EFFECTS OF BULK pH AND OF MONOVALENT AND DIVERENT CATIONS ON CHLOROPHYLL a FLUORESCENCE AND ELECTRON TRANSPORT IN PEA THYLAKOIDS

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Summary

Millimolar concentrations of monovalent cations enhance and divalent cations impede the redistribution (spill-over) of electronic excitation energy from Photosystem (PS) II to PS I in cation-depleted (sucrose-washed) thylakoids; this concept is based on chlorophyll a fluorescence and electron transport measurements over a narrow pH range around 7. We have tested the above concept in pea thylakoids over the pH range 5 to 9 by parallel measurements of various chlorophyll a fluorescence parameters (spectra, transients, and lifetimes at 77 K and 293 K, and polarization at 293 K) and of the rates of partial reactions of PSI and II.

Our results provide the following information.

(1) Mg²⁺ enhancement of fluorescence is maximum between 680 and 690 nm and minimum between 710 and 720 nm.

(2) The optimum conditions for the observation of the Mg²⁺-induced enhancement of fluorescence are: wavelength of emission, 685 nm; concentration of Mg²⁺, 10 mM, and pH, ~7.5.

(3) Mg²⁺ decreases the efficiency of excitation redistribution from PS II to PS I over the pH range 6 to 9.

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Abbreviations: DAD, diaminodurene; DBMIB, dibromothymoquinone; DCMU, 3-(3,4 dichlorophenyl)-1,1-dimethylurea or, diuron; DCIP (or DCPIP), dichlorophenol indophenol; PS I, Photosystem I; PS II, Photosystem II; Chl, chlorophyll.
(4) The antagonistic effects between Na\(^+\) and Mg\(^{2+}\) hold simultaneously for both the fluorescence intensity and lifetime, at physiological temperatures, only within the pH range 6 to 8.

(5) Mg\(^{2+}\) enhances the light-limited electron transport rate through PS II in the pH range 5.4 to 8.2 and decreases that through PS I at pH 7.1 and 8.2. The % increase in PS II is, however, about twice the % decrease in PS I.

Introduction

Monovalent and divalent cations play a crucial role in the regulation of net electronic excitation energy distribution between the two photosystems of green plant photosynthesis [1,2]. We present here the effects of varying the bulk H\(^+\) concentration in the suspension medium on this excitation energy regulation in pea thylakoids.

The purpose of the present investigation was three fold.

First, define the pH range over which the existing findings on cation-induced fluorescence changes hold, since most studies have been made over the narrow pH range of 7 to 8. This is particularly important since the pH of the intrathylakoid space (loculus) show large variations in normal operation [1].

Second, re-examine the antagonistic effects of Mg\(^{2+}\) on electron transport in photosystem (PS) I and PS II as a function of pH because the inhibiting effect [3] of Mg\(^{2+}\) at pH > 7.5 on PS I electron transport (measured as the rate of NADP\(^+\) reduction) turns into an enhancing effect at pH < 7.5 [4].

Third, provide a comprehensive study, through parallel measurements, of the pH dependence of the cation effects on various aspects of chlorophyll a fluorescence and electron transport in the two photosystems, in order to clarify the regulatory phenomenon of excitation energy distribution. Since Barber et al. [5] have reported that the cation effects on chlorophyll a fluorescence correlate with the membrane surface charge density, and that the electrophoretic mobility of the thylakoids is constant in the pH range 6 to 10, it was important to know whether or not the cation effects are also pH insensitive.

Materials and Methods

Thylakoids were prepared as described in Ref. 6. The final suspension medium contained 100 mM sucrose and 2 mM Tris adjusted to an appropriate pH with HCl or HNO\(_3\). Since the concentrated stock thylakoid membranes, suspended in 100 mM unbuffered sucrose, showed slightly acidic pH, the final measured pH of each sample was 0.4–0.8 unit lower than the pH of the dilution medium. Other sample details were as given in the legends of figures and tables. Diuron, when used, was added prior to cations, and, the measurements were made at least 10 min after incubation in the final suspension.

Chlorophyll a fluorescence spectra and transients were measured with an instrument described elsewhere [7,8]. For measurements at 77 K, 0.5-ml aliquots of thylakoid suspension were adsorbed onto two layers of cheese-cloth (0.3 mm thickness) and frozen in liquid nitrogen. All fluorescence spectra were corrected for monochromator transmission characteristics and photocathode
(S20) sensitivity. The degree of polarization of fluorescence was measured as described elsewhere [9,10]. Fluorescence lifetimes (τ) were measured by the phasen shift method using a mode-locked He-Ne laser (λ = 632.8 nm; modulation frequency = 75 MHz) [11]. With the exception of the fluorescence polarization (P) which utilized right angle geometry, all other fluorescence parameters were measured from the same surface as the incident irradiation.

Electron transport rates in the partial reactions were measured either optically, as the rate of bleaching of DCIPH₂ at 597 nm, or, as the rate of oxygen evolution or uptake, by a Clark-type electrode in a water jacketed chamber and an oxygen monitor (Yellow Springs Instrument, Model 53). The extinction coefficients of DCIP at different pH values were determined as in Ref. 12. Chlorophyll concentrations were determined by the method described in Ref. 13. The temperature was 23 ± 1°C.

Results

Cation effects on chlorophyll a fluorescence

Cation concentration curves for maximum yield of fluorescence in diuron-treated thylakoids

The optimum concentrations of cations to be used were determined from cation concentration dependence of the maximum steady-state yield (fluorescence intensity/absorbed intensity) of fluorescence, Fₐ (corresponding to the P level) at 685 nm in salt-depleted thylakoids ([Chl], 5 µg • ml⁻¹) treated with 3.3 µM diuron at pH 6.2, 7.1, and 8.6 (Fig. 1). With Fₐ for the salt-depleted sample in each case normalized to 1.0, the following is noted:

1. There are two phases (Fig. 1A) in the cation (Na⁺ and Mg²⁺) concentra-

Fig. 1. The maximum relative yield of fluorescence at 685 nm as function of the cation concentrations, for three pH values. (A) The effect of Na⁺ or Mg²⁺ over the concentration range 0–100 mM. (B) The fluorescence enhancement with increasing Mg²⁺ concentration between 0 and 10 mM replotted to demonstrate the pH dependent shift in [Mg²⁺] for half-maximum enhancement. Thylakoids suspended in 100 mM sucrose + 2 mM Tris-HCl (appropriate pH); [Chl] = 5 µg/ml; [DCMU] = 3.3 µM; temperature = 23°C. Excitation was at 636 nm (half-bandwidth, 8 nm).
tion curves for $F_m$. The maximum fluorescence, $F_m$, increases sharply as [Mg$^{2+}$] is increased from 0 to 5 mM, attaining a maximum at 6–8 mM, and then declines slightly from 10 to 100 mM. In the case of Na$^+$, however, $F_m$ shows an 'S'-shaped dependence on its concentration; in the 20–40 mM range (depending on pH), $F_m$ decreases slightly, but, beyond ~40 mM, it increases, saturating at ~100 mM (cf. Ref. 14). At ~100 mM, both Na$^+$ and Mg$^{2+}$ give the same $F_m$.

(2) $F_m$ in the presence of 10 mM Mg$^{2+}$ is highest at pH 7.1, followed by pH 6.2, and then pH 8.6 (Fig. 1). 10 mM Na$^+$, on the other hand, induces a greater decrease in $F_m$ at pH 8.6 than at pH 7.1; at pH 6.2, there is only a very slight change in $F_m$ (Fig. 1A).

(3) The half-saturation concentration for the Mg$^{2+}$-induced increase in $F_m$ shifts to lower values with increasing pH (Fig. 1B).

**pH dependence of cation effects on chlorophyll a fluorescence at room temperature**

*Emission spectra.* The room temperature emission spectra of thylakoid suspensions at pH 5.3, 6.3, 7.8 and 8.9 are similar to each other, although the relative enhancement by 10 mM Mg$^{2+}$ is different in each case. From 5.3 to 8.9 pH, the main emission peak is at ~685 nm with a smaller band at ~730 nm, as is known for chloroplasts at pH 7.0. The quotient of the emission intensity of a sample containing 10 mM Mg$^{2+}$ and 10 mM Na$^+$ to that of a sample without Mg$^{2+}$ but with 10 mM Na$^+$, at various pH values, is strongly dependent upon emission wavelength (Fig. 2). At pH 6.3 or greater, this ratio shows a large Mg$^{2+}$-induced enhancement between 670 and 690 nm; this enhancement declines monotonically beyond 690 nm to a minimum at 710–720 nm, followed by a rise, and, perhaps, a final decline beyond 750 nm. At pH 5.4, only a slight wavelength independent increase is observed.

*Maximum steady-state yield.* Figs. 1A and 2 show that the ideal conditions for the study of cation effects on chlorophyll a fluorescence in the pH range 6 to 9 are to measure the emission at ~685 nm in the presence of 10 mM cations. To determine the optimum pH, the maximum steady-state fluorescence yield ($F_m$) of the thylakoids suspended in cation-free Na$^+$ (10 mM), and Na$^+$ (10 mM) + Mg$^{2+}$ (10 mM) media in the presence of 3.3 μM diuron was measured (Fig. 3). The profiles of $F_m$ for the three conditions are different. In cation-depleted medium (open circles), $F_m$ shows an increase from pH 5.0 to a peak at pH 6.3, then a decline to a relative minimum at pH 7.7, and a slight rise thereon to pH 9. With the addition of 10 mM Na$^+$ (closed squares), $F_m$ shows a broad maximum around pH 5.7, intersecting the previous curve at pH ~6.1, so that $F_m$ (Na$^+$) is greater than $F_m$ (cation-free) at low pH and smaller at high pH. In the presence of 10 mM Mg$^{2+}$ (closed triangles), $F_m$ peaks at pH 7.7, with $F_m$ (pH 7.7) = 2$F_m$ (pH 5) ~ 1.5$F_m$ (pH 8.8). Addition of 10 mM Na$^+$ to salt-depleted thylakoids gives a ~20% increase in $F_m$ at pH ~5.0 but a ~35% decrease at pH 8.8, the transition over the pH range being almost linear. The enhancement in fluorescence yield upon the addition of 10 mM Mg$^{2+}$ to a sample containing 10 mM Na$^+$ rises dramatically from ~10% at pH 5.0 to >300% at pH 7.7, followed by a slight drop at pH 8.8.

*Transients.* To estimate how much effect cations have on the constant ($F_o$)
and variable ($F_v = F_m - F_o$) parts of fluorescence, transients were measured (Table I). In both the salt-depleted and the Na\textsuperscript+ samples, $F_o$ at pH 7.8, is lower than at pH 6.0 and pH 8.9. In the Na\textsuperscript+ + Mg\textsuperscript{2+} sample, $F_o$ at the two lower pH values is about the same, but is lower at the highest pH. $F_o$ decreases slightly upon Na\textsuperscript+ addition to salt-depleted thylakoids, for all 3 pH values but it increases significantly (~60%) upon the addition of Mg\textsuperscript{2+} only at pH 7.8 (cf. Ref. 15), no change being observed at pH 6.1 and 8.9.

### Table I

**CATION EFFECTS ON THE INITIAL AND THE MAXIMUM RELATIVE FLUORESCENCE YIELD AT 23°C AT 3 DIFFERENT pH VALUES**

Fluorescence was measured at 685 nm through a monochromator (band-pass, 6.6 nm). Excitation was with broad-band blue light, white light passed through Corning CS 3-73 + CS 4-96 filters. [Chl] = 5 μg/ml.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH 6.1 ± 0.2</th>
<th>pH 7.8 ± 0.1</th>
<th>pH 8.9 ± 0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_o^*$</td>
<td>$F_m$</td>
<td>$F_v/F_m$</td>
</tr>
<tr>
<td>Salt-depleted</td>
<td>10.0</td>
<td>20.7</td>
<td>0.52</td>
</tr>
<tr>
<td>10 mM NaCl</td>
<td>8.5</td>
<td>16.9</td>
<td>0.50</td>
</tr>
<tr>
<td>10 mM NaCl + 10 mM MgCl$_2$</td>
<td>8.6</td>
<td>20.1</td>
<td>0.57</td>
</tr>
</tbody>
</table>

$F_o^*$ also called the o level or 'constant fluorescence' is the initial yield of fluorescence upon illumination of a sample; $F_m$, also called P level fluorescence, the maximum yield of fluorescence attained during illumination, and the variable fluorescence $F_v = F_m - F_o$. 
At pH 7.8, the ratio of $F_v$ to $F_m$ (in Table I) shows cation-induced changes similar to those in Ref. 6 for the $\mu$s fluorescence transient induced by a single 10 ns flash: a decrease in the ratio with addition of Na$^+$ and an increase with a subsequent addition of Mg$^{2+}$. However, these effects are either diminished or absent at higher and lower values of pH.

**Lifetimes.** For both the salt-depleted and Na$^+$ samples, fluorescence lifetime of $F_m$ at 686 nm $\tau(F_{686, m})$ shows a general decline with increasing pH (Fig. 4). In the Na$^+$ + Mg$^{2+}$ sample, the pH profile of $\tau(F_{686, m})$ shows a broad peak around pH 7.5. The maximum relative fluorescence yields at 686 nm, $F_{686, m}$, simultaneously measured with $\tau(F_{686, m})$, show similar pH profiles. Also, the pH profiles of the $\tau(F_{686, m})$ in Fig. 4 closely resemble those from steady-state fluorescence yield measurements in diuron treated thylakoids (Fig. 3), suggesting that the pH profiles of $\tau$ may be diuron insensitive.

**Polarization.** The degree of polarization of fluorescence $(P)$ at 686 nm was measured at pH 6.6 and 9.0 (Table II). Results at pH 7.6 were reported earlier [9]. Addition of Na$^+$ induces a greater increase in $P$ of $F_{686, m}$ at pH 6.6 ($\sim$ 11%) than at pH 9.0 (4%). The Mg$^{2+}$-induced decrease in $P$ is about the same ($\sim$ 10–13%) at both pH values. These changes in $P$ are consistent with a Na$^+$-induced decrease in energy migration in PS II and a Mg$^{2+}$-induced increase in such migration, over the pH range 6.6 to 9.0 (cf. Ref. 9).

**pH dependence of cation effects on chlorophyll a fluorescence at 77 K**

**Lifetimes.** The $\tau$ at the P-level at 77 K was measured at 686, 695, and 730 nm for pH values of 6.2, 7.7, and 8.8. Values of $\tau$ at pH 7 were reported in Ref. 16. The addition of Na$^+$ to salt-depleted thylakoids causes decreases in $\tau(F_{686, m})$ and $\tau(F_{695, m})$: from 0.35 to 0.24 ns for $\tau(F_{686, m})$ and from 0.57 to 0.46 ns for $\tau(F_{695, m})$ at pH 7.7 and 8.8; at pH 6.2, there is no cation-induced change ($\tau(F_{685, m}) \approx 0.53$ ns; $\tau(F_{695, m}) \approx 0.7$ ns). Subsequent addition of Mg$^{2+}$ causes increases in $\tau(F_{685, m})$ from 0.51 to 0.83 ns (at pH 6.2), from 0.25 to 0.54 ns (at pH 7.7), and from 0.23 to 0.43 ns (at pH 8.8). The increases in

![Fig. 4. pH dependence of the lifetime of the maximum fluorescence at 686 nm, $\tau(F_{686, m})$, for the salt-depleted (○), Na$^+$ (■), and Na$^+$ + Mg$^{2+}$ (▲) samples. Fluorescence excitation was at 632.8 nm and emission was detected through an interference filter at 686 nm (half-bandwidth, 6.8 nm). Lifetimes were measured as described in Ref. [11].](image-url)
TABLE II
CATION EFFECTS ON THE POLARIZATION OF CHLOROPHYLL a FLUORESCENCE FOR TWO pH VALUES

Samples were 3 ml thylakoid suspensions in 100 mM sucrose + 2 mM Tris-HNO₃, [Chl] = 5 μg/ml, and [DCMU] = 3.3 μM. NaCl and MgCl₂ were added to the final concentration of 5 mM. Excitation was at 630 ± 2.5 nm and fluorescence was measured through a long-pass filter (Schott RG 665) and a 686 nm interference filter (half-bandwidth = 6.8 nm). The temperature was 24°C.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>Degree of polarization (%) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt-depleted</td>
<td>6.6</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>Na⁺</td>
<td>6.6</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>Na⁺ + Mg²⁺</td>
<td>6.6</td>
<td>2.8 ± 0.1</td>
</tr>
<tr>
<td>Salt-depleted</td>
<td>9.0</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>Na⁺</td>
<td>9.0</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Na⁺ + Mg²⁺</td>
<td>9.0</td>
<td>2.3 ± 0.1</td>
</tr>
</tbody>
</table>

* The degree of polarization (%) was calculated as \([(F_v - F_h)/(F_v + F_h)] \times 100\), where \(F_v\) and \(F_h\) are the intensities of vertically and horizontally polarized components of the fluorescence, when the actinic illumination is vertically polarized. Instrumental corrections were made as described by Wong et al. [10].

\(\tau(F_{695,m})\) are from 0.7 to 1.1 ns at pH 6.2, from 0.45 to 0.85 ns at pH 7.7, and from 0.48 to 0.81 ns at pH 8.8. The changes in \(\tau(F_{730,m})\) are small (≤15%). \(\tau(F_{730,m})\), at various pH values, are ~2.4 ns (pH 6.2), ~2.1 ns (pH 7.7) and ~1.8 ns (pH 8.8); there is a general decrease in \(\tau\) at all wavelengths with increasing pH.

**Emission spectra.** The cation effects on the emission spectra at 77 K, normalized to their relative \(\tau\) at 686 nm, are shown in Fig. 5. The typical three-band spectrum with maxima at 685 (PS II), 693–696 (PS II), and 735 nm (PS I) is obtained for all values of pH and the three cationic conditions. At pH 6.3,
~10 mM Na\(^{+}\) causes a slight decrease in \(F_{687}\) and a slight increase in \(F_{735}\) (see Ref. 17); divalent cations, on the other hand, cause a large increase in \(F_{684}\) and \(F_{693}\) and a large decrease in \(F_{735}\) (see Ref. 3). At pH 8.0 and 9.1, however, Na\(^{+}\) causes a large decrease in the (relative) increase in the fluorescence at 684 and 693–696 nm, but no change at 735 nm.

**Transients.** The fluorescence transients at 77 K have been used for evaluating the energy distribution and redistribution in the two photosystems at pH 7.8 (cf. Ref. 18). We present measurements of \(F_{o}\) and \(F_{m}\) at 690 and 730 nm at pH 6.2 and 8.8 (Table III). Here, the fluorescence at 690 nm, mainly from PS II, for the different samples was normalized by taking \(\tau(F_{690}) = [\tau(F_{686}) + \tau(F_{695})]/2\). Even the emission at 730 nm, mainly from PS I, has a ‘variable’ fluorescence \(F_{v}\) (10–20% of \(F_{m}\) at 730 nm); it is suggested [18] that this component results from energy transfer from PS II to PS I.

\(F_{v}/F_{m}\) at 690 nm (Table III), by an earlier analysis [19], is the product of the efficiency of excitation transfer from the antenna to the reaction center of PS II and the efficiency of back-transfer from the closed center, and is taken as an index of the extent of excitation cycling between the antenna and the reaction center. At both acid and basic values of pH, the addition of 10 mM Na\(^{+}\) to salt-depleted thylakoids lowers \(F_{v}/F_{m}\) (at 690 nm), and, thus, the energy cycling. However, the subsequent addition of 10 mM Mg\(^{2+}\), while increasing \(F_{v}/F_{m}\) at acid pH, causes a further decrease in the ratio at basic pH.

**Excitation distribution and redistribution in Photosystem II.** From the above data on \(\tau\) and transients, estimates (Table IV) of the energy distribution and redistribution parameters in PS II were made by a method similar to that described in Refs. 18 and 19, but to which measurements of \(\tau\) were incorporated for estimating the fractional absorption by each photosystem in individual thylakoid samples (cf. Ref. 20, and footnote in Table IV). Our findings are: (1) at pH 6.2, 10 mM Na\(^{+}\) induces a smaller decrease in \(\beta\) (the fraction of total absorbed quanta initially partitioned to PS II) than the increase induced

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**TABLE III**

CATION EFFECTS ON THE INITIAL AND THE MAXIMUM FLUORESCENCE AT 77 K AT 690 AND 730 nm FOR TWO pH VALUES

Thylakoid suspensions (0.5 ml each) in 100 mM sucrose + 2 mM Tris-HNO\(_3\) were adsorbed on two layers of cheese-cloth (0.3 mm thickness) and frozen at 77 K. Excitation was through a 636 nm interference filter (half-bandwidth = 8 nm), and fluorescence was measured through a Corning CS 2-59 glass filter and a monochromator set at 690 nm (band-pass = 10 nm). Chlorophyll concentration was 20 \(\mu\)g/ml, [NaCl] = [MgCl\(_2\)] = 9.8 mM. For meaning of symbols, see the legend of Table I.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>690 nm</th>
<th>730 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(F_{o})</td>
<td>(F_{m})</td>
</tr>
<tr>
<td>Salt-depleted</td>
<td>6.2</td>
<td>22.3</td>
<td>65.1</td>
</tr>
<tr>
<td>Na(^{+})</td>
<td>6.2</td>
<td>29.5</td>
<td>62.1</td>
</tr>
<tr>
<td>Na(^{+}) + Mg(^{2+})</td>
<td>6.2</td>
<td>36.6</td>
<td>100.0</td>
</tr>
<tr>
<td>Salt-depleted</td>
<td>8.8</td>
<td>19.9</td>
<td>47.7</td>
</tr>
<tr>
<td>Na(^{+})</td>
<td>8.8</td>
<td>24.0</td>
<td>36.4</td>
</tr>
<tr>
<td>Na(^{+}) + Mg(^{2+})</td>
<td>8.8</td>
<td>47.7</td>
<td>63.6</td>
</tr>
</tbody>
</table>
TABLE IV
ENERGY DISTRIBUTION AND REDISTRIBUTION PARAMETERS IN PHOTOSYSTEM II BASED ON 77 K FLUORESCENCE TRANSIENTS AND LIFETIMES FOR TWO pH VALUES

The fraction of total absorbed quanta initially partitioned to PS II (β) and the efficiencies for de-excitation of excited chlorophyll in the antenna complex of PS II, namely, thermal dissipation (ψ_D2), fluorescence (ψ_F2), and energy transfer to PS I (ψ_T(21)) and to reaction center II (ψ_T2), were calculated for individual samples, using fluorescence lifetime and transients measured at 77 K and equations described in Refs. 19 and 20. [Cations] ~ 10 mM.

<table>
<thead>
<tr>
<th>Sample</th>
<th>pH</th>
<th>β</th>
<th>ψ_F2</th>
<th>ψ_T(21)</th>
<th>ψ_D2 + ψ_T2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt-depleted</td>
<td>6.2</td>
<td>0.54</td>
<td>0.01</td>
<td>0.14</td>
<td>0.85</td>
</tr>
<tr>
<td>Na^+</td>
<td>6.2</td>
<td>0.52</td>
<td>0.02</td>
<td>0.24</td>
<td>0.74</td>
</tr>
<tr>
<td>Na^+ + Mg^{2+}</td>
<td>6.2</td>
<td>0.57</td>
<td>0.02</td>
<td>0.09</td>
<td>0.89</td>
</tr>
<tr>
<td>Salt-depleted</td>
<td>8.8</td>
<td>0.46</td>
<td>0.01</td>
<td>0.13</td>
<td>0.86</td>
</tr>
<tr>
<td>Na^+</td>
<td>8.8</td>
<td>0.51</td>
<td>0.02</td>
<td>0.23</td>
<td>0.75</td>
</tr>
<tr>
<td>Na^+ + Mg^{2+}</td>
<td>8.8</td>
<td>0.58</td>
<td>0.03</td>
<td>0.18</td>
<td>0.79</td>
</tr>
</tbody>
</table>

* β was calculated as 1 - α, where α is the fraction of total absorbed quanta initially partitioned to PS I. α was calculated according to the equation:

\[
\alpha = \frac{F_{730,\alpha}}{F_{730,\alpha} + F_{690,m} \cdot \tau(F_{730,m})},
\]

where \( F_{730,\alpha} \) = \( F_{730,\alpha} - (F_{690,\alpha}/F_{690,v}) \cdot F_{730,v} \); \( F_{730,\alpha} \) = 'constant' fluorescence at 730 nm; \( F_{690,o}, F_{690,v}, \) and \( F_{690,m} = \) 'constant', 'variable', and maximum fluorescence at 690 nm; \( \tau(F_{730,m}) \) and \( \tau(F_{690,m}) \) = lifetimes of \( F_m \) at 730 and 690 nm. \( \tau(F_{690,m}) \) was taken as \( \tau(F_{686,m}) + \tau(F_{695})/2 \).

by the subsequent addition of 10 mM Mg^{2+}; at pH 8.8, both Na^+ and Mg^{2+} induce approximately the same (~10%) increase in β. (2) The Mg^{2+}-induced decrease in the efficiency of energy redistribution from PS II to PS I, ψ_T(21), is ~60% at pH 6.2 and ~22% at pH 8.8. The sum of efficiencies of nonradiative processes other than energy transfer from PS II to PS I, ψ_D2 + ψ_T2, shows a slight decrease with Na^+ addition at both pH values; subsequent addition of Mg^{2+} causes some increase at pH 6.2, but no significant change at pH 8.8.

**Mg^{2+} effects on electron transport**

**Electron transport in light-limiting conditions**

The effects of cations at low light intensities, presented below, are related to the excitation energy distribution and redistribution discussed above; these results confirm the concept that cations indeed regulate excitation distribution between the two photosystems, and extend the validity of this concept to a wider range of pH values.

**PS II partial reaction: H_2O → DCIP.** It was necessary to confirm for our samples the reported cation effects before proceeding with new measurements on pH effects. The use of low actinic light intensities (linear portion of the light curves), high concentration of DCIP, and slow steady-state measurements, assured us that this was a PS II reaction (cf. Ref. 21). The Mg^{2+} enhancement of the rate of the H_2O → DCIP Hill reaction confirmed, in our preparations, most previous reports of the effects of this cation. However, additional results in Table V extend the validity of this effect to pH 5.4 and 8.2. In particular, we
TABLE V

Mg\(^{2+}\) EFFECT ON THE H\(_2\)O → DCIP ELECTRON TRANSPORT RATE UNDER LIGHT-LIMITING CONDITIONS FOR THREE pH VALUES

[Chl] = 10 μg/ml; [DCIP] = 30 μM; [NH\(_4\)Cl] = 9.8 mM; [Mg\(^{2+}\)] = 9.8 mM; full actinic intensity at 635 nm = 10 mW/cm\(^2\). DCIP reduction was measured as a bleaching at 597 nm, using a 3 ml suspension of thylakoids in a 1-cm path-length cuvette. Other details as given in the text.

<table>
<thead>
<tr>
<th>pH</th>
<th>Actinic intensity (%)</th>
<th>Electron transport rates (μeqiv./mg Chl/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>− Mg(^{2+})</td>
</tr>
<tr>
<td>5.4</td>
<td>75</td>
<td>5.1</td>
</tr>
<tr>
<td>7.3</td>
<td>12</td>
<td>5.4</td>
</tr>
<tr>
<td>8.2</td>
<td>12</td>
<td>37.1</td>
</tr>
</tbody>
</table>

note the 2-fold enhancement at pH 8.2 (at 12% intensity) of PS II reaction upon the addition of Mg\(^{2+}\); this suggests that in the absence of Mg\(^{2+}\), there must be a massive spill over of energy from PS II to PS I.

PSI partial reaction: DCIPH\(_2\) → methylviologen. The intent of these experiments was to test whether or not Mg\(^{2+}\) causes an equivalent reduction in the electron transport rate in PS I at pH above 7.5 [4], when NADP\(^+\) is replaced by methylviologen as electron acceptor. The results in Fig. 6 show that, at both pH 7.0 and 8.2, Mg\(^{2+}\) causes only a decrease in the PS I electron transport rate, but the % decrease, at low light intensities, is much smaller than the % increase in PS II reaction. More experiments, under identical conditions for PS II and PS I reactions, are required to obtain quantitative information. Hoch (Hoch, G., personal communication) has pointed out that our experiments should be repeated in the absence of ammonium chloride before firm conclusions are made. In addition, he suggests that in the absence of Mg\(^{2+}\), the energy ‘spilled over’ from PS II to PS I may be delivered to those PS I units that are not engaged in the non-cyclic electron flow!

Electron transport in light-saturating conditions

We emphasize here that the effects of cations on PS I and PS II reactions in saturating light, reported below, are not related to the excitation energy distribution and redistribution phenomenon, and are most probably due to effects on some dark reaction(s), including effects on the affinity of electron carriers to the membrane.

PS II partial reactions. H\(_2\)O → Fe(CN\(_6\))\(^{3-}\). At pH <7.8, Mg\(^{2+}\) enhances the electron transport rate, but, at pH >7.8, Mg\(^{2+}\) inhibits this rate; the maximum stimulation is ~30% at pH 7.0 (Fig. 7). (2) H\(_2\)O → DCIP. Only a slight Mg\(^{2+}\)-induced decrease is observed in this Hill reaction in the presence of DBMIB under light-saturating conditions (Table VI), the effect declining with increasing pH: ~10% at pH 6.4 and ~5% at pH 8.3.

PS I partial reactions. DAD\(_{red}\) → methylviologen. The saturation rates for electron transport from DAD/ascorbate to methylviologen are unaffected by Mg\(^{2+}\) at acid and neutral values of pH; at pH 8.0, Mg\(^{2+}\) induces a slight increase,
Fig. 6. Rates of Photosystem I partial electron transport under light-limiting conditions. Actinic light was at 636 nm (half-bandwidth, 8 nm) at a maximum intensity of 22 mW/cm². [Chl] ~25 µg/ml; [NH₄Cl] = 9.5 mM; [DCMU] = 4.8 µM; [DCIP] = 60 µM; [sodium ascorbate] = 1.9 mM; [methylviologen, MV] = 95 µM; [MgCl₂] (when added) = 9.5 mM; temperature = 25 ± 1°C. In this system, 1 mol of O₂ uptake is equivalent to 1 electron transferred.

Fig. 7. Rates of Photosystem II partial electron transport (H₂O to ferricyanide) under light saturation conditions. A relative rate of 100 denotes 144 µmol O₂ evolved/mg Chl/h. The results were the average of the absolute rates measured for three different thylakoid preparations; the error bars denote one standard deviation. [Chl] = 25 µg/ml; [NH₄Cl] = 5 µg/ml; [K₃Fe(CN₆)] = 0.75 mM; when added, [MgCl₂] = 9.8 mM. Actinic illumination for all measurements was from a tungsten lamp passed through a water filter and a Corning 3-73 filter; the irradiance at the sample was 175 mW/cm². The temperature was regulated at 25 ± 1°C.

TABLE VI

<table>
<thead>
<tr>
<th>Reaction</th>
<th>pH</th>
<th>Electron transport rates (μequiv./mg Chl/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-Mg²⁺</td>
</tr>
<tr>
<td>PS II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H₂O → DCPIP (+ DBMIB)</td>
<td>6.4</td>
<td>248</td>
</tr>
<tr>
<td></td>
<td>8.3</td>
<td>257</td>
</tr>
<tr>
<td>PS I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DADred → MV *</td>
<td>6.3</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>7.3</td>
<td>202</td>
</tr>
<tr>
<td></td>
<td>8.0</td>
<td>223</td>
</tr>
<tr>
<td>DCPIPH₂ → MV</td>
<td>7.1</td>
<td>316</td>
</tr>
<tr>
<td></td>
<td>8.2</td>
<td>630</td>
</tr>
</tbody>
</table>

* MV in this table stands for methylviologen.
if any, in the electron transport rate at pH 7.1, but gives a 10% decrease at pH 8.2 (Table VI).

Discussion

The maximum cation effects over the pH range 6 to 9 are observed (Fig. 1) at concentrations of \( \sim 10 \) mM, and, unless otherwise stated, it will be understood throughout this section that cations are added to this concentration.

The first point of interest was to determine the pH range over which the earlier conclusions regarding the role of Na\(^{+}\) and Mg\(^{2+}\) in the initial distribution and redistribution of electronic excitation in and between the two photosystems are valid. An earlier conclusion for spinach \([15,17]\) appears to hold in pea thylakoids only for pH >6.1 (also see Ref. 22). The Mg\(^{2+}\)-induced enhancement of fluorescence yield, however, holds from pH of \( \sim 5 \) to 9 (Figs. 3 and 4), with the maximum effect around pH 7.5. The fraction of total absorbed quanta initially distributed (or partitioned) to PS II, \( \beta \), is decreased by Na\(^{+}\) at pH >6.2 and increased by Mg\(^{2+}\) over the entire pH range 6 to 9. Na\(^{+}\)-induces an increase in the efficiency of excitation transfer from PS II to PS I, \( \psi_{T(21)} \), and Mg\(^{2+}\) induces a decrease in the efficiency of this transfer over the pH range 6.2 to 8.8. This conclusion derived from 77 K fluorescence (Table IV) is supported at room temperature by measurements on (a) the degree of polarization of fluorescence at 686 nm (mainly PS II) and at 712 nm (mainly PS I); (b) fluorescence lifetimes (Fig. 4); and (c) rates of electron flow at low light intensities (Table V and Fig. 6). Mg\(^{2+}\) causes a larger relative increase in PS II reaction than the relative decrease in PS I reaction; however, it should be noted that the inadequacy of electron transport data is that they do not provide any information regarding the fractional contributions of initial distribution of absorbed quanta and subsequent redistribution in the overall change.

The second point of interest was to examine the implications of the earlier report \([4]\) that the light-limited rate of electron transport from DCIPH\(_2\) to NADP\(^{+}\) is stimulated or inhibited by Mg\(^{2+}\) depending upon whether the bulk pH is below or above 7.5. EPR measurements \([23]\) of steady-state P-700 oxidation at low light intensities support the Mg\(^{2+}\) inhibition of electron transport through PS I from DCIPH\(_2\) to methylviologen at the pH used. We find here that Mg\(^{2+}\) induces only an \( \sim 35\% \) decrease in the light-limited electron transport rates from DCIPH\(_2\) to methylviologen in diuron treated thylakoids at pH 7.1 and 8.2 (Fig. 6). Thus, the conclusions regarding the stimulation of PS I reaction at pH <7.5 should be considered with great caution.

The third point of concern in this investigation was the pH sensitivity of the cation effects. Since the thylakoid surface is negatively charged \([24]\), protons should be expected to compete with cations if the effects of the latter are mainly electrostatic (cf. Ref. 5). Mohanty et al. \([25]\) showed that lowering the pH to 3.8 in oat thylakoids causes a decreased energy transfer from PS II to PS I just as Mg\(^{2+}\) does. Information accumulated in the present paper suggests the presence of two roles for pH in regulating the cation effects: (1) The increasing effectiveness of Na\(^{+}\) with decreasing pH (Fig. 1A) is taken to indicate the similarity of the effect of the two monovalent cations. The lowering of the half-saturation concentration for the Mg\(^{2+}\) effect with increasing pH (Fig. 1B) is
interpreted to indicate competition between H\(^+\) and Mg\(^{2+}\). (2) The pH dependence of the electrophoretic mobility of thylakoids shows an almost constant response between pH 6 and 10 [24]. Thus, the variety of pH dependences found in this study — for instance, the strong dependence on pH of the divalent cation effects on steady-state fluorescence yield (Figs. 3 and 4) — requires further study and explanation.

Finally, in Itoh’s concept [26], the site of ferricyanide reduction in system II exists inside the membrane with negative surface charges hindering the access of ferricyanide by electrostatic repulsion; cations would screen these membrane charges and, thus, increase the rate of reduction of ferricyanide in saturating light. This picture readily accounts for the Mg\(^{2+}\)-induced stimulation of ferricyanide reduction at pH <7.8 (Fig. 7). It also accounts for the absence of the stimulatory effect on the reduction of the neutral molecule like DCIP. It appears, however, that other factors limiting ferricyanide accessibility — e.g., membrane stacking — must be considered, at pH <7.8.

References

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