Redox signalling and the structural basis of regulation of photosynthesis by protein phosphorylation

J. F. Allen and A. Nilsson


In photosynthesis in chloroplasts and cyanobacteria, redox control of thylakoid protein phosphorylation regulates distribution of absorbed excitation energy between the two photosystems. When electron transfer through chloroplast photosystem II (PSII) proceeds at a rate higher than that through photosystem I (PSI), chemical reduction of a redox sensor activates a thylakoid protein kinase that catalyses phosphorylation of light-harvesting complex II (LHCII). Phosphorylation of LHCII increases its affinity for PSI and thus redistributes light-harvesting chlorophyll to PSI at the expense of PSII. This short-term redox signalling pathway acts by means of reversible, post-translational modification of pre-existing proteins. A long-term equalisation of the rates of light utilisation by PSI and PSII also occurs: by means of adjustment of the stoichiometry of PSI and PSII. It is likely that the same redox sensor controls both state transitions and photosystem stoichiometry. A specific mechanism for integration of these short- and long-term adaptations is proposed. Recent evidence shows that phosphorylation of LHCII causes a change in its 3-D structure, which implies that the mechanism of state transitions in chloroplasts involves control of recognition of PSI and PSII by LHCII. The distribution of LHCII between PSII and PSI is therefore determined by the higher relative affinity of phospho-LHCII for PSI, with lateral movement of the two forms of the LHCII being simply a result of their diffusion within the membrane plane. Phosphorylation-induced dissociation of LHCII trimers may induce lateral movement of monomeric phospho-LHCII, which binds preferentially to PSI. After dephosphorylation, monomeric, unphosphorylated LHCII may trimerize at the periphery of PSII.

Key words – Chloroplast light-harvesting complex, molecular recognition, photosynthesis, photosystem I, photosystem II, protein kinase/phosphatase, redox sensor/response regulator, redox signalling.

J. F. Allen and A. Nilsson (corresponding author, e-mail anders.nilsson@plantcell.lu.se), Plant Cell Biology, Lund Univ., Box 7007, SE-220 07 Lund, Sweden.

Abbreviations – CD, circular dichroism; CP29, chlorophyll binding protein 29 kDa; FTIR, Fourier transform infrared; LHCII, light-harvesting complex II; NMR, nuclear magnetic resonance; PSI, photosystem I; PSII, photosystem II.

State transitions and post-translational modification of LHCII

Short-term physiological adaptation occurs when plants, algae or cyanobacteria are moved between illumination conditions favouring either PSI or PSII (Bonaventura and Myers 1969, Murata 1969, Myers 1971). The mechanism of this adaptation involves redistribution of absorbed excitation energy between the two photosystems, such that the light-limited photosystem receives more energy while the light-saturated photosystem receives less. Thus energy transfer to PSI becomes favoured under PSII light, while energy transfer to PSII becomes favoured under PSI light. The state of adaptation to PSI light is referred to as the light 1-state, or state 1, and the state of adaptation to PSII light is referred to as the light 2-state, or state 2. The transitions between the two adaptation states are therefore described as light-state transitions, or state 1-state 2 transitions. A coupling between excitation energy distribution and redox changes was suggested as a general principle for state transitions by Murata (1969) and Duysens (1972). Phosphorylation of LHCII causes it
to redirect its light-harvesting function from PSII to PSI. Activation of the LHCII kinase by chemical reduction of an electron carrier at the level of plastoquinone (J. F. Allen et al. 1981) thus provides a specific molecular mechanism for the coupling of excitation energy distribution to inter-photosystem electron transport.

The general phenomenon of inter-photosystem redox control of the LHCII kinase is now well documented (reviewed by J. F. Allen 1992a, 1995, Bennett 1991). Recent investigations have attempted to focus on the precise site of redox control, but continue to produce apparently conflicting results. Vener et al. (1997) suggest that the primary site of control is the Qo site of the cytochrome b6f complex, while Race and Hind (1996) favour a point of control on the acceptor side of PSII, on the basis that protein kinase activity is retained in PSII core preparations. The enzymology of thylakoid protein kinases and phosphatases is reviewed elsewhere in this issue (Gal et al. 1997).

Molecular recognition and structural effects of phosphorylation of LHCII

The prevalent view of the molecular mechanism of phosphorylation in photosynthetic systems, has, until recently, been a direct interaction model, according to which the negatively charged phosphate group of LHCII is supposed to induce changes in long range electrostatic repulsion or attraction. However, this view of a single mechanism must change in the light of recent results from structural studies of phosphoproteins involved in photosynthesis. This change of view removes a conceptual barrier between regulation of ligand binding in soluble proteins and regulation of function and interactions of membrane bound proteins: covalent attachment of the phosphate group causes a structural change, as the negatively charged phosphate group attracts a cluster of positive charges on amino acid side chains that are otherwise at varying distances from the phosphorylation site. In a few cases the structural influence of protein phosphorylation has been described at atomic resolution (Barford et al. 1991, Hurley et al. 1990) while in other cases less defined protein or peptide structural changes have been resolved (Augusteyn et al. 1989, Buelt et al. 1992, Mortishire-Smith et al. 1995, Rajogopal et al. 1994, Ram-wani et al. 1989). Most of these phosphoproteins are soluble enzymes in which structural determination is inherently easier than in membrane proteins.

Two chlorophyll binding proteins of PSII have been subject to studies regarding structural effects upon phosphorylation, namely LHCII (LHCIIb or Lhcb2) (Nilsson et al. 1997) and the 29-kDa chlorophyll binding protein (CP29) (LHCIIa or Lhcb4) (Croce et al. 1996). For both proteins, structural changes are detected. The phosphorylation site of CP29 differs from that found in other thylakoid membrane phosphoproteins. CP29 is phosphorylated at position 83 whereas LHCII is phosphorylated close (within 6 residues) to its N-terminus. CP29 (re-
reviewed by Bassi et al. 1997) shows a long-range allosteric effect of phosphorylation in the hydrophilic domain, as the organisation of bound chlorophyll molecules is affected (Crocce et al. 1996). The non-phosphorylated structure of CP29 is assumed to resemble that of LHCII (Kühlbrandt et al. 1994) but no direct structural information is available today. However, more detailed structural information has been obtained about the LHCIIb phosphorylation mechanism (Nilsson et al. 1997). The major part of the non-phosphorylated structure of LHCII has been resolved by Kühbrandt and co-workers (Kühbrandt et al. 1994) and, based on that structure, a nonsilent mechanism of phosphorylation has been proposed (Nilsson et al. 1997). N-terminal phosphorylation at Thr-5 causes a local structural rearrangement, giving an α-helix-like structure, composed of 5–10 amino acid residues, around the phosphorylation site. This domain is then docked into the positively charged region between helices A and B, which protrudes into the stroma (Fig. 1A). The relocation of the domain is made possible by loss of specific lipid-protein interactions close to the phosphorylation site, probably caused by the α-helix formation. This structural rearrangement also causes dissociation of LHCII trimers and thereby detachment of LHCII from PSII (Fig. 1B). The lateral migration involved in state transitions may therefore be understood in terms of altered protein-protein interactions in the surface-exposed domain of LHCII, the regulator for the transfer of monomeric phospho-LHCII away from PSII and towards PSI. This post-translational control of LHCII protein sorting implies a highly specific, redox-controlled mechanism that secures signal transduction to the right target during short-term adaptations to environmental change.

Photosystem stoichiometry adjustment as redox control of gene expression

In addition to state transitions, there exists a functionally similar, though slower, redox regulation of the relative quantities of PSI and PSII. This long-term mechanism compensating for unequal utilisation of light by the two photosystems involves an increase in the relative quantity of PSI under illumination favouring PSI, and an increase in the relative quantity of PSI under illumination favouring PSII (Chow et al. 1990, Melis and Harvey 1980). A role for the redox state of the plastocyanin pool or cytochrome b/f complex is indicated by experiments using inhibitors in cyanobacteria (reviewed by Fujita et al. 1994) and by the observation that PSI light-harvesting complex mutants of diverse photosynthetic groups remain in a state in which PSI is abundant compared with PSI, and show no further changes in photosystem stoichiometry (Kim et al. 1993).

In cyanobacteria, cells grown in PSI light with a high PSII/PSI stoichiometry and cells grown in PSII light with a low PSII/PSI stoichiometry both exhibit state transitions, but the amplitude of the fluorescence changes is greater in PSI-light-grown cells with their larger PSII antenna and greater contribution of PSII variable fluorescence to total room-temperature fluorescence emission (J. F. Allen et al. 1989). These results indicate that state transitions and adjustment of photosystem stoichiometry may operate in parallel, both being triggered by changes of redox state of inter-photosystem electron carriers.

Figure 2 shows a schematic outline of the proposed inter-relationship between state transitions and photosystem stoichiometry adjustment. The short signal transduction pathway of state transitions (depicted in the upper

---

**Fig. 2. Redox control of photosystem stoichiometry by redox effects on expression of genes for components of the two photosystems.** In this scheme, photosystem stoichiometry adjustment and state transitions are complementary adjustments to the same redox signal. LHC is LHCII in chloroplasts, and a putative phycobilisome component in cyanobacteria and red algal plastids.

Physiol. Plant. 100, 1997
half of Fig. 2) can be viewed as the post-translational arm of an integrated set of redox responses (J. F. Allen 1995). The wide occurrence of redox control of gene expression in bacteria (J. F. Allen 1993a) together with the emerging evidence for redox control as one basis of photocontrol of gene expression in plants (C. A. Allen et al. 1995, J. F. Allen et al. 1995) supports a scheme for long-term responses such as that shown in the lower part of Fig. 2.

**A proposal for the mechanism of bifurcated redox signalling**

A more specific proposal for the mechanism by which the redox signal may lead to two complementary mechanisms of adaptation is shown in Fig. 3. One assumption of this model is that state transitions have always been important enough to photosynthetic organisms for their underlying mechanism to have been conserved in evolution (however, see Delphin et al. 1996). A second assumption is that state transitions, in the prokaryotic progenitors of chloroplasts as well as in modern cyanobacteria, involve a two-component signal transduction pathway. This second assumption is plausible in the sense that two-component redox signal transduction has already be described in other systems (J. F. Allen 1993a), and two-component systems are known to respond to a diversity of environmental signals (Stock et al. 1989), among which altered redox state of a photosynthetic electron carrier would be an unsurprising addition.

Two-component systems contain two components: a sensor and a response regulator. Response regulators are proteins that become phosphorylated on an aspartate on a surface-exposed loop. The phosphate group is transferred to the aspartate from a phosphohistidine side chain of the response regulator’s cognate sensor (Stock et al. 1989). The histidine of the sensor becomes phosphorylated in response to the relevant environmental signal. The sensor is thus a response regulator kinase, mediating transfer of phosphate from ATP to the response regulator if, and only if, environmental conditions are right. Certain sensors, notably ArcB of *E. coli* (Iuchi and Lin 1993), contain both sensor and response regulator domains, and the assumption can be made that a sensor’s response regulator domain serves to damp the signal passed to the authentic, discrete response regulator. The suggestion outlined in Fig. 3 is that the branch-point of the bifurcated redox signal transduction pathway of photosynthesis may be the transfer of the phosphate group of the sensor domain to an aspartate, either of its own response regulator domain, or of a separate response regulator.

The basis of the ‘decision’ taken by a photosynthetic system about the appropriate level of response to a redox signal, would, according to this proposal (Fig. 3), be a simple consequence of success or failure of the most rapid response in restoration of redox poise. If the putative redox sensor of two-light-reaction organisms contains both sensor and response regulator domains, and if the kinetically favoured pathway for phosphoryl transfer is intramolecular, then phosphorylation of the redox sensor’s own aspartate would produce a rapid response, which, in this context, is the state 2 transition. If full activation of the light-harvesting kinase then fails to restore redox poise, it will also fail to switch off the stimulus initiating phosphorylation of the sensor’s histidine. At the same time, the kinetically preferred intramolecular phosphoryl transfer from histidine is prohibited by the acceptor (aspartate) being already in its phosphorylated form. The kinetically less favoured, intermolecular phosphoryl transfer may then predominate. This second phosphoryl transfer is to the aspartate of the response regulator that controls gene expression. According to
this specific proposal (Fig. 3), photosystem stoichiometry adjustment is therefore initiated when either of the state transitions has reached the limit of its response.

In the branched redox signalling pathway deployed in oxygenic photosynthesis, there is a clear selective advantage in location of the genes whose expression is thereby regulated within the same cellular compartment as that in which the redox signal originates. The proposal that chloroplast, and, by the same argument, mitochondrial genomes have been retained, in evolution, to enable redox control of gene expression (J. F. Allen 1993a) has been discussed in a previous minireview (J. F. Allen 1995). Subsequent developments of the original hypothesis suggest that such an intracellular division of labour may have had additional and wide-ranging evolutionary consequences (J. F. Allen 1996, J. F. Allen and Raven 1996).

Acknowledgments – Research in the authors’ laboratory is supported by the Swedish Natural Science Research Council and by the Per-Eric and Ulla Schyberg Foundation. We also thank P. O. Arvidsson for drawing Fig. 1.

References
Duyens, L. N. M. 1972. 3-(3,4-Dichlorophenyl)-1,1-dimethylurea (DCMU) inhibition of system II and light-induced regulatory changes in energy transfer efficiency. – Biophys. J. 12: 858–863.

Edited by B. Andersson and C. Sundqvist

Physiol. Plant. 100, 1997
This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.