LIGHT-INDUCED REDOX CHANGES IN CHLOROPLAST CYTOCHROME *f* AFTER PHOSPHORYLATION OF MEMBRANE PROTEINS

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1. Introduction

Phosphorylation of chloroplast proteins by illumination of chloroplasts in the presence of ATP results in a decreased yield of chlorophyll fluorescence measured at room temperature [1–3], a decrease in the PSII/PSI emission ratio at −196°C [1–3], a decrease in the rate of PSII excitation [2,3] and an increase in the rate of PSI electron transport [3,4]. This indicates that phosphorylation causes a redistribution of absorbed excitation so as to increase the exciton density in PSI and decrease it in PSII [1–9]. A prediction of this proposal is that the behaviour of an electron transport chain component operating between PSII and PSI should differ when thylakoids are phosphorylated. A demonstration of an effect on such a component would also strengthen the argument that phosphorylation can regulate the efficiency of whole chain electron transport; all previous work has looked at properties of PSII or PSI 'separately' rather than as they function in series at either end of the 'Z' scheme. For these reasons, it was decided to study light-induced redox changes of cytochrome *f* in phosphorylated chloroplasts. As in [4], the experimental rationale was to pre-treat chloroplasts with light + ATP in order to phosphorylate LHCP and other thylakoid proteins and then to inhibit phosphatase with NaF. After this pre-treatment, the ATP can be removed by dilution and centrifugation, so allowing chloroplasts to be subsequently assayed in any chosen way. Here we show that the capability for photoreduction of oxidized cytochrome *f* using PSII (650 nm) light was impaired after phosphorylation, indicating a decreased exciton density in PSII. However, after inhibition of PSII by DCMU, there was no increase in the ability of 650 nm light to photo-oxidize cytochrome *f* in phosphorylated membranes. It is suggested that phosphorylation does not induce an increase in the amount of energy transferred to PSI from LHCP/PSII but decreases the fraction of absorbed radiation arriving at PSII by an effect just on the LHCP/PSII complex.

Abbreviations: PSI, photosystem I; PSII, photosystem II; F_m, maximum fluorescence level when all PSII traps are closed; F_o, minimum fluorescence level when all PSII traps are open; $F_v = F_m - F_o$; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; LHCP, light-harvesting chlorophyll protein; chl, chlorophyll

2. Materials and methods

Chloroplasts were isolated from peas and incubated under continuous illumination ± ATP as in [2]. After incubation, chloroplasts were diluted and centrifuged. All media contained 10 mM NaF [2,10]. The ATP-induced fluorescence decrease was assayed and generally found to be 25–35%. Cytochrome *f* redox changes were measured using a Bristol dual wavelength spectrophotometer. The cuvette (1 cm × 1 cm) was water-jacketed (21°C), stirred by a magnetic stirrer and could be illuminated at 90°C by either far-red (707 nm) or red (650 nm) light. The medium contained 2 μM gramicidin, 0.1 mM methyl viologen and chloroplasts at 80 μg chl/ml plus the usual ingredients [2]. Total amounts of cytochrome *f* were estimated using ferricyanide and ascorbate as in [11]. Light Intensities were measured with a Crump Quantum Meter (no. 550).

3. Results and discussions

Fig.1A shows the absorbance changes at 554 nm caused by far-red and red light in control, unphos-

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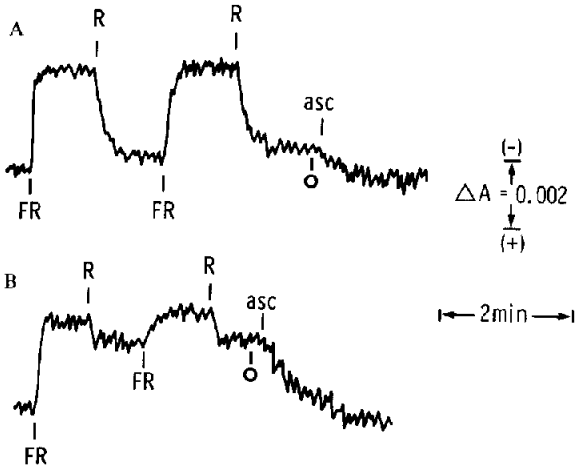

Fig.1. Absorbance changes at 554 540 nm in control (A) and phosphorylated (B) chloroplasts. The concentration of the added ascorbate was 4 mM. See section 2 for other details. 'O' refer to light 'off'. Light intensities were 5.2 $\mu E \cdot m^{-2} \cdot s^{-1}$ (707 nm) and 39 $\mu E \cdot m^{-2} \cdot s^{-1}$ (650 nm).

phorylated chloroplasts. Because cytochrome *b*-559 does not turnover under these conditions [12], only cytochrome *f* is seen here. A spectrum for the absorbance increase in red light is shown in fig.2 and has a peak at 554 nm, the α-band maximum of cytochrome *f* [13]. The clear 'push–pull' effects on the redox state of cytochrome *f* occur because far-red light is preferentially absorbed by PSI whilst red light is selectively absorbed by LHCP/PSII. In fig.1B, red light no longer caused a large increase in absorbance at 554 nm after phosphorylation. The difference in amplitude of absorbance change is due to a decreased amount of cytochrome *f* reduction (fig.2). The increased level of oxidized cytochrome *f* in red light in phosphorylated membranes is also shown by comparing the effects of adding ascorbate (to chemically reduce any remaining cytochrome *f*) as the red light is turned off; phosphorylated chloroplasts show a much bigger absorbance increase than controls (fig.1).

An interpretation of these data is that phosphorylation has caused a change in quantal distribution such that the red light absorbed by LHCP/PSII is transferred at greater rates to PSI, so changing the balance of PSII and PSI activity in favour of cytochrome *f* oxidation. Alternatively however, and having this same end result, phosphorylation may merely modulate the rate of PSII only. In effect, phosphorylation could affect the initial fraction of absorbed energy arriving at PSII. This was a possibility discussed in [14] concerning the effects of Mg^{2+} on fluorescence. To distinguish between these interpretations, phosphorylated and unphosphorylated membranes were inhibited with DCMU and the efficiency of red light in oxidizing cytochrome *f* measured. This would demonstrate if more red light was being transferred to PSI, since the redox state of cytochrome *f* in DCMU-inhibited chloroplasts should not be affected by any alteration in PSII activity. In the presence of DCMU, spillover of excitation from PSII to PSI has been demonstrated [14], and this assay should provide a means of testing whether phosphorylation causes an increase in spillover. As seen in fig.3, there is no increase in the ability of red light to photooxidize cytochrome *f* in phos-

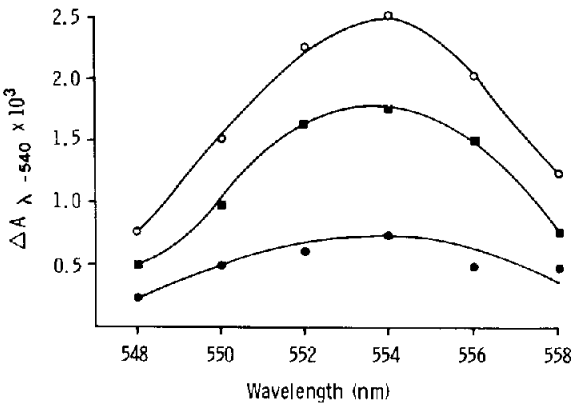

Fig.2. Spectrum of the amplitude of the absorbance increase in red light (see fig.1) for control (○) and phosphorylated (●) chloroplasts. The difference is represented by (■).

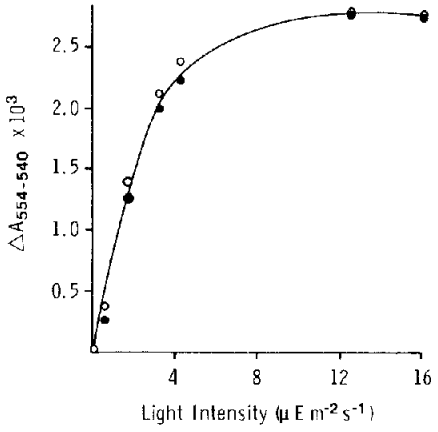

Fig.3. Amplitude of the absorbance decrease induced by different intensities of red light for control (○) and phosphorylated (●) chloroplasts. The chloroplasts contained 5 μM DCMU plus 4 mM ascorbate.

phorylated chloroplasts; this was observed over a complete range of light intensity, from <5–100% saturation of cytochrome *f* oxidation. In fact, there is a slight decrease in the ability of low intensity light ($<4\ \mu E \cdot m^{-2} \cdot s^{-1}$) to oxidise cytochrome *f* in the phosphorylated state. These data suggest that phosphorylation does not increase the probability that light absorbed by LHCP/PSII be transferred to PSI. Instead, it only decreases the rate of excitation of PSII or the fraction of incident radiation arriving at PSII.

The question arises as to how this result can be reconciled with earlier results discussed in terms of changes in the distribution of energy absorbed by LHCP [1–9]. Firstly, the decrease in room temperature fluorescence is clearly non-definitive in this regard. Similarly, the decrease in the PSII:PSI emission ratio at −196°C cannot be used to prove this kind of change in quantal distribution, as opposed to a change in fractional excitation of PSII. The decrease in rate of PSII excitation can also be explained by a selective effect on PSII without invoking increased energy transfer from LHCP to PSI. Perhaps less easily explained are reports of increases in PSI activity in low light after phosphorylation [3,4]. However, a decrease in the probability of absorption of light by PSII would mean an increased fraction of light absorbed by PSI perhaps by an amount sufficient to give a measurable increase in PSI activity under low light.

Thus phosphorylation would, in effect lead to a decrease in the light intensity at PSII. This would explain the fact that both F_v and F_0 are decreased by phosphorylation [2,3]; something hard to explain if the rate constant for energy transfer between PSII and PSI (k_T 11 → 1) were altered [15]. Similarly, it would explain why a residual effect of phosphorylation on the yield of PSII fluorescence persists after Mg^{2+} depletion. Finally, it means that the 'control' mechanism would be a simple 'feedback loop' in which high levels of reduced plastoquinone, by activating a protein kinase [3,5,7], cause a direct decrease in its rate of reduction by decreasing the rate of excitation of PSII. This model is, in addition, more easily reconciled with recent results on the location of the chlorophyll–protein complexes in the membrane system which would leave only a limited role for changes in spillover of excitation from LHCP/PSII to PSI during normal photosynthesis [15], unless phosphorylation were to induce migration of complexes from stacked to unstacked regions. Whatever the mechanism of the effects of phosphorylation, however, it is clear from these data that the process can serve to regulate non-cyclic electron transport.

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