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EFFECTS OF SODIUM AND MAGNESIUM CATIONS ON THE "DARK-" AND LIGHT-INDUCED CHLOROPHYLL *a* FLUORESCENCE YIELDS IN SUCROSE-WASHED SPINACH CHLOROPLASTS

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SUMMARY

The effects of Na^+ and Mg^{2+} on the "dark" level (O level) and light-induced (P level) fluorescence in sucrose-washed spinach chloroplasts were studied. Low concentrations of NaCl (2–10 mM) cause a significant decrease in both the O and P levels in the chlorophyll fluorescence transient. The effect on the O level may reflect changes in the bulk chlorophyll *a*. At 77 °K NaCl increases the *F*735/*F*685 emission peak ratio in dark-adapted and preilluminated chloroplasts, but has no significant effect on this ratio in sucrose-washed Photosystem II particles. This evidence is consistent with a sodium-induced excitation-energy distribution in favor of Photosystem I.

In the presence of $MgCl_2$, with or without NaCl, there is a slight decrease in the O and P level fluorescence as compared with the salt-free control, but an increase as compared with the NaCl-treated sample. Magnesium appears to override the sodium-induced changes. At low temperatures in chloroplasts and Photosystem II particles, $MgCl_2$ has different effects on the *F*735/*F*685 ratio apparently depending on the state of the membrane. Magnesium, however, always induces an increase in the *F*695/*F*685 ratio. These results suggest that magnesium may influence Photosystem II reaction centers as well as energy distribution between the two photosystems.

INTRODUCTION

To account for the relatively constant quantum yield for photosynthesis over the red region (600–680 nm) of the spectrum, a spillover mechanism was proposed whereby excess energy absorbed in Photosystem II could be transferred to Photosystem I (Myers [1]; see also Malkin [2]). Murata [3–5] obtained evidence to suggest that this spillover of excitation energy was controlled by divalent cations, particularly magnesium.

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Recently, Gross and Hess [6] found that upon the addition of low concentrations of sodium salts ($\approx$ 2 mM) to sucrose-washed spinach chloroplasts in a medium of low ionic strength, chlorophyll *a* fluorescence at room temperature decreased. When divalent cations were then added to the medium, fluorescence increased back to the control level. The addition of divalent cations prior to the addition of the sodium had little apparent effect on the fluorescence but it did prevent the sodium-induced decrease. At low temperature (77 °K), sodium decreased the *F*685 (fluorescence intensity at 685 nm) and *F*695 relative to the *F*735; upon further addition of divalent cations this was reversed. Gross and Hess concluded that mono- and divalent cations act as antagonists in the control of energy spillover between the two photosystems. Hence, according to these authors, in the chloroplast preparations of Murata [3–5] and other workers [7–10] where a magnesium-induced increase in the fluorescence was observed, sufficient sodium must have been present initially to promote maximum spillover so that upon the addition of magnesium, spillover was inhibited. At very high concentrations of sodium ($>$ 40 mM), the fluorescence increased back to the control level in accord with the results of Murata [11], suggesting that sodium at high concentrations behaves in a similar way as divalent cations.

In the work of Gross and Hess [6] only the total steady-state fluorescence was measured. It has been shown, however, that light induces membrane conformations different from those in the dark [12], which influence energy-transfer mechanisms [4]. Thus, it was considered necessary to look at the salt effects both in dark-adapted and preilluminated chloroplasts in order to gain a better insight into the function of monovalent and divalent cations in photosynthesis. In this paper we report the effects of sodium and magnesium on the "dark" level (O level) and light-induced (P level) fluorescence at room and liquid nitrogen temperatures. Sodium is shown to cause a significant decrease in both the O and P level fluorescence. Magnesium, on the other hand, appears to have a much more complex effect.

MATERIALS AND METHODS

Chloroplasts were isolated from market spinach and suspended in a medium of low ionic strength according to the method of Gross [13]. System II particles were prepared according to the procedure of Briantais [14]. After isolation the particles were washed twice and suspended in 350 mM sucrose. Reaction mixtures consisted, in addition to the chloroplasts or System II particles, of 0.2 mM Tris buffer (pH 7.8–8.2) and 100 mM sucrose. Chlorophyll concentration, as determined by the method of Arnon [15], was 5 or 10 μg/ml in all samples. Appropriate amounts of magnesium and sodium were added to samples from 100 mM stock solutions of the corresponding chloride salts.

Fluorescence was measured by a spectrofluorometer described by Shimony et al. [16]. The procedure used for measuring the fast chlorophyll *a* fluorescence transient is essentially the same as that given by Munday and Govindjee [17], while the procedure for the measurement of emission spectra is that given by Cho et al. [18]. The spectra were corrected for the spectral variation of the photomultiplier (EMI 9558B). Other details are given in the legends of the figures.

RESULTS

1. *Effects of sodium and magnesium on the chlorophyll a fluorescence transient*

We first set out to determine the effects of salts on fluorescence induction. The fast chlorophyll *a* fluorescence transient is characterized by an initial or O level at the onset of illumination, which represents the fluorescence before photochemistry and light-induced conformational changes have taken place, and by the steady-state or P level, which reflects the fluorescence after these events are complete. (For definition of the characteristic points see the review by Govindjee and Papageorgiou [19].)

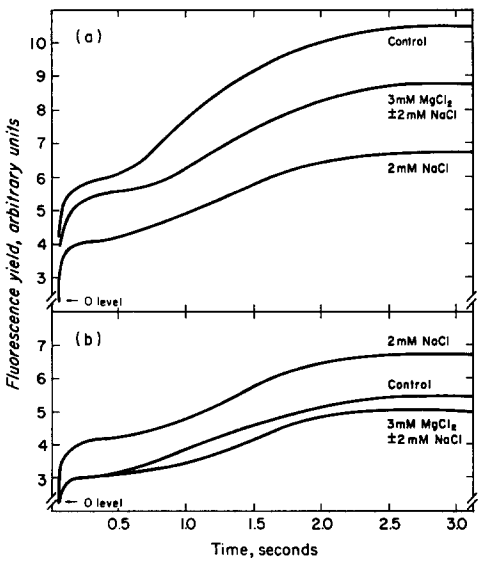

Fig. 1. (a) Time course of chlorophyll *a* fluorescence in sucrose-washed spinach chloroplasts in the presence and absence of sodium and magnesium. Fluorescence was measured at 685 nm (half-band width, 6.6 nm); excitation, broad-band blue light (CS 4-96 and CS 3-73); intensity, $1.2 \cdot 10^4$ ergs $\cdot$ $cm^{-2} \cdot s^{-1}$. The 4 ml reaction mixture consisted of 5 μg/ml chlorophyll, 0.2 mM Tris buffer, pH 7.8–8.2, and 100 ml sucrose. Appropriate amounts of 100 mM stock salt solutions were added to give final concentrations of 2 mM NaCl and 3 mM $MgCl_2$. Samples were kept dark until the time of measurement. (b) Fluorescence transients from (a) normalized at the 0 level.

Fig. 1a shows the fluorescence transient in the presence and the absence of sodium and magnesium. With 2 mM NaCl the P level is decreased by 37 % as compared with the control (Gross and Hess [6] found a 38 % decrease in the total fluorescence with 1.2 mM NaCl). In the presence of 3 mM $MgCl_2$, with or without 2 mM NaCl, there is also a decrease at the P level, although not as large as with NaCl alone. It is also apparent that the O level is affected by the salts as well: 2 mM NaCl causes a significant decrease at the O level, while 3 mM $MgCl_2$, with or without 2 mM NaCl, causes only a slight decrease.

The O level measurements in Fig. 1a may have been somewhat limited by the response time of the recorder. (An Esterline-Angus Model E-1101 S recorder was used.) To obtain more accurate O levels, additional measurements were made: (1) a

TABLE I

Comparison of 0 levels in sucrose-washed chloroplasts in the presence and absence of sodium and magnesium using three different methods of measurement.

Treatment	0 Level, Percent of control[1]		
	Measured from transients using Esterline-Angus, Model E-1105 Graphic recorder[2]	Measured from transients using Midwestern, Model 801 B, Oscillographic recorder[3]	Measured at low light intensities[4]
Control	100	100	100
2 mM NaCl	62	67	73
3 mM $MgCl_2$	88	86	85
2 mM NaCl+ 3 mM $MgCl_2$	84	88	88

[1] Conditions as in Figure 1

[2] 0 level measured after approximately 30 ms of illumination

[3] 0 level measured after approximately 4 ms of illumination

[4] Fluorescence measured at light intensity where there was no apparent transient. Conditions as in Fig. 1 except as follows: chlorophyll concentration, 10 μg/ml; excitation intensity, 32 ergs · $cm^{-2} \cdot s^{-1}$, half-band width of measuring monochromator, 9.9 nm.

Midwestern Model 801 B oscillograph recorder was employed; and (2) fluorescence was measured at very low light intensities where the variable fluorescence is non-existent and is comparable to the O level. Table I compares the results obtained by these three methods. Clearly, NaCl causes a significant decrease at the O level, while $MgCl_2$ induces only a small change. (The values quoted in Table I are the average of five different samples; in some cases, however, $MgCl_2$ had no apparent effect on the O level compared with the control.)

Fig. 1b shows the fluorescence transient curves from Fig. 1a normalized at the O level. If the differences throughout the fluorescence transients induced by the salts arise only from changes in the O level fluorescence, then we would expect the fluorescence transients of the salt-treated samples to fall on top of the control after normalization. However, as shown here, this is not the case. At the P level the sodium curve is higher, while the magnesium curve is lower than the control. (In some cases the $MgCl_2$-treated samples showed transient curves which were higher than the control at the P level, after normalization, depending on the effect at the O level.) These results imply that the salts have different effects on the O and P levels.

This last point is further demonstrated by the concentration curves shown in Figs 2 and 3. The change induced by NaCl in the fluorescence yield at the P level (see Fig. 2) follows the same pattern over the same range of concentrations as first reported by Gross and Hess [6] for the total fluorescence. However, the O level follows a similar (but not exactly the same) pattern. The greatest effect produced by NaCl on the O and P levels occurs at concentrations from 2–10 mM (Fig. 2a). At higher concentrations NaCl increases the fluorescence (Fig. 2b) as expected (see Murata [11]).

In the case of $MgCl_2$ (Fig. 3) there is a general decrease in the O and P levels, although of smaller magnitude than with NaCl. In the 1–10 mM concentration range (Fig. 3a), the effect saturates at about 1 mM showing half-saturation at about 0.2 mM.

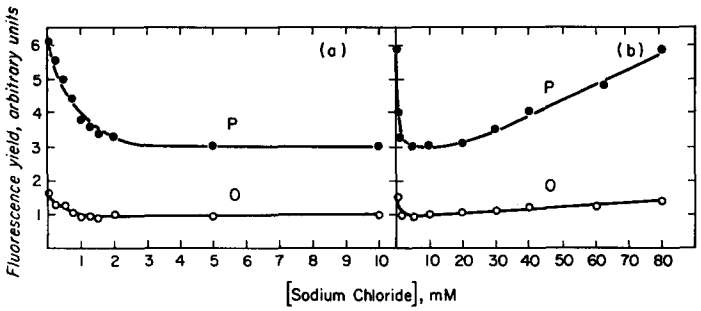

Fig. 2. Concentration curves showing the changes in the O and P level fluorescence as a function of NaCl concentration. O level was measured after 30 ms of illumination and P level after 3.5 s of illumination. Experimental conditions as in Fig. 1.

Gross and Hess [6] reported that the magnesium reversal of the sodium-induced decrease has a half-saturation value of 0.16 mM. However, the half-saturation value in the higher concentration range (Fig. 3b) is about 5 mM. Since the fluorescence versus magnesium chloride concentration curve is clearly biphasic (Fig. 3b), there must be at least two separate effects. High concentrations of $MgCl_2$ do not increase the fluorescence yield either at the O or P levels in contrast to NaCl (see Fig. 2b). The different effects of sodium and magnesium can also be seen in Fig. 4 which is a plot of the ratio of variable to total fluorescence.

Fig. 5 shows a concentration curve for NaCl up to 5 mM in the presence (dashed curves) and absence (solid lines) of 3 mM $MgCl_2$. The large sodium-induced decrease at both the O and P levels are almost eliminated by the presence of magnesium. However, the small magnesium-induced decrease occurs whether sodium is present or not as compared with the control having no salts.

The above results (Figs 1–5) show that (1) in this spinach chloroplast preparation magnesium and sodium affect the fluorescence yield at both the O and P levels, the sodium causing the most significant decrease and that (2) magnesium appears to counteract the sodium-induced decrease at both the O and P levels only to the extent of its own effect.

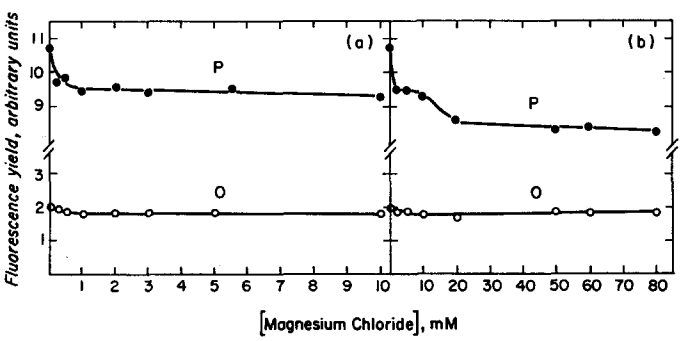

Fig. 3. Concentration curves showing the changes in the O and P level fluorescence as a function of $MgCl_2$ concentration. Other details as in Fig. 2.

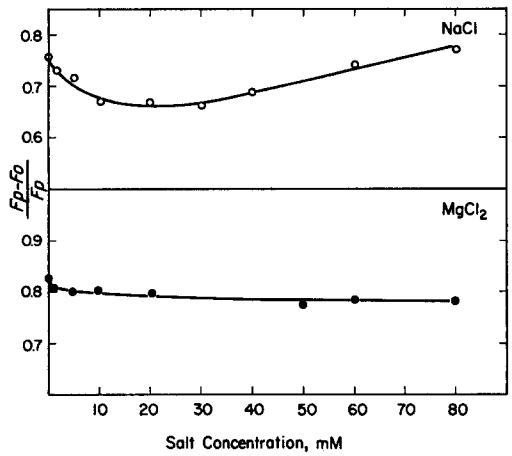

Fig. 4. Plot of ratio of variable ($F_p - F_0$) to total (F_p) fluorescence versus NaCl or $MgCl_2$ concentration. O and P level measurements from Figs 2 and 3 for NaCl and $MgCl_2$, respectively.

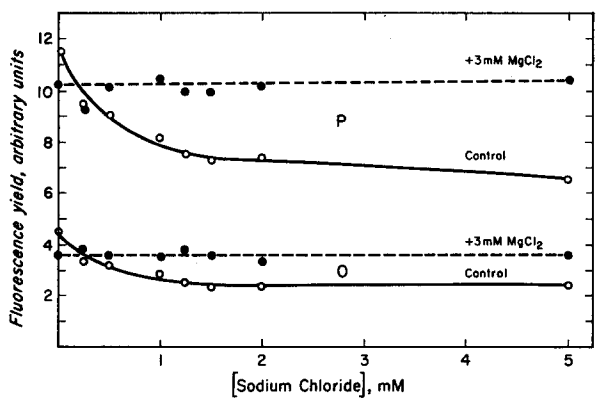

Fig. 5. Concentration curves showing the changes in the O and P level fluorescence as a function of NaCl concentration in the presence (●- - -●) and absence (○—○) of 3 mM $MgCl_2$. O level was measured after 30 ms illumination and P level after 3.5 s illumination. Experimental conditions as in Fig. 1.

2. Effects of sodium and magnesium on the emission spectrum at 77 °K

At liquid nitrogen temperatures the fluorescence emission spectrum of chloroplasts shows three peaks: *F*685, *F*695, and *F*735. It is now generally accepted that *F*685 and *F*695 arise primarily from Photosystem II while *F*735 arises primarily from Photosystem I [20–22]. The relative heights of these peaks have been used as an indicator of excitation energy distribution between the two photosystems [3]. When more quanta are distributed to Photosystem I the *F*735 emission is relatively higher and the *F*685 emission relatively lower than when more quanta are preferentially distributed to Photosystem II.

The *F*735/*F*685 and the *F*695/685 emission peak ratios at 77 °K, normalized to a control value of 1.00, are shown in Table II. Control and salt-treated samples were either dark-adapted or were given one minute preillumination in strong white light.

TABLE II

EMISSION PEAK RATIOS

The ratios were normalized to a control value of 1.00 for sucrose-washed spinach chloroplasts at 77 °K in the presence and absence of sodium and magnesium.

	*F*735/*F*685		*F*695/*F*685	
	Dark*	Light**	Dark	Light
Control	1.00	1.00	1.00	1.00
2 mM NaCl	1.22	1.25	1.04	1.02
3 mM $MgCl_2$	0.81	1.17	1.15	1.11
2 mM NaCl+ 3 mM $MgCl_2$	0.90	1.14	1.11	1.05

* Samples were kept dark-adapted at all times. Chloroplasts were preincubated with the salts for 3–5 min at room temperature before freezing. Exciting light, 435 nm, 6.6 nm half-band width plus CS 4-96 filter; intensity, 30 ergs · cm^{-2} · s^{-1}. Measuring wavelengths, variable, 6.6 nm half-band width, CS 2-61 filter before the analyzing monochromator. Chlorophyll concentration, 10 μg/ml. Each value represents an average of 6–11 different samples.

** Same condition as above except chloroplasts were preilluminated for 1 min in strong white light (200 W incandescent bulb) prior to freezing in the light.

The preilluminated samples exist in a state comparable to that from which the P level fluorescence is emitted; that is, Q (the primary electron acceptor of Photosystem II) is reduced and light-induced conformational changes have taken place. These changes are maintained upon freezing to 77 °K. The dark-adapted samples, on the other hand, exist in a state comparable to that from which the O level fluorescence is emitted and is kept that way since the intensity of the exciting light in the measurement of the emission spectra was kept low enough not to induce any variable fluorescence and reduce Q.

In samples frozen in the light the presence of 2 mM NaCl increases the *F*735/*F*685 ratio over that of the control. This means that the relative fluorescence from Photosystem I is proportionately greater than from Photosystem II. Similarly, with 3 mM $MgCl_2$ there is also an increase in this ratio over the control, but not as much as with NaCl. In the presence of both 2 mM NaCl and 3 mM $MgCl_2$ the ratio is approximately the same as with $MgCl_2$ alone. These results are consistent with the salt effects on the P level fluorescence at room temperature.

In dark-adapted samples we obtained *F*735/*F*685 ratios whose absolute values were, in overall, higher than those in preilluminated ones. For example, in the preilluminated control chloroplasts the average *F*735/*F*685 ratio was 1.74 while in the dark-adapted control chloroplasts the average *F*735/*F*685 ratio was 2.56. This has been observed previously and has been explained in terms of a light-induced conformational change in favor of a more equal distribution of quanta between the two photosystems, resulting in lower ratios in the light [4].

The dark-adapted chloroplasts show a higher (normalized) *F*735/*F*685 ratio (Table II) in the presence of 2 mM NaCl as compared with the control, similar to what is found in the preilluminated chloroplasts. However, in the presence of 3 mM $MgCl_2$ the *F*735/685 ratio is lower than in the control, a result opposite of what would be expected from the results at room temperature. Similarly, in the presence of

2 mM NaCl and 3 mM $MgCl_2$ the *F*735/*F*685 ratio is also lower than in the control. These data suggest that the magnesium effect on the O level state at 77 °K is different than at room temperature.

Table II also gives the *F*695/*F*685 ratios. Sodium apparently has no effect on this ratio either in dark-adapted or preilluminated chloroplasts. However, in all cases where 3 mM $MgCl_2$ is present there is a significant and consistent increase in this ratio. It has been suggested that the *F*695 emission arises from or is an indicator of the energy trap in Photosystem II [21–23]. These results may imply that magnesium is directly affecting these traps.

3. *Effects of sodium and magnesium on Photosystem II particles*

If the salts are affecting energy distribution between the two photosystems, then a priori, both photosystems need to be present in order to observe the salt-induced effects. Table III shows the results on Photosystem II particles prepared from spinach which were sucrose-washed to remove endogenous salts. The presence of 2 mM NaCl has no significant effect on the *F*735/*F*685 and *F*695/*F*685 ratios either in the dark-adapted or in preilluminated particles. This gives strong support to the idea that sodium affects the redistribution of light quanta between the two photosystems only when both photosystems are present. On the other hand, as in chloroplasts, magnesium shows a complicated effect. In the dark-adapted particles (O level state) there is no significant change in either *F*735/*F*685 or the *F*695/*F*685 ratio. But in the preilluminated particles magnesium produces a slight increase in the *F*735/*F*685 and *F*695/685 ratios. If the *F*735 emission is assumed to remain relatively constant in Photosystem II particles, then the increase in the *F*735/*F*685 ratio is due to a decrease in the *F*685 emission, and apparently the increase in the *F*695 emission arises at the expense of the *F*685 emission (see Fig. 6). Again, this may be due to an effect of magnesium directly on Photosystem II traps.

TABLE III

EMISSION PEAK RATIOS

The ratios were normalized to a control value of 1.00 for sucrose-washed photosystem II particles at 77 °K in the presence and absence of sodium and magnesium. Chlorophyll *a*/*b* ratios were 1.6–1.7. All other conditions as in Table II.

	*F*735/*F*685		*F*695/*F*685	
	Dark	Light	Dark	Light
Control	1.00	1.00	1.00	1.00
2 mM Na^+	0.96	1.01	0.96	0.99
3 mM Mg^{2+}	1.05	1.12	1.04	1.12
2 mM Na^+ + 3 mM Mg^{2+}	0.99	1.04	1.02	1.08

DISCUSSION

One of the main points we wish to emphasize here is that the cation effects on the O level fluorescence are significant. From the results in Table I, sodium causes a

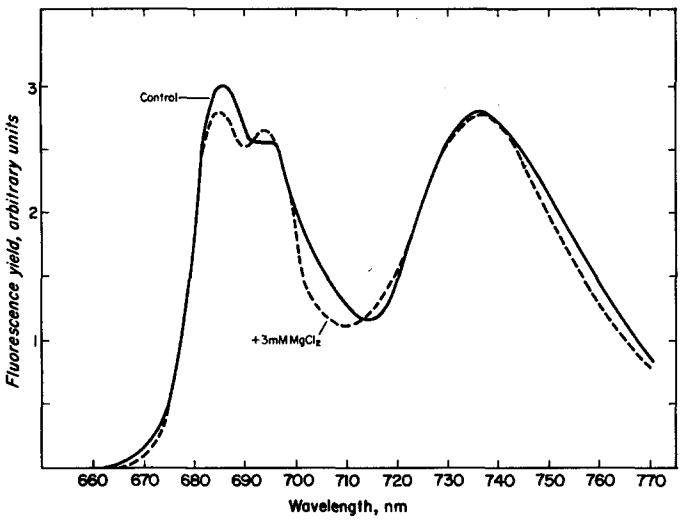

Fig. 6. Emission spectra at 77 °K of chlorophyll *a* fluorescence from Photosystem II particles. Chlorophyll *a*/*b* ratio was 1.65. Particles were preilluminated before freezing. All other conditions as in Table III. Spectra normalized at 735 nm. *F*735/*F*685 for the control was 0.906.

large (≈ 30 %) decrease in the O level which is concentration dependent (Fig. 2). This decrease in the O level probably reflects a direct effect on the bulk chlorophyll *a* molecules not involving the reaction center as at the P level. Magnesium, on the other hand, causes a small decrease in the O level as compared with the control, but a small increase as compared with the sodium-treated samples (Table I). Many investigators [3, 7–10] report no change or small increases in the O level fluorescence upon the addition of magnesium. These investigators used chloroplast preparations in which endogenous sodium salts were not removed; thus, the magnesium-induced increases they observed are consistent with our results. Since the magnesium effect was much greater at the P level, any small change(s) in the O level fluorescence were considered insignificant. However, in view of our data showing that the magnesium effect on the O level is intermediate between the salt-depleted control and the sodium-treated samples (Fig. 1) and that the magnesium counteracts the sodium effect (Table I, Fig. 5), we feel that the salt-induced changes in the O level fluorescence are also important.

The results reported here suggest that there are complex interactions of Na^+ and Mg^{2+} in chloroplasts and that each may show more than just one effect. Sodium at low concentrations most likely promotes energy distribution in favor of Photosystem I. However, at present there are at least two hypotheses to explain the role of magnesium in photosynthesis. In one hypothesis magnesium is also assigned the function of controlling spillover or energy distribution between the two photosystems, [3–6, 8, 9, 24–26] while in the second hypothesis magnesium is proposed to cause an increase in the number of active reaction center units [27, 28]. To account for the variable results with magnesium perhaps both mechanisms are involved. Presumably, one mechanism could predominate over the other depending on the state of the membrane (see Briantais et al. [9]) which may be controlled by such factors as light, redox state of Q and temperature.

It may be asked how magnesium can exert its influence through apparently entirely different mechanisms. Recently, Seely [29, 30] has proposed a theoretical model by which energy transfer within a photosynthetic unit can be controlled by the orientation of only a few pigment molecules. It may be possible that magnesium could alter the orientation of these pigments under certain membrane conformations to direct excitation energy from the bulk pigments either between the two photosystems or to unused Photosystem II reaction centers. Jennings and Forti [31] have suggested that the energy distribution between the two pigment systems may be controlled by a small amount of a chlorophyll *a* form absorbing at 695 nm; and, VanderMeulen and Govindjee [32] have suggested that the initial state of orientation of these specialized pigment molecules is species dependent, to account for the variable responses to divalent cations they observed for spinach and other plant species. As a working hypothesis, we propose that the key chlorophyll molecules controlling energy distribution between the two photosystems are complexed with a protein that changes conformation upon interaction with mono- and divalent cations and leads to a reorientation of these chlorophyll molecules. Further research with mutants or chloroplast preparations lacking various chlorophyll-protein complexes is needed to elucidate this point.

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