BBA 75258

THE MORPHOGENESIS OF THE BACTERIAL PHOTOSYNTHETIC APPARATUS

III. THE FEATURES OF A PHEOPHYTIN–PROTEIN–CARBOHYDRATE COMPLEX EXCRETED BY THE MUTANT M 46 OF *RHODOSPIRILLUM RUBRUM*

J. SCHICK* AND G. DREWS

Institut für Biologie II, Lehrstuhl für Mikrobiologie, Universität Freiburg i.Br., Freiburg i.Br. (Germany)

(Received November 27th, 1968)

SUMMARY

The mutant M 46 was selected from the wild strain F of *Rhodospirillum rubrum* after treatment with the mutagen 1-methyl-3-nitro-1-nitrosoguanidine. M 46 is unable to grow photosynthetically. It synthesizes a nonfunctional bacteriochlorophyll in small quantities under semiaerobic conditions. The characteristic bacteriochlorophyll *in vivo* absorption band at 880 nm is shifted to 872 nm, and the usual absorption band at 807 nm is completely absent. The mutant does not produce unsaturated carotenoids.

Under semiaerobic conditions in the dark a bacteriopheophytin–protein–carbohydrate-containing complex is excreted. This process is correlated with the bacteriochlorophyll synthesis but starts later. The complex was precipitated by ammonium sulfate, purified by gel filtration and density gradient centrifugation. In electron micrographs of the negatively stained complex long filamentous aggregates of the macromolecules are to be seen. The filaments have a diameter of 40 $\pm$ 5 Å. The sedimentation constant is 22.4 S; the diffusion constant is 1.24 F. The molecular weight was calculated to be $1.5 \cdot 10^6$. The complex consists of 21.5% bacteriopheophytin, 61.0% protein (the amino acid composition is given), 10.0% glucose and 7.5% glucosamine. The complex was split into subunits by treatment with sodium dodecylsulfate together with urea. The molecular weight of the subunit is lower than 100 000. We assume that the pigment complex may be the well known P 800, which apparently cannot be incorporated into the photosynthetic unit.

INTRODUCTION

The thylakoids (chromatophores) of *Rhodospirillum rubrum* are vesicular structures which are connected to each other and to the cytoplasmic membrane[1–3].

* Present address: Institute of Molecular Biophysics, Tallahassee, Fla. 32306, U.S.A.

The thylakoids are capable of ATP production by the process of cyclic photophosphorylation[4]. The morphogenesis of the bacterial photochemical apparatus is regulated by the partial pressure of oxygen in aerobic dark cultures and by the light intensity in anaerobic cultures[1,5–8]. Thylakoids and cytoplasmic membrane form a continuous membrane system with different protein patterns which are related to the function of membrane subunits[6,9–11]. Our knowledge about biosynthesis of the photosynthetic apparatus is restricted to the pathway of bacteriochlorophyll synthesis[12]. Bacteriochlorophyll is associated with different parts of the membrane. In addition to the light harvesting bacteriochlorophyll, which is the major component of bacteriochlorophyll, thylakoids contain lesser pigment components associated with photochemical reactivity. They are called P 800 and P 890 bacteriochlorophyll. These active complexes have been identified by characteristic reversible light-induced absorbance changes of the *in vivo* spectrum[13,16] and on the basis of a relative stability after treatment with iridic chloride[14,15].

Mutant strains have been isolated which are unable to grow photosynthetically because the active center is missing[17,18] or the tetrapyrrole biosynthetic pathway is blocked at an earlier stage of bacteriochlorophyll synthesis[19,20]. In most cases the accumulated precursors were extracted with solvents, identified and integrated in the pathway of the bacteriochlorophyll synthesis. The isolation of the membrane-bound pigment particles seems to be very difficult. A partial fractionation was achieved by detergent treatment[21,22]. KIHARA[23] and LASCELLES[24] have extracted pigment particles from growing cultures of *R. rubrum* and *Rhodopseudomonas spheroides*. They have not investigated the nonpigment component of the isolated macromolecules. However, analysis of these pigment complexes would seem to be useful in improving our knowledge of the biosynthesis and the association of the photosynthetically active thylakoid subparticles. For our investigations we have selected the mutant strain M 46 of *R. rubrum*, which is unable to grow photosynthetically. This strain is able to synthesize some parts of the photosynthetic apparatus and excretes into the medium a large pigment complex which may be related to the photosynthetic apparatus. In this paper we report results of studies on the disturbed production of thylakoids and pigments by M 46 and the isolation and analysis of the pigment complex.

METHODS

Growth conditions and isolation of mutant strains

As the parent strain, we used strain F of *R. rubrum* which is capable of aerobic growth in the dark. The bacteria were normally cultivated aerobically in R8ÄH medium using a rotary shaker[25,26]. R8ÄH is a synthetic medium containing malate as the only carbon source and enriched with yeast extract (0.1%). The conditions of semiaerobic growth have been described previously[5]. Normally the bacteria were cultured in 300-ml erlenmeyer flasks, which were filled up to the neck and stirred magnetically. The oxygen partial pressure in this culture is near 1 mm Hg.

100 ml of a rotary shaker culture at the end of the logarithmic phase of growth was harvested, washed and resuspended in Tris buffer, 0.05 M (pH 7.5), to a density of about 0.1 (660 nm, $d = 0.5$ cm). 10 μg/ml of the mutagen 1 methyl-3-nitro-1-nitrosoguanidine was added, and the mixture was incubated for 30 min on a rotary

shaker at 30° in the dark. Appropriate dilutions were spread over plates of R8ÄH agar medium. The plates were incubated aerobically in the dark. Faintly or greenish coloured pigment mutants were isolated. Potential mutants were replated to establish their purity and then checked for inability to grow anaerobically in the light.

Analytical methods

Protein. The protein content of fractions was determined according to the method of LOWRY *et al.*[27] with bovine albumin V as a standard.

Bacteriochlorophyll. Bacteriochlorophyll was determined in the methanol extract using the specific extinction coefficient 46.1 (ref. 28). Bacteriopheophytin was extracted by peroxide-free ether, and the concentration was calculated from the absorbance of the solution at 750 nm using the extinction coefficient 70.9 l/cm·g (refs. 29, 30, 55). The specific extinction coefficient of bacteriopheophytin bound to the pigment complex in an aqueous solution was found to be 35.1 l/cm·g at 793 nm. This value was used to calculate the concentration of the pigment complex.

Bacteriochlorophyll and its derivatives were chemically characterized by the hydrochloric acid number[31] and the MOLISCH[32] phase test.

Glucose. Glucose was determined by the anthrone method[33].

Diffusion and sedimentation measurements were performed by the use of the Spinco analytical ultracentrifuge, model E, fitted with double sector cells with capillars and 12-mm cells for diffusion and for sedimentation, respectively. The solvent was 0.015 M phosphate buffer at pH 7.8. The sedimentation was measured at 59780 rev./min and a temperature of 25°. The rotor speed during diffusion measurement was 8225 rev./min.

Chromatography. The pigments were chromatographically separated by a modified method of KIM[34] and EGGER[35]. The methanol extract of bacteria, to which ascorbate (0.025 M) had been added, was applied to plates covered with Kieselgur (Merck, Darmstadt) and impregnated with 7% triolein in light petroleum (b.p., 30–60°). The plates were developed with the solvent system methanol–acetone–water (20:4:3, by vol.). The chromatograms were run in a dark room at 6° for 40 min. The bands were scraped off, thoroughly vacuum dried, eluted with ether and characterized spectroscopically.

Amino acids and glucosamine were chromatographically separated and quantitatively measured by the method of MOORE, SPACKMAN AND STEIN[36]. Polyacrylamide-gel disc electrophoresis[37,38] was carried out in a Shannon apparatus. The concentration of acrylamide gel amounts to 2.6 to 5.6%.

Enzyme assays. The measurement of the rate of photophosphorylation has been described previously[26,39].

δ-Aminolevulinic acid synthetase and δ-aminolevulinic acid dehydratase were measured in cell-free extracts[40–42].

The activity of the succinate dehydrogenase was assayed by the method of BOLL[43].

Electron microscopy. A suspension of the pigment complex was dropped on carbon-coated grids. 30–60 sec later, the grids were touched with filter paper. The wet preparations were stained for 30–60 sec with solutions of uranyl formate (1%) or phosphotungstic acid (2%) adjusted to pH 7.0.

The bacteria were fixed with OsO_4 (ref. 44) and embedded in Epon. Ultrathin

sections were prepared with a LKB Ultrotome I, stained with lead hydroxide[45], and examined in a Siemens Elmiskop I a, fitted with an apparatus for cooling the sample space.

Preparation of the pigment complex. The mutant M 46 of *R. rubrum* was incubated in the dark at a very low oxygen partial pressure (about 1 mm Hg) at 30°. The culture was harvested when the absorption at 793 nm in the culture medium reached a maximum. This was the case about 72 h after the start of the incubation. The cells

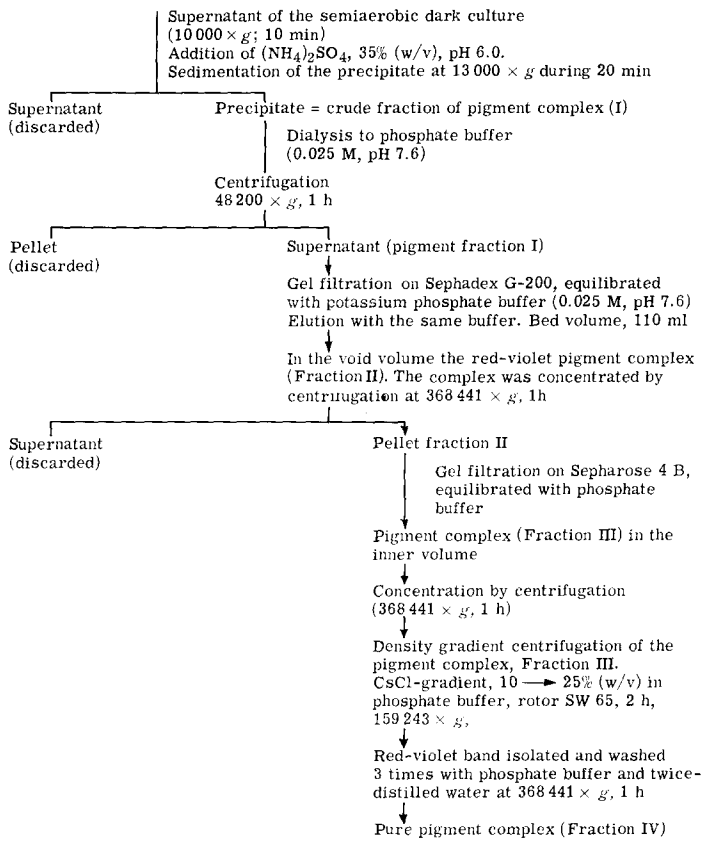


Fig. 1. Flow diagram of the preparation and purification of the pheophytin–protein–carbohydrate complex.

were separated by centrifugation (8000 × *g*, for 20 min), and the bacteriopheophytin complex was precipitated in the supernatant with ammonium sulfate. Further purification was performed by chromatography on Sephadex G-200 and Sepharose 4 B and density gradient centrifugation (Fig. 1). During the different steps of purification the pigment complex could easily be identified by the red-violet colour. The absorbance of the eluted fractions was measured at 793 and 280 nm. Chromatography on DEAE-cellulose is ineffective because the pigment complex is strongly adsorbed and not removable with buffer.

RESULTS

Growth and pigment production

The wild strain F and the mutant M 46 of *R. rubrum* grow in aerobic dark cultures at the same rate. The protein doubling time in the log phase of growth at 30° on the medium R8ÄH amounts to 4 h. Small amounts of coproporphyrine III, but never bacteriochlorophyll, are produced by both strains in aerated cultures. When cultures are made anaerobic and illuminated, strain F starts to synthesize bacteriochlorophyll and continues to grow after a lag phase of 3 to 4 h (ref. 1), while M 46 does neither. If the mutant is not able to synthesize a functional photosynthetic apparatus, we have to check the capacity of pigment and thylakoid production in semiaerobic dark cultures. The wild strain synthesizes all parts of the functional thylakoids[5,46] in slightly aerated dark cultures (pO_2 1–3 mm Hg). M 46 also produces bacteriochlorophyll, but the kinetics of production is quite different compared with the F-strain (Fig. 2). The synthesis of bacteriochlorophyll in M46 ceases during the logarithmic growth phase. The level does not exceed 2 μg bacteriochlorophyll per mg protein. In both strains the pigment production is repressed by high aeration and inhibited by chloramphenicol (0.7 μg/ml).

The pigments

The *in vivo* spectra of the isolated pigment particles of M 46 and F show some interesting features (Fig. 3). M 46 lacks unsaturated coloured carotenoids (solid curve in the region of 430 to 560 nm). The bacteriochlorophyll infrared absorption

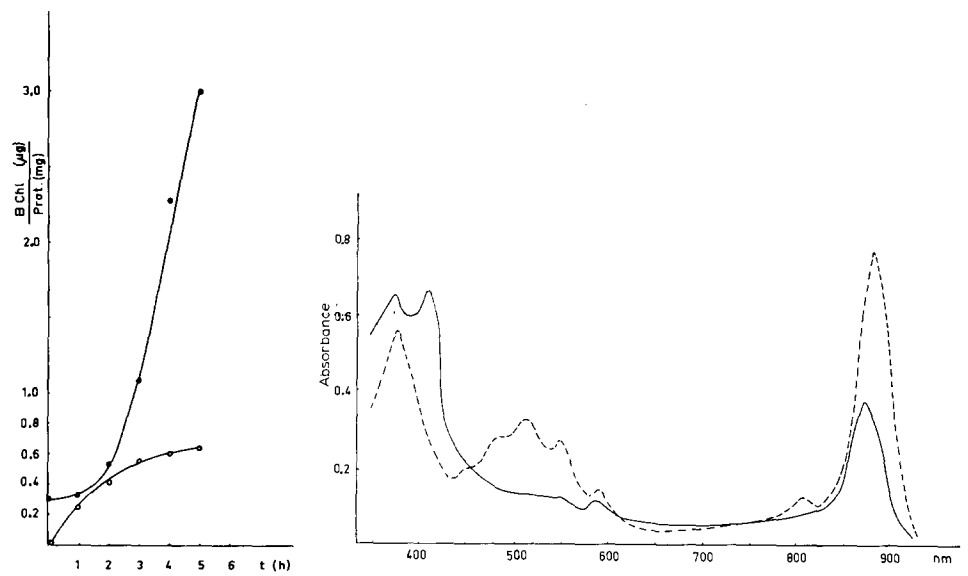


Fig. 2. Bacteriochlorophyll (Bchl) synthesis of *R. rubrum*, strain F (wild type) (●), and the mutant strain, M 46 (○), cultured semiaerobically in the dark in stirring flasks at 30°. Ordinate: the specific bacteriochlorophyll content of the bacteria. Abscissa: time of incubation.

Fig. 3. *In vivo* absorption spectra of the thylakoid fraction of strain F (– – – –) and strain M 46 (————).

maximum of the wild strain F at 880 nm (dashed curve) is shifted to 872 nm in the spectrum of the mutant (solid curve). The characteristic peak at 807 nm seems to be absent in the spectrum of M 46. The 880/376 ratio is distinctly lower in the mutant strain. A new peak at 412 nm has arisen which may be related to the presence of a tetrapyrrole compound. Taken together, these results imply that the mutant strain M 46 lacks coloured carotenoids and seems to be inhibited in the production of wild-type bacteriochlorophyll *a* complexes.

TABLE I

BACTERIOCHLOROPHYLL CONCENTRATION AND ACTIVITY OF δ-AMINOLEVULINIC ACID SYNTHETASE AND δ-AMINOLEVULINIC ACID DEHYDRATASE IN CELLS OF *R. rubrum* F AND M 46 (MUTANT STRAIN) AFTER 20 h GROWTH IN DARK CULTURES

Incubation	*Bacteriochlorophyll a* (*μg/mg protein*)		*δ-Aminolevulinic acid synthetase* (*μg aminolevulinic acid/mg protein per 30 min*)		*δ-Aminolevulinic acid dehydratase* (*μg porphobilinogen/mg protein per 30 min*)	
	F	*M 46*	*F*	*M 46*	*F*	*M 46*
Aerobic	—	—	5.32	5.53	3.10	2.96
Semiaerobic	14.41	1.04	6.64	6.58	3.00	2.92

In spite of the low production rate of bacteriochlorophyll in M 46 the specific activity of the first enzymes of the tetrapyrrole pathway in M 46 is nearly the same as in F (Table I). However, a feedback inhibition of these enzymes *in vivo* during the growth cannot be excluded by these experiments.

The differences in the *in vivo* spectra of M 46 and F may be due not only to changes in the active centre, but also to the bacteriochlorophyll moiety itself. Therefore, the methanolic extracts of both strains were investigated by thin-layer chromatography. The chromatograms of M 46 and of the wild strain F pigments were quite different. Besieps the absence of the three pink-coloured bands in the M 46 chromatogram, the R_F-value and the colour of the bacteriochlorophyll fractions were different. The absorption spectra of the bluish green band (strain F) and of the faster-moving

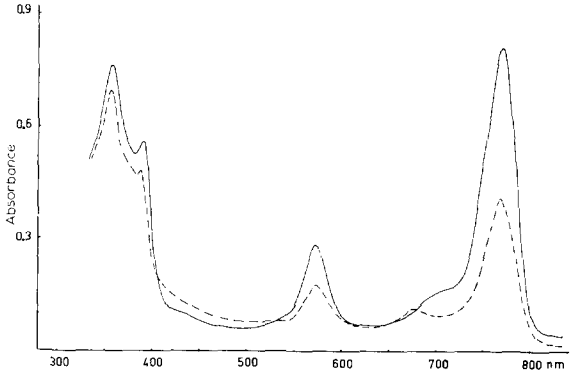


Fig. 4. Absorption spectra of etheral solutions of *R. rubrum* bacteriochlorophyll fractions, strain F and M 46 (– – – –).

yellowish green band (M 46) in ether are shown in Fig. 4. The spectrum of the wild-type fraction (solid curve) corresponds to bacteriochlorophyll *a*. The spectrum of M 46 bacteriochlorophyll (dashed curve) shows a peak at 678 nm in addition to the normal peak at 772 nm, the latter being smaller than the peak in the Soret region. This indicates that the bacteriochlorophyll of M 46 is different from that of the wild strain. It has been mentioned that M 46 is unable to grow photosynthetically. The spectroscopic data suggest that the bacteriochlorophyll is not able to operate. This assumption was confirmed by the negative result of measurements of the cyclic photophosphorylation: Strain F esterified 26.0 μg PO_4^{3-}/mg bacteriochlorophyll per 30 min, whereas strain M 46 exhibited no phosphorylating activity.

Cytology

Electron micrographs of thin section of F and M 46 differ in the development of the thylakoid reticulum[1] in semiaerobically grown dark cultures. The cells of F are filled up with thylakoids (Fig. 5), whereas the mutant cells contain only a few vesicles (Fig. 6). These micrographs show once more that bacteriochlorophyll and membrane synthesis in *R. rubrum* may be closely related. All the mutants investigated which are unable to grow photosynthetically and to synthesize bacteriochlorophyll lack thylakoids (G. DREWS, unpublished results). M 46 produces some nonfunctional bacteriochlorophyll and few thylakoids.

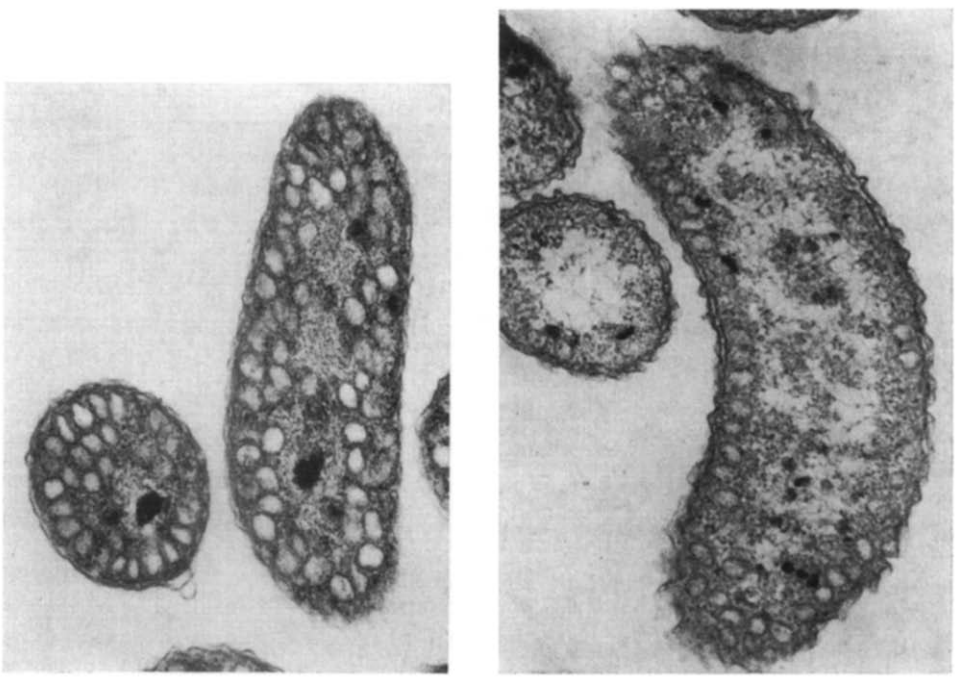


Fig. 5. Ultrastructure of *R. rubrum*, strain F, grown 24 h semiaerobically in the dark. The cytoplasma is packed with thylakoid vesicles. × 37500.

Fig. 6. Ultrastructure of *R. rubrum*, mutant strain M 46, growing 24 h semiaerobically in the dark. Only a few peripheral vesicular membrane structures are to be seen. × 45000.

Excretion of a pigment by M 46

The mutant strain M 46 produces a pigment besides bacteriochlorophyll when the cells are growing in semiaerobic conditions. The synthesis of this pigment runs parallel to the production of bacteriochlorophyll (Fig. 7), but the pigment is excreted in the medium. A more careful measurement of the pigment concentrations during the first 20 h shows that the pigment in the medium (solid curves) appears later than bacteriochlorophyll in the cells (Fig. 8). Since this pigment is not detectable within

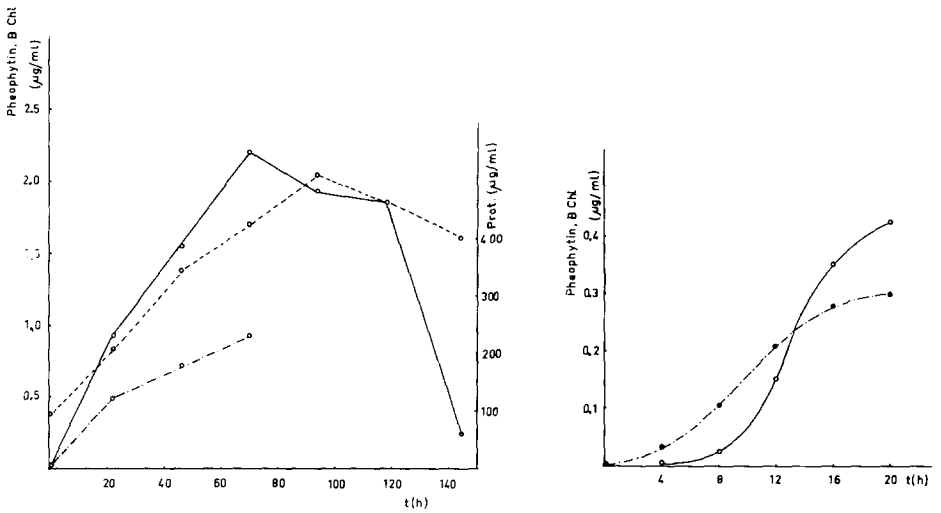


Fig. 7. Pigment, protein, and bacteriochlorophyl (BChl) synthesis of mutant strain M 46 in a semiaerobic culture at 30°; O———O, excreted bacteriopheophytin; O– – – –O, cell protein; O–·–·–O, intracellular bacteriochlorophyll. Abscissa: time of incubation.

Fig. 8. Kinetics of intracellular bacteriochlorophyll (BChl) synthesis (●–·–·–●) and bacteriopheophytin excretion (O———O) of mutant strain M 46. Semiaerobic dark conditions. Abscissa: time of incubation.

the cell, it can be concluded that the synthesis of the excreted pigment starts later than bacteriochlorophyll synthesis. The pigment is not released from the cells by autolysis because the pigment level is increased during the logarithmic phase of growth.

The characteristics of the excreted pigment complex

The pigment excreted by M 46 is sedimentable in the ultracentrifuge (4 h at 160000 × *g* or 1 h at 368000 × *g*) and precipitable with ammonium sulfate. On the basis of this observation we have enriched and purified the macromolecular pigment complex in a stepwise manner (Fig. 1). The ratio of relative absorbances at the wavelengths 793, 537 and 365 nm was a scale of purification (Table II). The chromatography on Sephadex G-200 separates the pigment complex from substances of lower molecular weight. The pigment complex is eluted as a sharply limited and red-violet-coloured fraction in the void volume of the column. On sepharose 4 B the pigment complex passes the column in the inner volume of the molecular sieve. The pigmented fraction migrates as a uniform band. The pigmented band from the CsCl gradient was isolated, washed three times and checked for purity.

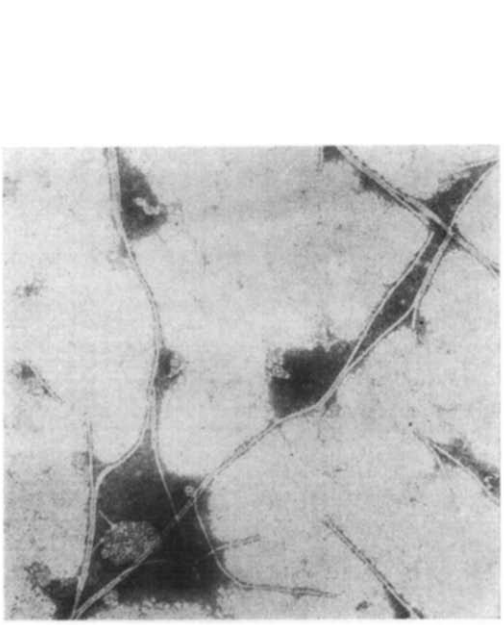
TABLE II

THE RELATIVE ABSORBANCE AT 3 CHARACTERISTIC WAVELENGTHS DURING THE PURIFICATION OF THE PIGMENT COMPLEX

Step of purification	*Absorbances of pigment complex at 537 and 365 nm related to 793 nm = 1*		
	793	*537*	*365*
Crude Fraction I, $(NH_4)_2SO_4$ ppt.	1	0.79	3.7
Fraction II (Sephadex G-200)	1	0.35	2.1
Fraction III (Sepharose 4B)	1	0.29	1.9
Fraction IV (CsCl gradient)	1	0.21	1.55

Criteria of purity of pigment complex

Electron micrographs of preparations of the pigment complex stained negatively with phosphotungstic acid or positively with uranyl acetate show uniform filamentous structures (Figs. 9 and 10). The ends of the filaments are frequently clustered. The diameter of the filaments is 40 Å ± 5 Å. The data of molecular weight determination and electrophoresis exclude the possibility that the filamentous structure seen in the electron microscope is the natural state. The filaments are likely molecules which have aggregated during the process of drying. The homogeneity of the isolated pigment complex was also checked by analytical ultracentrifugation. The purified pigment preparation represented a single sharp symmetrical peak. In the acrylamide gel electrophoresis the pigment complex moves as a uniform band (Fig. 11).



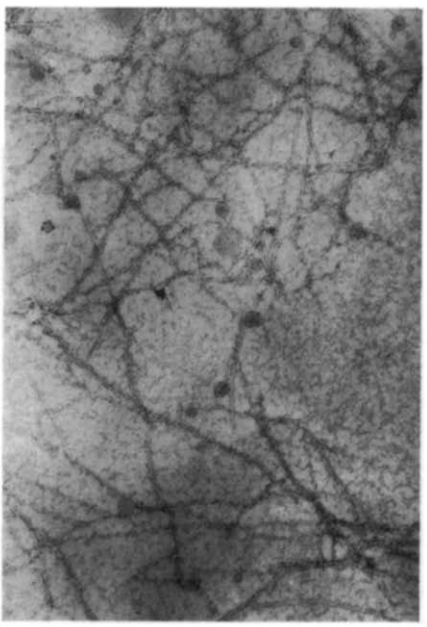


Fig. 9. Electron micrographs of the bacteriopheophytin complex, negatively stained with 2% phosphotungstate. × 100000.

Fig. 10. Electron micrograph of the pigment complex, stained with 1% uranyl formiate. × 100000.

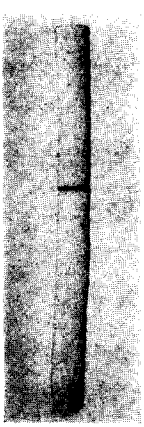
Calculation of molecular weight of the pigment complex

The pore diameter of acrylamide gel can be modified by variation of the acrylamide concentration. Therefore conclusions about the molecular size can be drawn from the ability of pigment complex molecules to pass through supporting gels of varying acrylamide concentration. The pigment complex enters a gel of 2.6% acrylamide and a pore diameter of about 50 Å (Fig. 11), but it scarcely penetrates a gel of 3.75% acrylamide. These results taken together with the fact that the pigment complex appears in the void volume of G-200 and in the inner volume of Sepharose 4 B indicate a molecular weight of about $1 \cdot 10^6$–$2 \cdot 10^6$.

From the data of sedimentation of the pigment complex in the analytical ultracentrifuge (Figs. 12 and 13) a sedimentation constant of 22.5 ± 1.3 S and a diffusion constant of 1.24 F extrapolated to infinite dilution was calculated. The small amount of available material excluded the experimental determination of the partial specific volume $\bar{v}$. Therefore an average value for protein was assumed. The molecular weight of the pigment complex was calculated from the Svedberg equation.

$$M_{SD} = \frac{s_0 R^0 T}{D_0(1 - \bar{v}\rho)} = 1.52 \cdot 10^6$$

$T = 298°K$; $\rho = 1.006$; $\bar{v} = 0.7$ ml/g; $s_0 = 22.5 \cdot 10^{-13}$; $D_0 = 1.24 \cdot 10^{-7}$; $R^0 = 8.313 \cdot 10^7$.



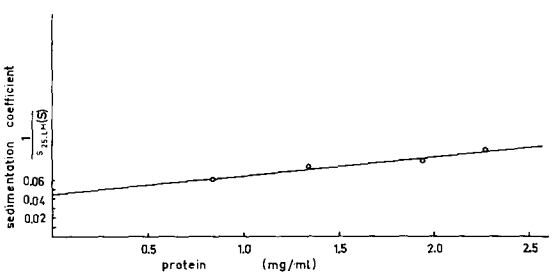


Fig. 11. Polyacrylamide disc gel electrophoresis of the pheophytin complex; acrylamide concn., 2.6% (pH 7.6).

Fig. 12. Extrapolation of the sedimentation constant of the pigment complex to infinite dilution (0.015 M phosphate buffer, pH 7.8). Rotor speed, 59780 rev./min; temp., 25°.

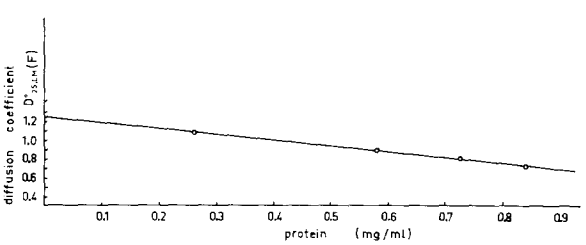


Fig. 13. Extrapolation of the diffusion constant of the pigment complex to infinite dilution at pH 7.8 (0.015 M phosphate buffer). Rotor speed, 8225 rev./min; temp., 25°.

The chemical composition of the pigment complex

The spectrum of an aqueous solution of the purified pigment complex shows two main peaks at 793/794 and 365 nm and one lower peak at 537 nm; small shoulders are to be seen at 300 nm and at 515 nm (Fig. 14). An ethereal solution is characterized by maxima at 750, 680, 620, 525, 384 and 357 nm (Fig. 14). This spectrum is identical with the published spectrum of bacteriopheophytin *a* (refs. 29, 30) and corresponds to the spectrum of bacteriopheophytin *a* which is prepared by HCl treatment of bacteriochlorophyll *a*. The reaction of the Molisch-phase test with the chromophoric group of the pigment complex is positive. This shows the presence of the isocyclic ring, V, of the tetrapyrrole. The hydrochloric acid number of the pigment is higher than 29.

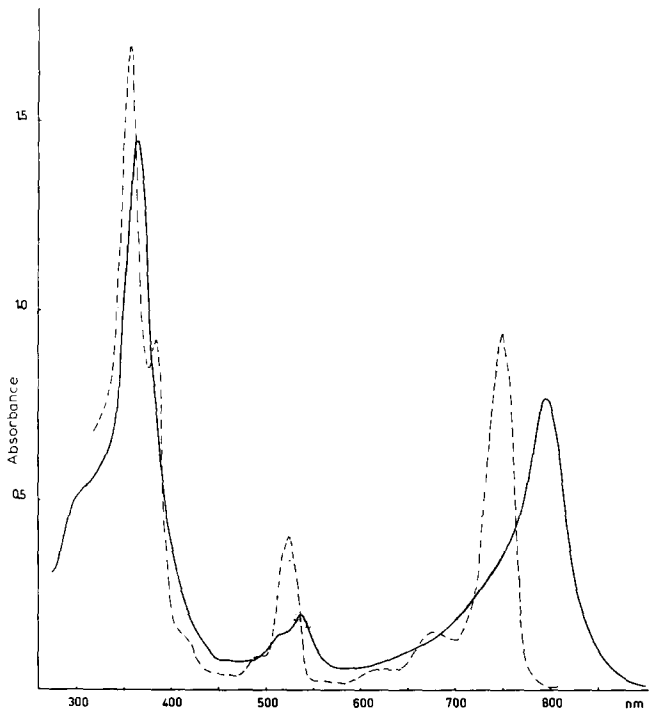


Fig. 14. Absorption spectrum of the purified bacteriopheophytin–protein–carbohydrate complex (———). – – – –, ethereal extract of this complex.

More than 2/3 of pheophytin is dissolved in the HCl phase if the concentration of HCl is 29% or higher. From these spectroscopical and chemical data it can be concluded that the chromophoric group of the pigment complex is bacteriopheophytin *a*.

The main constituent of the bacteriopheophytin complex is protein, which was routinely determined by the method of LOWRY *et al.*[27]. The data are is good agreement with the quantitative evaluation of the chromatographic analysis of amino acids. Morover, glucosamine could be identified by column chromatography. The second sugar we found is glucose, which was quantitatively determined by the anthrone method and identified by paper chromatography. Lipids or phospholipids are not detectable in the chromatograms of the lipid extracts by staining with rhodamin B or molybdate reagent. Succinate dehydrogenase, a typical component of thylakoids[46]

and of the cytoplasmic membrane[56], seems to be absent, too. The complex consists of 21.5% bacteriopheophytin, 61% protein and 17.5% carbohydrates. The molecular composition of the pigment complex was calculated from the quantitative data (Table III).

TABLE III

THE MOLECULAR COMPOSITION OF THE BACTERIOPHEOPHYTIN–PROTEIN–CARBOHYDRATE COMPLEX CALCULATED ON THE BASIS OF A MOLECULAR WEIGHT OF $1.5 \cdot 10^6$ AND THE QUANTITATIVE DATA OF AMINO ACID DETERMINATION

Protein	*Molecules per mole pigment complex*	*Weight (g)*
Lysine	988	144 500
Arginine	338	59 100
Aspartic acid	1300	173 000
Threonine	369	43 800
Serine	422	44 100
Glutamic acid	726	107 200
Proline	264	30 000
Glycine	847	63 600
Alanine	693	61 400
Valine	395	46 200
Methionine	50	7 530
Isoleucine	187	24 600
Leucine	341	44 600
Tyrosine	147	26 600
Phenylalanine	234	38 600
		914 830
Tetrapyrrole		
Bacteriopheophytin *a*	360	323 000
Carbohydrates		
Glucose	780	150 000
Glucosamine	640	113 000
		1 500 830

Degradation of the bacteriopheophytin complex

From the data of Table III it can be assumed that the pigment complex consists of subunits. Therefore we have tried to split the complex. Separation of subunits could not be achieved by 6 M urea or 5 M guanidine–HCl, but distinct dissociation of the pigment complex into subunits was observed after treatment with the detergents Triton X-100, Tween 80, and sodium dodecyl sulfate. The most effective and reproducible method was the application of 5 M urea together with sodium dodecyl sulfate (0.1%). The substance was no longer sedimentable at $331000 \times g$ after the treatment. The subunits show a uniform band in the acrylamide gel electrophoresis and a symmetrical narrow peak in the analytical ultracentrifuge. The sedimentation constant is $s_{20,w} = 1.85$. The molecular weight seems to be lower than 100000.

DISCUSSION

The mutant M 46 described in this paper is not able to synthesize unsaturated carotenoids. This kind of mutation is very often observed and does not impair the

morphogenesis and function of the photosynthetic apparatus aside from causing a higher sensitivity to light[12,57]. More important is the disturbance of the bacteriochlorophyll synthesis in M 46. This pathway seems to be impaired only in the last part of the magnesium branch because coproporphyrin, a bacteriochlorophyll-like substance, and pheophytin are synthesized. However, the production rate of these compounds is very low, and the kinetics of synthesis is changed in comparison with the wild strain, though a normal level of the first enzymes, δ-aminolevulinic acid synthetase and δ-aminolevulinic acid dehydratase, can be observed. The bacteriochlorophyll produced is not identical with the wild-type bacteriochlorophyll *a*. Moreover, the spectroscopically well-known active bacteriochlorophyll components P 800 and P 890 seem to be absent. The *in vivo* spectrum of M 46 corresponds in the infrared region to the spectrum of the mutant PM-8 of *Rps. spheroides*[17]. The mutant M 46 is unable to grow photosynthetically because all the normal bacteriochlorophyll components are absent.

As shown by many investigations the synthesis of bacteriochlorophyll and that of protein are likely to be coupled[47–49]. Enzymes of the bacteriochlorophyll pathway and structural proteins of the photosynthetic apparatus may be involved in this process of thylakoid morphogenesis[5,9,49,50]. All mutants investigated which are unable to produce bacteriochlorophyll have no thylakoids (G. DREWS, unpublished results). Therefore it seems reasonable to take into consideration the possibility that the synthesis of all macromolecules which constitute the photosynthetic apparatus proceeds only if the molecules can be combined to form the membrane. The production of the pheophytin complex by M 46 is closely related to the bacteriochlorophyll synthesis (Figs. 7 and 8). The special leakage of the cytoplasmic membrane for this big molecule of $1.5 \cdot 10^6$ molecular weight is remarkable. The spectrum of this excreted pigment complex is identical with that of B 794 described by KIHARA[23]. However, in contrast to the observations of KIHARA, the pheophytin complex is not sedimentable at 60000 × *g*. KIHARA assumes that B 794 is P 800, one of the active complexes in the photosynthetic apparatus of purple bacteria[13–16] and that the chromophore group of the complex exists as a bacteriopheophytin-containing complex when dissociated from the natural environment. The kinetic data of pigment production (Fig. 8) may suggest that the pheophytin complex of M 46 contains a false protein which is unable to associate with the membrane or that a coupling factor is missing which combines the pigment complex with the membrane. However, we know nothing about the genetic background and the biochemical steps of membrane synthesis in *R. rubrum*. Therefore, we are not able to substantiate the observations presented.

The morphogenesis of the thylakoids in higher plants is different from that of the bacterial system with regard to the process itself and its regulation[58,59]. It is noteworthy that the chloroplast development in higher plants is likewise not dependent upon the presence of carotenoids. However, grana formation takes place only in the presence of chlorophyll[51]. Chlorophyll is apparently not necessary for the process of lamellar formation, but the lamellar arrangement is coupled with the greening process.

Bacteria have no prolamellar body, and different stages of thylakoid arrangement have not been described. However, the cytoplasmic membrane and intracellular membrane structures can be fitted out with the system of photophosphorylation[5,9,54]. Thus a reorganisation of membrane subparticles seems to be possible[50]. Bacterio-

chlorophyll and the other components of the photosynthetic apparatus are necessary to build typical bacterial thylakoids. Mutants such as M 46 may be helpful in obtaining more information about the arrangements of membrane subunits to form a functional system.

ACKNOWLEDGEMENTS

This work was supported by the Deutsche Forschungsgemeinschaft. We thank Dr. C. Grisebach and H. de Klerk for criticism of the manuscript.

REFERENCES

1 G. DREWS AND P. GIESBRECHT, *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, 190 (1963) 508.
2 E. S. BOATMAN, *J. Cell Biol.*, 20 (1964) 297.
3 S. C. HOLT AND A. G. MARR, *J. Bacteriol.*, 89 (1965) 1413.
4 A. W. FRENKEL, *J. Am. Chem. Soc.*, 76 (1954) 5568.
5 M. BIEDERMANN, G. DREWS, R. MARX AND J. SCHRÖDER, *Arch. Mikrobiol.*, 56 (1967) 133.
6 J. OELZE, M. BIEDERMANN AND G. DREWS, *Biochim. Biophys. Acta*, 173 (1969) 436.
7 G. COHEN-BAZIRE AND W. R. SISTROM, in L. P. VERNON AND G. R. SEELEY, *The Chlorophylls*, Academic Press, New York, 1966, p. 313.
8 S. L. HOLT AND A. G. MARR, *J. Bacteriol.*, 89 (1965) 1421.
9 G. DREWS, M. BIEDERMANN AND G. SCHÖN, *Zentr. Bakteriol. Parasitenk. Abt. I Orig.*, 205 (1967) 38.
10 M. BIEDERMANN AND G. DREWS, *Arch. Mikrobiol.*, 61 (1968) 48.
11 D. L. KEISTER, in H. METZNER, *Progr. Photosynthesis Res., Proc. Intern. Congr. Photosynthesis Res., Freudenstadt, 1968*, Preprint No. 196.
12 J. LASCELLES, *Advan. Microbial Physiol.*, 2 (1968) 1.
13 L. N. M. DUYSENS, *Progr. Biophys.*, 14 (1964) 3.
14 R. K. CLAYTON, *Photochem. Photobiol.*, 5 (1966) 669.
15 R. K. CLAYTON, *Photochem. Photobiol.*, 5 (1966) 807.
16 W. J. VREDENBERG AND J. AMESZ, *Biochim. Biophys. Acta*, 126 (1966) 244.
17 R. K. CLAYTON AND W. R. SISTROM, *Photochem. Photobiol.*. 5 (1966) 661.
18 W. R. SISTROM, *Photochem. Photobiol.*, 5 (1966) 845.
19 J. LASCELLES AND T. ALTSHULER, *Arch. Mikrobiol.*, 59 (1967) 204.
20 O. T. G. JONES, *Biochem. J.*, 91 (1964) 572.
21 C. BRIL, *Biochim. Biophys. Acta*, 29 (1958) 458.
22 A. GARCIA, L. P. VERNON AND H. MOLLENHAUER, *Biochemistry*, 5 (1966) 2399.
23 T. KIHARA, Dissertation Abstracts, Vol. XXVII, No. 4, 1966 (Order No. 66–8905), cited in: T. KIHARA AND A. W. FRENKEL, in H. GEST, A. SAN PIETRO AND L.-P. VERNON, *Bacterial Photosynthesis*, Antioch Press, Yellow Springs, Ohio, 1963, p. 115.
24 J. LASCELLES, *Biochem. J.*, 100 (1966) 175.
25 G. DREWS, *Zentr. Bakteriol. Parasitenk. Abt. I Suppl.*, 1 (1965) 170.
26 G. SCHÖN AND G. DREWS, *Arch. Mikrobiol.*, 54 (1966) 199.
27 O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
28 H. C. SMITH AND A. BENITEZ, in K. PAECH AND M. V. TRACEY, *Moderne Methoden der Pflanzenanalyse*, Bd. IV, Springer, Berlin, 1955, p. 179.
29 H. C. SMITH AND A. BENITEZ, in K. PAECH AND M. V. TRACEY, *Moderne Methoden der Pflanzenanalyse*, Bd. IV, Springer, Berlin, 1955, p. 181.
30 H. C. SMITH AND A. BENITEZ, in K. PAECH AND M. V. TRACEY, *Moderne Methoden der Pflanzenanalyse*, Bd. IV, Springer, Berlin, 1955, p. 183.
31 R. WILLSTÄTTER AND W. MEIG, *Ann. Chem.*, 350 (1906) 1.
32 H. MOLISCH, *Ber. Deut. Botan. Ges.*, 14 (1896) 16.
33 T. A. SCOTT AND E. H. MELVIN, *Anal. Chem.*, 25 (1953) 1656.
34 W. S. KIM, *Biochim. Biophys. Acta*, 112 (1966) 392.
35 E. EGGER, *Planta*, 58 (1962) 664.
36 S. MOORE, D. H. SPACKMAN AND W. H. STEIN, *Anal. Chem.*, 30 (1958) 1185.
37 L. ORNSTEIN, *Ann. N.Y. Acad. Sci.*, 121 (1964) 321.
38 B. J. DAVIS, *Ann. N.Y. Acad. Sci.*, 121 (1964) 404.
39 G. DREWS, *Arch. Mikrobiol.*, 48 (1964) 122.

40 B. F. BURNHAM AND J. LASCELLES, *Biochem. J.*, 87 (1963) 462.
41 K. D. GIBSON, A. NEUBERGER AND G. H. TAIT, *Biochem. J.*, 83 (1962) 539.
42 G. DREWS AND J. OELZE, *Zentr. Bakteriol. Parasitenk., Abt. II*, 120 (1966) 1.
43 M. BOLL, *Arch. Mikrobiol.*, 64 (1968) 85.
44 A. RYTER, E. KELLENBERGER, A. BIRCH-ANDERSEN AND O. MAALØE, *Z. Naturforsch.*, 13b (1958) 597.
45 M. J. KARNOWSKY, *J. Biophys. Biochem. Cytol.*, 11 (1961) 729.
46 G. COHEN-BAZIRE AND R. KUNISAWA, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 1543.
47 M. J. BULL AND J. LASCELLES, *Biochem. J.*, 87 (1963) 15.
48 G. DREWS, *Z. Naturfsch.*, 21b (1966) 1224.
49 M. HIGUCHI, K. GOTO, M. FUGIMOTO, O. NANUKI AND G. KIKUCHI, *Biochim. Biophys. Acta*, 95 (1965) 94.
50 J. OELZE AND G. DREWS, *Biochim. Biophys. Acta*, 173 (1969) 448.
51 M. D. BACHMANN, D. S. ROBERTSON, C. C. BOWEN AND I. C. ANDERSON, *J. Ultrastruct. Res.*, 21 (1967) 41.
52 B. WALLES, *Hereditas*, 53 (1965) 247.
53 D. VON WETTSTEIN, *Brookhaven Symp. Biol.*, 11 (1958) 138.
54 G. DREWS, H. H. LAMPE AND R. LADWIG, *Arch. Mikrobiol.*, 65 (1969) 12.
55 W. J. WEIGL, *Am. Chem. Soc.*, 75 (1953) 999.
56 M. R. J. SALTON, *Ann. Rev. Microbiol.*, 21 (1967) 417.
57 J. CROUNSE, W. R. SISTROM AND S. NEMSER, *Photochem. Photobiol.*, 2 (1963) 361.
58 D. VON WETTSTEIN, *Brookhaven Symp. Biol.*, 11 (1958) 138.
59 G. W. BRYAN, A. H. ZADYLAK AND C. F. EHRET, *J. Cell Sci.*, 2 (1967) 513.