ANTAGONISTIC EFFECTS OF MONO- AND DIVALENT CATIONS ON POLARIZATION OF CHLOROPHYLL FLUORESCENCE IN THYLAKOIDS AND CHANGES IN EXCITATION ENERGY TRANSFER

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1. Introduction

Mono- and divalent cations affect several primary photoprocesses in thylakoids: initial energy distribution between the two pigment systems [1–7], excitation energy redistribution or 'spillover' from pigment system (PS) II to PSI [2,4,6–15], rate constant of thermal dissipative transitions [6,7,16,17], and activation of reaction center II [18–20]. Several of these effects may occur concurrently [2,6,7,17] with the largest effect on the excitation energy redistribution process [2,7]. It is generally accepted that excitation energy transfer in the photosynthetic system is by Förster's inductive resonance mechanism [21,22] in which the pair-wise transfer rate is dependent upon the distance ($\propto r^{-6}$, where r is the distance), the orientation factor, $\kappa^2(\kappa^2 \leq 4)$ between the donor and acceptor molecules, and the overlap of the donor fluorescence with the acceptor absorption spectrum [23,24]. Since the degree of polarization of fluorescence is an indicator of the extent of excitation energy migration and/or of the orientation of the molecules [22,25], we have measured, at room temperature, the effects of cations on chlorophyll *a* (Chl *a*) fluorescence polarization:

(i) At wavelengths selected to monitor preferentially PSII or PSI emission;
(ii) At 760 nm (to avoid artifacts due to scattering of excitation light) as a function of different wavelengths of excitation.

The results of this study are consistent with the following:

(1) There is an antagonistic effect of low concentrations (3–5 mM) of mono- and divalent cations on the excitation energy migration, Na^+ causing less energy migration among PSII units and more transfer to PSI – both effects being reversed by Mg^{2+};
(2) Divalent cations increase excitation energy transfer from chlorophyll *b* (contained in the light harvesting chlorophyll *a/b* protein complex, LHCP) to chlorophyll *a* in PSII. Conclusion (1) supports the hypothesis [8] of cation regulation by excitation energy redistribution from PSII to PSI, and conclusion (2) may be taken to be in agreement with the suggestion in [26] that divalent cations increase the coupling between LHCP and chlorophyll *a* of PSII (Chl a_{II}).

2. Materials and methods

Broken chloroplasts were isolated from leaves of week 2–3 pea seedlings by a modified method of that in [27], using a homogenizing medium of 350 mM sucrose and 50 mM Tris–HCl (pH 7.6). These chloroplast fragments (thylakoids) were osmotically shocked and washed 3 times with 100 mM unbuffered sucrose solution and stored in the same medium at 77 K at 1–1.5 mg chlorophyll/ml. Other

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details were as in [6]. Thylakoid suspensions for fluorescence polarization measurements were prepared by diluting the stock suspension with 100 mM sucrose containing ≤ 2 mM Tris–HCl, pH 7.6 or 7.9. Each of the 3 final samples (salt-depleted; Na^+; $Na^+ + Mg^{2+}$) contained 5 μg chlorophyll/ml and 3–5 μM (3,4-dichlorophenyl)-1,1-dimethylurea (DCMU), and the Na^+ and $Na^+ + Mg^{2+}$ samples contained, in addition, 3–5 mM NaCl and 3–5 mM NaCl + 3–5 mM $MgCl_2$, respectively. Ethanol (< 0.5% final vol.) added with DCMU was shown to have no effect on the parameters measured. Each sample was final pH 7.2–7.5.

Fluorescence was measured at right angles to the direction of incidence of the vertically polarized excitation light. The degree of polarization of fluorescence was calculated as $P = (F_{vv} - G \cdot F_{vh})/(F_{vv} + G \cdot F_{vh})$, where F_{vv} and F_{vh} are the polarized components of fluorescence intensities measured vertically (v, the second subscript) and horizontally (h) when the sample was excited with vertically (v, first subscript) polarized excitation, and $G = (F_{hv}/F_{hh})$ is the instrumental correction factor (obtained with 10^{-7} M Rhodamine 6G in glycerol) which equalizes the measurement sensitivities to vertically- and horizontally-polarized light for horizontally-polarized excitation. The excitation light was provided by a 200 W quartz–iodine lamp (GE Q 6.6 AT4/CL) through a Bausch and Lomb monochromator (model 33-48-45 – 0.5 m, 600 grooves/mm with a linear dispersion of 3.3 nm/mm slit) and Glan-Thompson polarizers.

The rationale for the choice of the measuring wavelengths at 685 nm (F685), 712 nm (F712), 730 nm (F730) and 760 nm (F760) was as follows: The emission spectrum of chlorophyll *a* fluorescence in thylakoids at room temperature shows a major band at ~685 nm with a minor band at ~740 nm (vibrational satellite of the 685 nm band). Although most of this fluorescence originates from PSII (see [28]), the fraction of PSII to PSI fluorescence attains a minimum in the 710–720 nm region [29–32]. From his studies on separated pigment systems, it was shown [32] that fluorescence from isolated PSI particles, at room temperature, has a broad peak in the 710–720 nm region. Thus, although F685, F730 and F760 represent mainly PSII, F712 would have a higher proportion of PSI fluorescence than at other wavelengths.

Fluorescence was detected through interference filters: maximum transmission at 686 nm (half-maximum bandwidth, HB, 6.8 nm), at 712 nm (HB, 5.2 nm), at 730 (HB, 8.4 nm), or at 762 nm (HB, 11.3 nm), together with sharp cutoff glass filters, Schott RG 665 or RG 10. The excitation spectrum of fluorescence polarization was measured for emission at 762 nm to avoid the entry of scattered exciting light (particularly in the 690–700 nm region) into our observations. All measurements were made at 24 ± 1°C.

3. Results

Table 1 shows the effects of mono- and divalent cations on the degree of polarization, *P*, of Chl *a* fluorescence at room temperature (excitation at 600 ± 8.3 nm) as a function of the emission wavelengths at 686 nm (F686; PSII), at 712 (F712; PSI) and at 730 (F730; PSII). The degree of polarization of F686, *P*(F686), changed from 2.2 ± 0.1% to 3.1 ± 0.3% to 2.0 ± 0.2% from salt-depleted to 5 mM NaCl to 5 mM NaCl + 5 mM $MgCl_2$ condition. This shows that the addition of 5 mM Na^+ increased *P* (F686) by 26–56% and the subsequent addition of 5 mM Mg^{2+} reversed this increase. Similar effects were

Table 1
Effects of Na^+ and Mg^{2+} on the degree of polarization of chlorophyll *a* fluorescence in the presence of 5 μM DCMU

Treatment	Degree of polarization (%)		
	P (F686)	*P* (F712)	*P* (F730)
Salt-depleted	2.2 ± 0.1	4.3 ± 0.3	2.9 ± 0.1
+ 5 mM NaCl	3.1 ± 0.3	2.3 ± 0.3	4.2 ± 0.2
+ 5 mM NaCl + 5 mM $MgCl_2$	2.0 ± 0.2	4.2 ± 0.3	3.8 ± 0.2

Fluorescence was excited at 600 nm (band pass, 16.5 nm) with vertically polarized light and detected through interference filters, at 686 nm (half-maximum bandwidth, HB, 6.8 nm), at 712 nm (HB, 5.2 nm) and at 730 nm (HB, 8.4 nm), with an EMI 9558 B (S-20 response) photomultiplier. Chloroplast samples were suspended in 100 mM sucrose containing 0.4 mM Tris–HCl at pH 7.6 concentration ~5 μg chlorophyll/ml. The results are the average of three experiments; the effects were further confirmed in two other measurements. The errors denote ± 1 SD

observed for P(F730). For F712, however, the addition of 5 mM Na^+ to salt-depleted thylakoids changed P from 4.3 ± 0.3% to 2.3 ± 0.3% (a decrease of 39–51%), which was then reversed by the addition of 5 mM Mg^{2+}. The above results suggest that Na^+ induces an increase in P from PSII and a decrease in that from PSI. Further addition of Mg^{2+} causes an antagonistic effect, i.e., a complete reversal of these effects.

In another set of experiments, the degree of polarization of F762, P(F762), was found to decrease with Mg^{2+} addition to the Na^+ samples at all excitation wavelengths (λ_{ex}) in the range $600 \leq \lambda_{ex} < 700$ nm. (The observation that the cation effects on P (F762) are similar to P(F686) and P(F730) suggests that F762 also reflects the fluorescence changes of Chl a_{II}.)

The excitation spectrum between 635 nm and 700 nm for the change in P(F762) of the $Na^+ + Mg^{2+}$ sample compared to the Na^+ sample (fig.1) shows negative bands with peaks at ~650 nm, ~675 nm, and

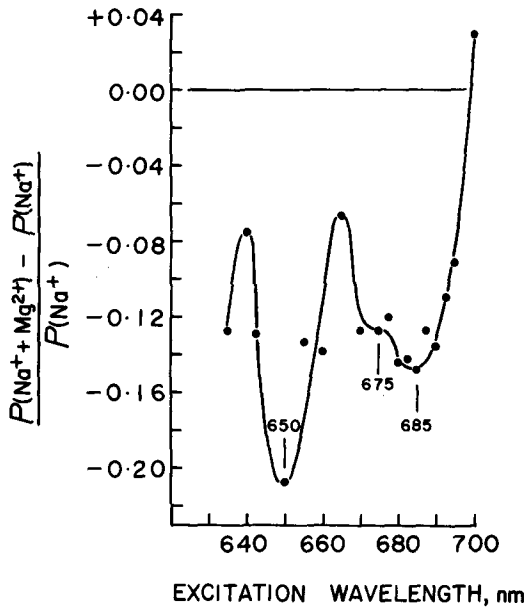

Fig.1. Excitation spectrum of the relative difference in the degree of polarization of F762 between the $Na^+ + Mg^{2+}$ sample and the Na^+ sample expressed as $[P(Na^+ + Mg^{2+}) - P(Na^+)]/P(Na^+)$. The excitation band pass was 5 nm. The fluorescence was detected through a combination of a Schott RG 10 glass filter and an interference filter at 762 nm (half-maximum bandwidth, 11.3 nm). Sample details are as given in the legend of table 1.

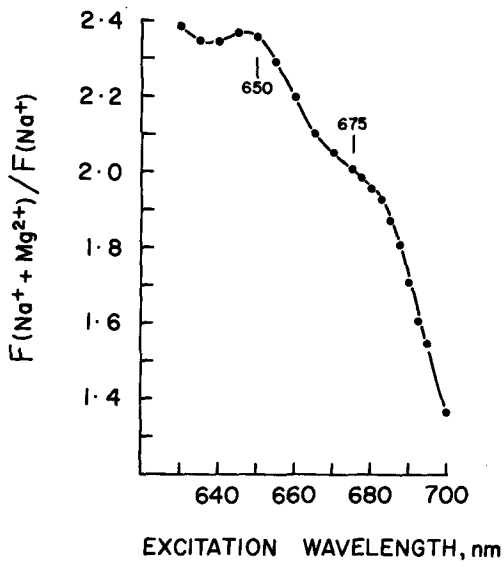

Fig.2. Excitation spectrum of the change in relative fluorescence yield of the $Na^+ + Mg^{2+}$ sample compared to the Na^+ sample expressed as $F(Na^+ + Mg^{2+})/F(Na^+)$. Other details are identical to those given in the legend of fig.1.

685 nm. The excitation spectrum for the Mg^{2+}-induced change in F762 (fig.2) shows a general enhancement of fluorescence yield by Mg^{2+} addition, with a peak at ~650 nm and a shoulder at ~675 nm. Our results on the Mg^{2+} effects in fig.1,2 are best explained in terms of:

(i) An increase in energy transfer from Chl b to Chl a_{II},

(ii) A decrease in transfer from PSII to PSI (see section 4).

Observations were also made on F730 with and without 43% (v/v) glycerol to reduce sample turbidity. Results very similar to those in fig.1,2 were obtained, although the Mg^{2+}-induced effects were diminished by 20–50%. The declining Mg^{2+} effect beyond 690 nm on P(F762) without glycerol and P(F730) with and without glycerol were indeed identical to those in fig.1 and are taken as confirmation that the observed changes are not the result of differences in sample scattering.

4. Discussion

This paper provides important experimental data

for the conclusion that cations cause changes in the excitation energy transfer in the photosynthetic system at physiological temperatures. This is shown by an increase and a decrease in the polarization of PSII and PSI fluorescence, respectively, by monovalent cations, and the reversal of these effects by further addition of divalent cations (table 1). Moreover, the increases in depolarization by divalent cations have maxima at ~650 nm (Chl *b*), ~675 nm and 685 nm for fluorescence measured at 760 nm (fig.1), as well as at 730 nm. At the same time, the fluorescence enhancement shows peaks at ~650 nm and ~675 nm. These data strongly suggest an increase in net energy transfer from LHCP (Chl *b* and Chl *a* 670) to Chl a_{II}. Changes in polarization of fluorescence excited at shorter wavelengths (in Chl *a* 670, Chl *b* 650,) have been suggested to be due to changes in excitation energy transfer between chlorophyll species with low mutual order [25,33]. Thus, the negative peaks at ~650 nm (due to Chl *b*) and at ~675 nm (due to Chl *a* 670) in the Mg^{2+}-induced changes in polarization of Chl a_{II} fluorescence (fig.1) and positive peaks at ~650 nm and ~675 nm in the Mg^{2+}-induced increase in relative fluorescence yield (fig.2) are interpreted to be due to an enhancement of energy transfer from these complexes (present in LHCP) to fluorescent Chl a_{II}. It is proposed that this is the process which increases the initial distribution of quanta from LHCP to PSII. The observation that the relative enhancement of fluorescence by Chl *b* is small (fig.2, ~6% for F730, not shown) is consistent with previous findings [2,4,6,7] that the Mg^{2+}-induced variations in the sensitization of PSII fluorescence is $\lesssim$ 20%. These changes are, perhaps, manifestations of the proposed Mg^{2+}-induced increase in the structure and energy coupling of LHCP with Chl a_{II} [26,34,35].

The presence of another band at 685 nm in fig.1 suggests that there is an additional effect on Chl *a* 685; this effect may be due to a change in the orientation of key chlorophyll *a* molecules that control PSII to PSI energy transfer, a concept suggested [36] (see also [37,38]). This explanation is also consistent with the concept presented in [33] that chlorophyll *a* molecules absorbing at $\gtrsim$ 680 nm have a high mutual order, so that increased energy transfer between these molecules have little consequence on the fluorescence polarization. Thus, changes in polarization of fluorescence with excitation at 685 nm may safely be taken as a reduction of mutual orientation of Chl *a* 685. Finally, since linear dichroism and fluorescence polarization spectral studies (cf. [39]) suggest that the shorter wavelength forms of chlorophyll *a* (absorbing $\lesssim$ 670 nm) are less aligned with respect to the membrane plane, i.e., sustaining a greater angle with the plane, we suggest that a reorientation of Chl *a* 685 closer to the membrane plane decreases the orientation factor [23] between Chl *a* 670 (in PSII) and Chl *a* 685 (in PSI) decreasing the rate and, hence, the amount of energy transfer from PSII to PSI.

Since the discovery [8,40] that divalent cations increase the relative Chl *a* fluorescence yield even in the presence of the electron transfer inhibitor DCMU, various investigators (reviewed in [14,15]) have attempted to find the molecular mechanism by which cations affect the photoprocesses in thylakoids. LHCP has been shown [41] to be necessary for the cation-induced regulation of the excitation energy transfer. Our results show that divalent cations affect Chl *b* and Chl *a* 675 (components in LHCP) in such a way that there is increased energy transfer from these chromophores to Chl a_{II} (increased energy coupling). In addition, it is seen that the mutual orientation of Chl *a* 685 is decreased by Mg^{2+}, supporting the hypothesis [36] of how cations may regulate excitation transfer from PSII to PSI. Decreased energy transfer from PSII to PSI seems to be accompanied by an increase in inter-unit transfer among PSII units (cf. [4]).

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