A DETERGENT-POLYACRYLAMIDE GEL SYSTEM FOR ELECTROPHORETIC RESOLUTION OF MEMBRANE AND WALL PROTEINS

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Received March 5, 1971

Summary: Membrane and envelope proteins are separated more clearly in a detergent than a non-detergent polyacrylamide gel electrophoretic system. The molecular weights of the several bands are judged by comparison to internal standards of known molecular weight. Micrococcus lysodeikticus membranes contain more proteins than either Erwinia sp. or Serratia marcescens, and nearly as many as the entire envelope complex of the latter.

Many investigators (1-16), including ourselves, have examined bacterial membrane and envelope complexes to determine the number and variety of protein components by polyacrylamide electrophoresis. The systems differ in numerous details of which, as shown in this manuscript, one of the more important is the addition of a detergent through comparison of the membrane of cell envelope complexes of three microorganisms--M. lysodeikticus, Gram-positive, Erwinia sp. and Serratia marcescens, Gram-negative, have been confirmed in detergent vs non-detergent containing electrophoretic systems.

Materials and Methods: M. lysodeikticus was grown in our defined medium (17), and membranes isolated as described previously (18). Erwinia sp. and S. marcescens were grown on nutrient agar for 24 hr at 25°C and 30°C respectively and envelopes obtained by breaking the cells in an X-press (0.0025M Tris buffer, pH 7.4), followed by centrifugation for 30 min at 54,500g.

All membrane and envelope preparations were washed three times in

cold 0.0025 M Tris buffer (pH 7.4) for 30 min at 54,500g, lyophilized to dryness, and stored at -30°C.

Polyacrylamide gel electrophoresis (detergent system) was performed using 10% acrylamide as described by Weber and Osborn (19) in glass tubes 75 mm long having an ID of 8.5 mm.

Samples (2.5mg/ml) were dissolved (37°C for 2 hr) in 0.01 M sodium phosphate buffer (pH 7.0), containing 1% sodium lauryl sulfate and 1% β-mercaptoethanol. Prior to electrophoresis, glycerol (3 drops/ml solvent buffer) was added and the solubilized sample (30 - 50 µl) then applied to the top of the gels. Buffalo Black in glycerol served as the marker dye; electrophoresis was performed at a constant current of 5.0 mA/tube for 5 - 6 hr.

Staining (Coomassie Blue) was also performed according to Weber and Osborn; however, destaining was modified in that it was accomplished at 37°C on a reciprocal shaker with periodic changes of the destaining solution until the gels cleared (usually 6 - 8 times).

For electrophoresis in a non-detergent system, we utilized 7% acrylamide gels in the pH 2.7 system (without stacking gel) described by Neville (20). Both chambers of the electrophoretic apparatus were filled with 10% acetic acid (21), and the lower electrode served as the cathode. Electrophoresis was accomplished in 40 min using a current of 2.5 mA/tube.

To avoid anomalies caused by persulfate (22), gels were pre-electrophoresed for 1 hr. After such pre-running, both chambers were emptied and refilled with freshly prepared 10% acetic acid.

Samples were solubilized (3mg/ml) in the solvent described by Takayama et al., (21): 60 - 70 µl (per gel) were used for electrophoresis. For staining, gels were immersed 1 hr in 7% acetic acid containing 1% Buffalo Black. Destaining was accomplished as above except 8% acetic acid was used in the destaining solution.

Electropherograms were made using a Canalco Model K densitometer.

Results and Discussion: Comparative protein profiles (electropherograms) obtained using the detergent and non-detergent gel systems are given in Figs. 1-6. Numbering of bands is based on resolution of stained proteins by densitometer scanning as well as direct observation of gels. Approximate molecular weights indicated on the detergent electropherograms were extrapolated from data shown in Figure 7.

Several general and specific points can be made.

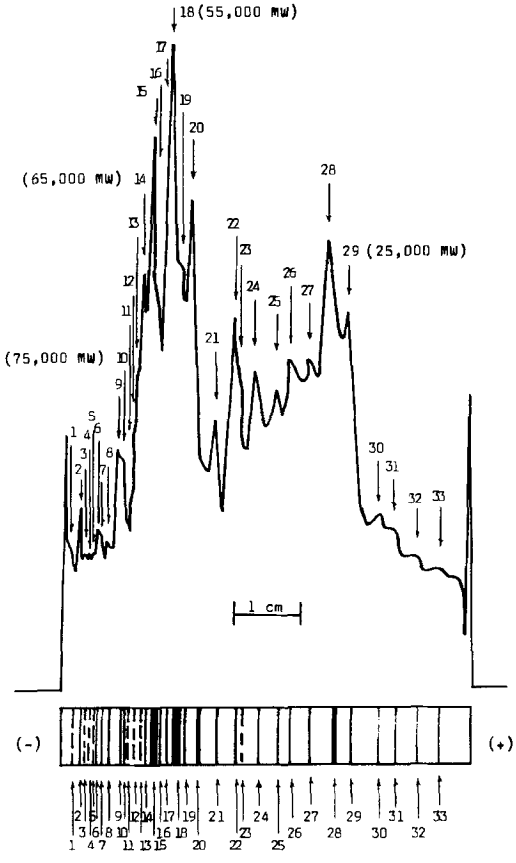

Fig. 1

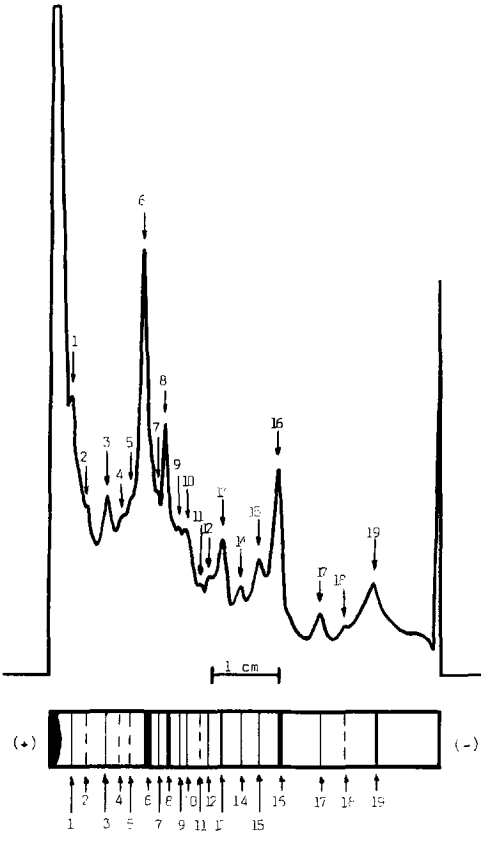

Fig. 2

Figures 1-6. Electropherograms and drawings of membrane or envelope fractions. Figs. 1-2, membranes from M. lysodeikticus; Figs. 3-4, envelopes from Erwinia sp.; Figs. 5-6, envelopes from S. marcescens. Profiles shown in Figs. 1, 3, and 5 were obtained using the detergent system; those in Figs. 2, 4, and 6 the non-detergent system.

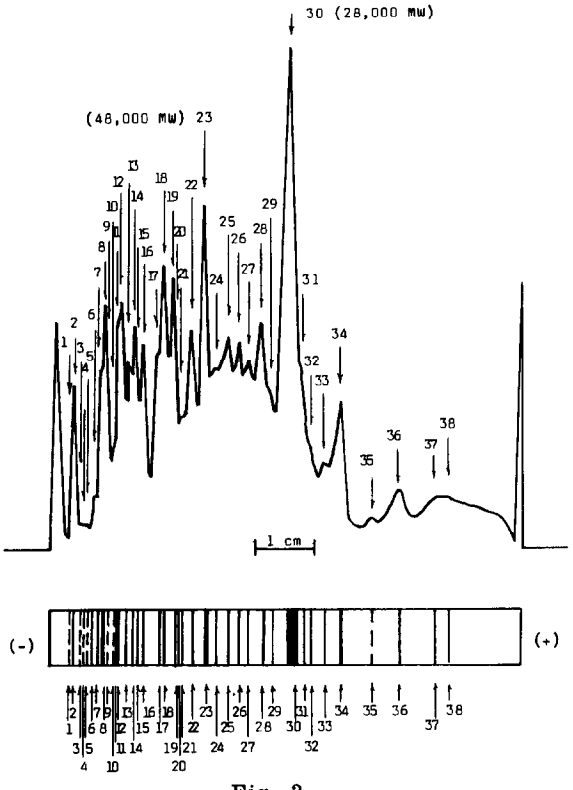

Fig. 3

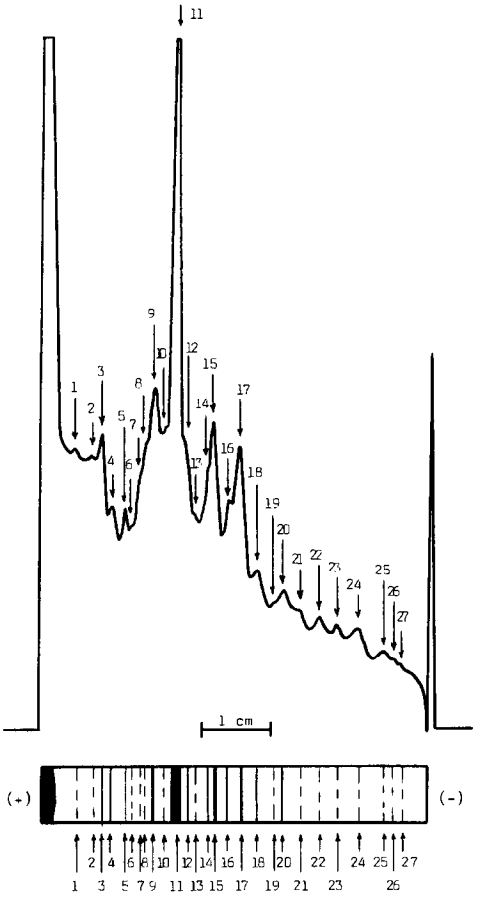

Fig. 4

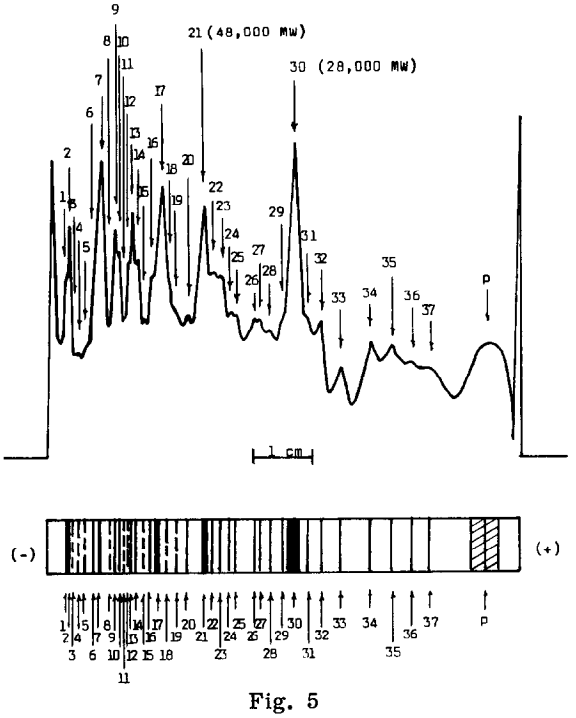

Fig. 5

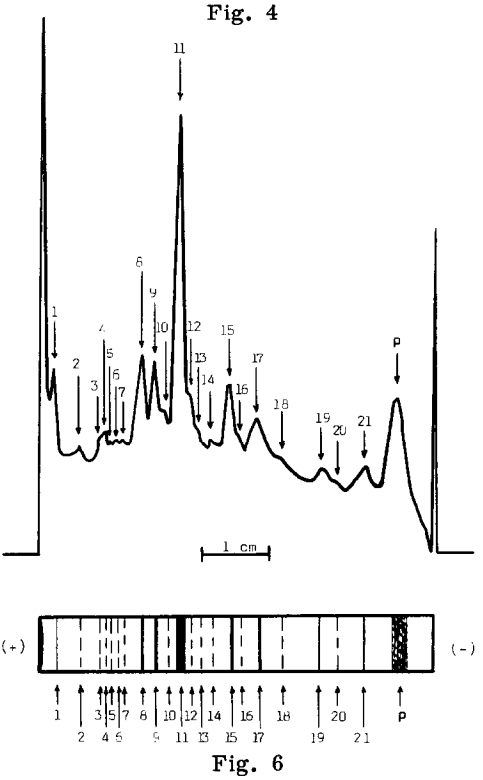

Fig. 6

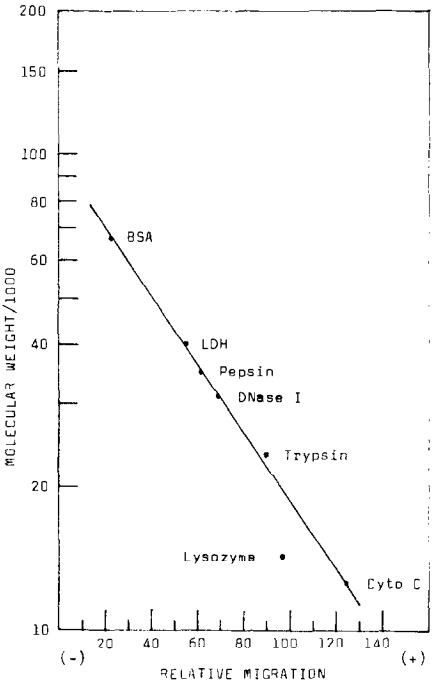

Figure 7. Relative migration of proteins having known molecular weights in the detergent system.

a) The membrane of the Gram-positive organism, M. lysodeikticus, as well as the envelopes (cell wall plus cell membrane) of the two Gram-negative organisms are very complex. Because the degree of complexity resolved by polyacrylamide gel electrophoresis is far greater than previously observed using the Model E ultracentrifuge (18), the membrane subunit concept, as previously envisaged, should be abandoned until data to permit a more meaningful definition can be obtained.

b) It has become very obvious to us that resolution of protein components from the membrane of M. lysodeikticus is far greater in the detergent gel system than in the non-detergent gel system. Although this conclusion also holds true for the Gram-negative organisms studied, the differences are not as striking.

We are not sure if the decreased number of proteins resolved using the non-detergent system is due to poor solubility in the Takayama

solvent (21), or increased dissociation by detergent of membrane protein complexes into a large number of peptides. It may be a combination of both activities; however, as shown by the gel representations in Figs. 2, 4, and 6 large amounts of protein never enter the non-detergent gels. Many of the proteins, which appear to be solubilized by the Takayama solvent, precipitate after being placed on top of the gels in the acetic acid buffer as soon as current is applied and mixing is initiated. This is particularly apparent when using the cell membrane of M. lysodeikticus and may indicate some type of fundamental difference between the membrane of a Gram-positive organism and the envelope complex of Gram-negative bacteria.

c) All bands shown stained either with Coomassie Blue or Buffalo Black and can therefore be considered to be completely or partially protein except the band labelled P in Figs. 5 and 6. This band contains prodigiosin, the red water-insoluble pigment of S. marcescens. As shown, this pigment runs ahead of all proteins and can be resolved using either gel system. Care must be exercised when using the detergent gel system, however, since the pigment usually runs off the gels during the running time necessary for satisfactory resolution of protein components. The carotenoid pigments of M. lysodeikticus behave in much the same way.

d) Utilizing what appear to be optimal conditions for gel electrophoresis (detergent system), the number of proteins that can be resolved from the cell membrane of the Gram-positive organisms (M. lysodeikticus) is almost equal to the number of proteins obtained from the entire envelope complex of the Gram-negative organisms. Study of additional organisms should allow us to determine if this observation constitutes a generalized truth.

e) Because the number of components that can be resolved from all organisms is greatest using the detergent gel system, this procedure represents the superior electrophoretic technique for study

of bacterial membrane proteins at this time. The number of proteins is large, but may still represent only a minimal number since it is possible that several are sufficiently close in molecular weight to resist resolution by this procedure.

We have tried, without success, to increase still further the number of proteins resolved by incorporating EDTA into the gels, solvent, and buffer. Also, although addition of β-mercaptoethanol to the gels increases sharpness of the separations, no increase in total number of proteins is apparent.

Increased resolution of proteins in the detergent gel system argues strongly in favor of this procedure; however, an additional reason for utilizing this technique relates to the ease of molecular weight determination. Estimates (included in Figs. 1, 3, and 5) reveal that proteins having a relatively wide range of molecular weight (about 15,000 to over 100,000 daltons) are present in the membrane and envelope complex of these bacteria. It may be unfortunate that resolution of proteins having molecular weights less than 10,000 daltons is not reliable in gels containing 10% or less acrylamide (19). Considering this possible drawback, the number of proteins that can be resolved seems to be fairly evenly distributed over the range of molecular weights shown.

ACKNOWLEDGMENT

This investigation was supported in part by grants from the National Institutes of Health (AI-02530), the National Science Foundation (GB-18,845) and a Public Health Service research career program award to E. A. G. (GM-13,968).

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