PHOSPHOPROTEINS IN THE PURPLE PHOTOSYNTHETIC BACTERIUM, RHODOSPIRILLUM RUBRUM

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

1. INTRODUCTION

The photosynthetic apparatus of purple photosynthetic bacteria is organised into photosynthetic units (PSU) each consisting of a reaction centre pigment-protein complex with its associated light-harvesting pigment-proteins (1). In what has been described as the cooperative state it is envisaged that excitation energy absorbed by the light-harvesting components of one PSU can be transferred to a neighbouring PSU (2). In the cooperative state (light + Mg^{2+}) increased phoshorylation of the B880- polypeptide of the light-harvesting complex isolated from Rhodospirillum rubrum has been demonstrated and suggested as a possible mechanism for regulating PSU cooperativity (3). In whole cells the phosphorylation of proteins of apparent molecular weight 13 and 10 kDa has also been shown to occur respectively under cooperative (light + Mg^{2+}) and non-cooperative (dark) conditions (4). It has been suggested that these phosphorylated polypeptides may be the B880- and B880- subunits of the light-harvesting complex (4,5). Here we describe experiments designed to evaluate this proposal.

2. MATERIALS AND METHODS

Growth of R.rubrum, sample preparation, SDS-PAGE and autoradiography were performed essentially as described (4). ^{32}P-labelling of whole cells was carried out in the light or dark in medium containg [^{32}P]orthophosphate. Chromatophores were prepared from ^{32}P-labelled cells as previously described (5). Chromatophores for photophosphorylation experiments were resuspended in 20 mM MOPS/20mM NaCl/4mM $MgSO_4$ (pH 7.9).

3. RESULTS AND DISCUSSION.

Incubation of R.rubrum cells with [^{32}P]orthophosphate resulted in a wide variety of proteins being phosphorylated, covering a wide range of molecular

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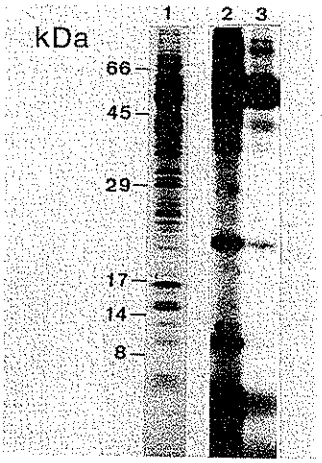

Fig. 1

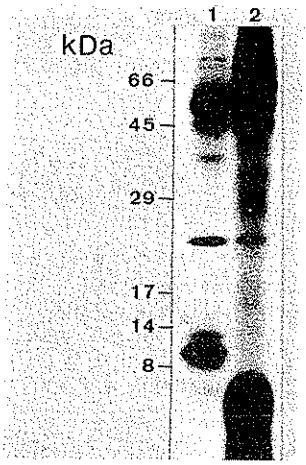

Fig.2

Figure 1: Lane 1: Whole cell protein from R.rubrum separated by SDS-PAGE and stained with Coomassie blue. Lanes 2 and 3: Autoradiograph showing ^{32}P-labelled protein from R.rubrum incubated with ^{32}P-phosphate for 6 hr in the light (lane 2) or in the dark (lane 3).

Figure 2: Fractionation of light grown ^{32}P-labelled cells. Autoradiograph appearance of soluble fraction (lane 1) and chomatophore fraction (lane 2).

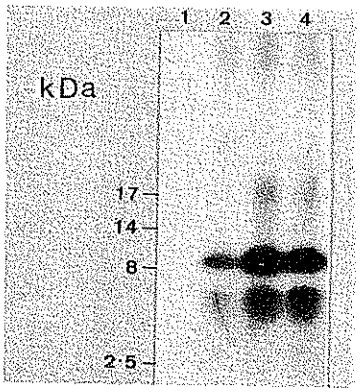

Fig. 3

Figure 3: Protein phosphorylation in chromatophores supplied with [^{32}P]orthophosphate and incubated in the light for 2 hr with various concentrations of ADP, ie. lane 1: 20 mM; lane 2: 200 uM; lane 3: 2 uM; lane 4: 20 nM.

weights. Labelling of proteins in the light (Fig. 1, lane 2) was generally more pronounced than in the dark (Fig. 1, lane 3) but the most striking differences were seen for bands of apparent molecular weight 4, 10, 13 and 22 kDa. Of particular interest are the phosphorylated species of 10 and 13 kDa. Under the conditions employed the 10 kDa protein was strongly labelled in the light whereas the 13 kDa band was weakly labelled , but in the dark neither species was phosphorylated to a detectable level. Both these labelled bands were coincident with Coomassie blue staining bands (Fig. 1, lane 1)

In order to test the proposal that the 10 and 13 kDa phosphoproteins are the subunits of the light-harvesting complex we labelled cells with [^{32}P]orthophosphate in the light and carried out a chromatophore preparation. Samples of soluble components (144,000g supernatent) and chromatophores were analysed by SDS-PAGE and autoradiography. Figure 2, lane 1 shows that the 10 and 13 kDa phosphoproteins were found exclusively in the soluble fraction. This result clearly does not support the suggestion that these phosphoproteins are the light harvesting polypeptides.

We are also evaluating a novel approach to the study of protein phosphorylation reactions in isolated chromatophores. The method involves incubation of chromatophores in the light with [^{32}P]orthophosphate and unlabelled ADP and relies on the ability of chromatophores to synthesize [^{32}P]ATP <u>in situ</u> by photophosphorylation. Figure 3 shows that, surprisigly, the major phosphorylated component observed is a band of 10 kDa. This conflicts with the fractionation experiment data described above and suggests the 10 kDa protein may be associated with the chromatophores. However, it is possible that the 10 kDa phosphorylated band observed here arises from a residual contamination of the chromatophore fraction with some soluble components. In the dark, or in the presence of excess unlabelled phosphate, labelled material was not detected.

In summary, we conclude that the 10 and 13 kDa phosphoproteins of <u>R.rubrum</u> are unlikely to be the subunits of the light-harvesting complex. It appears that both are soluble, cytoplasmic components but it is possible that the 10 kDa protein associates with chromatophores under certain conditions. Consequently, the role of these phosphoproteins in the regulation of PSU cooperativety needs to be re-evaluated.

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