Photosynthesis III. Structure and Molecular Organisation of the Photosynthetic Apparatus
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THYLAKOID PROTEIN PHOSPHORYLATION: A REGULATORY ROLE IN PHOTOSYNTHESIS

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Summary. The two major phosphoproteins of the thylakoid membrane are the 25 000 and 26 000-M_r light-harvesting chlorophyll a/b proteins. In the phosphorylated state, the light-harvesting complex transfers excitation energy preferentially to photosystem I, whereas in the dephosphorylated state the complex transfers excitation energy preferentially to photosystem II. The protein kinase responsible for the phosphorylation reaction is regulated by light through the redox state of plastoquinone. The phosphatase responsible for dephosphorylation is not regulated by light or electron transport. A model is proposed in which the kinase and phosphatase participate in the regulation of excitation energy transfer between the two photosystems.

Introduction. The discovery that isolated intact pea chloroplasts incorporate ^{32}P-orthophosphate into a range of thylakoid polypeptides (1) has led to a number of interesting developments. Firstly, the most heavily labelled phosphoproteins have been identified as the 25 000 and 26 000-M_r light-harvesting chlorophyll a/b proteins (2). These are the most abundant polypeptides of the thylakoid membrane and serve to organize about one-third of the chlorophyll a and most of the chlorophyll b of the membrane into specific light-harvesting antennae that transfer excitation energy to both photosystem I and photosystem II (3). Secondly, the next most abundant phosphoprotein has been tentatively identified as the 8 000-M_r proton-conducting channel of the chloroplast ATP synthase (4). Thirdly, the protein kinase responsible for phosphorylation of these polypeptides has been shown to be thylakoid-bound and to be activated by light-driven electron transport (4,5). Finally, the phosphatase responsible for dephosphorylation is also thylakoid-bound but is not affected by light, electron transport inhibitors or molecules such as ATP and ADP (6). It is however activated by Mg^{2+} ions and inhibited by F^- ions (6). In this article we propose a model which is consistent with these and more recent data. According to this model, thylakoid protein phosphorylation plays an important regulatory role in photosynthesis,

especially in the regulation of excitation energy distribution.

Chlorophyll Fluorescence. A key finding was that the light-harvesting chlorophyll a/b proteins are phosphorylated on a terminal segment that protrudes from the surface of the membrane and is accessible to trypsin in vitro (6). It is this segment that has been implicated in granal stacking and in the salt-induced redistribution of excitation energy between photosystem I and photosystem II (7). It seemed to us that introduction of phosphoryl groups into this region of the light-harvesting proteins might be as effective as changes in the ionic environment of the membrane in altering the distribution of excitation energy. Accordingly, in collaboration with K.E. Steinback and C.J. Arntzen, we investigated the effect of protein phosphorylation on the fluorescence properties of thylakoid membranes (8,9).

Several important findings emerged from this work. Firstly, when thylakoids were illuminated at room temperature under phosphorylating conditions (in buffer containing sorbitol, tricine, $MgCl_2$ and ATP), marked changes were observed over a period of minutes in the 77°K chlorophyll fluorescence emission spectrum. Relative to emission at 685 nm (predominantly derived from light-harvesting complexes and photosystem II), there was as much as a doubling of emission at 734 nm (derived predominantly from photosystem I). The rise in emission at 734 nm followed the same time-course as phosphorylation of the light-harvesting polypeptides (LHP) and required both ATP and illumination. Secondly, phosphorylation of thylakoid proteins caused a decrease in the variable component of fluorescence recorded at room temperature after the addition of DCMU. When phosphorylated membranes were returned to darkness to permit dephosphorylation prior to the recording of fluorescence, the variable component was found to have returned to its initial value. Thirdly, when thylakoids were illuminated in sorbitol/tricine/$MgCl_2$/KCl buffer without ATP, room temperature fluorescence (in the absence of DCMU) was approximately constant for 5-10 min. Addition of ATP (though not of ADP) caused a biphasic fluorescence quenching. The rapid phase of quenching was abolished by prior addition of the uncoupler nigericin but the slower phase was insensitive to uncouplers. Upon addition of DCMU to inactivate the kinase (5), the slow quenching was replaced by a slow rise in fluorescence, which was itself halted by addition of the phosphatase inhibitor fluoride. Fourthly, it was found that the strong reducing agent dithionite can replace light both in activating the kinase and in

eliciting the changes in the 77°K fluorescence emission spectrum. Our conclusion from these studies is that phosphorylation of the LHPs increases the proportion of excitation energy received by photosystem I from the light-harvesting chlorophyll a/b complexes. Conversely, dephosphorylation increases the proportion of excitation energy received by photosystem II from the light-harvesting complexes.

Kinase Activation. The ability of dithionite to replace light in the activation of the thylakoid protein kinase raised the possibility that activation might be a consequence of the reduction of a particular component of the electron transport chain. Several lines of evidence now point to plastoquinone as the crucial component (9,10). Firstly, pre-illumination of thylakoids with a series of single-turnover flashes reduces the plastoquinone pool and activates the kinase in parallel. Secondly, DBMIB, which inhibits plastoquinone oxidation at low concentrations and plastoquinone reduction at high concentrations, inhibits kinase activation at exactly the same concentration as it inhibits plastoquinone reduction. These results were obtained in collaboration with K.E. Steinback and C.J. Arntzen. Finally, redox titrations, conducted in collaboration with P. Horton, establish that the mid-point redox potential for kinase activation is exactly the same as the mid-point redox potential for the reduction of plastoquinone and for ATP-dependent fluorescence quenching (that is, an E_m at pH 7.8 of -10 mV). Thus, kinase activity is responsive to the redox state of the plastoquinone pool. Whether plastoquinone directly activates the kinase is not yet clear.

Our previous observation that NADPH and ferredoxin could also activate the protein kinase in darkness (5) may be explained by the ability of reduced ferredoxin to reduce plastoquinone (11,12). We do not believe that activation of the thylakoid-bound protein kinase is mediated by either the thioredoxin system or the 'LEM' system, both of which have been implicated in the control of stromal enzyme activity and require electron transport via components on the reducing side of photosystem I (13,14).

Feed-back Control. A model which incorporates these results is presented in Fig. 1. In it, the LHPs exist in a phosphorylated state in which excitation energy is transferred preferentially to photosystem I and in a dephosphorylated state in which excitation energy is transferred preferentially to photosystem II. Conversion from one state to the other

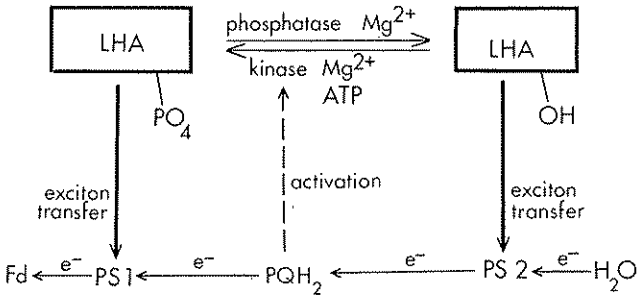

Fig. 1. Phosphorylation of polypeptides of the light-harvesting apparatus (LHA) places exciton transfer under the control of the redox state of plastoguinone. Reduced plastoquinone (PQH_2) activates a thylakoid-bound protein kinase which then phosphorylates the hydroxyl group (-OH) of threonyl residues. In the phosphorylated state, excitons are transferred preferentially to photosystem I (PS1), whereas in the dephosphorylated state they are transferred preferentially to photosystem II (PS2). Dephosphorylation is due to a thylakoid-bound phosphatase that is not under the control of plastoquinone.

is achieved by either the kinase (which requires ATP and Mg^{2+}) or the phosphatase (which requires Mg^{2+}). The rate of phosphorylation is controlled by the redox state of the plastoquinone pool. The rate of dephosphorylation is unaffected by redox poising.

This model contains all the elements demanded of a negative feed-back control system. If, under light of a certain quality, the entire pigment bed of the thylakoid transfers excitation energy in such a way that photosystem II is more active than photosystem I, the plastoquinone pool will become reduced and the kinase will be activated. Phosphorylation of the LHPs will then correct the imbalance by permitting increased excitation energy transfer to photosystem I at the expense of transfer to photosystem II. Should light quality then change such that the far-red component of incident light increases, the entire pigment bed would initially transfer excess excitation energy to photosystem I. The plastoquinone pool would then become oxidized, the kinase would be inactivated, and the phosphatase would dephosphorylate the LHPs, thereby increasing excitation energy transfer to photosystem II. This mechanism provides a possible basis for the state 1-state 2 transitions observed in algae (15,16). We are currently investigating this possibility in the green

alga Chlorella pyrenoidosa, in collaboration with W.P. Williams. It is interesting to note that another green alga, Euglena gracilis, displays kinetics of light-harvesting protein phosphorylation and dephosphorylation that are virtually identical to those displayed by isolated intact pea chloroplasts (17).

It is not yet clear exactly how the addition of a phosphoryl group to the LHP alters the distribution of excitation energy. Excitation energy transfer requires donor and acceptor chlorophylls to be closely spaced and appropriately oriented (3). Phosphorylation could modify the interaction between the LHP and the photosystems in its vicinity, weakening its affinity for photosystem II and strengthening its affinity for photosystem I. Alternatively, phosphorylation could cause the LHP to migrate from a region of the membrane enriched in photosystem II to one enriched in photosystem I. The direction of excitation energy transfer would then be a function of the abundance of each photosystem in the neighbourhood of the LHP.

Double Layer Theory. The ability of cations such as Mg^{2+} to increase the transfer of excitation energy from the LHP to photosystem II has been explained by Barber and co-workers in terms of double layer theory (18). In their treatment, a certain density of fixed surface negative charges must be screened by cations before protein particles in the membrane can adopt the configuration giving maximal transfer of excitation energy from the LHP to photosystem II. In the absence of Mg^{2+} ions, considerable transfer can take place to photosystem I but in the presence of 3-10 mM Mg^{2+} this transfer is much reduced. We suggest that even at 3-10 mM Mg^{2+} (that is, at the Mg^{2+} concentrations found in illuminated chloroplasts), the direction of excitation energy transfer can be regulated by changes in the surface negative charge density brought about by phosphorylation and dephosphorylation.

Double layer theory has also been applied to the binding of coupling factor (CF_1) to the thylakoid membrane. Telfer et al. (19) concluded that CF_1 binding only occurs when negative charges on CF_1 and its binding site (CF_o) are electrostatically screened by cations. By analogy with the LHP, we suggest that phosphorylation of the 8 000-M_r proton-conducting channel of CF_o (4) antagonizes the binding between CF_1 and CF_o and represents the physiological mechanism by which the coupling between electron transport and ATP synthesis is regulated.

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