Control of chloroplast electron transport by phosphorylation of thylakoid proteins

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Several chloroplast membrane proteins can become phosphorylated by a membrane protein kinase. The kinase is activated when the electron carriers (plastoquinone) between photosystem II and photosystem I are reduced. Phosphorylation of the light harvesting complex of photosytem II causes a decrease in energy transferred to photosystem II and an increase in the rate of excitation of photosystem I. This process, which is mediated by lateral diffusion of the phosphorylated complex serves to regulate the relative rates of electron transfer through the two photosystems. Phosphorylation may also have an important role in balancing the rate of excitation of photosystem II with the capacity for photosynthetic carbon metabolism and in controlling the functional state of photosystem II.

Chloroplast *Protein kinase* *Light harvesting complex* *Thylakoid membrane* *Electron transport* *Chlorophyll fluorescence*

1. INTRODUCTION

Chloroplast membranes possess a protein kinase and phosphatase system which can reversibly phosphorylate a number of polypeptides including the major light-harvesting chlorophyll protein known as LHC-II [1,2]. In the past 3 years, it has been learned how the kinase is activated, what the effects of phosphorylation of LHC-II are and how this process can affect the rate of electron transport as a result of changes in quantal redistribution. It seems that herein lies the only physiological mechanism yet identified that can provide control of photosynthetic energy transduction. It is the purpose of this article to review the pertinent features of this regulatory mechanism.

2. PROTEIN KINASE SUBSTRATES

Thylakoid protein kinase is ATP-dependent and will incorporate ^{32}P from [γ-^{32}P]ATP into several membrane proteins [2]. The enzyme will also use exogenous histone as a substrate [3]. The major physiological substrate in terms of the amount of ^{32}P incorporation is the M_r 25000–29000 polypeptides which comprise LHC-II [2]. Both of the major polypeptides are labelled with equal specific activity [4] and it is estimated that up to 50% LHC-II can become phosphorylated [5]. It is not known what the kinase concentration is in the membrane nor whether the same kinase phosphorylates all the potential phosphoproteins. All of the ^{32}P incorporated is attached to threonine residues present on the thylakoid surface [2,3]. In the case of LHC-II, the phosphothreonine is part of a positively charged hydrophilic segment which is trypsin-cleavable [2,6]. Besides LHC-II, a number of other polypeptides are phosphorylated most notably with an app. M_r of 9000 [1–3]. None of these polypeptides has been identified but they all separate with PS II upon detergent fractionation of membranes [8], and polypeptides of these sizes are seen in purified PS II fractions. In some species (e.g., spinach) the M_r 9000 polypeptide is incorporated with a higher specific activity than LHC-II (P. Horton and C. Foyer, unpublished) whereas in barley LHC-II is the only major phosphoprotein [5].

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3. ACTIVATION OF PROTEIN KINASE

Protein kinase is light activated and the light requirement is one of production of reducing power rather than ATP or an energized state [1]. Use of inhibitors such as DCMU and DBMIB as well as various electron donor–acceptor systems pointed to reduction of a component between PS II and PS I as being necessary for kinase activation [8,9] (this mode of activation is to be contrasted with the ferredoxin-mediated reductive activation of soluble stromal enzymes such as fructose 1,6-bisphosphatase [10]). Particularly definitive was the dark activation of the kinase by selective reduction of electron transport components. Thus, whilst ascorbate plus diaminodurene, which reduce cytochrome *f*, plastocyanin and P700 cause only marginal kinase activation, reduced tetramethylquinone, a reductant for plastoquinone, elicited maximal activation [11]. Potentiometric redox titration of chloroplasts also showed kinase activation when plastoquinone was reduced to plastoquinol [12,13]. Activation by plastoquinol was also suggested by 'titration' with light flashes, by the effects of low (cf. high) DBMIB concentration [9], and by antagonistic effects of PS I and PS II light [12]. Inhibition of activation by lypophilic maleilmides suggests the involvement of a membrane sulphydryl redox centre in the activation process [14]. In this respect, it should be stressed that it is not known how direct is the redox activation by plastoquinol, only that plastoquinone reduction is a prerequisite for activation.

4. PROTEIN PHOSPHATASE

Thylakoids possess a phosphatase which catalyses dephosphorylation of LHC-II and other polypeptides. The Mg^{2+} requirement is lower [15] and the rate of dephosphorylation higher [7] for LHC-II than for other polypeptides. Sodium fluoride completely inhibits phosphatase activity. Evidence from in vitro studies so far indicate that the phosphatase activity is not subject to regulation.

5. CHANGES IN CHLOROPHYLL FLUORESCENCE ASSOCIATED WITH PROTEIN PHOSPHORYLATION

An ATP-dependent decrease in yield of fluorescence from PS II chlorophyll has been observed [6,8,12] and this decrease correlates well with observed amount and rates of LHC-II phosphorylation. Associated with the fluorescence decrease from PS II is an increase in yield from PS I chlorophyll when assayed either at −196°C [6,8,12] or more recently at 20°C [16]. Generally the amount of quenching is 15–25% of the maximum yield, the extent of which may vary according to growth conditions and species (Horton, P., unpublished). Dephosphorylation of LHC-II correlates with a rise in PS II fluorescence and this rise is inhibited by the phosphatase inhibitor NaF [7,8]. Recent work with a barley mutant lacking LHC-II [5,17] and with partially developed barley also deficient in LHC-II [5] confirm the involvement of phosphorylation of LHC-II in the ATP-induced fluorescence changes.

6. MECHANISMS OF THE PHOSPHORYLATION EFFECT ON QUANTAL DISTRIBUTION

The fluorescence data suggest that phosphorylation causes a decrease in the rate of excitation of PS II and an increase in the rate of excitation of PS I. There are two mechanisms which may bring this about. Firstly, there may be an alteration in the initial partition of energy between PS II and PS I, a phenomenon determined in terms of α, the fraction of energy at PS I [18]. Alternatively, quantal redistribution can be brought about by a change in 'spillover' of energy from PS II to PS I [18].

Analysis of fluorescence induction at −196°C can give information on these processes. Phosphorylation appears to increase both α and spillover [5,19] and calculation shows that the combined effect is to increase the proportional excitation of PS I from 38–67% [5]. A parameter which defines the coupling between LHC-II and PS II was also found to decrease upon phosphorylation [19]. This latter change suggested that phosphorylation causes removal of LHC-II from association with PS II. Biochemical and structural information consistent with this idea has been recently obtained [20]. Thus an increase in concentration of LHC-II in stroma membranes occurs after phosphorylation, indicating its migration from the PS II-enriched granal membranes. Moreover, a particle size class seen in freeze fractures of thylakoids and

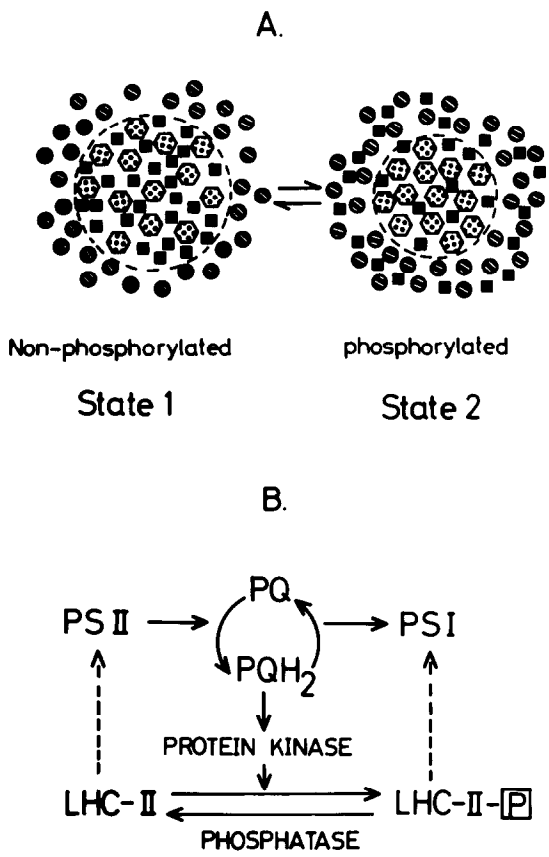

Fig.1. Control of State transitions by the plastoquinol activated protein kinase: (A) Diagrammatic surface view of a thylakoid membrane. Dotted line denotes area of membrane appression; hexagons LHC–PS II complex; circles PS I complex; Squares 'free' LHC-II; (B) Involvement of plastoquinone as a meter of PS II and PS I excitation rates and the function of the kinase in their control. Dotted lines indicate relative energy transfer probability.

thought to be LHC-II is seen to migrate from granal to stromal membranes upon phosphorylation. The pool of migratory LHC-II appears to be 'free' LHC-II that is not tightly bound to PS II.

Thus a postulated model for phosphorylation effects on the organization of thylakoid membranes would first propose that phospho-LHC-II would migrate from the vicinity of PS II to the PS I-enriched stromal membranes (fig.1A). This would increase α, the fraction of energy initially arriving at PS I. Such an event would lead to a PS II fluorescence change at room temperature that would not be quenching as such, but fluorescence lowering [8]. Thus both F_o and F_m would decrease as the change is only in the absorption cross section of PS II. Results of this kind were observed in [8]. However, a significant decrease in the F_m/F_o ratio which indicated an increase in dynamic quenching such as would occur if phosphorylation were to increase spillover, was observed in [21]. Thus, a second consequence of phosphorylation might be to cause an increase in the probability of PS II–PS I interaction. It has been observed that a small decrease in the amount of membrane appression results in phosphorylated samples [17,20]. This might allow greater opportunity for interaction between PS II and PS I at the edges of the stacks [20].

It seems therefore that there could be some biological variability in the observed effects of phosphorylation in terms of the blend of 'α' and 'spillover' change caused by LHC-II phosphorylation. Important factors would be the chlorophyll–protein composition of the membrane and the resulting forces involved in membrane organization [22]. It is of note therefore that if the potential for unstacking is increased by a decrease in Mg^{2+} to about 1 mM, then phosphorylation causes a large dynamic quenching indicative of an increased spillover (and unstacking), whereas at 5 mM Mg^{2+} a change consistent with just LHC-II uncoupling is seen.

The kinetics of fluorescence induction are also altered by phosphorylation. The induction curve of chloroplasts inhibited by DCMU is biphasic, consisting of a fast sigmoidal phase and a slow exponential one [24,25]. The sigmoidicity is almost certainly due to energy transfer between PS II centers [26–28] and probably requires the presence of the 'free' LHC-II referred to above [29]. Two effects of phosphorylation would be predicted according to the proposal for migratory LHC-II. First, the rate of fluorescence rise of the sigmoidal phase should decrease due to the decreased absorption cross section. Secondly, there may be a loss of connectivity between some of the PS II centers, thus giving rise to a decreased proportion of the sigmoidal phase in the induction curve. Both these effects have been observed [8,21,23]. There is variability in the extent of the latter change which might depend on the size of migratory LHC-II pool.

A model to explain the effects of phosphorylation on the organisation of the major chlorophyll–protein complexes is shown in fig.1A. This model is based on the ideas developed by Barber concerning the control of the lateral organisation of the thylakoid membrane by surface charge [22,30,33]. The primary effect of phosphorylation would be to insert charge in the electrically neutral appressed membrane region [22,30]. A phosphorylation-induced increase in membrane surface charge density has been measured [31,32]. The consequent increase in charge density would upset the balance of attractive and repulsive forces [33] and cause ejection of the charged LHC-II. Hence the area of stacked membrane would tend to decrease.

7. CONTROL OF ELECTRON TRANSPORT – THE STATE 1–2 TRANSITION

The 'Z' scheme for photosynthetic electron transport, which invokes 2 photosystems with different light-absorbing characteristics operating in series, ought to have a regulatory mechanism to allow balanced excitation rates of the two systems under conditions when the spectral composition of incoming irradiation varies. Only when these rates are balanced will radiation be used with maximal efficiency. Such a regulatory process was indeed observed in green algae [34] as well as in a variety of algae [34,35] and higher plants [17]. The essence of the process is that over-excitation with PS II (state 1) brings about a change in distribution of energy in favour of PS I (state 2) whilst over-excitation with PS I causes reversal (back to state 1). To sense the imbalance, a monitor of electron flow between PS II and PS I is required and the plastoquinone pool would seem ideal; thus over-excitation of PS II would result in a highly reduced plastoquinone pool, whilst over-excitation of PS I would cause its oxidation. The activation of protein kinase when plastoquinone is reduced provides a mechanism for the transition between state 1 and state 2, whilst inactivation (together with phosphatase activity) provides for reversal to state 1 (fig.1B).

The relationship between protein phosphorylation and state 1–2 transitions has been experimentally established by a variety of experiments using isolated chloroplasts. Thus PS II light activates the ATP-induced fluorescence decrease [12,37,38] and phosphorylation of LHCP [37]. PS I light causes dephosphorylation and a reversal to state 1 in a process inhibited by the phosphatase inhibitor NaF [38]. A chlorophyll *b*-deficient mutant of barley, which showed no state 1–2 transition in vivo, showed no ATP-induced fluorescence decrease in isolated thylakoids [17].

Protein phosphorylation also produces directly measurable effects on electron transport. Thus, an increase in quantum yield of PS I electron transport [32,39] and a decrease in quantum yield of PS II electron transport [7] result after phosphorylation. A decrease in rate of Q reduction after phosphorylation has been reported in [8]. All of this data is consistent with the fluorescence experiments described above and with the proposal that protein phosphorylation increases the rate of excitation of PS I at the expense of PS II. The control of relative rates of excitation of PS II and PS I was also shown by measurement of the redox state of Q and cytochrome *f*. Thus, it has been shown in [37] that phosphorylation increased the level of Q oxidation in PS II light whilst a similar change was seen for cytochrome *f* in [40].

8. CONTROL OF ELECTRON TRANSPORT – PS II FUNCTION

As mentioned earlier, a number of polypeptides in addition to LHC-II are phosphorylated and these are confined to PS II. In fact, in *Chlamydomonas*, the protein kinase itself co-purifies with the reaction center of PS II [41]. Several aspects of PS II function may be altered by phosphorylation. For instance, the binding affinity for DCMU is increased by phosphorylation [42]. This leads to a proposal that a major function of protein phosphorylation is to maintain the active organizational state of the PS II complex, that involves not just antenna–reaction centre interaction but also associations between other component polypeptides [41]. The idea that PS II is this kind of highly interactive unit has been proposed previously [43]. Whether phosphorylation has any function in the assembly and/or turnover of PS II components remains to be established but high kinase activities have been observed during the early stages of chloroplast development [44].

9. CONTROL OF ELECTRON TRANSPORT – A WIDER PERSPECTIVE

Spectral imbalance represents one in vivo situation in which the protein kinase would be important. However, it is clear, as pointed out in [8], that the redox state of plastoquinone will respond to changes in light intensity, rate of electron transport and energy state. For instance, it was shown in [45] that the redox state of Q varies in the period following illumination of whole leaves; an antiparallel relationship has been shown between the rate of CO_2 assimilation and the reduction level of Q in chloroplasts [46,47]. Thus, it would be predicted that protein kinase activity would also vary under these conditions. Indeed, the level of protein phosphorylation was maximal during the induction period of CO_2 fixation, but decreased as the rate of CO_2 fixation increased [48]. Recently, this work has been extended using a reconstituted chloroplast system and clear effects of light intensity and capacity for carbon assimilation on protein phosphorylation were observed [49].

The impression is that the level of protein phosphorylation responds to the balance between light intensity and capacity for NADPH consumption by CO_2 reduction; protein kinase thereby allows the thylakoid membrane to respond to the demands of stromal carbon metabolism. Thus during photosynthesis the chloroplast would strive towards (by controlling the level of protein phosphorylation) a condition in which the rate of excitation of PS II comes into balance with the capacity for carbon assimilation. This might have two advantages. Firstly, a protective effect on PS II might be achieved. Secondly, any increased demand for ATP and NADPH could be achieved by dephosphorylation and the subsequent increased rate of excitation of PS II.

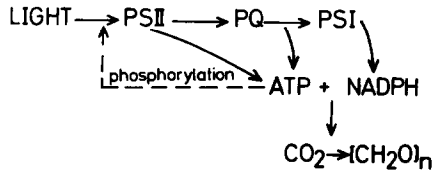

Fig.2. The possible role of protein kinase in controlling the rate of non-cyclic electron transport and photophosphorylation. The kinase enables feedback inhibition of products (ATP and NADPH) on the substrate (light). Rates of excitation of PS II will come into balance with the rate of carbon assimilation.

Recent experiments have also shown that protein kinase activity of thylakoids responds to the ATP/ADP ratio with ADP being a kinase inhibitor [50]. Using ribose-5-phosphate as an ATP sink, it has been shown how a low ATP/ADP ratio can elicit dephosphorylation even when the plastoquinone pool is highly reduced [49]. These effects of ATP/ADP fit in with the concept of a wider function for the kinase. Thus, kinase activity will be maximal when light intensity in PS II exceeds the ability of stromal enzymes to consume NADPH and ATP. Conversely the kinase activity will be minimal when NADPH and ATP consumption exceeds the rate of photosynthetic electron transport and photophosphorylation (fig.2).

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NOTE ADDED IN PROOF

Recently [51,52], two protein kinases have been purified from spinach chloroplasts with molecular weights of 25,000 and 38,000. It is not known as yet whether these kinase are involved in LHC-II phosphorylation but it clearly shows the heterogeneity of the kinase.

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