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A COMPARISON BETWEEN CATION AND PROTEIN PHOSPHORYLATION EFFECTS ON THE FLUORESCENCE INDUCTION CURVE IN CHLOROPLASTS TREATED WITH 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA

PETER HORTON and MICHAEL T. BLACK

Department of Biochemistry and ARC Research Group on Photosynthesis, The University, Sheffield S10 2TN (U.K.)

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Fluorescence induction curves in chloroplasts phosphorylated by the thylakoid protein kinase activated at low light intensity and high chlorophyll concentration have been measured. At 5 mM Mg^{2+}, phosphorylation did not preferentially quench variable fluorescence. At 1 mM, preferential quenching of variable fluorescence was observed, indicating a second effect of phosphorylation at low Mg^{2+} (Horton, P. and Black, M.T. (1982) Biochim. Biophys. Acta 680, 22–27). Comparison of the extent of fluorescence decrease and the resulting ratio of variable to maximum fluorescence after phosphorylation and after lowering Mg^{2+} concentration demonstrated a difference between these two mechanisms of lowering of fluorescence. The significance of these results in terms of how phosphorylation may alter membrane organization is discussed.

Introduction

Chloroplast membranes possess a protein kinase which phosphorylates several membrane polypeptides including those of LHCP, the major light-harvesting chlorophyll protein associated with PS II [1,2]. This phosphorylation process is thought to be involved in the regulation of quantal distribution between PS II and PS I [3–5]. This suggestion is based on the following effects of phosphorylation; the decrease in yield of fluorescence from PS II chlorophyll [3–6], a change in the ratio of PS II and PS I emission at −196°C [3–5], an increase in the rate of PS I electron transfer [7], a change in the redox state of cytochrome *Q* [8] and of Q [9] and changes in the kinetic rate constants for fluorescence induction at room temperature [5,10] and −196°C [11]. One area of uncertainty is the mechanism which brings about the phosphorylation-induced fluorescence decrease. In previous work we showed that both F_0 and F_m are decreased to the same extent after phosphorylation [4]. Work by Bennet et al. [3] and Kyle et al. [10], in contrast, showed a specific quenching of F_v. In our earlier work, protein kinase was activated by strong light and with dilute chlorophyll concentration, and under these conditions substantial loss of PS II activity occurs. It was possible that differential photoinhibition in minus- and plus-ATP samples was occurring so as to obscure the true effect of phosphorylation. This discrepancy is not trivial, since on the basis of our fluorescence data we

Abbreviations: PS, photosystem; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; Q, the quencher of fluorescence and primary acceptor of PS II; F_0, initial fluorescence level recorded. when Q is fully oxidized; F_m, final or maximal fluorescence level recorded when Q is fully reduced; F_v, $F_m - F_0$; LHCP, light-harvesting chlorophyll-protein complex associated with PS II.

proposed that phosphorylation causes detachment of LHCP from the PS II 'pool' [4]. Thus, phosphorylation may alter the initial partition of excitation between PS II and PS I rather than increase spillover from PS II to PS I [4,8]. We therefore decided to re-investigate the fluorescence induction curve after phosphorylation induced under milder conditions. In addition, a comparison between Mg^{2+} depletion and phosphorylation was undertaken under identical conditions; Mg^{2+} depletion is well known to quench preferentially F_v and increase spillover. Moreover, at low Mg^{2+} a different kind of phosphorylation effect may occur [7], which could serve as a useful comparison to the usual high Mg^{2+} case.

Materials

Osmotically shocked pea chloroplasts were prepared exactly as described previously [5]. Recording and analysis of fluorescence induction curves were performed using procedures described in detail [5,12]. Phosphorylation was induced by incubation for 10 min with 0.2 mM ATP at 150 μg/ml at 20°C and a light intensity of 35 W/m^2 of white light. Samples were dark adapted for 15 min before DCMU addition and fluorescence measurement. 10 mM NaF was present throughout.

Results

In a recent paper, it was shown that the effect of phosphorylation on F_m is enhanced when the Mg^{2+} concentration is lowered to 1 mM from 5 mM. It was postulated that at 1 mM Mg^{2+} an additional effect of phosphorylation comes into play which is really just an antagonism of Mg^{2+} screening [7]. In Fig. 1 are shown fluorescence induction curves recorded for chloroplasts incubated ± ATP at 1 and 5 mM Mg^{2+}. A decrease in F_0 as a result of phosphorylation occurs at both 5 and 1 mM Mg^{2+}. Lowering the Mg^{2+} concentration lowers the F_m of both phosphorylated and non-phosphorylated chloroplasts but does not significantly decrease F_0.

Measurement and semilogarithmic analysis of the area growth above the induction curve allow quantitation of the relative contribution of PS II$_\alpha$ and PS II$_\beta$ [5,10,13]. These centres differ in the

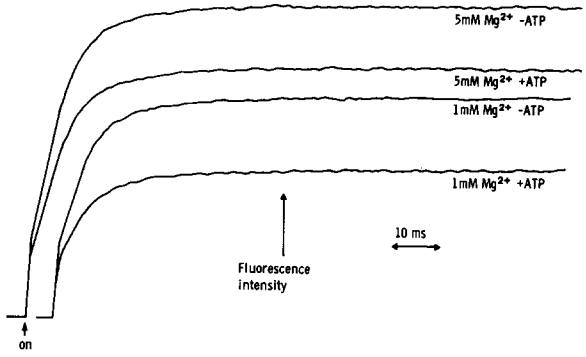

Fig. 1. Fluorescence induction curves of chloroplasts illuminated for 10 min ±ATP (0.2 mM) with 5 and 1 mM $MgCl_2$. Incubation medium contained sorbitol (0.33 M), NaCl (10 mM) and Hepes (50 mM) adjusted to pH 7.6. Intact chloroplasts were osmotically shocked in H_2O immediately prior to incubation. Samples were dark adapted for 15 min following incubation and diluted to 15 μg chlorophyll/ml into a medium containing 10 μM DCMU. Fluorescence induction curves were recorded as previously described [5,12].

degree of connectivity between the PS II reaction centres and in size and composition of their antenna chlorophyll [14,15]. Both phosphorylation [5,10] and cation depletion [10,17] have been shown to decrease the proportion of PS II$_\alpha$. Fig. 2 shows the results of this kind of analysis performed on chloroplasts phosphorylated at 5 and 1 mM. Both Mg^{2+} lowering and phosphorylation increase the proportion of PS II$_\beta$ in the induction curve. Table I describes the important parameters extractable from the induction curves.. Several features are to

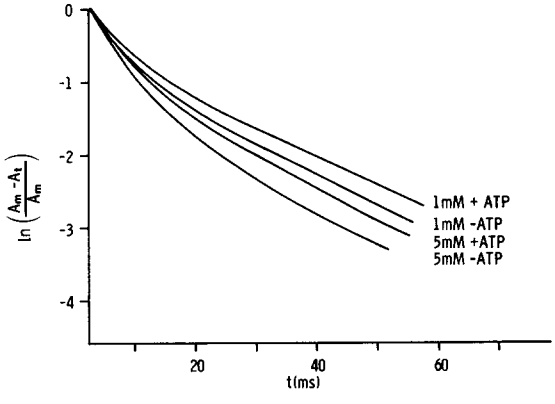

Fig. 2. Semilogarithmic plots of the area growth above the curves shown in Fig. 1. The analysis was based on that of Melis and Homann [13] as detailed previously [5]. A_m and A_t refer to the total area and area accumulated at time t, respectively.

TABLE I

INDUCTION PARAMETERS AFTER PHOSPHORYLATION AT HIGH AND LOW Mg^{2+}

Fluorescence induction parameters were obtained from the data in Figs. 1 and 2. β_{max} was determined from the y-axis intercept as described earlier [5,13].

Mg^{2+} (mM)	±ATP	F_0	F_m	F_v/F_m	β_{max}
5	−	11.9	48.4	0.75	0.39
5	+	9.9	39.1	0.75	0.47
1	−	11.8	35.8	0.67	0.53
1	+	9.8	22.5	0.57	0.60

be emphasised. Firstly, phosphorylation at 5 mM brings about a decrease in F_0 that is proportional to the decrease in F_m such that F_v/F_m is unchanged. In contrast, lowering of Mg^{2+} has a much smaller effect on F_0 even though the F_m decrease is actually a few percent greater than that caused by phosphorylation (compare the difference between 5 mM and 1 mM −ATP with the difference between 5 mM −ATP and 5 mM + ATP).

The decrease in F_v/F_m which accompanied a fluorescence decrease of 26% due to Mg^{2+} lowering was therefore to be contrasted with the unchanged F_v/F_m during a fluorescence decrease of 19% due to phosphorylation. Phosphorylation at 1 mM also causes F_0 to decrease, but here F_m decreases more so as to lower F_v/F_m. This decrease is consistent with the notion that phosphorylation raises the Mg^{2+} requirement for inhibition of spillover [7]; this leads, in effect, to fluorescence quenching due to phosphorylation that more closely resembles that due to cation lowering.

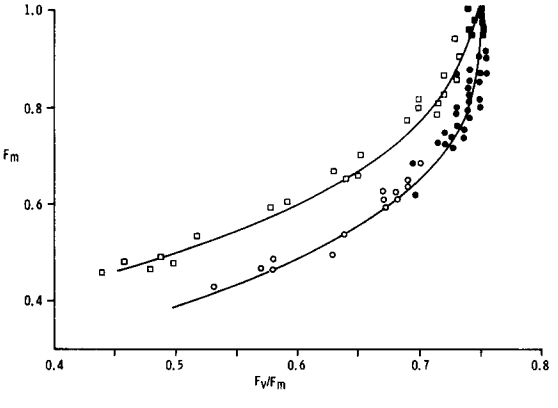

Fig. 3. A comparison of the decrease in F_m and F_v/F_m ratio as a result of different treatments: (■) unphosphorylated, 5 mM Mg^{2+}; (●) phosphorylated, 5 mM Mg^{2+}; (□) unphosphorylated at various Mg^{2+} concentrations from zero to 4 mM; (○) phosphorylated at various Mg^{2+} concentrations ranging from 1 to 4 mM. Data was derived from a series of chloroplast batches and several different experiments.

Fig. 3 represents an extension of the comparison between cation and protein phosphorylation effects to include a wider range of Mg^{2+} concentrations and extents of phosphorylation. Clearly with F_m decreases (due to phosphorylation) of as much as 30% only marginal decreases in F_v/F_m result. In contrast, degrees of Mg^{2+} depletion which decreases F_m by less than 20% result in significant decreases in F_v/F_m. Phosphorylation at low Mg^{2+} gives rise to an F_v/F_m decrease with the slope of that curve being very similar to that obtained from unphosphorylated samples.

Discussion

Data presented here substantiate the previously held notion that phosphorylation has two kinds of effect on the yield of PS II fluorescence. At saturating Mg^{2+} decreases in both F_0 and F_m occur. At lower Mg^{2+} in addition to a decrease in F_0, phosphorylation brings about a bigger decrease in F_m, so that F_v/F_m drops. This effect of phosphorylation occurs because of an increased Mg^{2+} requirement in phosphorylated membranes [7]. The decrease in fluorescence due to lowering of Mg^{2+} has generally been interpreted in terms of increased spillover from PS II to PS I [18,19]. As expected, preferential quenching of F_v results (see Table I and data in Refs. 19 and 20). This is to be contrasted then with the phosphorylation effect at 5 mM Mg^{2+}. The characteristics of the two effects of phosphorylation are seen in the curve in Fig. 3; thus, at high Mg^{2+} no F_v/F_m change occurs, but on lowering Mg^{2+}, decreases are seen.

The difference in characteristics of $-Mg^{2+}$ and phosphorylation-dependent fluorescence decrease implies a different mechanism. Such a difference is implicit in the membrane models of Barber [21] which ascribe the Mg^{2+} effects to screening charges on PS I proteins. Phosphorylation, of course is of

PS II proteins such as LHCP. This will have an effect by disturbing the delicate balance of attractive and repulsive forces in the stacked membrane regions [21].

The fluorescence measurements reported here and previously [4] are interpretable in terms of detachment of LHCP from the granal PS II [4]. As proposed by Barber [22], phosphorylated proteins might migrate away from the appressed membrane regions. This suggestion is supported by loss of chlorophyll from stacked regions after phosphorylation [9] and recent structural data indicating migration of a 'pool' of LHCP from the appressed membranes (Arntzen, C.J., personal communication).

Detachment of LHCP would, if it became either intrinsically non-fluorescent or coupled to PS I, give rise to a decrease in F_0, Moreover, the process would imply a preferential effect on PS II_α centres which are proposed to exist in a statistical pigment bed in the appressed membranes. A change in the ratio of PS II_α to PS II_β is caused by phosphorylation (Table I and Refs. 5 and 10). It is important to note that an increase in βmax in the semilogarithmic plot of area growth does not necessarily imply conversion of PS II_α into PS II_β, but could equally be accomodated by preferential 'quenching' of PS II_α. Detachment of LHCP should preferentially remove chlorophyll from PS II_α. Significantly, therefore, our earlier analyses showed that the rate of reduction of PS II_α was slower after phosphorylation [4]. PS II_α also retained its sigmoidicity. Loss of a periperhal amount of LHCP need not decrease sigmoidicity. For instance, *Phormidium* PS II particles show sigmoidal induction with an antenna size of only 40 chlorophylls [12]. A preferential 'quenching' of PS II_α by this mechanism might be responsible for the decreased proportion of PS II_α seen in the area growth curves, although this is difficult to assess because of the tendency for a larger area to result from the decreased absorption cross-section. Nevertheless, the loss of LHCP might also cause α-centres to become β-centres. At present it is hard to distinguish between these two notions. Barber has suggested that the whole phosphorylated LHCP-PS II complex migrates upon phosphorylation [22]; this scheme allows for unaltered PS II-PS II interaction of the α-centres remaining in the stacked regions [5] and implies that the displaced PS II units are β-centres.

The data reported here and in a previous publication are at variance with recent data from Arntzen and colleagues [10]. The data presented here suggest the difference may be biological rather than methodological. Thus, under incubation conditions virtually identical to those described in Ref. 10 this difference persists. Moreover, manipulation of Mg^{2+} levels under these conditions gives rise to a decreased F_v/F_m whereas phosphorylation does not. It is interesting that if we use 1 mM Mg^{2+} the effect of phosphorylation more closely resembles that seen by Kyle et al. [10]. Haworth et al. [11] have shown that both spillover and the initial distribution of quanta between PS II and PS I are altered by phosphorylation. Detachment of LHCP would only cause a change in the latter whereas the former might occur as a result of any consequent unstacking. It is possible therefore that the contribution from changes in the initial distribution of quanta and the amount of spillover vary in different systems. Specifically, the relative proportions of PS II, PS I and LHCP could determine what the effect of protein phosphorylation on the energy-transfer properties will be. Clearly, a comparative study of the effects of protein phosphorylation is warranted.

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