

REGULATION OF PHOTOSYNTHETIC UNIT FUNCTION BY PROTEIN PHOSPHORYLATION

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1. INTRODUCTION

Phosphorylation of LHC-II regulates distribution of absorbed excitation energy between photosystems I and II in green plants [1] by causing lateral migration of LHC-II from appressed regions of thylakoid membrane rich in PS II to unappressed regions rich in PS I [2-5]. This migration is thought to be a result of electrostatic repulsion between phosphorylated and therefore negatively charged LHC-II complexes on adjacent, appressed regions of membrane [3-5]. Regulation of the activity of the protein kinase by the redox level of plastoquinone completes a negative feedback loop which maintains balanced excitation energy distribution under physiological conditions [1, 2].

This model cannot apply to photosynthetic prokaryotes, however, since it depends on lateral heterogeneity in distribution of the two photosystems between appressed and unappressed regions of membrane. In cyanobacteria, for example, the photosystems are thought to be distributed homogeneously throughout unappressed thylakoids. Cyanobacteria also have no LHC-II and have phycobilisomes as alternative light-harvesting complexes. Nevertheless it is clear that cyanobacteria are able to perform physiological regulation of excitation energy distribution in the manner of green plants [6-9]. This is also true of red algae [10]. Although it has been argued that the absence of lateral heterogeneity rules out a protein phosphorylation mechanism for phycobilisome-containing organisms [11], cyanobacterial membrane protein phosphorylation has been demonstrated [12-15] and is accompanied by changes in excitation energy distribution [14, 15].

Phosphorylation of light-harvesting membrane proteins has also now been demonstrated in the purple photosynthetic bacteria *Rhodospirillum rubrum* [16-18] and *Rhodospseudomonas sphaeroides* [19]. Photosynthetic bacteria have a single type of reaction centre and obviously cannot control excitation energy distribution between photosystems I and II; the primary function of membrane protein phosphorylation in purple bacteria is thought to be control of cooperativity of individual photosynthetic units [17, 18, 20].

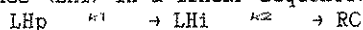
Here we describe a recently-proposed [21] common mechanism for effects of protein phosphorylation on excitation energy transfer in these apparently diverse systems, and discuss the possible identity of the 9 kDa phosphoprotein of chloroplast thylakoids in the context of the model's requirement for an intermediate complex that is phosphorylated and which therefore controls the association of LHC-II with photosystem II.

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2. THE MODEL

Our model has eight postulates, as follows.

(1) Excitation energy transfer from peripheral light-harvesting complexes (LHp) to reaction centres (RC) occurs via one or more intermediate light-harvesting complexes (LHi) in a linear sequence:



(2) Chlorophyll-binding polypeptides of both types of light-harvesting complex (LHp and LHi) are reversibly phosphorylated under physiological conditions.

(3) Phosphorylation of LHp and LHi causes a change in the balance of charge on their polypeptide chains at the membrane surface. This results in their mutual electrostatic repulsion with the repulsive force acting in a direction parallel to the membrane plane.

(4) The magnitude of the repulsive force between phosphorylated LHp and LHi exceeds the sum of the forces which otherwise hold the unphosphorylated complexes in close contact. The phosphorylated complexes therefore move apart, and this decreases the probability of excitation energy transfer between them, that is, k_1 is decreased.

(5) LHi is tightly bound to RC and excitation energy transfer from LHi to RC is unaffected by protein phosphorylation, that is, k_2 is constant.

(6) Where a number of reaction centres are connected by a common pool of LHp, the connectivity of these reaction centres is decreased by phosphorylation-induced mutual electrostatic repulsion of individual LHi and LHp complexes.

(7) Phosphorylation-induced mutual electrostatic repulsion of LHp and LHi decreases the effective absorption cross-section of individual photosynthetic units.

(8) Where two kinds of reaction centre compete for excitation energy from a pool of LHp, decreased excitation energy transfer to a reaction centre that has a tightly-bound and phosphorylated LHi permits increased excitation energy transfer to a reaction centre that has no phosphorylated LHi.

Figure 1 shows a diagram representing this general model as it applies in the ideal case of two cooperating photosynthetic units. The individual components of each photosynthetic unit are represented as if viewed in a direction parallel to the membrane plane. A protein kinase catalyzes phosphorylation of polypeptides of both LHp and LHi, leading to their electrostatic dissociation; the cooperativity of the two photosynthetic units is decreased. Reassociation of LHp and LHi results from their dephosphorylation which is catalyzed by a protein phosphatase. This in turn causes reconnection of the two units.

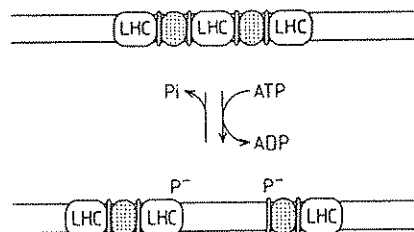


FIGURE 1. Control of cooperativity by phosphorylation and mutual electrostatic repulsion of peripheral (LHC) and intermediate light-harvesting complexes. The stippled area represents the reaction centre complex.

3. PURPLE PHOTOSYNTHETIC BACTERIA AND CYANOBACTERIA

The general model described above was formulated initially in an attempt to make sense of the role of membrane protein phosphorylation in photosynthetic prokaryotes [21]. Evidence for control of cooperativity by protein phosphorylation in purple photosynthetic bacteria is available and is consistent with the general model [17, 18]. Recent results from our own laboratory are presented elsewhere in these proceedings [20]. Likewise, evidence for control of excitation energy distribution by the redox level of plastoquinone in cyanobacteria [8] is consistent with our view that a protein phosphorylation mechanism is involved in cyanobacterial state transitions [9]. A key regulatory role of protein phosphorylation in this process is supported by the recent discovery in our laboratory of light-dependent phosphorylation of a component of the phycobilisome [15].

4. CHLOROPLASTS: THE ROLE OF THE 9 kDa PHOSPHOPROTEIN

A thylakoid membrane polypeptide of 9 kDa was reported as one of the substrates of chloroplast protein phosphorylation in the original studies of Bennett [22]. This polypeptide is phosphorylated in the same way as LHC-II [23], that is, in a light-dependent reaction [24] catalysed by a membrane-bound kinase [23, 24]. Dephosphorylation of the 9 kDa polypeptide is generally slower than that of LHC-II and requires higher Mg^{2+} concentrations but it is nevertheless thought to be catalysed by the same membrane-bound phosphatase [25].

We have recently reported the amino acid composition of the 9 kDa phosphoprotein of pea thylakoids [26]. The result is consistent with it being a chlorophyll-binding protein structurally related to LHC-II. By analogy with the known amino acid sequence of LHC-II and structural predictions based thereon, we have proposed [26] that the 9 kDa phosphoprotein has a structure similar to that of the part of LHC-II represented by its N-terminal, surface-exposed segment together with the first hydrophobic domain. The protein would then have a single membrane-spanning α -helix containing one cysteine and a chlorophyll-binding histidine. If this proposal is correct, then the 9 kDa phosphoprotein would be likely to be a nuclear-encoded protein synthesised cytoplasmically and imported into the chloroplast in precursor form. Furthermore, it should show sequence homology with LHC-II. The functional implications of our proposal are that the 9 kDa polypeptide is a component of an LHI complex which serves to link excitation energy transfer from LHC-II to P680. Regulation of excitation energy distribution in green plants could then involve mutual electrostatic repulsion between the phosphorylated forms of the 9 kDa polypeptide and LHC-II.

Farchaus and Dilley [27] describe an amino acid composition for the spinach 9 kDa phosphoprotein isolated from photosystem II particles. Their result is broadly in agreement with ours [26]. The N-terminal sequence of nine amino acids obtained by Farchaus and Dilley [27] shows homology with one predicted from the nucleotide sequence of an open reading frame in tobacco chloroplast DNA (M. Sugiura, personal communication). Amino acids 41 to 60 of this sequence predominantly have non-polar side chains, and this region corresponds exactly to a helical region according to our own structural predictions. The protein encoded in the chloroplast ORF is clearly a membrane protein of about the right size, but its structure differs from the one we propose [26]. In particular, it has no cysteine or histidine (we obtain

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1.53% and 2.93% respectively). The complete amino acid sequence of the protein must be determined directly in order to resolve this problem.

We believe that the balance of evidence currently favours a role for the 9 kDa phosphoprotein in regulation of excitation energy distribution in chloroplasts. This in turn allows us to view light-state transitions in green plants as a special case of a more widespread and fundamental phenomenon.

4. ACKNOWLEDGEMENTS

We thank R. J. Ellis (Warwick) and J. B. C. Findlay (Leeds) for helpful discussion, and M. Sugiura (Nagoya University) for sequence information prior to publication. Supported by grants to JFA from SERC, The Royal Society, and The Nuffield Foundation.

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