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Light-dependent phosphorylation of Photosystem II polypeptides maintains electron transport at high light intensity: separation from effects of phosphorylation of LHC-II

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Phosphorylation of chloroplast thylakoid membrane proteins, including LHC-II and PS II polypeptides D_1, D_2, CP43 and the *psbH* gene product, occurs under plastoquinone-reducing conditions. Dephosphorylation of LHC-II and PS II phosphoproteins proceeds under plastoquinone-oxidizing conditions, but with differing kinetics. This study shows that phosphorylation sites of PS II polypeptides are substrates for exogenous alkaline phosphatase activity, whereas those of LHC-II are not. By combined use of exogenous and endogenous phosphatase activities four types of thylakoid membrane are generated, with (i) both LHC-II and PS II polypeptides phosphorylated, (ii) only PS II polypeptides phosphorylated, (iii) only LHC-II phosphorylated and (iv) neither class of polypeptide phosphorylated. Examination of PS II electron transport in each membrane type shows that a mechanism involving phosphorylation of PS II polypeptides specifically is required for PS II function at high light intensity. Phosphorylation of LHC-II results in an inhibition of PS II electron transport at limiting light intensity which can therefore be attributed to decreased PS II absorption cross-section. Q_B binding is unaffected by phosphorylation in any membrane type, nor are changes in function at the donor side of PS II involved. The role of PS II polypeptide phosphorylation in energy dissipation and protection from photoinhibition is discussed.

Introduction

Higher plants and green algae are able to vary the relative distribution of absorbed excitation energy between the photosystems in response to varying light regime by the mechanism of State 1–State 2 transitions. Selective excitation of Photosystem II (PS II) gives rise to State 2, whereby excitation energy may be redistributed in favour of Photosystem I (PS I). Selective excitation of PS I gives rise to State 1, whereby excitation energy distribution to PS II is favoured (for a review see Ref. 1).

The molecular mechanism of the transition to State 2 involves phosphorylation of thylakoid membrane proteins, in particular LHC-II [2]. This protein phosphorylation, which has been observed both in vitro in higher plant and green algal chloroplast thylakoid membranes [2–4] and in vivo in green algae [3,4], results from the action of a protein kinase, the activity of which is stimulated by the reduction of an electron transport intermediate with a standard redox potential close to that of plastoquinone [5,6]. Net reduction of plastoquinone occurs as a result of the selective excitation of PS II which thereby induces phosphorylation of LHC-II. Phosphorylated LHC-II then migrates away from PS II, decreasing the antenna size of the photosystem [7]. This decrease in PS II absorption cross-section is manifested as decreased PS II fluorescence yield at room temperature [8] and at 77 K [9], diagnostic for

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3′,4′dichlorophenyl)-1,1-dimethylurea; DPC, diphenyl carbazide; DCPIP, 2,6-dichlorophenol indophenol; Hepes, *N*-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; I_{50}, concentration of inhibitor giving 50% inhibition of maximum rate; *I*, intensity of incident light; LHC-II, the chlorophyll *a*/*b*-binding light-harvesting complex of PS II; MV, methyl viologen; PAGE, polyacrylamide gel electrophoresis; PS I, Photosystem I; PS II, Photosystem II; Q_A, the quinone which is the primary stable electron acceptor of PS II; Q_B, the quinone which is the secondary stable electron acceptor of PS II; SDS, sodium dodecyl sulphate; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

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State 2. State 1 occurs when plastoquinone becomes oxidized; the kinase is inactive and a light-independent phosphatase activity dephosphorylates LHC-II, permitting its reassociation with PS II [10].

In addition to LHC-II, several other thylakoid membrane proteins which are integral components of PS II also undergo light-dependent phosphorylation [11,12]. The D_1 Q_B-binding and D_2 Q_A-binding proteins are both phosphorylated [13], as are the CP43 chlorophyll *a*-binding protein of PS II [12] and a distinctive 8.3 kDa polypeptide which is the product of the chloroplast *psbH* gene. The phosphorylation reactions for LHC-II and PS II polypeptides show differential sensitivity to kinase inhibitors [14,15] and their respective dephosphorylation reactions proceed with different kinetics: the dephosphorylation of LHC-II occurs with a half-time in the range 8–10 min, whereas that for the 8.3 kDa polypeptide may be as long as 40 min [10]. Only the kinetics of LHC-II phosphorylation and dephosphorylation follow closely the kinetics of room temperature fluorescence changes indicative of state transitions [16,17].

An inhibition of PS II electron transport is observed upon phosphorylation of thylakoid membrane proteins, an inhibition which persists even at saturating light intensity and cannot therefore be ascribed solely to the decreased PS II light-harvesting capacity resulting from LHC-II phosphorylation [18,19]. PS II phosphorylation has been suggested to have an additional, direct effect on PS II function [19]. These effects are proposed to include increased dark stability of the Q_B^- semiquinone [20], an increase in the capacity of herbicides to inhibit PS II [21] and a phosphorylation-induced decrease in the rate constant for Q_A to Q_B electron transfer and a concomitant increase in the stability of the Q_A^- semiquinone [22,23]. Thylakoid membrane phosphorylation has also been proposed to contribute to a mechanism for a hydroxylamine-sensitive cyclic electron transport pathway around PS II which may reoxidise Q_A^- [24].

Very few studies have been directed towards dissecting out the respective functions of LHC-II and PS II polypeptide phosphorylation, although the differential effects of kinase inhibitors have been used to demonstrate a correlation between inhibition of oxygen evolution and phosphorylation of the 8.3 kDa polypeptide specifically [25]. In this study it is demonstrated that the PS II phosphoproteins are substrates for an exogenous alkaline phosphatase activity, whereas LHC-II phosphoproteins are not. Since the dephosphorylation of LHC-II phosphoproteins by endogenous phosphatase activity occurs at a much higher rate than does dephosphorylation of PS II phosphoproteins [10], it is possible by combining the effects of exogenous and endogenous phosphatase activities to generate four types of thylakoid membrane: membranes in which only PS II polypeptides are phosphorylated; in which only LHC-II is phosphorylated; in which both classes of polypeptide are phosphorylated; and in which neither class of polypeptide is phosphorylated. Consideration of the functional properties of these membrane types makes it possible to distinguish between effects of altered excitation energy distribution mediated by LHC-II phosphorylation and additional effects mediated by phosphorylation of PS II polypeptides.

Materials and Methods

Chloroplasts were prepared by a method modified from that in Ref. 26 by homogenization of 12-day-old pea seedlings (variety Feltham First) in ice-cold buffer comprising 0.33 M sorbitol/5 mM $MgCl_2$/10 mM NaCl/2 mM EDTA/50 mM Hepes-NaOH (pH 7.6). The homogenate was filtered through muslin and the chloroplasts pelletted by centrifugation at $8000 \times g$ for 2 min at 4°C. Chloroplasts were lysed in 5 mM $MgCl_2$/50 mM Hepes-NaOH (pH 7.6) and the thylakoids pelleted at $8000 \times g$ for 5 min at 4°C. Membranes were resuspended to a chlorophyll concentration of 0.5 mg ml-1 in a buffer comprising 100 mM sorbitol/5 mM $MgCl_2$/5 mM NaCl/50 mM Hepes-NaOH (pH 7.6) and maintained in the dark, on ice, until required.

Phosphorylation of thylakoid membranes was induced by incubation in the above buffer in the presence of 400 μM ATP at room temperature for 20 min under incandescent light (approx. 130 $\mu E\ m^{-2}\ s^{-1}$). For dephosphorylation time-course experiments thylakoid membranes were phosphorylated as above but with the addition of [γ-^{32}P]ATP (New England Nuclear) to a specific activity of 100 nCi $(nmol\ ATP)^{-1}$. Complete initial dephosphorylation of LHC-II was ensured by incubation in the dark for 45 min prior to radiolabelling.

Dephosphorylation experiments were performed on membranes harvested by centrifugation at $8000 \times g$ for 5 min and resuspended to a chlorophyll concentration of 0.5 mg ml^{-1} in 100 mM sorbitol/5 mM $MgCl_2$/100 mM Tricine-NaOH (pH 8.2), and incubated in the light in the presence of bovine alkaline phosphatase (EC 3.1.3.1) (Sigma) at 5 units ml^{-1}, or in the dark. Aliquots were removed at prescribed times and the thylakoid proteins precipitated in 80% (v/v) acetone at −20°C. Proteins were solubilized in a buffer containing 50 mM dithiothreitol, 2% (w/v) SDS, 7.5% (v/v) glycerol, 50 mM sodium carbonate and Bromophenol blue dye, and analysed by SDS-PAGE on 10–25% acrylamide gradient gels with the buffer system described in [27]. Autoradiography of Coomassie brilliant blue-stained gels was performed at −85°C with pre-flashed film (Amersham Hyperfilm MP), with intensifying screens.

For electron transport measurements, thylakoids from a single batch and phosphorylated as above were harvested, resuspended into sorbitol-Tricine buffer, di-

vided into four lots and treated in the following ways:
(a) Resuspended directly in sorbitol-Tricine buffer in the presence of 10 mM sodium fluoride and maintained in the light for 20 min. These are subsequently referred to as Type 1 membranes.
(b) Removed to the dark for 20 min prior to the addition of sodium fluoride to 10 mM. These are referred to as Type 2 membranes.
(c) Incubated in the light for 10 min prior to the addition of alkaline phosphatase and a further 10 min incubation in the light. Finally, sodium fluoride was added as above. These are Type 3 membranes.
(d) Incubated in the dark for 10 min prior to the addition of alkaline phosphatase and a further 10 min dark incubation. Sodium fluoride was added as above after the 20 minute incubation. These are Type 4 membranes.

The light regime used to maintain LHC-II phosporylation had an intensity of 50 μE m^{-2} s^{-1} All four preparations were maintained at 18°C throughout the incubations.

The four membrane types were exposed to the alkaline sorbitol-Tricine buffer for the same period of time, before harvesting and resuspension into sorbitol-Hepes buffer at pH 7.6 in the presence of 10 mM sodium fluoride. Electron transport through PS II was measured spectrophotometrically as reduction of DCPIP (0.1 mM) with water or DPC (0.5 mM) as electron donors. Actinic light was provided by fibre-optic cable from a stabilized light source, screened by Corning 4-96 filter, giving maximum light intensity 382 μE m^{-2} s^{-1} DCPIP reduction was measured as absorbance change at 550 nm and rates expressed assuming an extinction coefficient of 6.8 mM^{-1} cm^{-1}. Chlorophyll concentration, determined using the equations in Ref. 28, was 50 μg ml^{-1}. Light intensity was varied by the use of neutral-density filters. PS I partial reactions were measured in a Hansatech DW2 oxygen electrode at 20°C as oxygen uptake by a methyl-viologen-mediated Mehler reaction in the presence of 20 μM DCMU, with ascorbate-reduced DCPIP as electron donor. Methyl viologen was at 50 μM final concentration, ascorbate at 5 mM and DCPIP at 0.1 mM. All electron transport measurements were made on uncoupled membranes in the presence of nigericin (2 μM added from 200 μM stock in ethanol) and supplemented with 10 mM KCl (added from 1 M stock).

Results and Discussion

Exogenous alkaline phosphatase had a striking effect on the phosphorylation status of thylakoid membrane proteins. Fig. 1 shows SDS-PAGE analysis and subsequent autoradiography of ^{32}P-radiolabeled thylakoid proteins exposed to the enzyme for varying times at (pH 8.2) in the light. It is clear that in as little as 4 min all phosphate groups were removed from phosphoproteins of relative molecular mass 43 kDa and in the range 30–34 kDa, corresponding to CP43 and the reaction centre proteins D_1 and D_2, respectively. After 10 min incubation in the presence of alkaline phosphatase all detectable radiolabeling was also removed from the 8.3 kDa *psbH* polypeptide. In contrast, phosphorylation of LHC-II remained at a high level throughout the alkaline phosphatase incubation. After removal with this enzyme, no radiolabeling subsequently reappeared on PS II polypeptides when thylakoid membranes were washed and resuspended in sorbitol-Hepes buffer at pH 7.6 and incubated in the light (Fig. 1, lane L), eliminating the possibility that phosphorylation of PS II polypeptides

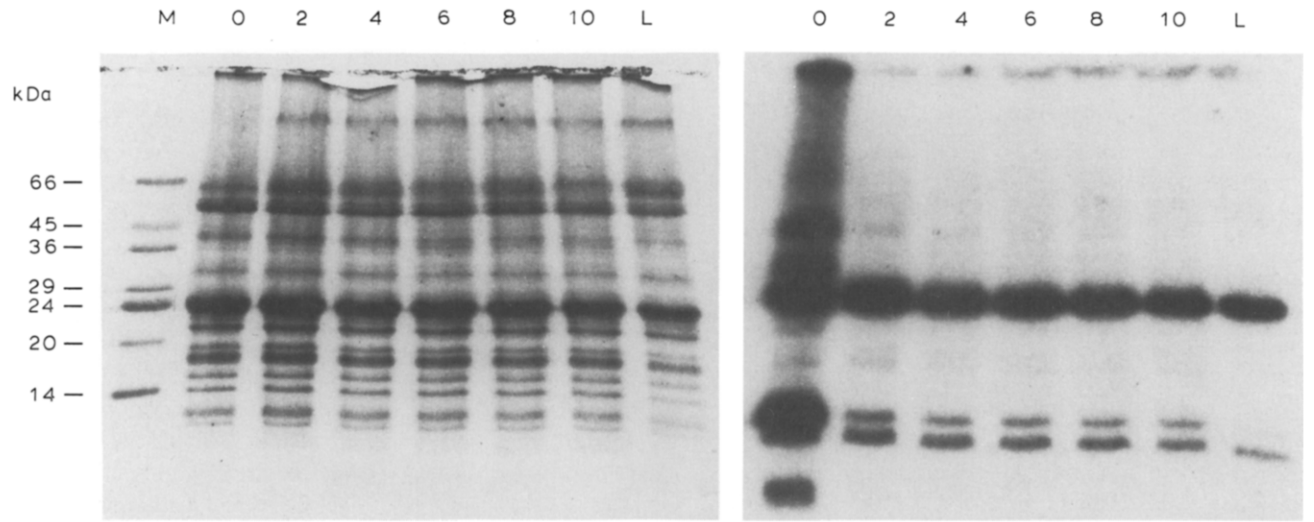

Fig. 1. SDS-PAGE (left) and subsequent autoradiography (right) of ^{32}P-radiolabeled thylakoid membranes incubated for various times in the presence of alkaline phosphatase at (pH 8.2). Samples were removed at time zero and after 2, 4, 6, 8 and 10 min. Lane L represents material treated with alkaline phosphatase for 10 min, washed and resuspended to pH 7.6 and reincubated in the light. Tracks were loaded with protein equivalent to 3 μg chlorophyll. Positions (M) and M_r of markers are indicated.

might arise through phosphate group transfer or exchange from LHC-II. A phosphate group transfer mechanism has been suggested on the basis of the observation that LHC-II deficient mutants of the green alga *Chlamydomonas reinhardtii* are unable to phosphorylate PS II polypeptides in vivo [29].

Dephosphorylation of LHC-II and of PS II polypeptides by endogenous phosphatase activity under dark conditions occurs with different kinetics [10], as demonstrated by the dephosphorylation time course for ^{32}P-radiolabeled thylakoid proteins shown in Fig. 2. From Fig. 2, which shows SDS-PAGE analysis and autoradiography of thylakoid proteins exposed to dark conditions for varying times, it is evident that after 16 min dark incubation at pH 8.2 essentially all phosphate groups were removed from LHC-II by endogenous phosphatase activity, whereas phosphorylation of PS II polypeptides remained largely unaffected.

By combining the differential effects of exogenous and endogenous phosphatase activities it is possible to generate four types of membrane:

Type 1 membranes, with both LHC-II and PS II polypeptides phosphorylated, are equivalent to the membranes removed at time zero (Figs. 1 and 2).

Type 2 membranes, with only PS II polypeptides phosphorylated, are membranes exposed to dark dephosphorylating conditions until dephosphorylation of LHC-II is complete (Fig. 2).

Type 3 membranes, with only LHC-II phosphorylated, are membranes exposed in the light to alkaline phosphatase activity for 10 min (Fig. 1).

Type 4 membranes, with neither class of polypeptide phosphorylated, are generated by the combined phosphatase effects illustrated in Figs. 1 and 2, by incubation in the dark in the presence of alkaline phosphatase (results not shown).

In order to eliminate variations in PS II function due to photoinactivation resulting from the light incubation used to maintain LHC-II phosphorylation and to ensure that valid comparisons could be made, Type 1 and Type 3 membranes were maintained under identical light and temperature conditions during incubation.

Separate and distinct kinases have been proposed to act on LHC-II and PS II polypeptides on the basis of the differential effects of kinase inhibitors upon the phosphorylation of these two classes of protein [14,15]. From the differing kinetics of dephosphorylation illustrated in Fig. 2 and the observation that exogenous alkaline phosphatase acts exclusively to dephosphorylate PS II polypeptides (Fig. 1), it seems probable that the two classes of phosphoprotein are also substrates for separate thylakoid membrane phosphatases of quite different specificity.

The differing electron transport properties of the four types of thylakoid membrane, measured as the light-dependent, PS-II-mediated reduction of DCPIP, with DPC as electron donor, are shown in Figs. 3, 4 and 5. Fig. 3a shows light-dependence curves for PS II electron transport reactions in Type 1 and Type 2 membranes, both types having phosphorylated PS II polypeptides but with only Type 1 membranes retaining phosphorylated LHC-II. Type 1 membranes showed lower rates of electron transport even at saturating light intensity, an observation illustrated further by Fig. 3b, which shows Eadie-Hofstee plots of rate V against V/I for the data in Fig. 3a. Kinetic plots for electron transport in Type 1 and Type 2 membranes appear to be biphasic, with distinct reaction components occurring at high and low light intensities. At low light intensity, rates for the two membrane types tend towards the same approximate V_{max} (68 μmol DCPIP (mg Chl)$^{-1}$ h^{-1}), indicating a light-saturable effect due to decreased

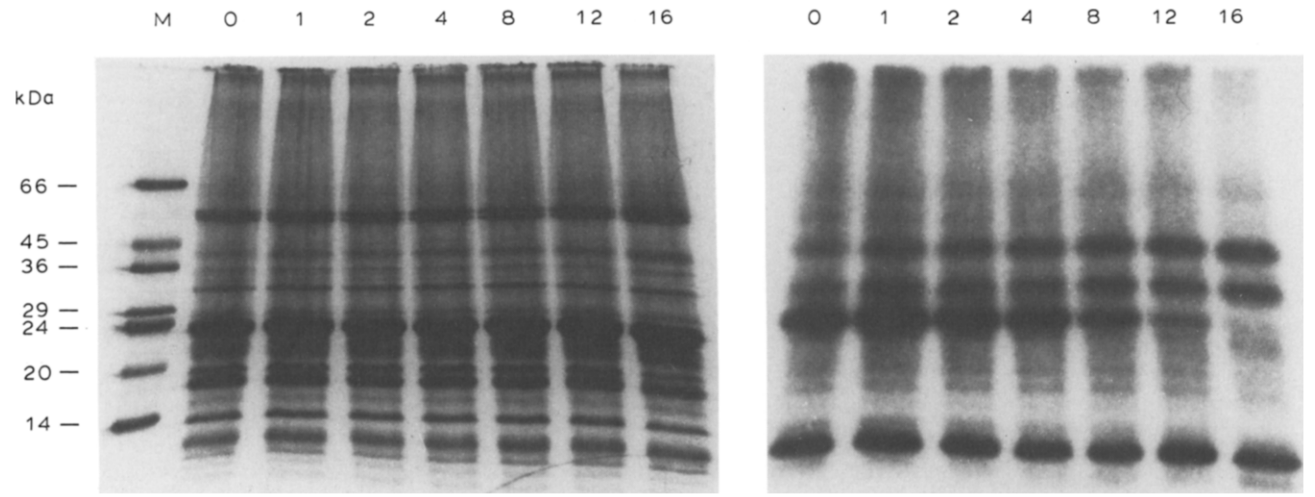

Fig. 2. SDS-PAGE (left) and autoradiography (right) of ^{32}P-radiolabeled thylakoid membranes incubated for various times in the dark. Samples were removed at time zero and after 1, 2, 4, 8, 12 and 16 min. Tracks were loaded with protein equivalent to 3 μg chlorophyll. Positions (M) and M_r of markers are indicated.

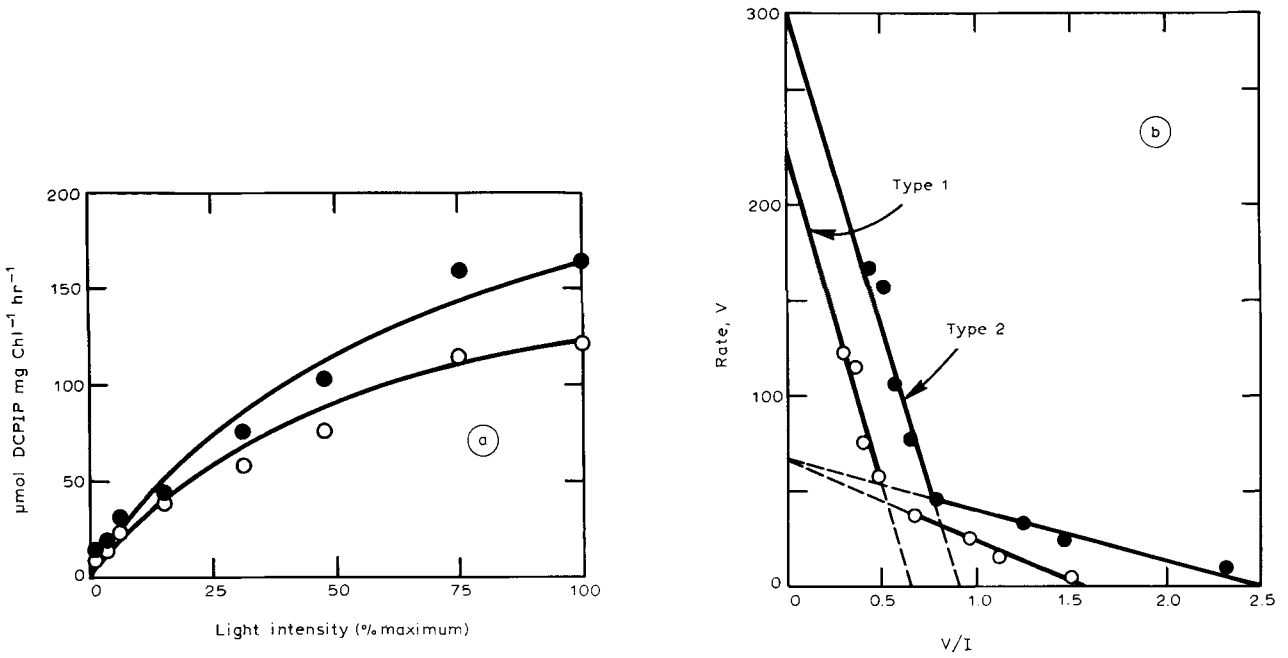

Fig. 3. (a) Light-dependence curves for PS-II-mediated electron transport from DPC to DCPIP for Type 1 (○)) and Type 2 (●) membranes, and (b) Eadie-Hofstee plots of rate V against V/I for the data in (a). Type 1 membranes have phosphorylated PS II and LHC-II, Type 2 membranes have phosphorylated PS II only. Data points are the means of four separate measurements.

PS II absorption cross-section as a result of LHC-II phosphorylation. At high light intensity, however, a non-saturable effect predominates, with rates tending towards different maxima with the same K_m.

The light conditions used to maintain LHC-II phosphorylation in Type 1 membranes were observed to give a decrease in the saturated PS II activity of only 3.5% with respect to the activity of the membranes after the initial phosphorylation incubation. Differences in activity between Type 1 and Type 2 membranes cannot therefore be attributed, except in small part, to photoinactivation resulting directly from the light incubation of the Type 1 membranes.

The removal of phosphate groups from PS II polypeptides by alkaline phosphatase activity resulted in a significant decrease in electron transport capacity at saturating light intensity. This is demonstrated by comparison of Fig. 3 with Fig. 4a, which shows light-dependence curves for Type 3 and Type 4 membranes. Neither type of membrane has phosphorylated PS II polypeptides, but Type 3 membranes retain phosphorylated LHC-II. An additional, superimposed inhibitory effect of LHC-II phosphorylation is also observed for Type 3 membranes, as demonstrated for Type 1 membranes. Consideration of Eadie-Hofstee plots for the data in Fig. 4a, shown in Fig. 4b, indicates that the superim-

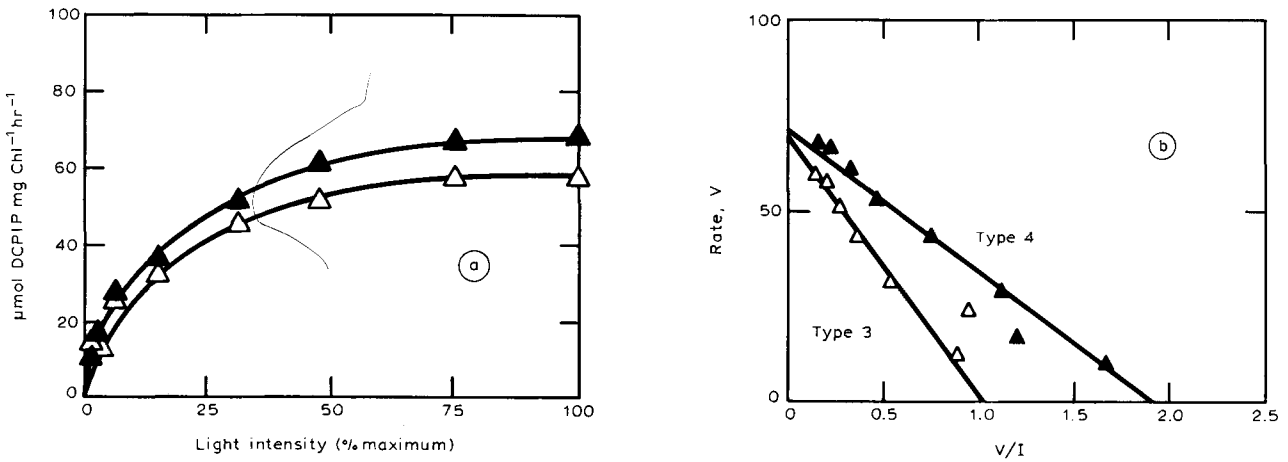

Fig. 4. (a) Light-dependence curves for PS-II-mediated electron transport from DPC to DCPIP for Type 3 (△) and Type 4 (▲) membranes, and (b) Eadie-Hofstee plots of rate V against V/I for the data in (a). Type 3 membranes have LHC-II phosphorylated only. Type 4 membranes have neither LHC-II nor PS II phosphorylated. Data points are the means of four separate measurements.

posed inhibitory effect of LHC-II phosphorylation occurs at limiting light intensity and is therefore due to decreased PS II light-harvesting. Rates for Type 3 and Type 4 membranes tend towards the same V_{max} of 70 μmol DCPIP (mg Chl)$^{-1}$ h^{-1}, comparable to the deduced value for V_{max} for the component occuring at low light intensity in Type 1 and Type 2 membranes.

Although a small difference in activity between Type 3 and Type 4 membranes would be expected to result from the light incubation of the Type 3 membranes used to maintain LHC-II phosphorylation, this cannot fully account for the observed differences. In addition, the observed kinetics for Type 3 and Type 4 membranes are not consistent with differences in activity resulting from a non-saturable photoinactivation effect exerted on Type 3 membranes.

By comparison of Figs. 3b and 4b It is clear that the inhibition of PS II activity resulting from the removal of phosphate groups from PS II polypeptides occurs to a greater extent at higher light intensities. This is illustrated in Fig. 5, which shows the nature of the change in the ratio of rates Type 1/Type 3 (both with phosphorylated LHC-II, Type 1 with phosphorylated PS II polypeptides) and Type 2/Type 4 (both with non-phosphorylated LHC-II, Type 2 with phosphorylated PS II), with varying light intensity. Both ratios change with the same relationship to light intensity, with the ratios increasing in magnitude with increasing light intensity, indicating relatively greater PS II electron transport under higher irradiance in membrane types containing phosphorylated PS II polypeptides.

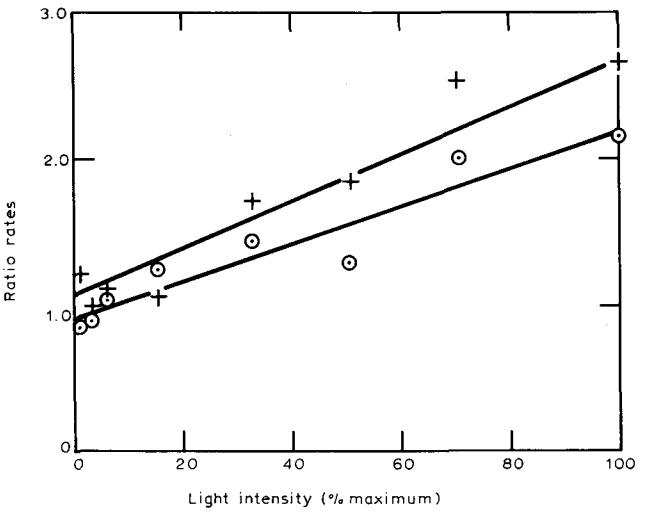

Fig. 5. The change in the ratio of rates for PS-II-mediated electron transport in membrane types with or without phosphorylated PS II polypeptides, as a function of light intensity. Regression analyses (least squares method) are fitted to each data set. Data, derived from Figs. 3 and 4, are for the ratio of rates Type 1/Type 3 (○) ($r = 0.96$, $P < 0.001$) and Type 2/Type 4 (+) ($r = 0.88$, $p < 0.005$). Type 1 membranes have phosphorylated LHC-II and PS II. Type 2 membranes have phosphorylated PS II only. Type 3 membranes have phosphorylated LHC-II only. Type 4 membranes have neither LHC-II nor PS II phosphorylated.

TABLE I

Changes in PS II and PS I electron transport partial reactions induced by phosphorylation of thylakoid membrane proteins

Summary of electron transport properties of the four types of thylakoid membrane detailed in the text, and listed 1–4. Type 1 refers to membranes with phosphorylated LHC-II and PS II. Type 2 membranes have phosphorylated PS II only. Type 3 membranes have phosphorylated LHC-II only. Type 4 membranes have neither LHC-II nor PS II phosphorylated. Rates were determined at a light intensity of 382 μE m^{-2} s^{-1}. Figures in parentheses describe each value as a percentage of the maximum rate achieved for each reaction. Rates given are means of four separate measurements.

	PS II reactions		PS I reaction
	$H_2O \rightarrow$ DCPIP (μmol O_2 (mg Chl)$^{-1}$h^{-1}	DPC $\rightarrow$ DCPIP (μmol DCPIP (mg Chl)$^{-1}$h^{-1})	Asc/DCPIP $\rightarrow$ MV/O_2 (μmol O_2 (mg Chl)$^{-1}$h^{-1})
Type 1	99	124 (74)	211 (97)
Type 2	–	168 (100)	218 (100)
Type 3	47	59 (35)	206 (94)
Type 4	–	70 (42)	213 (98)

As emphasized in previous reports [19], the inhibitory effect of thylakoid membrane protein phosphorylation cannot be at the water oxidation steps of PS II, since variations in functional properties are maintained even with DPC as electron donor. Nor was the effect of either phosphatase to inhibit generally electron transport reactions: PS I partial reactions were not influenced to the same extent as PS II partial reactions by phosphorylation status (Table I). This observation would also tend to indicate that some form of proteolytic degradation or other form of membrane degeneration is not responsible for the observed differences in PS II activity. Nor was any proteolysis evident from the SDS-PAGE analyses (Figs. 1 and 2).

Increased PS I electron transport might be anticipated for Type 1 and Type 3 membranes if phosphorylated LHC-II is able to act as an antenna for PS I in State 2 [30]. No significant effect of LHC-II phosphorylation on PS I electron transport was detected (Table I). Electron transport properties of the four membrane types are summarized in Table I.

The capacity of the herbicide DCMU to inhibit PS II electron transport was not altered by phosphorylation of either LHC-II or PS II. DCMU inhibition curves showed no appreciable change in I_{50} for any of the four classes of membrane, remaining within the range 55–60 nM, as illustrated in Fig. 6. This indicates that changes in PS II electron transport rates are not due to phosphorylation-induced changes in Q_B-binding. This contrasts with previous work [21] which demonstrated increased capacity of herbicides to inhibit PS II, but is consistent with the observations of Hodges and coworkers [15], who were of the opinion that changes in

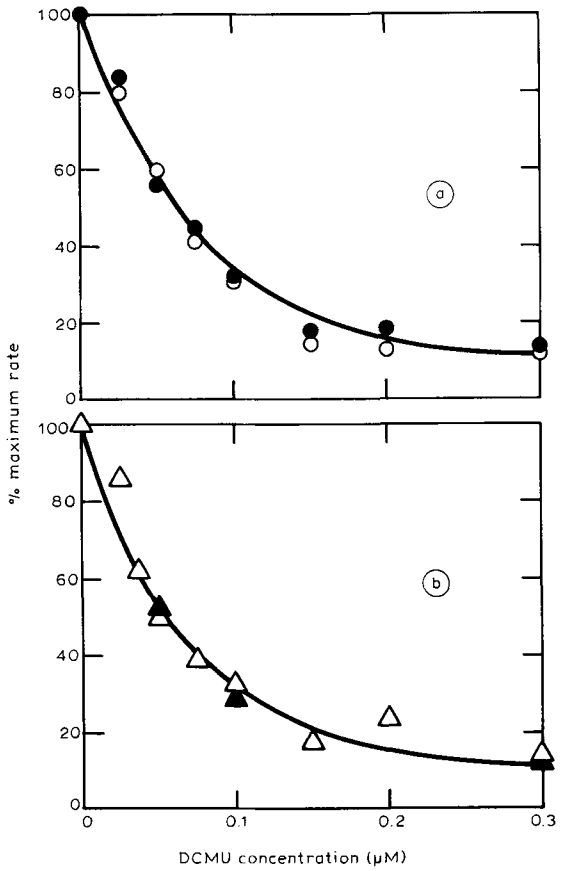

Fig. 6. DCMU inhibition curves for the four types of thylakoid membrane described in the text and in the legend to Fig. 5. (a) Type 1 (○) and Type 2 (●) and (b) Type 3 (△) and Type 4 (▲). PS-II-mediated electron transport rates for each membrane type, at various concentrations of DCMU, are expressed as percentage of the light-saturated rate for each type in the absence of DCMU. Maximum rates, in μmol DCPIP (mg Chl)$^{-1}$ h^{-1}, were:–Type 1, 111; Type 2, 149; Type 3, 52; Type 4, 64. Data points were the means of four separate measurements.

DCMU binding capacity reflected differences in redox state of plastoquinone between phosphorylated and non-phosphorylated membranes. Such differences were proposed to give rise to variations in competition between oxidized plastoquinone and DCMU. In this study, membrane samples were maintained under the same conditions after adjustment of the phosphorylation status and could therefore be expected to maintain the same redox state for plastoquinone.

Previous reports [18,19] have demonstrated phosphorylation-induced inhibition of PS II at saturating light intensity. The failure of saturating light to alleviate this inhibition was proposed to be due to a mechanism involving PS II phosphorylation acting directly upon PS II. This mechanism would supplement inhibition due to the decreased PS II absorption cross-section resulting from LHC-II phosphorylation and its subsequent migration. The inhibitory effect of LHC-II phosphorylation alone could therefore be presumed to be lost under saturating light conditions. Data from this study show this to be the case. Thylakoid membranes in which phosphorylation of PS II polypeptides does not occur (Type 4 compared to Type 3) still show inhibition of PS II electron transport at limiting light intensity. This effect can only be mediated through phosphorylation of LHC-II.

The molecular mechanism giving rise to LHC-II migration and decreased PS II absorption cross-section has been proposed to involve electrostatic repulsion between phosphate groups in a plane parallel to that of the thylakoid membrane [31]. The induction of this spatial separation between PS II and LHC-II in State 2 is, however, unlikely to occur by electrostatic repulsion between LHC-II and PS II phosphate groups since the inhibitory effect of LHC-II phosphorylation is observed even in the complete absence of PS II phosphorylation (Fig. 4). Electrostatic repulsion between phosphate groups of LHC-II complexes in non-mobile and mobile subpopulations [32] could occur, or alternatively phosphorylation-induced conformational change [33] might induce the separation of antenna complexes.

From the data presented it is evident that a mechanism involving phosphorylation of PS II polypeptides may be required for PS II electron transport at high light intensity, consistent with a role in protection from photoinhibition [34,35]. Indeed, the presence of bicarbonate ions has been shown to moderate photoinhibition and to inhibit specifically the phosphorylation of the 8.3 kDa PS II phosphoprotein [36].

Phosphorylation of PS II polypeptides has been proposed to decrease non-cyclic electron transport, but permit an energy-dissipating cyclic electron flow around PS II [24]. However, the data in this study conflict with the cause-and-effect relationship for PS II phosphorylation and PS II inhibition suggested by previous investigations. This study shows that after exposure to conditions giving rise to phosphorylation of PS II polypeptides, the presence of phosphate groups on PS II polypeptides is required for optimum PS II function specifically at high light intensity. A plausible explanation could be that a mechanism involving PS II phosphorylation prevents generation of the fully-reduced quinol Q_A^{2-} under high intensity illumination. Generation of this species has been observed as the first stage of PS II photoinhibition, and may result in loss of Q_A from the reaction centre [37]. The use of exogenous phosphatase activity may provide a useful tool with which to manipulate rapidly and specifically the phosphorylation status of reaction centre constituents and would enable investigations into possible changes in reaction centre conformation and photochemistry resulting from protein phosphorylation.

Phosphorylation of LHC-II will also act to moderate photoinhibition by decreasing PS II light-harvesting capacity. The functional effects of PS II and LHC-II phosphorylation may therefore be complementary in

effect but functionally distinct and probably regulated by different kinase and phosphatase systems.

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