Biochimica et Biophysica Acta 892 (1987) 68–74
Elsevier

BBA 42533

Formation of the B800–850 antenna pigment-protein complex in the strain GK2 of *Rhodobacter capsulatus* defective in carotenoid synthesis

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(Received 17 November 1986)
(Revised manuscript received 29 January 1987)

Key words: Reaction center; Pigment-protein complex; Carotenoid synthesis; Phototrophic bacteria, facultative; *(Rb. capsulatus)*

Lowering of oxygen tension in dark cultures of *Rhodobacter capsulatus* wild type induces formation of three pigment-protein complexes. The mutant strain GK2 formed the B800–850 antenna complex only when grown anaerobically in the light but not in the dark. Under phototrophic culture conditions bacteriochlorophyll, bacteriopheophorbid, and B800–850 polypeptides were synthesized in larger amounts than in chemotrophically grown cultures. The B800–850 complex of the mutant strain GK2 was formed in the absence of carotenoids. Messenger RNAs for reaction center B870 and B800–850 proteins were synthesized in chemotrophic and phototrophic cells. The formation of mRNA in GK2 differed quantitatively from the expression in the wild type strain.

Introduction

Synthesis and composition of the photosynthetic apparatus in *Rhodobacter capsulatus* and other facultative phototrophic bacteria is regulated mainly by the external factors, oxygen tension and light intensity [1–3]. Changes of oxygen partial pressure or light intensity affect via an unknown sequence of events (signal chain) the transcription of the genes for pigment-binding proteins [4,5] and of bacteriochlorophyll (BChl) genes [6] and modify the activity of enzymes for BChl synthesis [7]. Three pigment-protein complexes are formed in *Rhodobacter capsulatus* and other phototrophic bacteria; these are the reaction center (RC) and the two antenna pigment-protein complexes B870 and B800–850. In all three complexes two different polypeptides bind Bchl and carotenoids [8].

The B800–850 antenna complex of *Rb. capsulatus* contains, in addition to the two subunits α and β which bind 3 mol BChl and 1 mol carotenoid, the γ subunit which is not associated with pigment [9,10]. The B800–850 complex is dominant in cells growing phototrophically under low light intensity and varies in its relative amounts to the RC [2,11,12]. It is also present in semi-aerobically dark grown cells [1].

Mutant strains having defects in BChl synthesis do not contain pigment-protein complexes [13–15]. They synthesize, however, various pigment-binding polypeptides in low amounts which are detectable in the membrane fraction [14,16,17]. These proteins are not stably incorporated and disappear soon [16,18,19]. Carotenoid-less mutants of *Rb. capsulatus* and *Rb. sphaeroides,* having intact RC

Abbreviations: BChl, bacteriochlorophyll; RC, reaction center.

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and B870 complexes, have been described [14, 20–23]. All these mutant strains lack the B800–850 complex [15]. Some of the blue-green mutant strains of *Rb. capsulatus* contain the α and β subunits but not the γ subunit of B800–850 in the membrane [14,24]. Site-directed mutagenesis in carotenoid genes of *Rb. capsulatus* had pleiotropic effects in that also the expression of the γ subunit of the B800–850 complex was also suppressed [13,14]. The blue-green strain Ala^+ of *Rb. capsulatus* synthesizes only the α subunit of B800–850 [17].

The original carotenoid-less mutant R26 of *Rb. sphaeroides* did not contain the B800–850 complex. The derivative mutant R26.1 is still carotenoid-less and does not contain the B800 BChl absorption peak, but the B870 peak shifted to 860 nm [25]. The strain R26.1 contained the BChl-binding α and β proteins of B800–850 having a single amino acid exchange or missed the N-terminal methionine compared to wild type strain 2.4.1 [26]. A reconstitution of carotenoids into the light-harvesting complex of the mutant strain R26 failed to restore the 800 nm absorption [27].

These observations suggest that the carotenoids have an essential effect on the assembly and/or function of the B800–850 complex. In *Rb. capsulatus* a mutual relationship seems to exist between the γ protein and carotenoids for formation and stability of the B800–850 complex. In the mutant strains NK9 and NK19 of *Rb. capsulatus* [24] the synthesis of carotenoids and the γ polypeptide were reconstituted in a single step by conjugational transfer of a DNA fragment encoding enzymes of carotenoid synthesis (Boos, H., unpublished observation).

In the present study the formation of a B800–850 complex lacking carotenoids in the mutant strain GK2 will be described. This strain was constructed by transfer of photosynthetic genes of the plasmid pRCF 1002 to the photosynthetic inactive mutant strain Ala^+Pho^- [22,28]. The plasmid pRCF 1002 contains about 25 kb chromosomal DNA from the wild type strain 37b4. It was used to reconstitute different phototrophic negative mutant strains [22]. The partially reconstituted strain GK2 showed striking differences in its absorption spectra according to whether the cells were grown anaerobically in the light or semiaerobically in the dark. This phenotype was stable when the plasmid pRCF 1002 was replaced by a plasmid of the same incompatibility group not containing *Rb. capsulatus* DNA [22].

Materials and Methods

Materials

Radiochemicals were from Amersham-Buchler, Frankfurt, F.R.G. Enzymes were purchased from Boehringer, Mannheim, F.R.G., and used according to suppliers instructions.

Wild type strain 37b4 of *Rhodobacter capsulatus* (formerly called *Rhodopseudomonoas capsulata*) was deposited in Deutsche Sammlung für Mikroorganismen, Göttingen, DSM 938.

Culture conditions and in vivo labelling

Rhodobacter capsulatus was cultivated in a malate-salt medium, supplemented with 0.05% yeast extract (Difco) [29]. The induction of the photosynthetic apparatus by shifting cells from aerobic to semiaerobic conditions has been described previously [30]. For shifting cultures to phototrophic conditions, the cells from aerobic cultures were harvested, resuspended in fresh medium to an absorbance of 1.5 (660 nm, 1 cm) and incubated in 5-ml screw-cap flasks at 32° C in the light. In vivo labelling of cells was carried out at low oxygen tension (660 Pa) as described [31]. 5 ml of the cultures were incubated with 100 μCi [^{35}S]methionine (13.3 mCi/ml). After 1 min of pulse, unlabelled L-methionine was added to a concentration of 5 mM. Samples were mixed with an equal volume of an ice-cold solution containing 25 mM Tris-HCl (pH 7.6)/5 mM EDTA/15 mM NaN_3/1 mM phenylmethylsulfonylfluoride/100 μg/ml chloramphenicol (stop buffer).

Preparation, electrophoretic analysis and immunological detection of membrane proteins

Cells of *Rb. capsulatus* were harvested, washed twice in stop buffer and were sonicated five times for 15 s with 30 s breaks in an ice salt bath. The samples were centrifuged (20 min, $30000 \times g$), and the membrane fraction was separated from the supernatant by centrifugation at $200000 \times g$ for 1.5 h.

For analysis of membrane proteins SDS-polyacrylamide gradient gels (11.5–16.5%) were used [32]. The native pigment-protein complexes were separated by lithium dodecyl sulfate (LDS) -polyacrylamide gel electrophoresis at 0 °C [33]. Gels were fixed for 30 min in 10% acetic acid/50% methanol. Proteins were stained with Coomassie brilliant blue R250. For fluorography the gels were soaked in Amplify (Amersham-Buchler, Frankfurt) for 15 min, dried and exposed to Kodak X-Omat films at −70 °C.

Immunoprecipitation of pigment-binding proteins using specific antibodies and fixed cells of *Staphylococcus aureus* (Sigma, München) has been described [34]. Trnasfer of proteins from SDS-polyacrylamide gels to nitrocellulose was carried out according to Towbin et al. [35]. For the detection of the proteins with specific antibodies the enzyme-linked immunosorbent assay [36] was used. The quantitative immunoassay of pigment-binding proteins was done as described [5].

Quantification of mRNA levels

Total RNA of *Rb. capsulatus* was isolated [37] at different times after reduction of oxygen tension. Immobilization of the RNA to nitrocellulose,labelling of plasmid DNA and hybridization have been described previously [5]. Quantification of radioactivity was performed by scintillation counting of the nitrocellulose filters.

Pigment analysis

Analysis of pigments was performed using acetone/methanol (7 : 2, v/v) extracts from membranes, which were twice evaporated, redissolved and applied to silica gel thin-layer plates (Merck DC Fertigplatten Kieselgel 60) under a nitrogen stream.

The following solvent system was used for chromatography: petroleum ether (40–60)/benzene/acetone (110 : 95 : 5; v/v).

The pigments were identified by R_f values and absorption spectra in diethyl ether [10,38,39].

Since the 750 nm absorption of bacteriopheophorbid superimposed the 770 nm absorption of BChl, contents of these pigments were estimated from neutral and acidified extracts using millimolar absorption coefficients of 28.3 for bacteriopheophorbid and 2.7 for BChl at 525 nm [39].

Absorption spectra at 77 K were recorded with an Perkin-Elmer Spectrophotometer 330. Samples (0.5 mm film) in 50% glycerol (v/v) were frozen in liquid nitrogen and adapted to 77 K in a low-temperature spectroscopic device from Oxford Instruments. Fluorescence emission spectra were detected with a photomultiplier tube type S1 on a home-made spectrophotometer at 10 K. Samples were excited at 370 nm (GK2) or 380 nm (37b4).

Results

Low-temperature absorption spectra of membranes isolated from phototrophically or chemotrophically grown cultures of *Rhodobacter capsulatus* strain GK2 differed considerably (Fig. 1). The chemotrophically grown cells showed the typical absorptions of RC and B870 (880 nm at 77 K) complexes. The pigment content increased when the oxygen partial pressure was reduced in aerobically grown cultures, but the positions of the peaks did not change (not shown). Membranes of phototrophically grown cells showed absorption peaks at 797, 855 and 878 nm, indicating the presence of B800–850. The low 800 nm absorption points to a low B800–850/B870 ratio.

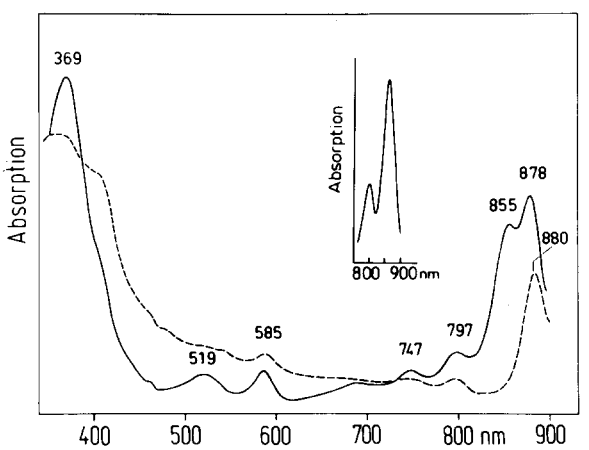

Fig. 1. Low-temperature (77 K) absorption spectra of membrane fractions isolated from *R. capsulatus* strain GK2 grown anaerobically in the light (————) or under low oxygen tension in the dark (- - - - - -). Chemotrophic membranes show the typical absorption of the RC (793 nm) and of the B870 (880 nm) complex. Phototrophic membranes have in addition an absorption at 855 nm. At 77 K NIR bands are shifted to the red. Inset: absorption spectrum of a membrane with a higher 800/850 nm ratio.

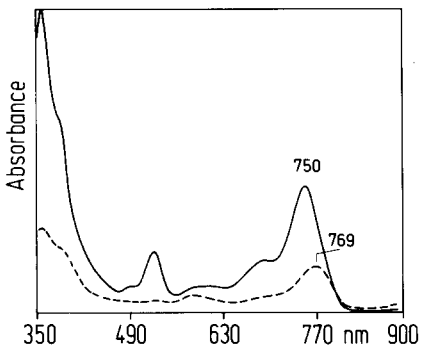

Fig. 2. Absorption spectra of methanol/acetone extracts from membranes isolated from GK2 cells grown anaerobically in the light (————) or under low oxygen tension in the dark (- - - - - -). The absorption maxima at 520 nm and 750 nm are typical for bacteriopheophorbid, the maximum at 770 nm for BChl.

In other membrane preparations (see inset to Fig. 1) we observed a higher 800/850 ratio. As observed with wild type, the 800 nm absorption band seems to be more labile. The 519 nm absorption band belongs to a BChl precursor.

Absorption spectra of methanol/acetone extracts (Fig. 2) showed the typical absorption maximum of BChl at 770 nm in extracts from chemotrophic membranes, but maxima at 520 and 750 nm in extracts from phototrophic membranes. In both cases absorption bands of carotenoids were missing. Two violet pigments were separated by thin-layer chromatography from extracts of phototrophic cells and were extracted with diethyl ether. They showed identical absorption spectra (maxima at 358, 385, 525, 625, 680, 749 nm; not shown) but different mobility on silica gel in hydrophobic solvents. The R_F values were 0.05 for bacteriopheophorbid and 0.2 for bacteriopheophytin. Based on millimolar absorption coefficietns of 67.5 at 749 nm [39] the ratio of bacteriopheophorbid to bacteriopheophytin was estimated to be 5 : 1 in phototrophic and 0.5 : 1 in chemotrophic membranes. The same ratio of BChl to bacteriopheophytin (4 : 1) was detected in both membrane types, whereas the ratio of BChl to bacteriopheophorbid was phototrophically 4 : 1 and chemotrophically 8 : 1. All pigments were found to be membrane associated after ultrasonic treatment and centrifugation.

Membranes of phototrophically grown mutant

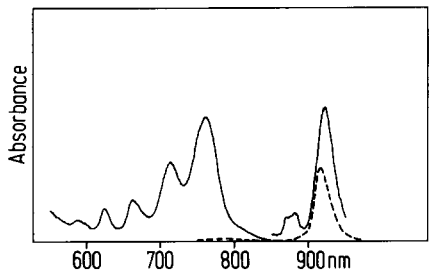

Fig. 3. Fluorescence emission spectra of membranes from wild type (- - - - - -) and from mutant strain GK2 (————) grown phototrophically, recorded at 10 K. Excitation at 380 nm (wild type) or 370 nm (GK2). Samples contained 50% (v/v) glycerol.

cells showed fluorescence emission at 625, 672, 720, 770, 880 and 920 nm, while membranes of wild type cells showed the emission band at 915 nm of the B870 complex (Fig. 3). The presence of the 880 nm emission band in the mutant strain suggested that the antenna complexes B870 and B800–850 are not completely energetically coupled [41]. Further emission peaks at 625, 672 and 720 nm indicate other tetrapyrroles besides BChl, bacteriopheophytin and bacteriopheophorbid (770 nm), possibly precursor or degradation products.

The polypeptides α, β and γ of the B800–850 complex were detected in SDS-polyacrylamide gels

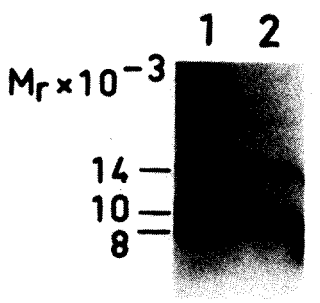

Fig. 4. The polypeptides α (10 K), β (8 K) and γ (14 K) of the B800–850 complex from wild type strain 37b4 and mutant strain GK2 were labelled by incuabtion of phototrophic cultures with [^{35}S]methionine. Membranes isolated from cells were solubilized under non-denaturating conditions and the complexes were exposed to antibodies against the B800–850 complex. The antigen-antibody complexes were precipitated with cells of *Staphylococcus aureus* and solubilized under denaturating conditions with SDS. Proteins separated by polyacrylamide gel electrophoresis were visualized by autoradiography. Lane 1, 37b4; lane 2; GK2.

of the GK2 membrane fractions and were identified by immunoprecipitation (Fig. 4).

All B800–850 polypeptides were stably inserted into the membranes as shown by pulse-chase experiments. Cells labelled under phototrophic growth conditions synthesized more B800–850-specific proteins in relation to other membrane proteins than cells labelled during chemotrophic growth (not shown).

Native pigment-protein complexes from *Rhodobacter capsulatus* wild type strain 37b4 and from mutant strain GK2 either grown phototrophically or chemitrophically were separated on LDS gels. The typical pattern of pigmented bands were obtained from 37b4 membranes under both growth conditions (Fig. 5). The characteristic absorption

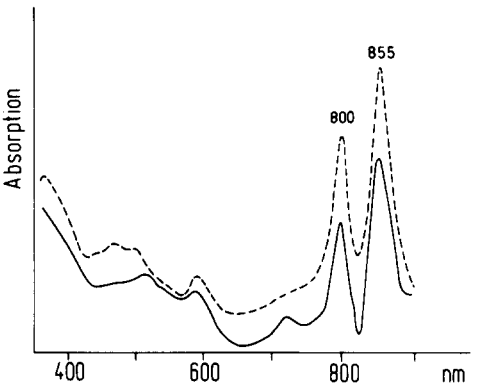

Fig. 6. Absorption spectra of band b (B800–850 complex) excised from native LDS gels shown in fig. 5. Membranes were isolated from strain 37b4 (- - - - - -) or from strain GK2 grown anaerobically in the light (————).

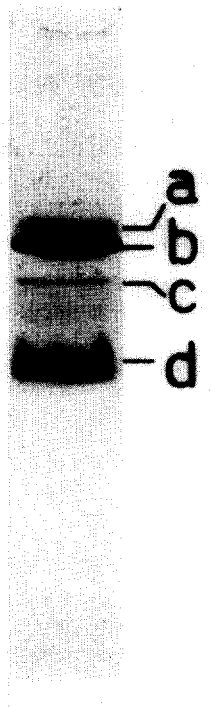

Fig. 5. Unstained pigment-protein complexes from the membrane fraction of *Rb. capsulatus* wild type separated by native LDS-polyacrylamide gel electrophoresis at 0 °C. Bands a and b are red coloured by carotenoids and contain the B800–850 complex. Band c is gree coloured and contains the RC complex. Pigment-binding polypeptides found in band d (red coloured) are associated with free BChl (absorption at 770 nm). The pattern of the GK2 membrane fraction was the same but much weaker because carotenoids were lacking and the concentration of complexes was lower.

spectrum of B800–850 was found in band b from 37b4 (Fig. 6). A very similar absorption but lacking carotenoids was detected in the respective fraction from phototrophic GK2 membranes (Fig. 6), whereas the chemotrophic GK2 membranes showed no B800–850 absorption in this fraction (not shown). The peak at 520 nm resulted from BChl precursors or degradation products (Figs. 1 and 3). After extraction of the pigment complexes from the LDS gel (band b) their composition was studied under denaturating conditions on SDS-polyacrylamide gels. Fractions from phototrophic GK2 membranes contained the three B800–850 polypeptides in much smaller amounts than fractions from 37b4. No B800–850-specific proteins occurred in the respective fraction from chemotrophic membranes, i.e., in chemotrophically grown cells, proteins are incorporated into the membrane in very low amounts but are not associated into B800–850 pigment-protein complex.

Expression of the genes for pigment-binding proteins

The amounts of B800–850 specific and RC/B870 specific mRNAs were determined in GK2 cells, shifted from aerobic to semiaerobic or phototrophic growth (Fig. 7). The increase of the mRNAs was under both conditions less than reported for wild type cells [5]. Especially the increase of B800–850-specific mRNA is low. The maxima of mRNA formation appeared later compared to those found in wild type cultures (60–90

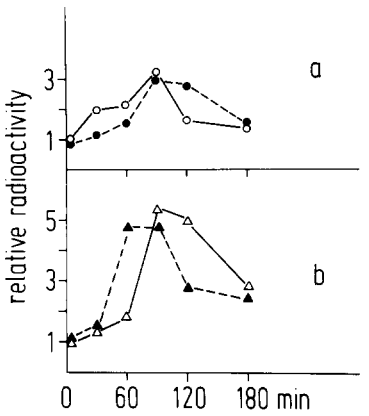

Fig. 7. Formation of photosynthetic mRNA in strain GK2. At time 0 aerobically precultivated cells were shifted to low oxygen tension in the dark (- - - - -) or anaerobic conditions in the light (————). Plasmids pVK1 (a, genes for B800–850) or pBBCl (b, genes for RC/B870) were hybridized against total RNA isolated at various time points after transition [5]. The plots represent an average of the relative increase of radioactivity of Northern filters from three single experiments. ○ ●, B800–850-specific RNA; △ ▲, RC/B870-specific RNA.

min instead 30–60 min). In cultures shifted to phototrophic growth the maximal amount of RC/B870- and B800–850-specific mRNAs were found at the same time after induction (Fig. 7). In cultures shifted to semiaerobic conditions the occurrence of B800–850-specific mRNA was delayed to RC/B870 mRNA, similar to that reported for the wild type. In both experiments the 770 nm absorption of methanol/acetone extracts from the cells increased 3-fold within 3 h (not shown) and the level of mRNA for RC/B870 was higher than for B800–850 polypeptides.

Discussion

In contrast to the wild type strain the absorption spectra of membrane fractions of phototrophically and chemotrophically at low p_{O_2} grown cells of the mutant strain GK2 differ considerably. Analysis of pigments and photosynthetic complexes revealed that: (1) phototrophic cultures of GK2 produced more bacteriopheophorbid than chemotrophic cultures, and (2) phototrophic cultures formed a B800–850 complex which is missing in chemotrophic cultures. Therefore, light has an additional effect on the expression of the pigment-protein components of the photosynthetic apparatus in GK2 cells compared to wild-type cells.

The kinetics of mRNA formation for pigment-binding polypeptides were similar whether the cultures were shifted to low oxygen tension in the dark or to anaerobic conditions in the light (Fig. 7). However, the increase of the RC/B870 and the B800–850 mRNAs in GK2 cells after induction was less than reported for the wild type strain [5].

It is clear that the mutant strain Ala^+Pho^-, which was the recipient for transconjugation with the plasmid pRCF 1002, was not reconstituted to a wild type as it was possible with strain Y5 [22]. It has to be studied whether the formation of the B800–850 complex in the strain GK2 is limited by the reduced BChl synthesis or by the lack of carotenoids, which may be of importance for the assembly and stability of B800–850 antenna complexes.

The results showed that coloured carotenoids are not obligatory for the assembly of the B800–850 complex.

Acknowledgements

We thank G. Ulrich, University Stuttgart Hohenheim, for supplying fluorescence emission spectra. The figures were prepared by J. Nährig. This work was supported by Deutsche Forschungsgemeinschaft and Fonds der Chemischen Industrie.

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