of the observed ∆Lk. In the absence of more refined data, it remains to be seen if the omission of these factors has any significant effect upon the predicted ∆Lk. A real residual discrepancy between the experimental determination of ∆Lk and that predicted from Eqn (3) could be ascribed to either of two possibilities: (1) the neglect of possible contributions from the linker region to ∆Lk and ∆Φ; and (2) the neglect of interaction terms between nucleosomes and the adjoining linker regions. The latter effect is almost certainly very small, in view of the independence of the measured value of ∆Lk upon m. On the basis of the first possibility, it is predicted that any additional contribution from the linker regions via ∆Φ would be in the direction of a reduction of h relative to h0. Alternatively, any additional contribution via ∆SLk would require right-handed supercoiling of the linker DNA.

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How does protein phosphorylation regulate photosynthesis?

John F. Allen

Phosphorylation of light-harvesting antenna proteins redirects absorbed light energy between reaction centres of photosynthetic membranes. A generally accepted explanation for this is that electrostatic forces drive the more negatively charged, phosphorylated antenna proteins between membrane domains that differ in surface charge. However, structural studies on soluble phosphoproteins indicate that phosphorylated amino acid side chains have specific effects on molecular recognition, by ligand blocking or by intramolecular interactions which alter protein structure. These studies suggest alternative mechanisms for phosphorylation in control of pairwise protein–protein interactions in biological membranes. Thus, in photosynthesis, the surface charge model is only one possible interpretation.

Since PSI and PSII are connected in series by the electron transport chain, they must have equal rates of electron transport, with maximum efficiency achieved only when they receive light energy at equal rates. Their intrinsic rates of energy capture will, however, vary according to light intensity and spectral composition, and their series connection may be modified by addition of more PSI turnover to produce extra ATP by cyclic photophosphorylation. The relative light-harvesting ability of the two photosystems must therefore be controlled.

Balancing the light-harvesting capacity of the two photosystems is achieved by redox activation of the LHClI protein


IN PLANTS and photosynthetic bacteria, phosphorylation of membrane proteins is ultimately responsible for many of the physiological responses to changes in incident light and redox poise. A major protein substrate of phosphorylation is the chloroplast light-harvesting chlorophyll a/b-binding complex known as light-harvesting complex II (LHClII), which binds perhaps half of the chlorophyll in nature, therefore absorbing half the light converted in photosynthesis. It has been known for over ten years that this light-harvesting complex changes its allegiance upon phosphorylation. In its unphosphorylated form, LHClII interacts specifically with photosystem II (PSII) of the photosynthetic electron transport chain, and the light energy it absorbs is converted into electrochemical potential at the PSII reaction centre. Upon phosphorylation of a threonine close to the amino terminus (Thr6 in the major pea polypeptide), LHClII complexes detach themselves from PSII, and reattach to PSI instead.

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OPEN QUESTION

How does protein phosphorylation regulate photosynthesis?
versely, oxidation of plastoquinone
now been critically tested. For example,
ances the energy distribution. Con-
PSI at the expense of PSII, which bal-
quence of PSII running faster than PSI.

The mobile antenna
Chloroplast thylakoids exhibit hetero-
genility in lateral distribution of PSI and
there is a greater density of PSI in
membrane vesicles that originate
from appressed domains of the thyla-
koid membrane (corresponding to
chloroplast grana or thylakoid stacks)
than in vesicles originating from
unstacked stroma-exposed thylakoids.
In contrast, PSI is present predomi-
nantly in unstacked thylakoids or grana
margins1. If phosphorylation of LHCCI polypeptides decreases the interaction of
LHCCI with PSII and increases its
interaction with PSI, then lateral re-
association of the three complexes must
occur, and movement of phospho-LHCCI
into PSI-rich domains now has exten-
sive experimental support2,6,8,9. The
ture domain organization of the thyla-
koid may be more complex than this
simple bipartite model suggests, with
six discrete domains being possible1.
The grana margin may therefore be an
important site of protein traffic and of
alteration in protein–protein inter-
actions6,7.

The surface charge hypothesis
Barber8 and Arntzen and co-workers9
have proposed a mechanism for the
functional effects of phosphorylation of
chloroplast LHCCI. In this hypothesis,
the primary effect of phosphorylation is
an increase in the negative charge on
the outer (cytoplasmic) surface of the
appressed domain of the thylakoid
membrane, and the mag-
nitude of the change is
sufficient to overcome the
attractive forces other-
wise holding together
LHCCI on adjacent, ap-
pressed thylakoid domains.

Dependence on lateral heterogeneity. Move-
ment of pigment–protein complexes
between discrete domains cannot occur
in laterally homogeneous membranes,
since they have no such domains. Miller
and Lyon11 go as far as to suggest that
protein phosphorylation evolved in
photosynthesis as a means for lateral
migration of LHClI to overcome the dif-
ficulties introduced by the stacked
membrane system'. Yet membrane pro-
tein phosphorylation seems to regulate
photosynthesis in laterally homogen-
eous, unstacked prokaryotic mem-
branes2 as well as in LHCCI-containing
algae that show no distinct thyla-
koid organization into grana and
stroma12.

The local charge hypothesis
Allen and Holmes suggested that the
electrostatic forces which are induced
by phosphorylation and which control
protein–protein interactions act in a
direction parallel to the membrane
plane13. This proposal removes the
requirement for distinct domains and replaces surface charge with more localized charge on individual protein complexes. This model can therefore accommodate prokaryotes and unstacked chloroplast thylakoids, circumventing the third problem of the surface charge model.

The local charge model also circumvents the problem of the non-specificity of surface charge. This is because individual complexes rather than whole domains would have their charge altered by phosphorylation. However, the requirement for this process to have specific effects on protein-protein interactions introduces the additional constraint that all participating complexes should be phosphorylated, since otherwise phosphorylation of a single protein would alter its interaction, non-specifically, with all its neighbours. This constraint is consistent with the multiple phosphorylations observed in chloroplasts, cyanobacteria and purple bacteria, and provides a basis for proposed identities and functions of purple bacterial chromatophore, cyanobacterial and chloroplast thylakoid phosphoproteins.

**Isocitrate dehydrogenase**

The quite unrelated, soluble enzyme isocitrate dehydrogenase has 416 residues and is inactivated by phosphorylation of Ser113. The molecular and structural basis of this effect is understood since structures at 2.5 Å resolution have been obtained for the *E. coli* enzyme both with and without substrate and in both phosphorylated and dephosphorylated forms. These structures show that the phosphorylation site is also part of the catalytic site, since one of the six hydrogen bonds formed between isocitrate and amino acid side chains is that between O4 of the α-carboxyl group of isocitrate and Ser113. The effect of phosphorylation of Ser113 is therefore to block substrate binding at the active site by short-range electrostatic forces. The phosphate group has minor and local effects on the position of other side chains near the substrate-binding site, but there is no general conformational change in the protein. The effect of phosphorylation in providing an electrostatic block to substrate binding can be mimicked in site-directed mutants where Ser113 is replaced by the acidic amino acids glutamate or aspartate, and neither of the mutants shows significant long-range structural alterations.

The mechanism by which phosphorylation exerts its functional effect on isocitrate dehydrogenase is therefore similar to the local charge model proposed specifically for light-harvesting membrane proteins of photosynthesis, though in the local charge model protein-protein interactions rather than protein-substrate interactions are blocked by phosphorylation. The membrane surface charge model has no counterpart in phosphorylation of...
isocitrate dehydrogenase, although isocitrate binding to the glutamate site-directed mutant can be induced at high ionic strength (6.9 M) and isocitrate concentration (100 mM), where isocitrate is sterically accommodated by movements of the side chain of Glu113 and the γ-carboxylate of isocitrate. This effect of forcing the formation of the enzyme-substrate complex may be comparable to the effect of altered cation concentration on chloroplast thylakoid structure and function.

Glycogen phosphorylase
Rabbit glycogen phosphorylase is activated by phosphorylation, and, like isocitrate dehydrogenase, structures have been solved from X-ray crystallography for both its phosphorylated and dephosphorylated forms. Barford et al. have recently reported structures at 2.9 Å resolution showing that phosphorylation of Ser14 at an allosteric site close to the amino terminus affects substrate binding; long-range protein structural effects cause rotation of the two identical 841-residue subunits, which exposes the catalytic site and also creates a high-affinity binding site for a cofactor, AMP.

The key event in activation of glycogen phosphorylase by phosphorylation of Ser14 is charge compensation, whereby the phosphate group shields basic amino acid side chains on either side of the phosphorylation site from each other. When these repulsive forces are neutralized, the amino-terminal segment containing the phosphoserine assumes an α-helical conformation that is absent from the dephosphorylated protein. The effect of this local change in secondary structure represented by helix formation is to move the whole amino-terminal segment from a peripheral position in the protein to a closer interaction, via salt bridges, with a number of initially remote, basic side chains, including Arg69 of the same chain and Arg43 of the opposing chain. Ser14 itself moves through about 36 Å, and Arg10 moves through 50 Å. Thus the tertiary and quaternary structural changes arising from phosphorylation of glycogen phosphorylase are generated initially by short-range electrostatic forces, and are reinforced by formation of salt bridges of about 2.6 Å between side chains that are in the region of 40 Å apart in the dephosphorylated form of the protein. Helix formation and some of the immediate tertiary structural changes are shown in Fig. 2.

The structure of LHCII
The best three-dimensional structure of LHCII currently available is one at 6 Å resolution and obtained by electron crystallography of dephosphorylated pea LHCII by Kühlbrandt and Wang. This shows three membrane-spanning helices and 15 chlorophylls. Helices I and III (termed B and A respectively by Kühlbrandt and Wang) are 49 Å and 46 Å long — longer than any helix described previously for a membrane protein — and must protrude beyond the membrane surface. They extend for 9 Å and 7 Å beyond the top of helix 2 (helix C). The 15 chlorophylls (chlorophylls a and b cannot be distinguished at 6 Å) are arranged on two levels, the upper level of eight chlorophylls being related by the local twofold symmetry found in helices I and III, indicating conserved chlorophyll-binding sites in the internally homologous polypeptide segments.

The structure of LHCIIb
Amino-terminal segments of pea LHCIIb and rabbit glycogen phosphorylase, showing phosphorylated amino acid residues (') and basic amino acid residues (bold) flanking the phosphorylation sites. Helix shows the extent of the α-helix induced by phosphorylation of glycogen phosphorylase. A segment of E. coli isocitrate dehydrogenase is also shown. Alignments of phosphorylation sites of the three proteins are made to permit comparison of the distribution of adjacent, basic amino acids.
The molecular recognition hypothesis

The molecular recognition hypothesis states that the electrostatic forces exerted initially by phosphorylation are entirely intramolecular, and ultimately lead to major structural changes that alter the interactions of membrane proteins through effects on the complementarity of their respective docking surfaces.

The principal proposals of the hypothesis are as follows. (1) Phosphorylation of membrane proteins reversibly increases fixed negative charge at the phosphorylation site. (2) The increased negative charge alters electrostatic interactions between the side chain of the phosphorylated amino acid and other amino acid side chains located in its immediate vicinity, within about 5 Å. (3) The negative charge of the phosphat group compensates for fixed positive charges on basic side chains, which would otherwise repel each other if brought close together (10–12 Å) in an α-helix. (4) Charge compensation usually occurs within one polypeptide segment, but may occur between polypeptides where a phosphorylation site is close to one of their amino termini. (5) Charge compensation permits a change in secondary structure of the polypeptide segment containing the phosphorylation site. In LHCII this change is formation of an α-helix. In the helix of phospho-LHCII, the phosphate group neutralizes the interaction of positive charges located on side chains 3–4 residues away on each side of the phosphorylation site, at about 5–6 Å (one helix turn) from the phosphorylation site and therefore 10–12 Å from each other. (6) The local change in secondary structure perturbs long-range interactions between side chains, and this perturbation causes a change in the tertiary structure of the hydrophilic domain. (7) The tertiary structural change may involve further electrostatic interaction between the phosphorylation site and distant basic side chains located either on the same or an adjacent polypeptide, bringing the nitrogen atoms involved to within 3.5 Å of oxygen atoms of the phosphate group. (8) Tertiary structural changes alter the shape of a surface of the phosphoprotein, which decreases its complementarity with that of a neighbouring protein complex (e.g. the inner LHCII pool or the PSII core antenna). (9) The decrease in complementarity decreases the sum of the various interactions holding the two proteins together. Their hydrophilic domains cease to bind together, and the two proteins then become free to diffuse independently of each other within the membrane. (10) If sufficient thermal energy is available, the two proteins become separated by lateral diffusion, and their functional interaction is prevented. For light-harvesting proteins, intermolecular excitation energy transfer is prevented. (11) Upon phosphorylation of Thr6 of pea LHCIIb [steps (1), (2)], charge compensation could be expected to occur between basic residues 2–3 and 8–9 [step (3)]. This could permit the amino-terminal segment of phospho-LHCII to form a helix approximately between residues 1 and 10 [steps (4), (5)], as depicted in Fig. 5. The threonine phosphate could then form salt bridges with Lys61 and Arg63 close to the top of membrane helix I, with Arg143, and with Lys178, 180 or 183 close to the top of membrane helix III. This would be expected to induce a large change in tertiary structure in the cytoplasmic (stromal) surface-exposed domain of LHCII. The membrane disposition of the LHCII polypeptide is intended to correspond to that proposed by Kühlbrandt and Wang on the basis of their three-dimensional electron density map at 6 Å resolution. Helices I, II and III (numbered from the amino terminus) probably correspond to helices B, C and A respectively. A and B are longer than C, and are tilted with respect to the membrane plane, and protrude from the membrane. A and B also have hook-like extensions at the stromal side of the membrane, and together with the hooks show a twofold symmetry about an axis perpendicular to the membrane plane. These regions may correspond to the polypeptide segments showing internal sequence homology, and contain the basic side chains proposed here as possible ligands for the threonine phosphate. See also Fig. 3.
The molecular recognition model for phosphorylation-induced changes in the organization of the chloroplast thylakoid. PSII centres may be connected laterally and transversely for excitation energy transfer by docking of LHCII complexes with the PSII core antenna system (diagonally hatched) and with each other (left-hand side). This brings the acceptor side of PSII reaction centres into opposition. Phospho-LHCII has a decreased affinity for the PSII core (lateral protein–protein interactions) and for itself (transverse protein–protein interactions), and therefore becomes free to diffuse independently of PSII within the membrane, eventually to dock instead with the PSI antenna system (right-hand side). In contrast to the surface charge model (Fig. 1), only thermal energy is required for dissociation of phospho-LHCII from PSII. The connectivity and antenna size of PSII units are decreased, and the loss of adhesion contact surfaces may cause some transverse separation of adjacent thylakoids of the grana stack. A proportion of PSII reaction centres ceases to be in opposition. The altered shape of the block representing LHCII is intended to convey a structural change in the surface exposed domain (Fig. 5), electrostatic blocking of protein-protein interactions by the phosphate groups, or a combination of both.

Prospects for the molecular and structural basis of regulation

With only two exceptions – glycogen phosphorylase and isocitrate dehydrogenase – the structural basis of regulation by protein phosphorylation in general is an open question. Photosynthesis is a particularly interesting example for a number of reasons. First, the process itself regulates the primary events in energy capture by life on earth, and modifies the function of the world’s most abundant membrane protein complex, one visible in lunar photography, and without which life would be possible but unimaginably different from that which we know. Second, phosphorylation of LHCII guides protein–protein recognition rather than protein–small-molecule recognition, and specifically concerns membrane proteins. As such, this regulation may have features in common with control of function of receptors and components of cellular recognition and signalling pathways. Third, since the widely accepted surface charge model faces problems of its own, and since recent findings show that phosphorylation initially perturbs short-range electrostatic forces (operating typically over distances of no more than 10 Å), modification of local protein–protein recognition may be a viable alternative to the surface charge model. The relative strengths of these two different mechanisms in the actual redistribution of light energy in green plants is a subject for future research.

Progress in understanding regulation of photosynthesis by protein phosphorylation will depend upon a concerted effort of protein engineering along the lines already set out so clearly for *E. coli* isocitrate dehydrogenase. For this to occur the existence of the problem must be appreciated – we cannot expect its solution to appear by accident. In addition, the problem has wide implications for control of protein–protein and protein-DNA interactions. Membrane phosphoproteins act as environmental sensors and initiate adaptive responses by controlling gene expression. Photosynthesis itself may be an archetype of processes where post-translational and transcriptional levels of response can be brought into play.

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References


Figure 6

The molecular recognition model for phosphorylation-induced changes in the organization of the chloroplast thylakoid. PSII centres may be connected laterally and transversely for excitation energy transfer by docking of LHCII complexes with the PSII core antenna system (diagonally hatched) and with each other (left-hand side). This brings the acceptor side of PSII reaction centres into opposition. Phospho-LHCII has a decreased affinity for the PSII core (lateral protein–protein interactions) and for itself (transverse protein–protein interactions), and therefore becomes free to diffuse independently of PSII within the membrane, eventually to dock instead with the PSI antenna system (right-hand side). In contrast to the surface charge model (Fig. 1), only thermal energy is required for dissociation of phospho-LHCII from PSII. The connectivity and antenna size of PSII units are decreased, and the loss of adhesion contact surfaces may cause some transverse separation of adjacent thylakoids of the grana stack. A proportion of PSII reaction centres ceases to be in opposition. The altered shape of the block representing LHCII is intended to convey a structural change in the surface exposed domain (Fig. 5), electrostatic blocking of protein-protein interactions by the phosphate groups, or a combination of both.