# Biosynthesis of pigment–protein complex polypeptides in bacteriochlorophyll-less mutant cells of *Rhodopseudomonas capsulata* YS

Roland Dierstein

*Institut für Biologie 2, Mikrobiologie, Albert-Ludwigs-Universität, Schänzlestraße 1, D-7800 Freiburg, FRG*

Received 22 June 1983

Bacteriochlorophyll-less mutant cells of *Rhodopseudomonas capsulata* YS were capable of synthesizing pigment–protein complex polypeptides under conditions permitting the formation of the photosynthetic apparatus in the wild type. Individual polypeptides were identified by immunoprecipitation. Pulse-chase experiments revealed that the polypeptides did not accumulate in the membranes but underwent rapid degradation. The data suggest that bacteriochlorophyll is needed to stabilize the polypeptides of pigment–protein complexes.

*Bacteriochlorophyll* *Pigment–protein complex* *Biosynthesis regulation*
Rhodopseudomonas capsulata

## 1. INTRODUCTION

The intracytoplasmic membranes of *Rhodopseudomonas capsulata* contain three different pigment–protein complexes, two of which functionally interact to collect light energy. These are the light-harvesting (LH) complexes I and II [1–3], and the reaction center (RC) complex [4] which, in concert with the photosynthetic electron transport and phosphorylation, convert light energy into biochemically utilizable form. The biosynthesis of these pigment–protein complexes is controlled mainly by the oxygen tension [5]. Recent studies have shown that lowering of the oxygen tension in the culture medium leads to an increase in *messenger* RNA which bound to DNA fragments most probably coding for only the proteins which form complexes with bacteriochlorophyll *a* (Bchl) and carotenoids [6]. The data clearly demonstrated that the initiation of the biosynthesis of pigment–protein complexes is due to a derepression of particular genes. Nevertheless, it still remained obscure, how protein synthesis is coordinated with the synthesis of the pigments to form functional pigment–protein complexes.

Since the RC and LH pigment–protein complexes have already been isolated [1,4], studies on the biosynthesis of polypeptide components could be undertaken by the use of immunochemical methods coupled with pulse-chase experiments [7,8]. In a previous report, it was shown that the biosynthesis of pigment–protein complex polypeptides immediately stopped when the synthesis of Bchl was inhibited at the level of $\delta$-aminolevulinate dehydratase [8]. The data indicated that the availability of Bchl was crucial to the assembly of pigment–protein complex polypeptides into membranes during the depression of genes involved in the synthesis of protein components of the photosynthetic apparatus. More insight into this particular problem was expected by studies on mutants defective in Bchl synthesis. Here, results obtained with a photosynthetically negative mutant, YS, of *Rhodopseudomonas capsulata* will be reported. In

*Abbreviation:* $M_r$, relative molecular mass

*Published by Elsevier Science Publishers B.V.*
00145793/83/$3.00 

an earlier work, this mutant, apparently depleted of all pigment–protein complexes, was used to reconstitute with high efficiency the wild type in a single step using a gene transfer agent [9]. As the gene transfer agent is known to have only a DNA molecule of $M_r$ $3.6 \times 10^6$ [10], it was not conceivable that the genes of altogether 8 pigment–protein complex polypeptide subunits, in the range of $M_r$ 28000 and $M_r$ 7000 [2–4], and those of the defective pigment synthesis could be genetically restored in a single transduction step. Mutant YS was analyzed in detail, as it obviously showed pleiotropic effects. These could reflect control mechanisms involved in the regulation of the synthesis of pigment–protein complexes by (i) coordinating the production of pigments and proteins and (ii) ensuring a proper assembly of these constituents into functional membranes.

## 2. MATERIALS AND METHODS

### 2.1. *Bacterial strains and incubation conditions*

*Rhodopseudomonas capsulata* strain 37b4 (wild type, German collection of microorganisms, Göttingen, Nr. 938) and the photosynthetically negative mutant strain YS produced by treatment of wild type cells with *N*-methyl-*N*-nitroso-*N'*-nitroguanidine [9] were cultured under conditions of reduced oxygen tension in a modified malate medium [11]. Yeast extract was replaced by a mixture of 19 amino acids (without methionine) at a concentration of 0.5 mM, each. Pulse-chase experiments were conducted in a 12-ml conical vessel at 30°C and 70 Pa $pO_2$, as described previously [7]. 4–8 ml of bacterial suspension, containing $8 \times 10^9$ cells per ml, were employed. After adjusting the temperature and oxygen tension [$^{35}$S]methionine (1000 Ci/mmole) was added to the suspension to give a concentration of 13 nM. After incubation for 3 min, unlabeled *L*-methionine was added in excess (to 0.5 mM). At the times indicated in the figures, 2-ml samples were withdrawn and poured onto equal volumes of ice-cold 25 mM Tris–Hcl/5 mM ethylenediaminetetraacetic acid (EDTA)/15 mM $NaN_3$ (pH 7.6) containing 10 $\mu$l of a mixture of protease inhibitors in dimethylsulfoxide per ml (phenylmethylsulfonyl fluoride, *L*-tosylamide-2-phenylethylchloromethyl ketone, and *N*-tosyl-*L*-lysylchloromethane at 0.1 M, each) (Stop buffer).

### 2.2 *Membrane preparation*

Cells were harvested by low-speed centrifugation and washed thrice in Stop buffer. They were resuspended to a density of $4 \times 10^9$ cells per ml in Stop buffer containing 10 $\mu$g/ml deoxyribonuclease, and lysed by 4 periods of 15 s sonications in a salt/ice bath. The cells were kept for 1 min in the salt/ice bath between the sonications. Membranes were sedimented from clarified lysates (after $30000 \times g$ for 20 min) by centrifugation at $200000 \times g$ for 2 h. For preparation of unlabeled membranes, the cell density was 10-fold.

### 2.3. *Immunoprecipitation*

Membrane suspension (20 $\mu$l) with 200000 cpm of $^{35}$S in Stop buffer were mixed with 20 $\mu$l of a solution of 4% sodium dodecylsulfate ($NaDodSO_4$), and incubated at 65°C for 30 min. The mixture was diluted with 1 ml of 80 mM Tris–acetate/1 mM EDTA/15 mM $NaN_3$/1% Triton X-100, pH 8.5 (Triton buffer) and centrifuged at $15000 \times g$ for 30 min. The supernatant was diluted to 10 ml with Triton buffer, mixed with 0.1–0.2 ml of the desired antisera, and incubated overnight at room temperature. Then, 0.2 ml of a 10% (w/v) suspension of fixed *Staphylococcus aureus* cells [12] was added, and the mixture was shaken at room temperature for 6 h. The cells were washed thrice in Triton buffer. The detergent was omitted in the last wash. The antigen was extracted for 5 min at 97°C with 50 $\mu$l of Laemmli's solubilization buffer [13] containing 6 M urea. Cells were removed by centrifugation, and the supernatant was analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis.

### 2.4. *Gel electrophoresis*

Polyacrylamide11.5–17% gradient gel slabs were used in the Laemmli system [13]. Samples were heated at 65°C for 30 min. Gels were stained with Coomassie Brillant Blue. Dried gel slabs were analyzed by fluorography, using the autoradiography enhancer Enlightning (New England Nuclear Inc., Boston MA).

### 2.5. *Extraction of pigments and proteins*

Cells (1 g of wet weight) were extracted with 10 ml of ice-cold acetone/methanol (7:2, v/v). Organic solvent soluble proteins were extracted from lyophylized, radioactively labeled mem-

branes ($10^6$ cpm) with 5 ml of a chloroform/methanol mixture (1:1, v/v). After clarification by centrifugation at 25000 × *g* for 30 min, the solvent was evaporated and the dried material solubilized in sample buffer [13].

Published procedures were used to prepare antisera [7], to measure protein [14], and to take spectra of absorbance [15] and fluorescence emission [16] at −196°C. Radioactivity was meassured by liquid scintillation counting in Ready-Solv HP (Beckman Instruments Inc., Irvine CA).

## 3. RESULTS AND DISCUSSION

### 3.1. *Spectroscopic analysis of pigments*

Low-temperature absorbance spectra of membranes of the mutant YS grown under conditions of reduced oxygen tensions did not reveal any near-infrared absorbance which could be related to either the RC or the LH pigment–protein complexes of wild type cells (fig.1a). This demonstrates that membranes isolated from the cells of the mutant YS in the steady state of growth, do not contain any protein bound Bchl. However, the shoulders on the spectra at 460 nm, 485 nm and 520 nm, respectively, indicate the presence of typical wild type carotenoids (fig.1a). Scanning of extracts of whole cells in acetone/methanol (7:2, v/v) also failed to detect Bchl absorbance which is expected at 770 nm (fig.1b). In the region of carotenoid absorption, a maximum at 480 nm was detected which could be related to spheroidenone, the major carotenoid component in chemotrophic cells of *Rhodopseudomonas capsulata* [2]. Fluorescence emission spectra of whole cells of the mutant YS, taken at −196°C revealed a shoulder at 710 nm (not shown). This emission was related to 2-desacetyl, 2-α-hydroxyethylbacteriochlorophyllid or the magnesium free derivative 2-desacetyl, 2-α-hydroxyethylbacteriopheophorbid in *Rhodopseudomonas sphaeroides* mutants [17]. 2-desacetyl, 2-α-hydroxyethylbacteriochlorophyllid is the presumptive precursor of bacteriochlorophyllid [18]. Obviously, under conditions which derepress the synthesis of various components of the photosynthetic machinery, the mutant YS was capable of synthesizing carotenoids and tetrapyrrole derivitives up to a level which, according to the present day knowledge, is expected two steps before Bchl.

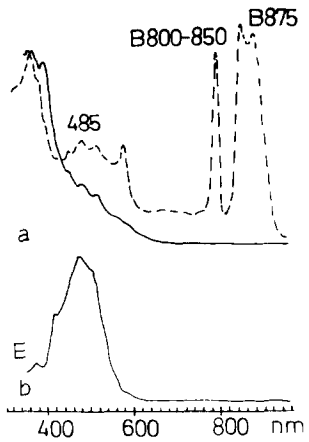


Fig.1. Mutant YS does not contain bacteriochlorophyll. Absorption spectra of membranes taken at −196°C (a) and of cell extracts at room temperature (b). – – –, *Rhodopseudomonas capsulata* wild type strain 37b4; ——, phototrophically negative mutant YS. E, extract of whole cells with acetone/methanol (7:2, v/v). B875, light-harvesting pigment–protein complex I; B800–850, light-harvesting pigment–protein complex II.

### 3.2. *Membrane protein patterns*

In an earlier work on genetic transfer, we have shown that the protein pattern of mutant YS differs drastically from that of the wild type *Rhodopseudomonas capsulata* in the region of low molecular mass polypeptides [9]. Fig.2A shows this pattern in a continuous polyacrylamide gradient which had higher resolution below the apparent $M_r$ of 14000 than that reported earlier [9]. Besides the RC pigment-protein complex polypeptides ($M_r$ 28000, 24000, and 20500) [4], the polypeptides of the LH pigment–potein complex I ($M_r$ 12000 and 7000) [3] and II ($M_r$ 14000, 10000, and 8000) [2] could be separated in preparations from the wild type (lanes 1 of fig.2A and 2B). These polypeptides were either missing from, or present in low quantities in the membranes of steady-state grown cells of the mutant YS (fig.2A, lane 2). In vivo labeling with [$^{35}$S]methionine was carried out to make the detection of membrane proteins easier. To minimize degradation during the processing of the samples, the freshly harvested cells were immediately treated with inhibitors of respiration and protease activities. Pulsing of derepressed cells of the mutant YS with radioactive methionine resulted, surprisingly, in a pattern of newly synthesized membrane polypeptides which was qualitatively

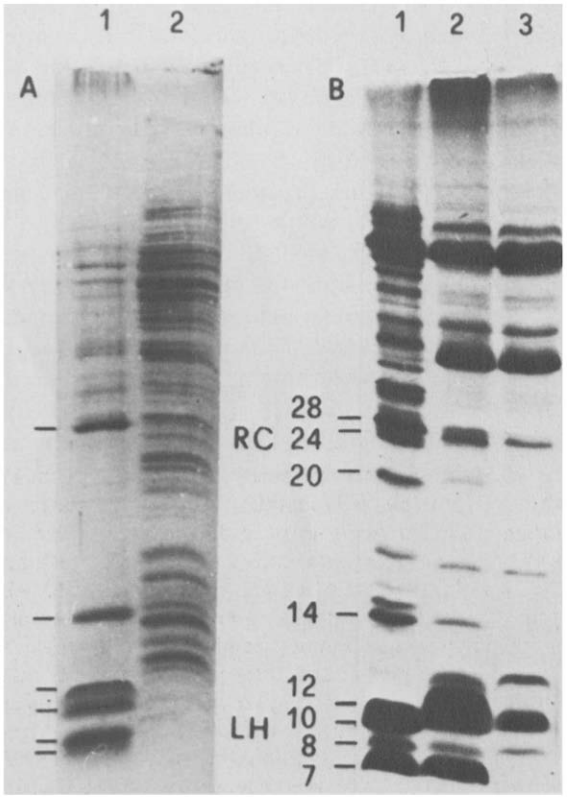


Fig.2. (A), Prominent polypeptides are missing from the membrane protein pattern of mutant YS. Na DodSO$_4$/polyacrylamide gel electrophoresis of membrane samples from cells grown under conditions of reduced oxygen tension. Gels were stained with Coomassie Brillant Blue. 50 $\mu$g of protein were applied per slot. Lane 1, *Rhodopseudomonas capsulata* wild type strain 37b4; lane 2, mutant YS. (B), Changes in the membrane pattern of mutant YS during a pulse–chase experiment. Membrane proteins of wild type strain 37b4 (lane 1) and mutant YS (lane 2) were pulse-labeled with [$^{35}$S]methionine as described in Materials and Methods. Then, the label was chased with an excess of unlabeled L-methionine for 10 min (lane 3). Fluorogram after Na DodSO$_4$/polyacrylamide gel electrophoresis. 65 000 cpm were applied per slots 1 and 2, and 50 000 cpm per slot 3. The numbers indicate the apparent $M_r \times 10^{-3}$.

very similar to that of the wild type, especially in the low molecular mass region (fig.2B, lanes 1 and 2). However, this pattern was drastically changed during a chase period of 10 min, when prominent bands running to the positions of the RC and LH pigment–protein complex polypeptides disappeared (fig.2B, lane 3).

### 3.3. *Identification of pigment–protein complex polypeptides*

Antibodies raised against RC and LH pigment–protein complex II were used to identify single polypeptides in the mutant YS. The anti-RC immunoglobulin detected the large subunit of the RC pigment–protein complex in the membranes of YS (fig.3, lane 3). This antiserum was already described to precipitate specifically the large RC polypeptide ($M_r$ 28 000) from wild type membranes [7]. An additional minor band with an apparent $M_r$ between 12 000 and 10 000, which is also found in precipitates from wild type membranes, remained

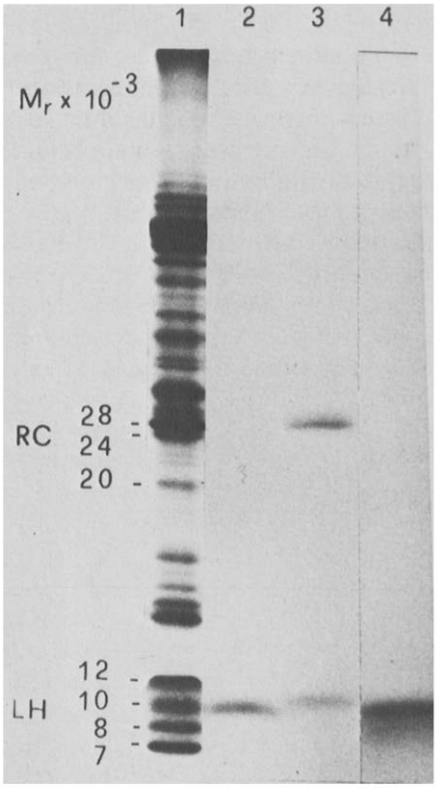


Fig.3. Identification of the large polypeptide subunits of the reaction center and light-harvesting pigment–protein complex II in the membrane fraction of mutant YS. Immunoprecipitates with anti-light-harvesting complex II antiserum (lane 2), and anti-reaction center antiserum (lane 3), as well as the chloroform/methanol (1 : 1, v/v) extract (lane 4) of [$^{35}$S]methionine labeled YS membranes were analyzed on Na DodSO$_4$/polyacrylamide gel electrophoresis followed by fluorography. As a comparison, the wild type membrane was co-electrophoressed (lane 1).

unidentified. Most likely, this band represents traces of the large polypeptide of LH pigment–protein complex I ($M_r$ 12000) which was found as a contaminant during the isolation of the RC complex [4]. The precipitate obtained from YS membrane extracts by the use of anti-LH pigment–protein complex II serum, showed a band with an apparent $M_r$ of 10000 (fig.3, lane 2). This result was indistinguishable from that obtained with precipitates from extracts of wild type membranes (not shown). The large subunit of LH pigment–protein complex II is the most hydrophobic polypeptide of the LH pigment–protein complexes of this organism, with only 27% polarity [19]. It was described to be selectively soluble in chloroform/methanol (1:1, v/v) [20]. Analysis of the organic solvent extract of mutant YS membranes again revealed the band with an $M_r$ of 10000 (fig.3B, lane 4). Thus, the 10000-$M_r$ polypeptide of the mutant was not only immunoreactive but also showed the solubility characteristics of the large LH pigment–protein complex II polypeptide. The data clearly show that mutant YS is able to synthesize polypeptides of the RC and those of the LH pigment–protein complex II. Considering that the radioactive membrane proteins of mutant YS, migrated exactly to the positions of the wild type pigment–protein complex polypeptides (fig.2B, lane 1 and 2), it is concluded that the mutant was able to synthesize other pigment–protein complex polypeptides as well.

The stability of the large polypeptides of the RC ($M_r$ 28000) and LH pigment–protein complex II ($M_r$ 10000) was followed in a pulse–chase experiment. Fig.4 shows the decrease in the contribution of immunoprecipitable radioactivity to total membrane radioactivity during the chase period. From these data half-lives for the large RC and the large LH pigment–protein complex polypeptide subunits were estimated, which roughly correspond to 12 min and 8 min, respectively. To distinguish between the possibilities of either excretion or turnover of the polypeptides, the culture medium was tested for the appearance of radioactive proteins during the chase period. Immunoprecipitation failed to detect the 28000 and 10000 polypeptides. Obviously, these polypeptides underwent a rapid turnover in the mutant cells which observed for the first time for pigment–protein complexes of *Rhodopseudomonas capsulata*. As a consequence,

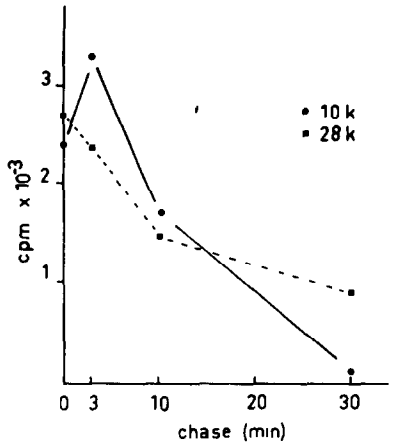


Fig.4. Disappearance of newly synthesized pigment–protein complex polypeptides from membranes of mutant YS. To aliquots of radioactively labeled YS membranes (200000 cpm, each), taken at 0, 3, 10, and 30 min after the start of chase, 10 $\mu$g of unlabeled wild type membrane proteins were added. Complex polypeptides were immunoprecipitated using anti-light-harvesting complex II and anti-reaction center antisera, and the radioactivity of the precipitates was determined by liquid scintillation counting. 10 kDa, large polypeptide of the light-harvesting pigment-protein complex II, 28 kDa, large polypeptide of the reaction center complex.

the polypeptides did not accumulate in the mutant membrane (fig.2A, lane 2).

The results obtained with the wild type strain and the mutant, respectively, strongly suggest that the presence of Bchl was crucial to the stabilisation of pigment–protein complex polypeptides, thus controlling the correct assembly of polypeptides into membranes during the formation of pigment–protein complexes. The observation that pigment-binding proteins are turning over does not seem to be unique to *Rhodopseudomonas,* as it was recently reported for barley that chlorophyll *b* may be needed to stabilize thylakoid membrane polypeptides which bind this photosynthetic pigment [21].

## ACKNOWLEDGEMENT

The work was supported by a grant (Di 298/1-1) of the Deutsche Forschungsgemeinschaft.

## REFERENCES


[1] Feick, R. and Drews, G. (1978) Biochim. Biophys. Acta 501, 499–513.

[2] Shiozawa, J.A., Welte, W., Hodapp, N. and Drews, G. (1982) Arch. Biochem. Biophys. 213, 473–485.

[3] Peters, J. and Drews, G. (1983) FEMS Microbiol. Lett. 17, 235–237.

[4] Nieth, K.F., Drews, G. and Feick, R. (1975) Arch. Microbiol. 105, 43–45.

[5] Schumacher, A. and Drews, G. (1978) Biochim. Biophys. Acta 501, 183–194.

[6] Clark, G. and Marrs, B.L. (1982) in: Abstr. IV Int. Symp. on Photosynth. Prokaryotes, Bombannes, France, pp. D15.

[7] Dierstein, R., Schumacher, A. and Drews, G. (1981) Arch. Microbiol. 128, 376–383.

[8] Dierstein, R. and Drews, G. (1982) in: Cell Function and Differentiation, Part B (Akoyunoglou, G. et al. eds) pp. 247–257, Alan R. Liss, New York.

[9] Drews, G., Dierstein, R. and Schumacher, A. (1976) FEBS Lett. 68, 132–136.

[10] Marrs, B.L. (1974) Proc. Natl. Acad. Sci. USA 71, 971–973.

[11] Drews, G. (1965) Zbl. Bakt. I, Suppl. 1, 170–178.

[12] Kessler, S.W. (1975) J. Immunol. 115, 1617–1624.

[13] Laemmli, U.K. (1970) Nature 227, 680–685.

[14] Bradford, U.M. (1976) Anal. Biochem. 72, 248–254.

[15] Firsow, N.N. and Drews, G. (1977) Arch. Microbiol. 115, 299–306.

[16] Garcia, A.F., Drews, G. and Reidl, H.H. (1981) J. Bacteriol. 145, 1121–1128.

[17] Kaiser, G.H. (1981) Thesis, Stuttgart.

[18] Richards, W.R. and Lascelles, J. (1969) Biochemistry 8, 3473–3482.

[19] Tadros, M.H., Suter, F., Drews, G. and Zuber, H. (1983) Eur. J. Biochem. 129, 533–536.

[20] Tadros, M.H., Zuber, H. and Drews, G. (1982) Eur. J. Biochem. 127, 315–318.

[21] Bellemare, G., Bartlett, S.G. and Chua, N.H. (1982) J. Biol. Chem. 257, 7762–7767.