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COMPARISON OF PURPLE MEMBRANE FROM *HALOBACTERIUM CUTIRUBRUM* AND *HALOBACTERIUM HALOBIUM*

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SUMMARY

Direct comparison of purple membrane preparations from *Halobacterium cutirubrum* and *Halobacterium halobium* was carried out. Both preparations were found to be essentially identical with respect to their molecular weight, retinal content, lipid composition, fingerprinting of peptides from peptide digestion, electron micrographs and X-ray diffraction patterns, and behaviour as a light-activated proton pump. Thus, there would appear to be no species differences in the purple membranes from these two bacteria.

INTRODUCTION

A purple membrane was first isolated by Stoeckenius and colleagues from a species of extremely halophilic bacteria, *Halobacterium halobium* [1–3]. The purple color could be attributed to the presence of a retinal-protein complex, bacteriorhodopsin, of which the protein component is the only protein in this membrane fragment [3, 4]. The purple membrane appears to function as a light-driven proton pump, and the cells use the resulting chemiosmotic gradient for ATP synthesis [5, 6].

Recently, the purple membrane was isolated from another species of halophile, *Halobacterium cutirubrum* [7, 8], and appears to be present in several other species as well [9]. Analysis and characterization of the purple membrane from *H. cutirubrum* [8] showed that it differed in several respects (e.g. molecular weight, amino acid composition, retinal : protein molar ratio) from that of *H. halobium* [3]. These results raised the question whether species differences occur in purple membranes from different halophilic bacteria. This question could only be settled by direct comparison of the two membranes, and the results of such a comparison are presented in this communication.

MATERIALS AND METHODS

Purple membrane from *H. halobium* was prepared as described by Stoeckenius and co-workers [3] and that from *H. cutirubrum* as described by Kushwaha et al. [8].

Extraction and chromatography of lipids, extraction and estimation of retinal, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the membrane protein were carried out as described previously [8]. Pepsin digestion of the delipidated protein [8] in 10 % formic acid and fingerprinting of the peptides was carried as described elsewhere [10]; amino-terminal groups were determined by the dinitrophenol procedure [11].

Electron microscopy and X-ray diffraction were carried out as described elsewhere [4]. Incorporation of isolated purple membrane in a model system of lipid vesicles was done essentially as described previously [12]; the vesicles were subsequently separated from free lipid in a sucrose density gradient. For flash spectroscopy a spectrophotometer of our own design was used (Lozier, Bogomolni and Stoeckenius, to be published). It has a time resolution of 10 μs.

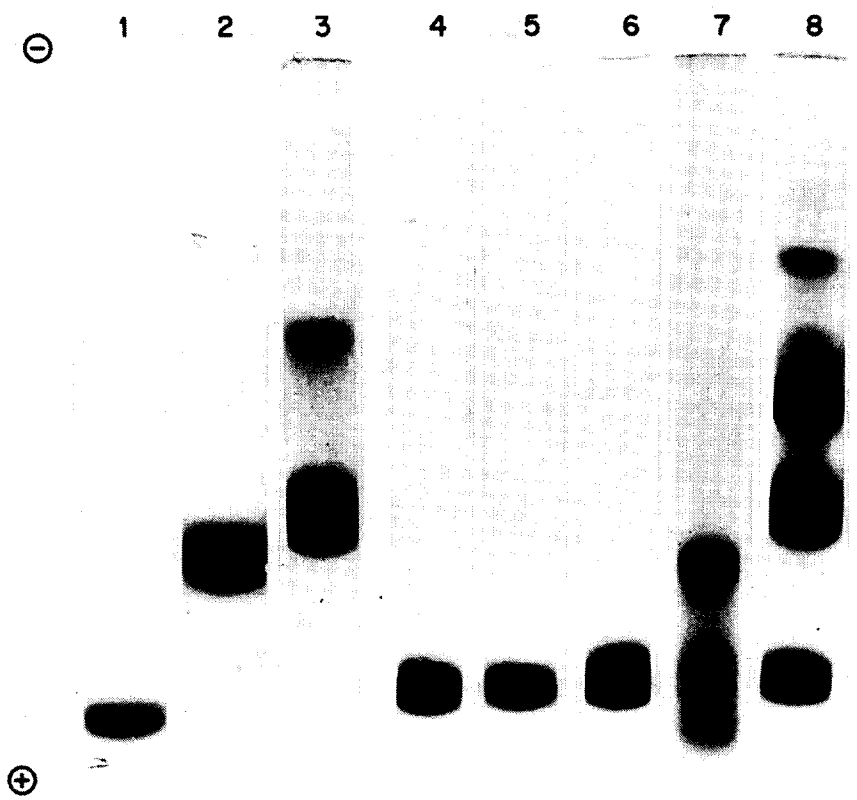

Fig. 1. Disc-gel electrophoresis on 7 % polyacrylamide of purple membranes from *H. cutirubrum* and *H. halobium* in sodium dodecyl sulfate. (1) ribonuclease A (M_r 13 700); (2) chymotrypsinogen (M_r 25 000); (3) ovalbumin (M_r 45 000); (4) purple membrane from *H. halobium*; (5) purple membrane from *H. cutirubrum*; (6) mixture of 4 and 5; (7) mixture of 1, 2, 4 and 5; (8) mixture of 3, 4, 5 and bovine serum albumins (monomer, M_r 66 000 and dimer M_r 132 000).

RESULTS AND DISCUSSION

Both purple membrane preparations migrated identically as a single sharp band on disc-gel electrophoresis on 7 % polyacrylamide gel, when run separately or in admixture (Fig. 1). From the plot of migration distance against log molecular weight (Fig. 2) a molecular weight of 19 300±200 was obtained for either preparation. This value agrees with the value (19 600±800) found previously [8] for the purple membrane from *H. cutirubrum*, but is significantly lower than that (26 000) reported previously [3] for the *H. halobium* preparation. The discrepancy in the molecular weight may have been due to the lack of sufficient standard marker proteins in the original sodium dodecyl sulfate gel electrophoresis of the *H. halobium* purple membrane. It is clear that both preparations have identical molecular weights, but the question concerning which absolute value is correct must await further analysis, e.g. sedimentation analysis, amino acid sequencing, etc.

The purple membrane preparations from *H. cutirubrum* and *H. halobium* were found to have essentially the same content of retinal (0.47 % and 0.49 %, respectively) corresponding to a retinal : protein molar ratio of 2.3 and 2.2, respectively, assuming a protein molecular weight of 20000. These ratios correspond well with the value found previously for the *H. cutirubrum* preparation [8] but differ from the 1 : 1 ratio reported for the *H. halobium* preparation [3]. The reason for this descrepancy is not yet known, but it should be mentioned that no residual bound or free retinal was detected in the protein remaining after the extraction procedure. It is possible that retinal may have been lost from the membrane during the preparation procedure, but this must then have occurred with both preparations to the same extent. Another possible explanation is that some of the retinal is destroyed during the extraction procedure; however, this possibility was eliminated since quantitative recoveries of known amounts of retinal added to the membrane preparations were obtained.

The lipids extracted from either preparation showed the same lipid components

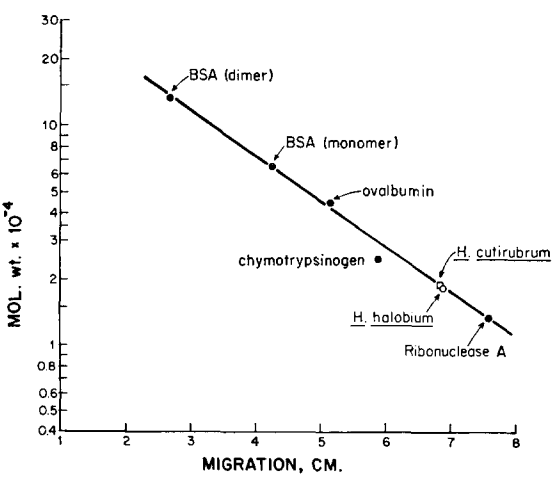

Fig. 2. Plot of migration distance vs. logarithm of molecular weights of purple membranes from *H. halobium* and *H. cutirubrum* and calibration proteins after electrophoresis on 7 % polyacrylamide gel in sodium dodecyl sulfate. BSA, bovine serum albumin.

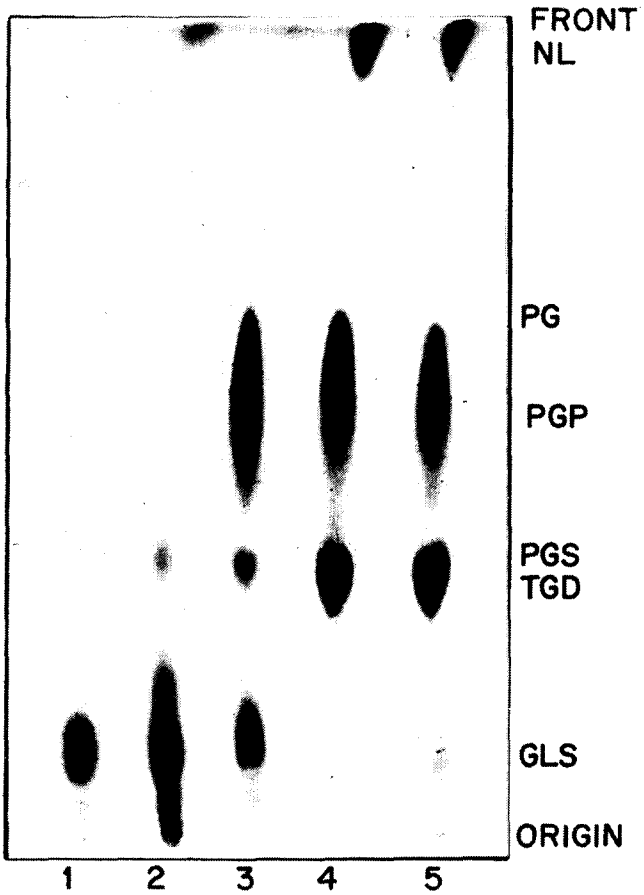

Fig. 3. Thin-layer chromatogram on silica gel H of total lipids of purple membranes from *H. halobium* and *H. cutirubrum* in solvent system chloroform/90 % acetic acid/methanol (30 : 20 : 4, v/v). (1) pure and (2) crude glycolipid sulfate; (3) total polar lipids from cells of *H. cutirubrum*; (4) total lipids of purple membrane from *H. cutirubrum*; (5) total lipids of purple membrane from *H. halobium*. Identity of spots: GLS, glycolipid sulfate; TGD, triglycosyl diether; PGS, phosphatidylglycerosulfate; PGP, phosphatidylglycerophosphate; PG, phosphatidylglycerol; NL, neutral lipids.

when chromatographed on thin-layer plates on silica gel H in chloroform/acetic acid/methanol (30 : 20 : 4 v/v) as shown in Fig. 3. The major components were the diphytanyl ether analogs of phosphatidylglycerophosphate, phosphatidylglycerosulfate, phosphatidylglycerol, glycolipid sulfate and its breakdown product triglycosyl diphytanyl ether, essentially as was found previously [8]. Neutral lipids were present in both preparations but were not further analyzed.

The proteins obtained from either preparation gave very similar peptide patterns after pepsin digestion. All major peptides (spots 1–19, Fig. 4) were common to both preparations with the reservation that peptides numbers 7 and 11 appeared as major spots in *H. halobium* and as minor ones in *H. cutirubrum*. The large number of hydrophobic peptides formed is noteworthy.

Amino terminal group analysis by the dinitrophenol procedure failed to reveal

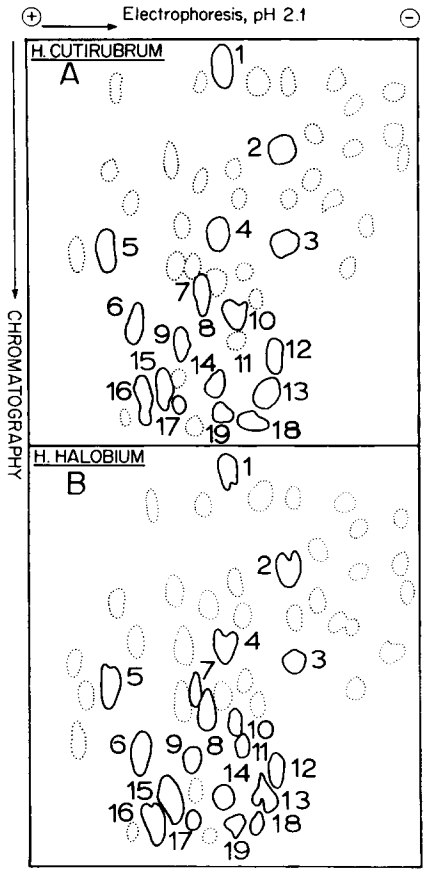

Fig. 4. Fingerprints of the soluble peptides formed by pepsin digestion (24 h at 37 °C in 10 % formic acid) of the purple membranes from (A) *H. cutirubrum* and (B) *H. halobium*. Bold spots and dotted spots indicate major and minor peptides, respectively. Except for minor peptides 7 and 11 in *H. cutirubrum*, only the major peptides (spots 1–19) have been numbered in both the peptide maps. Peptides were electrophoresed in first direction at 3000 V for 1 h at pH 2.1 on Whatman 3MM paper in Varsol-cooled tanks, then chromatographed in second direction in butanol/acetic acid/water/pyridine (60 : 12 : 48 : 40 by vol.).

an N-terminal amino acid residue, suggesting that the N-terminal residue might be blocked.

Electron micrographs and X-ray diffraction patterns of the two preparations were indistinguishable. They showed the typical difference in the surface pattern of the inner and outer membrane surface, reflecting the asymmetry of the membrane and revealing the planar lattice of the bacteriorhodopsin molecules (Fig. 5).

Absorbance changes of the *H. cutirubrum* membrane after excitation with a 1 μs flash at 575 nm were recorded at 400, 540 and 640 nm. The time course was the same as that observed with the *H. halobium* membrane, indicating that the same photoreaction cycle occurs in both preparations (Fig. 6).

In the vesicle system obtained from isolated *H. cutirubrum* purple membrane

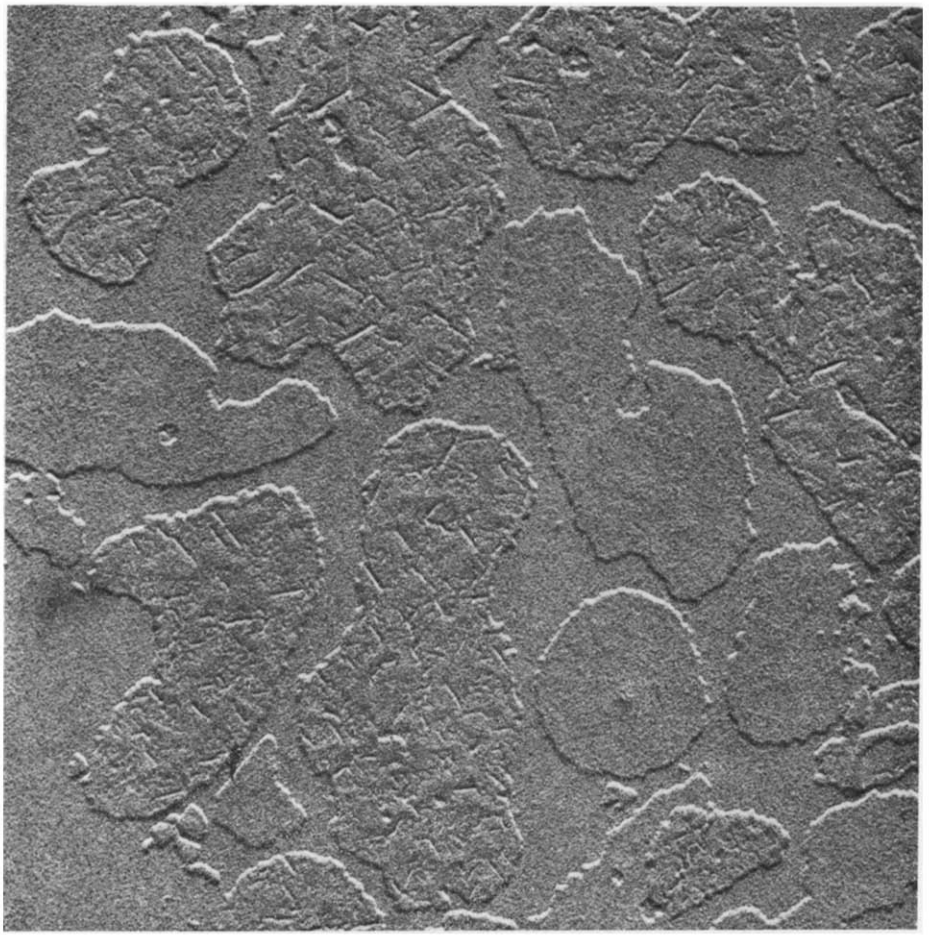

Fig. 5. Electron micrograph of isolated purple membrane from *H. cutirubrum* sprayed onto freshly cleaved mica and shadowed with platinum carbon. Magnification 51 100 ×.

and purified soy bean phosphatidylcholine, light induces an alkalinization of the medium, which relaxes rapidly in the dark. This shows that *H. cutirubrum* purple membrane translocates protons during illumination and in the same direction as *H. halobium* purple membrane (Fig. 7).

The results presented show that the two purple membrane preparations are essentially identical with respect to molecular weight, retinal content, lipid composition, fingerprinting of peptides from pepsin digestion and behaviour as light-activated proton pump. Thus, there would appear to be no species differences in purple membrane preparations, at least with respect to those from *H. halobium* and *H. cutirubrum*. However, final verification of identity of these two preparations must await determination of their amino acid sequence.

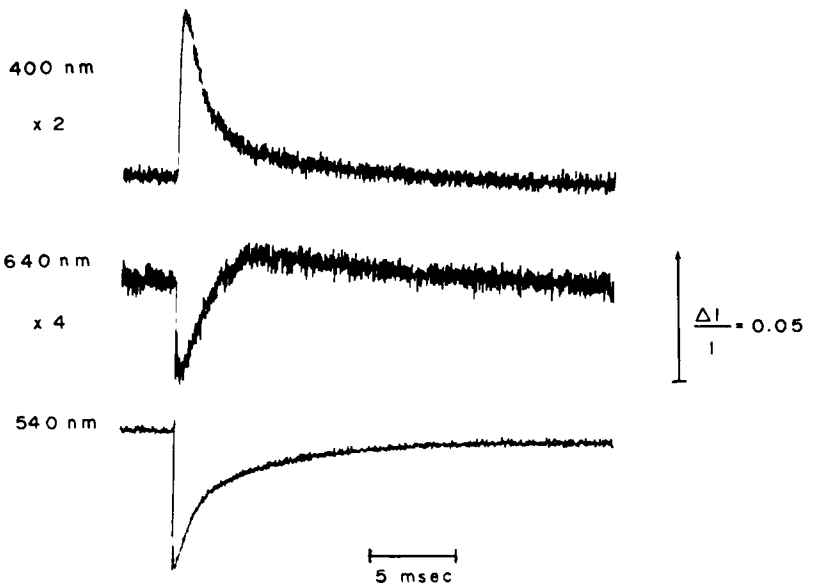

Fig. 6. Absorbance changes of isolated purple membrane from *H. cutirubrum* after exposure to a 1 μs light flash. The membrane was suspended in basal salt solution at pH 6.5. The traces at 400 and 640 nm are recorded at 2× and 4× higher sensitivity than the change at 540 nm. An increase in absorbance is indicated by the arrow on the scale mark.

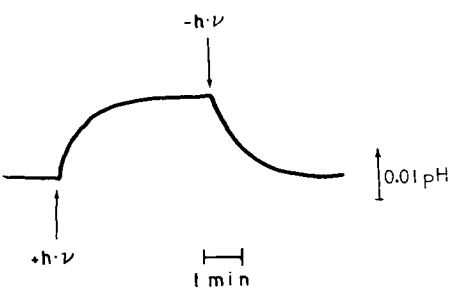

Fig. 7. Record of the pH change in an illuminated suspension of *H. cutirubrum* purple membrane reconstituted with soybean phosphatidylcholine to form vesicles and suspended in 75 mM K_2SO_4 at pH 5.0, protein concentration 0.04 mg/ml.

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