Rapid Microprocedure for Isolating Detergent-Insoluble Outer Membrane Proteins from Haemophilus Species

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A rapid microprocedure for isolating detergent (sodium N-lauroyl sarcosinate)-insoluble major outer membrane proteins from Haemophilus species produced results qualitatively identical to those obtained with a commonly used preparative isolation procedure. Proteins isolated by both procedures were compared by sodium dodecyl sulfate-polyacrylamide gel electrophoresis after staining with Coomassie brilliant blue R-250. The time for outer membrane protein isolation was substantially reduced with the rapid procedure, allowing a larger number of membrane preparations to be obtained rapidly for routine analysis.

Membrane proteins are important determinants of pathogenicity in many bacteria. Haemophilus species can be subtyped (2, 3, 12, 18) and often differentiated (4) on the basis of these proteins. In general, gram-negative bacterial outer membrane proteins are isolated by preparative techniques (9-11, 13, 22, 24), some of which require an initial enrichment step that exploits a selective solubility of cytoplasmic membrane proteins in a particular detergent (1, 5-7, 21, 22). The conditionally insoluble membranes contain integral and likely some peripheral proteins which are released by relatively small changes in pH or ionic strength and are referred to as outer membrane proteins and, more appropriately, detergent-insoluble membrane proteins (6, 15). Phenotypic and epidemiologic information can be obtained by comparing the protein profiles of these sodium dodecyl sulfate (SDS)-solubilizable membranes (6, 8) after SDS-polyacrylamide gel electrophoresis (PAGE) and staining (2, 4, 12, 14, 15, 18, 23).

We compared the membrane protein profiles of three species of Haemophilus obtained by a rapid microprocedure (rapid outer membrane protein [ROMP] procedure) with profiles obtained by a commonly used preparative isolation procedure (4) to determine whether the two procedures produced comparable results.

MATERIALS AND METHODS

Bacterial cultures and cell harvest. H. influenzae type b KC525 and H. aegyptius NCTC 8502 were obtained from the culture collection of the Bacterial Reference Laboratory, Meningitis and Special Pathogens Branch, Division of Bacterial Diseases, Center for Infectious Diseases, Centers for Disease Control, Atlanta, Ga., and H. parainfluenzae ATCC 7857 was obtained from the Sexually Transmitted Diseases Laboratory Program, Center for Infectious Diseases. Cells stored at -70°C were inoculated onto chocolate agar plates (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 37°C for 24 h in a candle extinction jar. Isolated colonies (n = 2 to 4) were inoculated into 10 ml of brain heart infusion broth (Difco Laboratories, Detroit, Mich.) supplemented with 5 μg each of hemin and NAD (Sigma Chemical Co., St. Louis, Mo.). The inoculated tubes were slowly rotated (100 rpm) overnight (circa 18 h) at 37°C with the caps loosened; after 18 h of growth, the cultures were in the stationary phase. A chocolate agar plate was streaked from each culture and incubated as described above to check for purity. The optical density of each culture was measured and adjusted to between 0.5 and 0.6 at 600 nm by dilution with sterile supplemented brain heart infusion broth. A 10-ml sample of the adjusted bacterial suspension was centrifuged at 5,000 × g for 10 min at 4°C. The supernatant fluid was decanted, the pellet was suspended in 1.5 ml of cold (4°C) 10 mM HEPES (N-2-hydroxyethylpiperezine-N'-2-ethanesulfonic acid) buffer (pH 7.4), and the cell suspension was transferred to a 1.5-ml centrifuge tube. The cells were then washed once by centrifugation at 15,600 × g for 2 min at 4°C in a microcentrifuge (Brinkmann Instruments, Inc., Westbury, N.Y.), and the supernatant fluid was decanted. The cell pellets were used immediately or stored at -70°C.

Preparative outer membrane protein and SDS-PAGE. The procedures for detergent-insoluble membrane protein preparation (outer membrane proteins), SDS-PAGE (4), and gel analysis (19) have been previously described.

ROMP procedure. Sodium N-lauroyl sarcosinate (Sarkosyl; Pfaltz and Bauer, Stamford, Conn.)-insoluble proteins (referred to as outer membrane proteins) were obtained from the cell pellets by a modification of the procedures of Filip et al. (6) and Portnoy et al. (20). Cells pellets were suspended in 1 ml of 10 mM HEPES buffer (pH 7.4) and, while kept on ice, sonicated (six bursts, 10 s each at 40 W) with a Sonifer cell disruptor (Heat Systems Co., Melville, N.Y.) fitted with a microtip. The unbroken cells and debris were removed by centrifugation at 15,600 × g for 2 min at 4°C. The supernatant fluid was transferred to a 1.5-ml centrifuge tube, and the cell membranes were sedimented from the supernatant fluid by centrifugation at 15,600 × g for 30 min at 4°C in a microcentrifuge (Brinkmann). The supernatant fluid was decanted, and the cell membrane pellets were thoroughly suspended in 0.2 ml of 10 mM HEPES buffer (pH 7.4) by repeated pipetting. The cytoplasmic membranes were solubilized by addition of an equal volume of 2% Sarkosyl in 10 mM HEPES (pH 7.4) and incubated at room temperature for 30 min with intermittent mixing. The outer membranes were then pelleted by centrifugation as described above; the supernatant fluid was decanted, and the membranes were washed once (without resuspending the pellet) with 0.5 ml of 10 mM HEPES buffer. The membrane pellets were then suspended in 50 to 100 μl of 10 mM HEPES buffer.

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HAEMOPHILUS OUTER MEMBRANE PROTEIN ISOLATION

The commonly used preparative procedures for outer membrane protein isolation are somewhat cumbersome since they are time consuming and require relatively large amounts of cells and expensive equipment (an ultracentrifuge) (2, 4, 5, 15). The ROMP procedure required approximately 2 h to complete; the other procedure we used (4) required, on average, five times as long to prepare an equivalent number of preparations. For most replicate analyses there is no need to isolate preparative amounts of membrane protein since the anionic dye Coomassie brilliant blue R-250, the most commonly used protein stain, can detect as little as 0.5 µg of protein in a sharp band (8 resolved by SDS-PAGE, and silver staining is approximately 100 times more sensitive (17). The ROMP procedure allows large numbers of membrane preparations to be obtained rapidly for routine outer membrane protein analysis without requiring large amounts of cells or an expensive ultracentrifuge. This allows outer membrane protein analysis to be used, when appropriate, in the diagnostic laboratory as an adjunct to epidemiologic investigations.

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LITERATURE CITED