SEPARATION OF LIPIDS FROM PROTEINS AND CELL MEMBRANES BY DISC ELECTROPHORESIS

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During the course of an investigation of the behavior of disaggregated bacterial membranes on disc electrophoresis (M. R. J. Salton, Park E. Trefts and Margreth D. Schmitt, unpublished observations) we noticed that the yellow carotenoid of the detergent-treated membrane fractions of Micrococcus lysodeikticus moved as a band through the acrylamide gel. This band preceded the tracking dye through the gel on electrophoresis under the conditions described by Ornstein (1964) and Davis (1964) and the bands were particularly sharp and well-defined when deoxycholate was used in the preparation of the disaggregated membrane fractions. It occurred to us that this phenomenon may provide the basis for a general method for the electrophoretic separation of lipids from proteins and that it may even permit the separation of lipids from cell membranes.

To test the possibility of separating lipids from proteins, disc gel electrophoresis was carried out in the Model 12, Canalco equipment (Canal Industrial Corporation, Rockville, Md.) using the standard 7% polyacrylamide

gel separating at pH 9.5, the stacking gel at pH 8.9, with the tris-glycine buffer prepared according to the Canalco formulations. Solutions of bovine serum albumin and rabbit serum (from an immunized animal) were prepared by dissolving or diluting the proteins into 0.1M tris buffer, pH 7.5 and into 0.1M tris buffer containing sodium deoxycholate at a final concentration of 1% w/v. Lecithin and the total lipid extracts from Micrococcus lysodeikticus membranes were dissolved in 1% deoxycholate in buffer, to give solutions containing final concentrations of 1% w/v with respect to the lipids when the solutions were mixed with the proteins. The solutions (proteins in buffer alone, proteins in 1% deoxycholate, proteins in 1% deoxycholate together with 1% lipid) were finally mixed with an equal volume of 40% sucrose and 0.15 ml portions of each was layered on to the top of the stacking gel. Protein concentrations were adjusted so that each sample loaded on to the gel contained about 200-300 μg protein. Thus the amount of deoxycholate added in the sample was 750 μg. Electrophoresis was usually carried out at 2 ma per column for 55-65 minutes at room temperature. The gels were extruded from the columns and fixed and stained in 0.5% aniline black in 7% acetic acid. Fig. 1 illustrates the typical results obtained with bovine serum albumin (gels A-C) and rabbit serum (gels E-H) with the lipid-deoxycholate controls shown in gels D and I. The deoxycholate and lipid-deoxycholate bands were revealed as opaque zones on fixation in the acetic acid-dye mixture, with a relatively small affinity for the dye compared to the protein bands. The amount of deoxycholate in the sample could be reduced to 1/3rd (as in gels E-H) without affecting the separation. However, inclusion of deoxycholate (0.1%) in the gels and buffer gave very poor resolution. The lipid bands could also be revealed by staining with Oil Red O. It is of

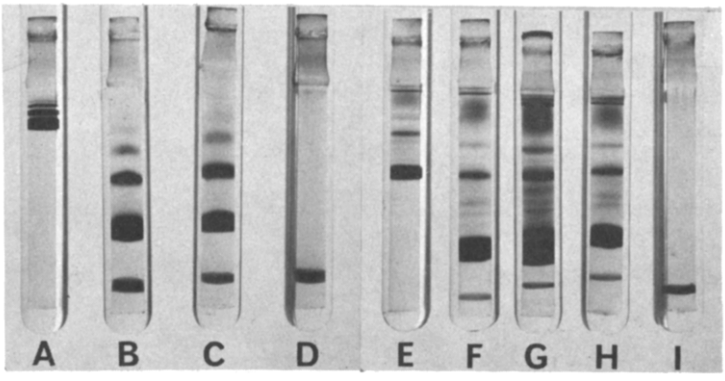

Fig. 1 Gels stained with aniline black after disc electrophoresis for 55-65 minutes at 2 ma/column. A. bovine serum albumin; B. bovine serum albumin + deoxycholate; C. bovine serum albumin + deoxycholate + lecithin; D. deoxycholate + lecithin; E. rabbit serum; F. rabbit serum + deoxycholate; G. rabbit serum (double quantity) + deoxycholate + lecithin; H. rabbit serum + deoxycholate + membrane lipid; I. deoxycholate + membrane lipid.

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interest to note that the deoxycholate has increased the mobility of the proteins without causing any major distortions of the patterns of protein bands.

That the lipid does indeed migrate through the polyacrylamide gel, perhaps as a complex (micellar?) with the deoxycholate, was confirmed by using ^{32}P-labelled lipid extracted from isolated Micrococcus lysodeikticus membranes prepared from the organism grown in the presence of radioactive phosphate. The ^{32}P-labelled lipid was dissolved in the deoxycholate as described above, mixed with bovine serum albumin, layered on the top of the stacking gel and electrophoresis carried out at 2 ma per column for approximately 60 minutes. Gels were frozen by placing them on the surface of a

steel block in a bath packed with crushed, solid CO_2 and they were sliced in the frozen state. The thin gel slices were crushed, dispersed on planchettes, dried and the radioactivity in each determined. Fig. 2 illustrates the separation of the ^{32}P-labelled lipid as a sharp 'peak' of radioactivity. In addition to demonstrating the separation of ^{32}P-labelled phospholipids in the total lipid fractions extracted with organic solvents from the bacterial membranes, we have also obtained very similar results with ^{14}C-palmitic acid and ^{14}C-cholesterol in this system.

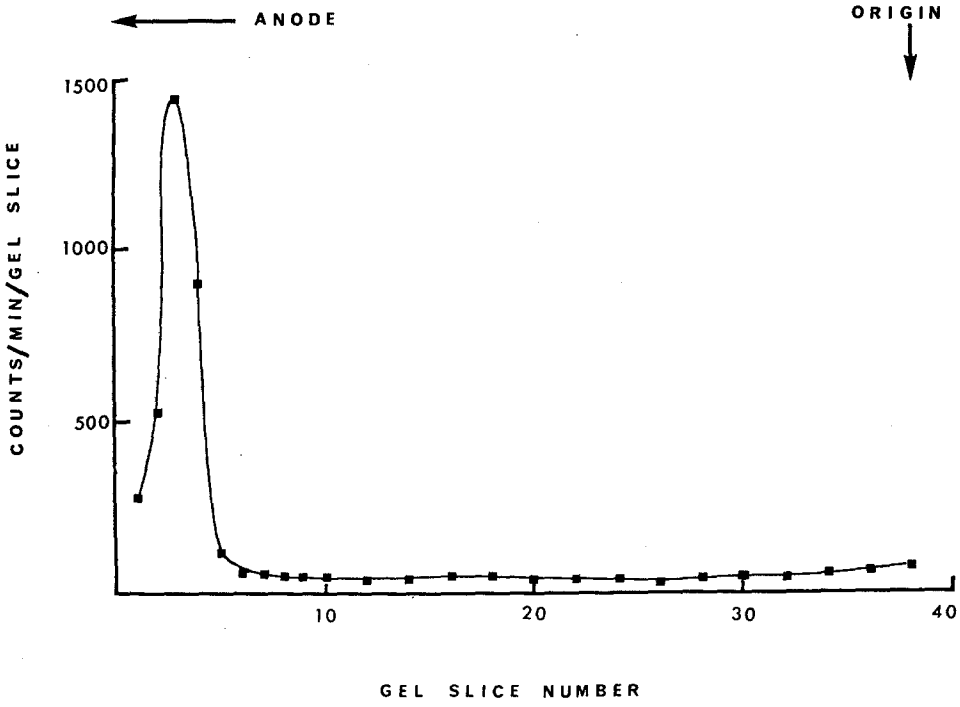

Fig. 2 Disc gel electrophoresis of ^{32}P-labelled lipid extracted from Micrococcus lysodeikticus membranes, dissolved in deoxycholate and electrophoresed in the presence of bovine serum albumin. Protein bands are in the zone from gel slice number 9 to 25.

Finally, in order to determine whether or not it is possible to electrophorese away the lipid from cell membranes, ^{32}P-labelled membranes of

Micrococcus lysodeikticus were dispersed by sonication (Salton and Netschey, 1965), mixed with deoxycholate and incorporated into a sample gel. After polymerization of the sample gel, the stacking gel and separating gels were formed in the usual way and electrophoresis was performed for 40 minutes at 4 ma/column. The gels were frozen, sliced and assayed for radioactivity. The results shown in Fig. 3 demonstrate a very clean separation of the lipid, with little residual lipid left in the sample gel. From our extensive studies on the disc electrophoresis of membrane proteins, we can conclude that little, if any, phospholipid is covalently linked to protein in these membranes.

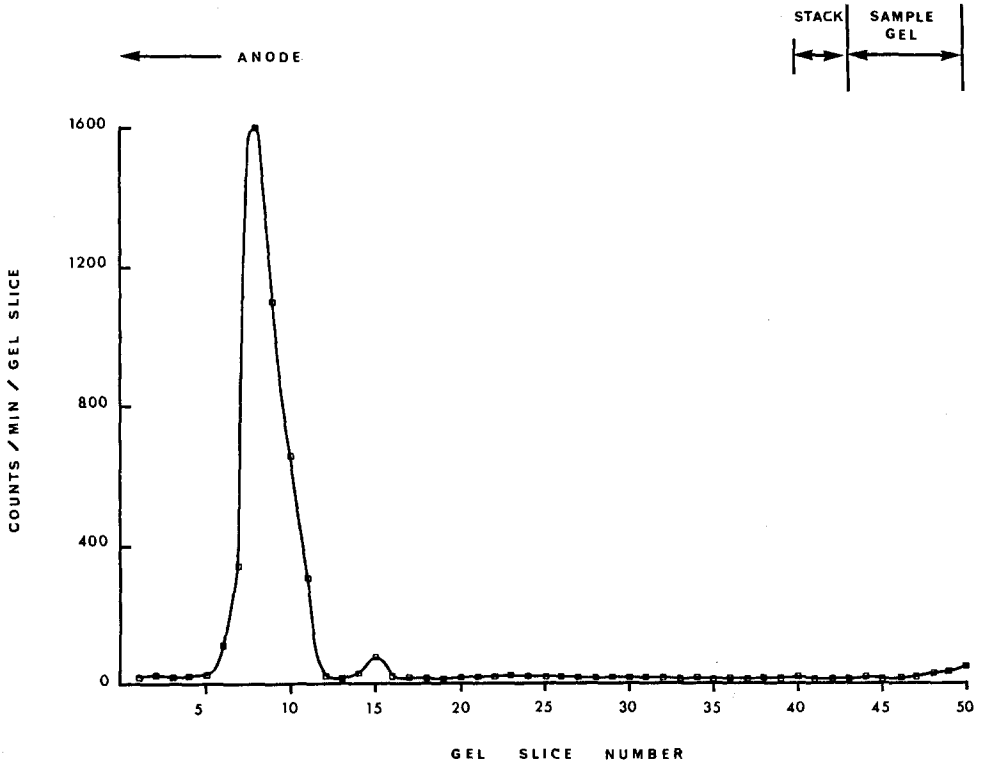

Fig. 3 Disc gel electrophoresis of ^{32}P-lipid from sonicated Micrococcus lysodeikticus membranes mixed with deoxycholate and incorporated into the sample gel. Electrophoresis carried out for 40 minutes at 4 ma/column.

Thus, by the inclusion of deoxycholate in the sample it is possible to effect an electrophoretic separation of lipid and other "lipid-soluble" constituents such as carotenoids, sterol and free fatty acid, from proteins and from cell membranes. This method may have potentialities for separation of lipids from tissues and isolated organelles and may provide functional entities which would not survive "defatting" with organic solvents.

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