Membrane protein phosphorylation in the cyanobacterium *Synechococcus* 6301

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Phosphorylation of thylakoid membrane proteins of higher plants has been studied in a number of laboratories in recent years (Haworth *et al.*, 1982; Allen, 1983; Barber, 1983; Bennett, 1983; Horton, 1983). A major substrate for this reaction is light-harvesting chlorophyll *a*/*b*-binding protein. Control of the membrane-bound protein kinase catalysing this reaction by redox state of plastoquinone (Allen *et al.*, 1981, Horton *et al.*, 1981) permits the physiological regulation of distribution of absorbed excitation energy that can be demonstrated experimentally as state-1–state-2 transitions. We have undertaken preliminary studies designed to resolve the significance of membrane protein phosphorylation in photosynthetic prokaryotes. Here we report results obtained with the cyanobacterium *Synechococcus* 6301 (*Anacystis nidulans*). The following paper (Holmes *et al.*, 1986) reports results obtained with the purple bacterium *Rhodopseudomonas sphaeroides*.

The cyanobacteria, like higher plants, regulate distribution of absorbed excitation energy between Photosystems I and II (Fork & Satoh, 1983). However, they lack light-harvesting chlorophyll *a*/*b*-binding protein and therefore the model devised for higher plants cannot apply. The cyanobacterium *Synechococcus* 6301 nevertheless phosphorylates a number of thylakoid membrane proteins both *in vivo* and *in vitro*.

Fig. 1(*a*) shows results of *in vivo* labelling experiments in which *Synechococcus* cells were grown on [^{32}P]P_i. Radioautography of SDS/polyacrylamide slab gels of thylakoid proteins and soluble proteins obtained by fractionation of labelled *Synechococcus* cells shows major phosphorylated bands with apparent molecular mass of 22.5, 15 and 13.5 kDa in thylakoids and 65, 18.5 and 10.5 kDa in the soluble fraction. There are also minor phosphorylated bands at 62 and 18 kDa in thylakoids. Of these, the 18.5 kDa polypeptide in both thylakoids and the soluble fraction and the 15 kDa polypeptide in thylakoids are phosphorylated in illuminated cells but not in cells incubated in the dark. The presence of 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) appears to inhibit phosphorylation to a small extent.

Light-harvesting and DCMU-sensitive phosphorylation of the 25 kDa light-harvesting polypeptide of pea thylakoids (Bennett, 1979) provided the first evidence for redox control and hence for the role of this reaction in regulation of excitation energy distribution (Bennett *et al.*, 1980). It remains to be established that the 18.5 and 15 kDa phosphoproteins of *Synechococcus* thylakoids have a similar control mechanism and functional significance, but we propose, on the basis of this preliminary evidence, that the 18.5 and 15 kDa phosphoproteins of *Synechococcus* are involved in regulation of excitation energy transfer from light-harvesting phycobiliproteins to the reaction centre of Photosystem II. A linker protein of molecular mass 18.3 kDa forms part of the phycobilisome core in *Synechococcus* (Glazer, 1983), and this linker protein may be identical with the 18.5 kDa phosphoprotein. We propose that regulation of coupling between the phycobilisome and the antenna chlorophyll

Abbreviations used: SDS, sodium dodecyl sulphate; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

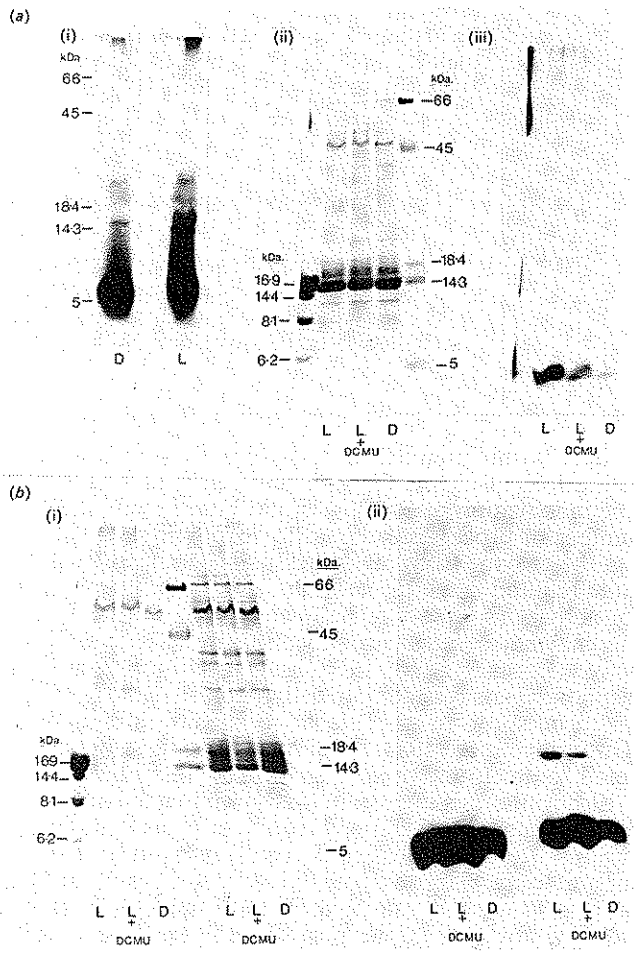

Fig. 1. *Phosphoproteins from cells and from isolated thylakoids of Synechococcus 6301*

(*a*) *Synechococcus* cells were grown for 3 days in medium containing [^{32}P]orthophosphate (100 μCi/ml of culture). Cells were incubated for 90 min under three different conditions: light (L), light + 50 μM-DCMU (L + DCMU) and dark (D). Membrane and soluble fractions were separated by centrifugation after osmotic lysing of sphaeroplasts formed by lysozyme digestion of the cell wall (Hellingwerf *et al.*, 1975). Protein was precipitated with 5% trichloroacetic acid. Protein pellet was washed with buffer then treated overnight with inorganic pyrophosphatase to remove polyphosphates. The pellet was washed with 80% acetone at −20°C, dissolved in SDS sample buffer, then treated with ribonuclease overnight, before SDS/polyacrylamide-gel electrophoresis on a 10–30% gradient. Phosphoproteins were detected by radioautography using Kodak X-OMAT S film. (i) Radioautograph of membrane fraction (gels not shown). (ii) Gel of soluble fractions polypeptides showing molecular mass markers. (iii) Radioautograph of (ii). (*b*) Membranes were prepared by French press or as for membrane fraction of whole cells. Membranes were incubated for 10 min, in the presence of [γ-^{32}P]ATP, under the same condition as whole cells then treated with trichloroacetic acid, 80% acetone and SDS sample buffer before SDS/polyacrylamide-gel electrophoresis, and radioautography, as for the whole cells. (i) Gel of membrane polypeptides; left-hand side, French press membranes; right-hand side, sphaeroplast-prepared membranes. (ii) Radioautograph of (i).

proteins of Photosystem II occurs by means of the mutual electrostatic repulsion of the 18.5 kDa linker phosphoprotein in the phycobilisome core and the 15 kDa phosphoprotein in the membrane. This may prove to be a general mechanism for control of excitation energy transfer in phycobilisome-containing organisms.

Synechococcus thylakoids prepared by osmotic lysis of sphaeroplasts (obtained in turn by lysozyme digestion of the cell wall) show protein kinase activity and are able to phosphorylate a number of polypeptides using exogenous [γ-^{32}P]ATP. Results of such labelling *in vitro* are shown in Fig. 1(*b*). Minor phosphorylated bands occur at 82, 64, 55, 39.5 and 12.5 kDa and a major band at 15 kDa. The 15 and 12.5 kDa bands are phosphorylated in the light but not in the dark, with DCMU having a slight inhibitory effect. Absence of the 18.5 kDa phosphoprotein supports our suggestion that this is a component of the phycobilisome since the phycobilisomes are mostly washed off the membranes during preparation. Protein phosphorylation *in vitro* has previously been reported for thylakoids of another cyanobacterium, *Fremyella displosiphon* (Schuster *et al.*, 1984).

Thylakoid membrane fragments prepared by French press treatment of *Synechococcus* cells (England & Evans, 1981) show virtually no protein phosphorylation (Fig. 1*b*) and no ATP-induced fluorescence changes at room temperature or 77 K (results not shown). It has been suggested (E. H. Evans, personal communication) that such thylakoids are inside-out with respect to their orientation *in vivo*. Comparison of sphaeroplast-prepared thylakoids with French-press thykaloids (Fig. 1*b*) thus supports the conclusion that the protein kinase activity and phosphorylating polypeptide segments of membrane proteins are exposed only on the cytoplasmic side of the thylakoid membrane.

We suggest that redox-controlled protein phosphorylation is directly responsible for state-1–state-2 transitions in phycobilisome-containing organisms, with electrostatic decoupling of the phycobilisome from Photosystem II acting instead of the 'lateral shuffling' of light-harvesting chlorophyll *a*/*b*-binding protein. Studies of Biggins *et al.* (1984) with the red alga *Porphyridium cruentum* failed to reveal major differences in ^{32}P-labelling between cells stably adapted to lights 1 and 2. This finding does not, in our view, preclude a protein phosphorylation mechanism for the transition between states 1 and 2, contrary to the conclusion of Miller & Lyon (1985). A further implication of a protein phosphorylation mechanism for state-1–state-2 transitions in phycobilisome-containing organisms is that lateral heterogeneity in the distribution of thylakoid components is not required. Lateral shuffling of a light-harvesting complex between discrete membrane regions in higher plants would then be a secondary effect of phosphorylation, with the primary effect, in all photosynthetic systems, being control of coupling of excitation energy transfer within the photosynthetic unit.

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Membrane protein phosphorylation in the purple photosynthetic bacterium *Rhodopseudomonas sphaeroides*

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Membrane protein phosphorylation is involved in regulation of distribution of absorbed excitation energy in photosynthesis in plants and green algae (Allen, 1983; Bennett, 1983). The same reaction may also regulate photosynthesis in cyanobacteria though with different membrane protein substrates (Sanders *et al.*, 1986). Purple photosynthetic bacteria have only one kind of photochemical reaction centre with associated light-harvesting pigments systems (Drews, 1985). They are therefore unable to perform the phosphorylation-dependent adaptive responses to changing spectral quality that are known as state-1–state-2 transitions. Here we report a direct demonstration of membrane protein phosphorylation in cells and chromatophore membranes of a purple bacterium and suggest a role for this reaction in regulating excitation energy transfer within a single photosystem.

Growth of the purple, non-sulphur species *Rhodopseudomonas sphaeroides* type 2.4.1. on medium containing [^{32}P]P_i gives several radioactively labelled bands on SDS/polyacrylamide gels (Fig. 1). The bands are pronase-sensitive, indicating that they are polypeptides. Polypeptides of the following molecular masses have been seen to be labelled: 59, 54, 45, 25, 21, 18, 16, 13.5 and 12 kDa. Using [γ-^{32}P]ATP, incorporation of ^{32}P into polypeptides can be seen in chromatophore preparations of *Rps. sphaeroides*, indicating that protein kinase activity is retained during isolation of these membranes. Fig. 1 shows a radioautograph from an experiment where chromatophores were labelled *in vitro* and the

Abbreviation used: SDS, sodium dodecyl sulphate.