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PHOSPHORYLATION OF CHLOROPLAST THYLAKOIDS DECREASES THE MAXIMUM CAPACITY OF PHOTOSYSTEM-II ELECTRON TRANSFER

P. HORTON and P. LEE

Research Institute for Photosynthesis and Department of Biochemistry, University of Sheffield, Sheffield, S10 2TN (U.K.)

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Phosphorylation of chloroplast thylakoid polypeptides by the light-activated protein kinase was found to decrease the light-saturated rate of whole chain and Photosystem-II electron transport. This decrease in electron-transport capacity was reversible and was found to correlate with the phosphorylation-induced decrease in chlorophyll fluorescence.

Introduction

Chloroplast thylakoids contain protein kinase activity which results in the phosphorylation of several polypeptides, including those comprising the LHCP and several others including ones with molecular weights of 9000 and 32000 (see Refs. 1 and 2 for reviews). It is generally thought that the functional significance of protein phosphorylation is to regulate the relative rates of excitation of PS II and PS I, principally by controlling their absorption cross-sections [1,3–5].

However, recent data has provided evidence for other effects of phosphorylation on electron transfer which are not explainable by alteration in the distribution of excitation energy. Thus sensitivity to DCMU is increased [6], charge density around Photosystem II is enhanced [7,8], B^- is stabilized [9] and a dissipative cyclic pathway around PS II is increased [10]. Some workers have found an ATP-induced fluorescence change (normally a marker for the change in excitation distribution) that is not associated with an elevated rate of photon delivery to PS I [11]. These data tend to suggest that protein phosphorylation may directly affect PS II. In this paper, measurement of light-saturated rates of electron transfer through PS II are shown to be reversibly inhibited by reversible protein phosphorylation.

Materials and Methods

Chloroplasts were isolated from peas and incubated with and without ATP as previously described [3]. Rates of electron transfer of phosphorylated and nonphosphorylated chloroplasts were measured using standard procedures; PS-II electron transfer from water to oxidized diaminodurene (DAD) followed the procedure of Izawa et al. [12]. PS-I electron transfer with ascorbate plus DAD as the donor and methyl viologen as the acceptor used the procedure of Ort and Izawa [13] as described in Ref. 8. Whole chain electron transfer used methyl viologen (0.1 mM) as acceptor in a medium containing NaN_3 (0.2 mM)

Abbreviations: PS I, II, Photosystem I, II; LHCP, light-harvesting chlorophyll protein; B, the secondary quinone acceptor of Photosystem II; DCMU, 3-(3',4'-dichlorophenyl)-1,1-dimethylurea; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; DAD, diaminodurene; DCIP, 2,6-dichlorophenolindophenol.

and NH_4Cl (5 mM). The rate of DCIP reduction was measured as described previously [14] in the presence and absence of diphenyl carbazide. The assay medium contained 0.33 M sorbitol, 5 mM $MgCl_2$, 2 mM EDTA, 1 mM $MnCl_2$ and 50 mM Hepes adjusted to pH 7.6 with KOH. Chloroplasts were diluted to 50 μg/ml for whole-chain and PS-II assays but to 25 μg/ml for the PS-I and DCIP assays. O_2 uptake or evolution was measured in a modified Hansatech O_2 electrode [15]. Heat-filtered red actinic illumination was defined by RG610 + Balzars K65 filters and its intensity was adjusted using neutral density filters.

Results

The light-saturated rates of whole-chain electron transfer (H_2O to methyl viologen) were found to be lower in thylakoids pre-illuminated with ATP compared to those incubated without ATP. (Table I). In Fig. 1a it is seen that the difference in rate is less pronounced under low intensity of light than at light saturation. Similar results were obtained using DCIP as an electron acceptor (Fig. 1b). When DAD, an electron acceptor specific to PS II [13], is used the inhibitory effect of ATP at light saturation is still clearly observed (Fig. 1c). However, in this assay, the slope of the light-intensity curve is also diminished in ATP-treated chloroplasts and a larger change in rate is observed under light limitation than at light saturation. This

TABLE I

EFFECT OF ILLUMINATION WITH ATP ON THE LIGHT-SATURATED RATES OF ELECTRON TRANSFER THROUGH PS II AND PS I

For details see materials and methods. Whole chain (H_2O to MeV) values are given ±S.E.M. (95% confidence limits) and are means of 21 separate experiments. PS-I values are means of five determinations with the range given in brackets.

	Rate of electron transfer (μmol O_2 per mg Chl per h)	
	PS II + PS I	PS I
+ATP	102.8 ± 8.2	323 (316–335)
−ATP	124.2 ± 8.8	315 (296–341)

is indicative of the change in rate of excitation of PS II which occurs after pre-illumination with ATP [3,16,17]. In contrast to the decrease in the light-saturated rates of PS II and of whole chain electron transfer which occur after ATP treatment, measurement of the maximum rate of PS-I transfer (DAD/ascorbate to methyl viologen) was unchanged (Table I).

The decrease in the maximum rate of PS II electron transfer resulted from phosphorylation of thylakoid proteins. Thus, parallel measurements of ^{32}P incorporation into thylakoid proteins showed extensive phosphorylation of LHCP and other polypeptides [18]. Three other observations con-

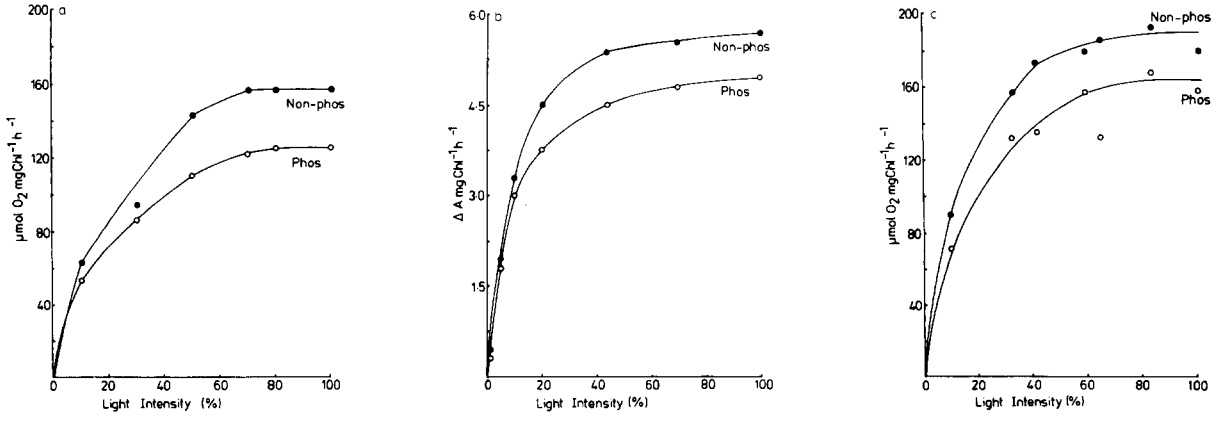

Fig. 1. Effect of phosphorylation on the rate of electron transfer. Phosphorylated (Phos) and unphosphorylated (Non-phos) chloroplasts were prepared as described in the methods. Assays were (a) H_2O to methyl viologen in the presence of uncoupler, (b) H_2O to DCIP in the presence of uncoupler and (c) H_2O to DAD/ferricyanide. In (b) rates are expressed as $\Delta A_{550-500}$. Saturating-light intensity was 350 $W \cdot m^{-2}$.

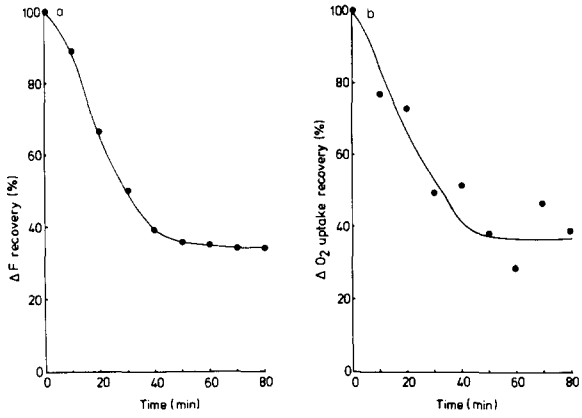

Fig. 2. Influence of dephosphorylation on the difference in fluorescence (a) and electron-transfer capacity (b). Chloroplasts were phosphorylated by illumination with ATP and allowed to dephosphorylate by incubation in darkness. The percentage differences between the phosphorylated samples and an unphosphorylated control, treated identically, were then plotted against dephosphorylation time. Each data point is the mean of four values recorded during four different experiments.

firmed the involvement of protein phosphorylation in the electron-transport effect. Firstly, the effect of ATP was reversible upon dark incubation, a half-time of approx. 20 min being seen (Fig. 2a); dephosphorylation of thylakoids is similarly slow [17–19]. Secondly, it was found that NaF, an inhibitor of phosphatase action, prevented the increase in the light-saturated rate of electron transfer that occurs during dark incubation (Table II). Thirdly, the effect of ATP on electron-transfer capacity was always associated with a decrease (15–20%) in the maximum yield of chlorophyll fluorescence which has been clearly shown to be associated with protein phosphorylation [3,20–22].

Several polypeptides in addition to LHCP are phosphorylated upon illumination of the pea thylakoids used in these experiments [18]. In particular a 9 kDa polypeptide which is a component of PS II [17] is a major phosphoprotein. Experiments were performed to investigate whether phosphorylation of LHCP was involved in the decrease in PS-II electron-transfer rate or whether the 9 kDa polypeptide could be implicated. It has been shown that the dephosphorylation of the 9 kDa phosphoprotein is slower than that of LHCP [17–19] and that an increase in yield of PS-II fluorescence-correlates with the dephosphorylation of LHCP rather than that of the 9 kDa polypeptide [17,18]. Fig. 2b shows results of assays of chlorophyll fluorescence made on the same chloroplast samples used for electron transfer assays; there is clearly a close correlation between the reversal of the electron-transfer effect and the dephosphorylation of LHCP that causes the fluorescence increase.

The ATP concentration can be used to control the degree of phosphorylation of LHCP and the 9 kDa polypeptide, the $S_{0.5}$ for the former being approx. 50 μM and for the latter approx 0.3–0.4 mM [18]. The ratio of the electron-transfer effect at high ATP to that seen at low ATP can therefore be used to investigate the involvement of the 9 kDa polypeptide in the effect on PS II electron

TABLE II

EFFECT OF THE PROTEIN PHOSPHATASE INHIBITOR, NaF, ON THE LIGHT-SATURATED RATE OF ELECTRON TRANSFER

Thylakoids were illuminated for 10 min with 0.2 mM ATP and then incubated ± NaF for a further 30 min in darkness. NaF was added to the dephosphorylated sample and electron-transfer assays were performed as for Fig. 1a. Each value is the mean of five assays performed on two separate experiments. The range is given in brackets.

	Rate of electron transfer μmol O_2 per mg Chl per h)
+ NaF (phosphorylated)	76 (75–76)
− NaF (dephosphorylated)	91 (89–92)

TABLE III

EFFECT OF ATP CONCENTRATION ON THE CHANGES IN FLUORESCENCE AND ELECTRON TRANSFER CAPACITY

Chloroplasts were illuminated for 5 min in the presence of 1.0 mM and 0.2 mM ATP, as described in Ref. 18. Each data value is the means of four assays. ΔF and ΔO_2 refer to the differences in maximum fluorescence and light-saturated rates of electron transport between phosphorylated and non-phosphorylated chloroplasts expressed as a percentage of the nonphosphorylated values.

[ATP] (mM)	ΔF	ΔO_2
1.0	22	16
0.2	17	12
Ratio 1.0/0.2	1.29	1.33

TABLE IV

EFFECT OF HYDROXYLAMINE ON THE RATE OF ELECTRON TRANSFER IN PHOSPHORYLATED AND NON-PHOSPHORYLATED THYLAKOIDS

The rate of DCIP reduction was measured in saturated light as described in Fig. 1b. NH_2OH (10 mM) was added 10 s prior to illumination and the rate then measured over the first 10 s. Each value is the average of eight assays performed on two different batches of phosphorylated and nonphosphorylated chloroplasts, with ranges given in brackets.

	Rate of DCIP reduction ($\Delta A_{550-500}$ per mg Chl per h)		Difference (%)
	Nonphosphorylated	Phosphorylated	
Minus NH_2OH	72.9 (71–73)	60.0 (59–61)	17.7
Plus NH_2OH	56.7 (54–58)	51.9 (49–53)	8.5

transfer. As seen in Table II, the decrease in electron-transfer rate responds in the same way to ATP concentration as does the fluorescence yield. If the 9 kDa polypeptide were to be involved in the electron-transfer effect, a 1.0 : 0.2 ratio of larger than 2 [18] would be predicted instead of the value of 1.3 obtained.

Addition of the PS-II donor, diphenyl carbazide did not increase the rate of electron transfer in either the phosphorylated or non-phosphorylated thylakoids, suggesting a lesion in the water oxidation was not involved (data not shown). However, addition of hydroxylamine, though slightly inhibitory to both chloroplast samples, was able to halve the observed difference in rate between phosphorylated and non-phosphorylated chloroplasts (Table IV).

Discussion

The data presented here show that phosphorylation of thylakoid proteins causes a decrease in the electron-transfer capacity of PS II (and whole chain) electron transfer. The decrease is reversible and is correlated with the fluorescence decrease brought about by phosphorylation.

This correlation, observed in terms of dephosphorylation kinetics and [ATP] requirement, suggests that LHCP phosphorylation is involved since other work has indicated an association between LHCP phosphorylation and the fluorescence change [17,18]. Conversely other polypeptides which become phosphorylated such as the 9 kDa polypeptide are dephosphorylated more slowly than LHCP [17,18] and, in the case of the 9 kDa polypeptide, have a higher [ATP] requirement [18].

The mechanism of the decrease in rate appears not to be due to an inhibition of electron donation from water splitting. The unchanged ratio of variable-to-maximum fluorescence (F_v/F_m) after phosphorylation [3–5] indicates no decrease in the yield of photochemistry, unless several factors are changing which co-incidentally balance out. The partial elimination of the difference by addition of NH_2OH suggests the involvement of a dissipative back-reaction [10]. Indeed, an elevated extent of a NH_2OH-sensitive back-reaction has been reported after phosphorylation [10]. However, the NH_2OH effect on electron transfer, shown in Table II, is complicated by its inhibitory effect and the data could easily be interpreted as differential sensitivity to NH_2OH. Nevertheless, it is clear that phosphorylation decreases the electron-transfer capacity of PS II, and that this effect is presumably related to a series of observations. which point to altered PS-II properties. Besides the elevated back-reaction [10], the stability of B^- is enhanced [9], sensitivity to DCMU is elevated [6] and affinity for charged oxidants is reduced [7]. These observations indicate that an alteration in the Q/B region of electron transfer occurs after phosphorylation.

The inhibitory effect of phosphorylation on PS II has several important implications. Firstly, conditions normally associated with photo-inhibition (i.e., excess illumination over dissipative capacity of electron transfer) would lead to a phosphorylation-induced decrease in electron transfer. This effect could, in fact, be regarded as a reversible photo-inhibition. Possibly, this reversible effect affords partial protection against irreversible damage to PS-II centres during strong illumination. Secondly, interpretation of the physiological role of protein phosphorylation requires careful examination. The involvement of protein phosphorylation in the state transitions under light-limiting conditions is well established both from in vitro [22] and

in vivo experiments [23]. The results presented here suggests an additional regulatory role for protein phosphorylation under conditions of high irradiance. The effects on both capacity and absorption cross-section of PS II as a result of phosphorylation are additive and would represent a powerful feedback response to overreduction of the plastoquinone pool. A reduction of PS II capacity may be a part of the process which changes the balance in rates of electron transfer in PS II and PS I. Whilst this may seem to represent a decrease in efficiency of noncyclic electron flow, that of cyclic electron flow around PS I could be enhanced as a result of better 'poising' (i.e., prevention of overreduction). Low concentrations of DCMU, for instance, have been shown to increase cyclic electron flow in intact chloroplasts [24,25] and protein phosphorylation may well have a similar effect. This role for protein phosphorylation, though presently only tentative, is supported by recent work on maize chloroplasts which revealed maximum phosphorylation under conditions of high ATP demand [26].

In contrast to the results described here, Farchaus et al. [16] and Steinback et al. [17] did not observe a decrease in the maximum rate of PS-II electron transfer upon phoshorylation. The reason for this difference is unexplained at present. If protein phosphorylation were to delay the onset of photo-inhibition, the phosporylation-induced difference in the maximum rate of PS II may disappear. However, in Ref. 16 careful precautions were taken in minimize photo-inhibition. It should be noted, however, that in Ref. 16 light-saturated PS-II rates equivalent to only approx. 75 μmol O_2 per mg CHl per h were reported, whereas in our experiments rates of 100–150 were recorded. Perhaps the expression of the effect of phosphorylation on light-saturated electron transfer requires thylakoids which show an electron-transfer capacity closer to that occurring in vivo.

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