Acrylamide Gel Electrophoresis of Group A Streptococcal Cell Walls

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Patterns obtained by acrylamide gel electrophoresis of group A streptococcal cell walls were characteristic for M types 1, 2, 3, 4, and 5, respectively. Best results were obtained with cell walls which had been solubilized with a phenol-acetic acid-water mixture. The method will make it possible to compare group A streptococci of epidemiological and clinical significance and to separate components for more critical analysis.

Recognition of specific serological types of group A streptococci is based on cell wall proteins. One of these, M protein, is an alcohol-soluble, acid- and heat-resistant antigenic protein which can be denatured by proteolytic enzymes and is associated with virulence of the organism (5). Besides the M protein, various other cell wall proteins, such as T and R, have been described (4, 11). The present investigation showed that patterns obtained by acrylamide gel electrophoresis of streptococcal cell walls were characteristic for selected M types of group A streptococci. Proteins from whole cells and cell walls were extracted by acid-heat and by phenol-acetic acid-water. Electrophoretic patterns for each protein preparation were compared.

MATERIALS AND METHODS

Representative strains of M types 1, 2, 3, 4, and 5 from the collection of the National Streptococcal Disease Center (NSDC) were used. The serological group and M and T type were confirmed on all strains at the beginning of the study (3, 8, 10). Acid-heat extracts of whole cells were prepared according to Lancefield’s method (3).

For cell wall preparations, the streptococci were
grown 18 hr in 3 liters of Todd-Hewitt Broth (Difco). Between 1.0 and 2.0 g of cells were harvested and washed three times in 0.01 M phosphate-buffered saline, pH 7.2, and frozen at -20 C until ready for use. They were then disrupted for 5 min in a Braun (Bronwill Scientific, Rochester, N.Y.) homogenizer (1). After disruption, the cellular material was separated from the glass beads by filtration through a Buchner funnel with Whatman no. 41 filter paper. The cell walls were then separated by centrifugation at 34,800 X g for 30 min in a Sorvall RC2-B refrigerated centrifuge. After treatment with pancreatic deoxyribonuclease and pancreatic ribonuclease (Sigma Chemical Co., St. Louis, Mo.; reference 6), the purified cell walls were washed three times with sterile distilled water and frozen at -20 C. The amount of cell wall material collected varied from 400 to 800 µg. The protein content of the cell walls was determined by the method of Lowry et al. (7) and adjusted by the addition of distilled water so that equal concentrations were tested for each streptococcal type. Acid-heat extracts of the cell wall material were prepared and the presence of M antigen was determined (10). The extracts were saved for electrophoresis at a later time. The protein of the cell wall material was solubilized with phenol-acetic acid-water (2:1:0.5, v/v/v; reference 9).

The apparatus for acrylamide gel disc electropho-

![Densigraphs of protein bands on acrylamide gels of types 1 to 5. Numbers on abscissae and ordinates do not represent unit values but are included for comparing patterns among types.](http://aem.asm.org/)
resis was fabricated as described by Davis (2). Electrophoresis of acid-heat extracts of whole cells and cell walls was performed according to the methods of Davis (2) and the following modifications of Rottem and Razin (9). The gel contained 8.4% (w/v) acrylamide in final concentration, 1.0 ml of freshly prepared 0.5% (w/v) ammonium persulfate, and 0.025 ml of \( N,N',N''-\)tetramethylethylenediamine. The buffer used was 10% acetic acid (9). Electrophoresis was carried out at room temperature for 90 min at a constant current of 5 ma/tube. All runs were repeated at least twice. While immersed in ice water, the gels were removed from the tubes. The gels were stained for 2 hr with 1% naphthol blue black in 7% acetic acid; then destained electrophoretically in 7% acetic acid for 30 to 40 min at a constant current of 5 ma/tube. Densicord readings and Polaroid photographs of all preparations were made.

RESULTS AND DISCUSSION

Electrophoretic patterns characteristic of solubilized cell wall preparations of strains of M types 1 through 5, respectively, are shown in Fig. 1. Their corresponding densigraphs are shown in Fig. 3. Careful examination of areas designated in Fig. 1 indicates that different patterns can be demonstrated for each M type. The number of bands lying in each area, their spacial relationships, and their intensities are peculiar to each M type. Electrophoretic patterns of cell wall proteins of four different strains of M type 4 are shown in Fig. 2.

The results obtained in these studies provide further evidence that group A streptococci can be subdivided into types dependent upon the presence of characteristic protein constituents in the cell wall. Acrylamide gel electrophoresis apparently provides a method for comparing strains of group A streptococci of epidemiological and clinical significance and for separating components for more critical analyses.

LITERATURE CITED