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THE FORMATION OF BACTERIOCHLOROPHYLL · PROTEIN COMPLEXES OF THE PHOTOSYNTHETIC APPARATUS OF *RHODOPSEUDOMONAS CAPSULATA* DURING EARLY STAGES OF DEVELOPMENT

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Summary

Cells of *Rhodopseudomonas capsulata* cultivated at an oxygen partial pressure of 400 mmHg in the dark contained 0.1 nmol or less total bacteriochlorophyll per mg membrane protein. The bacteriochlorophyll was found in the reaction center (10 pmol bacteriochlorophyll/mg membrane protein) and in the light harvesting bacteriochlorophyll I but not in the light harvesting bacteriochlorophyll II. Formation of the photosynthetic apparatus in those cells was induced by incubation at a very low oxygen tension in the dark. Reaction center bacteriochlorophyll and light harvesting bacteriochlorophyll increased three fold after 60 min of incubation at 1—2 mmHg (pO_2). Light harvesting bacteriochlorophyll II increased strongly after 60 min and became dominating after 90 min of incubation. The total bacteriochlorophyll content doubled every 30 min, but synthesis of reaction center bacteriochlorophyll proceeded at much lower rates. Consequently the size of the photosynthetic unit (total bacteriochlorophyll/reaction center bacteriochlorophyll) increased from 15 to 52 during 150 min of incubation. The proteins of the photosynthetic apparatus were synthesized concomitantly with bacteriochlorophyll.

Cells which were incubated at 0.5 mmHg (pO_2) do not grow but form the photosynthetic apparatus. During the first hours of incubation light harvesting bacteriochlorophyll I and reaction center bacteriochlorophyll were the dominant bacteriochlorophyll species, but light harvesting bacteriochlorophyll II was synthesized only in small amounts. Total bacteriochlorophyll and reaction center bacteriochlorophyll increased from 30 min up until 210 min of incubation more than 10 fold. The final concentrations of total bacteriochlorophyll and reaction center bacteriochlorophyll were 8.6 nmol and 0.26 nmol per mg membrane protein, respectively. The three protein components of the reaction centers (mol. wts. 28 000, 24 000 and 21 000) and the protein of the light harvesting I complex (mol. wt. 12 000) were incorporated simultaneous-

ly. The protein of band 1 (mol. wt. 14 000) which was present in the isolated light harvesting complex II, was synthesized only in very small amounts. The proteins of bands 3 and 4 (mol. wt. 10 000 and 8000) however, which were shown to be associated with light harvesting bacteriochlorophyll II, were synthesized in noticeable amounts as was light harvesting bacteriochlorophyll II. In addition a protein with an apparent molecular weight of 45 000 showed a strong incorporation of ^{14}C-labeled amino acids. This protein comigrates with one protein which was found to be associated with a green pigment excreted during incubation at 0.5 Torr into the medium. The in vivo-absorption maxima of this pigment complex were 660, 590, 540, 417 and 400 nm. The succinate oxidase and the NADH oxidase seemed to be incorporated into the newly formed intracytoplasmic membrane only in very small amounts. Thus, reaction center and light harvesting bacteriochlorophyll and their associated proteins were simultaneously synthesized, whereas light harvesting complex II is the variable part of the photosynthetic apparatus.

Introduction

The photosynthetic units of *Rhodopseudomonas* (R.) *capsulata* and *R. sphaeroides*, are localized on intracytoplasmic membranes [1] and are known to contain several species of bacteriochlorophyll [2]. The photochemically active reaction centers of both species have been isolated and characterized [3—7]. Associated with the reaction center are two species of light-harvesting bacteriochlorophyll with different spectral characteristics [8–13]; light harvesting bacteriochlorophyll I shows a single major absorbancy peak at approximately 876 nm and light harvesting bacteriochlorophyll II have absorbance maxima at 802 and 855 nm [10].

Kinetic studies have shown that the formation of light harvesting bacteriochlorophyll of *R. capsulata* is accompanied by incorporation of two or more polypeptides into the intracytoplasmic membranes [14—16]. Light harvesting bacteriochlorophyll was isolated from a carotenoid-less mutant strain and found to be associated with a protein of an apparent mol. wt. of 11 500 [17, 18]. The light harvesting bacteriochlorophyll II complex was isolated from the phototroph negative mutant Y5. The complex contained three proteins with mol. wts. of approximately 14 000, 10 000 and 8000 [18].

The rate of formation of the photosynthetic apparatus and of intracytoplasmic membranes in *Rhodospirillaceae* is correlated with the oxygen tension in the culture [14,16,19—24]. This article describes the biosynthesis of reaction center and light harvesting complexes I and II in cultures of *Rhodopseudomonas capsulata*, in which the oxygen partial pressure was reduced from 400 to 1—2 or 0.5 Torr. It will be shown that during this transient state of membrane morphogenesis pigment complexes were synthesized in different rates.

Materials and Methods

Organisms. R. capsulata, strain 37b4 (German collection of microorganisms, Göttingen, strain number 938) was used in these studies. The strain has been described elsewhere [25].

Culture conditions. The bacteria were precultivated aerobically in the dark in RÄ medium [26] with 0.1% casamino acids (Difco) for three passages in 500 ml Erlenmeyer flasks with baffles. The flasks were filled with 150 ml medium and agitated at 30°C on a rotary shaker. The last passage was used as inoculum for aerobic culture in a 5500 ml turbidostat fitted with a device for regulation of oxygen-partial pressure (Eschweiler and Co., Kiel, G.F.R.) as previously described [16]. Cells were grown at 400 mmHg oxygen tension. The bacteria were harvested, washed in phosphate buffer (0.05 M, pH 7.6) at 4°C and immediately frozen in liquid nitrogen. The frozen cells were stored at −80°C for induction experiments.

The induction of the photosynthetic apparatus was at first performed semiaerobically in the dark according to the method of Nieth and Drews [15] in 100 ml Erlenmeyer flasks. After thawing the bacteria were resuspended in RÄ medium containing 0.1% casamino acids and adjusted to a final absorbance (A) of 1.0 (0.5 cm pathway, wavelength 660 nm). The oxygen partial pressure was about 1—2 mmHg. Induction experiments with an oxygen partial pressure of 0.5 mmHg were done in a 2 l laboratory fermenter (Eschweiler and Co., Kiel) with an automatic oxygen partial pressure regulation device suitable for regulation at very low pO_2 (ref. 16, and Dierstein unpublished). After the pO_2 of the medium had been adjusted to 0.5 mmHg, the bacteria were added to the culture vessel up to a final absorbance of 1.0 ($A_{660}^{0.5\ \mathrm{cm}}$). At 30 min intervals an appropriate volume was taken out and chilled on crushed ice. The cells were washed once at 4°C and were kept on ice until needed.

Radioactive labeling of the membrane proteins was performed in the RÄ medium supplemented with 0.05% casamino acids and 1.5 μCi ^{14}C/ml amino acid mixture, New England Nuclear, Boston Mass.).

Analytical procedures. Carotenoids and bacteriochlorophyll were extracted from whole cells with an acetone/methanol mixture (7 : 2; v/v). The mM extinction coefficients of $\epsilon = 128\ mM^{-1} \cdot cm^{-1}$ for carotenoids (at 484 nm) and $\epsilon = 76\ mM^{-1} \cdot cm^{-1}$ for bacteriochlorophyll (at 772 nm) were used in estimating carotenoids and bacteriochlorophyll in vitro [27]. Bacteriochlorophyll content of membranes was calculated from the in vivo absorption of the membrane fraction at 860 nm using the coefficient $\epsilon = 127\ mM^{-1} \cdot cm^{-1}$ [27]. Reaction center bacteriochlorophyll was calculated from $\Delta A_{880\,nm}$ reduced minus oxidized. The extinction coefficient $\epsilon = 113\ mM^{-1} : cm^{-1}$ was used [27].

Absorbance spectra of membranes at 77°K were measured in a clear Dewar flask filled with liquid nitrogen. A dense paste of homogenized membranes in 50% glycerol was layered as a thin film (approx. 0.15 mm) on slides and placed in a disposable cuvette. The spectral measurements were done with a Cary 14 R spectrophotometer.

Photophosphorylation was measured according to Cusanovich and Kamen [28]. A Perkin-Elmer double beam spectrophotometer, model 356 was used for estimation of the light induced absorbance changes of cytochromes c_2 and b and reaction center bacteriochlorophyll. The method is described elsewhere [29,30].

Protein was measured by the method of Lowry et al. [31].

The preparation of a crude membrane fraction is described elsewhere [16]. The crude membranes were washed with phosphate buffer (0.05, pH 7.6) con-

taining 5 mM EDTA, and purified on a continuous linear sucrose gradient (60 30% w/v) with a Beckman SW 41 Ti rotor at 150 000 × *g* for 15 h.

Membrane proteins were separated by SDS-polyacrylamide gel electrophoresis on 1 mm slab gels [32]. A continuous 10% to 15% acrylamide, 5—20% sucrose gradient was prepared. Samples of 20—30 μg protein were loaded in each trough. Gels were stained with 0.04% Coomassie brillant Blue R 250 (Serva-Heidelberg, G.F.R.) in a mixture of H_2O/isopropanol/acetic acid (65 : 25 : 10, v/v/v) and destained in water/methanol/acetic acid (80 : 10 : 10, v/v/v).

Autoradiographic studies were carried out by exposing Osray M-3 film (Agfa-Gevaert) to dried gels for 5—14 days.

The chemicals used for gel electrophoresis were of the purest grade available.

NADH- and succinate-oxidase activities in the crude membrane fraction were determined by polarographic O_2-measurement using a YSI 53 biological oxygen monitor (Yellow Springs Instruments Co., Ohio). The oxygen uptake was calculated on the basis of an oxygen concentration of 237 mM in the air saturated buffer at 30°C [16,33]. NADH dehydrogenase (EC 1.6.99.3) and succinate dehydrogenase (EC 1.3.99.1) were measured as described previously [34].

Results

The aerobic preculture

The rate of formation of bacteriochlorophyll in growing cultures of *Rhodospirillaceae* increases when the oxygen partial pressure is lowered [1]. The threshold of pO_2 for bacteriochlorophyll formation is strain specific. Bacteriochlorophyll is formed in cells of *R. capsulata* 37b4 even at the oxygen tension of 100 mmHg [35]. The bacteriochlorophyll synthesis is, however, repressed and the growth rate slightly reduced when the cells are cultivated at 400 mmHg [14]. For this reason all cells of *R. capsulata* used in induction experiments were precultivated in a turbidostate at 400 mmHg pO_2. These exponentially growing cells contained no measurable bacteriochlorophyll in the acetone-methanol extract. The membrane fraction isolated from these cells contained about 0.1 nmol bacteriochlorophyll/mg protein. The content of reaction center bacteriochlorophyll was about 10 pmol bacteriochlorophyll/mg protein. The absorption spectrum showed a small peak at 875 nm which is referred to light harvesting bacteriochlorophyll I. Peaks at 800 and 850 nm (light harvesting bacteriochlorophyll II) were not detectable. The concentration of carotenoids in this membrane fraction was found to be 1—2 nmol/mg protein.

Formation of the photosynthetic apparatus at the oxygen tension of 1—2 mmHg

Cells precultivated at 400 mmHg (pO_2) were resuspended in fresh medium so as to give final absorbance of 1.0 and incubated in the dark at a pO_2 of 1—2 mmHg.

The growth rate of these bacteria was a third of the maximal rate. During the first 30 min after induction only light harvesting bacteriochlorophyll I (Fig. 1) and reaction center bacteriochlorophyll (Table I) were detectable. The total amount of bacteriochlorophyll does not increase during this period. Afterwards the bacteriochlorophyl content increased strongly: at 60 min of incubation

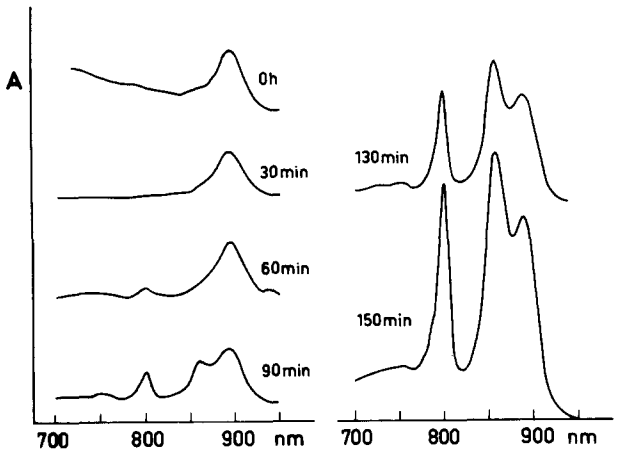

Fig. 1. Near-infrared absorption spectra of membrane fraction of *Rhodopseudomonas capsulata* 37b4 at temperature of liquid nitrogen. Samples were prepared as described in Materials and Methods. Culture conditions: preculture: turbidostate, 400 mmHg (pO_2), dark; induction: 1—2 mmHg (pO_2), Erlenmeyer flasks, dark, 30°C.

reaction center bacteriochlorophyll and light harvesting bacteriochlorophyll I had increased three fold (Table I). The small peak at 800 nm after 60 min and the peak at 857 nm after 90 min of incubation show that synthesis of light harvesting bacteriochlorophyll II had begun (Fig. 1).

From 90 min to 150 min of incubation light harvesting bacteriochlorophyll II became the dominant bacteriochlorophyll species. The total bacteriochlorophyll content doubled every 30 min (Table I), reaction center bacteriochlorophyll synthesis, however, proceeded at much lower rates. Consequently the size of the photosynthetic unit, i.e. the ratio of total bacteriochlorophyll to reaction center bacteriochlorophyll, increased from 15 to 52 during this experiment (see Table I).

Membrane proteins were synthesized together with bacteriochlorophylls as demonstrated by autoradiography of the banding patterns in polyacrylamide

TABLE I

BACTERIOCHLORPHYLL IN MEMBRANES OF *RHODOPSEUDOMONAS CAPSULATA* 37b4

Preculture: 400 mmHg (PO_2), dark, 30°C. Induction: 1—2 mmHg (PO_2), dark, 30°C. The experiment is described in Materials and Methods. Bchl = bacteriochlorophyll, LH = light harvesting, RC = reaction center.

Time (min) after induction	pmol Bchl / mg protein	pmol RC Bchl / mg protein	Total Bchl / RC Bchl	LH I/LH II	
				894 nm / 857 nm	800 nm / 857 nm
0	136	9.3	14.5		
30	150	9.5	15.8	6.8	
60	502	25.8	19.5	4.3	0.75
90	1132	32	35.3	1.3	0.75
130	2352	52.6	44.7	0.7	0.8
150	2804	53.4	52.5	0.7	0.9

gels. A protein with an apparent molecular weight of 12 kdalton was labeled strongly by ^{14}C-labeled amino acids during the first 90 min of induction. This protein was shown to be associated with light harvesting bacteriochlorophyll I [17,18,36]. In correlation with the spectral data, proteins, associated with light harvesting bacteriochlorophyll II (8,10 and 14 kdaltons, ref. 18, 36) were incorporated into the membrane mainly during the last period of incubation. The synthesis of reaction center and light harvesting I associated proteins continued during the whole experiment (data not shown here). Measurable rates of photophosphorylation were observed in the newly synthesized membranes after 130 min of incubation (14.5 nmol ATP · μmol^{-1} bacteriochlorophyll). The presence of a functional reaction center as well as the light-induced reduction of cytochrome c_2 and oxidation of *b*-type cytochromes during the early phase of incubation (data not shown here) make it appear likely that the potential for photophosphorylation exists much earlier.

The formation of the photosynthetic apparatus at the oxygen tension of 0.5 mmHg

Cells of *R. capsulata*, precultivated at 400 mmHg oxygen tension were resuspended in fresh medium to give a final absorbance of 1.0. The oxygen partial pressure of the culture was adjusted to 0.5 mmHg. Under these conditions the cells ceased to grow. The number of viable bacteria per ml were constant over a period of 4 h (Fig. 3). At the beginning of the experiment only the bacteriochlorophyll species light harvesting I (absorption maximum: 895 nm at 77°K; Fig. 2) and reaction center bacteriochlorophyll were spectroscopically detectable. In contrast to the results of the previous experiment light harvesting bacteriochlorophyll I remained the dominating bacteriochlorophyll species during an incubation period of 4 h (data not shown). The main infrared absorption peak shifted to 885 nm (Fig. 2). Peaks at 800 nm and a shoulder at 850 nm indicate that small amounts of light harvesting bacteriochlorophyll II (approx. 20% of total bacteriochlorophyll) were synthesized. Total bacteriochlorophyll

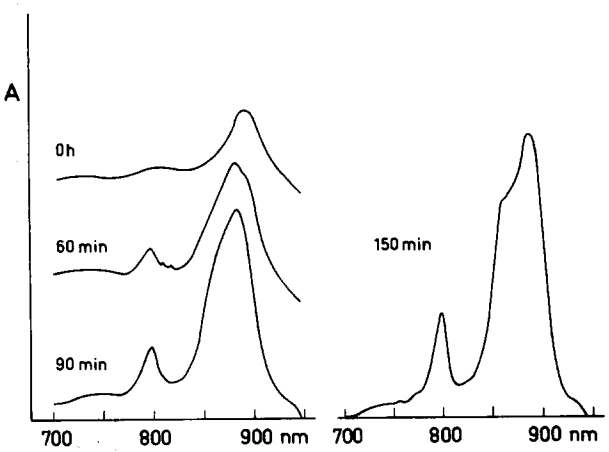

Fig. 2. Near-infrared absorption spectra of membrane fraction of *Rhodopseudomonas capsulata* at temperature of liquid nitrogen (approx. 77°K). Culture conditions: preculture: turbidostate, 400 mmHg (pO_2), 30°C, dark, induction: 0.5 mmHg (pO_2) ± 0.1 mmHg 30°C, dark.

TABLE II

MEMBRANE DIFFERENTIATION AFTER LOWERING OF OXYGEN TENSION

Preculture: 400 mmHg (PO_2), dark; Induction: 0.5 mmHg (PO_2), dark. PSU = photosynthetic unit, n.d. = not determined. Activity of enzymes was calculated on basis of membrane protein, Bchl = bacteriochlorophyll, RC = reaction center.

Induction (min)	Cell protein mg/ml	Total Bchl (nmol per mg membrane protein)	RC-Bchl (nmol per mg membrane protein)	Size of PSU total Bchl / RC-Bchl	nmol cyt c_2 reduced / mol RC-Bchl	Oxidases nmol O_2 / min mg protein		Dehydrogenases nmol substrate / min mg protein	
						Succinate	NADH	Succinate	NADH
0	n.d.	n.d.	n.d.	n.d.	n.d.	24.4	19.6	53.7	20.3
30	0.526	0.66	0.03	24.3	1.3	31.4	16.1	84.8	23.2
60	0.529	1.68	0.06	29.3	1.3	31.5	17.5	95.2	26.6
90	0.552	2.78	0.09	31.2	1.2	27.1	18.4	125.9	29.7
120	0.552	5.57	0.15	37.7	1.0	20.3	15.7	108.5	24.1
150	0.600	7.95	0.23	34.2	1.0	23.1	13.8	72.4	22.4
180	0.600	8.69	0.26	32.7	0.8	21.6	13.2	8.48	22.0
210	0.600	8.61	0.26	32.5	0.7	21.4	13.1	90.7	22.9

and reaction center bacteriochlorophyll increased from 30 min to 210 min of incubation (Table II) more than 10 fold. The final concentration of bacteriochlorophyll was found to be 8.6 nmol total bacteriochlorophyll/mg membrane protein and 0.26 nmol reaction center bacteriochlorophyll/mg membrane protein. The kinetics of light harvesting and reaction center bacteriochlorophyll synthesis on basis of membrane protein can be represented by a sigmoidal curve (Table II). The ratio of light harvesting bacteriochlorophyll I (*B*-870) to reaction center bacteriochlorophyll remained nearly constant at about 30 during the incubation period. The relatively low concentration of light harvesting bacteriochlorophyll II does not contribute substantially to the size of the photosynthetic unit.

Under experimental conditions of growth limitation carotenoid synthesis began after a lag period of 30—60 min whereas bacteriochlorophyll synthesis was observable earlier (Fig. 3). Steady state concentrations of pigments were not attained during the experimental period.

Specific activities of both succinate dehydrogenase and NADH-dehydrogenase increased during the first period of induction process and decrease later on (Table II). Specific activities of succinate oxidase and NADH oxidase in the membrane fraction fluctuate during the first 120 min and reach constant levels after a slight decrease (Table II). The labeling patterns of the proteins of the photosynthetic apparatus show that the three reaction center proteins (mol. wts. 28, 24 and 21 kdalton, refs. 6 and 15) were incorporated synchronously into the membrane (Fig. 4). As expected the light harvesting I protein (mol. wt. 12 kdalton, see refs. 17, 18, 36) was synthesized simultaneously with light harvesting bacteriochlorophyll I (Fig. 2) and reaction center (Fig. 4). As shown in Fig. 2 light harvesting bacteriochlorophyll II was synthesized in a relatively low rate during the whole period of incubation. The proteins which were detected in the light harvesting comples II [44] (bands 1, 3 and 4, [18,36]), were synthesized from the beginning but in different rates (Fig. 4). Light harvesting bands 3 and 4 in polyacrylamide gels were labeled strongly, whereas band 1 showed weak labeling. Another unexpected result was the strong synthesis of a protein with an apparent mol. wt. of about 45 000 (Fig. 4). This protein runs to the same position as one protein which was found to be associated with a green pigment excreted into the medium during incubation at 0.5

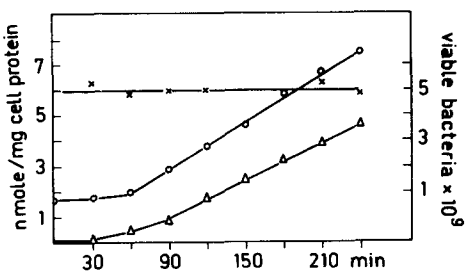

Fig. 3. Kinetics of bacteriochlorophyll and carotenoid formation in cells of *Rhodopseudomonas capsulata* and the number of viable (Colony-forming) bacteria cultivated at 0.5 mmHg (pO_2) in the dark at 30°C. ○———○, carotenoid; △———△, bacteriochlorophyll concentrations per cell protein; X———X, number of viable cells per ml.

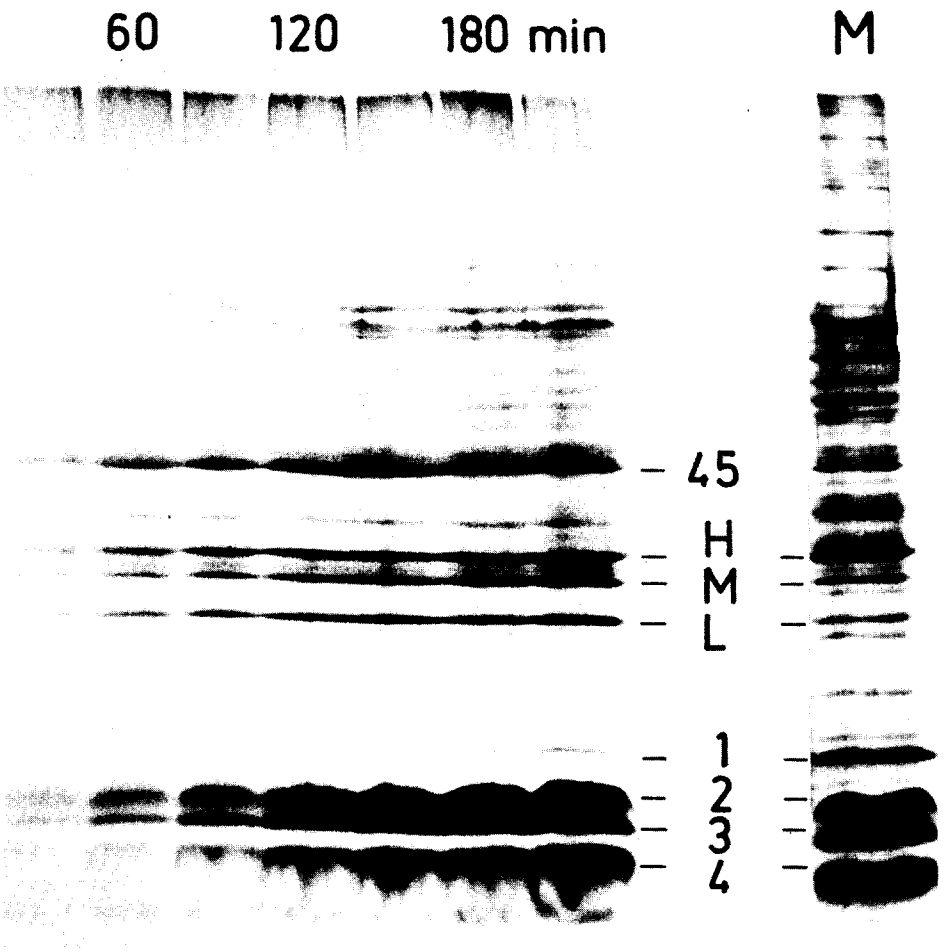

Fig. 4. Autoradiogram of polyacrylamide slab gel of membrane fractions from cells harvested after incubation at low oxygen pressure at the time as indicated on the top of the gel. The gel M at the right shows the stained protein patterns of purified intracytoplasmic membranes of *R. capsulata* grown anaerobically in the light. 45 refers to a protein of a mol. wt. of approx. 45 000, which runs to the same position as a polypeptide of the green pigment complex excreted into the medium after induction. L, M and H denote the polypeptides which form the reaction center and 1, 2, 3 and 4 refers to the light-harvesting bacteriochlorophyll associated proteins. Band 2 was found to be associated with light harvesting bacteriochlorophyll I, whereas bands 1, 3 and 4 are ascribed to light harvesting bacteriochlorophyll II. The autoradiogram shows a synchronous labeling of reaction center polypeptides and light harvesting bacteriochlorophyll I associated protein 2. Light harvesting bacteriochlorophyll II proteins are assembled asynchronously. Bands 3 and 4 are labeled strongly while band 1 is labeled poorly by ^{14}C-labeled amino acids.

Torr. The in vivo-absorption maxima of this pigment complex were 660, 590, 540, 500, 417 and 400 nm. It remains to be determined whether this pigment-protein complex is a precursor of one of the bacteriochlorophyll complexes in the membrane or a by-product formed under the stress of low oxygen tension.

Photochemical activity was found during all stages of formation of the photosynthetic apparatus. Approximately 1 mol cytochrome c_2 was reduced per mole reaction center in each membrane fraction (Table II). However, the

reduction of cytochrome c_2 proceeded slowly, indicating a low coupling of electron transport to reaction center.

Discussion

The aerobic preculture at a pO_2 of 400 mmHg provided cells with a very low bacteriochlorophyll content, allowing studies on the early events of formation of the photosynthetic apparatus. Low amounts of bacteriochlorophyll even after several generations of aerobic culture, have been found in other *R. capsulata* strains [12]. The relatively high pO_2 threshold for bacteriochlorophyll formation in *R. capsulata* differs significantly from that of *Rhodospirillum rubrum*, which does not synthesize bacteriochlorophyll at oxygen tensions above 60 mmHg [22]. Though *R. capsulata*, grown at 400 mmHg (pO_2), contains small amounts of bacteriochlorophyll, light harvesting bacteriochlorophyll II synthesis is totally suppressed.

After induction of the morphogenesis of the photosynthetic apparatus at a pO_2 of 1—2 mmHg, the reaction center and light harvesting bacteriochlorophyll I are synthesized first, followed by an increasing synthesis of the light harvesting bacteriochlorophyll II complex (Fig. 1). The stepwise formation of these pigment complexes proceeds as a simultaneous incorporation of bacteriochlorophyll and of the respective proteins into the intracytoplasmic membrane. Based on mutant studies it has been proposed that the coupled synthesis of reaction center and light harvesting I proteins is due to a genetic linkage [36]. A similar pattern of assembly of the photosynthetic apparatus has been observed with *Rhodopseudomonas sphaeroides* [24], a photosynthetic bacterium which also has two light harvesting bacteriochlorophyll complexes [11].

An initial decrease of the size of the photosynthetic unit (total bacteriochlorophyll/reaction center bacteriochlorophyll) was observed in an earlier study with *R. capsulata* [15], whereas in the present study an immediate increase of the size of the photosynthetic unit was shown. In the previous investigation the initial concentration of total bacteriochlorophyll in the cell was somewhat higher. The size of the photosynthetic unit in these bacteria was found to be greater than 100, indicating the presence of light harvesting bacteriochlorophyll II. Subsequent to induction, reaction center and light harvesting bacteriochlorophyll I were synthesized first and consequently the photosynthetic unit was reduced in size [15]. The molar ratio of light harvesting bacteriochlorophyll I to reaction center bacteriochlorophyll in *Rhodospirillum rubrum* and *R. sphaeroides* was found to be constant under various steady-state conditions [11]. In *R. capsulata* we found a constant ratio of 30 : 1 for light harvesting bacteriochlorophyll I/reaction center bacteriochlorophyll when the light harvesting bacteriochlorophyll II synthesis was reduced by the oxygen tension of 0.5 mmHg (Table II). This reduced rate of light harvesting bacteriochlorophyll II synthesis is not yet understood. It may be that due to the very low ATP production (low energy charge) not only growth but also light harvesting bacteriochlorophyll II formation is suppressed. The question remains open whether the excretion of a green bacteriochlorophyll precursor complex into the medium at a pO_2 of 0.5 mmHg in the wild type strains 37b4 and the

mutant A1a$^+$ (not shown here) is a consequence of inhibition of light harvesting II incorporation.

It was shown that the isolated light harvesting bacteriochlorophyll II complex from the strains 37b4 of *R. capsulata* contains three proteins [18]. The light proteins of this complex (8 and 10 kdaltons) were synthesized concomitantly with light harvesting bacteriochlorophyll II (Fig. 4), while the 14 kdalton protein was only weakly labeled. This result is in agreement with very recent observations which indicate that light harvesting bacteriochlorophyll II is associated with the light proteins in the complex [44]. It remains to be studied whether the 14 kdalton protein participates in the process of light harvesting formation.

The results show that in *R. capsulata* the formation of the photosynthetic apparatus is adapted to different growth conditions by variability of the size of the photosynthetic unit, which is mainly managed by an independent regulation of the synthesis of the light harvesting bacteriochlorophyll II complex (ref. 11, 16, and Drews, 1977, in press).

At least during the first stage of induction no significant turnover of membrane protein was observed [38]. Under conditions of growth limitation under low aeration (0.5 mmHg, pO_2) reaction center and light harvesting bacteriochlorophyll I complexes seem to be the dominating species which are incorporated in substantial amounts into the photopigment depleted membrane. These pigment complexes seem to be sufficient to establish an active photosynthetic apparatus in a membrane active in oxidative phosphorylation. This idea is supported by the observations that the same coupling factor is active in oxidative phosphorylation and photophosphorylation [39,40] and that reaction center can be coupled efficiently to electron transport in phototroph negative membranes [29,41,42]. Photophosphorylation, however, was not detectable during the first 60 min of induction, possibly because of the high scattering effect of the sample. The slow reduction of cytochrome c_2 and the small size of the photosynthetic unit, however, seem to indicate that the structural arrangement and the component stoichiometry are not yet optimal in membranes at an early stage of cell differentiation.

During incubation at the pO_2 of 0.5 mmHg the activities of respiratory enzymes show fluctuations (Table II) which cannot be interpreted at yet. The activities calculated on the basis of total membrane bacteriochlorophyll decrease. However, the total amounts of respiratory enzymes per cell might be constant or slightly increased.

The highest activity of NADH oxidase is localized in the cytoplasmic membrane, whereas intracytoplasmic membrane has lower activities [14]. Intracytoplasmic membranes were formed during incubation at pO_2 0.5 mmHg (results not shown here) and consequently the portion of intracytoplasmic membrane in the total membrane fraction increased. It was shown that in cells of *Rhodospirillum rubrum* NADH- and succinate oxidizing electron transport systems are distributed unequally during membrane differentiation [43]. The pattern of distribution of respiratory enzymes during membrane differentiation in *R. capsulata* requires further studies.

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