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Identification and Characterization of Peptidoglycan-Associated Proteins in *Neisseria gonorrhoeae*

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The principal proteins associated with *Neisseria gonorrhoeae* peptidoglycan (PG), as identified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, are the following: two proteins at approximately 90 kilodaltons (kDa), single major species at both 60 and 44 kDa, a 34- to 36-kDa protein, and three proteins between 28 and 32 kDa. A protein analogous to *Escherichia coli* Braun lipoprotein was not detected with gonococcal cell wall preparations. The identity of the PG-associated proteins was confirmed immunologically with antibody generated against purified cell walls. Two types of protein species, dithiothreitol extractable (the majority) and alklylation dependent (primarily the 34- to 36-kDa protein), appeared to be associated with the *N. gonorrhoeae* cell wall fraction. It was found that a crucial step in the extraction of the proteins from the PG fraction was the inclusion of an acetone-water wash of the purified PG pellet. Studies with cell wall preparations obtained from *N. gonorrhoeae* intrinsically labeled with 32P revealed that the acetone wash was removing phospholipid from the cell wall fraction and thus facilitating protein extraction. Autoradiographic analysis with PG material derived from 125I-surface-labeled cells indicated that the 44-kDa protein is exposed on the surface of the organism even when associated with the PG layer. Radioimmunoprecipitation with anti-PG antibody confirmed these findings. Lectin analysis (wheat germ agglutinin conjugated to horseradish peroxidase) suggested that the 34- to 36-kDa protein is covalently attached to the PG layer.

Gonorrhea is a disease solely of human beings and is caused by the gram-negative diplococcus *Neisseria gonorrhoeae*. The biology of *N. gonorrhoeae* is complex, probably reflecting the need of the organism to survive in various anatomical niches while withstanding a variety of host defenses within the human body. The organism is a typical gram-negative bacterium, with an inner cytoplasmic membrane, a thin peptidoglycan (PG) layer, and an outer membrane (OM) containing protein, lipopolysaccharide, and phospholipid. Various OM proteins, including the major OM protein P.I (2, 19), the opacity-associated protein(s) P.II (30), and several less-well-defined protein components that include P.III (18), H.8 antigen (6, 29), and a 44-kilodalton (kDa) protein (35), have been characterized. In contrast to the situation with other gram-negative organisms (4), a PG-associated protein analogous to Braun lipoprotein (BLP) has not been found in *N. gonorrhoeae* (15, 34). Nonetheless, gonococci are believed to possess a PG-associated protein(s) after in vitro growth in acidic medium (pH 6) (13, 14). However, the identity of this protein(s) is complicated because of the extreme insolubility of the *N. gonorrhoeae* PG-protein complex.

The association of protein with the cell wall fraction (i.e., the fraction remaining after exhaustive extraction with detergent) under acidic conditions may represent an important structural modification that is relevant to the survival of gonococci in the human body (8). Several studies have addressed the effects of pH on the biology of gonococci (12-14, 22, 25, 32, 33). One found that the organism had an enhanced ability to adhere to host cells at a low pH (25). Also, structural analysis of gonococcal PG (12, 32, 33) demonstrated that the maximum autolytic activity occurred when cells were grown in vitro at pH 9. This effect could be suppressed when cells were grown at pH 6 in the presence of Mg²⁺ ions or reduced 50% at pH 6 in the absence of the cation (32). Similarly, it was found that penicillin-induced lysis could also be prevented by growth at a low pH in the presence of Mg²⁺ (33). These results suggest that the decrease in the autolytic activity of the PG at a low pH was due in part to an increased stability of the cell, possibly through the association of protein with the PG (13, 14).

We looked for the PG-associated protein(s) in *N. gonorrhoeae* for another reason. PG isolated from 125I-surface-labeled cells contained significant amounts of radioisotope which remained with the PG even after extensive solubilization. This result suggested that surface-exposed proteins were associated with the PG and suggested a means by which we could purify surface-exposed OM proteins from crude OM-protein-PG mixtures by selectively hydrolyzing the proteins away from the PG in a relatively pure state. In this study we identify the proteins which are associated with gonococcal PG; at least one of these proteins is surface exposed. The removal of phospholipid from the cell wall fraction was found to greatly facilitate the solubilization and extraction of these PG-associated proteins. These findings constitute preliminary evidence for the covalent association of a 34- to 36-kDa protein with the PG.

**MATERIALS AND METHODS**

**Strains.** *N. gonorrhoeae* CS7 (13, 14), a gift from Tom Dougherty (Rockefeller University, New York, N.Y.), was primarily used in this study. Strains JS1, JS3, FA635, and FA638 have been described elsewhere (19). Growth on solid medium utilized the clear typing medium described by Swanson (30). Single colonies were passaged daily and grown at 36.5°C in a 5% CO₂ atmosphere. In all cases only transparent colonies were selected. For the majority of the experiments described, cells were grown in liquid medium. In this case, bacteria were swabbed from a 14-h plate and suspended in prewarmed medium to an optical density at 600
acted with buffered saline. The lectin partially blocked overnight as described (13, 14) by adjusting the potassium salt ratios. The cells were grown in conical flasks at 37°C by using a wrist-action shaker. Growth was monitored by measuring the optical density in a Coleman 2 spectrophotometer.

**Radiolabeling of gonococci.** Surface iodination of *N. gonorrhoeae* with 125I was done by the iodogen (Sigma Chemical Co., St. Louis, Mo.) procedure as described by Judd (18). For the intrinsic labeling of phospholipid with H32PO4, bacteria were grown in liquid medium containing 2 μCi of H32PO4 per ml. The cells were labeled continuously throughout growth. Labeling was terminated by pouring late-log-phase cultures over ice, with excess radiolabel being removed by washing the cells with phosphate-buffered saline.

**Preparation of purified cell walls.** Purified cell walls were prepared as described by Gomez-Miguel and Moriyon (11) for the preparation of *Brucella abortus* PG. For large-scale isolation of cell walls, 1-liter cultures were used. The cells were harvested by centrifugation and refixed at 100°C for 20 min in 10 ml of 4% sodium dodecyl sulfate (SDS)-50 mM sodium acetate (pH 5.6) with stirring. The pellet was collected by centrifugation (10,000 × g) and extracted two more times under identical conditions. The resulting pellet was washed six times with 50 mM sodium acetate (pH 5.6). The washed pellet was extracted three times with acetone-water (6:1 [vol/vol]). The acetoacetone-washed residue was collected by centrifugation at 10,000 × g for 20 min.

**Extraction of acetone-washed cell walls.** Detergent extraction of cell walls was carried out overnight at 37°C on a rocking platform. Cell walls were extracted with 4% SDS in the presence of either 8% 2-mercaptoethanol (Sigma) or 5 mM dithiothreitol (Sigma). In some cases reduced sulphydryl groups were alkylated with iodoacetamide (Sigma) before detergent extraction. Generally, alkylation of a 50-µl sample (approximately 250 µg of protein) was done with 10 µl of 100 mM iodoacetamide in the dark at room temperature for 3 h. Alkylated cell walls were pelleted and suspended for further extraction.

**Preparation of anti-CS7 cell wall antibody.** Antiserum was generated in a rabbit by inguinal and axillary injections of purified cell walls in complete Freund adjuvant. These injections were followed 1 week later with intramuscular and subcutaneous injections in incomplete Freund adjuvant and subsequent intravenous inoculations at approximately 1-week intervals.

**Electrophoresis.** SDS-polyacrylamide gel electrophoresis (PAGE) was done by the method of Laemmli (20) at a constant power of 10 W. The gels were fixed and stained in 25% isopropanol–5% acetic acid containing 0.05% Coomassie brilliant blue. Autoradiographs of gels were done with XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) with high-speed intensifying screens at −70°C.

**Immunoblotting.** Immunoblotting was performed exactly as described by Judd (19) with the low-phosphate buffer system (pH 8) of Batteiger et al. (1).

Protein immobilized on nitrocellulose after transfer was also probed with the lectin wheat germ agglutinin (WGA) conjugated to horseradish peroxidase. The blots were initially blocked overnight in 0.05% Tween in phosphate-buffered saline. The lectin conjugate (3 µl; 1 mg/ml; Sigma) was reacted with the paper for 2 h at room temperature. The blots were washed extensively with phosphate-buffered saline-Tween and developed by the same method as that used for antibody detection. To assess competitive inhibition of WGA, we preincubated lectin preparations overnight at room temperature in various sugar solutions. Each lectin–sugar solution was used to probe proteins immobilized on nitrocellulose. The following sugar solutions were used: 50 mM glucose, 50 mM N-acetylglucosamine, or the soluble fraction resulting from a 2-day lysozyme (derived from *Streptomyces globisporus* [Miles Scientific, Div. Miles Laboratories, Inc., Naperville, Ill.]) hydrolysis at 37°C of purified gonococcal PG (a [1H]GlcNH2-labeled PG sample was used as an internal control to monitor the extent of hydrolysis that was >90%).

**Radioimmunoprecipitation.** Radioimmunoprecipitation with anti-CS7 cell wall antiserum was performed exactly as described by Swanson and Barrera (31). Whole, intact gonococci were surface iodinated and reacted with antibody before solubilization with Zwittergent 3-14. The surface-labeled proteins were precipitated with Sepharose 4B-CLB beads coated with protein A (Sigma).

**Phospholipid analysis.** 32P-labeled phospholipids were extracted from whole cells by the procedure described by Bligh and Dyer (3). 32P-labeled phospholipids were also isolated from the pooled acetone washes of the cell wall material. Phospholipids were prepared for paper chromatography with Whatman SG81 paper by drying the acetone samples in vacuo and suspending the anhydrous phospholipids in a small volume of chloroform. The chromatography solvent systems used were chloroform–methanol–2,6-dimethyl-4-heptanone–acetic acid–water (35:10:50:25:4) in the first dimension and chloroform–methanol–2,6-dimethyl-4-heptanone–pyridine–0.5 M NH4Cl (pH 9.8) (60:30:45:70:12) in the second dimension. The phospholipids were visualized by autoradiography of the chromatograms as described above.

**RESULTS**

**Identification of PG-associated proteins in *N. gonorrhoeae*.** From purified acetone-extracted PG, we were unable to extract protein by strictly nondetergent means (NaCl, EDTA and urea extraction). However, extraction of acetone-washed PG at 37°C overnight with 4% SDS containing either 8% 2-mercaptoethanol or 5 mM dithiothreitol resulted in the liberation of several protein species from the PG-protein complex (Fig. 1A). Principal proteins associated with CS7 cell walls were two at approximately 90 kDa, one each at 60 and 44 kDa, and three between 28 and 32 kDa. Little protein could be extracted when the acetone wash was omitted before detergent extraction (Fig. 1A, lane B) or in the absence of a reducing agent. These results suggest the involvement of disulfide linkages in the PG-protein complex. Tryptic digestion of *N. gonorrhoeae* cell wall material with the same conditions as those described by Braun and Rehn (5) failed to liberate a protein analogous to the Braun lipoprotein of *Escherichia coli* cell walls, while *E. coli* treated in an identical fashion yielded a single major species at approximately 14 kDa (Fig. 1A, lane E). In contrast, lysozyme digestion of *N. gonorrhoeae* PG facilitated the liberation of all the PG-associated protein from the PG-protein complex (data not shown). The Western blot (immunoblot) in Fig. 1B shows the association of H.8 antigen with gonococcal PG. The blot also demonstrates that H.8 antigen tended to accumulate with the PG fraction as cells entered the stationary phase of growth (this occurred after approximately 10 h). H.8 antigen was not released by
the action of trypsin, as assessed by Western blot analysis. PG-protein material probed with a P.III-specific monoclonal antibody (MC01) proved to be negative.

When purified PG fractions were obtained from surface-labeled bacteria, a single protein was found to be readily iodinated, even though closely associated with the PG fraction; this was the 44-kDa OM protein (Fig. 2A and D). Strain FA638 was unusual in that the 44-kDa OM protein was not surface exposed when associated with the PG fraction; for this strain it was found that a 60-kDa protein was surface labeled (Fig. 2E). For strain FA635, both the 44- and 60-kDa proteins were found to be accessible to surface labeling (Fig. 2D).

**Analysis of acetone-soluble material.** The crucial feature of this rigorous extraction procedure was found to be the acetone-water washing step, suggesting that this step was extracting a lipidlike substance—possibly phospholipid. Isolation of purified PG from cells intrinsically labeled with $^{32}P$ revealed that approximately 1% of the total $^{32}P$ counts remained with the cell wall fraction after extensive 4% SDS treatment. Analysis of the acetone-water washes for the presence of phospholipids by two-dimensional paper chromatography demonstrated a single phospholipid associated with CS7 PG (Fig. 3B). Identical results were also found for strain JS1. A comparison of Fig. 3B with the phospholipid profile obtained from the extraction of whole cells (Fig. 3A) tentatively identified this lipid as phosphatidylglycerol. Trace amounts of several other lipids were also found. The phospholipid(s) associated with the PG fraction was noncovalently bound to protein found in the PG complex, judging from the lack of a signal from any protein in this fraction when $^{32}P$-labeled cell wall material was separated by SDS-PAGE and then autoradiographed (Fig. 3C).

**Immunological confirmation of the identity of gonococcal PG-associated protein.** Antiserum was generated in a single rabbit to purified CS7 PG preparations that had not undergone acetone extraction and was screened against various whole-cell lysates by immunoblotting (Fig. 4B). The predominant antigens recognized by this antiserum were a 30-kDa protein, lesser bands at approximately 36, 40, and 56 kDa, and multiple bands upward from 60 kDa. The spectrum resembled the SDS-PAGE profile presented in Fig. 1A, except for the lack of recognition of the 90-kDa proteins. The same antiserum shown in Fig. 4B was used to radioimmunoprecipitate CS7 surface-labeled proteins. The autoradiograph in Fig. 4C demonstrates that the antiserum recognized and precipitated surface-labeled 44-, 60-, and 90-kDa proteins from intact gonococci.

**Lectin characterization of PG-associated protein.** If protein is covalently bound to the PG-protein complex, as for BLP in E. coli (4), then a PG disaccharide subunit might associate with a specific N. gonorrhoeae protein, and this band should react with WGA (10). A 34- to 36-kDa protein in whole-cell lysate fractions reacted with the WGA conjugate when electrobotted onto nitrocellulose (Fig. 5). No other components in the whole-cell lysates were recognized by the WGA conjugate under the conditions described. The results of the competitive inhibition studies (Fig. 5A, C, and D) of WGA demonstrated that the hydrolysate obtained from lysozyme digestion of gonococcal PG was a more potent inhibitor than that resulting from treatment with 50 mM N-acetylglucosamine (apparently reflecting the lower equilibrium constant of the former [10] for WGA). The inclusion of 50 mM glucose in the reaction mixture caused no inhibition of WGA binding as expected; likewise, concanavalin A-conjugated peroxidase (specific for glucose) failed to react. When acetone-washed PG fractions were alkylated with iodoacetamide before detergent extraction, a 36-kDa protein that reacted with the WGA conjugate was extracted (Fig. 5E). This protein failed to react with a P.I-specific monoclonal antibody (4G5; Genetic Systems, Seattle, Wash.). The above data suggest that a protein with a variable molecular mass (between 34 and 36 kDa, depending upon the strain) is covalently attached to the PG fraction.

**DISCUSSION**

Previous work (13, 14) had demonstrated that protein associated with the N. gonorrhoeae PG fraction when cells were grown under acidic conditions (pH 6) in vitro. From the data presented in this report, it is clear that N. gonorrhoeae possesses PG-associated proteins, as defined in the literature for other gram-negative organisms (4, 5, 9, 11, 24). The multiple species associated with the gonococcal PG fraction consisted of several OM proteins (e.g., the 44-kDa protein) and several other proteins, presumably periplasmic in origin. Identical results were found for all strains that we tested, regardless of the culture conditions. However, as previously described (13, 14), there was considerable strain variation...
with regard to the amount of protein associated with the PG fraction. A direct assessment of protein concentration by colorimetric determination or determination of specific activities with a radiolabel was found to be impractical, primarily because of the extreme insolubility of the complex in that complete solubilization was never achieved. This can be best appreciated in Fig. 2, in which the surface-labeled samples still emitted a considerable signal at the interface of the stacking and separating gels. However, we believe that complete solubilization of the OM was achieved during the preparation of the PG fraction because lipopolysaccharide could not be demonstrated by silver staining, immunological probing, or autoradiography of samples intrinsically labeled with $^{32}$P (Fig. 3C). However, this may reflect the greater degree of solubility of certain components than of others.

These results are in contrast to those reported in the mid-1970s by two groups (15, 34) who were unable to detect proteinaceous material in association with their purified PG preparations by amino acid analysis. Several reasons may account for this disparity. (i) In the unsuccessful studies, the bacteria were grown in media buffered at pH 7. Hebler et al. (13, 14) have since demonstrated that the optimum culture conditions for protein association occur at pH 6, the pH at which the cells were grown for the majority of the experiments reported here. (ii) In the unsuccessful studies, the bacteria were harvested during exponential growth. The immunoblot in Fig. 1B indicates that a greater degree of protein association occurs upon entry into the stationary phase of growth (beginning at 10 h in Fig. 1B). Similar results have also been reported for another gram-negative bacte-

![Fig. 2. SDS-PAGE analysis of cell wall fractions isolated from surface-labeled strain JS1 (A), strain JS3 (B), strain CS7 (C), strain FA635 (D), and strain FA638 (E). PG preparations were obtained from surface-labeled cells as described previously (18) and extracted with detergent in the presence of a reducing agent. WC, Whole-cell lysate; P, PG fraction. Arrows indicate surface-labeled PG-associated proteins. MW, Molecular weights in thousands.](image)

![Fig. 3. Analysis of $^{32}$P-labeled phospholipids isolated from a purified cell wall fraction of strain CS7. Strain CS7 was labeled continuously with $^{32}$P until the late log phase. Cell walls were isolated and washed with acetone-water (6:1 [vol/vol]). The acetone-water fractions were pooled and dried in vacuo, and the phospholipids were analyzed by two-dimensional paper chromatography. PE, Phosphatidylethanolamine; PG1, phosphatidylglycerol; CL, cardiolipin; LPS, lipopolysaccharide; PI, OMP protein P.I. (A) Analysis of phospholipids derived from whole cells (3) (trace amounts of an additional phospholipid were also seen close to the origin but were omitted from this particular figure). (B) Analysis of phospholipids contained in the acetone-water wash of strain CS7. Trace phospholipids are indicated by the arrows. (C) Autoradiograph of an SDS-PAGE gel of the $^{32}$P-labeled cell wall material (Coomassie staining of this fraction revealed the presence of proteins). Lanes: 1, $^{125}$I-surface-labeled whole-cell preparation; 2, whole-cell preparation intrinsically labeled with $^{32}$P; 3, $^{32}$P-labeled PG preparation.](image)
rrium, *Proteus mirabilis* (9). (iii) They could possibly have been using a strain that did not possess PG-associated protein. Hebeler et al. (13, 14) have also demonstrated that the amount of protein association appears to be somewhat strain specific. Indeed, one of the strains that was reported to lack PG-associated proteins (RUG 40) was later shown to possess these proteins. Thus, the use of an inappropriate strain, exacerbated by the use of unfavorable conditions, may account for the previously unsuccessful demonstrations of PG-associated proteins in *N. gonorrhoeae*.

Initial attempts (13, 14) to identify *N. gonorrhoeae* PG-associated proteins were probably thwarted because of the extreme insolubility of the complex. After considerable effort, we found that a crucial step in the solubilization, extraction, and manipulation of these proteins was the inclusion of an acetone-water wash of the cell wall fraction before extraction with detergent (compare lane B with lane C in Fig. 1A). A possible explanation for the insolubility problem of the PG-associated proteins could well be the presence of phospholipid in the purified cell walls (Fig. 3). Proteins that are tightly complexed with phospholipids are not unknown, an example being the membrane penicillinase found in *Bacillus licheniformis* (21). The association of phospholipid with the cell wall fraction is no doubt enhanced...

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**FIG. 4.** Immunological analysis of gonococci with antibody raised against purified CS7 PG. (A) Whole-cell lysates of strains JS1 (lane 1), JS3 (lane 2), CS7 (lane 3), FA635 (lane 4), and FA638 (lane 5) electrobotted onto nitrocellulose and stained with India ink for comparison. MW, Molecular weight markers; numbers at left and right indicate molecular weights in thousands. P.I., OMP protein P.I. (B) Immunoblot with anti-CS7 cell wall antibody (1:50 dilution). Strains were as in panel A. Preimmune serum was negative under identical conditions. (C) Radioimmunoprecipitation of CS7 125I-surface-labeled proteins (31) with the anti-CS7 PG antibody. Lanes: I, whole-cell lysate preparation of surface-labeled bacteria; II, surface-labeled proteins precipitated with the anti-CS7 PG antibody; III, preimmune serum control. P.I., OMP protein P.I.

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**FIG. 5.** Lectin analysis of gonococcal proteins probed with WGA conjugated to horseradish peroxidase. Whole-cell lysates of *N. gonorrhoeae* JS1 (lanes 1), CS7 (lanes 2), and FA638 (lanes 3) and various cell wall fractions obtained from strain CS7 were electrobotted onto nitrocellulose and probed for the presence of PG subunits associated with GC proteins by using the PG-specific probe WGA (10). (A) WGA in the presence of 50 mM glucose. (B) Concanavalin A conjugated to horseradish peroxidase (specific for glucose). (C) WGA in the presence of 50 mM N-acetylglucosamine (50% inhibitory concentration [10]). (D) WGA in the presence of the supernatant fraction resulting from a 2-day lysozyme hydrolysis of gonococcal PG (percentage of hydrolysis that was >90%, as determined with a labeled internal control). (E) WGA probing of various CS7 cell wall fractions. WC, whole-cell lysate; P, cell wall fraction; 2ME, 2-mercaptoethanol.
by the O acetylation of the gonococcal PG sugar backbone itself (26, 27). Interestingly, we also found an increase in the percentage of O acetylation of PG with growth under acidic conditions (data not shown). O acetylation is also known to increase upon entry into the stationary phase (27). Both of these conditions correlate with elevated levels of proteins associated with the cell wall fraction, as determined by SDS-PAGE analysis. Consequently, one can envision that gonococcal cell walls are particularly hydrophobic in character, and this may enhance the hydrophobic barriers found in the OM or provide additional ones (22).

Our attempts to demonstrate in *N. gonorrhoeae* a protein that is equivalent to the bound form of BLP were unsuccessful (Fig. 1A). In addition, we were unable to demonstrate the association of lipoprotein with gonococcal cell walls, based on fluorescent microscopic analysis of cell wall material isolated from \[^{3}H\]palmitate-labeled cells. However, a recent study (29) of H.8 antigen demonstrated that this protein is a lipoprotein. Other work analyzing \[^{3}H\]palmitate-labeled whole-cell lysates (7) suggested that a 44-kDa protein and several proteins between 28 and 32 kDa are lipoproteins (results that we were able to confirm). Whether the proteins demonstrated in this study correspond to those believed to possess fatty acid must also await further clarification.

The data presented in Fig. 5E suggest that the integrity of the PG-protein complex is maintained by disulfide interactions in that iodacetamide treatment is required to release a protein from the PG cell wall fraction that contains a PG subunit. The lack of recognition by the P.I-specific monoclonal antibody 4G5 argues that this protein is not P.I but is a protein of similar molecular weight. The fact that the lectin reacts with the 34- to 36-kDa protein in the whole-cell lysate fractions (interestingly, in a stairlike pattern reminiscent of P.I) suggests that a pool of these molecules attached to a PG subunit but unattached to the murein sacculus may exist. An analogous situation has also been found for BLP in *E. coli* (23), in which nascent BLP attached to a PG disaccharide subunit has been isolated away from the PG layer in the OM fraction. Consequently, this may provide gonococci with a mechanism with which they can associate a protein with the PG layer when conditions dictate (such as growth under acidic conditions). Such a protein is most likely a periplasmic protein, judging from the lack of recognition in the radioimmunoprecipitation experiment.

An initial thrust of this study was to identify the surface-exposed proteins associated with the PG layer in the hope of possibly using this association as a means to rapidly purify OM proteins of interest. With regard to this point, we believe that we have been successful in identifying at least one major surface-exposed OM protein, this being the 44-kDa protein (Fig. 2 and 4C). Additional proteins (60 and 90 kDa) may also be surface exposed, judging from the precipitation data in Fig. 4C. However, the 60-kDa protein is not present in purified OM preparations (16), suggesting that it may be a periplasmic protein that has been accidentally labeled, possibly by iodine gaining access through the porin molecule during the labeling process. Recently, a surface-exposed PG-associated protein was demonstrated in *Haemophilus influenzae* (24) and is now believed to be an important surface antigen for the organism. The original observations demonstrating the presence of this protein in *H. influenzae* resulted from radioimmunoprecipitation experiments with antisera generated against purified cell walls. Whether the 44-kDa protein or any other PG-associated protein in gonococci is an important surface antigen must await further study. However, evidence suggests that a 44-kDa protein is an antigen recognized by convalescent-phase antisera (35), a protein of similar molecular weight has been implicated in serum resistance (17), and a 44-kDa surface-exposed protein is also believed to bind the lysosomal enzyme cathepsin G (28). Consequently, *N. gonorrhoeae* PG-associated proteins may also represent important surface antigens, similar to the situation with *H. influenzae*.

**LITERATURE CITED**


