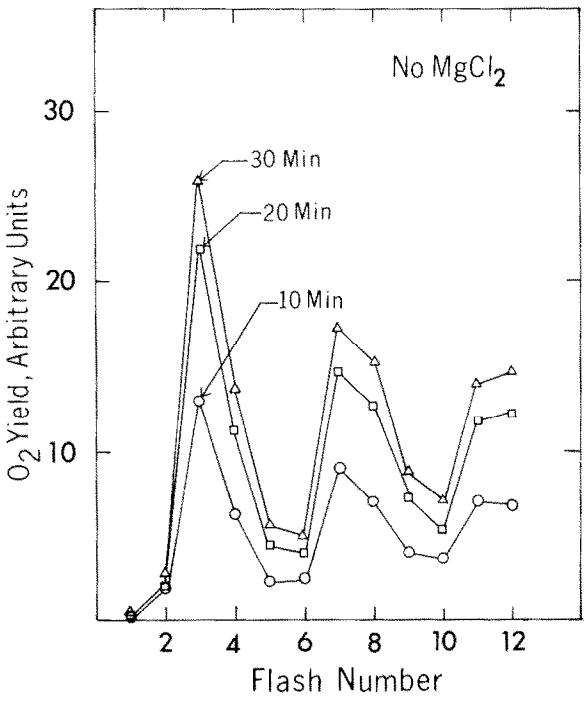


Fig.3. Same conditions as in fig.2, except these cation-depleted chloroplasts had no $MgCl_2$ added.

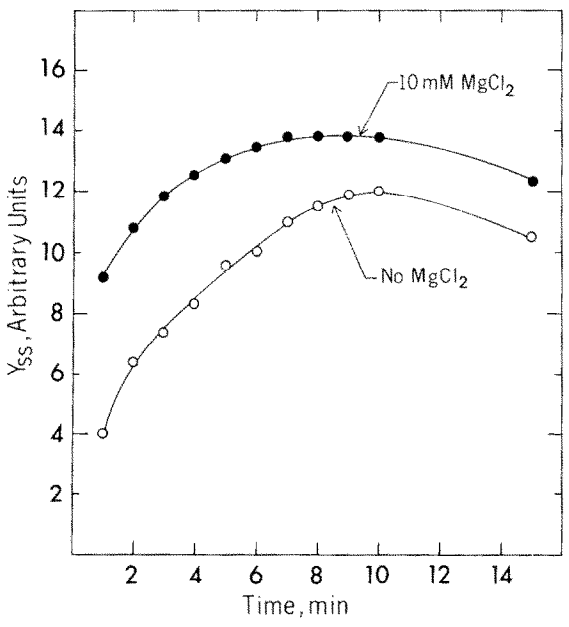


Fig.5. Steady-state oxygen flash-yield ($Y_{SS}$) *vs* sample incubation time on the electrode. A series of 24 flashes was given at the time interval indicated. $Y_{SS}$ was obtained by averaging the flash-yield on flashes 20–24. Data are shown for divalent cation-depleted chloroplasts with 10 mM $MgCl_2$ (•) or without $MgCl_2$ (○). Flashes were given at a rate of 2 Hz.

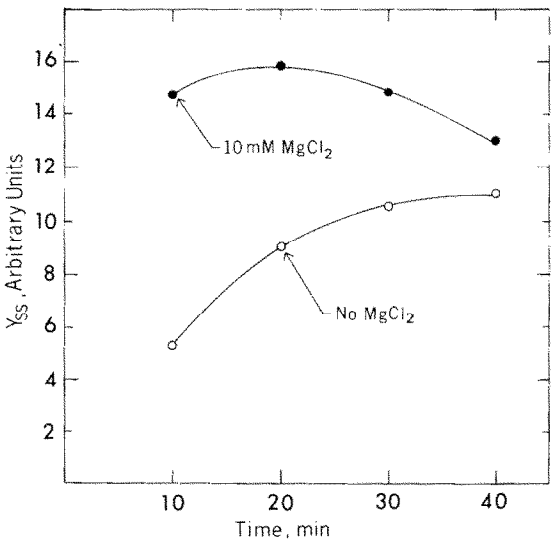


Fig.4. Steady-state oxygen flash-yield ($Y_{SS}$) *vs* sample incubation time of the electrode. $Y_{SS}$ was obtained by averaging the flash-yields on flashes 30–34, following dark adaptation. Data are shown for divalent cation-depleted chloroplasts with 10 mM $MgCl_2$ (•) or without $MgCl_2$ (○). Flashes were given at a rate of 2 Hz.

evolution flash-yield measured after 10 min incubation on the rate electrode (cf. fig.4). It was felt that perhaps in these divalent cation-depleted samples the increase in oxygen flash-yield was more rapid than the 10 min incubation. To check this possibility, a series of 24 flashes was given once a minute beginning 1 min after the sample was placed on the electrode. The oxygen yields on flashes 20–24 were averaged to give an estimate of the steady-state yield at these 1 min intervals. These data are plotted in fig.5 and show that a very rapid increase in oxygen flash-yield occurs for these samples. After 1 min on the electrode, the samples with $MgCl_2$ present had oxygen flash-yields ~2.2-fold greater than the cation-depleted samples. By 10 min on the electrode, this ratio had decreased to 1.2.

### 3.3. *Divalent salt effects on the rate electrode sensitivity*

When performing the rate electrode experiments, $MgCl_2$ in the upper chamber of the electrode was changed from 0 to 10 mM depending on the sample being measured (see section 2). It is possible that this

media change affects the electrode sensitivity. To test this possibility, chloroplasts with 10 mM $MgCl_2$ were incubated on the electrode for 10 min, and their steady-state oxygen flash-yield was determined. The medium in the upper chamber of the electrode was then rapidly replaced with medium without 10 mM $MgCl_2$, a procedure that takes ~15 s to complete, and the steady-state yield was immediately determined. The time (15 s) for which $MgCl_2$ was absent was short enough to suggest that no substantial amount of $MgCl_2$ depletion occurred in the sample. The ratio of the steady-state oxygen yield observed was 1.17 ± 0.5, with the yield being greater when the upper chamber had 10 mM $MgCl_2$ present. The 1.17-fold increase in steady-state yield is attributable to a change in electrode sensitivity.

### 3.4. *Divalent salt effects on chloroplast settling rate*

One possible explanation for the effects seen in fig.3–5 might be that the presence or absence of divalent salts alters the settling rate of the chloroplast on the platinum surface of the rate electrode and this in turn changes the amplitude of the signal.

The settling rate was checked by centrifuging 10 ml chloroplast for 8 min at 1000 × *g*. The centrifugation was stopped at 1 min intervals and the optical density of the top 2 ml of the sample was measured. In fig.6 data is shown for chloroplasts with or without divalent cations present. A much slower settling rate is observed for chloroplast depleted of divalent cations.

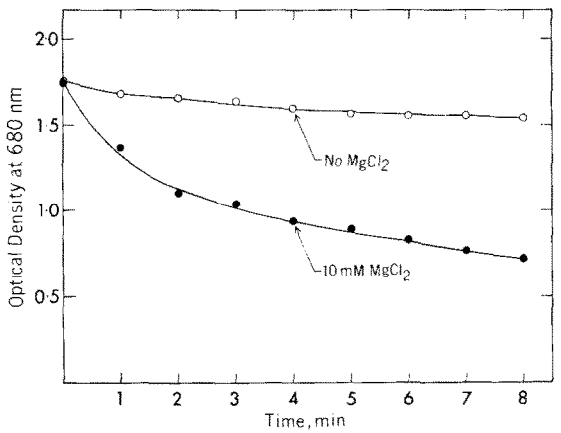


Fig.6. Optical density at 680 nm as a function of time of centrifugation of chloroplasts with or without $MgCl_2$ present. The sample (10 ml) was centrifuged at 1000 × *g* for the times indicated and aliquots of 2 ml were collected from the supernatant. Measurements were made with a Cary model 219 spectrophotometer with a 1 cm pathlength cuvette. Data is shown for chloroplasts with 10 mM $MgCl_2$ (•) or without $MgCl_2$ (○).

Table 1
Effects of electrode orientation on oxygen flash-yield

| Electrode orientation before the measurement | $Y_{ss}$ (arbitrary units) |
|---|---|
| 10 min upright | 3.56 |
| 10 min upside down | 0.23 |
| 8 min upside down, 2 min upright | 0.25 |
| 5 min upside down, 5 min upright | 2.23 |

Oxygen yield measurements were made for samples that had been incubated on the electrode for 10 min in the dark. The electrode was oriented either upright or upside down for the times indicated. The steady-state yield ($Y_{ss}$) is the oxygen flash yield that occurs after 30 flashes when the oscillations have damped out

To determine if settling had any effect on the signal size of the rate electrode, measurements were made with the electrode kept in different orientations for 10 min preceding the measurement. When the electrode is positioned upright the platinum surface is beneath the chloroplasts and gravity causes the chloroplasts to settle on the platinum surface. When it is upside down the chloroplasts will not settle on the platinum surface. The results of this type of experiment are shown in table 1. Positioning the electrode for 10 min upside down causes a 15-fold decrease in signal size. This loss of signal can be reversed to a large extent by having the electrode upright for 5 min (last line of table 1).

## 4. Discussion

The oxygen evolution activity measured with a concentration electrode does not show a significant sensitivity to divalent cations. This shows that PS II reaction centers remain unchanged by divalent cations. This view has also been stressed in [9] based on divalent salt effects on the amplitude of electrochromic changes, on the amount of reduced primary donor and oxidized primary acceptor, and on oxygen evolution measurements with a concentration electrode.

We show (fig.2–4) that apparent stimulation of oxygen evolution activity by divalent cation [8] is observed only if the sample is on the electrode for short periods of time. This stimulation of oxygen evolution activity is not observed with longer incubation time on the electrode (fig.4,5). It is shown here that

this increase in oxygen yield is due to the settling of the chloroplasts on the platinum surface of the electrode. Different samples settle at different rates, and divalent cation-depleted chloroplasts settle slower than normal chloroplasts (fig.6, table 1). Chloroplast membranes isolated in 'low salt' medium are unstacked and loosely held together [14,15], which might explain the slower settling time.

In all these data, even after sufficient incubation time had been allowed for complete settling, the samples with $MgCl_2$ present had ~1.2-fold greater oxygen flash-yield. It is believed that this small residual effect of divalent salts is actually an alteration in the electrode response and not a change in PS II activity.

## Acknowledgement

R. K. thanks Dr Govindjee for the use of his laboratory equipment.

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