MOLECULAR STRUCTURAL EFFECTS OF PROTEIN PHOSPHORYLATION IN REGULATION OF PHOTOSYNTHESIS

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1. INTRODUCTION

Phosphorylation of membrane proteins is responsible for a wide range of responses of the photosynthetic apparatus to changes in incident light and redox poise [1]. In chloroplasts, phosphorylation modifies polypeptides of the LHC II light-harvesting complex, CP43 of the PS II core antenna system, the PS II reaction centre polypeptides D1 and D2, and a PS II-associated membrane protein of 73 amino acids termed the 9 kDa phosphoprotein. Phosphorylation of chloroplast LHC II is under redox control at the level of plastoquinone, and functions in regulation of the relative antenna size of the two photosystems. In cyanobacteria, phosphorylation of polypeptides at 15, 18.5, 85 and 120 kDa correlates with regulation of relative antenna size. Phosphorylation of the cyanobacterial 15 kDa polypeptide shows redox control and sensitivity to protein kinase and phosphatase inhibitors identical to that of chloroplast LHC II, and identical to energetic decoupling of the phycobilisome from photosystem II. A soluble 13 kDa phosphoprotein in cyanobacteria is a signal-transducing P_{II} protein whose modification *in vivo* indicates a link between photosynthetic electron transport and transcriptional control of gene expression [1].

2. PROTEIN PHOSPHORYLATION AND MEMBRANE SURFACE CHARGE

According to the "surface charge" hypothesis, the primary effect of phosphorylation of LHC II to increase in the negative charge on the outer (cytoplasmic) surface of the appressed domain of the thylakoid membrane, and the magnitude of the change is sufficient to overcome the attractive forces otherwise holding together LHC IIs on adjacent, appressed thylakoid domains. The phosphorylated complexes then migrate laterally into unappressed thylakoid domains, where increased distance and screening by stromal cations serve to minimize the repulsive forces acting between them [2-3]. The surface charge hypothesis does not obviously account for control of protein-protein interactions by phosphorylation of proteins in unstacked, laterally homogeneous membrane systems, and suggests that phosphorylation of photosynthetic membrane proteins evolved after the lateral separation of the photosystems [4].

3. INTERMOLECULAR EFFECTS OF PHOSPHORYLATION: LOCAL CHARGE ON INDIVIDUAL MEMBRANE PROTEINS

An alternative proposal is that the electrostatic forces which are induced by phosphorylation and which control protein-protein interactions act in a direction parallel to the membrane plane [5] This proposal removes the requirement for distinct domains and replaces surface charge with more localised charge on

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individual protein complexes. In contrast to the surface charge model, the local charge model can therefore accommodate prokaryotes and unstacked chloroplast thylakoids.

Isocitrate dehydrogenase

E. coli isocitrate dehydrogenase is inactivated by phosphorylation of serine 113. The molecular and structural basis of this effect is understood since structures at 2.5 Å resolution have been obtained for the enzyme both with and without substrate and in both phosphorylated and dephosphorylated forms [6]. 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 the γ-carboxyl group of isocitrate and serine 113. The effect of phosphorylation of serine 113 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 conformation change in the protein. The mechanism by which phosphorylation exerts its functional effect on isocitrate dehydrogenase therefore shows certain similarities with the local charge model proposed specifically for light-harvesting membrane proteins of photosynthesis [5], though in the local charge model protein-protein interactions rather than protein-substrate interactions are blocked by phosphorylation.

4. THE MOLECULAR RECOGNITION HYPOTHESIS

The molecular recognition hypothesis [1,7-8] states that the electrostatic forces exerted initially by phosphorylation are entirely intramolecular, and lead ultimately to major structural changes that alter the interactions of membrane proteins through effects on the complementarity of their respective docking surfaces. One possibility is intramolecular charge compensation and helix formation [1,8], where the negative charge of the phosphate 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. Such a local change in secondary structure may be expected to cause a change in the tertiary structure of the hydrophilic domain, in turn altering the complementarity of the docking surface of the phosphorylated membrane protein relative to that of its neighbours in the membrane, such as other protein complexes of photosystem II and photosystem I.

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 [9]. Structures at 2.9 Å resolution show that phosphorylation at an allosteric site affects substrate binding by means of long-range protein structural effects which cause rotation of the two identical subunits, exposing the catalytic site and also creating a high-affinity binding site for a cofactor, AMP. Each subunit has 841 residues. The phosphorylation site is serine 14, close to the N-terminus. The key event in activation of glycogen phosphorylase by phosphorylation of serine 14 is charge compensation, whereby the phosphate group shields basic amino acid side chains on either side of the phosphorylation side from each other. When these repulsive forces are neutralised, the N-terminal segment containing the phosphoserine is able to assume an α-helical conformation that is absent from the

dephosphorylated protein. The effect of the local change in secondary structure represented by helix formation is to move the whole N-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 arginine 69 of the same chain and arginine 43 of the opposing chain. Serine 14 itself moves through about 36 Å, and arginine 10 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.

5. COMPARISON OF PHOSPHORYLATION SITE SEQUENCES.

Fig. 1 shows the amino acid sequences of the segments containing the phosphorylation sites of pea LHC II*b*, rabbit glycogen phosphorylase and *E. coli* isocitrate dehydrogenase. It is seen that LHC II resembles glycogen phosphorylase in the distribution of basic side chains on either side of the phosphorylation site, a necessary condition for charge compensation by the phosphate group. *Helix* indicates the segment of glycogen phosphorylase that forms a helix upon phosphorylation. It is also seen that both glycogen phosphorylase and LHC II have their phosphorylation sites close to the N-terminus, indicating the possibility that the N-terminal segment moves upon phosphorylation and forms interactions with side chains of the same or other polypeptides that are remote from the unphosphorylated N-terminus [8,10]. In contrast, isocitrate dehydrogenase has a much more central phosphorylation site, and one with only a single neighbouring basic side chain. Thus, from sequence comparisons alone, LHC II shows greater similarity with glycogen phosphorylase than with isocitrate dehydrogenase, and contains the structural features necessary for intra-molecular charge compensation and altered molecular recognition.

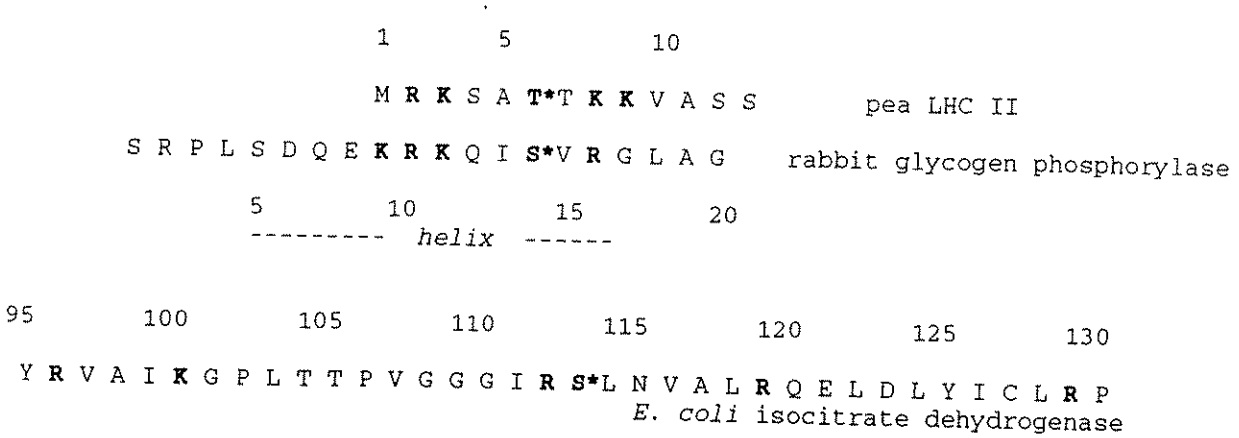

FIGURE 1. Segments of pea LHC II*b*, rabbit glycogen phosphorylase, and *E.coli* isocitrate dehydrogenase, showing phosphorylated amino acid residues (*) and basic amino acid residues (bold) flanking the phosphorylation sites. *Helix* shows the extent of the α-helix induced by phosphorylation of glycogen phosphorylase.

6. SECONDARY STRUCTURAL PREDICTIONS.

Computer structural predictions on LHC II have been carried out to see if replacement of Thr6 with Glu might mimic the proposed effect of phosphorylation [1]. This replacement increases helix probability for each amino acid from 1 to 11. This is consistent with an increase in helical content for the first 20 amino acids of LHC IIb. A control "experiment" is to apply the same predictions to the N-terminal segment of glycogen phosphorylase with the corresponding substitution of Glu for Ser14. This produces a similar result [1], and for glycogen phosphorylase helix formation is, of course, the correct answer [9]. It should also be noted that one might expect only moderate helix probability in any segment where the helix must be broken and remade.

7. 2-D PROTON NMR OF SYNTHETIC PEPTIDES.

2-D NMR spectra of the synthetic LHC II peptide RKSATTKKVASSGSP and of the T5E "mutant" RKSAETKKVASSGSP show no cross-peaks in that arise between main-chain protons, as would be observed if either formed a helix (Forsén S., Spangfort, M. and Allen, J.F., unpublished). The molecular recognition model involving helix formation [1,9] would predict a helix in the latter peptide if no long-range forces are involved in helix stabilization and if glutamate successfully mimics phosphothreonine. Corresponding spectra for the glycogen phosphorylase peptides SRPLSDQEKRKQISVRGLAG and RPLSDQEKRKQIEVRGLAG will be obtained, which will test the validity of this approach to probing altered states of protein folding upon replacement of phosphorylation sites with negatively charged side chains. If a helix is observed in the glycogen phosphorylase S14E "mutant" but not in the "wild type", then a corresponding helix formation in LHC II will be very difficult to reconcile with existing data on the synthetic analogues of LHC II.

8. FUTURE STUDIES

With only two exceptions, glycogen phosphorylase and isocitrate dehydrogenase, the structural basis of regulation by protein phosphorylation in general is an open question. Progress in will depend upon a concerted effort of protein engineering, following the precedent set by *E. coli* isocitrate dehydrogenase [6]. The most promising application in photosynthesis is to LHC II, since rapid progress is being made on the structure of the dephosphorylated protein [10].

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