125I-Peptide Mapping of Protein III Isolated from Four Strains of Neisseria gonorrhoeae

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Gonococcal outer-membrane protein I (PI) and PIII were isolated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis from reduced and unreduced whole-cell and outer-membrane lysates of four strains of nonpiliated (P-), transparent (O-) Neisseria gonorrhoeae. These proteins were radioiodinated and digested with a-chymotrypsin. The resultant 125I-peptides were then resolved by high-voltage thin-layer electrophoresis, followed by ascending thin-layer chromatography, and visualized by autoradiography. Results corroborated previous observations regarding the structural relationships of PIs having different apparent subunit molecular weights. All PIIIs had very similar apparent primary structures, regardless of the strain from which they were isolated, the source (i.e., whole cells or outer membranes), or the reduction state of the sodium dodecyl sulfate lysates. By the techniques used, it appeared that PIII is structurally similar in all of the gonococcal strains studied, even though each strain had structurally unique PIs.

The gonococcal outer membrane (OM) contains several proteins which are exposed on the cell surface. These proteins have been classified into several groups (28) on the basis of their occurrences and their behaviors in sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE). The major, or principal, OM protein (10), protein I (PI), is present in all gonococci studied (15, 16, 20, 22). The apparent subunit molecular weight (aMW) of PI varies from strain to strain (11, 22, 23) but shows no variation among different phenotypes within a single strain (15, 16, 22, 31). The aMW of PI is not altered by varying the solubilization temperature (5, 18, 24) or by the presence (or absence) of 2-mercaptoethanol (2ME) in the solubilization mixture (18).

In a previous study, the technique of 125I-peptide mapping (23) was used to investigate the structures of three PIs of different aMWs isolated from 10 gonococcal strains. A seemingly high degree of structural homology was found for not only PIs having the same aMW but also for PIs having aMWs of 34,000 (34K) and 33K; several 32K PIs were closely related to each other but distinct from the 34K and 33K PIs. Immunoprecipitation experiments have shown that rabbit antisera raised against OM (18) or whole gonococci (27) react with homologous PI in OM vesicles or on intact cells, respectively, suggesting that PI could contribute to serotype-specific reactions (3, 9, 11).

PIIs are a heterogeneous family of OM proteins which can vary widely in aMW, presence, and number within a single strain (9, 16, 20, 22, 31, 32). They are distinguished by an increase in aMW when solubilized in SDS at 100 versus 56°C (i.e., heat modifiable [5, 9, 24, 31]). The presence of some of these proteins, which all have somewhat similar primary structures as shown by 125I-peptide mapping (24), correlates with gonococcal colony opacity (22). The gain or loss of PIs occurs at a high rate, necessitating single-colony transfer on an appropriate medium to maintain the desired phenotype (21). These proteins have extensive surface exposure (1, 5, 6, 26) and have been associated with gonococcal aggregation (20), susceptibility of gonococcus to killing by serum (2), and interactions with eucaryotic cells (8, 13, 25, 29). Immunoprecipitation with antisera raised against whole gonococci has shown that rabbit immunoglobulin G can combine with PIIIs in situ on intact organisms (27), demonstrating that these proteins, like PIs, are immunogenic and antigenic and may, therefore, contribute to serotype-specific reactions.

PIIIIs (5, 18) are characterized by an increase in aMW when solubilized in the presence of 5 to 8% 2ME as compared with solubilization without 2ME (i.e., 2ME modifiable) [18]. The aMWs of the unreduced (30K PIII) and the reduced (31K PIII*) forms of PIII are the same in all strains and intrastrain phenotypes studied to date (27). PIIIs, unlike some PIs and all PIIIs, are very resistant to exogenous proteolytic cleavage.
(1, 26) and are weakly iodinated by lactoperoxidase (5). Iodogen (17), a smaller surface-reactive catalyst, more efficiently radiolabels PIIIs (unpublished data), suggesting that PIIIs may have less surface exposure and perhaps less accessibility to surface-reactive agents than do PIIs and PIIIs.

PIII has been shown to exist in heteropolymers with PI in whole cells (WCs) and OM vesicles by both cross-linkage studies (18, 19) and radioimmunoprecipitation (18, 27). Since PI and PIII seem to coimmunoprecipitate, it is difficult to establish whether antibodies react directly with PIII or whether PIII is merely an "innocent bystander" immunoprecipitated by immunoglobulin G-PI complexes. Since PIIIs appear to be found in all of the strains and phenotypes studied to date, seem to be exposed on the gonococcal surface, and are intimately associated with PI, they may contribute either cross-reactive serotypic antigens, if alike, or strain-specific antigens, if different, to the various immunological reactions described for gonococci and gonococcal OM vesicles (17).

In this study, the structures of PIIIs isolated from four gonococcal strains, chosen because the PI of each strain has a different aMW, were investigated by the technique of 125I-peptide mapping. PI and PIII were obtained from each strain by SDS-PAGE of unreduced or reduced lysates from WC and OM preparations. The protein bands were excised, radioiodinated, and digested with a-chymotrypsin. Resultant 125I-peptides were resolved by high-voltage electrophoresis, followed by ascending thin-layer chromatography (TLC). The migration of the 125I-peptides was then visualized by autoradiography, yielding characteristic "fingerprints" for the proteins under investigation. Results confirmed that there are two PI structural homology groups, one containing the higher aMW PIIs and the other containing the lower aMW PIIs; all PIIIs appeared to be structurally similar in all four strains studied.

**MATERIALS AND METHODS**

**Bacteria. Neisseria gonorrhoeae** strains JS1 (original designation, F62), JS2 (original designation, 10677-3), JS3 (original designation, 120176-3), and JS4 (original designation, MSL-7040, 1972) were grown on clear typing medium (21) as previously described (21). Organisms were grown at 36°C in 5% CO2 for 18 h. Nonpiliated (P−), transparent (O−) organisms were used throughout this study.

**OM preparations.** OM vesicles were prepared by shaking whole N. gonorrhoeae in 0.1 M Tris–1 M NaCl–0.02% sodium azide buffer (pH 8.0) at 43°C with 0.3-mm-diameter glass beads. Vesicles were then isolated by differential centrifugation and column chromatography (Sepharose 6B; Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) (23) and stored at −20°C until used.

**SDS-PAGE.** The proteins used in 125I-peptide mapping were separated on 15% acrylamide (acylamide/N,N′-methylenebisacrylamide ratio, 30:0.8) slab gels by the Tris-glycine system of Laemmli (14). WC or OM samples were solubilized at 100°C in 10% (wt/vol) SDS–10% (vol/vol) glycerol–0.1 M Tris (pH 6.8) solubilizing solution, either with or without 8% 2ME. Samples were electrophoresed at 45 mA until the tracking dye (bromophenol blue) had migrated approximately 75 mm. The gels were then fixed in 7% acetic acid–25% 2-propanol, stained with 0.2% Coomassie brilliant blue, and destained until the background cleared. The low-molecular-weight markers Phosphorylase B (94K), bovine serum albumin (68K), ovalbumin (43K), carbonic anhydrase (30K), soybean trypsin inhibitor (21K), and lysozyme (14.3K) (Bio-Rad Laboratories, Richmond, Calif.) were included in each gel.

**2-D SDS-PAGE.** Two-dimensional (2-D) SDS-PAGE was used to visualize the change in aMW of PIIIs in the presence of 2ME. WC or OM strains were solubilized in 10% (wt/vol) SDS–10% glycerol–0.1 M Tris (pH 6.8) without 2ME and subjected to SDS-PAGE as described above. One of two duplicate lanes of each strain was excised from the gel and soaked in 0.2% (wt/vol) SDS–8% (vol/vol) 2ME–0.1 M Tris (pH 6.8) for 1 h at 56°C. The gel lanes of each strain, which were cut so as to include only the region containing PI and PIII, were then inserted, at a 90° angle to the first dimension electrophoresis, into a slab gel apparatus which had a prepolymerized 15% running gel in place. A stacking gel consisting of 4% agarose–0.2% (wt/vol) SDS–0.1 M Tris (pH 6.8) was held at 56°C until needed. Immediately before use, 2ME was added to 1%. This solution was then pipetted around the reduced gel lane and allowed to solidify. This was then electrophoresed, and the gel was fixed and stained as described above.

**Radioiodination and protease treatment of protein bands.** The radioiodination and a-chymotrypsin digestion of proteins in bands excised from acrylamide gels were done by the procedures described by Elder et al. (4) and Swanson (23).

**Electrophoresis and chromatography.** The 125I-peptide fragments resulting from α-chymotryptic proteolysis were suspended in a solution of L-leucine–L-arginine–L-tyrosine (0.2 mg/ml each) to yield approximately 30,000 cpm/μl. A 2-μl amount of this mixture was spotted onto a Polygram R Cel 300 (Brinkmann Instruments, Inc., Westbury, N.Y.) thin-layer cellulose sheet (two samples per sheet). High voltage thin-layer electrophoresis (TLE) was carried out at a constant 1,200 V for 45 min on a TLE 20 apparatus (Savant Instruments, Inc., Hicksville, N.Y.) under Varsol cooled to 10°C by a circulating cooling bath. Electrophoresis buffer was a solution (pH 3.7) of water-acetic acid- pyridine (200:10:1). After electrophoresis, the sheet was air dried and cut down the center line. Each half was then turned 90° and subjected to TLC, which was allowed to proceed until the solvent front (N-butanol–pyridine–water–acetic acid, 13:10:8:2) was within 2 to 3 mm of the sheet top. The sheets were dried, sprayed with 0.25% ninhydrin in acetone to locate the amino acid markers, and applied to X-ray film (XAR-5; Eastman Kodak Co., Rochester, N.Y.). The 16-h exposure was enhanced by using a Cronex (Du Pont Co., Wilmington, Del.) intensifying screen at −76°C.
peptide mapping: A, for PIII) lysates I'-L', Pllls; PIII* strains (See Fig. PI; b, JS2 lysates electrophoresed PIII* strains JS1, JS2, JS3, JS4. (See Fig. PI; JS2). lysates were mixed). The appropriate digest of each strain was digested with 2ME and subjected to SDS-PAGE. The aMW of each strain was estimated to be as follows: JS1, 36.5K; JS2, 36K; JS3, 35.4K; and JS4, 35K. These aMWs were higher than previously published values (23) and more in agreement with estimates of other investigators for PIs from these strains owing to the current use of different molecular-weight markers. There was no alteration of aMW of any of the PIs when solubilized either with or without 2ME. Each PI band (Fig. 1, bands A–D and 1B, bands a–d) was excised and examined by 125I-peptide mapping.

The PIIIs of each strain can be clearly seen in the SDS-PAGE gel of the OM lysates (Fig. 1B). Comparison of the unreduced and reduced preparations shows the increase in aMW in the presence of 2ME which characterizes these proteins. The aMW of the unreduced PIIIs was estimated to be 30.4K (30K PIII), whereas that of the reduced form had an aMW of 31.4K (31K PIII*).

The PIIIs were much less apparent in gels of WC lysates than they were in OM lysates. Nevertheless, 31K bands could be identified in the reduced WC preparations, which showed a
slightly increased staining intensity (Fig. 1A, bands I'-L') when compared with the unreduced preparations. These bands had the same aMW as that of 2ME-modified PIIIs seen in OM lysates (Fig. 1B) and correlated in aMW with the PIII* seen above the diagonal in Fig. 1C and with surface-iodinated PIIIs (12). In addition, the PIII* spot above the diagonal in Fig. 1C was confirmed to be surface iodinatable by 2-D SDS-PAGE of surface-iodinated WC lysates (data not shown) and of surface-labeled OM lysates (data not shown). Based on these correlations, these bands were designated as reduced 31K PIIIs. A prominent 30K band could be seen in all strains in both the unreduced and reduced WC lysates (Fig. 1A, bands E–H and E'–H'). In addition, bands corresponding in aMW to reduced PIIIs, not present in unreduced OM lysates, were seen in all unreduced WC lysates (Fig. 1A, bands I–J). These bands showed slight differences in aMW (<500) from strain to strain which did not occur in any of the 30K PIIIs or the 31K PIII*s. The fate of 31K PIII bands in the reduced preparations is not clear. A faint band directly above the 31K PIII* of the JS2, 2ME-reduced WC lysate may correspond to the JS2 31K PIII; however, it seems likely that the 31K PIII* bands overlay the 31K PIII bands in reduced WC lysates. All of the above PIIIs bands were excised and examined by 125I-peptide mapping.

2-D SDS-PAGE of WC lysates. To confirm that 2ME modification of PIII occurred in WC lysates, 2-D SDS-PAGE was performed on WC lysates of each *N. gonorrhoeae* strain. The 2-D SDS-PAGE of this experiment (Fig. 1C) shows that 2ME modification of PIII, as evidenced by the single spot falling well above the diagonal, occurs in the WC lysates of all four strains. Similar results were observed in 2-D gels of OM vesicles (data not shown).

125I-peptide mapping. Those bands indicated in Fig. 1 were excised and subjected to 125I-peptide mapping. The 125I-peptide maps of PIs from lysates of WCs and OM from all four strains are shown in Fig. 2A and B. 125I-peptide maps in Fig. 2, 3, and 4 are shown in groups of four, with JS1 and JS2 preparations on the upper left and right, respectively, and JS3 and JS4

![Image](http://iai.asm.org/DownloadedFromhttp://iai.asm.org)
FIG. 3. α-Chymotryptic $^{125}$I-peptide maps of PIII from P$^{-}$O$^{-}$ WC's of N. gonorrhoeae strains JS1, JS2, JS3, and JS4. (A) Unreduced PIII (30K PIII); (B) unreduced proteins (31K PIII) corresponding in aMW to reduced PIII (31K PIII$^*$); (C) reduced PIII (30K PIII$^*$) corresponding in aMW to 30K PIII; (D) 31K PIII$^*$'s. Letters on each map correspond with bands shown in Fig. 1. Arrow indicates a variable peptide as discussed in the text. TLC, Ascending thin-layer chromatography.
preparations on the lower left and right, respectively. The direction of high-voltage TLE is always towards the top of each figure, and the direction of ascending TLC is toward the left (Fig. 2A). The double $^{125}$I-peptides shown in the upper right corner of each map are felt to be artifactual, since they occur in maps of unrelated, nongonococcal proteins (data not shown).

Virtually identical $^{125}$I-peptide maps of PI for each *N. gonorrhoeae* strain were obtained from SDS-PAGE gels of WC lysates and OM preparations (Fig. 2A and B). The directions of high-voltage TLE and TLC procedures. These variations were reflected in the migration of amino acid markers (data not shown).

The $^{125}$I-peptide maps of JS1 and JS2 PIs were very similar, with two relatively acidic, hydrophilic $^{125}$I-peptides (as described by Tsai et al. [30]) occurring only in the JS2 PI (arrows, Fig. 2A and B). The maps of JS3 and JS4 PIs were quite different from those of JS1 and JS2 PIs but were very similar to one another; however, the JS3 PI had one acidic, hydrophilic and two approximately neutral, hydrophilic $^{125}$I-peptides (30) not seen in the JS4 PI (double arrows, Fig. 2A and B). In addition, three hydrophilic peptides (30; stars, Fig. 2A and B) were more intensely labeled in the JS3 PI. Despite the different overall $^{125}$I-peptide maps of the JS1 and JS2 PIs as compared with the JS3 and JS4 PIs, several peptides appeared to be shared by all PIs, suggesting that some structural homology exists among all PIs. These results corroborated earlier observations (23) regarding the structural relationships of PIs.

$^{125}$I-peptide mapping of PIIIs. The $^{125}$I-peptide maps of PIIIs isolated by SDS-PAGE of both WC (Fig. 3A–D) and OM (Fig. 4A and B) lysates were quite distinct from any of the P1 $^{125}$I-peptide maps. All of the PIIIs showed a remarkable degree of similarity both within a given strain and among different strains. Each group of four $^{125}$I-peptide maps represents the same aMW PIII type, either reduced or unreduced, isolated from each of the four strains. The preparations are designated as follows: 30K PIII, 30K bands from WC and OM lysates.
FIG. 5. α-Chymotryptic 125I-peptide maps of mixtures of PIIIs isolated from WC lysates of \( \text{P}^+ \text{O}^- \text{N. gonorrhoeae} \) strains JS1, JS2, JS3, and JS4. (A) Mixture of 30K PIIIs from each strain; (B) mixture of 31K PIIIs from each strain; (C) mixture of 30K PIII* from each strain; (D) mixture of 31K PIII* from each strain; (E) mixture of JS1 30K PIII, 31K PIII, 30K PIII*, and 31K PIII*; (F) composite α-chymotryptic 125I-peptide map of PIII. ●, Common to PIIIs; ●, varies; ●, weak or variably resolved; ●, absent from 31K PIIIs; ●, artifact; ●, varies as a group in TLE migration. Arrow indicates a variable peptide as discussed in the text. TLC, Ascending thin-layer chromatography.

subjected to SDS-PAGE in the absence of 2ME (Fig. 3A and 4A); 31K PIII, ~31K bands from WC lysates subjected to SDS-PAGE in the absence of 2ME (Fig. 3B); 30K PIII*, 30K bands from WC lysates subjected to SDS-PAGE in the presence of 2ME (Fig. 3C); 31K PIII*, 31K bands from WC and OM lysates subjected to SDS-PAGE in the presence of 2ME (Fig. 3D and 4B).

Comparison of PIIIs within each strain. The structural relationships of the various PIIIs found in each strain could be ascertained by comparing 125I-peptide maps in the same relative positions within the different PIII groupings (e.g., to compare JS1 PIIIs, see Fig. 3A, upper left, and Fig. 3B, upper left). The overall 125I-peptide map patterns of all WC PIII forms within each strain suggest that all of these proteins have very similar primary structures. The 30K PIII, the 30K PIII*, and the 31K PIII* were found to be identical by 125I-peptide mapping for one strain by mixing experiments (Fig. 5E). The 31K PIIIs, though identical among strains (Fig. 3B and 5B), were different from 30K PIII, 30K PIII*, and 31K PIII*. Several peptides were absent from 31K PIII type as shown in Fig. 5F, a composite PIII 125I-peptide map.

The apparent identities of 125I-peptides from 30K PIII and 31K PIII* of OMs were also confirmed by carrying out the 2-D separation on
mixtures of the PIII types of all four strains (data not shown). The slightly different positioning of some of the peptides in the $^{125}$I-peptide maps of the OM PIIIIs as compared with the WC PIII maps (see Fig. 5F) is believed to be owing to technical variations inherent in such complex procedures.

There are some intensity differences between the WC and OM PIII peptide maps, with several of the OM PIIIs peptides being more weakly labeled than those in the WC PIII preparations. However, increased exposure times of the OM PIII maps confirmed that all of the peptides seen in WC PIII maps were present in the OM PIII preparations as well. Mixing experiments of JS1 PIIIs from WCs and OM showed that all but three of the peptides migrated to the same location, with only the three peptides in the lower right of each PIII map being blurred (data not shown). Therefore, it appears that the OM PIIIs are not significantly different from the same PIII types found in WCs.

**Interstrain comparison of PIIIs.** Interstrain comparisons of each PIII type are made by observing the four $^{125}$I-peptide maps within each group (e.g., 30K PIIIs, Fig. 3A and 31K PIIIs, Fig. 3B). It appears that each PIII type is structurally identical in all strains (confirmed by mixing experiments) in both WC PIIIs (Fig. 3A–D and 5A–D) and OM PIIIs (Fig. 4). One peptide, marked by an arrow in all PIII maps, varied in occurrence (or possibly intensity), especially in the WC PIII maps. It was generally more apparent in the 30K PIIIs than in the 31K PIIIs. As noted above for JS1, the $^{125}$I-peptide maps of 30K PIIIs, 30K PIIIs*, and 31K PIIIs* were very similar in both WCs and OM’s of strains JS2, JS3, and JS4. The 31K PIIIs, which were the same in all strains, differed from the other PIIIs in that four peptides seen in all other PIIIs were absent from the 31K PIII preparations. Four peptides, denoted by open triangles in the PIII composite map (Fig. 5F), showed some variation as a group in TLE migration. However, since the migration of these peptides varied as a group in individual PIII preparations while behaving identically in mixed $^{125}$I-peptide maps, the observed variation in migration of these peptides is felt to reflect technical variations rather than unique peptides (see Discussion).

The above results support the following conclusions. (i) $^{125}$I-peptide maps obtained for PI and PIII from SDS-PAGE of WC lysates correspond to $^{125}$I-peptide maps of the same proteins in OM preparations. (ii) PIII appears to be similar or identical in all strains studied, regardless of the PI type. (iii) 2ME modification of PIII occurs in both WC and OM lysates. This modification seems less complete in WC lysates than in OM preparations. (iv) The primary structure of PIII as revealed by $^{125}$I-peptide mapping is not significantly altered as a result of 2ME modification. (v) A unique protein of ~31K occurs in WC lysates in the absence of 2ME. It exhibits slight aMW differences among strains and is similar in structure to PIII. (vi) The four PIs in this study can be categorized into two $^{125}$I-peptide map homology groups, one containing the two higher aMW PIs and the other containing the two lower aMW PIs. Despite these similarities, all PIs do have differences in their primary structures.

**DISCUSSION**

Interest in PIII has increased since previous studies have shown that PI and PIII exist as a heteropolymeric unit in situ in both intact *N. gonorrhoeae* (19) and OM vesicles (18). Immunoprecipitation experiments have shown that PI and PIII coprecipitate (18, 27), prompting speculation about the role of PIII in immunological reactions on the surface of *N. gonorrhoeae*. In this study, the technique of $^{125}$I-peptide mapping has been used to study the structural relationships of PIIIs from four strains of *N. gonorrhoeae*. PI and PIII bands from SDS-PAGE were obtained from both WC lysates and OM vesicles of each strain and were subjected to $^{125}$I-peptide mapping. Several modifications of the procedure resulted in enhanced resolution of the $^{125}$I-peptides generated by α-chymotrypsin digestion. The use of the Savant TLE 20 apparatus allowed more efficient cooling than did a flatbed apparatus and resulted in more consistent migration and greater resolution of $^{125}$I-peptides in the electrophoresis step. Precise temperature control during TLE is very important. Temperature variation is accompanied by a change in the pH of the running buffer, and this change alters the charge on all of the peptides. If a peptide undergoes a change charge (i.e., deprotonates) at a pH that is close to that of the TLE running buffer, small pH fluctuations can have a profound effect on the electrophoretic mobility of the peptide. The four peptides in the PIII $^{125}$I-peptide maps, which show some variation in TLE migration, illustrate this point. Mixing experiments (Fig. 5) clearly demonstrated that these peptides are identical in the different preparations; yet even with the improved cooling provided by the Savant apparatus, they showed some variation from run to run.

Peptide mapping of PIs of the four strains extended previous studies; although all PIs shared some structural similarity, two homology groups, one containing the 36.5K and 36K PIs and the other containing the 35.4K and 35K PIs, were apparent. It is interesting that the peptides which were unique to the individual PIs were all hydrophilic. If they represent surface-exposed portions of the PI molecules, they may contrib-
ute to antigenic differences observed among the PI types.

The 125I-peptide mapping patterns obtained for the various PIIIs indicated that, with the exception of the 31K PIII, all PIIIs seemed virtually identical. There were no observed peptides which were unique to one strain, as were seen in the 125I-peptide maps of PIs. One peptide in PIIIs isolated from WCs did seem to vary in occurrence (or possibly intensity). This peptide was not observed in PIIIs isolated from OM vesicles. With this exception, the PIIIs from OMs appeared to be very similar to those derived from WCs. Similarly, reduction did not significantly alter 125I-peptide patterns of PIII, indicating that no major change in primary structure accompanies the increase in aMW of 2ME-modified PIIIs and supporting the suggestion that PIII has an internal disulfide linkage which, when cleaved in reducing environments, allows the molecule to more fully unfold, thereby increasing the Stoke’s radius and the aMW (18).

Conversion of 30K PIII to 31K PIII* by 2ME was clearly evident in OM vesicles (Fig. 1B) but was less easily visualized in WC lysates. One-dimensional electrophoresis (Fig. 1A) showed that only a small amount of PIII was converted to the higher aMW form. However, 2ME treatment of the one-dimensional SDS-PAGE, followed by 2-D electrophoresis (Fig. 1C), showed that the majority of the PIII material was converted to the 31K PIII* form, suggesting that only a partial conversion of PIII from 30K to 31K occurs in WC lysates in the presence of 2ME. This could indicate that WCs have many more sites competing for the available reducing agent or that PIII is less accessible to reduction in WC preparations owing to decreased penetration of the 2ME or limited exposure of PIII to the reducing environment.

The 31K PIII, which was present in unreduced WC lysates, was similar to the other PII types but lacked several peptides seen in the other PIIIs. This protein was not readily radiiodinated by surface-reactive agents (unpublished data), nor was it found in OM lysates, suggesting that the 31K PIII may not be a constituent of blebbed OM vesicles. It may represent a precursor of PIII or a modified form of PIII which functions in a different capacity than does PIII. The fate of the 31K PIII in reduced WC lysates is not clear. It seems likely that the 31K PIII* band overlays the 31K PIII band and that the 125I-peptide map of 31K PIII* is actually a composite of the two proteins. Certainly, further study is necessary to elucidate the role of the 31K PIII.

There were a few 125I-peptides which appeared to migrate to the same location in the PI and PIII maps. Similar observations have been made regarding structural relationships between PIIIs and PIs (24), indicating that a certain amount of structural homology exists among all of the N. gonorrhoeae OM proteins studied by 125I-peptide mapping. However, the differences between the PI and PIII maps demonstrate that PIII is not a cleavage product of PI. PIII also appears to be quite different than the PIIIs so far studied (24; unpublished data).

On the basis solely of structural data, it is difficult to predict what the immunological relationships of PIIIs from different strains may be. It has been shown that PIIIs having similar 125I-peptide maps have different peptides exposed on the bacterial surface (7). Furthermore, PIIIs which had similar structures had different antigenicity as assessed by radiodeterminoprecipitation of WCs; the observed differences in antigenicity correlated with the surface exposure of different peptides (J. Swanson, O. Barrera, and R. C. Judd, manuscript in preparation). Whereas PIIIs may have different peptides exposed on the surface when associated with different PI types, the virtually identical 125I-peptide maps of these proteins suggest that all PIIIs will have the same surface exposure.

The nature of PIII surface exposure in both WCs and OM vesicles can be investigated by extrinsic 125I labeling, followed by peptide mapping procedures similar to those discussed in this study. This information may help elucidate further the contribution of PIII to the structure and immunobiology of the gonococcus.

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LITERATURE CITED