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EARLY FORMATION OF INTRACYTOPLASMIC MEMBRANES IN *RHODOSPIRILLUM RUBRUM*

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SUMMARY

Cytoplasmic and intracytoplasmic membranes were isolated from *Rhodospirillum rubrum* by equilibrium sucrose density gradient centrifugation. Immediately after the induction of photosynthetically active intracytoplasmic membranes, bacteriochlorophyll is incorporated predominantly into the cytoplasmic membrane. With increasing pigment concentrations the newly arising intracytoplasmic membranes become sites of preferential bacteriochlorophyll incorporation. During this process the infrared absorption band of the pigment shows a red shift. The shift is more pronounced with intracytoplasmic than with cytoplasmic membranes. Pulse-chase of cytoplasmic membrane proteins reveals that such proteins become constituents of intracytoplasmic membranes.

INTRODUCTION

Phototrophically grown cells of *Rhodospirillum rubrum* exhibit two types of membranes which can be distinguished on the basis of their structure and predominant functions [1]. Cytoplasmic membranes contain the respiratory chain. Intracytoplasmic membranes, on the other hand, are sites for photosynthetic activities. Under typical chemotrophic conditions, i.e. high aeration in the dark, no intracytoplasmic membranes can be found. However, after transfer from high to low aeration in the dark or to anaerobiosis in the light, cells synthesize intracytoplasmic membranes, including the photosynthetic apparatus [1, 2].

Currently there are two hypotheses concerning the origin of intracytoplasmic membranes. First, cytoplasmic membranes provide sites for intracytoplasmic membranes to condense de novo from their constituents in constant proportions [3]. The second hypothesis postulates that, at least initially, parts of the cytoplasmic membrane are used for intracytoplasmic membrane formation [1, 4]. This paper presents new evidence supporting the latter hypothesis.

MATERIAL AND METHODS

R. rubrum, strain FR1, was grown with high aeration in the dark in medium RÄH [5]. Cells were harvested during the logarithmic phase of growth, sedimented by

centrifugation, resuspended in 2 l of fresh culture medium (0.7 mg protein/ml), and incubated in a cylindrical vessel (11.5 cm diameter) at 30 °C in the dark. Low aeration was achieved by constant stirring. For pulse-chase experiments, high-aerated cells were resuspended in 1 l of fresh medium plus 50 μCi (U-^{14}C)-labeled proteinhydrolyzate (Radiochemical Centre, Amersham, U.K.) and highly aerated for another 45 min. After harvesting, one half of the labeled cells was subjected to membrane isolation. The second half was resuspended in 2 l of fresh culture medium supplemented with 0.5 % casamino acids and grown with low aeration in the dark.

All steps for membrane isolation were done at 4 °C. Cells were harvested at various times, resuspended in Tris/HCl buffer (0.02 M; pH 7.6) containing 3 mM EDTA, and homogenized by two passages through a French pressure cell at 16 000 lb/inch^2. Material sedimenting between 64 000 $\times g$ and 113 000 $\times g$, both for 60 min, was used as the enriched-membrane fraction. After washing, this fraction was subjected to further purification by equilibrium sucrose density gradient centrifugation for 36 h at 123 000 $\times g$. Sucrose gradients (25–50 %, w/w) were prepared with Tris/HCl buffer without EDTA (0.02 M; pH 7.6). For preparation of highly purified intracytoplasmic membranes, Ficoll density gradient centrifugation [5] was performed before centrifugation of the membranes in sucrose gradients. Following centrifugation, density gradients were fractionated. The distribution of membranes was determined spectrophotometrically with a Cary 14 R, measuring membrane-bound cytochrome absorption at 412 nm (corrected for light scattering) or absorption of membrane-bound bacteriochlorophyll at 875 nm. Determinations of protein, bacteriochlorophyll and radioactivity [6] as well as membrane solubilization and polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate [7] were done as described previously.

RESULTS

Measuring the incorporation of bacteriochlorophyll, the prominent constituent of the photosynthetic apparatus, into the membranes should be one way to answer the questions about the origin of intracytoplasmic membranes. Such experiments are feasible since the isolation and characterisation of cytoplasmic membranes from *R. rubrum* have been described recently [8]. Samples were taken before and at two different times after transfer of the cells to low aeration and prepared for membrane separation by sucrose density gradient centrifugation. Membrane fractions of highly aerated cells are composed only of cytoplasmic membranes which band at a buoyant density of $\rho = 1.146$ (ref. 8 and Fig. 1A). Transfer to low aeration induces the synthesis of bacteriochlorophyll [2]. At very early stages bacteriochlorophyll is incorporated predominantly into the cytoplasmic membrane fraction (Fig. 1B). Considerably lower amounts of this pigment are detectable within a band arising at a density of $\rho = 1.165$, characteristic of intracytoplasmic membranes [9]. It becomes dominating as the cellular bacteriochlorophyll contents increase (Fig. 1C). The clear-cut difference in buoyant densities indicates that both membranes form individual fractions.

In separate experiments, spectra of the membrane fractions were recorded. Fig. 2 shows that the infrared absorption peak of bacteriochlorophyll shifts towards longer wavelengths as the pigment level increases. At low cellular pigment concentrations, the positions of infrared absorption peaks are below 875 nm (Fig. 2B). At

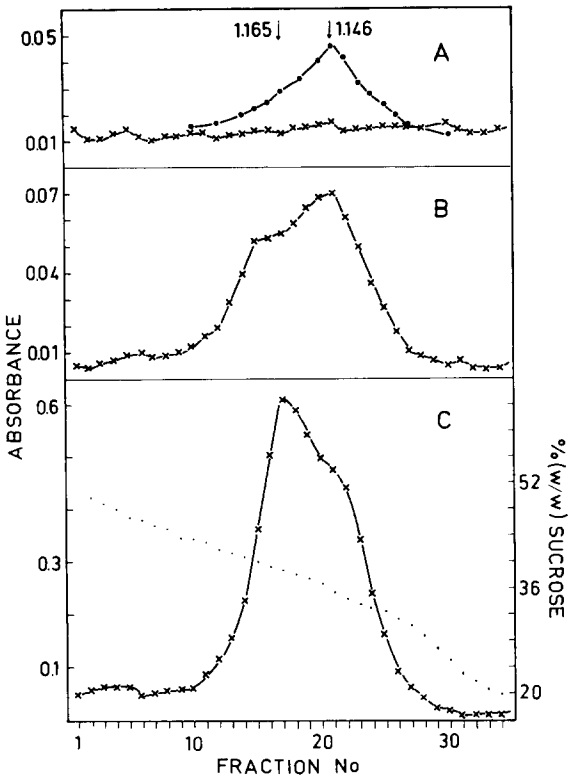

Fig. 1. Sucrose density gradient separation of membranes isolated from *R. rubrum* at various cellular bacteriochlorophyll levels: (A) bacteriochlorophyll not detectable, (B) 0.3, and (C) 2.5 μg bacteriochlorophyll/mg protein. The bacteriochlorophyll contents (B, C) were measured at 875 nm (× – ×), membrane-bound cytochromes were determinable only in the absence of bacteriochlorophyll (A) at 412 nm (● – ●). Sucrose concentrations are shown by the dotted line (C); buoyant densities (g/cm^3) of the membrane fractions (A–C) are indicated by the arrows (A).

increased pigment concentrations the peaks shift above 875 nm (Fig. 2C). Membranes of fully developed cells exhibit the well known bacteriochlorophyll absorption maximum at 880 nm (Fig. 2D). In addition, there are spectral differences between membranes from cells at the same developmental stage. At a low cellular pigment level, cytoplasmic and intracytoplasmic membranes isolated after sucrose density gradient centrifugation reveal absorption maxima at nearly identical wavelengths (Fig. $2B_1$, $2B_2$). At higher pigment levels the absorption maxima of intracytoplasmic membranes are shifted to a higher wavelength (Figs. $2C_1$, $2C_2$).

The participation of cytoplasmic membrane proteins in the formation of intracytoplasmic membranes was checked with pulse-chase experiments. Cytoplasmic membrane proteins were labeled in highly aerated cells with radioactive amino acids. After this, cells were washed, resuspended in fresh culture medium supplemented with casamino acids and then transferred to low aeration. The latter step induces intracytoplasmic membrane formation [2, 10]. Cells were harvested at a bacteriochloro-

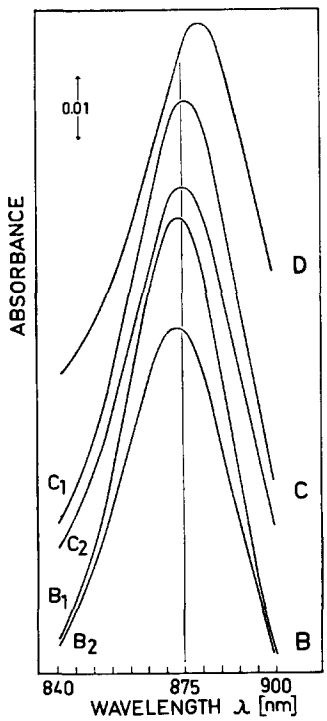

Fig. 2. Infrared absorption spectra of membrane-bound bacteriochlorophyll at various cellular bacteriochlorophyll levels: (B) 0.46, (C) 4.0, (D) 12.0 (μg bacteriochlorophyll/mg protein). Intracytoplasmic membranes (B_1, C_1, D) cytoplasmic membranes (B_2, C_2).

phyll content of 2.7 μg per mg protein. Membranes were isolated from high-aerated as well as low-aerated cells as outlined under Material and Methods.

Protein patterns of cytoplasmic membranes are characterized after gel electrophoresis by high molecular weight proteins (bands 1–6, Fig. 3A, and ref. 8). Proteins migrating in the region of bands 8–10 are characteristic cell wall contaminants [8]. The typical cytoplasmic membrane proteins are found in the purified intracytoplasmic membranes (Fig. 3B). In addition, intracytoplasmic membranes are characterized by the three reaction-center proteins (bands 11, 14 and 15, see ref, 7). Autoradiograms demonstrate that the characteristic proteins of cytoplasmic membranes (Fig. 3a) are exclusively labeled in the intracytoplasmic membrane fraction (Fig. 3b). Proteins of the reaction center complex (bands 11, 14 and 15) which are predominantly synthesized under low aeration (ref. 10 and Oelze, J., unpublished) contain no detectable radioactivity. This shows that no proteins were labeled during the period of chase.

DISCUSSION

Bacteriochlorophyll synthesized immediately after the transfer of *R. rubrum* from high to low aeration is incorporated into cytoplasmic membranes. However, subsequently, bacteriochlorophyll is incorporated into the newly arising intracytoplasmic membranes. The red shift observed simultaneously for the bacteriochlorophyll

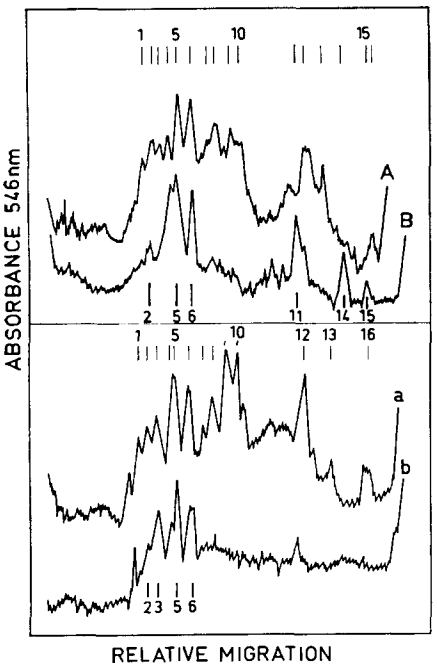

Fig. 3. Pulse chase of cytoplasmic membrane proteins into intracytoplasmic membranes. Membrane proteins were separated by electrophoresis in 10 % polyacrylamide gels. Protein patterns (A, B) and autoradiograms (a, b) were scanned densitometrically. Enriched membrane fractions (A, a), containing cytoplasmic membranes, were isolated from bacteriochlorophyll-free cells. Intracytoplasmic membranes (B, b) were isolated from cells containing 2.7 μg bacteriochlorophyll/mg protein by means of Ficoll and sucrose equilibrium density gradient centrifugation (Material and Methods).

infrared absorption peak can be explained on the basis of recent findings by Lehoczki and Csatorday [11]. These authors reported a slight red shift of the chlorophyll *b* red absorption maximum upon increasing the pigment content in Triton X-100 micelles. Accordingly, the different bacteriochlorophyll spectra lead to the following interpretations. First, under the conditions applied, a low bacteriochlorophyll content is an intrinsic property of cytoplasmic membranes and not caused by contamination with intracytoplasmic membranes. Second, the total red shift indicates that the interaction between bacteriochlorophyll molecules and the membrane changes gradually, supporting the idea that intracytoplasmic membranes are formed through a differentiation process [1]. The early intracytoplasmic membranes do not differ significantly from cytoplasmic membranes with respect to the position of the bacteriochlorophyll absorption peak. This suggests that the pigment concentrations of both membranes are within comparable ranges. Bacteriochlorophyll synthesized later is incorporated predominantly into intracytoplasmic membranes, measurable as the red shift of the infrared absorption band.

To study the origin of intracytoplasmic membranes, chase experiments were performed with pulsed cytoplasmic membrane proteins. There is always a possibility that the results obtained for intracytoplasmic membranes might be influenced by cytoplasmic membrane contaminants. But the distinct properties, i.e. densities as well

as infrared spectra, of the membranes strongly suggest that the two membranes are not cross contaminated significantly. Nevertheless, intracytoplasmic membranes were subjected to thorough purification with Ficoll gradient centrifugation [5] and subsequent equilibrium sucrose density gradient centrifugation. After this, intracytoplasmic membranes formed the only band present in sucrose gradients. The pulse chase clearly indicates that intracytoplasmic membranes contain labeled proteins exclusively derived from cytoplasmic membranes.

Thus, the data confirm the hypothesis that early intracytoplasmic membranes arise by invagination of cytoplasmic membranes [1]. During further development, however, intracytoplasmic membranes are differentiated and extended by preferential incorporation of specific constituents, e.g. those necessary for the photosynthetic apparatus [1].

Finally, a phenomenon should be mentioned which cannot be explained on the basis of the data presented. It is assumed that intracytoplasmic membranes are formed through a process of differentiation. Accordingly, there should exist intermediates exhibiting densities between those of cytoplasmic membranes and fully developed intracytoplasmic membranes. But, while with Ficoll gradients the sedimentation properties of intracytoplasmic membranes depend on their developmental stage [6, 12] no such dependency was observable with sucrose gradients.

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