Phosphorylation of chloroplast thylakoid membrane proteins does not increase the absorption cross-section of photosystem 1

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Direct measurements on the effective absorption cross-section of photosystem (PS) 1 were obtained with control (light – ATP) and phosphorylated (light + ATP) chloroplast thylakoids from spinach. The rate of light absorption by PS1 was invariant in control and phosphorylated thylakoids, suggesting a constant functional antenna size for this photosystem. We conclude that phosphorylated chlorophyll *a–b* light-harvesting complex from PS2 is not functionally connected with the antenna pigment of PS1.

P700 kinetics *Fluorescence induction* *Phosphorylation* *State transition* *Spinach thylakoid*

1. INTRODUCTION

The phenomenon of state 1–state 2 transition in vivo is assumed to reflect an autoregulatory mechanism by which the chloroplast thylakoid membrane can alter the distribution of absorbed quanta between the two photosystems [1,2]. Early investigations into the biochemical mechanism for state transitions suggested the involvement of cations, mainly Mg^{2+} [3,4]. Since the observation of reversible light-induced phosphorylation of thylakoid membrane proteins [5–7] however, an alternative mechanism has been suggested [8,9]. In this scheme, the phosphorylation of chl *a–b* LHC from PS2 is believed to cause the migration of a small 'pool' of chl *a–b* LHC from grana to stroma lamellae [10,11]. Thus, the effective absorption cross-section of PS2, found largely in granal regions [12], would be significantly decreased. Simultaneously, the absorption cross-section of PS1, found in stroma lamellae [12], would correspondingly increase.

Analyses of low temperature (77 K) fluorescence emission from thylakoid membranes [13,14] were interpreted in support of such changes in the absorption cross-section of the two photosystems. Yet, low temperature fluorescence emission is at best an indirect probe of the photosynthetic light-harvesting complex organization. Measurement of partial electron transport reactions has been used to test the activity of the two photosystems more directly. Authors in [15] measured the relative quantum yield of PS2-dependent electron flow ($H_2O \longrightarrow$ DPIP) and found it to be decreased by about 30% in phosphorylated membranes.

A smaller effect on PS2 (a rate decrease of about 18%) was reported in [16], assaying H_2O to DAD. These authors [16] also reported a concomitant 14% increase in PS1 rates ($DPIPH_2 \longrightarrow$ MV). Thus, within the accurary of their experimental procedure [16], the thylakoid membrane phosphorylation effect on the rate of PS1 was statistically small (89 ± 11 *vs* 104 ± 12) and became manifested only by assuming a previously unreported photoinhibitory effect on PS1. Using a similar method [17] it was also noted that phosphorylation induced an 18% increase in PS1 rates, but that was

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Abbreviations: DPIP, 2,6-dichloroindophenol; MV, methylviologen; DCMU, 3-(3′,4′-dichlorophenyl)-2,2-dimethyl urea; DAD, diaminodurene; chl, chlorophyll

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observed only at low (in the vicinity of about 9 $W.m^{-1}$) red actinic light intensities. Such measurements are indirect because they monitor the end result of electron flow through several components of the photosynthetic electron transport chain. Until now, no direct measurement on the rate of light absorption by PS1 has been reported for control and phosphorylated thylakoids.

The rate of light absorption by PS1 is proportional to the incident light intensity and to the effective absorption cross-section of the PS1 reaction centers [18,19]. The latter parameter defines the antenna size of the light-harvesting pigments associated with PS1. Under weak illumination conditions, the rate of P700 photooxidation is limited by the rate of light absorption by PS1. In principle then, one can obtain an accurate and direct estimate of the antenna size of the light-harvesting pigments of PS1 from measurements of the kinetics of P700 photooxidation [20]. In this study we applied the kinetics of P700 photooxidation as a direct measure of the effect of protein phosphorylation on the absorption cross-section of PS1. Within the experimental error of our procedure (± 10%) the PS1 chlorophyll antenna was the same in control and phosphorylated thylakoids.

2. MATERIALS AND METHODS

Chloroplasts were isolated from hydroponically-grown spinach leaves in buffer containing: 10 mM tricine–NaOH (pH 7.8), 0.4 M sorbitol, 10 mM NaCl and 2 mM $MgCl_2$. The membranes were washed in a similar buffering system, except that sorbitol was omitted. For phosphorylation, the washed thylakoids were finally resuspended in 10 mM tricine–NaOH, 0.1 M sorbitol, 10 mM NaCl and 2 mM $MgCl_2$ to a 200 μg chl/ml. The thylakoid membranes were then divided into two aliquots. The first was treated with ATP (Sigma), final concentration 100 μM, and incubated at room temperature in dim cool white light [15] for 20 min. The second aliquot was incubated in the light without ATP. After the incubation, NaF was added, final concentration 10 mM, and the membranes pelleted at 10000 × g for 10 min. The pellet was washed in buffer containing 10 mM NaF instead of 10 mM NaCl and finally resuspended to the original 200 μg chl/ml.

Phosphorylation of membranes was tested by measuring room temperature fluorescence induction parameters as in [18]. The kinetics of P700 photoconversion were measured at various light intensities as in [19].

3. RESULTS AND DISCUSSION

Recently, controversy has arisen concerning the nature of fluorescence quenching induced by the phosphorylation of thylakoid membrane proteins [21–23]. The work in [7] and [21] indicated a specific quenching of F_v, when measured in the presence of 5 mM $MgCl_2$. In [22,23], in contrast, a quenching of both F_o and F_{max} in phosphorylated membranes was observed, so that the F_m/F_o ratio remained unaltered, but these workers do report a specific quenching of F_v in 1 mM $MgCl_2$ [23]. Here, we have used an intermediate concentration of 2 mM $MgCl_2$ to observe the effects of phosphorylation on F_v. The data in fig.1 show fluorescence induction kinetics from Light – ATP (control) membranes and Light + ATP (phosphorylated) membranes. A reversible 20–30% quenching of F_v was observed in the phosphorylated membranes, consistent with the results obtained with spinach in 5 mM $MgCl_2$ (submitted). These results are in agreement with [7,21], but contrast with those in [22,23].

Fig.2 shows the kinetic traces of P700 photooxidation in control and phosphorylated membranes.

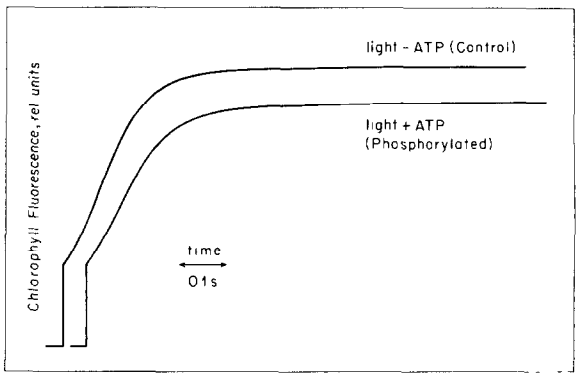

Fig.1. Room temperature fluorescence transients of Light – ATP and Light + ATP membranes in the presence of 2 mM $MgCl_2$. The reaction mixture contained 20 μM DCMU and 100 μg chl/ml.

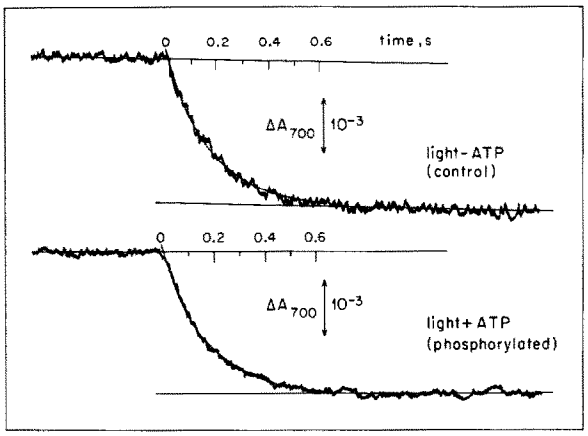

Fig.2. Kinetics of P700 photoconversion of Light – ATP and Light + ATP spinach thylakoids in the presence of 2 mM $MgCl_2$. The reaction mixture contained 200 μg chl/ml, 20 μM DCMU and 200 μM methylviologen.

Table 1

Experimental values of K_{P700} for control and phosphorylated membranes at various light-intensities

	Light intensity of actinic beam ($\mu E.m^{-2}.s^{-1}$)		
	50	95	130
K_{P700} s^{-1} control	4.0 (3)	7.6 (8)	10.3 (4)
K_{P700} s^{-1} phosphorylated	4.2 (3)	7.3 (8)	10.3 (4)
Δ	6%	−4%	0%

Numbers in parentheses indicate the number of individual measurements; for other experimental conditions see fig.2

From these data we have calculated [19] the rate-constant of P700 oxidation under both conditions (fig.3). From the semilogarithmic plots of fig.3 it is clear that the rate of P700 photooxidation is not increased in phosphorylated membranes. This result demonstrates that no increase in the PS1 absorption cross-section occurs under phosphorylated conditions. To investigate this possibility further we repeated the experiments at different light intensities. The results of such determinations are summarized in table 1. The value of the rate constant K_{P700} changed in proportion to the actinic light intensity, thus verifying the limiting nature of our actinic light intensity conditions. At low (50 $\mu E.m^{-2}.s^{-1}$), intermediate (95 $\mu E.m^{-2}.s^{-1}$), and higher (130 $\mu E.m^{-2}.s^{-1}$) actinic light intensities, the respective values of the rate constant K_{P700} are essentially the same in both control and phosphorylated thylakoids. Although phosphorylation has reportedly increased basal PS1 electron transport rates by 14–18% in the past [16,17], the direct determinations of the PS1 antenna size, as measured by the rate constant K_{P700} in this study, failed to show the expected enhancement of light absorption for phosphorylated membranes. The data of fig.3 and table 1 show that within experimental error the antenna size of PS1 remained unchanged upon phosphorylation of thylakoid membrane proteins. Our results indicate that phosphorylated chl *a–b* LHC from PS2 has not become associated with PS1.

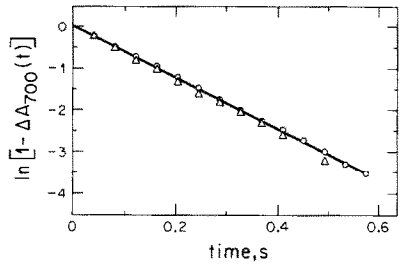

Fig.3. First-order analysis of the kinetics of P700 photooxidation, calculated from data in fig.2: (○) Light – ATP; (△) Light + ATP.

4. CONCLUSIONS

Phosphorylation of spinach thylakoids in the presence of 2 mM $MgCl_2$ resulted in a 20–30% quenching of F_v. However, we are unable to report a corresponding increase in the absorption cross-section of PS1 under the same conditions. Our experimental approach was a critical test of the hypothesis that phosphorylated chl *a–b* LHC from PS2 becomes functionally connected with the PS1 complex. Thus, the phosphorylation of the thylakoid membrane proteins appear to affect PS2

photochemical parameters only. As a consequence of our measurements, previous models on the significance of thylakoid membrane phosphorylation [8,9] should be critically re-examined.

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