PROTEIN PHOSPHORYLATION: A MECHANISM FOR CONTROL OF EXCITATION ENERGY DISTRIBUTION IN PURPLE PHOTOSYNTHETIC BACTERIA

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1. INTRODUCTION

In higher plant chloroplasts, the regulation of energy transfer within the pigment matrix is known to involve the redox-controlled phosphorylation of LHC-II (1-3). Evidence from Loach and co-workers (4) and from Holuigue et al (5) indicates that phosphorylation of light-harvesting polypeptides also occurs in purple photosynthetic bacteria and that this plays a part in regulating energy transfer between photosynthetic units. We have recently (6) suggested a general role for protein phosphorylation in the regulation of energy transfer in both prokaryotic and eukaryotic systems. We have proposed a model in which phosphorylation of polypeptides of pigment-protein complexes gives rise to a mutual electrostatic repulsion of neighbouring complexes parallel to the plane of the membrane. Figure 1 shows how this model would apply to an organism such as Rhodospirillum rubrum with one type of reaction centre and one type of light-harvesting complex. We have identified phosphorylated polypeptides in both cyanobacteria (7) and purple photosynthetic bacteria (8) as possible energy transfer regulation sites between pigment-protein complexes. The work discussed below extends these observations to Rds. rubrum.

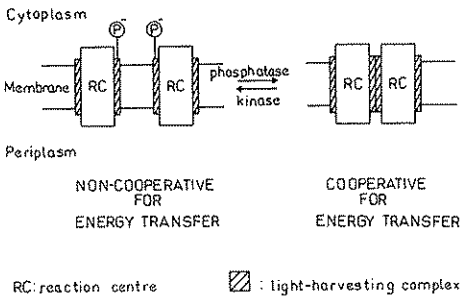

Figure 1. Model for the regulation of energy transfer between photosynthetic units in Rds. rubrum

2. PROCEDURE

2.1 Materials and methods

Cells of Rds. rubrum were grown and chromatophores isolated as in (9,10). Conditions for incubation of cells with photosynthetic units cooperating or non-cooperating in energy transfer were similar to those developed by Loach et al (4), ie 20mM KPi, pH 6.8, 2 hours incubation in the dark for non-cooperative cells and 20mM KPi, 20mM $MgSO_4$, pH 6.8, 2 hours incubation in the light for cooperative cells. For whole cell labelling experiments, cells were grown overnight in a 7 ml bottle with 1 mCi ^{32}P-Pi. The cells were pelleted in an Eppendorf centrifuge and

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resuspended under the conditions given, in the presence of a further 250 μCi ^{32}P-Pi per ml of original culture. Samples were precipitated in 5% trichloroacetic acid and SDS-PAGE performed, using an 11.5% to 16.5% gel (8).

In vivo bacteriochlorophyll fluorescence was measured, at right angles, using a fluorimeter constructed in this department. Filter combinations of Corning 4-96 with Calflex C and Scott 891 nm interference filter with Wratten 88A were used on the exciting light and the photodiode respectively.

3. RESULTS AND DISCUSSION

3.1 We have used the transient rise of the *in vivo* bacteriochlorophyll fluorescence yield, on a millisecond time scale, to monitor the extent to which energy is able to pass between photosynthetic units (11); as the probability of transfer increases, the curve of the transient becomes more sigmoidal in appearance (12). Figure 2 compares the transients in whole cells of *Rds. rubrum* incubated as above. Under conditions of light+Mg^{2+} the transient is clearly more sigmoidal than under conditions of dark-Mg^{2+}.

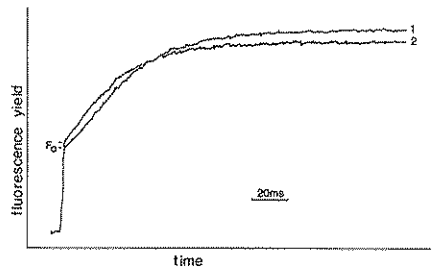

Figure 2.
Fluorescence yield transients
Rds. rubrum cells
1) Light+Mg^{2+}
2) Dark-Mg^{2+}

3.2 Cells were also incubated, in the presence of ^{32}P-Pi, under conditions giving different degrees of cooperativity. Analysis by SDS-PAGE and autoradiography (Fig. 3) shows that several pronase sensitive bands are phosphorylated with M_r 51 kDa, 20.5 kDa, 17 kDa, 13 kDa and 10.5 kDa. Of these labelled polypeptides, the 17 kDa and 13 kDa were phosphorylated only in the cooperative cells, whereas the 10.5 kDa polypeptide was phosphorylated strongly in non-cooperative cells but only weakly in cooperative cells. In contrast, the labelled bands of M_r 51 kDa and 20.5 kDa were phosphorylated to a similar extent under both incubation conditions. The 13 kDa and 10.5 kDa bands appearing on the autoradiograph correspond with bands staining with Coomassie Blue on the gel.

3.3 Chromatophores of *Rds. rubrum* were incubated with [γ^{32}P]-ATP and the polypeptides analysed by SDS-PAGE and autoradiography as for whole cells (Figure 4). Several components were phosphorylated, including polypeptides of M_r 13 kDa and 10.5 kDa which were strongly labelled after illumination for 15 minutes, but only weakly labelled in the dark. In the presence of 2 mM potassium ferricyanide only the 13 kDa polypeptide was phosphorylated. 5 mM sodium dithionite inhibits labelling of most low molecular weight components as was previously reported in chromatophores of *Rhodopseudomonas sphaeroides* (8). This effect of dithionite may be a direct effect on the kinase(s), possibly a direct

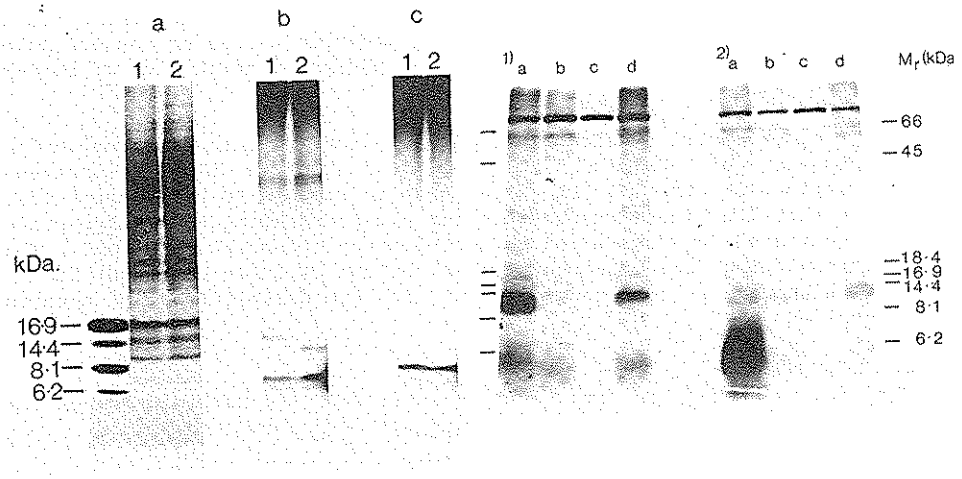

Figure 3. a) gel stained with Coomassie Blue; b) autoradiograph of (a); c) autoradiograph of pronase-treated samples of cells of Rds. rubrum incubated: 1) Light+Mg^{2+}; 2) Dark-Mg^{2+}.
Figure 4. Autoradiograph of chromatophores of Rds. rubrum incubated for 15 minutes with [γ^{32}]-ATP, with (2) or without (1) 144,000 g supernatant fraction, as follows, a) light; b) dark; c) dark + 5mM sodium dithionite; d) dark + 2mM potassium ferricyanide. Chromatophores were made and incubated in 20 mM KPi, 20 mM $MgSO_4$, pH 6.8.

reduction of the enzyme. Adding back the supernatant gives strong phosphorylation of a polypeptide of M_r 10.5 kDa; the phosphorylation of this polypeptide is not inhibited by the addition of dithionite. Phosphorylation of 13 kDa and 11 kDa components in Rds. rubrum chromatophores has also been observed by Holuigue et al (5). However, in their experiments, they observed that phosphorylation of the 13 kDa polypeptide required the presence of both a membrane fraction and a soluble fraction, in contrast to the results reported here.

3.4. In conclusion we have confirmed that cooperative behaviour is associated with phosphorylation of a 13 kDa polypeptide which we identify as B880-α (as has been previously suggested by Loach et al (4)) and that non-cooperative behaviour is associated with phosphorylation of a 10.5 kDa polypeptide, which we provisionally identify as B880-β. In terms of the model presented above, mutual electrostatic repulsion between phosphorylated B880-β would, by increasing the distance between photosynthetic units, then provide a plausible mechanism for inhibiting energy transfer between those units. Phosphorylation of B880-α, which may occur on the periplasmic side of the membrane (4), could then reinforce

the effect of the dephosphorylation of B880-β. This reinforcement could possibly involve neutralising an existing positive charge on the molecule on the opposite side of the membrane to the phosphorylation side of B880-β on the cytoplasmic site.

The chromatophore studies reported above confirm that phosphorylation of components of Mr 13 kDa and 10.5 kDa is light activated. Phosphorylation of the 13 kDa polypeptide is responsive to ferricyanide, suggesting that the kinase responsible for phosphorylating this polypeptide may be activated by oxidation of an electron transport component. The factors regulating the phosphorylation of the 10.5 kDa component remain unclear, although a component present in the soluble fraction appears to be involved

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