EVIDENCE FOR THE FORMATION OF MEMBRANOUS CHROMATOPHORE PRECURSOR FRACTIONS IN RHODOPSEUDOMONAS SPHEROIDES.

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Summary

Several membrane fractions have been isolated from Rhodopseudomonas spheroides which had been harvested at various intervals during the adaption from aerobic-dark to semiaerobic-light growth conditions. The membrane fractions were separated by sucrose density gradient ultracentrifugation. As a result of labeling experiments with L-(U-^{14}C)proline, it was suggested that some of these fractions may represent discrete intermediate stages in the formation of the membranous photosynthetic apparatus in R. spheroides. This formation may be summarized as follows: Cell membrane → prephore fraction → prochromatophore fraction → chromatophore.

As part of a study of changes in membrane proteins during the morphogenensis of the photosynthetic apparatus in Rhodopseudomonas spheroides, membrane preparations were studied by sucrose density gradient ultracentrifugation at various times during the adaptation from aerobic-dark to semiaerobic-light growth conditions. Gorchein et al.(1) have shown that R. spheroides grown heterotrophically in pure oxygen contained no chromatophores. Cessation of oxygenation induced an adaptive response, chromatophores were formed, and the culture began to grow phototrophically in the light within 24 hr (1). Gorchein et al. (2) have also shown that the chromatophore protein contained about twice the level of proline as did cell membrane protein. We have grown R. spheroides in pure oxygen and resuspended the cells in fresh medium containing radioactive proline as an indicator of increased photosynthetic membrane protein synthesis. The internal membranes were analyzed at

various times during this adaptation. The results have indicated that these membranes undergo a number of alterations in chemical composition resulting in changes in their density. Various membrane fractions have been characterised as to the time of their appearance during adaptation, their position in a sucrose density gradient, and their Bchl[1] content. The radioactive proline incorporation studies have allowed us to propose a precursor relationship between some of these membrane fractions and the mature chromatophore during the adaptive formation of the photosynthetic apparatus in R. spheroides.

Methods

R. spheroides N.C.I.B. 8253 was grown for 18 hr at 30° in the dark by bubbling oxygen into 1 l. cultures of the bacterium in medium MG of Lascelles (3). The cells were then centrifuged, resuspended in 3 l. of fresh medium MG, and sealed in a 5 l. flask. Experiments with a Beckman model 777 oxygen electrode have shown that such a culture rapidly (in 5 min) becomes semi-aerobic with an oxygen partial pressure of 3 mm Hg. At this point 4 μmole of L-(U-^{14}C)proline (5 μCi/μmole; New England Nuclear Corp., Boston, Mass.) was added and a 500 ml sample was withdrawn by nitrogen displacement. The cells were harvested by centrifugation for 15 min at 10,000g. The approximate time that the sample was in contact with the radioactive proline before centrifugation was 5 min; it was termed the zero hr sample. The remainder of the culture was stirred magnetically at 30° under 300 ft candles illumination. Additional 500 ml samples were withdrawn at 2, 6, and 20 hr and centrifuged as above. All samples were resuspended in 10 ml of 0.05 M potassium phosphate buffer (pH 7.0 and disrupted by sonicating twice for 1 min at 0° with a Biosonik III sonicator at 70 kc. Lysis in a French pressure cell at 20,000 psi produced similar results. Unbroken cells and cell debris were removed by centrifugation for 15 min at 12,000 g. The supernatants were then centrifuged for 100 min at 100,000g. The pellets were resuspended in 5 ml of 0.05 M potassium phosphate buffer (pH 7.0) and layered on top of 30 ml of linear sucrose density gradients formed between 25 and 55% (w/v) sucrose by an ISCO model 570 gradient former. Centrifugation was carried out for 15 hr at 63,600g in a Beckman model L2-65B ultracentrifuge with a 60 Ti rotor. After centrifugation, the gradients were fractionated into twelve 3 ml samples with an ISCO model 182 fractionator. The total protein in each fraction was measured by the method of Lowry et al.(4). The visible and near-infrared spectrum of each fraction was recorded on a Cary model 14 spectrophotometer and Bchl was estimated by the method of Gorchein et al.(2). The measurement of radioactivity was accomplished on a Beckman model LS-200B scintillation counter in 10 ml solutions of 5% PPO phosphor (Aldrich Chemical Co., Inc., Milwaukee, Wisc.) in toluene.

[1] The following abbreviations are used: Bchl, bacteriochlorophyll; PHB, poly-β-hydroxybutyrate.

Electron microscopy was performed on a RCA model EMU-3H microscope at 50 keV. All samples were negatively stained using 1% phosphotungstic acid neutralized with sodium hydroxide. The samples were examined on 200 mesh nickel grids coated with carbon and collodon.

Results and Discussion

The results are given in Fig 1-3. Fig 1 shows the rate of radioactive incorporation into the membranous fractions.

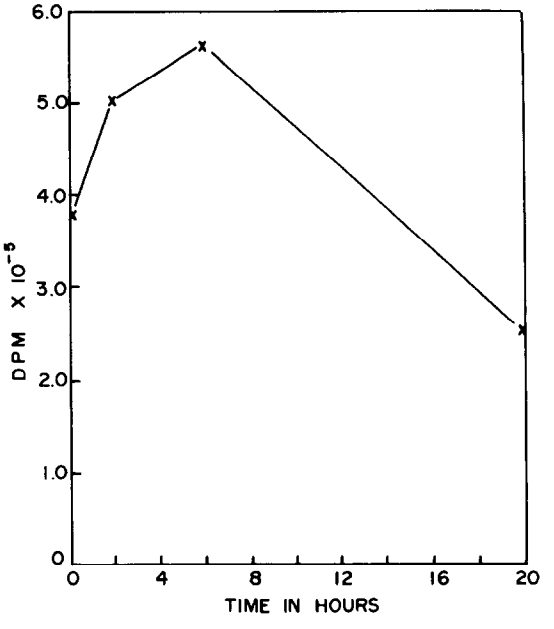

Figure 1: The total activity in dpm x 10^{-5} contained in the membrane preparations at each of the four times examined during the adaptation of R. spheroides from heterotrophic to phototrophic growth conditions in the presence of L-(U-^{14}C)proline.

Fig 2 shows the protein content and specific activity (in dpm/mg protein) and Fig 3 shows the specific Bchl content (in nmole/mg protein) of each 3 ml sample at each time examined. It was found that mature chromatophores occupied a position at 38-39% sucrose under these centrifugation conditions; they are not, however, visible until the 20 hr sample. At 0 hr the protein assays indicated that the majority of the particulate

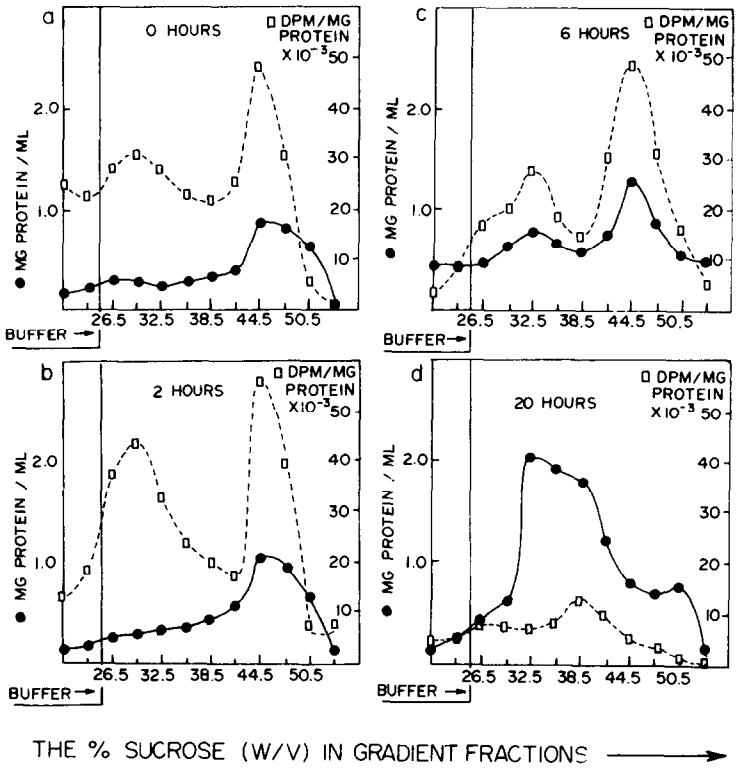

Figure 2: Sucrose density gradient separation of the membrane preparations of Fig 1. The protein content (-●-) in mg/ml and the specific activity (-□-) in dpm x 10^{-3}/mg protein of each 3 ml fraction is shown at each of the four times examined.

protein occupied a position at 44-46% sucrose (Fig 2). A smaller band was also located at 26-30% sucrose. Both of these bands were significantly labeled after 5 min of contact with the radioactive proline. In fact, approximately 2/3 of the total label incorporated appeared during this time period (Fig 1). Neither of these bands was pigmented (Fig 3). Electron microscopic examination of negative stains of similar bands observed during semiaerobic-dark growth of R. spheroides revealed that the 26-30% sucrose band consisted of 1500-2500 Å in diameter raised spherical particles similar in appearance to vesicles observed in both R. rubrum (5-7) and R. spheroides

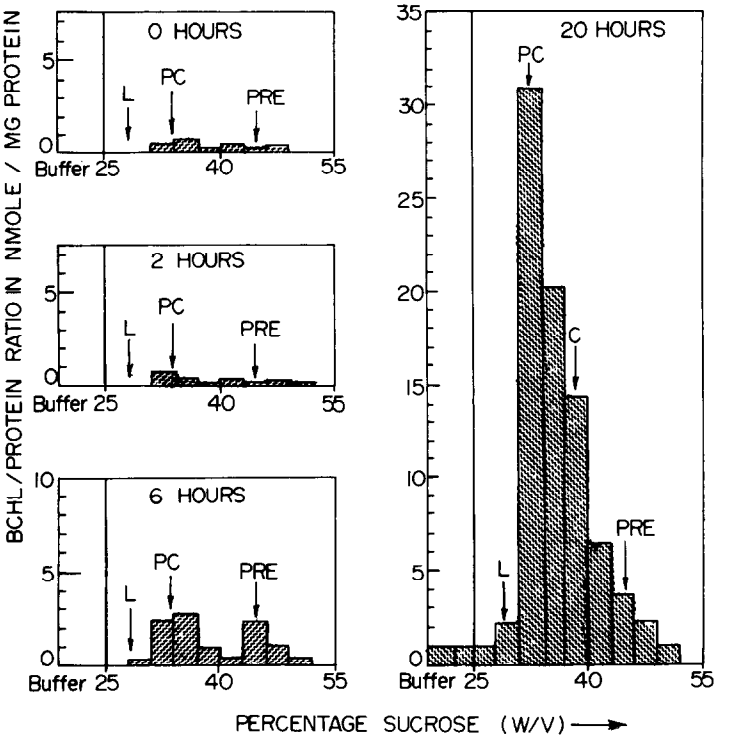

Figure 3: Sucrose density gradient separations of the membrane preparations of Figure 1. The Bchl/protein ratio in nmole/mg protein of each 3 ml fraction is shown at each of the four times examined. L is the light fraction, PC is the prochromatophore fraction, PRE is the prephore fraction, and C is the chromatophore fraction.

(8-10). These latter vesicles were thought to contain storage PHB or glycogen (8) and were shown to be membrane bound (7). In some cases they appeared to be continuous with the cell membrane (9). Gorchein (11) has confirmed that PHB accumulates in R. spheroides during aerobic growth and has shown that it and storage carbohydrate are reduced during adaptation to phototrophic conditions (1). Since the 26-30% sucrose fraction never contained any pigment, it is likely that these particles were storage vesicles. This fraction has been termed the "light fraction".

Electron microscopic observations also revealed that the 44-46% sucrose band consisted of raised spherical particles of a diameter similar to that of chromatophores (800-1000 Å) but without the characteristic "flattened-disc" appearance (12). In addition, many ribosome-sized particles (200 Å) were present. Other workers (5, 13, 14) have noticed non-chromatophore particles or vesicles in photosynthetic bacteria but have not ascribed any particular function to them. The fraction did not however contain any cell wall or cell membrane fragments. It was termed the "prephore fraction".

After 2 hr the specific activity of both the light and the prephore fractions had increased, indicating continued synthesis of their proteins (Fig 2). Neither contained a significant amount of pigment at this time (Fig 3). After 6 hr the specific activity of the light fraction had decreased significantly, whereas the specific activity of the prephore fraction remained high. A new band appeared at 32-33% sucrose in the gradient (Fig 2). This band was termed the "prochromatophore fraction". Fig 3 indicates that both the prephore and prochromatophore fractions contained Bchl also both contained carotenoids. In addition, prephore fractions have been observed to contain peaks of between 395 and 415 nm in addition to the normal Bchl Soret, indicating the possible accumulation of porphyrin precursors of Bchl. Thus, the prephore fraction was the likely site for the initiation of Bchl synthesis. After 20 hr, the prephore band had largely disappeared; however, the prochromatophore band had increased (Fig 2). In addition, chromatophores were also present in the gradient at 38-39% sucrose. The radioactivity data indicated that the membrane proteins of highest specific activity were now found in the

chromatophore band (Fig 2). Fig 3 shows that the Bchl/protein ratio of the prochromatophore band had risen elevenfold, indicating that the majority of Bchl synthesis had occurred in the prochromatophore. Additional protein synthesis during the conversion of the prochromatophore to the chromatophore may have caused the Bchl/protein ratio to be lower in the latter fraction. The ratio in the prephore fraction remained at a fairly low value.

Since the light fraction is likely a storage vesicle, its function in chromatophore morphogenesis is uncertain. However, our data indicate that the morphogenesis may proceed as follows: prephore fraction membranes are formed first between 0 and 2 hr after the start of adaptation. Between 2 and 6 hr, Bchl synthesis ensues in the prephore membranes. Shortly thereafter, these are converted to prochromatophore membranes, perhaps by incorporation of lipids. After 6 hr the Bchl content of the prochromatophore membranes increases and shortly thereafter these are converted to chromatophores, perhaps by additional protein synthesis. We do not know at present what structure these non-chromatophore membrane fractions have within the cell during the adaptive formation of the photosynthetic apparatus.

Acknowledgements

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