# ISOLATION OF A "BASIC MEMBRANE" FRACTION ENRICHED IN AN ORNITHINE-CONTAINING LIPID, FROM A BLUE-GREEN MUTANT OF *RHODOSPIRILLUM RUBRUM*

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Received 17 September 1970

## 1. Introduction

An ornithine-containing lipid (OCL) that lacks phosphorus has been observed in some photosynthetic bacteria [1–5]. It has been found among the phospholipids of different subcellular fractions of *Rhodospirillum rubrum*, namely the chromatophores, and also in non-pigmented, aerobically grown organisms. Gorchein [1] suggests that this lipid could be a membrane-structural component, at least in anaerobically grown organisms.

On the other hand, De Pinto [2] observed that unlabelled ornithine added to a culture containing ($^{14}C_5$) ornithine yields no dilution of the isotope in the lipid fraction. This lack of turnover of the OCL may suggest a structural role for this compound. The intracellular distribution of this lipid [3, 5] and its physical and chemical properties, are consistent with a possible structural role within the cytoplasmic membrane system of the non-sulphur purple bacteria.

We attempted to prove this hypothesis by looking for the distribution of the OCL in different subchromatophore fractions, from a non-carotenoid-containing mutant of *Rhodospirrilum rubrum* (a blue-green, BG 1).

The cell membrane repeatedly treated with Triton X-100 yields an insoluble lipoprotein fraction. This fraction presents some properties characteristic of the so called "structural proteins" and was enriched in OCL, the only lipid detectable by thin-layer chromatography (TLC).

## 2. Materials and methods

All reagents and chemicals used were of analytical grade.

The *Rhodospirillum rubrum* BG 1 cells were grown in a malate medium, according to Newton [6]. The cells were harvested after 48 hr of growth (late log phase) and the membranes prepared by osmotic shock, according to Robish and Marr [7]. The membranes were repeatedly treated with Triton X-100 in a discontinuous gradient of sucrose, keeping a constant ratio of 50 mg of detergent/mg of bacteriochlorophyll (BChl). By this method a soluble fraction (Pheo) and two particulate fractions formed by light particles (L) and heavy particles (H) were obtained after each treatment (fig. 1).

Triton X-100, an alkylphenoxypolyethoxyethanol, is a non-ionic detergent which was purchased from Rohm and Haas Co., Philadelphia, Pa. This detergent reportedly has 9–10 oxyethylene groups per molecule, making an average molecular weight of 625.

Acetone-methanol (7:2, v/v) was used to extract BChl from the particles. The concentration of BChl was determined as described by Clayton [8]. Bacteriopheophytin (BPh) was detected by its absorption peak at 752 nm. Proteins were assayed in the different fractions by the method of Lowry et al. [9]. The lipids were extracted according to Bligh and Dyer [10], washing the chloroform phase as described by Folch et al. [11]. In the lipid extracts were assayed ornithine (Orn), by the method of Chinard [12], and phosphorus (P), by a combination of the methods of Barlett [13]

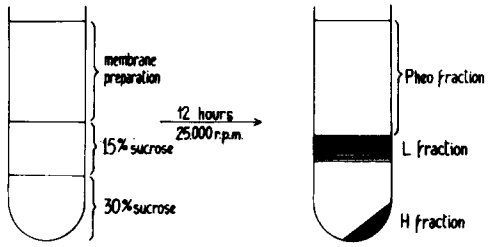


Fig. 1. Centrifugation of *R. rubrum BG1* membrane fractions on a discontinuous sucrose gradient consisting of two layers (15% and 30% sucrose). The amount of Triton X-100 was 50 mg of detergent/mg BChl. The tubes were centrifuged at 25,000 rpm in the 30 rotor of a Spinco L2 ultracentrifuge.

and Fiske-Subbarow [14]. Different lipids were separated by TLC according to Stahl [15] and detected by iodine vapours, ninhydrin spray and molybdenum spray [16].

## 3. Results

The cytoplasmic membrane, after 10 or 12 extractions with Triton X-100, yields a fraction which still contains BChl. We call this insoluble fraction, "basic membrane" (BM). Additional extractions of it, yield no more L particles, but disrupt the "basic membrane" into heterogeneous fragments.

Both L and H particles contain lipids and BChl. Only the first soluble fraction (Pheo 1) contains BPh and all the polar lipids, including OCL. Subsequent Pheo fractions are pigment-free, although polar lipids may be detected. All these fractions contain proteins.

In the cell membrane, the P/protein ratio is greater than either the Orn/protein, or the BChl/protein ratios (fig. 2). These ratios are smaller for the $H_1$ particles (obtained in the first extraction) and diminish in the succesive extractions of the H fractions. In the BM fraction, P/protein and Orn/protein ratios reach approximately the same value, and both are smaller than the BChl/protein ratio.

Under the electron microscope, H particles appear membranous-like, as was shown by García et al. [17]. The P/Orn, P/BChl and Orn/BChl ratios, for the different H fractions, are plotted in fig. 3. Both the P/Orn and P/BChl ratios fall with successive extractions, while the Orn/BChl ratio is practically constant after the second extraction.

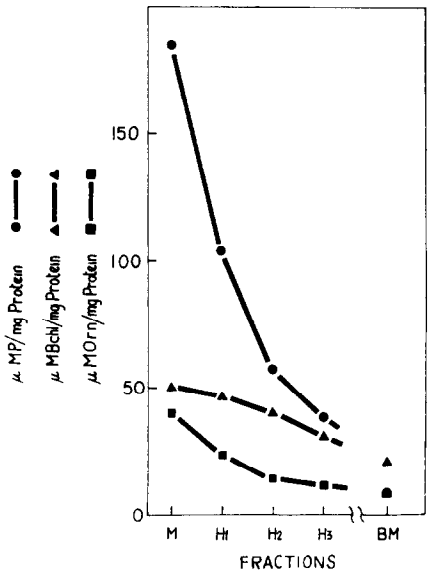


Fig. 2. P/protein, BChl/protein and Orn/protein ratios in the cytoplasmic membrane and in the succesive heavy fractions (H) obtained by repeated treatment with Triton X-100.

In the BM fraction, the OCL is the only one that can be detected by TLC (fig. 4). Nevertheless, small quantities of lipid phosphorus, generally below the sensitivity of the quantitative method are present. In this fraction, approximately 5–6% proteins, 2% BChl, 1.5% OCL and 0.2–0.3% of lipid phosphorus from the cell membrane, are present.

## 4. Discussion

The results suggest that the interaction between polar groups of membrane-bound lipids and that of the detergent, may result in a selective extraction. Thus, at this pH, phospholipids present a higher polarity than OCL, and are preferentially extracted, On the other hand, phospholipids could be more weakly bound than OCL and BChl to the "basic membrane". This could mean that both types of lipids have a different environment and function. The OCL and BChl in the "basic membrane" fraction should have similar hydrophobic characteristics.

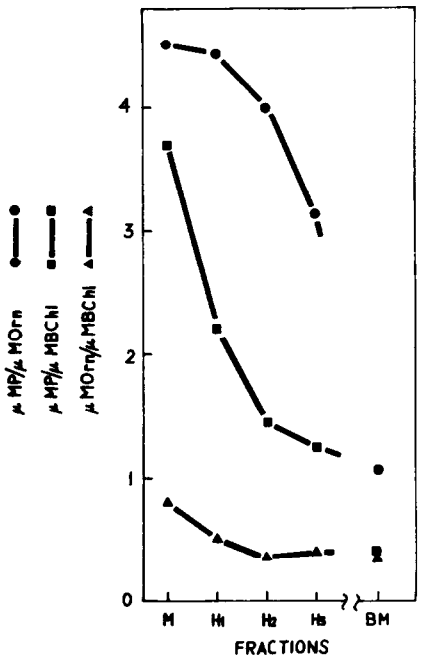


Fig. 3. P/Orn, P/BChl and Orn/BChl ratios in the cytoplasmic membrane and in the successive heavy fractions (H) obtained by repeated treatment with Triton X-100.

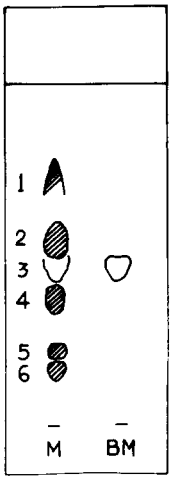


Fig. 4. TLC of the lipids extracted from the cytoplasmic membrane (M) and from the "basic membrane" (BM) fraction of *R. rubrum BG1*. Adsorbent Silica Gel G. Solvent system: chloroform–methanol–water (65:25:3.8, by vol). The lipids were detected with ninhydrin (spots 2 and 3 positive) and the molybdenum spray (dashed spots). Spot 1, diphosphatidyl glycerol; 2, phosphatidyl ethanolamine; 3,ornithine-containing lipid; 4, phosphatidyl glycerol; 5 and 6, lysophospholipids.

The BChl is only extracted in the L particles. Although the first extraction shows a Pheo fraction rich in BPh, this compound does not appear again in solution. We believe that the BPh of the first soluble fraction (Pheo 1) is pre-existent. This belief is based on the fact that subsequent extractions with the same detergent in the same conditions do not produce additional BPh. In addition, from the 48 hr cultures of the wild-type strain of *R. rubrum*, Kihara and Frenkel [18] isolated BPh-containing particles.

Furthermore, it is important to mention that in the L particles the ratio Orn/BChl is constant and similar to that found in the original membrane. The P/BChl ratio in these particles diminishes in the successive extractions, but is similar to the corresponding H fractions.

The experimental results suggest that by repeated treatment of the cytoplasmic membrane with Triton X-100 it would be possible to distinguish three different types of molecular complexes: 1) The BPh pre-existent, extracted by the first treatment with detergent, and some soluble proteins; 2) L fractions, heterogeneous, particulated, with a high content of lipids and photosynthetic properties [17]; 3) The "basic membrane", enriched in proteins and OCL, the only lipid detectable by TLC. This fraction, highly insoluble, does not produce more L particles by further treatment with detergent. This suggests that the "basic membrane" has a support function and could be related to the so-called "structural protein". The remaining BChl and OCL, not extractable by detergent, would be surrounded, in the "basic membrane", by a different environment than the lipids previously extracted. The most superficial polar lipids would be extracted by the action of the detergent. The hydrophobic interactions of their hydrocarbonated chains would carry into solution the less polar lipid (OCL). The L particles would also expose the polar groups of the phospholipids, giving them the nature of a charged micelle and facilitating their extraction by the detergent. The ornithine-containing lipid, could play an important role in the cohesion between these micelles and the "structural protein", being bound to them probably by hydrophobic bonds.

## Acknowledgements

This investigation was supported by grants 3372a/68 (A.F.G.) and 3697/69 (E.A.R.) from the Consejo Nacional de Investigaciones Científicas y Técnicas (R. Argentina). One of the authors (E.A.R.) is Career Investigator of this Institution.

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