HOW DOES PROTEIN PHOSPHORYLATION CONTROL PROTEIN-PROTEIN INTERACTIONS IN THE PHOTOSYNTHETIC MEMBRANE?

JOHN F ALLEN, Department of Pure and Applied Biology, University of Leeds, Leeds LS2 9JT, England

1. THE STRUCTURE AND PHOSPHORYLATION OF LHC II

The light-harvesting chlorophyll a/b complex (LHC II) of green plant chloroplasts accounts for about half the chlorophyll and a third of the protein of the thylakoid membrane (1,2). For each polypeptide of between 24 and 27 kDa, the complex contains 4 chlorophyll a molecules, 3 chlorophyll b molecules and 1-2 xanthophyll molecules. Each polypeptide is encoded by one of a family of nuclear genes.

The consensus view of the membrane disposition of the LHC IIb polypeptide is three membrane-spanning alpha-helices (3,4), with an extensive amino-terminal sequence on the stromal side of the membrane and a shorter carboxy-terminal sequence on the inside thylakoid surface. The amino-terminal structure on the outside is most likely the one contributing to the larger, 20 A surface-exposed extension, with a smaller, 7 A surface-exposed extension on the inside.

It is thought that the amino-terminal surface-exposed regions of the complex are sites of membrane adhesion that cause thylakoid stacking (5). This is consistent with the three-dimensional structure proposed by Kuhlbrandt on the basis of electron microscopy of two-dimensional crystals (6). This structure has three-fold rotational symmetry and a platform at one surface that could provide for interaction with a neighbouring platform through van der Waals' forces.

Proteolysis of thylakoid membranes shows that phosphorylation of LHC II polypeptides (7) occurs on the amino-terminal surface-exposed segment that is also required for membrane stacking, at one or both of adjacent threonines in positions six and seven in pea (8).

It is widely held (5,7,9) that phosphorylation alters the net electrical charge of LHC II at the membrane surface, causing a change leading to electrostatic repulsion that can overcome the resultant of van der Waals' and other forces otherwise holding neighbouring LHC IIs at 180^0 to each other on a common axis perpendicular to each membrane plane. This may be described as the "surface charge" hypothesis. It states that inter-

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molecular, inter-membrane forces control the LHC II-PS II interaction and it is these that are modified, directly, by protein phosphorylation. This model takes cation effects on stacking and on excitation energy transfer as a model for effects of phosphorylation.

An alternative suggestion (10) is that electrostatic repulsion between phosphorylated LHC II and a phosphorylated intermediate light-harvesting complex of PS II serves to detach the mobile LHC II pool from the PS II core and thereby functionally to disconnect PS II centres. This may be termed the "mutual electrostatic repulsion" hypothesis. It states that inter-molecular, intra-membrane forces control the LHC II-PS II interaction and are modified by protein phosphorylation. This model has the advantage over the surface charge hypothesis that thylakoid lateral heterogeneity in distribution of PS I and PS II is no longer a necessary condition for effects of phosphorylation on energy transfer and cooperativity. Unlike the surface charge hypothesis, the mutual electrostatic repulsion hypothesis can accommodate effects of phosphorylation in prokaryotic and other membrane systems devoid of lateral heterogeneity (10).

2. HYPOTHESIS: MOLECULAR RECOGNITION

I now propose a third possibility. I suggest that phosphorylation itself has a negligible direct effect on the electrical interactions of neighbouring membrane proteins, and that it is the sum of individually weak inter-molecular forces that is disrupted by phosphorylation at an allosteric site. I therefore suggest that the direct effect of phosphorylation is on intra-molecular forces in the hydrophilic domain of membrane proteins. The electrical effects of altered cation concentration may thus have served as a rather misleading model for phosphorylation effects.

I propose that the predominantly hydrophilic surface-exposed structures assumed in vivo by the 20 A amino-terminal segments of LHC IIb and by similar segments of other membrane-intrinsic proteins of PS II possess complemetary recognition and docking structures that determine their respective interactions in the aqueous phase. Recognition and docking of these complementary surfaces above the membrane surface will serve to guide the interaction of each protein's hydrophobic structures buried in the membrane. By this means phosphorylation of surface-exposed amino acids will control the function of the proteins' hydrophobic domains in inter-molecular excitation energy transfer.

This "molecular recognition" model for structural and functional effects of protein phosphorylation does not require direct control by phosphorylation of forces

holding neighbouring complexes together. The electrostatic effect of the phosphate group may be exerted purely on amino acid side chains within the same polypeptide, and may alter molecular recognition by steric effects that distort the docking surface otherwise complementary to that of the neighbouring protein complex.

This hypothesis is testable. It specifically predicts a three-dimensional structure for the 20 A stromal-surface extension of LHC IIb that includes a docking surface for a neighbouring, complementary structure. It also predicts a conformational change upon phosphorylation that distorts the docking surface, thereby changing the binding constant of the two polypeptides and hence also of the two complexes of which each forms a part.

Conformational changes have lost some of their mystical associations in recent years. From X-ray crystallography there is now an atomic-level resolution model for both the active, phosphorylated and inactive, dephosphorylated forms of the enzyme glycogen phosphorylase (11). From comparison of these structures, it is seen that the effect of phosphorylation of serine-14 of each subunit is to create an ordered helical conformation at each amino-terminus which in consequence binds more closely to the surface of the glycogen phosphorylase dimer. This produces rotation of each subunit about an axis perpendicular to the axis of symmetry of the dimer. This structural change clearly alters substrate binding at the catalytic site, even though the catalytic pyridoxal phosphates are located more than 30 A from the phosphoserine (12).

3. PREDICTIONS

Phosphorylation of LHC II and of other thylakoid and chromatophore membrane proteins does not have to work in the same way as that of the soluble enzyme glycogen phosphorylase. Nevertheless, I should like to suggest that the eventual solution of the structure of an antenna complex will show a docking surface for a reaction centre core component, and that the structure of this surface will be altered by covalent modification at a site remote from both the docking surface itself and the site of excitation transfer between chromophores. Complementary docking surfaces on the same protein are also to be expected, since their modification by phosphorylation would then permit altered connectivity between photosynthetic units (10). As allosteric proteins, antenna complexes will always be oligomeric (12).

It is possible in the case of purple bacterial reaction centres that a docking surface could be capable of resolution in the existing X-ray crystal structures

(13,14). From the above considerations one would predict its location in the large cytoplasmic-surface-exposed domain of the H-subunit.

The "molecular recognition" hypothesis proposed here for control of photosynthetic unit function by protein phosphorylation is testable in principle, but probably not in practice on data available at the present time. In this event I should like to recommend an aphorism of Myers, who also helped cause all these problems (15), and whose view is amply demonstrated by developments subsequent to the paper in which it is stated (16). "The test of a concept, like the question of pregnancy in the human female, is not current majority opinion but the test of time".

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