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Evidence for N-Terminal Exposure of the Protein IA Subclass of *Neisseria gonorrhoeae* Protein I

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The JS3 and FA638 strains of *Neisseria gonorrhoeae* bear a protein IA subclass of protein I (P.I). The purified P.Is of surface-labeled strains JS3 and FA638 were cleaved with the N-terminal degradation enzyme leucine amino peptidase (LAP), and the resultant fragments were separated in sodium dodecyl sulfate-polyacrylamide gels. Autoradiography demonstrated that the surface radiolabel was absent in a LAP-generated P.I peptide that was about 1,900 daltons lower in apparent molecular mass than the native P.I in both strains. Moreover, the 4G5 monoclonal epitope, known to be located on the surface of the organism, was also absent in the LAP-generated P.I peptide that was about 1,900 daltons less in apparent molecular mass than the original P.I of strain FA638. These data strongly suggest that the N terminus of the P.IA subclass is exposed on the surface of the bacterium and that this region represents about 5%, or 15 to 20 amino acids, of the total protein.

The orientation of the protein IA (P.IA) subclass of protein I (P.I) in the outer membrane of *Neisseria gonorrhoeae* appears to be different than that of the protein IB (P.IB) subclass. Proteolytic enzyme digestion of intact organisms cleaves a central region of the P.IB subclass leaving two membrane-bound fragments (1, 4), whereas in organisms with the P.IA subclass the P.I is much less susceptible to proteolytic cleavage (1, 4). Proteinase K (PK), a relative nonspecific protease, does cleave P.IA, yielding a single membrane-bound fragment that is about 1.5 kilodaltons (kDa) less than the intact protein, suggesting that one terminus of the protein is exposed on the surface of the organism (1).

The P.IA subclass is of particular interest because of its possible relationship to serum resistance (6, 7) and disseminated gonococcal infection (5). Therefore, the orientation and the exposure of molecule may play an important role in mediating the ability of the organism to invade deeper tissues. In this study I endeavored to demonstrate that the N terminus of P.IA is the exposed portion of the molecule and that this region carries an immunologically active epitope. Surface-radiolabled P.IAs of *N. gonorrhoeae* JS3 and FA638 were cleaved by the N-terminal degradation enzyme leucine amino peptidase (LAP). Resultant fragments of the FA638 P.IA were electroblotted and probed with both the 4G5 monoclonal (15) and polyclonal antibodies. Results demonstrate that both the surface label and the 4G5 epitope were absent in a LAP-generated fragment that was about 1.9 kDa less than the native P.I, indicating that the N terminus of the P.IA subclass of P.I is exposed on the surface. These results were confirmed by ^{125}I-labeled and surface-labeled peptide mapping.

MATERIALS AND METHODS

Bacteria. *N. gonorrhoeae* JS1 and JS3 were the generous gift of John Swanson, Rocky Mountain Laboratory, Hamilton, Mont. Strains FA635 and FA638 (serum-sensitive and -resistant transformants, respectively, derived by J. Cannon and P. F. Sparling, University of North Carolina, Chapel Hill, N.C.) were provided by Keith Joiner, National Institute of Allergy and Infectious Disease, Bethesda, Md. Strain JS1 has a P.IB subclass of P.I (8) and is in the WII/III coagglutination group (12). Strains JS3, FA635, and FA638 have P.Is in the P.IA subclass (8) and are in the WI coagglutination group (12) (serovaring was kindly performed by Joan Knapp, Centers for Disease Control, Atlanta, Ga.). All bacteria were grown on GC typing media (14) in 5% CO_2 for 18 h at 36.5°C.

Radioiodination. Bacteria were swabbed from plates and suspended in Dulbecco-modified phosphate-buffered saline (PBS; pH 7.4) to an optical density at 600 nm (OD_{600}) of 0.68 at 600 nm. A total of 1.5 ml of this suspension was centrifuged, and the bacterial pellet was washed 3 times in Dubbecco-modified PBS. The washed organisms were suspended in 150 μl of Dulbecco-modified PBS and radiolabeled with ^{125}I by the iodogen method described previously (9).

Electroblotting. Whole-cell lysates of strains JS1, JS3, FA635, and FA638 were separated by 15% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) as described previously (8). Lysates were transferred to nitrocellulose paper (NCP) (HAHY-Millipore; Millipore Corp., South San Francisco, Calif.) by electroblotting at 27 V and 0.8 A for 3 h in 20 mM phosphate buffer (pH 8.0) in a Transblot chamber (Bio-Rad Laboratories, Richmond, Calif.) with tap water cooling. The blots were blocked in 100 mM PBS–0.05% Tween 20 (pH 7.4) (2) and then were probed with anti-FA638 whole-cell antibody (1:100), anti-FA638 P.I antibody (1:100), or the 4G5 monoclonal antibody (1:10). Antibody binding was assessed by incubation with protein A (PA)-horseradish peroxidase (HRP; 1:2,000; Sigma Chemical Co., St. Louis, Mo.) or goat anti-mouse-immunoglobulin G (IgG)-HRP (1:2,000 Hyclone Laboratories, Logen, Utah). Following the washes 4-chloro-1-naphthol (Sigma) was added as color reagent (60 mg of 4-chloro-1-naphthol in 20 ml of methanol, diluted to 120 ml in 500 mM NaCl–20 mM Tris hydrochloride (pH 7.2)–100 μl of 30% H_2O_2). The reaction was stopped after 20 min by placing the NCP in distilled H_2O.

PK cleavage. Intact cells of strain FA638 were subjected to surface cleavage by PK (Calbiochem-Behring, La Jolla, Calif.) essentially as described by Barrera and Swanson (1). A cleavage time of 15 min at an enzyme concentration of 20 μg/ml was chosen because it left the majority of the P.I intact while it generated a PKs fragment (1) in an amount similar to

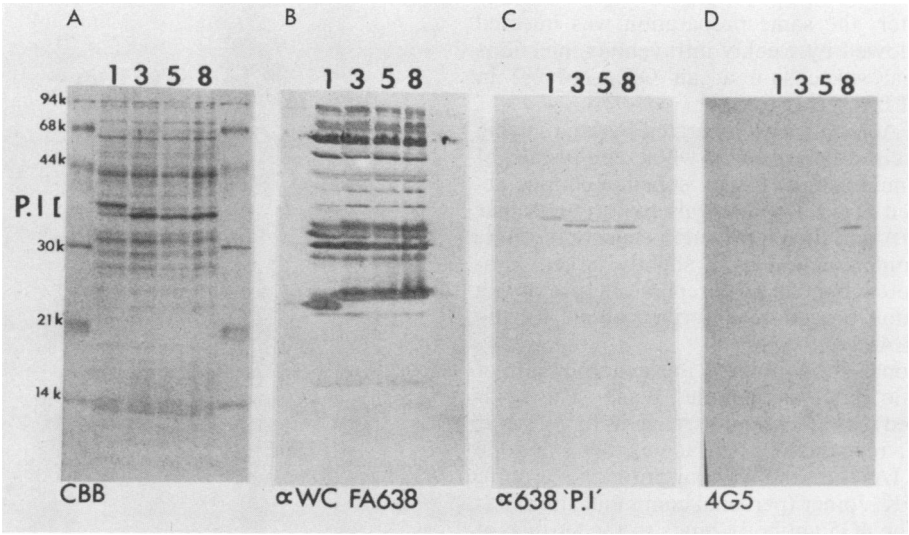

FIG. 1. (A) A CBB-stained 15% SDS-PAGE gel of whole-cell lysates of *N. gonorrhoeae* JS1 (lane 1); JS3 (lane 3), FA635 (lane 5), and FA638 (lane 8). (B) Immunoblot of the preparations shown in panel A incubated with rabbit anti-FA638 whole-cell antiserum (α WC FA638) (1:100) and probed with PA-HRP. Note the lack of antibody binding to either the homologous P.I or any of the heterologous P.Is. There was no visible reaction in control blots. (C) Immunoblot of the preparations shown in panels A and B incubated with rabbit anti-FA638 P.I antiserum (α638 'P.I') (1:100) and probed with PA-HRP. Note that all the P.Is bound the antibody. There was no visible reaction in control blots. (D) Immunoblot of the preparations shown in panels A to C incubated with the 4G5 anti-P.IA monoclonal antibody probed with goat anti-mouse-IgG-HRP. The specificity of the 4G5 monoclonal antibody for the FA638 P.I. is shown. Molecular mass markers were from a low-molecular-mass marker kit (Bio-Rad) and are presented in kilodaltons. P.Is is designated P.I.

that of the first LAP fragment reported in this study (see Fig. 4).

Purification of P.I. The bacterial pellet of a 1.5-ml suspension of bacteria at an OD_{600} of 0.68 was radiolabeled and solubilized in 200 μl of SDS solublizing solution (8). A total of 100 μl of this preparation was loaded onto a preparative 15% SDS-PAGE gel and subjected to electrophoresis at 10 W of constant power for 2.5 h. The gel was fixed, strained with Coomassie brilliant blue (CBB), and destained in 25% isopropanol–7% acetic acid as described previously (8). The P.I band was excised, soaked for 30 min in 40% ethanol–5% acetic acid, and then layered onto the stacking gel of a 10% SDS-PAGE gel and reseparated. The gel was then overlayed with NCP and electroblotted at 27 V and 0.8 A for 3 h in 20 mM phosphate buffer (pH 8.0) in a Transblot (Bio-Rad) chamber with tap water cooling.

The P.I band was located by staining the NCP in 0.1% naphthol blue black (Allied Chemical Co., New York, N.Y.) in distilled H_2O. The P.I band was excised and the protein was boiled off the NCP in 250 μl of 2% SDS in distilled dH_2O for 30 min. Recovery by determination of the radioactivity was about 75%. The P.I was then precipitated by the addition of 1.25 ml of absolute ethanol (final concentration, 83.3% ethanol), followed by centrifugation for 1 h at 10,000 × *g* (recovery, ~95% by determination of radioactivity). The P.I pellet was then rehydrated in 250 μl of Dulbecco-modified PBS (pH 7.4) containing 20 μg of porcine microsomal LAP (Sigma) per ml and incubated at 37°C. In some experiments 50-μl fractions of the digestion mixture were removed at 30-min intervals and solublized by boiling for 30 min after 50 μl of SDS solublizing solution was added twice. Twenty microliters of these preparations was then separated on a 15% SDS-PAGE gel. In other experiments, half of the preparation was removed after 1 h of digestion, and the remainder was removed after 5 h. Controls included LAP at time zero and at the end of incubation in the absence of P.I and P.I incubated in the absence of LAP.

Analysis of LAP fragments. Fragments of the strain JS3 P.I generated by LAP cleavage for 0.5, 1, 1.5, 2, and 2.5 h plus controls were separated by electrophoresis in a 15% SDS-PAGE gel, fixed, stained with CBB, and destained. This gel was then subjected to autoradiography (8) for 20 h at −70°C with a Lightning-Plus intensifying screen (Du Pont Cronex; E. I. du Pont de Nemours, Inc., Wilmington, Del.) and X-AR5 film (Kodak, Rochester, N.Y.).

Fragments of FA638 P.I generated by LAP cleavage for 1 and 5 h plus controls were separated by electrophoresis in a 15% SDS-PAGE gel. Four separate gels were run. One was fixed, stained with CBB, and destained. The second was stained by the silver staining procedure described by Tsai and Frasch (16). The third gel was electroblotted onto NCP in 20 mM phosphate buffer (pH 8.0). The NCP was then blocked for 1 h in 100 mM PBS–0.05% Tween 20 (pH 7.4). This was then incubated for 3 h at room temperature with rabbit anti-FA638-P.I antibody (1:100 dilution in 100 mM PBS–0.05% Tween 20). Following three washes in 100 mM PBS–0.05% Tween 20, PA-HRP was added (1:2,000) and incubated at room temperature for 1 h. Following the washes 4-chloro-1-naphthol was added as the color reagent for the PA-HRP. The reaction was stopped after 20 min by placing the NCP in distilled H_2O. The dried NCP was then subjected to autoradiography.

The fourth gel was electroblotted and blocked as described above. The blot was then incubated with the 4G5 monoclonal antibody (1:10 dilution in 100 mM PBS–0.05% Tween 20) for 16 h at 4°C. Following washing the NCP was incubated at room temperature for 1 with goat anti-mouse-IgG-HRP (1:2,000; Hyclone). Antibody binding was assessed as described above. The dried NCP was then subjected to autoradiography.

Antibodies. Antibody to whole cells of strain FA638 was generated in New Zealand rabbits. One milliter of bacterial suspension (OD_{600} of 0.2) was mixed with 1 ml of incomplete Freund adjuvant (IFA) and was injected in the hind foot-

pads. One week later, the same preparation was injected intramuscularly, followed by weekly intravenous injections of 1 ml of bacterial suspension at an OD_{600} of 0.2 in Dulbecco-modified PBS.

Antibody to the FA638 P.I was generated by immunizing a rabbit with P.I excised from gels of whole-cell lysates of FA638. Primary immunization was by subcutaneous injection in IFA, followed at weekly intervals by intramuscular injection of P.I in IFA and then intravenous injection of P.I. There was some contamination by a slightly larger, non-outer-membrane-protein band in this preparation, prompting the double purification procedure described above for the cleavage of P.I by LAP.

The 4G5 monoclonal antibody was the generous gift of Milton Tam, Genetic Systems, Seattle, Wash. This antibody, which is used as a screening reagent for serovar determinations (15), recognizes a surface-exposed epitope on the majority of P.IAs (i.e., the WI coagglutination group). Moreover, work by K. Joiner (personal communication) has demonstrated that the 4G5 antibody binds to the surface of intact cells of the FA638 strain and actively fixes complement, confirming the surface exposure of the 4G5 epitope.

Peptide mapping. To confirm that LAP-generated protein fragments were indeed from P.Is, the control P.I, the residual P.I at the same apparent molecular mass as the original P.I after 0.5 h of cleavage (JS3 only), and the LAP-generated P.I fragment which immediately underlied the native P.I (approximately 1.9 kDa less in apparent molecular mass in both JS3 and FA638) were subjected to ^{125}I-labeled peptide mapping as described previously (8). The ^{125}I-labeled peptide map of strain JS3 was compared with a surface-labeled peptide map (9) of P.I to confirm that surface peptides were indeed removed by LAP cleavage.

RESULTS

In Fig. 1A is a CBB-stained SDS-PAGE gel of four strains of *N. gonorrhoeae* shown for reference. JS1 is a well-characterized laboratory strain bearing the P.IB subclass of P.I. JS3 is also a well-characterized laboratory strain bearing a P.IA that does not bind the 4G5 monoclonal antibody (Fig. 1D). Strains FA635 and FA638 are transformants that were derived by J. Cannon and P. F. Sparling. FA635 is a serum-sensitive P.IA-bearing strain (6) which does not bind the 4G5 monoclonal antibody (Fig. 1D). FA638 is a serum-resistant strain (J. Cannon, personal communication; K. Joiner, personal communication) which binds the 4G5 monoclonal antibody. Molecular size markers were obtained from a low-molecular-weight marker kit (Bio-Rad).

In Fig. 1B an immunoblot with rabbit anti-FA638 whole-cell antibody is shown. Note that despite extensive antibody binding, no reaction is seen with the homologous FA638 P.I or with any other P.Is. This lack of immunogenicity and antigenicity is one of the unusual characteristics of P.IAs.

In Fig. 1C is shown an immunoblot with rabbit anti-FA638 P.I antibody. Note that all the P.Is bound this antibody, confirming previous observations by me (8) and others (12, 13) that all P.Is share some structural homology. Because of this universal binding, this antibody must be recognizing deeper, nonexposed regions of the P.I molecules.

In Fig. 1D the specificity of the 4G5 monoclonal for a unique epitope on the FA638 P.I is shown. This epitope is located within a surface-exposed region of the molecule (15; K. Joiner, personal communication). This antibody can thus be used to locate the area within the molecule where the surface region is found by observing how far into the molecule LAP must cleave before antibody binding is lost.

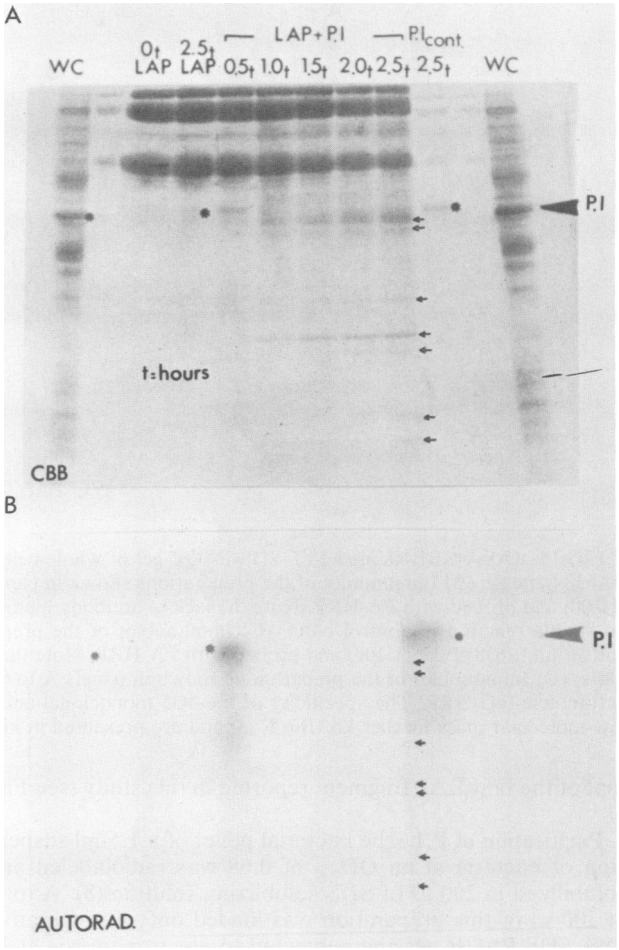

FIG. 2. (A) A CBB-stained 15% SDS-PAGE gel of strain JS3 whole-cell (WC) lysates; LAP at 0 (0_t LAP) and 2.5 h (2.5_t LAP) incubation; and LAP-cleaved strain JS3 P.I (LAP-P.I) after 0.5, 1.0, 1.5, 2.0, and 2.5 h of incubation and P.I control after 2.5 h of incubation ($P.I_{cont.}$ 2.5_t). Note the increasing appearance of LAP-generated P.I fragments as the incubation time increases (arrows). P.I is designated by the arrowhead and asterisks. (B) An autoradiogram of the CBB-stained gel shown in panel A. Symbols are the same as described for panel A.

In Fig. 2A is shown a CBB-stained SDS-PAGE gel of LAP cleavage of purified P.I of strain JS3. The LAP, which is present in great excess over P.I, does not appear to cleave itself (compare LAP at time zero with LAP at 2.5 h). The LAP bands fell well above P.I and its fragments. Cleavage of P.I was evident after 0.5 h of incubation with LAP. As the incubation time increased, more LAP-generated fragments became apparent. The first band to be seen was about 1.9 kDa smaller than the original P.I band (35.6 kDa original P.I; 33.7 kDa underlying band derived by scanning densitometry from a separate gel; data not shown). The bands of LAP-generated fragments probably resulted from the discontinuous nature of LAP cleavage. The enzyme especially has difficulty clearing proline. In Fig. 2B is shown an autoradiogram of the same gel. Note that all radiolabel was lost after 1 h of cleavage and that the first underlying band had no discernible residual label.

In Fig. 3 are shown ^{125}I-labeled peptide maps of the control P.I, P.I after 0.5 h of cleavage, the first underlying LAP-generated P.I band at 1 h of cleavage (Fig. 2A), and a

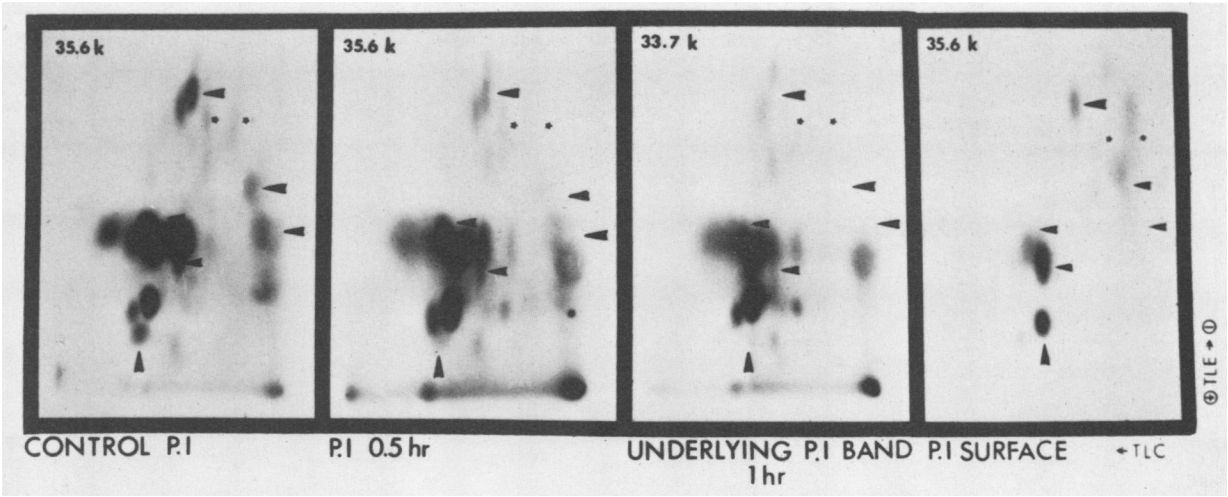

FIG. 3. ^{125}I-labeled peptide maps of the 35.6-kDa P.I control (2.5_t, Fig. 2A), the 35.6-kDa P.I after 0.5 h of LAP cleavage (Fig. 2A), and the 33.7-kDa underlying P.I band after 1 h of LAP cleavage (LAP P.I 1.0_t, Fig. 2A) of strain JS3. Also shown is a surface peptide map of the 35.6-kDa P.I of strain JS3. Peptides that were removed for diminished by LAP cleavage are identified by arrowheads and asterisks. These correspond well with peptides (arrowheads and asterisks) that were identified as surface peptides, indicating that the 33.7-kDa LAP-generated band is indeed derived from P.I and that LAP cleaved the surface label from the N terminus of the molecule. Abbreviations: TLE, thin-layer electrophoresis; TLC, thin-layer chromatography.

surface peptide map of strain JS3 P.I. Surface peptides that were clearly absent or greatly diminished in the 33.7-kDa underlying band are shown in Fig. 3, confirming that LAP removed the surface region of the P.I molecule after it cleaved about 1.9 kDa from the N terminus. Note that the bottom arrowhead in Fig. 3 identifies a peptide which corresponds exactly with the one identified by Barrerar and Swanson (1) that was lost by PK cleavage when the same mapping technology was used.

To further confirm that the N terminus is indeed the exposed region of the P.IA subclass, a second, immunological approach was used. The 4G5 monoclonal antibody epitope is known to be surface exposed. In Fig. 4 are shown the results of cleavage of intact surface-radioiodinated strain FA636 bacteria with PK and results of LAP cleavages of purified surface-radiolabeled P.I. By comparing loss of label with loss of the 4G5 monoclonal antibody binding, the surface-exposed region of P.I could be located.

In Fig. 4A is shown a CBB stain of an SDS-PAGE gel of whole cells, PK-treated whole cells, purified P.I control after 5 h of incubation without LAP, P.I after 1 h of incubation with LAP, P.I after 5 h of incubation with LAP, LAP control after 5 h of incubation in the absence of P.I, and molecular mass markers in kilodaltons. Five hours of incubation was chosen after time course studies similar to those presented in Fig. 2 showed that strain FA638 P.I was cleaved more slowly that strain JS3 P.I (data not shown). This is also true of other P.IAs (e.g., P.I of strain FA635 was very resistant to LAP cleavage; data not shown). At 5 h of cleavage much of the P.I was uncleaved, but cleavage products were being generated. Because CBB did not clearly stain cleavage products, silver staining was used to identify bands (Fig. 4B). The PKs (1) fragment in the PK-digested bacteria and LAP cleavage products after 5 h in the CBB and silver-stained gels are shown. The first visible LAP cleavage product is indicated by arrowheads in Fig. 4A to E. There are a variety of odd things visible in these gels. For example, there is a silver staining band at about 13 kDa in the P.Ic lane which does not correspond to bands in any other lane. There are also bands that are slightly larger in apparent molecular mass than P.I (visible in the silver-stained gel) which must result from LAP cleavage (they weakly bound the anti-FA638 P.I antibody; Fig. 4D) because they were absent in the P.Ic and LAPc lanes. Moreover, the P.I LAP-5 h lane shows an extra LAP band in the >100-kDa region. Despite these oddities, LAP did cleave P.I, generating a variety of cleavage products which were very similar to those seen in the P.I gel of strain JS3 (Fig. 2A).

In Fig. 4C is shown an autoradiogram of the silver-stained gel (Fig. 4B). All gels and blots gave the same autoradiographic results (data not shown). Note that the PKs fragment retained the radiolabel, as reported previously (1), while none of the LAP-generated bands, including the first underlying band had radiolabel, confirming the results seen in Fig. 2B for strain JS3 P.I.

In Fig. 4D is shown an immunoblot of the same preparations probed with the anti-FA638 P.I antibody. All the intact P.I peptides strongly bound the antibody, as did the PKs fragment (and several lower apparent molecular mass cleavage bands not previously reported). Note that the LAP-generated bands also bound the antibody, confirming that they are P.I fragments. The lower apparent molecular mass LAP-generated P.I fragments passed through the NCP, a common problem with low apparent molecular mass peptides.

In Fig. 4E is shown a revealing blot. The 4G5 monoclonal antibody bound to the intact P.I in the whole cell and PK lanes, as it did in the P.Ic and 1-h LAP lanes. It did not bind to the PKs fragment nor did it bind to any of LAP-generated fragments. In fact, the P.I peptide in the 5-h LAP lane had sufficient cleavage to almost completely abbrogate any 4G5 binding, indicating that the 4G5 epitope may be at the extreme N terminus of the molecule.

^{125}I-labeled peptide maps of the control P.I (primary structure) and the first underlying LAP-generated P.I fragment (LAP band I) excised from the CBB-stained gel (Fig. 4A) are shown in Fig. 5. These peptide maps confirm that the first underlying LAP-generated band is indeed a cleavage product of FA638 P.I. Peptides which are absent or diminished following LAP cleavage are also shown.

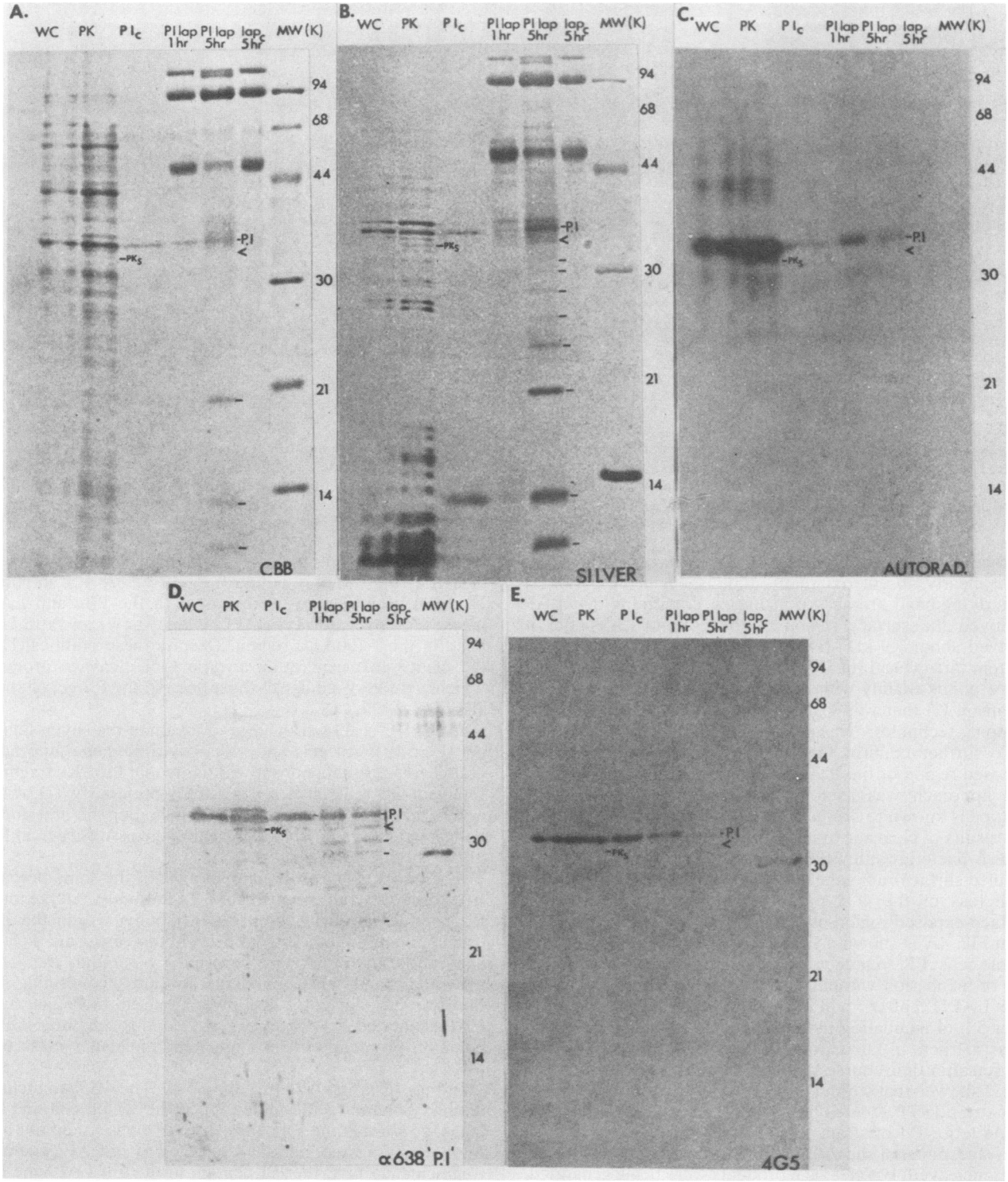

FIG. 4. (A) A CBB-stained 15% SDS-PAGE gel of lysates of strain FA638 surface radiolabeled whole cells (WC), a PK digest of whole cells, P.I control (P.Ic) after 5 h incubation in the absence of LAP, P.I incubated with 25 μg of LAP per ml for 1 h, P.I incubated with 25 μg of LAP per ml for 5 h, LAP control (LAP_c 5 h), and molecular mass (MW) markers in kilodaltons (k). The PKs band is designated with a bar in the PK lane, as are LAP-generated P.I fragments in the P.I LAP 5 h lane. An arrowhead points to the first LAP-generated band underlying the intact P.I. (B) A silver stain of the preparations shown in panel A. Silver staining labeled bands that were very poorly stained with CBB. (C) An autoradiogram of the silver-stained gel shown in panel B. The PKs band (bar in PK lane) retained surface label, as did all the intact P.I bands, yet the first LAP-generated band lost all label. (D) An immunoblot of the preparations shown in panels A to C probed with rabbit anti-FA638 P.I antibody (α638 'P.I'). The intact P.Is, the LAP-generated bands, and the PKs fragments (bars) all bound the antibody, indicating that the fragments are of P.I origin. (E) An immunoblot of the preparations shown in panels A to D probed with monoclonal antibody 4G5. Only the intact P.Is bound the 4G5 monoclonal antibody, while neither the PKs (bar) nor the underlying P.I fragment (arrowhead) bound the antibody. Note that the 30-kDa (Bio-Rad low-molecular-mass marker kit) bound both the rabbit α638 'P.I' antibody and the 4G5 monoclonal antibody, indicating shared epitopes between FA638 P.I and carbonic anhydrase. There were no reactions with normal rabbit serum, normal mouse serum, or conditioned tissue culture medium controls (data not shown).

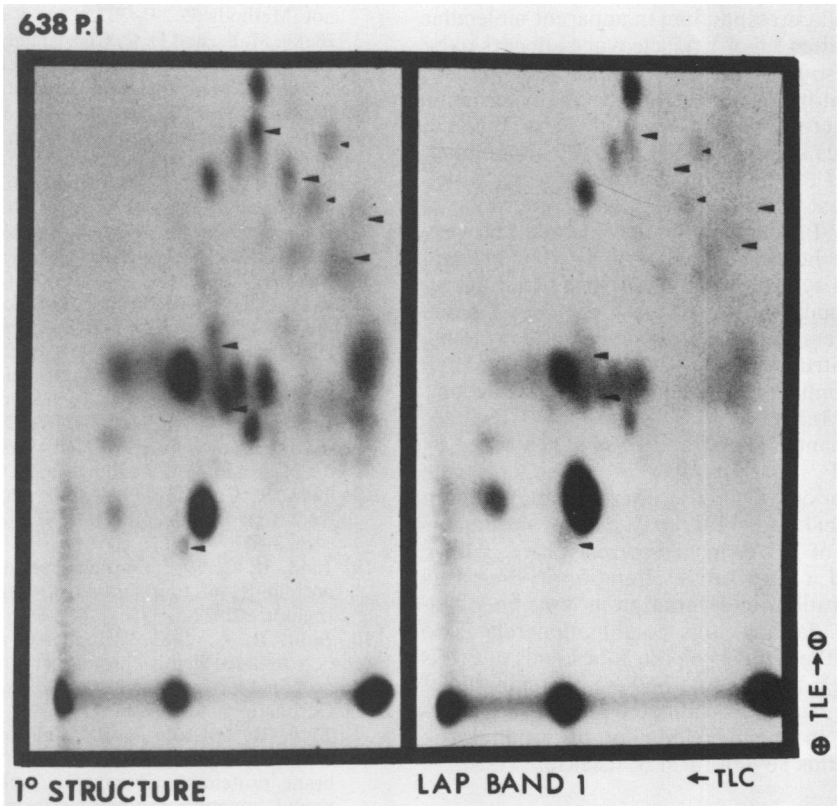

FIG. 5. ^{125}I-labeled peptide maps of P.Ic. (Fig. 4A) (primary [1°] structure) and the underlying LAP-generated P.I band (LAP Band 1) excised from the P.I LAP-5 h lane (arrowhead, Fig. 4A) from the CBB-stained gel. Arrowheads designate peptides that were lost or diminished after LAP cleavage. Abbreviations: TLE, thin-layer electrophoresis; TLC, thin-layer chromatography.

The data presented above indicate evidence that both the 4G5 epitope and the surface-radiolabeled portion of the P.IAs are removed after LAP cleaves approximately 1.9 kDa of the N terminus of the molecule. This region represents about 5% of the total molecule, or about 15 to 20 amino acids.

DISCUSSION

The demonstration that LAP removes both surface radiolabel and the 4G5 monoclonal antibody epitope after it cleaves about 1.9 kDa of the molecule presents strong evidence that the P.IA subclass is oriented in the outer membrane in such a way that the N terminus of the molecule is exposed. This region may represent a rather small portion of the total molecule (about 5% or 15 to 20 amino acids). The small exposure may explain, in part, the poor immunogenicity of the P.IAs in general (Fig. 1B) and their resistance to proteolytic degradation in situ (1, 4).

The association of the P.IA subclass with serum resistance (6, 7) and disseminated gonococcal infection (5) suggests that this minimal exposure may, in some way, protect the organism from being killed by serum factors such as serum proteases. Of four P.IA-bearing strains I have tested, (JS3, JS4, FA635, and FA638), all were resistant to in situ cleavage by pronase, α-chymotrypsin, trypsin, *Staphylococcus aureus* V8 protease, and LAP. Only PK cleaved their P.Is, and it cleaved them at different rates. The strains had the following increasing resistance to cleavage: JS3 < FA638 < JS4 << FA635 (unpublished data). Perhaps the P.IA orientation is a way of minimizing the availability of the protein to antibody or complement binding. K. Joiner (personal communication), however, has demonstrated that the 4G5 monoclonal antibody binds to P.I of FA638 in situ and fixes complement, yet the organism is not lysed, indicating that other factors may also be involved in serum resistance.

Absolute proof that the N terminus is the exposed region must await either results of pulse-labeling experiments or successful N labeling. However, identical experiments with strain JS1 P.IB gave expected results (i.e., the central region of the molecule was exposed). That work will be the subject of a future report. Attempts to cleave P.Is from the C terminus with carboxypeptidases, although not yet completely successful, indicate that LAP is not contamined with C-terminal attacking proteases. Cocleavage of surface-radiolabeled P.IAs with 1 μg of trypsin or α-chymotrypsin with 25 μg of LAP resulted in a mixture of fragments all with apparent molecular masses under 10 kDa, suggesting that LAP is free of endopeptidases as well. This technique therefore appears to present a useful method for the mapping of immunological epitopes within protein molecules. Moreover, demonstration that all surface label is removed by LAP and that this correlates with primary structural and surface peptide mapping results confirms that surface iodination and peptide mapping are valid techniques for investigating surface exposure of proteins.

It seems fortuitous that the N terminus is the exposed region of P.IAs. Because the 4G5 monoclonal antibody failed to bind to either the PKs fragment (about 1.5 kDa smaller than native P.I) or the first LAP-generated P.I fragment (about 1.9 kDa smaller than native P.I), the 4G5 epitope must reside near the N terminus. Moreover, the 4G5

epitope in the P.I band corresponding in apparent molecular mass with native P.I after 5 h of LAP cleavage appears to be sufficiently damaged so as to greatly diminish 4G5 binding, suggesting that the epitope resides at the extreme N terminus of the molecule. Therefore, sequencing of the first 20 amino acids at the N terminus should provide both the immunologically active region of the molecule and that portion which may mediate other host-parasite interactions. Generation of peptidic fragments by P.I enzymatic cleavage and recovery by high-performance liquid chromatography (10, 11) and generation by in vitro synthesis will allow for further immunological and functional analyses of these proteins. I have, as yet, had little success in the N labeling of these proteins, either in vivo or in vitro, which is what prompted the LAP approach, but I am confident that sequencing can be accomplished successfully (3).

A pair of transformants (FA635 and FA638) generated by J. Cannon and P. F. Sparling, one of which is serum sensitive and the other of which is serum resistant, and both of which have identical pedigrees, may help in the understanding of the role of P.IAs in serum resistance. I have already demonstrated a very subtle alteration in their P.Is (manuscript in preparation) and variation in their lipooligosaccharide migration. Perhaps this combination relates to serum resistance. Further studies on the N terminus of P.IAs may reveal information leading to a better understanding of how *N. gonorrhoeae* causes disseminated gonococcal infection. This will help in the development of prophylactic measures to prevent this severe form of disease.

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