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SOLUBILIZATION AND ELECTROPHORETIC ANALYSIS OF *STAPHYLOCOCCUS AUREUS* MEMBRANE PROTEINS

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The protein composition of homogeneous *Staphylococcus aureus* 6538P cytoplasmic membranes was examined under denaturing electrophoretic conditions. A comparative analysis on the effectiveness of a variety of membrane solubilizing agents revealed the membrane protein extracts to be qualitatively similar as determined electrophoretically but different in the quantity of protein released; Zwittergent-314, sodium dodecyl sulfate, Triton X-100, Nonidet P-40, and sodium deoxycholate all proved to be effective solubilizing agents under the conditions examined. Fifty-five to sixty protein components were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis from homogeneous late-exponential phase membranes. The profile was unaffected when phenylmethylsulfonyl fluoride was included during membrane isolation and solubilization. Analysis of the solubilized membrane proteins by two-dimensional gel electrophoresis demonstrated in excess of 100 membrane protein components in a pH gradient between 3.5 to 7.7. The profile consisted of a heterogeneous mixture of mostly acidic components with isoelectric points between pH 4 and 5 and relative molecular weights between 158000 and 35000. Periodic acid-Schiff staining following sodium dodecyl sulfate gel electrophoresis revealed six to ten reactive bands with two of these bands also exhibiting a reaction with concanavalin A.

Introduction

The separation and enumeration of membrane proteins is a fundamental step in the study of the structure-function relationships that exist in bacterial membranes. Analysis of membrane proteins by high resolution sodium dodecyl sulfate [1] and two-dimensional gel electrophoresis [2,3] have aptly demonstrated the protein composition from several bacterial cytoplasmic membranes in addition to delineating the biochemical characteristics of proteins in this structure. At present, however, the protein complexity of the *Staphylococcus aureus* cytoplasmic membrane has not been fully established largely due to the difficulty in preparing homogeneous membrane preparations. We have previously demonstrated the usefulness of *S. aureus* 6538P in examining the non-denaturing electrophoretic profile and biological activities of the cytoplasmic membrane proteins [4,5].

In this communication, we extend the survey of *S. aureus* membranes by resolving the protein and protein subunit composition of these preparations under denaturing conditons. The solubilization sensitivity of *S. aureus* membranes, the approximate isoelectric points and relative molecular weights of the resolved proteins, and the reactivity of the separated membrane components to glycoprotein staining methods is described.

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Abbreviations: TEMED, *N,N,N',N'*-tetramethylethylene diamine; PMSF, phenylmethylsulfonyl fluoride.

Materials and Methods

Membrane preparation. Cytoplasmic membranes were isolated from late-exponential phase (8.5 h) *S. aureus* 6538P as described [5].

Sodium dodecyl sulfate polyacrylamide gel electrophoresis. SDS-polyacrylamide gel electrophoresis analysis of membrane proteins was performed as described by Laemmli [6] with the modifications described by Anderson and Anderson [7]. A slab gel of polyacrylamide was formed between two vertical glass plates (190 × 160 mm) separated by rubber strips (190 × 15 × 2 mm) on three sides of the mold. One glass plate was notched at the top (130 × 20 mm section) allowing for buffer contact with the gel in the vertical electrophoresis apparatus. Components for the separating gel included: 16.25 ml of 30% acrylamide + 0.8% *N,N*-methylenebisacrylamide, 12.5 ml running gel buffer (1.5 M Tris-HCl/0.4% (w/v) SDS, pH 8.8), 20 ml distilled water, 0.2 ml 10% (w/v) ammonium persulfate and 0.020 ml *N,N,N',N'*-tetramethylethylene diamine (TEMED); values for total acrylamide concentration (T) and gel porosity (C) are expressed as described by Hjerten [8]. The separating gel ($T = 10\%$; $C = 2.5\%$) was polymerized for 1 h. A 10 mm stacking gel ($T = 4.5\%$, $C = 2.5\%$) was molded above the separating gel with 0.9 ml 30% acrylamide + 0.8% *N,N*-methylenebisacrylamide, 1.5 ml stacking gel buffer (0.25 M Tris-HCl/0.2% (w/v) SDS, pH 6.8), 3.6 ml distilled water, 0.01 ml (w/v) 10% ammonium persulfate, and 0.005 ml TEMED.

All protein samples subjected to SDS-polyacrylamide gel electrophoresis were mixed with SDS-treatment buffer (0.01 M Tris-HCl, pH 7.0/2% (w/v) SDS/5% (v/v) 2-mercaptoethanol) in sealed 1.5 ml polyethylene microcentrifuge tubes and heated at 100°C for 2 min [16].

Samples were layered into the pockets and overlaid with SDS-polyacrylamide gel electrophoresis electrode buffer (0.025 M Tris-HCl/0.192 M glycine/0.1% (w/v) SDS, pH 8.3). Electrophoresis was performed at a constant current of 30 mA for 5.5 h. Gels were stained with 0.2% (w/v) Coomassie brillant blue R-250 in 50% ethanol and 5% acetic acid; destaining was performed in a series of ethanolic-acetic acid solutions. The following proteins (Pharmacia Fine Chemicals; Piscataway, NJ) were electrophoresed as described for SDS-polyacrylamide gel electrophoresis and served as relative molecular weight (M_r) standards: aldolase, 158000; transferrin, 90000; γ-globulin (H chain), 50000; ovalbumin, 45000; chymotrypsinogen, 25000; ribonuclease A, 13700.

Two-dimensional gel electrophoresis. Membrane proteins were separated by two-dimensional gel electrophoresis based on the method described by O'Farrell [9] and modified by Anderson and Anderson [10]. Isoelectric focusing in the first dimension was performed in glass rods (130 × 2.5 mm) containing the polyacrylamide gel (110 × 2.5 mm). Gel components for isoelectric focusing ($T = 4\%$, $C = 6\%$) included: 5.5 g urea, 1.33 ml 30% acrylamide + 1.8% *N,N*-methylenebisacrylamide, 2 ml 10% (v/v) Nonidet P-40, 2 ml distilled water, 0.5 ml pH 3.5–10 ampholyte, 0.05 ml pH 5–7 ampholyte, 0.025 ml 10% (w/v) ammonium persulfate, 0.01 ml TEMED. The glass rods were filled with this solution, overlaid with 8 M urea, and allowed to polymerize for 1–2 h. Following polymerization, the urea was removed and the gel overlaid with lysis buffer (0.6 M urea/2% (v/v) ampholytes pH 3.5–10/4% (v/v) Nonidet P-40) and equilibrated for 1 h. This solution was removed before sample application.

All samples subjected to isoelectric focusing in two-dimensional gel electrophoresis analysis were mixed with 2% (w/v) SDS, 5% (v/v) 2-mercaptoethanol, 20% (v/v) glycerol and heated to 100°C for 2 min. After cooling, urea was added to saturation (760 mg urea to 1 ml sample volume). Electrode solutions for isoelectric focusing consisted of 20 mM NaOH (cathode) and 10 mM H_3PO_4 (anode). The samples were focused for 18 h at 260 V. Following isoelectric focusing, the gels were extruded into SDS-equilibration buffer (0.125 M Tris-HCl, pH 6.8/2% (w/v) SDS/5% (v/v) 2-mercaptoethanol/10% (v/v) glycerol) for 15 min at 37°C and used immediately or frozen at −70°C. For pH gradient determination, one unequilibrated focused gel was sliced into 5 mm sections, placed into tubes containing 1 ml of degassed distilled water, and the pH determined as described [9].

Second dimensional electrophoresis of the equilibrated first dimension isoelectric focusing gel was performed as described for one-dimensional SDS-

polyacrylamide gel electrophoresis with the following modification: the isoelectric focusing gel was placed on top of the stacking gel in SDS-polyacrylamide gel electrophoresis and sealed into place with 1% agarose in SDS-polyacrylamide gel electrophoresis electrode buffer. Electrophoresis and staining were performed as described for SDS -polyacrylamide gel electrophoresis.

Periodic acid-Schiff staining in SDS-polyacrylamide gel electrophoresis. Following electrophoresis, the gels were processed according to Fairbanks et al. [11] for periodic acid-Schiff staining of glycoproteins. Samples to be compared by the two methods for glycoprotein staining were electrophoresed in the same slab so that a side-by-side comparison could be made of the protein components.

The proteins were fixed in the gel matrix by overnight incubation in 25% isopropyl alcohol-10% acetic acid. The gel was rinsed in 10% isopropyl alcohol-10% acetic acid for 8 h, followed by washes in 10% acetic acid for 36 h. The gel was then processed by the following steps: (1) 0.05% (w/v) periodic acid (2 h); (2) 0.5% (w/v) sodium arsenite in 5% acetic acid (30 min); (3) 0.1% (w/v) sodium arsenite in 5% acetic acid (20 min); (4) 5% acetic acid (20 min); (5) Schiff reagent (overnight); (6) 0.1% (w/v) sodium metabisulfate in 0.01 M HCl. A known glycoprotein control was electrophoresed and processed simultaneously. The periodate step was omitted as an additional control.

Concanavalin A binding in SDS-polyacrylamide gel electrophoresis. Following electrophoresis, the gels were processed according to Kelly and Cotman [12] for concanavalin A binding to glycoproteins. The proteins were fixed in the gel matrix by overnight incubation in 25% isopropyl alcohol-10% acetic acid. The gel was then processed as follows: (1) three washes (2 h each) in concanavalin A buffer (0.4 M NaCl/50 mM sodium phosphate, pH 6.5); (2) a 20 min incubation in concanavalin A buffer containing concanavalin A (0.75 mg/ml); (3) six washes (10 min each) in concanavalin A buffer; (4) a 15 min incubation in a solution of horseradish peroxidase, type VI, (65 μg/ml) in NaCl-phosphate buffer (0.1 M NaCl/50 mM sodium phosphate, pH 7.0); (5) six washes (15 min each) in NaCl-phosphate buffer; (6) a 15 min incubation in NaCl-phosphate buffer containing 500 μg/ml benzidine with 0.25 ml of 3% (w/v) H_2O_2. A glycoprotein control was electrophoresed and processed as the membrane proteins. Further controls included omitting the concanavalin A and peroxidase treatments for comparison.

Protein determination. Prior to protein determination, membranes were treated with 5% (w/v) SDS for 30 min at room temperature. Protein was assayed by the method of Lowry et al. [13] modified according to Wang and Smith [14] to facilitate determinations in the presence of detergent. All protein extracts containing any of the detergents used in this study were assayed by this method. The absorbance at 750 nm was measured against a dilution blank containing the same amount of the individual detergent. Bovine serum albumin was used as the standard in this analysis.

Results

Solubilization of proteins from S. aureus 6538P membranes and analysis by SDS-polyacrylamide gel electrophoresis

In order to select an agent for optimal membrane solubilizing potential and electrophoretic

TABLE I

SOLUBILIZATION OF MEMBRANE PROTEINS FROM ISOLATED *S. AUREUS* 6538P MEMBRANES BY VARIOUS AGENTS

Membranes (5.5 mg protein/ml) were treated with the respective agent (final detergent concentration = 0.8%, v/v) in 0.05 M Tris-HCl (pH 7.5) for 30 min at 20°C and centrifuged at 45000 × *g* for 1 h. The supernatant was assayed for protein as described in the text. Values represent the mean of duplicate determinations from two separate membrane preparations.

Agent	% Protein solubilized
Triton X-100	26.9
Nonidet P-40	23.4
Zwittergent-314	28.3
Sodium deoxycholate	22.9
Sodium dodecyl sulfate	27.3
Tween 80	14.8
Brij 56	10.8
Brij 58	17.0
Brij 96	15.8
Urea (3 M) [a]	18.5
EDTA (2.5 mM) [a]	7.7

[a] Final solubilizing concentration.

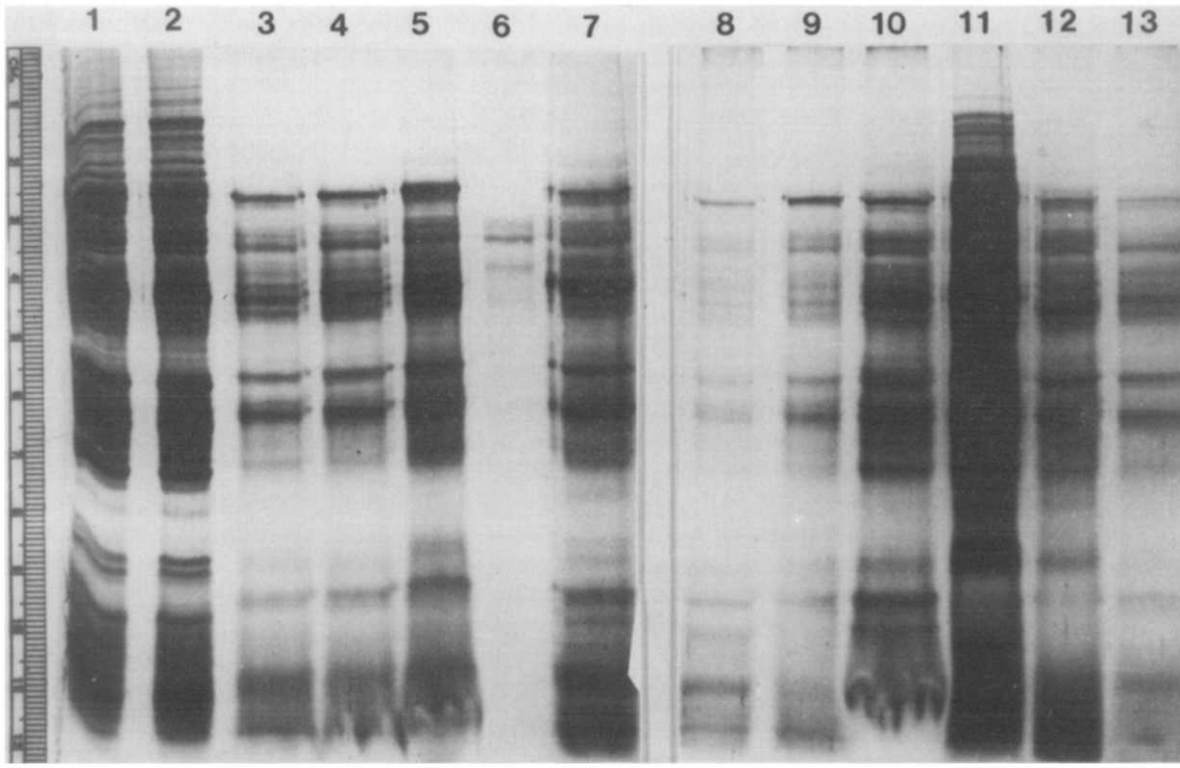

Fig. 1. SDS-polyacrylamide gel electrophoresis of membrane proteins solubilized by different chemical agents. Isolated *S. aureus* 6538P membranes were solubilized with various agents and 150 μg of the protein solubilized was electrophoresed by SDS-polyacrylamide gel electrophoresis ($T=10\%$, $C=2.5\%$). Prior to electrophoresis, the extracts were dialyzed for 6 h against 0.01 M Tris-HCl (pH 7.0), 0.1% SDS, and 0.1% 2-mercaptoethanol to achieve a similar ionic composition in each extract. Each sample was then mixed with SDS-treatment buffer (2% SDS/5% 2-mercaptoethanol) and heated at 100°C for 2 min. Lanes from left to right: 1 and 2, isolated membranes solubilized directly in SDS-treatment buffer without prior detergent treatment; 3, Nonidet P-40; 4, Triton X-100; 5, Zwittergent-314; 6, EDTA, 2.5 mM; 7, Urea. 3 M; 8, Brij 56; 9, Brij 58; 10, sodium deoxycholate; 11, SDS; 12, Brij 96; 13, Tween 80.

compatability, isolated membrane preparations were treated with various solubilizing agents of different chemical classes. Chemical and electrophoretic examination of the detergent extracts permitted a rapid comparison of their effectiveness against *S. aureus* membrane preparations. *S. aureus* 6538P membrane suspensions were treated with the respective agent (0.8%, v/v) for 30 min at room temperature. The membrane protein solubilized was defined as that which failed to sediment at $45000 \times g$ for 1 h. In every case after solubilization a small pellet was obtained following centrifugation. Of the non-ionic detergents used, Triton X-100 and Nonidet P-40 released 26.9% and 23.4%, respectively, of the membrane protein (Table I). The anionic detergents, sodium deoxycholate and sodium dodecylsulfate also favorably solubilized the membrane releasing greater than 23% of the membrane protein. The other non-ionic detergents, urea, and EDTA all released less than 18.5% (7.7–18.5). An amphoteric surfactant, Zwittergent-314 released 28.3% of the membrane protein under these conditions.

Following solubilization, the extracts were further analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1). Judging from the intensity of the stained protein bands and their respective number, the gel profiles roughly approximated the amount of protein solubilized by each agent as determined chemically. Triton X-100, Nonidet P-40, Zwittergent-314 and sodium deoxycholate solubilized a similar range of polypeptides that differed slightly

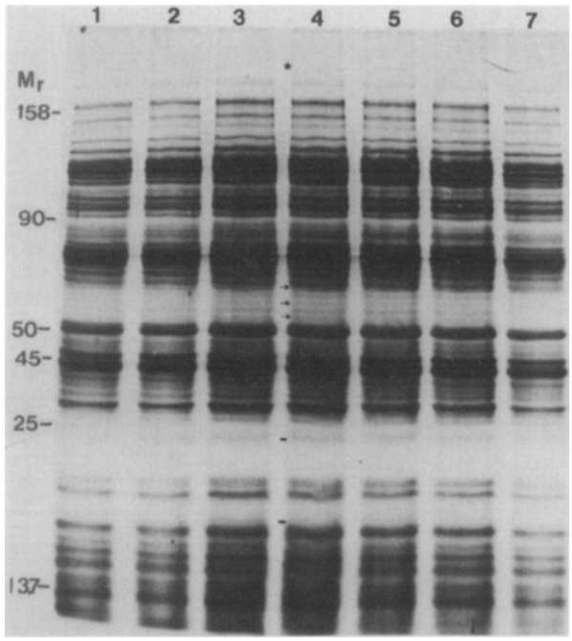

Fig. 2. SDS-polyacrylamide gel electrophoresis of *S. aureus* 6538P late exponential phase membrane proteins. Lanes 1–4 contain exponential phase membrane protein (1 and 2, 70 μg; 3 and 4, 140 μg). Lanes 5–7 contain exponential phase membrane protein solubilized as described in the text but in the presence of PMSF (5 and 6, 140 μg; 7, 70 μg). Molecular weight markers are indicated to the left ($M_r \times 10^{-3}$). The arrows indicate bands present in late-exponential membrane proteins that were missing from membranes prepared from stationary phase cells (12 h). The bars indicate the relative positions of newly appearing stationary phase membrane proteins.

in the presence of an additional band or bands. SDS solubilization completely overloaded the lane, while Brij 56, Brij 58, Tween 80, and EDTA did not shown extensive solubilization. Although solubilization with urea released only 18.5% of the membrane protein, the profile showed a range of bands similar in resolution to the anionic and non-ionic detergent treatments.

Direct SDS-polyacrylamide gel electrophoresis examination of membrane proteins

Following these initial observations on the effects of various solubilizing agents on the *S. aureus* 6538P membrane preparations, late-exponential phase membranes were treated directly in SDS-treatment buffer and electrophoresed in SDS-polyacrylamide gel electrophoresis slabs; SDS appeared to have the best potential in releasing membrane proteins. Under these conditions, the *S. aureus* 6538P membranes were resolved into 55–60 Coomassie blue-stained bands (Fig. 2). The treatment completely solubilized the membrane and most of the sample was able to enter the gel as judged by the lack of staining at the gel interface. Certain bands in the gel were more prominent with respect to staining (relative molecular weight (M_r): 35000, 45000, 50000, 75000, 125000). Some higher molecular weight components (*) were found between the gel application slot and the 158000 marker. Several diffuse bands were located below 25000 and two bands were located between the lowest molecular weight standard and the dye front.

To examine the possibility that some proteolysis may have occurred during cellular fractionation thereby affecting the electrophoretic profiles, the serine-type protease inhibitor [15] phenylmethylsulfonyl fluoride (PMSF, 0.1 mM) was added to the buffers used in membrane isolation. In addition, to dismiss the possibility of proteolytic breakdown during membrane solubilization, the membrane preparations were mixed with PMSF (20 parts protein: 1 part PMSF) prior to solubilization. There were no visible changes in the electrophoretic patterns detectable by this technique with or without the protease inhibitor (Fig. 2).

Two-dimensional gel electrophoresis examination of S. aureus membrane proteins

To further delineate the protein species in the *S. aureus* 6538P membrane, the components were resolved using isoelectric focusing (first dimension) and SDS-polyacylamide gel electrophoresis (second dimension). The solubilization conditions as described by O'Farrell facilitated the most effective solubilization and distribution of protein components. Under these conditions, in excess of 100 spots were demonstrated following Coomassie blue staining (Fig. 3). Most of the spots were focused in the pH range 4.04–4.84 and had approximate molecular weights between 158000 and 35000. The spots appeared to differ in quantity judging by the intensity of staining. Components present in greater proportions gave the characteristic 'teardrop' shape as has been noted [9]. Increased

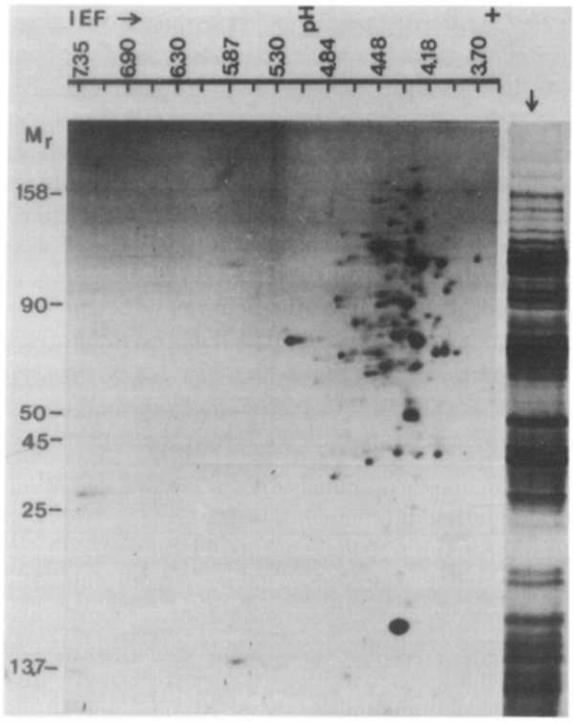

Fig. 3. Two-dimensional gel electrophoresis profile of *S. aureus* 6538P late exponential phase membrane proteins. Isolated membrane preparations were solubilized in 2% SDS, 5% 2-mercaptoethanol at 100°C for 2 min. The extract (140 μg membrane protein) was separated by isoelectric focusing (IEF) ($T=4\%$, $C=6\%$; first dimension) in a 3.5–7.7 pH gradient (top; left to right). Following isoelectric focusing, the gel was equilibrated for 15 min as described in the text and placed onto an SDS-polyacrylamide gel electrophoresis slab ($T=10\%$, $C=2.5\%$) for second dimension electrophoresis (top to bottom). A one-dimensional SDS-polyacrylamide gel electrophoresis lane (140 μg protein) was run simultaneously adjacent to the slab (right). M_r values indicated are as described in the text ($M_r \times 10^{-3}$).

membrane protein loads overshadowed many less intense spots although the two-dimensional profile shown represents the maximum resolution under these conditions. A lane of solubilized membrane protein (140 μg) was co-electrophoresed in the second dimension without prior isoelectric focusing (indicated to the right side of the gel in Fig. 3). The areas of banding in the SDS-polyacrylamide gel electrophoresis lane closely paralleled the areas and locations of the spots in the 2D gel profile.

A schematic of the two-dimensional gel electrophoresis profile is presented (Fig. 4) and represents a composite of three determinations. Although some variability was noticed, the spots were superimposable if the identical membrane isolation conditions and electrophoretic conditions were followed. A number of spots (B13.7; D13.7; and E13.7) ran close to the dye front. Two components were found with isoelectric points less than 4.00 (A125; A45). Although most components were found between pH 5.30 and 4.04, a few less distinct spots were found outside this area (7.35,35; 5.87,13.7). These regions did not attain the characteristic spot appearance but remained diffuse and slightly streaked even at increased membrane protein loads. It was possible that this system did

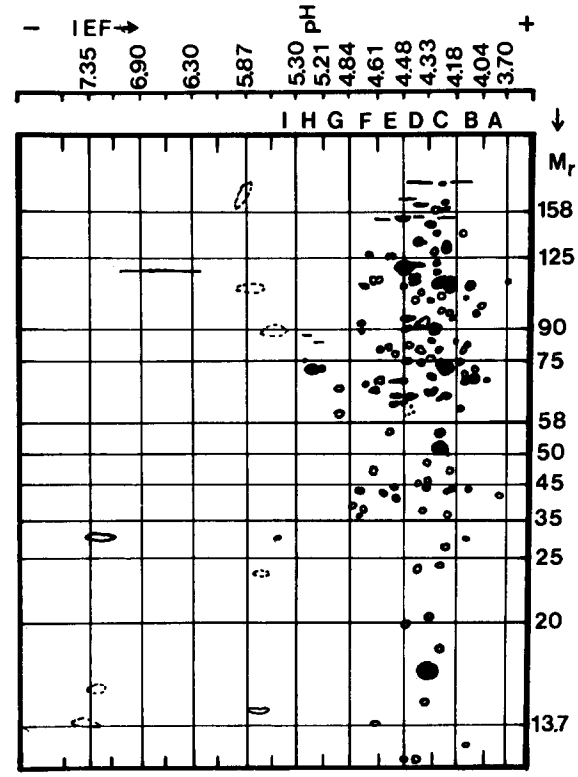

Fig. 4. Schematic of the *S. aureus* 6538P late-exponential phase membrane protein map in two-dimensional gel electrophoresis. The vertical lines that divide the map are based on the pH measurements from the isoelectric focusing (IEF) dimension (letter designations for individual ranges), while the horizontal lines represent the relative molecular weights. Major spots are shown in solid black and the spots of medium intensity are shown in dashed outline. The M_r values are as described in text; the M_r values of 125000, 75000, 58000, 35000, and 20000, represent the extrapolated values obtained from the curve relating M_r to electrophoretic mobility.

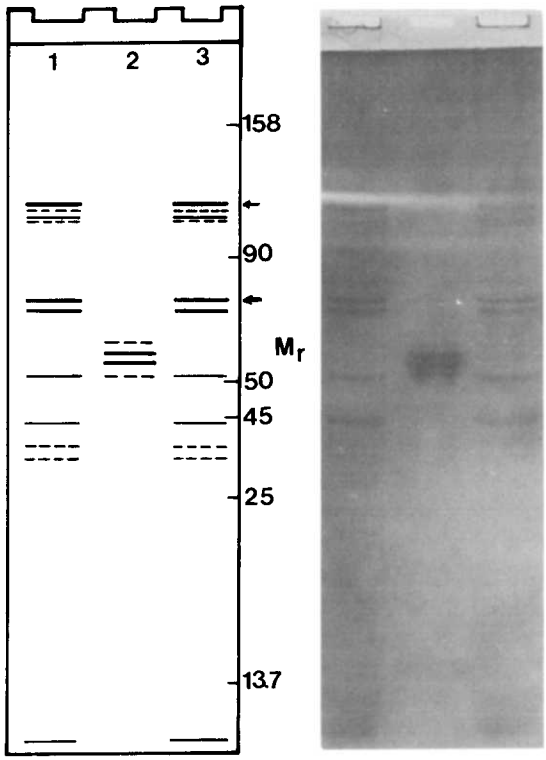

Fig. 5. Periodic acid-Schiff staining of membrane proteins in SDS-polyacrylamide gel electrophoresis. The SDS-polyacrylamide gel electrophoresis gel was processed following electrophoresis by the periodic acid-Schiff reaction as described in Materials and Methods. The actual periodic acid-Schiff processed gel is shown on the right with six deeply staining bands and four less intense bands (indicated in the schematic as dashed lines). Lanes 1 and 3, 140 μg protein; lane 2, 60 μg of a control glycoprotein. A faint band can be seen at the dye front in the membrane protein lanes. The arrows indicate the bands also showing a positive concanavalin A-binding reaction.

not optimally resolve basic protein components which may have resulted in this type of appearance. However, the integrity of several basic protein controls treated and electrophoresed in an identical manner was unaffected in this system.

Glycoprotein staining of electrophoresed protein components

In an effort to delineate the membrane proteins by classes, two procedures for tentatively identifying carbohydrate-containing proteins were attempted. The periodic acid-Schiff treatment resulted in the detection of several Schiff positive bands in SDS-polyacrylamide gel electrophoresis (Fig. 5). These bands ranged between 35000 and 125 000. The marker proteins used for the Coomassie blue stained gels were included in the accompanying schematic and serve only as references as it is recognized that glycoprotein electrophoretic mobilities may not reflect accurate molecular weights [16]. When the periodate treatment was omitted, no Schiff positive bands were detected in the membrane protein profile or with the glycoprotein control.

The results of the concanavalin A-binding procedure for carbohydrate detection are shown in Fig. 6. Although the gel became slightly darkened during development, two reactive bands were visualized in each lane. Based upon their electrophoretic mobilities, it is likely that these bands corresponded to a periodic acid-Schiff reactive band seen in Fig. 5 (arrows). The dye front also demonstrated a concanavalin A-binding compo-

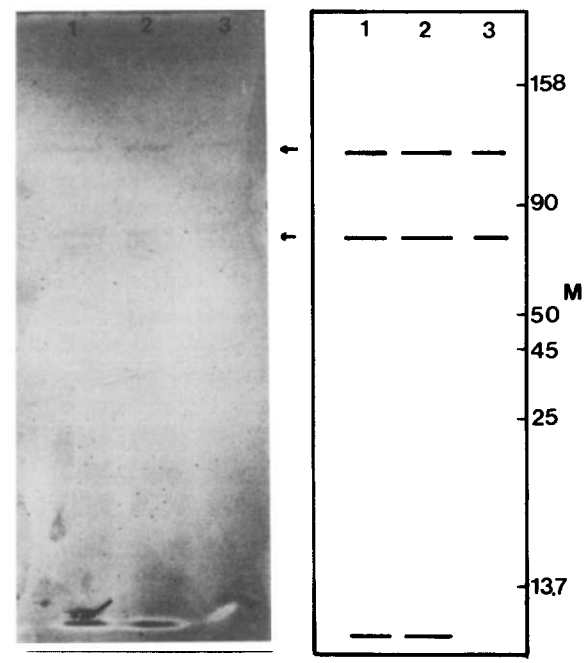

Fig. 6. Concanavalin A-binding to membrane proteins in SDS-polyacrylamide gel electrophoresis. The SDS-polyacrylamide gel electrophoresis gels were processed following electrophoresis by the concanavalin A-binding reaction as described in Materials and Methods. Lanes 1 and 2, 140 μg protein; lane 3, 70 μg protein. In addition to the two reactive bands (arrows) in the upper part of the gel, a concanavalin A-binding component can be seen at the dye front (bottom).

nent. The omission of concanavalin A or peroxidase during the processing failed to give rise to any reactive bands in the membrane protein profile or with the glycoprotein marker.

Discussion

A fundamental concern of this investigation was to investigate the gross protein composition of homogeneous *S. aureus* 6538P cytoplasmic membranes as delineated by denaturing electrophoretic methods. The preliminary solubilization analysis of isolated *S. aureus* membranes by various dissociating agents demonstrated a differential sensitivity of the staphylococcal membrane to these agents. The low concentration of detergent (0.8%) initially used was found satisfactory in judging the overall membrane dissociation. At this concentration, the detergent bound to the membrane in low amounts without total disintegration and extracted similar protein components with varying efficiencies. Detergents of the neutral (Triton X-100 and Nonidet P-40), anionic (SDS and sodium deoxycholate), and zwitterionic (Zwittergent-314) classes each facilitated effective protein release in agreement with other membrane solubilization analyses [17–19].

All of the detergent extracts were uniformly compared in SDS-polyacrylamide gel electrophoresis allowing for the rapid comparison of the number of components released. Based on the number of bands resolved, the *S. aureus* membrane appeared to release similar components regardless of detergent class. However, the extent of protein release through the disruption of the lipid bilayer was more related to detergent class. While the zwitterionic agent proved an efficient solubilizer, this detergent interfered with first dimensional isoelectric focusing and could not be utilized. The combination of SDS, Nonidet P-40, and urea facilitated the optimum resolution of the membrane proteins by two-dimensional gel electrophoresis. There appeared to be predominance of certain components based on the staining intensity in SDS-polyacrylamide gel electrophoresis and confirmed by two-dimensional gel electrophoresis. These data suggest that the *S. aureus* 6538P membranes may contain a few proteins which make up a large percentage of the total protein composition [2]. The degree of complexity found here is not uncommon as 60 components have also been resolved in *Streptococcus pyogenes* membranes by SDS-polyacrylamide gel electrophoresis [20]. Three exponential phase membrane proteins were absent from the stationary phase profile, with two additional lower molecular weight components detected. These stationary components may have represented breakdown of higher molecular weight components or new proteins synthesized between growth phases. The two-dimensional gel electrophoresis analysis of *S. aureus* membrane proteins represents the only investigation thus far reported demonstrating the complexity of the cytoplasmic membrane proteins from Gram-positive cocci by this technique. The results show that the majority of membrane protein components have relatively low isoelectric points (p*I* 4–5) and range in M_r values from 158000 to 35000. The overall composition of the membrane proteins resolved here is comparable to the results obtained in other studies with Gram-negative cytoplasmic membrane preparations from *Escherichia coli* and *Salmonella typhimurium* [2,3]. The effects of the denaturing conditions on the isoelectric points of some of the membrane protein components must be recognized when evaluating the two-dimensional gel electrophoresis profile. The high concentration of urea may induce slight deviations in isoelectric point determination as has been noted by O'Farrell [9].

The presumptive identification of membrane glycoproteins in the membrane was based on the reactions using concanavalin A binding and periodic acid-Schiff staining techniques. The results of the concanavalin A binding experiments revealed two reactive bands that apparently contained sugar residues in conformations facilitating an interaction with concanavalin A. The results of periodic acid-Schiff staining demonstrated six to ten reactive bands. It is noteworthy that membrane protein components, plus a dye front component, reacted positively with both methods. Owen and Salton [21] have shown as many as five membrane proteins in *Micrococcus lysodeikticus* demonstrating major reactions with lectins. The identification of *S. aureus* 6538P membrane glycoproteins in this analysis is supported by findings from other studies with *S. aureus* membrane proteins. Aasjord and

Grov [22] have shown a membrane glycoprotein exhibiting antigenic determinants in both the protein and sugar residues. In addition, the presence of a *S. aureus* membrane glycoprotein responsible for the development of delayed hypersensitivity in mice has been documented [23]. The identification of glycoproteins in a bacterial membrane is particularly significant and warrants further investigation.

The results in this work have presented a detailed denaturing profile of the membrane proteins from *S. aureus*. These efforts should contribute to a further understanding of membrane protein involvement in such cell-related properties as the sensitivity to chemical agents and, in particular, the binding and effects of different molecules to specific membrane protein components in Staphylococci.

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