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.

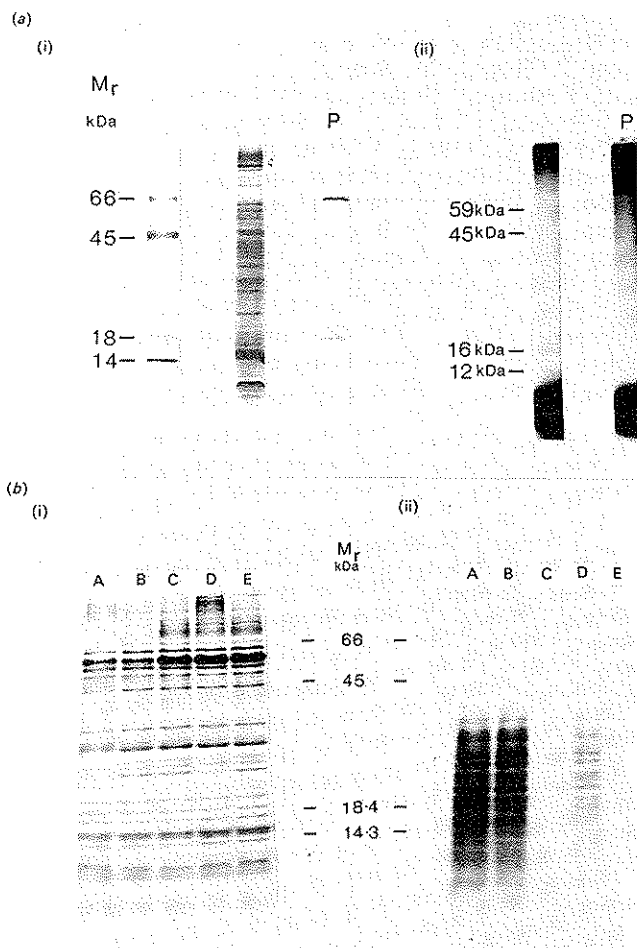

Fig. 1. *Phosphoproteins from cells and from isolated chromatophores of Rhodopseudomonas sphaeroides 2.4.1.*

(*a*) Whole cells, grown overnight with 1 mM-[^{32}P]orthophosphate, were precipitated with 5% trichloroacetic acid and the pellet treated as in Sanders *et al.* (1986) but without incubation with pyrophosphatase. SDS/polyacrylamide-gel electrophoresis was carried out on an 11.5–16.5% gradient. After electrophoresis, the gel was heated at 90°C in 16% trichloroacetic acid for 45 min (as in Manai & Cozzone, 1979) to remove polyphosphates and nucleic acids. Samples treated with pronase were treated for 6 h with 100 μg of pronase/ml at 30°C (P). (i) Gel. (ii) Radioautograph. (*b*) Chromatophores, prepared by French press treatment, were incubated with [γ-^{32}P]ATP in the presence of the following: (A) 2 mM-potassium ferricyanide, (B) no addition but sample aerated before reaction started, (C) 0.5 mM-duroquinol, (D) 2 mM-sodium succinate, (E) 5 mM-sodium dithonite. The samples were then treated as above but without incubation with ribonuclease or treatment of the gel with hot trichloroacetic acid. (i) Gel. (ii) Radioautograph.

samples subjected to SDS/polyacrylamide-gel electrophoresis; prominent bands can be seen with molecular masses 70, 55, 51 and 38 kDa, together with a number of polypeptides of lower molecular mass, including several in the region 6–12 kDa where polypeptides from light-harvesting complexes are known to run (Drews, 1985).

Labelling *in vitro* of the lower molecular mass polypeptides appears to be under redox control: oxidizing conditions (2 mM-ferricyanide or prolonged aeration of the sample) favour phosphorylation, while reducing conditions (5 mM-dithionite or 5 mM-duroquinol) favour dephosphorylation.

How general is control of photosynthetic unit function by protein phosphorylation?

It seems likely that protein phosphorylation is involved in regulation of excitation energy transfer in photosynthetic prokaryotes as well as in green plants. It is possible that redox-controlled protein phosphorylation causes electrostatic decoupling of the phycobilisome from Photosystem II during state-1–state-2 transitions in phycobilisome-containing organisms (Sanders *et al.*, 1986).

The nature of any redox control of protein phosphorylation in purple bacteria remains to be clarified. In view of the cyclic character of electron transport, a quinol-activated protein kinase would not be expected to respond to illumination in precisely the same way in bacteria as in higher plants. However, increased light intensity and therefore reaction centre turnover will cause net reduction of the ubiquinone pool where this precedes the rate-limiting electron-transfer step. It is therefore reasonable to explore a possible redox control mechanism analogous to that found in higher plants.

The overall regulatory role of bacterial protein phosphorylation must clearly differ from that found in plants, though phosphorylation of one or more light-harvesting proteins would be expected to alter excitation energy transfer within and between photosynthetic units. We are currently investigating a possible role in regulation of co-operativity. If both the direction of redox control (Fig. 1*b*) and the effect of phosphorylation on co-operativity (Loach *et al.*, 1984) were the reverse of the relationships obtaining in Photosystem II then the overall effects would nevertheless be similar: increasing light intensity would decrease co-operativity and vice versa. It is however difficult to see how increased negative charge resulting from phosphorylation could increase rather than decrease co-operativity. Clearly further investigation of functional effects of phosphorylation is now required.

We propose that control of coupling of excitation energy transfer within the photosynthetic unit is the primary effect of protein phosphorylation in all photosynthesis systems, and that state-1–state-2 transitions reflect special cases found in oxygen-evolving organisms where decreased transfer to one type of reaction centre can be accompanied by increased transfer to the other.

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