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Regulation of thylakoid protein phosphorylation by high-energy-state quenching

Kevin Oxborough, Pam Lee and Peter Horton

Department of Biochemistry and Research Institute for Photosynthesis, University of Sheffield, Western Bank, Sheffield S10 2TN, England

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The possible co-regulation of light-induced protein phosphorylation by the redox state of plastoquinone and the transthylakoid ΔpH was investigated in isolated pea chloroplasts. Incorporation of γ-^{32}P-labelled ATP into the LHC-II and 9 kDa phosphoproteins in the coupled state was reduced by 54 and 28%, respectively, when compared to the uncoupled state. Selective inhibition of energy-state-dependent quenching of chlorophyll fluorescence by antimycin A in the coupled state greatly reduced this inhibition of protein phosphorylation and subsequent quenching of chlorophyll fluorescence. These results suggest that the protein kinase(s) involved in phosphorylation of the LHC-II and 9 kDa phosphoproteins is regulated by either the energy state of the thylakoid membrane or the redox state of some component of the electron-transport chain, rather than by ΔpH per se.

Protein phosphorylation; Chloroplast; Photosynthesis; Chlorophyll fluorescence

1. INTRODUCTION

A number of thylakoid proteins have been shown to be reversibly phosphorylated under certain conditions (reviews [1–3]). In illuminated isolated chloroplasts, the highest levels of phosphorylation are seen within the polypeptides of the light-harvesting complex (LHC-II) which have molecular masses between 25 and 29 kDa. The protein kinase involved is light-activated, LHC-II phosphorylation having been shown to be controlled by the relative rates of excitation of photosystem (PS) II and PS I. A clear relationship between the redox state of the plastoquinone pool and kinase activity has also been established. The phosphorylation-induced shift in relative absorbance cross-section in favour of PS I provides a mechanism for the correction of spectral imbalance and the elimination of 'Emerson enhancement'.

An additional source of protein phosphorylation regulation may be the extent of the transthylakoid ΔpH. Evidence for this comes from experiments using intact chloroplasts in which protein phosphorylation was enhanced by an increase in the demand for ATP [4] or by a partial uncoupling by nigericin [5]. A dual control of protein phosphorylation, by redox and energy states within the chloroplast, is consistent with the concept of LHC-II phosphorylation enhancing ATP production through stimulation of either cyclic electron flow around PS I or a protonmotive 'Q' cycle [6,7]. Recent work, which demonstrates that the level of enhancement seen in isolated chloroplasts can be changed by the imposition of different ATP:NADPH demands [7,8], is consistent with this proposal since state transitions are, by definition, the elimination of enhancement.

Although there is good evidence for a correlation between the transthylakoid ΔpH and protein phosphorylation, the 'directness' of this interac-

Correspondence address: K. Oxborough, Department of Biochemistry and Research Institute for Photosynthesis, University of Sheffield, Western Bank, Sheffield S10 2TN, England

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tion remains unclear because of the changes in adenylate status and metabolite levels occurring in intact chloroplast. The primary aim of this study is to investigate the effect of ΔpH on protein phosphorylation induced by exogenous ATP in metabolically inactive chloroplasts.

2. MATERIALS AND METHODS

Intact pea chloroplasts were prepared according to Cerovic and Plesnicar [9]. The final assay medium contained 150 mM sorbitol, 10 mM KCl, 1 mM EDTA, 50 mM Hepes buffer, 10 mM NaF and 0.5 mM phloridzin. The low osmotic strength of this medium (35% isotonic) produces swollen chloroplasts which are freely permeable to ATP. Swollen chloroplasts were chosen in preference to broken chloroplasts because they consistently show higher levels of qE for the same ΔpH. Actinic illumination of 1080 $\mu E \cdot m^{-2} \cdot s^{-1}$ was provided by a 150 W Schott lamp through an RG610 (red) glass filter. Chlorophyll fluorescence and 9-amino-acridine fluorescence were measured using apparatus similar to that in [10] except that a Walz PAM chlorophyll fluorimeter was used.

Protein phosphorylation was assayed using the γ-^{32}P-labelled ATP incorporation technique described in [11].

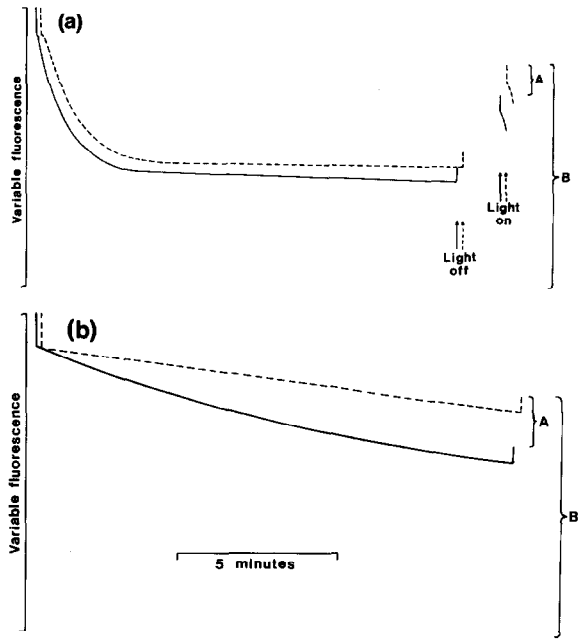

Fig.1. Chlorophyll fluorescence recorded in the presence (——) and absence (– – –) of ATP in the coupled (a) and uncoupled state (b). Saturating pulses of light were given at the start and end of each run to reverse photochemical quenching. In (b), qE was reversed by a 1.5 min dark period. qT was taken as A/B where A represents quenching induced by ATP and B denotes the yield of variable fluorescence at the point of calculation. qE was given by the fluorescence recovered during the dark period divided by the variable fluorescence yield.

3. RESULTS AND DISCUSSION

Previous studies of ATP-induced phosphorylation have been carried out on uncoupled broken chloroplasts in the absence of an artificial electron acceptor. Under these conditions, phosphorylation of thylakoid membrane proteins in the light results in a quenching of chlorophyll fluorescence (qT) which is irreversible in the presence of the phosphatase inhibitor, NaF [12]. In the coupled state, light-induced formation of a transthylakoid ΔpH induces 'energy-dependent' quenching of chlorophyll fluorescence (qE) which is associated with changes in thylakoid organisation [25]. qE and qT can be separated by allowing ΔpH (and consequently qE) to reverse during a 2 min dark period at the end of each run. qT was calculated from the level of fluorescence after this dark period (see fig.1). These data indicate that the presence of a transthylakoid ΔpH has suppressed the extent of ATP-induced fluorescence quenching.

It has been demonstrated that the addition of antimycin A can, under certain conditions, completely inhibit qE formation with no apparent effect on the establishment of the transthylakoid ΔpH [13,14]. By taking advantage of this phenomenon, the three possible combinations of ΔpH and qE were established: (A) uncoupled (with 2 μM nigericin), no antimycin A ($-\Delta$pH, $-qE$); (B) uncoupled, +2.5 μM antimycin A ($-\Delta$pH, $-qE$); (C) coupled, no antimycin A ($+\Delta$pH, $+qE$); (D) coupled, +2.5 μM antimycin A ($+\Delta$pH, $-qE$). B was used to ensure that any effect of antimycin A on protein phosphorylation in the coupled state could be attributed to its effect on qE.

Table 1

Levels of energy-dependent quenching (qE), 9-aminoacridine fluorescence quenching, ATP-induced quenching (qT) and phosphorylation of LHC-II and 9 kDa proteins

Condition	qE	9-AA	qT	Phosphorylation (cpm)	
				LHC-II	9 kDa
(A) Uncoupled, −ant. A	−	−	16.9 (100)	521 (100)	45 (100)
(B) Uncoupled, +ant. A	−	−	16.5 (97)	541 (104)	50.5 (112)
(C) Coupled, −ant. A	26.6	17	11 (64)	238 (46)	32.5 (72)
(D) Coupled, +ant. A	−	18	13.2 (74)	515 (99)	40 (89)

The phosphorylation results listed are the means of four replicates. The fluorescence data shown are from single replicates on the same batch of chloroplasts as was used in the phosphorylation experiment. Fluorescence measurements made on two different batches of chloroplasts showed an identical pattern. The method of calculation of qE and qT is described in the legend to fig.1. Numbers in parentheses indicate % of control (C); ant. A, antimycin A

Table 1 shows the levels of phosphorylation of the major pea thylakoid phosphoproteins (LHC-II and the 9 kDa protein) for the four conditions described above. The calculated levels of ΔpH, qE and qT are also shown. Whilst the level of LHC-II phosphorylation in D ($+\Delta$pH, $-qE$) is comparable with that seen in A and B ($-\Delta$pH, $-qE$) LHC-II phosphorylation in C ($+\Delta$pH, $+qE$) is inhibited by over 50% when compared to conditions where qE is absent. These results clearly demonstrate the suppression of LHC-II phosphorylation in the presence of ΔpH, whilst strongly suggesting that the effector is the organisational state of the thylakoid, monitored by qE, rather than ΔpH per se.

It is interesting to note that, although the fluorescence data show a lower value for qT in C ($+\Delta$pH, $+qE$) this inhibition is only around 33% of the uncoupled values (A,B). Also, whilst qT is significantly higher in D ($+\Delta$pH, $-qE$) than in C, it is still approx. 25% lower than in A and B. These results might be explainable in terms of the observation that only a proportion of the phosphorylated LHC-II migrates from the appressed to non-appressed regions of the thylakoid membranes [15] and that only limited phosphorylation is required for maximum fluorescence quenching [16].

It is also worth noting that the level of qT in each condition is closer to the level of phosphorylation of the 9 kDa protein than of LHC-II. The results of all previous work have led to the conclusion that only LHC-II is involved in alteration of excitation energy distribution (e.g. [17]). This observation is relevant to the suggestion that both the LHC-II and 9 kDa phosphoproteins need to be phosphorylated for qT formation to occur [28]. Similarly, differential sensitivity of the phosphorylation of LHC-II and the 9 kDa phosphoproteins to DBMIB suggests differences in their regulatory mechanisms [19] which may account for the differences seen here, between the fluorescence and LHC-II phosphorylation results.

Under most conditions, redox state and energy state show a high degree of interdependency. Previous work has indicated that the redox state of the plastoquinone pool and the energy state of the chloroplast are both important in the regulation of protein phosphorylation. The present results demonstrate a close association between energy state and the level of protein phosphorylation and could be taken as evidence for a direct inhibition of

the protein kinase by the energy state of the thylakoid membrane.

An alternative explanation for this association could be derived from the DBMIB studies mentioned above, and the fact that antimycin A is a well-documented inhibitor of *b*-type cytochrome oxidation. Since high ΔpH inhibits plastoquinone oxidation at the inner site of the cytochrome *b/f* complex, it seems feasible that a component of the cyclic electron-transport chain around PS I (possibly cytochrome *b*-563) may provide a link between the energy state of the chloroplast and the protein kinase.

Irrespective of whether the protein kinase is inhibited directly by high energy state or indirectly through the redox state of a component of cyclic electron flow, it seems quite evident that low redox potential and high energy state, respectively, provide feed-forward and feed-back control of protein phosphorylation, which in turn regulates electron flow in response to irradiance and metabolic state [6,7].

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