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Phosphorylation of the light-harvesting polypeptide LHI α of *Rhodobacter capsulatus* at serine after membrane insertion under chemotrophic and phototrophic growth conditions

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Abstract

The kinetics of protein phosphorylation was studied in cells of phototrophic cultures and in dark-grown cells induced to form the photosynthetic apparatus by lowering of the oxygen tension. Cells of *Rhodobacter capsulatus* grown in a malate medium with 0.2 mM potassium phosphate were shifted to semiaerobic conditions, and $^{32}PO_4^{3-}$ or [^{35}S]Met was added 25 min after induction. The label of both radioactive precursors appeared in the membrane fraction about 20 min after addition. The maximum of ^{32}P was found after 1 h of labeling in the α polypeptide of the light-harvesting (LH) complex I (B870). The LHI α protein was phosphorylated after insertion into the membrane. Chloramphenicol inhibited the phosphorylation of LHI α but not of phospholipids. The steady-state level of phosphorylation was higher in anaerobic cultures grown at the low light intensity of 2000 lux than in cultures grown at high light intensity of 35 000 lux. The phosphate label did not change significantly during a chase with unlabelled phosphate for 2 h. The phosphoamino acids in LHI α were detected with monoclonal antibodies and radioautography of labeled and hydrolyzed LHI α. Serine was shown to be the amino acid with the highest phosphate content; threonine and tyrosine were weakly phosphorylated. From the positions of these three amino acids in LHI α it was concluded that serine-2, which is exposed on the cytoplasmic side of the membrane, is the main phosphorylated amino acid. P-threonine and P-tyrosine are exposed on the periplasmic surface of the membrane.

Keywords: Protein phosphorylation; Light-harvesting protein; Phosphoserine; Phosphothreonine; Phosphotyrosine; (*R. capsulatus*)

1. Introduction

1.1. Enzymatic of protein phosphorylation and dephosphorylation

Protein phosphorylation is an important mechanism of sensing and signal transduction in eukaryotic and prokaryotic organisms [1,2]. It is also widespread in photosynthetic organisms. Several thylakoid proteins of eukaryotes [3] and prokaryotes [4] were found to be phosphorylated. The protein phosphorylation is catalyzed by protein kinases, enzymes that transfer a phosphate group from a phosphate donor, which is generally the γ phosphate of ATP or GTP, onto an acceptor amino acid in a substrate protein [5]. Protein kinases are classified by the amino acyl acceptor groups ((i) serine/threonine; (ii) tyrosine; (iii) histidine, arginine or lysine; (iv) cysteine; (v) aspartic acid or glutamic acid residues) [5]. The phosphate residue is removed by phosphatases. Like kinases, phosphatases are amino-acid-specific. Phosphoserine phosphatases have a weak phosphothreonine activity, while phosphotyrosine phosphatases are tyrosine specific [6]. Phosphorylation increases the negative charge of proteins, which affects conformation, protein–protein interaction and catalytic activity of the protein.

1.2. Phosphorylation of thylakoid proteins

Phosphorylation of light-harvesting (LH) proteins results in a change of conformation and organization of reaction center/LH complexes. This has an influence on

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the intensity of chlorophyll fluorescence, which indicates a change of the efficiency of excitation energy distribution [4]. Protein phosphorylation was shown to be regulated by light via redox control of the thylakoid kinases [4]. The kinase was activated by phosphorylation, if a one-electron carrier was reduced ($E_m \approx 40$ mV). Other proteins were phosphorylated when the kinase was dephosphorylated ([4] and Allen, J.F., personal communication). Several thylakoid phosphoproteins function as redox sensors for activation or inactivation of kinases. Other phosphoproteins can regulate the transcription of chloroplast genes via a signal chain (Allen, personal communication). The phosphatases are not under redox control. The steady-state level of LH-chlorophyll-protein complex II phosphorylation was under the control of two different kinase/phosphatase systems, which were regulated by the degree of membrane energetization and the plastoquinone pool, respectively [7].

1.3. Protein phosphorylation of the bacterial photosynthetic apparatus

The function of LH-protein phosphorylation in purple bacteria is unknown [8–11]. The phosphorylation seems to be under redox-control [4,9–13]. Recently, a soluble protein kinase specific for serine residues was purified from *Rhodospirillum rubrum* [14]. In *Rhodobacter capsulatus* the α protein of the LHI (B870) core antenna complex was the only polypeptide of the membrane-bound pigment-protein complexes which was phosphorylated in vivo and in vitro [10]. In order to find out the mechanism of protein phosphorylation and its function for anoxygenic photosynthesis, we have studied the process in cells induced to form the photosynthetic apparatus by lowering the oxygen tension in dark chemotrophic cultures and by variation of the light intensity under anaerobic conditions. We will show that the LHIα protein is phosphorylated after incorporation of the newly synthesized α-protein in the membrane. High light intensity reduced the rate of protein phosphorylation. Phosphorylated and unphosphorylated α-proteins migrate with different velocities in SDS-polyacrylamide gels. The kinase which phosphorylates LHIα seems to be a membrane-bound, serine-specific enzyme.

2. Materials and methods

2.1. Strains and culture conditions

The strains of *Rhodobacter capsulatus* used in this study are listed in Table 1. The bacteria were grown under aerobic or semiaerobic conditions at 30°C in the dark and anaerobically at low (2000 lux) and high (35 000 lux) light intensities, respectively, in the malate-minimal medium RÄ [15].

2.2. Induction to form the photosynthetic apparatus and pulse labeling

The formation of the photosynthetic apparatus was induced by lowering of the oxygen tension. The pulse-chase experiments were performed as described [16] with the following modifications. The aerobically grown cells were resuspended in 35 ml of RÄ medium containing 0.2 mM potassium phosphate. 5 mCi of $^{32}PO_4^{3-}$ and 0.5 mCi [^{35}S]methionine, respectively, were added after 25 min of induction. At different times of incubation at 30°C after addition of radioactivity, 6 ml samples were withdrawn and rapidly mixed with 6 ml of stop buffer [16]. Phototrophically grown cells were incubated in glass tubes in a water bath at 30°C. The cell suspension was covered with a layer of mineral oil, incubated for several hours in the dark to consume the residual oxygen in the medium, and illuminated at different light intensities. When the $A_{660\ nm}$ of the culture reached 1.0, 1 mCi carrier-free $^{32}PO_4^{3-}$ was added to 10 ml of culture. After 6 h of incubation under anaerobic light conditions, the cells were harvested and the membrane fraction was isolated.

2.3. Preparation of membranes

The cells were spun down at 4°C in the rotor SS34 of a Sorvall refrigeration centrifuge and washed two times in phosphate buffer (100 mM, pH 7.8). They were resuspended in Tris-HCl buffer (pH 7.6), and broken by ultrasonication for 4 times 15 s in an ice bath. Cell debris was sedimented at 30 000 × *g* for 20 min, and the supernatant was layered on top of 6 ml 25% sucrose in Tris-HCl buffer and centrifuged for 17 h at 180 000 × *g* in a Beckman

Table 1
R. capsulatus strains used

Bacterial strains *R. capsulatus*	Genotype	Phenotype	Ref.
U43 (pTX35)	*Δpuf*, pTJS133	RC$^+$, LHI$^+$, LHII$^-$	[25]
U43 (pTPR8)	U43 (pTX35)*ΔpufA*	RC$^+$, LHI$^-$, LHII$^-$	[21,24]
U43 (pTPR9)	U43 (pTX35)*ΔpufB*	RC$^+$, LHI$^-$, LHII$^-$	[21,24]
37b4	wild type	wild type, no capsule	DSM 938
Y5		RC$^-$,LHI$^-$,LHII$^+$	Barry Marrs [26]

ultracentrifuge. The pelleted membranes (heavy membrane fraction) were resuspended in 0.1 ml of sample buffer for SDS-PAGE. The supernatant consisting of the top 4 ml soluble fraction and the light membrane fraction in the lower part of the supernatant were precipitated by addition of 10% final concentration of TCA in 100 mM sodium dihydrogen phosphate. After 1 h on ice, the samples were centrifuged and the pellets were resuspended in 50 μl of sample buffer. The different fractions contained similar amounts of protein.

2.4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The proteins were separated by Tricine-SDS PAGE [17]. The gels were stained in a mixture of acetic acid/methanol/dist. water (1:5:4; v/v) with Coomassie brilliant blue R250 (0.3% w/v), destained in acetic acid/methanol/dist. water (1:3:6) and dried under vacuum. The dried gels were exposed to Hyperfilm beta max single-coated films by direct contact to the dried gel. To discriminate between ^{32}P and ^{35}S, an aluminium foil was placed between film and gel.

2.5. Isolation of LHI polypeptides

The polypeptides LHI α and β were extracted from freeze-dried membranes of the strain U43 (pTX35) with chloroform/methanol/ammonium acetate and separated by chromatography on Sephadex LH60 using the same solvent [18].

2.6. Determination of phosphorylated amino acid

Cells of *R. capsulatus* U43 (pTX35) were radiolabeled in vivo with [^{32}P]P_i. The membrane fraction was isolated as described and the proteins separated by SDS-PAGE. The LHI α protein band was transferred to Immobilon (PVDF) membrane, pretreated with methanol and afterward with transfer buffer (0.192 M glycine, 25 mM Tris, 0.1% SDS). After blotting for 2 h at 5 mA/cm^2 the PVDF-membranes were washed with distilled water and dried. The strips of PVDF membranes with the blotted LHI α protein were hydrolyzed in 6 M HCl at 110°C for 2 h. The hydrolysate was dried in a Speed-Vac concentrator, resuspended in 5 μl H_2O, spotted on a cellulose thin-layer plates (20 × 20 cm, 0.1 mm thick, Merck, Darmstadt) and separated by chromatography in the first direction with isobutyric acid/NH_4OH, 5 + 3 vols., and in the second direction with 2-propanol/HCl (25%)/H_2O, 7 + 1.5 + 1.5 vols. Routinely, one-dimensional chromatography was performed with isopropanol/HCl (25%)/H_2O, 7:1.5:1.5 vols. After the run the plates were dried and the ^{32}P-labeled products were detected by radioautography on Hyperfilm β-max, Amersham. *O*-Phospho-L-serine, *O*-phospho-L-threonine and *O*-phospho-L-tyrosine (Sigma), which were added to the hydrolysate as marker, were detected by staining with ninhydrin.

In a second series of experiments the phosphoamino acids were detected by monoclonal antibodies against phosphoamino acids. The proteins were resolved by Tricine SDS-PAGE and blotted on nitrocellulose (BA85)-membranes (Schleicher and Schuell). To avoid unspecific binding the membranes were treated for 45 min with buffer 2 (0.1 M Tris, 0.1 M NaCl, 2 mM $MgCl_2$, 0.05% Triton X-100, 2% BSA, w/v (pH 7.5)) and thereafter washed two times for 10 min with the same buffer without BSA (buffer 1). The antibodies (anti-phosphoserine, anti-phosphothreonine, anti-phosphotyrosine, Sigma) were diluted according the instruction of the manufacturer in buffer 2, and the membranes were washed in buffer 1 for 10 min. The specific immunoreaction was visualized with alkaline phosphatase coupled to secondary anti-mouse-IgG according to the instructions of manufacturer. The membranes were washed with buffer 3 (0.1 M Tris, 0.1 M NaCl, 5 mM $MgCl_2$ (pH 9.5)) and stained (10 ml buffer 3, 10 μl nitroblue tetrazolium chloride (50 mg/ml) 10 μl 5-bromo-4-chloro-3-indoyl phosphate (50 mg/ml). The reaction was finished with stop buffer (20 mM Tris-HCl (pH 7.5)/5 mM EDTA).

3. Results

3.1. The kinetics of protein phosphorylation

To learn more about the function of protein phosphorylation, the kinetics of incorporation of [^{35}S]methionine and of [^{32}P]P_i into the light-harvesting (LH) proteins was analyzed. The radioactive tracers were added after preincubation of cells for 25 min under semiaerobic conditions to induce the formation of the pigment-protein complexes of the photosynthetic apparatus. The phosphate concentration in the medium of the preculture was reduced to 0.2 mM to optimize the incorporation of [^{32}P]P_i. The low phosphate concentration, however, reduced the growth rate and the time for incorporation of radioactivity into the proteins. The ^{32}P and the ^{35}S label were measurable in the α-protein after about 20 min of incubation. The maximum of label appeared in the heavy membrane fraction after 60 min (Fig. 1b, lane 6). Radioactive phosphate was also incorporated into the phospholipid fraction (Fig. 1b). Labeling of LHI α with $^{32}PO_4$ in the light membrane fraction was not observed before 60 min (Fig. 1b, lane 8). The relative positions of the LHI and LHII α and β polypeptides on the SDS-PAGE were shown in Fig. 1a. The Coomassie brilliant blue-stained LHI α polypeptide band in the SDS-PAGE gels was relatively broad, and after labeling with ^{35}S or ^{32}P the LHI α band was split in more than one band (Fig. 1b). This may be caused by a different degree of phosphorylation. In order to study whether the radioactive ^{32}P label was equally distributed over the population of

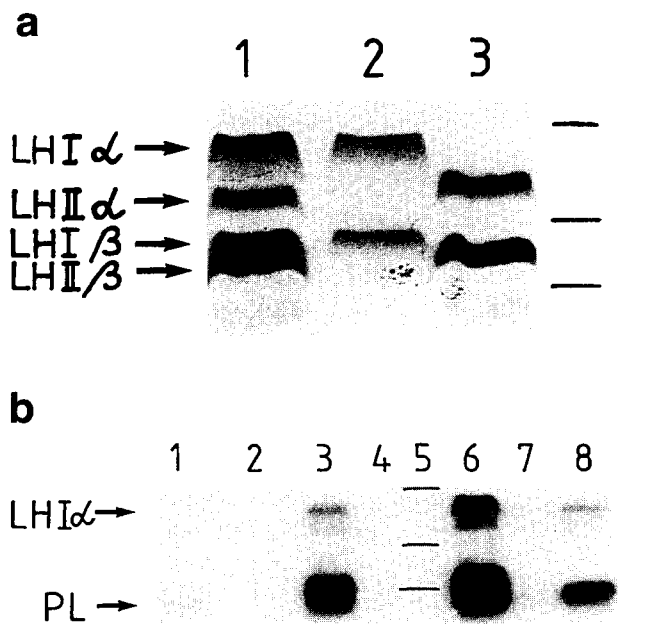

Fig. 1. (a) Polypeptide pattern of the light-harvesting complexes I and II. The membranes were isolated and purified from the cells by sucrose density centrifugation, applied to SDS-PAGE and the proteins stained with Coomassie brillant blue. Lane 1, proteins from the wild type, strain 37b4; lane 2, LHI α,β from strain U43 (pTX35); defective in LHII, and lane 3, proteins from strain Y5 showing LHII $\alpha\beta$ only. Horizontal lines on the right side indicate positions of molecular mass markers of 8.16, 6.21 and 3.46 kDa from top to the bottom. (b) Kinetics of incorporation of ^{32}P-labelled inorganic phosphate into the membrane and the soluble fraction of strain U43(pTX35) after preincubation for 25 min under low oxygen tension in the dark. [^{32}P]P_i was added after preincubation under semiaerobic conditions. PL, phospholipids. Lane 1, heavy membrane fraction after 20 min of labeling, lane 2, supernatant fraction after 20 min of labeling, lane 3, heavy membrane fraction after 40 min of labeling, lane 4, supernatant fraction after labeling of 40 min, lane 5, mol. mass markers 8.16, 6.21 and 3.46 kDa. lane 6, heavy membrane fraction after 60 min of labeling, lane 7, supernatant fraction after 60 min of labeling, lane 8, light membrane fraction after 60 min of labeling.

LHI α molecules, membranes were isolated from cells grown under anaerobic light conditions in the presence of [^{32}P]P_i. The polypeptides LHI α and β were extracted from the freeze-dried membranes by organic solvent and isolated by chromatography (see Section 2). The fractions of the purified proteins were applied to SDS-PAGE (upper panel of Fig. 2) and the LHI α and β polypeptides identified by N-terminal sequence determination (not shown here). The autoradiography of the gels seen in the upper part of Fig. 2 is depicted in the lower part of Fig. 2. The results showed that the maximal protein content of the fractions from the chromatography did not coincide with the maxima of radioactivity (compare upper and lower panel of Fig. 2). The highest concentration of LHI α protein was found in fraction 108 by Coomassie brilliant blue staining, but the highest ^{32}P radioactivity was found in fractions 142–146 of LHI α (Fig. 2). No radioactivity

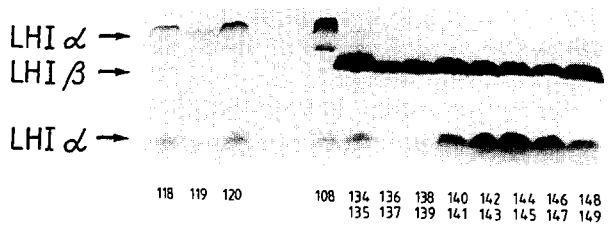

Fig. 2. Determination of proteins (Coomassie brilliant blue staining) and [^{32}P] label (radioautography) in the polypeptides α and β of LHI from U43 (pTX35). The cells were grown photosynthetically while being labeled for 2 h with [^{32}P]P_i. The membranes were isolated and the LHI proteins extracted from membranes with chloroform/methanol/ammonium acetate and separated by chromatography on Sephadex LH60 in the same solvent (see Section 2). Upper part: Coomassie brilliant blue-stained fractions from the column chromatography separated by SDS-PAGE and stained with Coomassie brilliant blue. Lower panel: Autoradiography of the gels seen on the upper part. Only LHI α was labeled. Fractions having a high protein content such as 108 (upper part) have a low ^{32}P radioactivity (lower part) and vice versa, fractions 142–146 showed a strong phosphorylation but weak Coomassie staining in LHI α.

was detectable in LHI β. We do not know why the LHI α polypeptides were so differently labeled. The phosphate bond in LHI α was resistant to boiling in 16% TCA for 1 h.

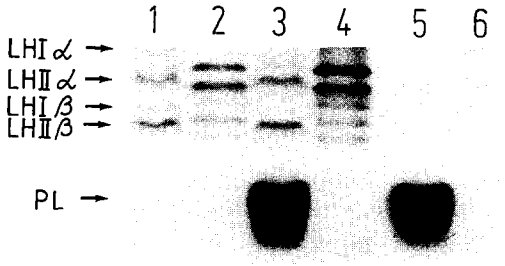

Fig. 3. Inhibition of incorporation of $^{32}PO_4$ into the membrane-bound LHI α in presence of chloramphenicol. Cells of U43 (pTX35) were preincubated for 20 min anaerobically at low light intensity in presence of [^{35}S]methionine; chloramphenicol was added and cells were incubated 40 min. Then $^{32}PO_4$ was added and incubation continued for 60 min. Membrane and supernatant fractions were isolated and the proteins separated by SDS-PAGE. Lanes 1–2 show autoradiograms of [^{35}S] labeled proteins and lanes 2–6 labeling with [^{32}P]P_i. In lanes 3 and 4 the proteins were double-labeled with ^{35}S and ^{32}P, in lanes 5 and 6 the ^{35}S label is discriminated from lanes 3 and 4 and only the phosphate label is visible. Lane 1, Proteins of membrane fraction after 20 min labeling with ^{35}S; lane 2, proteins of supernatant fraction after 20 min labeling with ^{35}S; lane 3, proteins of membrane fraction, 20 min [^{35}S]Met, 40 min additional incubation with 100 μg chloramphenicol/ml and further incubation for 60 min after addition of [^{32}P]P_i; lane 4, proteins from the supernatant, cells incubated as for lane 3; lane 5, proteins from membrane fraction of cells incubated as described for lane 3, but radioactivity of ^{35}S was shielded from the film by aluminum foil; lane 6, proteins of supernatant fraction of cells incubated as described for lane 3, but radioautography shows only label from ^{32}P as in lane 5. The figure shows that chloramphenicol inhibited phosphorylation of LHI α but not of phospholipids.

3.2. Protein is not phosphorylated in the presence of chloramphenicol

Cells were incubated under anaerobic-low-light conditions in the presence of [^{35}S]methionine. After 20 min of labeling (Fig. 3, lanes 1 and 2), chloramphenicol was added to a final concentration of 100 μg/ml. 40 min after addition of chloramphenicol [^{32}P]P_i was added and incubation was continued for an additional 60 min. Chloramphenicol inhibited the phosphorylation of LHIα and other proteins in the soluble fraction (Fig. 3, lanes 5 and 6), but phospholipids were labeled (Fig. 3, lanes 3 and 5). Control experiments showed that protein synthesis was completely inhibited by chloramphenicol under these experimental conditions. This result indicated that only the nascent LHIα protein was phosphorylated during incorporation into the membrane, presumably by a membrane-bound kinase.

3.3. Phosphorylation under high and low light intensities

Membrane differentiation and synthesis of LH complexes are strongly affected by the light intensity [19,20]. In order to study the effect of light on the phosphorylation of LHIα in anaerobic cultures of strain U43 (pTX35; LHII$^-$), the cells were grown at 2000 and 35 000 lux and incubated in presence of [^{32}P]P_i for several hours. Equal amounts of bacteriochlorophyll (BChl) of the membrane fractions were applied to each slot. Therefore, the densities of labeling as shown in Fig. 4, lanes 1 and 3, are directly comparable. The steady-state level of LHIα-bound phosphate was higher in low-light-grown cells than in high-light-grown cells. A very weak label was found in the supernatant of cell extract from low-light-grown cells due to a small contamination of the supernatant with membrane pieces (Fig. 4, lane 4). In conclusion, strong light inhibits phosphorylation of LHIα.

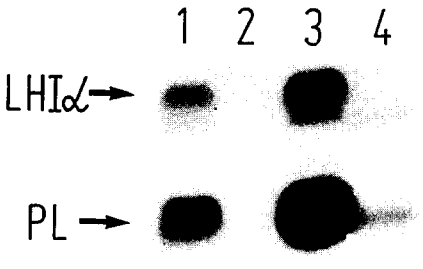

Fig. 4. In vivo labeling of cells of strain U43 (pTX35) with [^{32}P]P_i under anaerobic, high-light (35 000 lux; lanes 1 and 2) and low-light conditions (2000 lux, lanes 3 and 4), respectively. After incubation for several hours, membrane (lanes 1 and 3) and supernatant fractions (lanes 2 and 4) were applied to SDS-PAGE and autoradiography. Equal amounts of bacteriochlorophyll of membrane fractions and equal amounts of protein of supernatant fractions were applied to the slots.

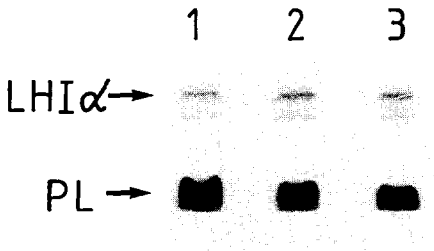

Fig. 5. Pulse-chase experiment with U43 (pTX35). Cells were induced by lowering of the oxygen tension in the dark for 25 min, then incubated with [^{32}P]P_i for an additional 30 min; afterwards, the [^{32}P]P_i in the medium was diluted with non-radioactive phosphate and the membrane fraction was isolated, subjected to SDS-PAGE and radioautographed. Lane 1 immediately after addition of cold phosphate, lane 2 after 60 min chase and lane 3 after 120 min of chase.

3.4. The turnover of LHIα phosphorylation

In the experiments described above, steady state levels of phosphorylation were measured. The low level of phosphorylation as shown in Fig. 1b could be affected by the rate of dephosphorylation. In the following experiment cells of U43 (pTX35) were induced by incubation at low oxygen tension in the dark for 30 min. Afterwards, the cells were incubated under the same conditions with [^{32}P]P_i in RÄ-medium containing 0.2 mM phosphate. After 30 min of labeling, unlabeled phosphate was added to a concentration of 100 mM. After 1 and 2 h of chase, equal amounts of membrane protein were applied to the slots of SDS-PAGE. As shown in Fig. 5, the ^{32}P label in LHIα did not change significantly. These results indicate a low turnover rate of LHIα phosphorylation under constant conditions of incubation.

The presence of phosphorylated LHIα restricted to the membrane fraction and LHIβ only in the supernatant [20] could be interpreted as a transfer of the $^{32}PO_4$ from the β to the α protein. To test this hypothesis the phosphorylation was studied in strains U43 (pTPR9) and U43 (pTPR8) which are deleted for one of the genes, *pufB* or *pufA*, coding for LHIβ or LHIα, respectively. Both strains were unable to form the LHI (B870) complex. LHIβ was reported to be incorporated into the membrane in the absence of LHIα [21], but the incorporation of LHIα into the membrane by the mutant U43 (pTPR9), defective in LHIβ, could not be determined for certain [21]. The incorporation experiments described in Fig. 1b were repeated with the mutant strains U43 (pTPR9) and U43 (pTPR8). LHIα of the strain U43 (pTPR9) was expressed and incorporated into the membrane and phosphorylated in the absence of LHIβ. The protein LHIβ of the strain U43 (pTPR8) was also incorporated into the membrane in the absence of LHIα, but, as expected, LHIβ was not phosphorylated (results not shown).

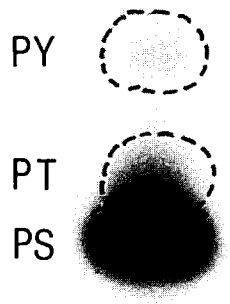

Fig. 6. Radioautography of phospho amino acids, separated by thin-layer chromatography in one dimension. The LHI proteins from cells incubated with $^{32}PO_4^{3-}$ were separated by SDS-PAGE and transferred from the gel to PVDF-membranes. The strip with the LHI α protein was treated and hydrolyzed as described in Section 2. 5 μl of the hydrolysate was applied to cellulose DC sheets. The labeled phospho amino acids serine, threonine and tyrosine were identified by co-chromatography of phospho amino acids which were stained with ninhydrin. PS, phospho serine; PY, phospho tyrosine; PT, phospho threonine. The positions of the ninhydrin stained phospho amino acids are indicated by the dotted circles.

3.5. The determination of the phosphoamino acids

The phosphoamino acids were detected by radioautography of the ^{32}P-labeled amino acids (see Section 2). The LHI α protein band was cut out of the gel and hydrolyzed for 2 h with HCl. The time for the hydrolysis was a compromise between optimum time to release all bound amino acids and a minimum to avoid destruction of phosphoamino acids during hydrolysis. Therefore, a quantification of the phosphoamino acids was not possible. Serine showed the strongest label. Tyrosine and threonine were only weakly labeled (Fig. 6). This result was confirmed by reaction with monoclonal antibodies: all three amino acids reacted positively with the specific antibodies, but *O*-phospho-L-serine showed the strongest response, while *O*-phospho-L-threonine and *O*-phospho-L-tyrosine reacted weakly positive (Fig. 7). From the observation that LHI α protein was phosphorylated after insertion into the membrane (Fig. 1B and Ref. [10]) it is concluded that serine is phosphorylated by a membrane-bound serine-protein kinase. The phosphate group was not removed by treatment of membranes with alkaline phosphatase.

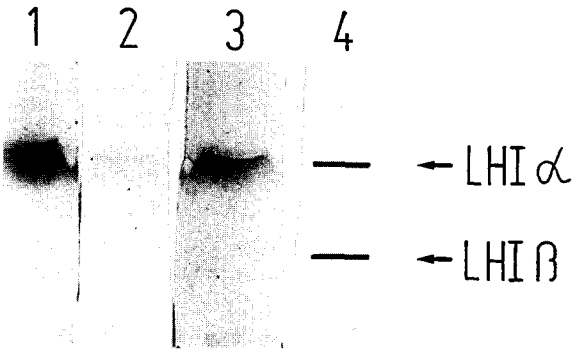

Fig. 7. Immunoblot of phospho amino acids. The non-radioactive labeled membrane proteins were separated by SDS-PAGE and blotted to nitrocellulose membranes. The phosphorylated amino acids of the LHI α protein were detected with monoclonal antibodies and visualized with anti mouse IgG coupled with alkaline phosphatase as described in Section 2. Lane 1, anti phosphoserine; lane 2, anti phosphotyrosine; lane 3, antiphosphothreonine; lane 4, positions of the LHI α and β polypeptides as detected by blotting on nitro cellulose membrane and staining with ponceau S. LHI β did not show any reaction with the antibodies.

4. Discussion

4.1. The kinetics of the LHIα phosphorylation

The α polypeptide of the light-harvesting complex I (B870) was found to be the only phosphorylated protein of the antenna complexes of *Rhodobacter capsulatus* grown under phototrophic conditions. The LHI α protein was also phosphorylated under in vitro conditions with [γ^{32}P]ATP or ADP plus [^{32}P]P_i under conditions of photophosphorylation [10]. The present study showed that LHI α is phosphorylated in cells grown in the dark under low oxygen tension, inducing the formation of the pigment-protein complexes. The phosphorylation seems to be directly coupled to or to follow immediately the insertion into the membrane, because the labeling with $^{32}PO_4^-$ and [^{35}S]methionine appeared at about the same time. The slowdown of incorporation of ^{35}S-labeled proteins compared with previous published experiments [16] can be attributed to the decrease of phosphate concentration in the medium by a factor of 125 (Fig. 1b) [16]. Inhibition of protein synthesis by chloramphenicol inhibited phosphorylation of LHI α but not of phospholipids. This, together with the observation that LHI α was never found phosphorylated in a soluble, unbound form in the supernatant, leads to the conclusion that de novo protein synthesis and insertion into the membrane are prerequisites for phosphorylation of LHI α. It is unlikely that the kinase is inhibited by chloramphenicol because, if inducible, the enzyme could be formed during the induction period in absence of chloramphenicol. The kinase is presumably membrane-bound and specific for the substrate.

4.2. The influence of light

Phosphorylation of proteins of the photosynthetic apparatus was reported to be regulated by redox control of the kinase [4,7–10]. During the present work it was observed that phosphorylation was stronger at low light than under high light intensities in anaerobic cultures (Fig. 4). Under in vitro conditions phosphorylation was stimulated by addition of ferricyanide, presence of air and absence of the soluble fraction [10]. These results are in accordance with the idea that phosphorylation affects the organization of the LHI complex and its position relative to the reaction center.

4.3. The site of phosphorylation

In order to identify the phosphorylated amino acids in LHIα, the protein was hydrolyzed and either the ^{32}P-labeled amino acids were detected by radioautography in TLC-plates or else the phosphoamino acids were identified with monoclonal antibodies on the proteins separated by SDS-PAGE. Serine showed the strongest radioactive label, but threonine and tyrosine were labeled weakly as well (Fig. 6). Phosphotyrosine is the least stable phosphoamino acid under acid hydrolysis, phosphothreonine is more stable than phosphoserine. Taking this into consideration, it is concluded that serine is the dominant phosphoamino acid in LHIα. These results were confirmed by an immunological test (Fig. 7). There is only one serine residue in LHIα which is exposed on the cytoplasmic surface of the membrane (Ser-2). Ser-37, Thr-38, -45 and -48 and Tyr-53 are on the periplasmic site of the membrane [23]. The results suggest that the strongly phosphorylated serine is at position 2 and is phosphorylated by a membrane-bound serine protein kinase. The mechanism and the relevance of the phosphorylation of the periplasmatically located amino acid residues has to be studied further. It has to be determined whether the low phosphorylation of these amino acids is due to another kinase located in the periplasm or on the periplasmic site of the membrane. The only serine residue in the LHIα of *Rhodospirillum rubrum* which was reported to be phosphorylated [11,14] is located near the C-terminal. A tyrosine phosphorylation was reported for a 13 kDa protein of *R. rubrum* [12]. It is strange that the phosphoserine in *R. rubrum* localized in the inside of chromatophores is easily dephosphorylated by alkaline phosphatase, but the phosphate group bound to serine in *Rb. capsulatus*, located on the outside of vesicles, could not be removed by treatment with phosphatase.

4.4. The role of protein phosphorylation

Since LHIα is the only phosphorylated protein in *Rb. capsulatus* and is part of the core complex, the conformation of the complex and possibly the oligomeric organization and position relative to the reaction center may be influenced as suggested for the LHCII complex of plants [4]. Similarly, in the rabbit glycogen phosphorylase and the LHCII complex the positively charged amino acids could be neutralized by phosphorylation of amino acids in the immediate vicinity. There is a lysine residue in position 3 of LHIα which follows Ser-2. When after insertion of LHIα, Ser-2 is phosphorylated the conformation of the protein, especially of the N-terminal region is changed, which may contribute to the assembling process and binding to the reaction center. Also excitation transfer from the B870 antenna to the reaction center might be affected [22]. The distribution of excitation energy under different conditions and the assembly of LHI should to be studied in mutant strains defective in the kinase. The results shown in Fig. 1b and 5 suggest that the turnover rate of the phosphoamino group is relatively low, but it should be determined whether this is true after changes of culture conditions.

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