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ANALYSIS OF CHLOROPHYLL FLUORESCENCE INDUCTION CURVES IN THE PRESENCE OF 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA AS A FUNCTION OF MAGNESIUM CONCENTRATION AND NADPH-ACTIVATED LIGHT-HARVESTING CHLOROPHYLL *a*/*b*-PROTEIN PHOSPHORYLATION

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The effect of Mg^{2+} concentration and phosphorylation of light-harvesting chlorophyll *a*/*b*-protein on various chlorophyll fluorescence induction parameters of isolated pea thylakoids has been studied. (1) Lowering the Mg^{2+} concentration from 3 to 0.4 mM decreases only the variable fluorescence (F_v) and the area above the induction curve while at the same time increasing the slow exponential component of the rise (β_{max}). (2) A further decrease in Mg^{2+} concentration from 0.4 to 0 mM decreases the initial (F_0) fluorescence level such that the ratio F_v/F_m increases slightly as does the area above the induction curve and β_{max}. (3) Thylakoid membranes, phosphorylated at 5 mM Mg^{2+}, show an equal decrease in F_v and F_0, no change in the area above the induction curve and an increase in β_{max}. At 2 mM Mg^{2+}, however, phosphorylation induced a more extensive quenching of F_v so that the F_v/F_m ratio was lowered and the area above the induction curve decreased while β_{max} increased. (4) When phosphorylated membranes were subsequently suspended in an Mg^{2+}-free medium the effect on F_0 due to phosphorylation was found to be additive to that due to the absence of Mg^{2+}. The effect of membrane phosphorylation on fluorescence is discussed in relation to the control of excitation energy distribution and shows that different mechanisms operate depending on the background Mg^{2+} levels. At high Mg^{2+} the phosphorylation seems to affect the absorption cross-section of Photosystem II while at lower Mg^{2+} levels there is an additional effect of increased spillover from Photosystem II to I.

Introduction

There has been some confusion in the literature concerning the relationship between cation- and protein phosphorylation-induced changes of chlorophyll fluorescence from isolated thylakoid membranes. It is generally agreed that both processes reflect changes in energy distribution between PS II and PS I. The phosphorylation mechanism involves a membrane-bound kinase controlled by the redox state of the plastoquinone pool [1,2]. The phosphorylated species is the light-harvesting Chl *a*/*b* pigment-protein complex often denoted LHCP. The confusion arises because of conflicting experimental observations which do not clearly distinguish to what degree the change in light distribution involves direct energy transfer from PS II to PS I (spillover) and changes in the absorp-

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; F_m, maximum chlorophyll fluorescence yield; F_0, initial chlorophyll fluorescence yield; $F_v = F_m - F_0$ (variable chlorophyll fluorescence yield); LHCP, light-harvesting chlorophyll *a*/*b*-protein; Chl, chlorophyll; PS, photosytem; Tricine, *N*-tris(hydroxymethyl)methylglycine.

tion cross-section of the two photosystems. Horton and Black [3–5] found distinct differences between the chlorophyll fluorescence changes induced by Mg^{2+} as compared with those induced by phosphorylation which has led them to favour the concept that protein phosphorylation itself, unlike the cation-induced changes, brings about a redistribution of energy to PS I by increasing its absorption cross-section. They base their conclusion on the observation that at 5 mM Mg^{2+} the effect of phosphorylation is to lower both the variable (F_v) and initial (F_0) fluorescence by an equal extent. Their results contrast with the work of Kyle et al. [6] and Haworth et al. [7] who present evidence that phosphorylation not only increases the absorption cross-section of PS I but also brings about changes in spillover from PS II to PS I. Kyle et al. [6] did not, however, observe a decrease in the F_0 level for room-temperature fluorescence as was reported by Horton and Black [4].

Horton and Black [8] have since demonstrated an interaction between the cation and phosphorylation effect such that at lower Mg^{2+} levels phosphorylation alters not only the absorption cross-section but also the degree of spillover. It has been proposed that these changes are brought about by the phosphorylation of LHCP which becomes detached from the PS II core complex and migrates from appressed to non-appressed regions of the thylakoid membranes [9].

In attempting to interpret the effect of LHCP phosphorylation on excitation energy redistribution between PS I and PS II we have reanalysed the effect of Mg^{2+} on chlorophyll fluorescence induction curves in the presence of DCMU and compared it to the effect of LHCP phosphorylation, induced under conditions which maintained the plastoquinone pool in a reduced state without the necessity of long-term illumination. The rationale of this approach was to avoid the inconsistencies of the earlier analyses of LHCP phosphorylation on chlorophyll fluorescence which could in part have been due to non-specific light damaging effects occurring during prolonged periods of excessive illumination used to maintain kinase activity [8].

Materials and Methods

Chloroplasts retaining their outer membranes were prepared from pea leaves as previously described by Nakatani and Barber [10] and resuspended as a concentrated stock in a medium containing 0.33 M sorbitol (pH 7.5, Tris) and 3 mM $MgCl_2$. The chlorophyll concentration was determined by the method of Arnon [11].

To investigate the effect of protein phosphorylation on chlorophyll fluorescence phosphorylated and control membranes were prepared as follows. Intact chloroplasts were subjected to an osmotic shock at twice the final Mg^{2+} concentration (or in minus Mg^{2+} samples containing 20 mM NaF) for 15 s and double-strength medium was then added to give final concentrations as follows: 30 μg Chl/ml, 10 mM Tricine (pH 8.2, KOH), 0.33 M sorbitol, 0–5 mM $MgCl_2$, 0.5 mM NADPH, 4.2 μM *Spirulina maxima* ferredoxin. 10 mM NaF and 0.15 mM ATP were added where indicated and when ATP was present an equimolar amount of $MgCl_2$ was also added to compensate for the chelation of free Mg^{2+} by the nucleotide.

Normally phosphorylated samples are those which were incubated at room temperature with NaF and ATP for 60 min in the dark while non-phosphorylated samples are those which were incubated without ATP but in the presence of NaF (see also legend to Table I). The dephosphorylated samples had no NaF added until the end of the 60 min dark incubation. In all three treatments 1 mM $NADP^+$ was added after 30 min to reoxidise the plastoquinone pool and thus allow dephosphorylation in the samples containing no NaF. After 60 min dark incubation, the samples were diluted to 5 μg Chl/ml in 10 mM Tricine (pH 8.2, KOH), 0.33 M sorbitol, 10 mM NaF, 0–5 mM $MgCl_2$, 2.5 μM valinomycin, 2.5 μM nigericin, 0.17 mM methyl viologen and 1 mM $NADP^+$ for analysis. We found it necessary to reoxidise the plastoquinone pool, and to add an electron acceptor for PS I in order to allow analysis of β_{max} by the method of Melis and Homann [12].

To investigate the effect on chlorophyll fluorescence due to changes in Mg^{2+} concentrations chloroplasts were osmotically shocked at various Mg^{2+} concentrations and the final composition of the reaction was as follows: 5 μg Chl/ml, 10 mM

Tricine (pH 8.2, KOH), 0.33 M sorbitol, 0–5 mM $MgCl_2$ and 5 mM KCl. Under these conditions the total monovalent cation level was approx. 15 mM which is lower than that present in the phosphorylation media (approx. 20 mM). In these experiments there was no long incubation period as described for the protein phosphorylation experiments.

All diluted samples were dark treated for 4.5 min, 20 μM DCMU was added and chlorophyll fluorescence was measured after a total dark time of 5 min. The induction curves were initiated by the opening of a Compur mechanical shutter (less than 1 ms rise time) and illuminating the samples with blue-green light transmitted by 4 mm Schott BG18 glass filters (8 $W \cdot m^{-2}$). The resulting fluorescence signal was detected via a photomultiplier (EMI 9558) protected by a Balzer 686 nm interference filter. The photocurrent generated was passed to a Datalab Transient Recorder (DL905) coupled to a Datalab Display Module (DL403B). The fluorescence changes were recorded on a Hewlett Packard X-Y Display Unit (1340A) and analysed by a Datalab Function Module linked tc a Lear and Siegler Display Terminal (ADM 3A). The analysis was based on the method of Melis and Homann [12].

Results

Because of the inconsistencies in the reports of the effect of Mg^{2+} and phosphorylation treatments on F_0 [4,6,7,13–16] we investigated the accuracy of our measuring system for detecting the initial chlorophyll fluorescence level by measuing induction curves as a function of light intensity in the presence of DCMU. We found that decreasing the exciting light intensity brought about an equal lowering of F_v and F_0 (all intensities used were sufficient to reduce fully Q in the presence of DCMU) so that the ratio F_v/F_m remained constant. This shows that the apparatus used does not overestimate F_0 at the expense of F_v. This might have occurred, as the light intensity was increased, if the rise time of a fast F_v component had come within the range of the mechanical shutter's opening time.

It was found that as F_v decreased the kinetics of

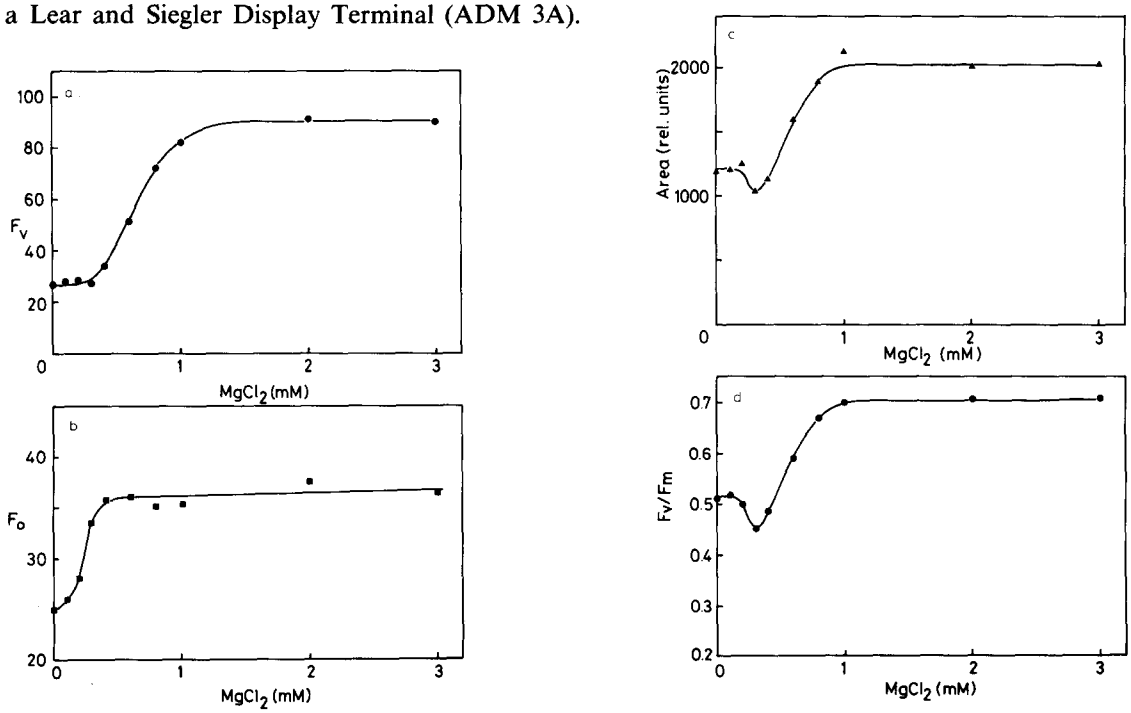

Fig. 1. The effect of $MgCl_2$ levels on (a) the variable fluorescence (F_v) (b) the initial fluorescence (F_0), (c) the area above the induction curve (A_{max}) and (d) the F_v/F_m ratio, of thylakoid membranes in the presence of 20 μM DCMU. The exciting light intensity used was 8 $W \cdot m^{-2}$ and measurements were made at room temperature.

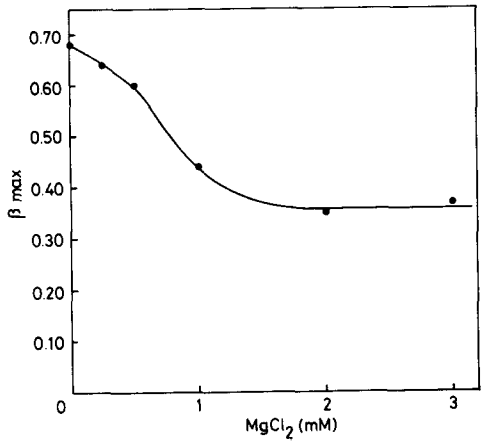

Fig. 2. The effect of the presence of varying $MgCl_2$ levels on the chlorophyll fluorescence parameter, β_{max}, which represents the intercept of the linearly extrapolated slow, exponential phase with the ordinate at zero time. This gives a relative measure of the total contribution of the exponential phase to the overall kinetics and is thought to indicate PS II connectivity [6].

the fluorescence rise became slower and as a consequence there was no observed change in the absolute area above the induction curve (A_{max}).

We then investigated various fluorescence parameters as a function of $MgCl_2$ concentration: Fig. 1a and b shows that lowering the Mg^{2+} concentration not only decreases F_v but also F_0. However, the Mg^{2+} concentration requirement for the decrease in F_0 ($C_{1/2} = 0.24$ mM) was lower than for the decrease in F_v ($C_{1/2} = 0.66$ mM). In Fig. 1c it can be seen that lowering the Mg^{2+} concentration from 5 to 0.4 mM decreases only the variable component of fluorescence (plotted as F_v/F_m) and this was accompanied by a similar decrease in A_{max} (Fig. 1d). However, below 0.4 mM Mg^{2+}, F_0 is decreased: proportionally more than F_v and hence there is a small increase in both F_v/F_m and A_{max}.

We have also measured, using the analysis of Melis and Homann [12], the parameter β_{max} as a function of Mg^{2+} concentration (see Fig. 2). As noted by others [6,12], this parameter (which is a measure of the exponential growth in the normal-

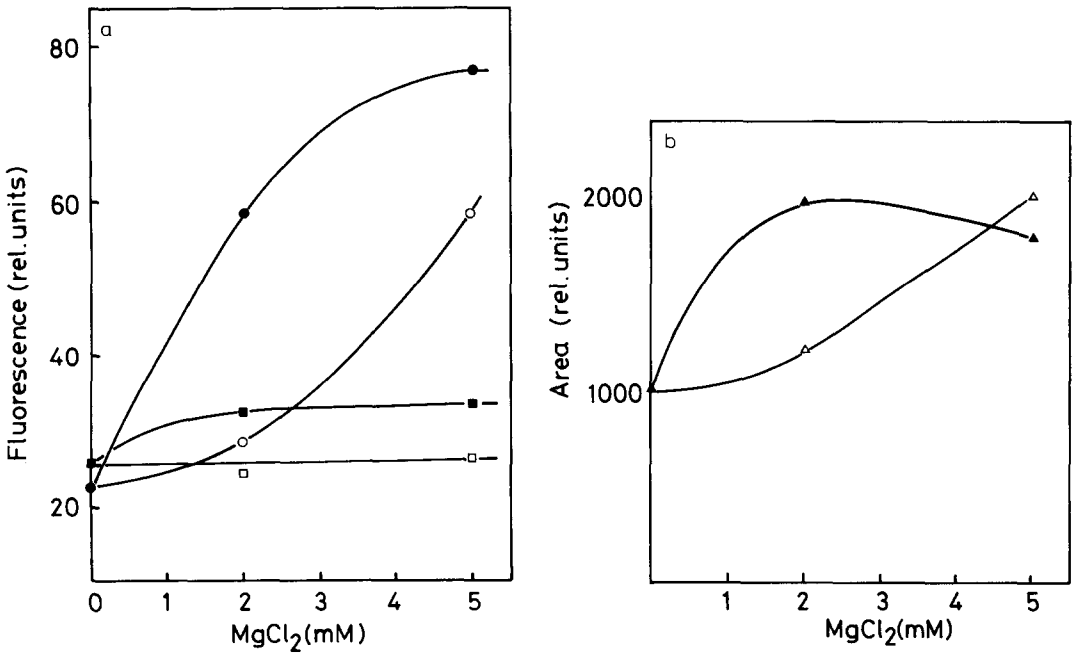

Fig. 3. (a) The effect of phosphorylation on the chlorophyll fluorescence parameters F_v (open circles) and F_0 (open squares) at 5 and 2 mM $MgCl_2$ compared with the non-phosphorylated fluorescence parameters F_v (closed circles) and F_0 (closed squares) at 5, 2 and 0 mM $MgCl_2$. All measurements were made at room temperature using the experimental conditions described in Materials and Methods. The light intensity was 8 $W \cdot m^{-2}$. (b) Plots of the area above the fluorescence induction curve for the conditions described in a; phosphorylated (open triangles), non-phosphorylated (closed triangles).

ized area above the chlorophyll fluorescence induction curve) decreases with increasing Mg^{2+} concentrations. It can be seen that the values of β_{max} decrease with increasing Mg^{2+} concentrations whether the fluorescence changes are brought about by an increase in F_0 (0 to 0.4 mM Mg^{2+}) or by an increase in F_v (0.4 to 3 mM Mg^{2+}).

In order to compare the changes in fluorescence induction kinetics induced by phosphorylation of LHCP with those induced by Mg^{2+} we utilized NADPH and ferredoxin to activate the protein kinase in the dark [17,18]. After 60 min incubation diluted samples were dark adapted and their fluorescence induction kinetics, in the presence of DCMU, were measured. In Fig. 3, F_v and F_0 values are given for membranes phosphorylated (incubated with ATP) or non-phosphorylated (incubated without ATP) at 2 and 5 mM $MgCl_2$. Phosphorylation at both Mg^{2+} levels decreases F_0 to a value comparable with the F_0 value obtained in the absence of Mg^{2+}. At 5 mM Mg^{2+}, phosphorylation brings about a decrease in the F_v and F_0 levels such that the F_v/F_m ratio for phosphorylated and non-phosphorylated membranes is constant (see also Table I) as reported by Horton and Black [4]. Moreover, at the higher Mg^{2+} concentration, the areas above the induction curve also remain constant for the phosphorylated and non-phosphorylated states (Fig. 3b). However, at 2 mM $MgCl_2$, phosphorylation induces a preferential quenching of F_v relative to F_o such that the F_v/F_m ratio is lowered. At this Mg^{2+} level phosphorylation also leads to a decrease in the area above the induction curve compared with the non-phosphorylated sample. These observed changes at 2 mM $MgCl_2$ due to phosphorylation are more like the fluorescence changes observed at low Mg^{2+} concentrations under non-phosphorylating conditions (Fig. 1).

To elaborate on the experiments shown in Fig. 3 we have checked the reversibility of the effects by using dephosphorylated membranes. These were obtained by introducing excess $NADP^+$ into the darkened reaction vessel after a 30 min period of phosphorylation in the absence of NaF. During the following 30 min dark period dephosphorylation of LHCP occurred as indicated by a return of the fluorescence characteristics to those of the

TABLE I

THE EFFECT OF PHOSPHORYLATION AND DEPHOSPHORYLATION OF THYLAKOID MEMBRANES ON VARIOUS FLUORESCENCE PARAMETERS.

Comparison of the fluorescence parameters F_0, F_v, β_{max} and F_v/F_m for pea thylakoid membranes which were phosphorylated (Phos), non-phosphorylated (NPhos) and dephosphorylated (DPhos) at 5 and 2 mM $MgCl_2$ with membranes suspended in the absence of Mg^{2+}. NPhos (b) and Phos (ii) were achieved by the procedures outlined in Materials and Methods. NPhos (a) represents samples which were incubated for 60 min in the presence of ATP in the presence of ferredoxin and $NADP^+$. After 60 min of this treatment NADPH and NaF were added to balance the overall content of the medium relative to those of the other experiments. Phos (i) represents samples which were incubated in the presence of ATP for 30 min but in the absence of NaF. After 30 min incubation, NaF and $NADP^+$ were added to inhibit phosphatase and kinase activity, respectively.

[$MgCl_2$] (mM)	Treatment		F_0	F_v	β_{max}	F_v/F_m
5	NPhos	(a)	36.5	70.8	0.31	0.66
	NPhos	(b)	41.5	86.0	0.21	0.67
	Phos	(i)	31.0	67.3	0.45	0.68
	Phos	(ii)	31.0	62.3	0.47	0.67
	DPhos		38.8	75.5	0.29	0.66
2	NPhos	(a)	34.0	75.5	0.46	0.69
	NPhos	(b)	37.3	79.3	0.47	0.68
	Phos	(i)	29.0	24.0	0.73	0.46
	Phos	(ii)	30.0	26.0	0.74	0.46
	DPhos		30.3	65.0	0.46	0.68
0	NPhos		29.0	19.0	0.81	0.40

TABLE II

COMPARISON OF THE EFFECT OF THE ABSENCE OF Mg^{2+} ON FLUORESCENCE OF PHOSPHORYLATED AND NON-PHOSPHORYLATED THYLAKOID MEMBRANES

Pea thylakoid membranes were phosphorylated (Phos) or non-phosphorylated (Nphos) at 5 mM $MgCl_2$ for 50 min as described in Materials and Method. Fluorescence induction was then measured as previously described either in the presence of 5 mM $MgCl_2$ ($+Mg^{2+}$) or after addition of sufficient EDTA to chelate the Mg^{2+} present in the diluted sample ($-Mg^{2+}$). Fluorescence induction of thylakoids initially incubated in the absence of Mg^{2+} was essentially the same as that seen in the NPhos samples in which Mg^{2+} had been chelated by EDTA.

	F_v/F_m		F_0		Decrease in F_0 due to $-Mg^{2+}$ (%)
	$+Mg^{2+}$	$-Mg^{2+}$	$+Mg^{2+}$	$-Mg^{2+}$	
NPhos	0.64	0.39	46.5	27.3	41
Phos	0.64	0.40	36.0	22.0	39
Decrease in F_0 due to Phos (%)	–	–	23	19	

non-phosphorylated samples. Presumably, excess $NADP^+$ induces on oxidation of the plastoquinone pool in the dark which inactivates the kinase, allowing dephosphorylation by the membrane-bound phosphatase [19]. Appropriate controls were set up for comparison as described in the legend to Table I. The data of Table I confirm the observations in Fig. 3 for the changes in the F_v/F_m ratio at the two background $MgCl_2$ levels. Table I also gives β_{max} values where it can be seen that this parameter increases with phosphorylation of the membrane at both concentrations of Mg^{2+} but with the greatest effect being observed at the lower level of Mg^{2+} (2 mM).

The data of Figs. 1 and 3 show that both the absence of Mg^{2+} and LHCP phosphorylation lead to a decrease in F_0. In order to determine whether these decreases were the same or independent effects, induction curves were measured in the absence of Mg^{2+} after samples were phosphorylated or non-phosphorylated at 5 mM Mg^{2+}. Table II shows that on phosphorylation there is a decrease in the minus Mg^{2+} F_0 level which is lower than that found for non-phosphorylated membranes. This additional change is also accompanied by a change in the F_v/F_m ratio to a level similar to that seen in the minus Mg^{2+} control. These results suggest that the change due to phosphorylation is different to the minus Mg^{2+} effect. This independence is emphasised by the fact that the percentage decrease in F_0, due to phosphorylation, is very similar plus and minus Mg^{2+} and the percentage decrease, due to the absence of Mg^{2+}, is very similar with and without LHCP phosphorylation.

Discussion

To attain maximal rates of non-cyclic photosynthetic electron flow at limiting light intensities there must be a balance in the excitation of PS I and PS II. It seems that plants have a mechanism to regulate such a balance which is detected on changing the spectral quality of the light falling upon them (see Ref. 20). The regulatory process has become known as the State 1–State 2 transition since its discovery by Bonaventura and Myers [21].

There are two ways in which the variation of light distribution between PS II and PS I has been considered: (a) by regulating the transfer of energy from PS II-LHCP complexes to neighbouring PS I complexes; (b) by changing the absorption cross-sections of PS II and PS I. The first mechanism (spillover) has been supported by a number of studies on thylakoids subjected to different cation levels [22–24]. It has been postulated that under low ionic conditions, when thylakoids unstack, the PS II-LHCP and PS I complexes randomise in the plane of the membrane and hence increase the

probability of energy transfer from PS II to PS I [25]. Addition of cations leads to the formation of granal (stacked) and stromal lamellae (unstacked) and a decrease in the PS II-LHCP to PS I energy transfer. The argument presented [25] was that salt addition leads to lateral segregation of the PS II-LHCP and PS I complexes to different domains, a conclusion supported by membrane fragmentation studies [26]. Reports that removal of cations lowers the F_0 as well as F_v have led some authors to the conclusion that removal of cations decreases the absorption cross-section of PS II as well as increasing energy transfer from PS II to PS I [13,27].

The results shown in Fig. 1 suggest that the cation effect is indeed more complex than suggested by Barber [25]. The difference in sensitivity of F_v and F_0 to Mg^{2+} concentration indicates that two independent control mechanisms may be involved. At very low Mg^{2+} levels F_0 is changed, suggesting a change in absorption cross-section of PS II. Such an observation is not expected for a change in spillover. The latter effect is associated with only a change in F_v, which was seen at the higher Mg^{2+} concentrations (Fig. 1a).

A possible model to explain the effect of low Mg^{2+} levels on fluorescence is one in which interaction between the PS II core and LHCP complex is reduced due to increased coulombic repulsion which might be expected to occur under conditions of very poor electrostatic screening [28]. A model in which cations enhance PS II to LHCP coupling and thus increase the probability of exciton transfer to PS II has been proposed by Butler [27]. This effect seems to be in addition to the cation-induced changes in spillover observed at higher Mg^{2+} levels. It may be controlled by local charge characteristics of certain protein complexes while segregation of PS I and PS II-LHCP into domains is thought to be controlled by overall surface charge characteristics of the thylakoid membrane [28].

Cation control of chlorophyll fluorescence characteristics may thus be visualised as follows. Initially, as the Mg^{2+} concentration is lowered from a saturating level, PS II-LHCP and PS I complexes randomise and hence spillover to PS I increases. This is seen as a decrease in F_v/F_m and A_{max}. At very low Mg^{2+} concentrations the PS II-LHCP interaction decreases, leading to a decrease in the absorption cross-section of PS II. As a consequence of this the degree of spillover will now decrease and hence lead to an increase in F_v/F_m and A_{max}. Such an increase in both these parameters was indeed seen as the Mg^{2+} level was lowered from 0.5 to 0 mM (Fig. 1c and d).

Henkin and Sauer [15], using a modulated technique, found very similar results to those shown in Fig. 1. They showed that the Mg^{2+} stimulation of background fluorescence (F_B) saturated at 0.5 mM Mg^{2+} while the Mg^{2+} stimulation of F_v saturated at 2.5 mM Mg^{2+}. However, they interpreted their results solely in terms of a change in PS II absorption cross-section.

In has been proposed that in vivo it is changes in the degree of LHCP phosphorylation which regulate excitation energy distribution between the two photosystems (see Ref. 1) rather than light-driven fluctuations in cation levels. The data presented show that at 5 mM Mg^{2+}, LHCP phosphorylation brings about an equal decrease in F_v and F_0 (see Fig. 3 and Table I) which confirms the results of Horton and Black [4]. Moreover, we found that the area above the induction curves for these conditions remained constant. These observations support the idea that in the presence of 5 mM Mg^{2+}, the sole effect of phosphorylation is to bring about a change in the PS II absorption cross-section, as suggested by Horton and Black [4,8]. We also agree with Horton and Black [8] that at lower Mg^{2+} levels (2 mM) phosphorylation preferentially quenches F_v relative to F_0 even though the latter is comparable to the F_0 observed for phosphorylation at 5 mM Mg^{2+}. Because of this the F_v/F_m ratio and area above the induction curve decrease to levels similar to those observed at zero Mg^{2+} (cf. Fig. 1).

From our results it is likely that both mechanisms (type a and b), for the regulation of energy distribution, may be operative depending on the background level of 'screening' cations. At 5 mM Mg^{2+} when screening is high, the phosphorylation-induced quenching is less extensive than at lower Mg^{2+} levels and seems to be due to a change in absorption cross-section of PS II. This could be accomplished by the detachment of phosphory-

lated LHCP from the PS II due to increased coulombic repulsion in a manner similar to that proposed above for the very low Mg^{2+} conditions. It has been suggested that the detached phosphorylated LHCP diffuses laterally in the membrane in such a way as to facilitate LHCP-PS I interaction [4,9]. At lower Mg^{2+} (e.g., 2 mM), where the phosphorylation-induced changes are more like the changes induced by salts, the detachment of LHCP from PS II is probably not the only cause of the fluorescence quenching. It seems likely that in this situation when electrostatic screening is weaker the phosphorylation process induces a greater lateral randomisation of complexes so that energy transfer from PS II-LHCP complexes to PS I may occur in addition to changes in the absorption cross-section of the two photosystems due to LHCP detachment. This argument is consistent with the finding that phosphorylation at 2 mM Mg^{2+} causes substantial unstacking similar to that observed by lowering the background cation levels (unpublished observation).

A simple model in which both phosphorylation and absence of cations bring about detachment of LHCP from PS II and hence increase the probability of direct transfer of excitons from LHCP to PS I predicts that these two effects should not be additive. In fact, we found (Table II) that the two effects were independent, i.e., phosphorylated membranes showed a further decrease in both initial and variable fluorescence when suspended in the absence of Mg^{2+}, such that the F_v/F_m ratio remained unchanged. This confirms and extends the results of Horton and Black [5], who demonstrated that phosphorylation induces a similar percentage decrease in F_m whether the thylakoids are suspended at 5 or 0 mM Mg^{2+}. The fact that we found no change in the F_v/F_m ratio emphasises that the phosphorylation-induced decrease is due to a change is absorption cross-section, as is a component of the cation-induced effect, but that these are independent processes. It would therefore seem that phosphorylated LHCP shows a greater degree of exciton transfer to PS I than that brought about by the decreased interaction of non-phosphorylated LHCP and PS II at zero Mg^{2+}.

The idea that both LHCP phosphorylation and cation level may control the relative spatial organisation of pigment-protein complexes within the membrane seems to be expressed by the magnitude of the β_{max} component, obtained by analysing the fluorescence induction curves [12]. From our results it is interesting to note that this parameter is sensitive to changes brought about by either preferential F_v quenching or when F_0 and F_v are equally quenched (see Fig. 2 and Table I). These findings are consistent with the concept that β_{max} represents the relative amount of kinetically separate PS II units but they emphasise that this parameter may not be attributed to a specific type of structural PS II unit.

At present, it is unclear whether the in vivo State 1–State 2 transition is accomplished solely by changes in absorption cross-section or by a combination of this and spillover due to membrane phosphorylation as proposed by Haworth et al. [9]. The precise mechanism may be highly dependent on the cation levels in the chloroplast stroma and probably will be influenced by the relative amounts of PS I, PS II and LHCP complexes present in the membrane as a whole. These latter quantities will vary according to growth conditions [29].

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