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THE EFFECT OF MAGNESIUM AND PHOSPHORYLATION OF LIGHT-HARVESTING CHLOROPHYLL *a*/*b*-PROTEIN ON THE YIELD OF P-700-PHOTOOXIDATION IN PEA CHLOROPLASTS

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The yield of P-700 photooxidation has been studied in isolated chloroplast membranes by measuring the extent of the flash-induced absorption increase at 820 nm (ΔA_{820}) in the microsecond time range. The extent of ΔA_{820} induced by non-saturating laser flashes was increased by the following treatments. (1) Suspension of chloroplast membranes in Mg^{2+} free medium (plus 15 mM K^+) which leads to unstacking of grana (as detected by a decrease in chlorophyll fluorescence). (2) Reduction of Q, the primary acceptor of Photosystem II, in the presence of 20 μM 3-(3,4 dichlorophenyl)-1,1-dimethylurea by a saturating xenon flash, fired 300 ms before the laser flash. (3) Phosphorylation of light harvesting chlorophyll *a*/*b*-protein complex, which occurs in the presence of ATP after activation of protein kinase in the dark with NADPH and ferredoxin. We conclude that the Mg^{2+} concentration, the redox state of Q and the protein-phosphorylation all can control the photochemical efficiency of P-700 photooxidation in isolated chloroplasts, and we discuss these results in relation to control of excitation energy distribution between the two photosystems. We also discuss the significance of these results in relation to the regulation of photosynthetic electron transport in vivo.

Introduction

Photosynthetic organisms in which two photosystems cooperate in series during photosynthetic electron transport are able to adapt to light of different wavelengths, so as to maximize photochemical efficiency when light is limiting (see Ref. 1). When light is preferentially absorbed by Photosystem II (PS II), adaptation leads to a condition known as State 2, while excess PS I light induces State 1 [2]. These state changes were observed in vivo in experiments in which oxygen yield was measured both by a modulated oxygen electrode [2] and recently by photoacoustic spectroscopy [3]. In order to understand this phenomenon, it is assumed that the quantum efficiency of both photosystems changes: for example, during adaptation towards State 2, there is an increase in PS I activity at the expense of PS II which leads to an increase in photochemical efficiency of the complete electron transport chain. Experiments with isolated chloroplast membranes have indicated that changes in quantum efficiency can be induced by varying the cation level of the suspension medium

Abbreviations: Chl, chlorophyll; DCMU, 3-(3′,4′ dichlorophenyl)-1,1-dimethylurea; DPIP, 2,6-dichlorophenolindophenol; F_m, maximum chlorophyll fluorescence yield; F_0, initial chlorophyll fluorescence yield; F_v, $F_m - F_0$; LHCP, light-harvesting chlorophyll *a*/*b*-protein complex; PS, Photosystem; Q, primary acceptor of Photosystem II; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

and by reversible phosphorylation of light-harvesting chlorophyll *a/b*-protein (LHCP) complex (see Ref. 4).

Previously, changes in the amount of energy going to PS I, induced either by changing cation levels or by LHCP-phosphorylation, have been determined both by measurement of chlorophyll fluorescence and steady-state electron transfer rates. Criticisms of the former method are that it is an indirect measure of PS I excitation when performed at room temperature because it only measures excitation of PS II, and that fluorescence measurements at 77 K may not relate to physiological temperatures [5,6].

Study of the effect of cations on steady-state electron transfer rates has yielded conflicting results because cations also affect interaction of intrinsic and extrinsic electron transport components (see Ref. 7). However, in experiments designed to eliminate secondary electron-transfer steps which might be affected by changes in cation level, Melis and Ow [8] found no effect of Mg^{2+} concentration on the initial rate of photooxidation of P-700 despite the fact that chlorophyll fluorescence from PS II was changed as expected for increased energy transfer to PS I. On the other hand, in careful electron transport studies phosphorylation of LHCP has been shown to bring about a 10–15% increase in efficiency of PS I activity [9,10].

In this paper, we have used a more direct approach to investigate possible changes in the photochemical efficiency of PS I in response to the effect of cations, LHCP-phosphorylation and Q reduction. We have examined their effects on the extent of the flash-induced absorbance change at 820 nm (ΔA_{820}) which in the microsecond time range has been attributed essentially to $P\text{-}700^+$ [11]. We have found that absence of Mg^{2+} and phosphorylation of LHCP increase the photochemical efficiency of P-700 oxidation induced by non-saturating flashes.

Materials and Methods

Biological material

Intact chloroplasts were isolated from the leaves of 10–14-day-old pea plants as described in Ref. 12, resuspended in 0.33 M sorbitol and 3 mM $MgCl_2$, brought to pH 7.5 with Tris base and stored on ice. Chlorophyll was determined by the method of Arnon [13].

For spectroscopic measurements, stacked (plus Mg^{2+}) or unstacked (minus Mg^{2+}) thylakoids were produced by osmotic shock of the intact chloroplasts in the reaction cuvette, as described by Telfer et al. [12]. The final composition of the reaction medium, in 2.5 ml, was as follows: 0.33 M sorbitol, 10 mM Tricine, 10 mM KOH, 5 mM KCl, brought to pH 8.2 with HCl. 5 mM $MgCl_2$ was added where indicated.

To investigate the effect of the redox state of Q on absorption measurements, the following additions were made: 1 mM sodium ascorbate, 20 μM DPIP and 20 μM DCMU. Samples were incubated in the dark for 5 min at room temperature (approx. 21°C).

For dark activation of the protein kinase catalysing phosphorylation of LHCP, the following additions were made: 0.5 mM NADPH, 6 μM *Spirulina maxima* ferredoxin, 5 mM glucose, 100 μg/ml glucose oxidase, 200 μg/ml catalase, 0.5 μM nigericin, 0.5 μM valinomycin, 10 mM NaF (in place of 5 mM KCl) and 0.15 mM ATP. Phosphorylated samples (plus ATP and Mg^{2+}), non-phosphorylated samples (minus ATP but plus Mg^{2+}) and unstacked samples (minus ATP and Mg^{2+}) were incubated in the dark, at room temperature for 50 min before measurement of flash-induced absorption changes. The chlorophyll concentration was 40 μg/ml.

Chlorophyll fluorescence measurements (to check for unstacking [14] and phosphorylation of LHCP [15]) were carried out on the samples used for absorption measurement after dilution with reaction medium (plus Mg^{2+} and NaF, where applicable) to a chlorophyll concentration of 10 μg/ml.

Absorption measurements at 820 nm

The absorption increase at 820 nm was measured as previously described [11,16]. The electrical bandwidth was 100 Hz–1 MHz. Measurements were carried out at room temperature in a 10 × 10 mm cuvette. The thylakoid suspension was excited with a dye laser flash ($t_{1/2} = 15$ ns; broad band at approx. 600 nm; $E_{max} \approx 10$ mJ). The laser flash was attenuated with controlled neutral density

filters and homogenized by a piece of ground glass located 8 mm in front of the cuvette. The flash frequency was 0.1 Hz and signals are the average of four or eight flashes. In order to reduce Q, samples were subjected to a saturating xenon flash (Stroboslave, white light) 300 ms before the laser flash.

Chlorophyll fluorescence

Chlorophyll fluorescence was measured with the same optical set-up as used for the ΔA_{820} measurements. The thylakoid samples were excited with the dye laser flash attenuated 650 times. Fluorescence was measured with a silicon photodiode protected by a Corning glass filter, CS 2-64 and a 685 nm interference filter (half band width, 3 nm). The Q oxidized (F_0) and Q reduced (F_m) states were achieved as described for the absorption measurements.

Results

First we checked that both phosphorylation of LHCP and absence of Mg^{2+} lowered chlorophyll fluorescence yield as previously reported (e.g., Ref. 12). Table I shows that this is the case; LHCP-phosphorylation decreased flash-induced F_v and F_0 equally (F_v/F_m was essentially unchanged) and absence of Mg^{2+} preferentially affected F_v (F_v/F_m decreased). The large effect of absence of Mg^{2+} on F_m is an indication that the chloroplast membranes were unstacked by this treatment [14].

Fig. 1 shows the absorption change at 820 nm induced by non-saturating ($I = 5\%$) and saturating flashes ($I = 44\%$) when Q was in the oxidized state. The time resolution was such that P-680$^+$ (which decays in the submicrosecond time scale) should not have been detected [11]. The decay typically consists of three kinetic components ($t_{1/2}$; $a = 10$–15 μs; $b \sim 200$ μs; $c = 4$–10 ms) which can all be attributed to P-700$^+$ reduction, but the decay of the slow phase is probably due mainly to the a.c.-coupling of the amplifier. Complete reduction of P-700$^+$ must have occurred between laser flashes, as there was no decrease in net signal size between the first and subsequent flashes (data not shown).

Fig. 1 also compares the ΔA_{820} of unstacked

TABLE I

THE EFFECT OF ABSENCE OF Mg^{2+} AND PHOSPHORYLATION OF LHCP ON FLASH-INDUCED CHLOROPHYLL FLUORESCENCE PARAMETERS

In experiment (i), thylakoids were incubated (after osmotic shock of intact chloroplasts, plus or minus 5 mM $MgCl_2$) at a chlorophyll concentration of 10 μg/ml for 5 min in the dark before measurement of F_0. The F_m measurements were made on the same sample after reduction of Q as described in Materials and Methods. Experiment (ii) was carried out as described in Materials and Methods. $+Mg^{2+}$: stacked thylakoids; No Mg^{2+}: unstacked thylakoids; $+Mg^{2+}$, Phos: phosphorylated thylakoids. Chlorophyll fluorescence was measured in arbitrary units.

Expt.	Additions	F_0	F_m	F_v/F_m
(i)	$+Mg^{2+}$	32	111	0.71
	No Mg^{2+}	23	38	0.39
(ii)	$+Mg^{2+}$	38	110	0.65
	No Mg^{2+}	26	42	0.38
	$+Mg^{2+}$, Phos	28	76	0.63

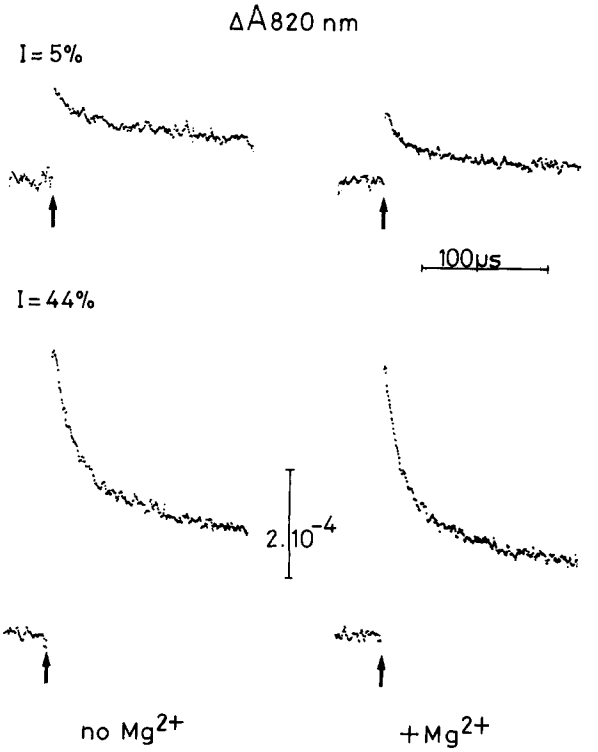

Fig. 1. The effect of 5 mM $MgCl_2$ on the absorption change at 820 nm induced in thylakoids by either non-saturating ($I = 5\%$) or saturating ($I = 44\%$) laser flashes.

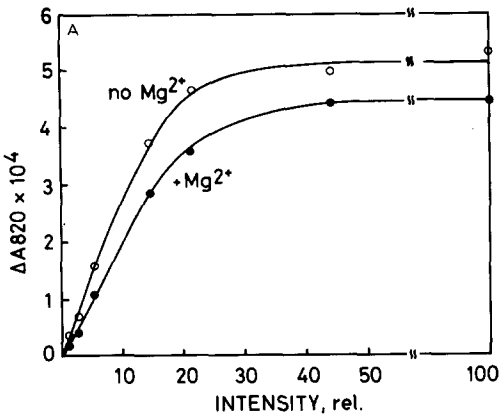

Fig. 2. (A) The effect of 5 mM $MgCl_2$ on the initial extent of the absorption change at 820 nm in thylakoids, measured as a function of laser flash intensity. (B) This shows the percentage increase in signal size due to the absence of Mg^{2+}.

(no Mg^{2+}) and stacked (plus 5 mM Mg^{2+}) thylakoids at both flash intensities. It can be seen that absence of Mg^{2+} increases the extent of ΔA_{820} induced by a non-saturating flash. Surprisingly, there was also a small increase (approx. 10%) in the signal induced by a saturating flash.

Fig. 2A shows the initial extent of ΔA_{820} as a function of flash intensity, in the presence and absence of Mg^{2+}. Again the increase in signal due to absence of Mg^{2+} can be seen at all flash intensities. However, Fig. 2B shows that the percentage increase was considerably greater under non-saturating conditions.

Table II shows the effect of reduction of Q (plus DCMU and xenon flash) on the 820 nm absorbance change both plus and minus Mg^{2+} and at low and high laser flash intensities. At saturating intensity ($I = 100\%$) we found that the effect of reducing Q was to lower the ΔA_{820} signal by approx. 10% whether Mg^{2+} was present or not. However, low intensity flashes ($I = 6.4\%$) gave

TABLE II

THE EFFECT OF THE REDOX STATE OF Q ON THE INITIAL EXTENT OF THE ABSORPTION CHANGE AT 820 nm AT HIGH AND LOW FLASH INTENSITY

Samples were prepared as described in Materials and Methods. Standard errors and numbers of replicates as indicated. The relative effect at $I = 6.4\%$ was calculated as follows: ΔA_{820} ($I = 6.4\%$)/ΔA_{820} ($I = 100\%$) expressed as a percentage of the + Mg, Q experimental condition.

Experimental condition	ΔA_{820} ($\times 10^4$)		
	$I = 6.4\%$	$I = 100\%$	Relative effect at $I = 6.4\%$
$+Mg^{2+}$, Q	1.49 ± 0.07(5)	5.76 ± 0.16(6)	100
$+Mg^{2+}$, Q^-	1.64 ± 0.05(4)	4.94 ± 0.05(4)	128
No Mg^{2+}, Q	2.08 ± 0.12(5)	6.49 ± 0.13(6)	124
No Mg^{2+}, Q^-	2.25 ± 0.08(4)	5.96 ± 0.10(4)	146

increased signal sizes when Q was reduced.

Column 3 of Table II shows the relative ΔA_{820} induced by a flash of low intensity after normalisation of the signals induced by saturating flashes. Both absence of Mg^{2+} and Q reduction, independently, increased the relative yield of ΔA_{820}. In these experiments, complete rereduction of P-700$^+$,

TABLE III

COMPARISON OF THE EFFECT OF ABSENCE OF Mg^{2+} AND PHOSPHORYLATION OF LHCP ON THE INITIAL EXTENT OF THE ABSORPTION CHANGE AT 820 nm AND THE MAXIMAL FLUORESCENCE YIELD (F_m)

The relative percentage effects of Mg^{2+} absence and LHCP-phosphorylation were calculated as follows: ΔA_{820} ($I = 5.3$ or 12%)/ΔA_{820} ($I = 100\%$) expressed as the percentage increase above the $+Mg^{2+}$ experimental condition. Other details as described in Table I.

Experimental conditions	F_m	ΔA_{820} ($\times 10^4$)		
		$I = 5.3\%$	$I = 12\%$	$I = 100\%$
$+Mg^{2+}$	100	1.34	2.62	6.07
No Mg^{2+}	34	2.05	3.45	6.42
$+Mg^{2+}$, Phos	79	1.64	2.91	6.19
Rel. percentage increase due to absence of Mg^{2+}		45	27	
Rel. percentage increase due to Phos of LHCP		17	10	

in between flashes, was ensured by the presence of DPIP and ascorbate.

The decrease in the maximum signal size brought about by DCMU plus a xenon flash suggests that P-680$^+$ might be contributing to the signal, in the absence of DCMU, when the PS II trap is open. This could be due to slow electron donation from H_2O in a small fraction of PS II centres. When thylakoids were treated with potassium ferricyanide, to oxidize P-700 in the dark, the remaining flash-induced ΔA_{820} mainly consisted of a rapidly decaying component ($t_{1/2} \simeq 10$ μs) which was abolished by DCMU. This component was approx. 6–10% of the total signal. Thus, the difference in total signal size, plus and minus DCMU and xenon flash treatment is probably mainly due to P-680$^+$.

Fig. 2 and Table II establish that absence of Mg^{2+} and reduction of Q independently increase the photochemical efficiency of P-700 photooxidation. Next, we investigated the effect of phosphorylation of LHCP on this process. In order to avoid any photoinhibitory effects, we activated the protein kinase in the dark by the method of Telfer et al. [12]. Thylakoids were incubated at room temperature for 50 min with NADPH, ferredoxin and an oxygen trap. NaF was also present to inhibit phosphatase activity [17]. Phosphorylated samples were those with added ATP. Control samples with no ATP (non-phosphorylated) and no Mg^{2+} were also subjected to the same incubation treatment.

Table III compares the effect of the absence of Mg^{2+} with the effect of LHCP-phosphorylation on both ΔA_{820} and F_m. The fluorescence data show that phosphorylation decreased F_m as expected (see Table I). It can be seen that LHCP-phosphorylation brings about an increase in the relative yield of ΔA_{820}, induced by non-saturating flashes, but that at each flash intensity it is a smaller increase than that seen in the absence of Mg^{2+}. In this experiment Q was oxidized. A similar increase in yield of ΔA_{820} was observed when Q was reduced by DCMU plus xenon flash treatment (data not shown).

Discussion

The data presented here show a variety of ways in which the yield of the flash-induced absorption change at 820 nm of isolated chloroplast membranes can be changed. We found that Mg^{2+} depletion of the suspending medium, which lowered chlorophyll fluorescence, increased the initial extent of ΔA_{820} measured in the microsecond time range. This signal has been ascribed largely to P-700$^+$ [11]. An increase in signal size was observed both with saturating and non-saturating flashes. However, the effect at low flash-intensities was considerably greater than that induced by saturating flashes (see Fig. 2). We conclude that this increase in quantum yield of ΔA_{820}, measured in the absence of Mg^{2+}, is the result of a change in excitation energy distribution in favour of PS I.

This conclusion depends on the assumption that the 820 nm absorption change, in the microsecond to millisecond time range, is due mainly to P-700$^+$. We found, by ferricyanide treatment, that when Q was oxidized, approx. 10% of the signal could be attributed to P-680$^+$. The P-680$^+$ contribution to the total signal, induced by saturating flashes, was about the same both in the presence and absence of Mg^{2+} and therefore does not explain the difference in total signal size induced by Mg^{2+}. Possible explanations for this difference are that it may be due to inactivation of a fraction of PS I centres by Mg^{2+} or it may be an experimental artifact related to the large difference in scattering properties of stacked and unstacked thylakoids.

Mg^{2+} depletion increased the yield of ΔA_{820} whether Q was oxidized or reduced (Table II). This is in agreement with the conclusions of Satoh et al. [18] based on electron transport data, and of Butler [19] based on 77 K fluorescence analyses that changes in energy distribution can be controlled by Mg^{2+} even when the PS II traps are open.

In addition to the Mg^{2+} effect, we also found that the redox state of Q itself controlled the flash yield of P-700$^+$. On reduction of Q, there was an increase in the efficiency of P-700 photooxidation, both in the presence and absence of Mg^{2+}, provided that the flash intensity was limiting. This also confirms the conclusions of Satoh et al. [18] and Butler [19] that the yield of energy transfer from PS II to PS I increases on closing the PS II traps. The conclusions of Satoh et al. [18] were derived from data on the effect of the redox state

of Q on the electron transport activity of PS I and the initial rate of P-700 photooxidation at 77 K.

The full extent of the effect of Q reduction on the yield of P-700 photooxidation was seen only after correction for the small contribution by P-680$^+$ to the total ΔA_{820} signal, which was seen when Q was oxidized. This correction is valid as the laser flash, at 600 nm, excites PS I and PS II more or less equally [20].

Finally, we studied the effect of phosphorylation of LHCP on the extent of ΔA_{820}. Again, we found an increase in the efficiency of P-700 photooxidation, although it was not as marked as that seen in the absence of Mg^{2+} (Table III) or on reduction of Q (Table II). Because of the effect of LHCP-phosphorylation on chlorophyll fluorescence (equal quenching of F_v and F_0 – see Table I and Ref. 12) we suggest that the photochemical efficiency of PS I is increased due to an increase in the absorption cross-section of PS I rather than to a change in energy transfer from PS II to PS I. This suggestion is supported by the fact that the increase was independent of the redox state of Q. It should be borne in mind that measurement of an increase in the quantum yield of P-700 photooxidation alone only indicates increased-excitation energy distribution in favour of PS I and does not directly give information on the mechanism of energy redistribution.

The results presented here lead to the conclusion that absence of Mg^{2+}, closing of the PS II traps and phosphorylation of LHCP all result in increased excitation energy distribution in favour of PS I. This is in agreement with the conclusions of many others which were based on less direct or less physiologically relevant measurements [5,6,9,10,18,21]. However, they do not agree with the conclusions of Melis and Ow [8,22] that neither Mg^{2+} concentration nor the redox state of Q affects the photochemical efficiency of P-700 photooxidation. The reason for this discrepancy is not clear, but it should be noted that the experiments of Melis and Ow [8,22] were carried out differently from ours. Their effects were measured on the initial rate of photooxidation of P-700 induced by a weak measuring beam in thylakoids which had been treated with KCN to inhibit electron donation to P-700 by plastocyanin.

Finally, we conclude that in isolated chloroplast membranes excitation energy distribution between the photosystems can be controlled by the cation level of the thylakoid suspension medium and by phosphorylation of LHCP. In vivo, there is evidence that PS I activity increases after adaptation to light initially preferentially absorbed by PS II both in algae and in higher plants [1,2,23]. The data on LHCP-phosphorylation presented here is strong evidence in favour of the hypothesis that this process is the biochemical mechanism which controls State 1–State 2 transitions in vivo [24–26].

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