

Stimulation of a cyclic electron-transfer pathway around photosystem II by phosphorylation of chloroplast thylakoid proteins

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Fluorescence induction kinetics of phosphorylated and unphosphorylated chloroplast thylakoid membranes have been studied under low light intensity. Hydroxylamine is able to enhance the fluorescence yield to a greater extent in phosphorylated membranes in both the presence and absence of DCMU. Examination of the light intensity dependence of the fluorescence yields leads to the conclusion that protein phosphorylation enhances the efficiency of a NH_2OH -sensitive cyclic electron-transfer pathway around photosystem II.

Photosynthesis

Chloroplast

*Protein phosphorylation
Chlorophyll fluorescence*

Thylakoid

Photosystem

1. INTRODUCTION

The protein kinase found in chloroplast thylakoids catalyses the ATP-dependent phosphorylation of several membrane polypeptides including those of LHC-II and a number of photosystem II components (reviews [1,2]). The protein kinase is reversibly activated by reduced plastoquinone, so providing a basis for regulation of photosynthetic electron transfer [1–5]. The principal effects of protein phosphorylation have been considered mainly in terms of light harvesting; thus, protein phosphorylation results in a redistribution of excitation away from PSII in favour of PSI. This has been measured by changes in the yields of PSII and PSI fluorescence [3,4,6,9] and the quantum efficiencies for PSII and PSI partial reactions [8,9]. However, several other effects

have been reported which suggest that protein phosphorylation may regulate photosynthesis by direct alteration of PSII as well as by these effects on light harvesting. Thus, it has been observed that:

- (i) The affinity for the PSII herbicide DCMU is enhanced by phosphorylation [10];
- (ii) The charge density around Q, the primary acceptor of PSII is increased by phosphorylation [11];
- (iii) The stability of the species B^- , the two electron gate of PSII, is enhanced by phosphorylation [12].

Here, we present evidence which suggests that the efficiency of a dissipative pathway around PSII, involving Q oxidation via the oxidising side of PSII is enhanced by protein phosphorylation.

2. MATERIALS AND METHODS

Pea chloroplasts were isolated as in [13]. Protein phosphorylation was carried out at 20°C by incubation for 25 min at 100 μg chl/ml in the presence of 0.2 mM ATP. The medium contained 0.33 M sorbitol, 10 mM NaCl, 5 mM MgCl_2 , 10 mM NaF and 50 mM Hepes (pH 7.6). Illumina-

Abbreviations: PSII, photosystem II; PSI, photosystem I; Q, the primary acceptor of PSII whose redox state controls the yield of fluorescence; F_0 , the level of fluorescence when Q is oxidised; F_{mv} , $F_m - F_0$, the variable fluorescence; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; LHC-II, the light harvesting complex associated with PSII

tion was provided by tungsten lamps filtered by Corning 4-96 filters and 10 cm of H₂O giving an intensity of 36 W.m⁻². At the end of the incubation, the thylakoids were centrifuged and resuspended to about 2 mg/ml. All media contained NaF. Chlorophyll fluorescence was assayed in a stirred cuvette at 20°C, excitation being provided by weak blue light (Corning 4-96 + OCLI Blue Dichroic Filters) and detected front face by an EMI 9558B photomultiplier blocked by a Schott RG665 cut-off filter.

3. RESULTS AND DISCUSSION

Fig.1. demonstrates the fluorescence induction kinetics of uncoupled chloroplasts excited by weak light in the absence of DCMU. The steady-state yield under these conditions will depend on 3 variable factors:

- (1) the exciton density in PSII;
- (2) the relative electron fluxes through PSII and PSI;
- (3) dissipation of excitation by re-oxidation of Q by the oxidising side of PSII, the so-called PSII back reaction [14].

Comparing phosphorylated and non-phosphorylated chloroplasts it is seen that the steady-state yield of fluorescence is decreased by about 40% by phosphorylation. Looking at only the variable components it is seen that phosphorylation causes a 4-fold decrease in the steady-state fluorescence level (compare F in fig.1A,B). Because of the non-linear relationship between F_v and $[Q]$ the change in the level of reduced Q may be as high as 6-fold. In this experiment, the change due to phosphorylation observed in F_o was 14% and in F_m 18%. (F_m was the value measured in the presence of 20 μ M DCMU, 10 mM NH₂OH and dithionite.) Thus the several-fold increases in the level of Q oxidation in the phosphorylated sample cannot be accounted for solely by the change in exciton density in PSII (mechanism 1 above). Therefore the fluorescence yields in fig.1 have to be accounted for by increased rates of Q oxidation as a result of phosphorylation, either by an increased efficiency of transfer to PSI or by increased rate of back reaction. NH₂OH is known to inhibit back reaction [15] and addition of this reagent causes a much larger proportional change in the phosphorylated compared to the

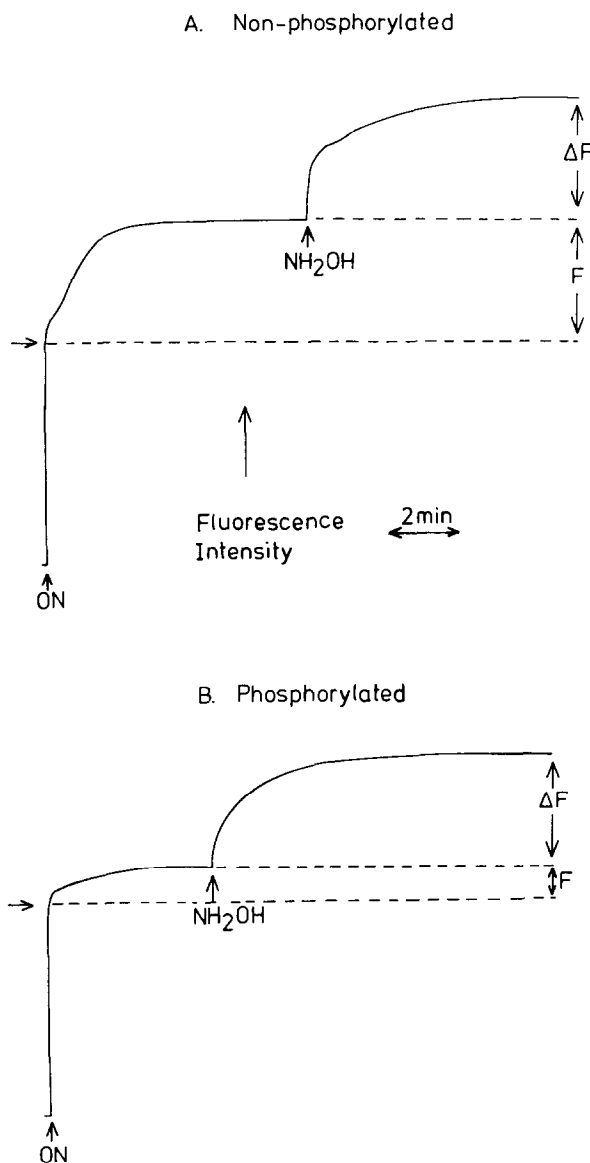


Fig.1. Fluorescence induction in non-phosphorylated (A) and phosphorylated thylakoids (B). Light intensity, 3 W.m⁻²; chlorophyll, 10 μ g/ml; NH₂OH added to 10 mM; ΔF and F were used to compute the extent of back reaction. The lowest dotted line is the F_o level. Phosphorylated and non-phosphorylated refers to thylakoid samples illuminated with and without ATP, respectively.

non-phosphorylated samples. Thus $\Delta F/F$ is about 1 in non-phosphorylated thylakoids, increasing to 3 upon phosphorylation. This suggests that an increased rate of back reaction is a factor con-

tributing to the lower fluorescence yield seen in phosphorylated chloroplasts. To assess the effects of changes in PSII exciton density and the relative electron flux through the two photosystems on this assay for back reaction, two experiments were performed:

(1) The NH_2OH -induced fluorescence rise was measured as a function of light intensity (fig.2A). The phosphorylation effect is largest at low light intensity with $\Delta F/F(\text{phos}) : \Delta F/F(\text{non-phos})$ being 3.2 at 5% decreasing to 1.8 at 100% light. Values of 1.8 were maintained at light intensities up to $3 \times$ those shown in fig.2A. The shapes of the curves in fig.2A suggest that changes in PSII exciton density of $>50\%$ would be required to account for the changes in the recorded values for back reaction purely in terms of light harvesting changes. Clear-

ly, this is therefore an untenable explanation for the enhanced stimulation of ΔF by phosphorylation.

(2) More difficult to assess are the effects of exciton distribution and accompanying changes in the rate of Q oxidation via PSI. In fig.2B the assay is repeated in the presence of DCMU. Lower light was used here to establish quantitatively reliable values for ΔF due to NH_2OH . Again, the enhancement of the $\Delta F/F$ value is seen in phosphorylated samples. In the presence of DCMU changes in electron flux to PSI are eliminated and examination of fig.2B indicates an exciton density change of 30–40% to account for the enhanced $\Delta F/F$ due to phosphorylation. It is of note that the $\Delta F/F(\text{phos}) : \Delta F/F(\text{non-phos})$ is 1.8 ± 0.2 for data in fig.2B and is virtually intensity-independent;

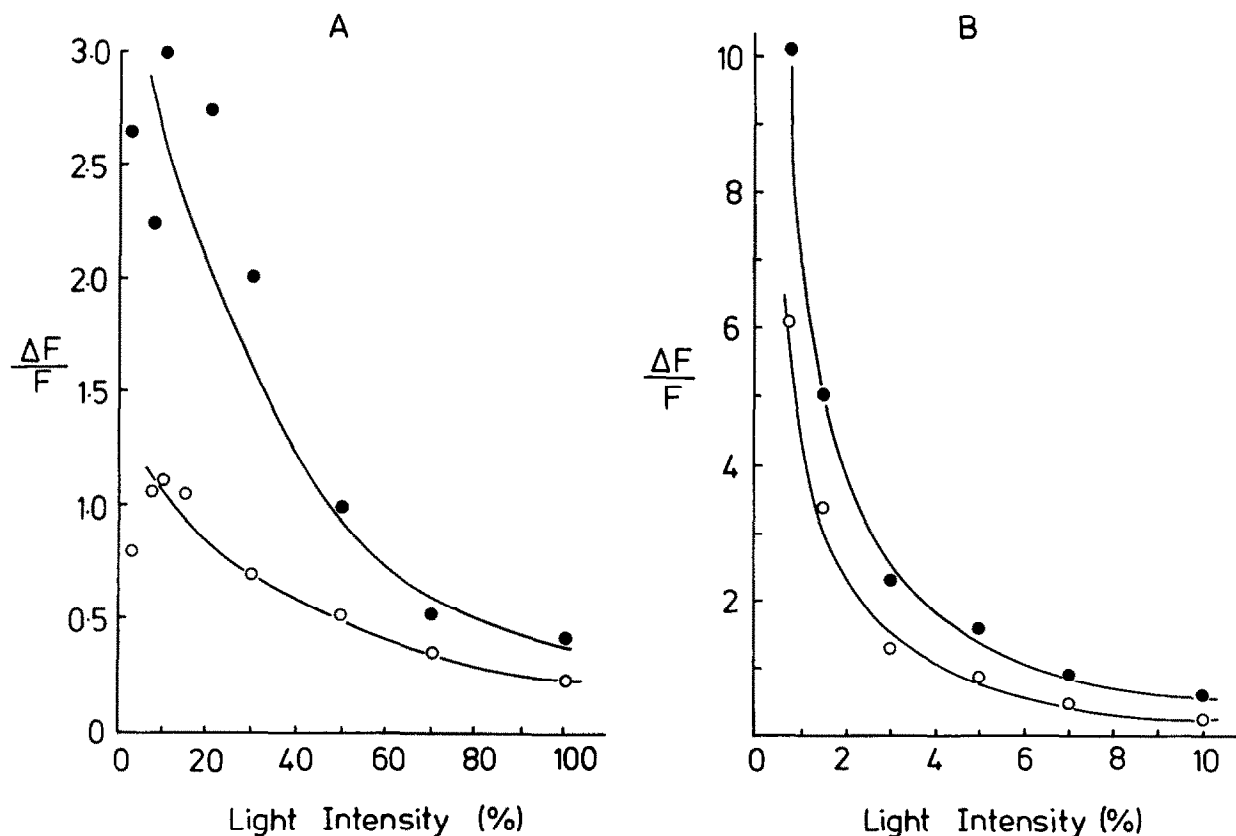


Fig.2. Dependence of the amount of fluorescence quenching due to back reaction upon light intensity. Conditions as in fig.1. In (B), 20 μM DCMU was included; 100% intensity was 12 $\text{W} \cdot \text{m}^{-2}$; (●) phosphorylated; (○) non-phosphorylated. $\Delta F/F$ was computed as illustrated in fig.1.

this value is the same as that approached in high light in the absence of DCMU (fig.2A). In these experiments proportionally more F_v was removed by illumination in the presence of DCMU for non-phosphorylated as compared to phosphorylated membranes. This, in itself, is further evidence for an enhanced back reaction in the phosphorylated chloroplasts [15].

Since the enhanced degree of NH_2OH -induced ΔF is maintained, at least in part, in the presence of DCMU and because a change in exciton density in PSII nearly twice that observed would be required to provide a trivial explanation for these results, it is concluded that protein phosphorylation enhances the efficiency of a cyclic pathway for Q oxidation. This pathway may well have DCMU sensitive and insensitive components. The enhanced cycling would presumably involve the reduction of oxidising equivalents generated by PSII (reduction of these by NH_2OH is the proposed mechanism by which the back reaction is suppressed [15]). Such a process could account for the increased stability of B^- in phosphorylated chloroplasts [12]. In experiments in which the redox state of cytochrome f was measured in phosphorylated chloroplasts, it has been found that, whilst phosphorylation drastically decreased electron flux from PSII to cytochrome f , attempts to explain this effect totally by quantal redistribution proved unsuccessful [17]. It is possible that an enhanced back reaction could provide an explanation for these data.

At this stage it is impossible to assign a physiological role for a phosphorylation-enhanced PSII cycle. At first sight this would seem to lead to less efficient photosynthesis. However, it is clear that protein phosphorylation is maximal whenever light input exceeds the capacity for dissipation of electron pressure [1,18]. Thus, a PSII cycle could be an additional mechanism, other than the changes in exciton distribution, to provide a protection against the potentially photo-inhibitory effect of excessive light intensity. However, this study was carried out at very low light intensity and the available evidence suggests that PSII back reactions or cycles may be of only minor importance in strong light [19]. Conversely, in very strong light, when H_2O splitting may become limiting, cyclic reactions will be able to compete

successfully for electron donation to P680 [20]. Our current work indicates that photo-inhibition can be partially prevented by prior phosphorylation of the thylakoids.

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