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Protein phosphorylation in *Rhodospirillum rubrum*: further characterization of the B873 kinase activity

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Protein phosphorylation of the B873 light-harvesting complexes in the phototrophic bacterium, *Rhodospirillum rubrum* G9, is an important means to regulate energy transduction. The B873 kinase activity is found in the water-soluble fraction and phosphorylates not only the α- and β-subunits but also a third, new component, Ω, which copurifies with the B873 complex. The B873 kinase is unaffected by the presence of cAMP but is weakly inhibited by ADP and AMP. Mg^{2+} stimulates the kinase reaction but the transition metal ions Cu^{2+} and Zn^{2+} ellicit the most active phosphorylation. The B873 kinase also phosphorylates in vitro purified B873 light-harvesting complexes from *R. rubrum*. The extent of phosphorylation of the B873 complexes in intact chromatophores is dependent on the redox state of the quinone pool as well as of the cytochrome bc_1 complex. Fluorescence measurements of the B873 complexes of isolated chromatophores confirmed the functional significance of the phosphorylation events observed.

Introduction

Many cellular processes such as sensing and signal transduction are now known to be regulated by protein phosphorylation [1–5]. In plants and cyanobacteria, light energy distribution between photosystems is also controlled by protein phosphorylation [5–7].

Although protein phosphorylation has been demonstrated in phototrophic bacteria such as *Rhodospirillum rubrum*, *Rhodobacter sphaeroides*, or *Rhodomicrobium vannielii* [8–12], its role in the regulation of photosynthesis of purple non-sulphur bacteria has been little studied so far. Loach and co-workers showed that the photosynthetic units of *R. rubrum* show cooperative energy transfer between light-harvesting complexes when the chromatophores are illuminated in the presence of Mg^{2+} and ATP [8–13]. These workers were able to isolate a phosphorylated 10-kDa polypeptide from illuminated chromatophores, which was assumed to correspond to the α-subunit of the B873 complex [8]. A change in the fluorescence kinetics of the B873 complex after illumination of the chromatophores in the presence of Mg^{2+} and ATP was also demonstrated by Holmes and Allen [14]. They suggested that the 13-kDa and 10-kDa polypeptides which were assigned to the α- and β-polypeptides of the B873 complex were phosphorylated in the light in the presence of ATP. The extent of phosphorylation was modulated by the redox balance of the quinone pool of the chromatophore membrane [15]. Furthermore, they suggested that a membrane-bound kinase might be responsible for the phosphorylation of the B873 complex. However, the addition of the supernatant fraction obtained by ultracentrifugation of a crude extract from cells of *R. rubrum* also enhanced the phosphorylation of the B873 complex. Unfortunately, the phosphorylated polypeptides could not be unambiguously identified. Furthermore, Holmes and Allen [15] observed the phosphorylation of an unknown component of low molecular mass (4 kDa). We have recently identified the 4 kDa component to be a small, very hydrophobic polypeptide, omega (Ω) which copurifies with the complex [16,17]. Although the precise function of the Ω polypeptide is not known, we will present evidence that it plays a role in the regulation of fluoresence energy transfer among B873 complexes.

Following the work of Holmes and Allen [15], we will show that not only the α- and β-polypeptides, but also the Ω-polypeptide is phosphorylated by a water-soluble protein kinase which in turn is regulated by the

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Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCCD, dicyclohexylcarbodiimide; TCA, trichloroacetic acid; cAMP, 2′,3′-cyclic AMP; DTT, dithiothreitol; BChl, bacteriochlorophyll; BPh, bacteriopheophytin.

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redox state not only of the quinone pool but also by the redox state of the cytochrome bc_1 complex.

A preliminary account of this work has been published previously [17].

Materials and Methods

Growth of the bacteria

R. rubrum G9 was grown anaerobically in the light in 100-ml bottles using a modified Sistrom medium [18]. Cells were harvested in the mid-log phase and used immediately.

Preparation of chromatophores

Chromatophores were prepared by the method of Snozzi and Bachofen [19] with the following modifications. Pelleted fresh cells (2 g) were suspended in 10-ml extraction buffer (50 mM Tris-HCl pH 7.5 containing 5 mM $MgSO_4$, 1 mM DTT and 10 mM PMSF). After addition of a grain of DNase the suspension was passed three times through a French Press and then centrifuged at 30 000 $\times g$ for 20 min at 4°C to remove cell debris. The supernatant was centrifuged at 100 000 $\times g$ for 1 h at 4°C. The supernatant obtained (henceforth referred to as the 'supernatant fraction') was recentrifuged to remove contaminating membranes, then frozen rapidly and stored at −70°C.

The 100 000 $\times g$ pellet ('the chromatophores') was homogenized using a precision Potter homogenizer (Reichelt, Switzerland) in 20 mM Tris-HCl pH 7.5 containing 5 mM $MgSO_4$ and 1 mM DTT, centrifuged again at 100 000 $\times g$ and then resuspended by homogenization in the same buffer. The homogenization step causes the chromatophores to rupture transiently, thus allowing their content (including the water-soluble cytochrome c_2) to be released. The washed chromatophores were applied to a sucrose gradient (0.4–2 M) in the same buffer and centrifuged for 22 h at 26000rpm in a Beckmann SW28 rotor. The cytochome c_2 content of the purified chromatophores was routinely checked by SDS-PAGE followed by haem staining. Usually only traces of cytochrome c_2 were found for all the chromatophore preparations reported here. The blue-green band containing the chromatophores (henceforth referred to as 'the chromatophore fraction') was harvested, frozen in liquid nitrogen and stored at −70°C.

Protein determinations of the supernatant and chromatophore fractions were performed by the modified Lowry method of Peterson et al. [20] using bovine serum albumin as a standard.

Determination of B873 kinase activity

The standard conditions for the determination of kinase activity were modified to be performed in a 1.5 ml Eppendorf tube: chromatophores and/or the supernatant fraction (30 μg protein of each fraction respectively) were added to the incubation buffer (50 mM Tris-HCl pH 7.5, 20 mM $MgCl_2$, 2 μg/ml venturicidin and 100 μM DBMIB) and incubated in the dark for 15 min at 30°C. The kinase reaction was initiated by the addition of 10–25 μCi [γ-^{32}P]ATP and 0.5 mM unlabelled ATP to yield a final reaction volume of 0.5 ml. The tubes were then illuminated for 30 min at 30°C, then the reaction was terminated by the addition of 50 ml 72% TCA. The TCA-treated assays were incubated for 10 min at room temperature and then centrifuged for 20 min at 3000 $\times g$. The supernatant was discarded and the pellet washed with 0.5 ml 20 mM Tris-HCl pH 7.5. The washed pellet was then dissolved in 20 ml SDS-sample buffer (56 mM Tris-HCl pH 6.8, 10 mM EDTA, 5% glycerol, 2% SDS, 50 mM DTT), heated for 90 s at 100°C and then applied to a 15% SDS-acrylamide gel prepared according to Laemmli [21]. After running, the gel was stained with Coomassie blue and then autoradiographed (Fuji film) at −70°C for 2–3 days.

Modulated fluorescence measurements of chromatophore preparations

Modulated fluorescence measurements were performed using a Hansatech modulated fluorescence apparatus [22]. The chromatophore suspension (5 ml) was pippetted into a temperature-controlled cell and allowed to equilibrate for 5 min, after which the ground level fluorescence (F_o) was recorded. The variable fluorescence (F_v), due to saturation of the available reaction centres, was recorded after excitation with actinic light. The fluorescence signal was measured using an infra-red filter (Kodak Wratten 89b) with a cut-off below 850 nm.

Results

Optimization of the assay conditions for the B873 kinase

(a) Assignment of the phosphorylated key components

Fig. 1(a,b) shows typical autoradiographs of ^{32}P-labelled chromatophores after performing the standard assay. We have indicated 3 regions of the autoradiogram profile which have been used to optimize the standard assay conditions. The 55-kDa band is similar to that observed by Turner and Mann [12] for *Rhodomicrobium vannielii* and is present in the cytoplasmic fraction (the supernatant). We identify this band provisionally as the Rubisco subunit of *R. rubrum*. The 13-kDa and 10-kDa bands present in the chromatophore fraction correspond to the α- and β-polypeptides, respectively, of the B873 complex, by comparison to the isolated complex [23]. As judged from the autoradiogram the α- and β-polypeptides often appear not to be resolved in this gel system. When the intensity of the α/β-region was analysed by digital process-

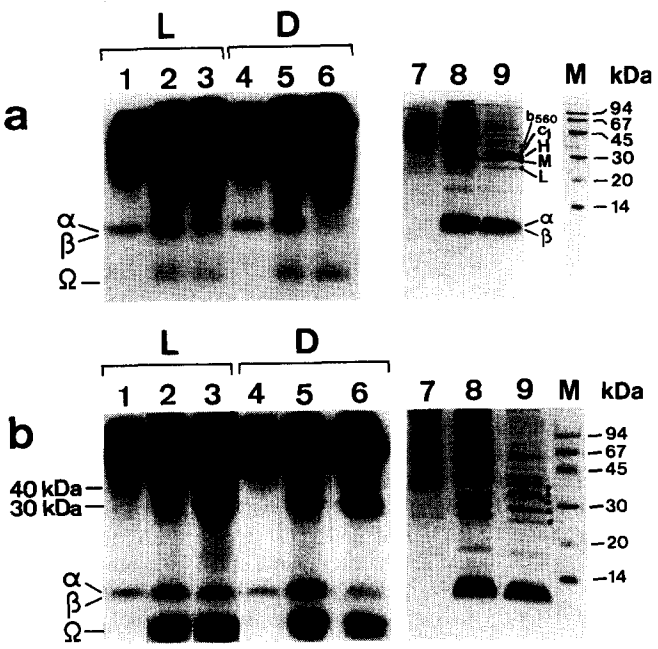

Fig. 1. Effect of illumination upon the phosphorylation of purified chromatophores. Conditions for the phosphorylation reactions: (a) no DBMIB added; (b) 100 μM DBMIB added. Phosphorylation reactions were performed in the light (L) or in the dark (D) as indicated. Samples in the phosphorylation reaction were: lanes 1,4,7, supernatant fraction alone; lanes 2,5,8, chromatophores plus supernatant fraction; lanes 3,6,9, chromatophores alone. Lanes 1–6 show the autoradiograms after SDS-PAGE, and lanes 7–9 show the Coomassie blue-stained gel corresponding to lanes 4–6 of the autoradiogram. The amount of protein applied to the gel was 30 μg for each fraction. The location of the α- and β-polypeptides of the B873 complex and of the Ω-component are indicated. In addition the positions of the L, M and H subunits of the reaction centre as well as of cytochrome c_1 are shown. The expected migration of cytochrome b-560 is indicated. The autoradiogram shown in Fig. 1(a) was overexposed in order to develop the somewhat weak signal due to the α- and β-, and Ω components of the B873 complex.

ing, the data clearly showed in all cases two overlapping phosphorylated bands which correspond exactly to the observed positions of α- and β-polypeptides as determined by Coomassie blue staining of the SDS-gel. Furthermore, the α- and β-polypeptides were distinguished visually from the Coomassie-stained gel in most separations. The third component indicated, Ω, is a new protein component of the chromatophores which copurifies with the B873 complex and which we assume to be associated with it [16,17]. The function of this component is at present unknown. The separation of water-soluble from membrane fractions as indicated by the near-IR absorption spectra and SDS-gel profile was not always successful, due to incomplete sedimentation of small membrane vesicles, particularly when large amounts of protein were employed. However, small scale preparations usually led to a good separation of chromatophore and water-soluble fractions. For preparations where a good separation had been achieved, the addition of the water-soluble fraction to the chromatophores was essential to demonstrate phosphorylation of the light-harvesting complexes. Our chromatophore preparation is more homogeneous than that of Holmes and Allen [15] who used only a single ultracentrifugal step to prepare the chromatophore fractions, as indicated by the presence of the 55 kDa band in the latter preparation. Furthermore, it should be noted that only minute amounts of kinase, which almost always contaminate the chromatophore fraction, are sufficient to catalyse a significant phosphorylation.

(b) Buffer

The pH dependence of B873 phosphorylation showed an optimum at pH 7.5 in both Tris-HCl and imidazole-HCl buffer systems, the activity in the latter being somewhat weaker (data not shown). Phosphate buffer proved unsuitable, resulting later in protein aggregates accumulated at the top of the gel.

We have routinely used Mg^{2+} as a cofactor in our standard assay system. The optimum concentration of Mg^{2+} was 20 mM, and the optimal concentration of ATP was 0.5 mM, sufficient to ensure maximal phosphorylation of the B873 complexes. Preliminary studies suggest the apparent K_m of the B873 kinase activity to be less than 100 μM (data not shown). The stability of the added ATP in the light-dependent phosphorylation assay was measured with a coupled assay of hexokinase and glucose-6-phosphate dehydrogenase [24]. No significant non-enzymatic breakdown of ATP was observed. Enzymatic breakdown of ATP due to the endogenous ATPase of the chromatophores was blocked with venturicidin. Substitution of venturicidin by DCCD or vanadate was not successful.

In most of the work we have included 100 μM DBMIB as an electron transport inhibitor unless otherwise stated. Under these conditions the phosphorylation of α, β, and Ω polypeptides was always reproducibly high although the dependence of phosphorylation upon illumination was abolished. The addition of 10 mM DTT to the storage buffer for membrane and soluble fractions had no apparent effect upon B873 phosphorylation.

(c) Dependence of B873 phosphorylation upon illumination

Fig. 1(a) shows a typical autoradiogram of isolated chromatophores after phosphorylation, conducted under light or dark conditions and in the presence or absence of added supernatant. In these experiments DBMIB was absent. We confirm the observation of Holmes and Allen [15] that a kinase activity in the supernatant fraction stimulates the phosphorylation of the B873 α- and β-polypeptides. However, phosphorylation with the supernatant fraction alone also re-

vealed a soluble phosphorylated component which migrates at the position of the α-polypeptide, thus complicating the interpretation of the effect of supernatant. This component, which is hardly visible on the Coomassie-stained gel, was also present on the gels shown by Holmes and Allen [15].

Illuminated chromatophores consistently show a slightly stronger phosphorylation than those incubated in the dark. An exact repetition of the experiments of Allen and Holmes [15], using $MgSO_4$ instead of $MgCl_2$, and a single ultracentrifugal step to prepare the chromatophores, yielded the same results.

Phosphorylation of whole cells according to Loach et al. [8] and Holmes and Allen [14] in the light or dark in the presence of 20 mM Mg^{2+} and 1 mCi of inorganic [^{32}P]phosphate yielded similar results to those observed for chromatophores phosphorylated in vitro. More inorganic phosphate was incorporated into the various cell components including the B873 polypeptides, but no other components were found to become labelled compared to chromatophores.

When various factors affecting B873 phosphorylation were investigated, large variations in the degree of phosphorylation in the absence of DBMIB were observed between different preparations. By contrast, when 100 μM DBMIB was added to the kinase reaction mixture the phosphorylation profiles were highly reproducible, and thus DBMIB was added routinely to the standard assay mixture. Fig. 1(b) shows that in the presence of DBMIB and added supernatant, the protein pattern of the chromatophores exhibits a strongly phosphorylated band with a molecular weight of approx. 30 kDa, corresponding to the position of cytochrome c_1 (Fig. 1b (lanes 2,3,5,6)). The reaction centre subunits are not phosphorylated although the H-subunit, running slightly below cytochrome c_1 may be weakly labelled. Another phosphorylated component of the chromatophores with a molecular mass of approx. 40 kDa is labelled only when the chromatophores are illuminated during the reaction. This 40-kDa component is located exclusively in the chromatophore fraction and might correspond to the cytochrome b-560 of the cytochrome bc_1 complex [25].

Finally, comparison of Figs. 1(a) and (b) shows that whereas the α- and β-polypeptides of the B873 complexes are more strongly phosphorylated than the Ω component in the absence of DBMIB, in its presence the phosphorylation of Ω is maximal and higher than α and β.

(d) Metal cofactor dependence of the B873 kinase activity

Fig. 2 shows a series of metal cofactors used to activate the B873 kinase activity. Interestingly, the transition metal ions Cu^{2+} and Zn^{2+} were significantly more active than either Mg^{2+} or Mn^{2+}. Ca^{2+} exhibited

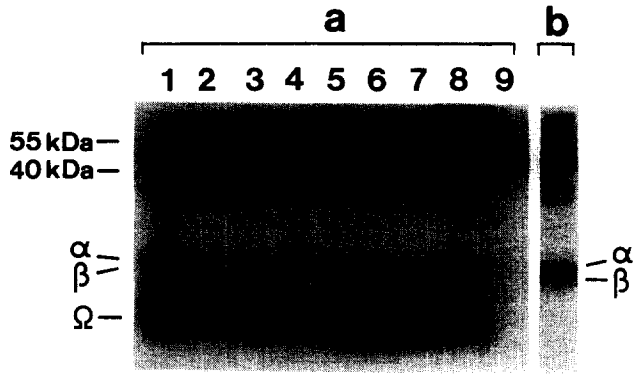

Fig. 2. Dependence of the B873 kinase activity upon the metal cofactor. Chromatophores were phosphorylated in the presence of added supernatant by the standard assay. The following metal cofactors were added to final concentration of: (1) 10 mM $CuCl_2$; (2) 10 mM $CaCl_2$; (3) 10 mM $MnCl_2$; (4) 10 mM $MgCl_2$; (5) 5 mM $MnCl_2$ and 5 mM $MgCl_2$; (6) 10 mM $ZnCl_2$; (7) 10 mM $MnCl_2$ followed by the addition of 10 mM $ZnCl_2$ after illumination; (8) conditions as in (7) but in the presence of 5 U alkaline phosphatase with 10 mM $ZnCl_2$; (9) control without metal cofactor. Autoradiograms are shown in (a), a representative Coomassie blue-stained lane is shown in (b).

only a weak activation effect. Combination of either Mg^{2+} and Mn^{2+} or Mg^{2+} and Zn^{2+} did not increase the activation above the sum of the two determined separately. In these experiments sub-optimal concentrations (5 mM) of metal ions were employed in order to maximize differences in activity.

We also attempted to induce a dephosphorylation of the phosphorylated B873 complex by the addition of Zn^{2+} and calf intestinal alkaline phosphatase (Fig. 2, lane 8). In the event, no dephosphorylation of either α, β, or Ω of the B873 complex was observed. In the presence of Zn^{2+} and Cu^{2+}, respectively, additional phosphorylated bands appeared in the 25 kDa region, which could not be identified.

(e) Regulation of the B873 kinase activity by ADP and AMP

Fig. 3 shows the effects of the addition of 0.1–0.4 mM ADP or AMP to the kinase assay mixture containing chromatophores and supernatant fractions prior to illumination. Increasing amounts of ADP progressively inhibit the phosphorylation of α-, β-, and Ω-polypeptides (Fig. 3, lanes 2–4), and the same trend is observed in the presence of AMP (Fig. 3 (lanes 5,6). In contrast, the addition of cyclic 3′-5′ AMP had no effect upon the phosphorylation of the α-, β-, or Ω-polypeptides (lane 7).

(f) Substrate specificity of the supernatant B873 kinase activity

Fig. 4 compares intact chromatophores, purified B873 complexes and histone V-S as substrates for the supernatant kinase activities. The α-, β-, and Ω-polypeptides of purified B873 complexes were phospho-

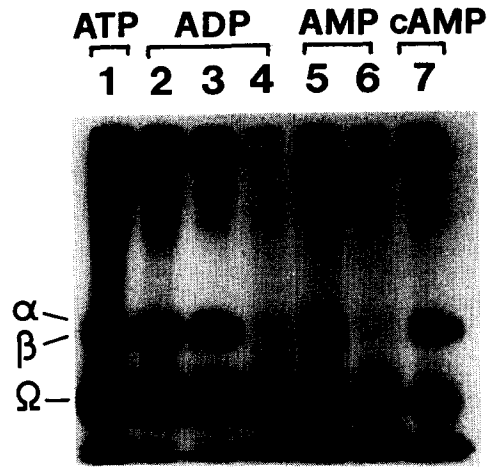

Fig. 3. Regulation of the B873 kinase activity by adenine nucleotides. Purified chromatophores were phosphorylated in the presence of added supernatant in the standard assay with the following additions: (a) 0.1 mM ADP; (b) 0.2 mM ADP; (c) 0.4 mM ADP; (d) 0.2 mM AMP; (e) 0.4 mM AMP; (f) 0.1 mM cAMP. The pH of the nucleotide stock solutions were adjusted to pH 7.0 before addition to the phosphorylation assay.

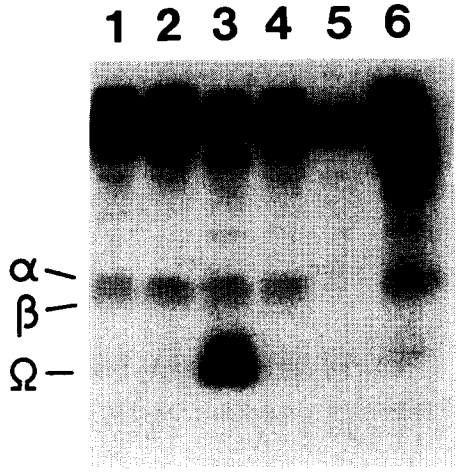

Fig. 5. Effect of inhibitors of electron transport upon the phosphorylation of B873 complexes. The standard assay was performed in the presence of: (1) 1 μM antimycin; (2) 100 μM antimycin; (3) 100 μM DBMIB; (4) 5 mM sodium ascorbate; (5) 5 mM sodium dithionite; (6) 5 mM potassium ferricyanide.

rylated identically to those in the chromatophore membranes. Histone V-S was also strongly phosphorylated and can thus be utilized efficiently as a substrate by the purified B873 kinase.

(g) Effects of electron transport inhibitors upon the B873 kinase activity

Fig. 5 shows the effects of various redox reagents and electron transport inhibitors upon the phosphorylation of the B873 components. As observed by Holmes and Allen [15], the addition of sodium dithionite completely abolished the phosphorylation of α-, β-, and Ω-polypeptides. After extensive oxidation of the membrane with $K_3(Fe(CN)_6)$, however, both α- and β-polypeptides were still phosphorylated whereas the Ω-polypeptide incorporated only little phosphate. Similarily, the addition of the reducing agent, sodium ascorbate, abolished the phosphorylation of Ω whilst leaving the phosphorylation of α and β unaffected. Inhibition of electron transport with antimycin also prevented the phosphorylation of Ω, whereas upon the addition of DBMIB all three polypeptides were strongly phosphorylated. In contrast to Holmes and Allen [15], a differential phosphorylation of the α- and β-polypeptides upon varying the redox state of the membrane was not found.

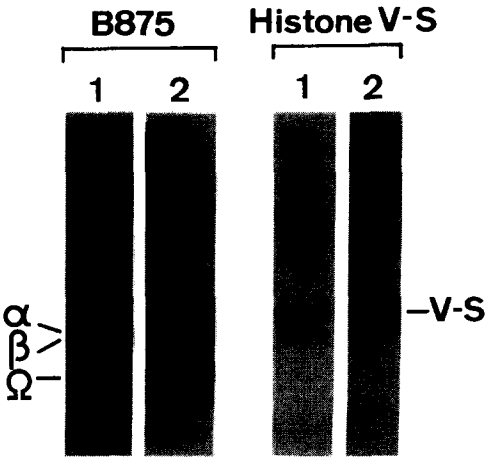

Fig. 4. Substrate specificity of the kinase activity in the supernatant fraction. Substrates (1) isolated B873 complexes (no detergent) or (2) histone V-S in the presence of supernatant fraction. Coomassie blue-stained gels in (a); autoradiogram in (b).

(h) Effects of phosphorylation upon the modulated fluorescence of the chromatophore membranes

Fig. 6 shows the effect of illumination during phosphorylation upon the modulated fluorescence profile of purified chromatophores. Under a variety of conditions, the variable fluorescence (F_v) was markedly decreased after phosphorylation during illumination. Table I summarises the effects of varying the reaction conditions of the phosphorylation reaction upon the modulated fluorescence profile. The variable fluorescence (F_v) observed from isolated and 'non-phosphorylated' chromatophores which have been inhibited by DBMIB was rather high (Fig. 6, trace a) and in all cases, was decreased when in vitro phosphorylation was performed prior to fluorescence measurement. F_v was markedly decreased when the chromatophores were phosphorylated in the light (Table I, samples 2,4,6), but approached the value observed under non-

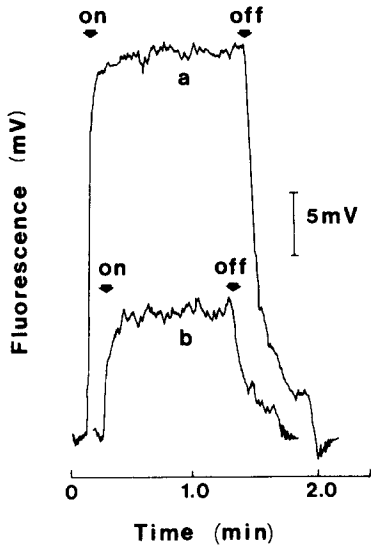

Fig. 6. Effect of protein phosphorylation on the fluorescence signal (F_v) of chromatophores in the absence of added supernatant: (a) in the absence of DBMIB and ATP; (b) after a phosphorylation reaction in the presence of DBMIB and ATP. The ground level fluorescence (F_o) is due to the modulating measuring beam, and the steady-state fluorescence signal observed after excitation with actinic light (on, light switched on; off, light switched off) is referred to as F_v in the text.

phosphorylated conditions when the chromatophores were phosphorylated in the dark. The presence of added supernatant had relatively little effect upon F_v (Table I, lanes 2–5). Although the effects of phosphorylation, presence of added supernatant, and illumination upon F_v largely paralleled those observed for F_o, the ratio F_v/F_o varied. A correlation between the changes of fluorescence, the redox state of a specific component, and the degree of phosphorylation is not possible at present. For instance, the level of fluorescence in the absence of DBMIB and illumination (Table I, lane 3), where α-, β-, and Ω-components of the B873 complex were only weakly phosphorylated, was very similar to that observed in the presence of DBMIB (Table I, lane 5) where all three components were labelled.

TABLE I

Modulated fluorescence measurements of chromatophores from R. rubrum

Modulated fluorescence was measured under various conditions as indicated in the table. The observed phosphorylation of the α-, β-, and Ω polypeptides under the conditions before the measurement is indicated. (+), phosphorylated; (−), not phosphorylated; (+/−), weakly phosphorylated.

Sample	Reaction conditions				Fluorescence measurements			Phosphorylation	
	Light	ATP	DBMIB	supernat.	F_o (mV)	F_v (mV)	F_v/F_o	$\alpha+\beta$	Ω
1	+	−	+	−	21	8	1.33	+	±
2	+	+	−	+	8	5	0.63	+	−
3	−	+	−	+	12	25	2.1	±	−
4	+	+	+	+	5	4	0.8	+	+
5	−	+	+	+	18	26	1.44	±	+
6	+	+	+	−	6.5	8	1.23	+	+
7	−	+	+	−	13	22	1.69	−	+

Discussion

The present study confirms and extends the work of Allen and co-workers [11,14,15] who showed for the first time that the phosphorylation of the B873 light-harvesting complexes of *R. rubrum* depends upon the redox state of the chromatophore membrane, probably at the level of ubiquinone. However, there are a number of significant differences between the study of Holmes and Allen [15] and the results presented here.

First, the chromatophore as well as the supernatant fractions were purified extensively by repeated homogenization and ultracentrifugation. The chromatophores contained only traces of cytochrome c_2, thereby minimizing effects attributable to an intact cyclic electron transport chain. In addition, the supernatant fraction was purified extensively by repeated ultracentrifugation so as to remove small chromatophore vesicles. These differences are significant since only minute amounts of kinase protein are sufficient to catalyse a light-dependent phosphorylation [17].

Secondly, in our assay procedure, which is similar to that of Holmes and Allen [15], we have omitted the final extraction with 80% acetone after pelletting the protein by TCA precipitation, since the Ω component is soluble in acetone [16] and the extraction of BChl or BPh from chromatophores by this procedure almost always leads to some extraction of B873-α as well. The effect of phosphorylation of α- and β-polypeptides upon their solubility in 80% acetone has not been determined.

In agreement with Holmes and Allen [15], the 13-kDa (B873-α) and 10-kDa (B873-β) polypeptides are heavily phosphorylated when the chromatophores are incubated with Mg^{2+} and ATP in the light. However, also in the dark both polypeptides become labelled. A major difference between our findings and that of Holmes and Allen [15] is that we find no evidence for differential phosphorylation of the α- and β-polypeptides dependent upon the illumination or redox state of the membrane. In fact the major differences in phosphorylation occur at the level of the component Ω. In this context the effects of redox reagents or electron transport inhibitors upon B873 phosphorylation are particularly illustrative. Complete reduction of the membrane abolished phosphorylation of α-, β-, and Ω-polypeptides, whereas complete oxidation al-

lowed the phosphorylation of α- and β-polypeptides, while the Ω-polypeptide became only weakly labelled. Addition of DBMIB, an electron transport inhibitor, caused all three components to be phosphorylated. The DBMIB effect shown here is different from that observed by Holmes and Allen [15] where DBMIB abolished the phosphorylation of the α- and β-polypeptides. Unfortunately, the mechanism of inhibition of electron transport by DBMIB is not well-defined; in fact, preincubation of chromatophores with 100 μM DBMIB does not appear to affect the dithionite-$K_3Fe(CN)_6$ redox spectrum of the cytochrome bc_1 complex (data not shown). It may well be that DBMIB behaves as a diverter of electrons and is able to influence the $[QH_2]/[Q]$ ratio in this way. However, this would not affect the general conclusion presented here: that changes of the redox state of the quinone pool affect the phosphorylation of the various components of the chromatophore membrane. Upon the addition of antimycin A, which blocks the reduction of the Rieske Fe-S complex by cytochrome *b*-560, we observe strong phosphorylation of the α- and β-polypeptides with only weak or no labelling of the Ω polypeptide. By contrast, the autoradiographs of Holmes and Allen [15] show the Ω-polypeptide to be heavily phosphorylated under these conditions. At present we are unable to explain the discrepancies, which are possibly due to some detailled difference in methodology (growth conditions, extraction procedures).

The effects of phosphorylation upon B873 function can be measured by studying the fluorescence of chromatophores under different conditions. Using this technique two parameters are important, the ground fluorescence, F_o, reflecting the background fluorescence due to the modulated measuring beam, and F_v, the variable fluorescence observed upon illuminating the chromatophore suspension with saturating actinic light. Although there is some variation in F_o, the effects of phosphorylation are seen in F_v which provides a measure of energy transfer and quenching within the complexes. We have measured F_o and F_v under a number of conditions which are pertinent to defined phosphorylation states (Table I) and a number of conclusions may be drawn: (a) in the absence of ATP (line 1) the variable fluorescence is maximal. We assign this state as being dephosphorylated (see also Ref. 8). Upon the addition of ATP, the variable fluorescence is decreased by a factor of approx. 3 when the phosphorylation is conducted in the light but remains almost unchanged in the absence of illumination (lines 2 and 3). The quenching of fluorescence we assume to be due to an as yet unknown mechanism which leads to an increase in the efficiency of energy transfer and perhaps of B873 coupling to the reaction centres; (b) although the kinase activity is predominantly found in the supernatant, the addition of the supernatant fraction does not further decrease the fluorescence changes due to phosphorylation. This is probably due to the fact that only traces of B873 kinase contaminating the membrane fraction are sufficient to allow phosphorylation to occur. However, we cannot necessarily exclude the presence of an additional membrane-bound B873 kinase which has not been identified so far; (c) the presence of DBMIB causes some modulation of both the basal (F_o) and variable (F_v) fluorescence without severely affecting the relative changes observed in the presence or absence of illumination during the phosphorylation reaction. This effect may be due primarily to the variable phosphorylation of the Ω polypeptide.

We have also shown that the ATP-dependent B873 phosphorylation is relatively insensitive to the presence of other nucleotides such as ADP, AMP and cAMP (Fig. 3). Only at high ADP concentrations (> 0.4 mM) could a significant inhibition of the B873 kinase activity be observed. In view of the estimated K_m of the reaction (in the μM range), the inhibiting effect of ADP and AMP is probably not important physiologically.

The B873 kinase activity in the supernatant shows an unusual metal dependence; although Mg^{2+} is able to activate the kinase, much higher activities are observed in the presence of transition metal ions such as Mn^{2+}, Zn^{2+}, and Cu^{2+} (Fig. 2). To our knowledge this metal ion specificity (also observed for the isolated B873 kinase, [17]) is novel among eucaryotic and prokaryotic kinases examined so far. By contrast Ca^{2+} appears to only weakly substitute for Mg^{2+} in the kinase assay.

Examination of the data shown in Figs. 1–5 demonstrates that many higher molecular mass components in the supernatant become phosphorylated under the same conditions as those for phosphorylation of the B873 complexes. One of the most prominent phosphorylated bands has a molecular mass of 55 kDa and probably corresponds to ribulose bisphosphate carboxylase (Rubisco). Under many conditions, the B873 complexes were not phosphorylated, whereas the 55-kDa band of the supernatant was highly labelled. This suggests the presence of at least two distinct kinase activities. In addition, the membrane fraction shows bands of higher molecular weight which are phosphorylated under certain conditions, some of these, probably cytochrome c_1 and *b*-560 components, by the B873 kinase. If so, this could provide the molecular basis for the redox control of B873 complex phosphorylation. At present, the unambiguous assignment of these bands and their function must await further clarification.

As shown by Holmes and Allen [15], the redox state of the membrane dominates the phosphorylation of the B873 complexes in vivo. When the quinone pool is reduced the B873 complex is less phosphorylated than under oxidising conditions. However, the comparison

of phosphorylation in the presence of sodium dithionite and sodium ascorbate suggests that not only the redox balance of the quinone pool but also that of the cytochrome bc_1 complex may be involved in regulation. Whereas sodium dithionite completely reduces the cytochrome bc_1 complex of *R. rubrum*, sodium ascorbate reduces only the cytochrome *b*-560 component but not the cytochrome c_1 [25]. Since the cytochrome bc_1 complex can be isolated as a supramolecular complex together with reaction centres and the B873 complex [26], a direct physical interaction between these complexes could affect the binding and activity of the B873 kinase by means of a redox-induced conformational change. The redox-regulated control of light-harvesting complex phosphorylation is not new: a similar effect has been observed for the plant light-harvesting complexes and the cytochrome *bf* complex [6], and recently, Drews and co-workers [27] have shown that the phosphorylation of the α-polypeptide of the B875 complex of *Rhodobacter capsulatus* may be under redox control.

Redox control of B873 phosphorylation provides a link to the regulation by light intensity [6]. Under conditions of high light, the quinone pool and cytochrome bc_1 complex will be essentially reduced, thus preventing extensive phosphorylation of the complexes. As shown by fluorescence measurements, the dephosphorylated complexes have a high intrinsic fluorescence, and are thus an indication of a decrease in the efficiency of energy transfer to the reaction centres. Conversely, under low light conditions the B873 complexes are highly phosphorylated, thus increasing the efficiency of energy transfer to the reaction centres. Clearly phosphorylation only modulates function, as completely dephosphorylated complexes are still able to transfer light energy to the reaction centres. Phosphorylation may provide a mechanism of adaptation to different light intensities under varying environmental conditions.

We have not been able to demonstrate the physiological *dephosphorylation* reaction in vitro so far, even though chromatophores can be dephosphorylated by the addition of alkaline phosphatase to phosphorylated membranes.

In summary, the phosphorylation phenomena observed in vitro for isolated cellular fractions are highly complex and possibly a variety of kinases are involved in the regulation and redox control of photosynthesis. The delineation of the gene products involved and their role in regulation will be of great importance for understanding the regulation of light energy transfer in photosynthesis.

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References

1 Cozzone, A.J. (1988) Ann. Rev. Microbiol. 42, 97–125.
2 Stock, J.B., Ninfa, A. and Stock, A.M. (1989) Microbiol. Rev. 53, 450–490.
3 Saier Jr., M.H., Wu, L.-F. and Reizer, J. (1990) Trends Biochem. Sci. 15, 391–395.
4 Wanner, B.L. (1992) J. Bact. 174, 2053–2058.
5 Allen, J.F. (1983) CRC Crit. Revs. in Plant Sciences Vol I, 1–22.
6 Allen, J.F. (1992) Biochim. Biophys. Acta 1098, 275–335.
7 Allen, J.F. (1992) Trends Biochem. Sci. 17, 12–17.
8 Loach, P.A., Parkes, P.-S. and Bustamante, P. (1984) Adv. Photosynth. Res. (Sybesma, C. (ed.)) Vol. II Martinus Nijhoff Publishers, pp. 189–197.
9 Holuigue, L., Lucero, H.A. and Vallejos, R.H. (1985) FEBS Lett. 181, 103–108.
10 Vallejos, R.H., Holuigue, L., Lucero, L.H. and Toruella, M. (1985) Biochem. Biophys. Res. Commun. 126, 685–691.
11 Holmes, N.G., Sandres, C.E. and Allen, J.F. (1986) Bioch. Soc. Trans. 14, 66–68.
12 Turner, A.M. and Mann, N.H. (1986) J. Gen. Microbiol. 132, 3433–3440.
13 Zebrower, M. and Loach, P.A. (1981) In Energy coupling in Photosynthesis (Selman, Selman-Peimer, eds.), Elsevier, Amsterdam, pp. 333–340.
14 Holmes, N.G. and Allen, J.F. (1986) FEBS Lett. 260, 144–148.
15 Holmes, N.G. and Allen, J.F. (1988) Biochim. Biophys. Acta 935, 72–78.
16 Ghosh, R., Tschopp, P., Eicher, S. and Bachofen, R (1990) In Current Research in Photosynthesis (Baltscheffsky, M., ed.) Vol. II, Elsevier, Amsterdam, pp.77–80.
17 Ghosh, R., Ghosh-Eicher, S., DiBerardino, M. and Bachofen, R. (1994) Biochim. Biophys. Acta 1184, 28–36.
18 Lueking, D.R., Fraley, R.T. and Kaplan, S. (1978) J. Biol. Chem. 253, 451–477.
19 Snozzi, M. and Bachofen, R. (1979) Biochim. Biophys. Acta 546, 236–247
20 Peterson, G.L. (1977) Anal. Biochem. 83, 346–356.
21 Laemmli, U.K. (1970) Nature 277, 680–685.
22 Setlik, I., Waldburger-Schlapp, M. and Bachofen, R. (1990) In Current Research in Photosynthesis (Baltscheffsky, M., ed.) Elsevier, Amsterdam, Vol II, pp. 341–344.
23 Ghosh, R., Hauser, H. and Bachofen, R. (1988) Biochemistry 27, 1004–1014.
24 Lamprecht, W. and Trautschold, I. (1962) Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.), Chemie Verlag, Weinheim.
25 Kriacunas, A., Yu, L., Yu, C.A., Wy, R.M. and Knaff, D.B. (1989) Biochim. Biophys. Acta 976, 70–76.
26 Ghosh, R., Kessi, J., Hauser, H., Wehrli, E. and Bachofen, R. (1990) In Molecular Biology of Membrane-Bound Complexes in Phototrophic Bacteria (G. Drews, and E.A. Dawes, eds.) Plenum Press, pp.245–251.
27 Cortez, N., Garcia, A.F., Tadros, M.H., Gad'on, N., Schiltz, E. and Drews, G. (1992) Arch. Microbiol. 158, 315–319.