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^{125}I-Labeled Peptide Mapping of Some Heat-Modifiable Proteins of the Gonococcal Outer Membrane

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Gonococci from opaque colonies have cell wall outer membrane proteins that are lacking from organisms which form transparent colonies. These "colony opacity-associated" proteins are among a group of "minor" proteins that exhibit heat modification of their apparent subunit molecular sizes, are easily extracted by deoxycholate, have apparent subunit molecular weights varying from 24,000 to 29,000 and are exposed on the surfaces of gonococci. Other minor proteins found on gonococci are the "leukocyte association proteins," whose presence correlates with reactivities of gonococci with human neutrophils. Several of the colony opacity-associated proteins and leukocyte association proteins were subjected to ^{125}I-peptide mapping of protein bands separated by polyacrylamide electrophoresis in the presence of sodium dodecyl sulfate. The structural similarities and differences among these heat-modifiable surface proteins were studied, as well as their similarities with the major protein of the gonococcal outer membrane. A relatively high apparent degree of structural homology is found among the heat-modifiable proteins from different strains of opaque colony gonococcal forms. There is also some apparent structural homology for ^{125}I-peptides of heat-modifiable versus major proteins of the gonococcal outer membrane.

Relatively few proteins are prominent in gonococcal cell wall outer membranes subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE); these can be categorized as either major proteins (MPs) or heat-modifiable proteins (hmP's). An MP is present as one of several subunit molecular weight forms in all gonococcal outer membranes examined (8, 9, 10, 14, 15, 27); there is no variation in the apparent subunit size of the MP for different colony phenotype preparations within a single strain (10, 23, 24, 26). The MP is quantitatively dominant in many outer membrane preparations and is thought to be an important contributor to the serotype specificity demonstrable with whole gonococci or cell wall outer membrane preparations (2, 13, 15). MP is labeled with ^{125}I by lactoperoxidase-catalyzed radioiodination of intact gonococci (10, 16, 24); however, it is relatively resistant to proteolytic attack by trypsin (22, 23), and it is not readily solubilized or extracted from outer membranes by deoxycholate (DOC) (9, 24). These observations suggest that MP is relatively deeply embedded in the outer membrane but has a portion exposed on the gonococcal exterior (13, 26). No change in apparent molecular weight is found for the MP when outer membranes or whole organisms are subjected to different degrees of heating during solubilization for SDS-PAGE (9, 13, 24). Taken together, the above observations suggest an analogy between the MP of gonococci and the "porins" of enterobacteria (1, 19, 20); however, the gonococcal MP has not been shown to act as a porin, nor have two different MP species been found in a single gonococcal strain, as is the case for porin proteins in some *Escherichia coli* K-12 preparations (3).

The hmP's vary considerably in occurrence, abundance, and apparent subunit size among different gonococcal preparations (18, 23, 24). Some dark, opaque colony forms yield whole cell lysate or outer membrane preparations in which one or more hmP's are actually equal to MP in abundance (10); in whole cell lysates or outer membranes of light, transparent colony preparations, these hmP bands are not usually visible by SDS-PAGE (18, 24). The hmP's vary in their apparent molecular weights from strain to strain and among intrastrain variants (18, 25). Variation in apparent molecular weights of hmP's also depends on the heating conditions used during solubilization of the proteins for SDS-PAGE (6, 9, 27). These hmP's are readily radioiodinated by lactoperoxidase treatment of intact gonococci and are also susceptible to proteolysis during trypsin treatment of whole organisms (25, 26).

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In these respects the gonococcal hmP's are similar to *tolG* protein, which is located in the outer membranes of some *E. coli* strains (4, 21).

My interest in the gonococcal outer membrane hmP's derives from observations which correlate particular biological activities with the presence or absence of these proteins in or on the gonococcal outer membrane. Both the degree of intergonococcal aggregation and the degree to which gonococci interact with human neutrophils appear to correlate with the presence of particular, different hmP's on the gonococcal surface (23, 27).

In this work the structures of gonococcal outer membrane hmP's were studied by ^{125}I-peptide mapping. The predominant hmP bands occurring in opaque colony outer membrane preparations were compared for different gonococcal strains, and these colony opacity-associated hmP's (hmP_{OP}'s) were compared with hmP's whose presence correlates with enhanced leukocyte association (LA) (hmP_{LA}'s) of LA+ gonococci. The comparative ^{125}I-peptide mapping of MPs and hmP's is presented for two strains. Finally, the specificity of the ^{125}I-peptide method was studied by carrying out ^{125}I-peptide analysis on proteins having known amino acid compositions.

MATERIALS AND METHODS

Organisms and medium. Gonococci were grown on a clear solid typing medium whose composition has been described previously (23). Laboratory strains F62 and MS11 were used, as were several strains more recently isolated from patients. The majority of recent isolates were obtained from the Salt Lake City, Utah, area and are designated by the date and order of isolation; e.g., 52077-5 is the fifth isolate obtained on 20 May 1977. All gonococci were serially passaged as colonies that were dark and very opaque. In addition, two strains were obtained from L. W. Mayer from a collection of isolates at the University of Washington; these are designated KH7189 and KH6305 and correspond to strains used in a study by Hildebrandt et al. (11). Cultivation was in 5% CO_2 at 36°C for 21 to 24 h before study.

Cell wall outer membrane preparations. Gonococci were scraped off solid medium into phosphate-buffered saline and were pelleted by centrifugation. Outer membranes were collected from these pelleted gonococci by agitation of the organisms in tris(hydroxymethyl)aminomethane-NaCl-sodium azide at 43°C, differential centrifugation, and chromatography on Sepharose 6B in the same buffer. The outer membrane fragments were pelleted from void volume fractions by ultracentrifugation and were then subjected to solubilization in DOC and ultracentrifugation as described previously (26). The pellet and supernatant fractions after DOC incubation and ultracentrifugation were carefully separated. For MS11 LA+-derived membranes, DOC-soluble material was subjected to chromatography on Sephadex G150 in the presence of DOC, as previously described (26).

Proteins of known amino acid composition. Chicken egg white lysozyme, chicken ovalbumin, and bovine pancreatic ribonuclease were obtained at the highest purity available from Worthington Biochemicals Corp. Ribonuclease and ovalbumin from Pharmacia Corp. were also utilized, as was lysozyme purchased from Sigma Chemical Co.

SDS-PAGE. All proteins subjected to ^{125}I-peptide mapping were separated on 12.5% acrylamide (ratio of acrylamide to *N,N'*-methylenebisacrylamide, 30:0.8) slab gels in the tris(hydroxymethyl)aminomethane-glycine system described by Laemmli (17) after the specimens were solubilized by boiling for 10 min in SDS, unless otherwise noted. Protein bands were visualized in the slab gels by staining with Coomassie brilliant blue and destaining in 25% isopropanol-10% acetic acid.

Radioiodination and protease treatment of protein bands. Gels containing bands for further study were soaked in 7% acetic acid overnight, and then ^{125}I-peptide maps were obtained essentially by the method of Elder et al. (5), as described in a previous publication from this laboratory (26), with the following modifications. After radioiodination, the gel fragments were washed in 15% methanol for 4 to 20 h (approximately 250 ml/specimen, two changes of methanol) and dried down. The radioiodinated proteins were subjected to digestion only in α-chymotrypsin.

Electrophoresis and chromatography. The dried residue containing ^{125}I-peptides was taken up in 10 μl of water, and 2 liters of a solution containing L-leucine, L-arginine, and L-tyrosine (1 mg of each per ml in water) was added. After mixing, 6 μl of this solution was spotted onto a thin-layer cellulose sheet (Polygram R cell 300; Brinkmann Instruments Inc.). Two specimens were spotted onto each sheet (20 by 20 cm). Electrophoresis was carried out for 1 h at a constant voltage of 100 V and 0°C in a pH 3.7 solution containing water, acetic acid, and pyridine (200:10:1). After air drying, the cellulose sheet was split so ascending chromatography proceeded 10 cm for each specimen in *n*-butanol-pyridine-acetic acid-water (260:200:40:160). The sheets were dried, sprayed with 0.25% ninhydrin in acetone to locate the added amino acids, dried, and applied to X-ray film (XM-2, Kodak). A 1- to 3-day exposure at room temperature was usually adequate for satisfactory definition of the majority of radioiodinated moieties in autoradiograms. All photographs of ^{125}I-peptide map autoradiograms were taken with a Polaroid MP-3 camera and Polaroid T52 film and identical exposure and reduction factors.

RESULTS

SDS-PAGE of outer membranes from opaque and transparent variants. Cell wall outer membranes were obtained from both opaque and transparent nonpiliated colony forms of strain F62 gonococci and were incubated with DOC. The pellet and supernatant fractions were subjected to SDS-PAGE after solubilization in SDS (Fig. 1). A 34,000-dalton MP (MP34) band was present in all fractions

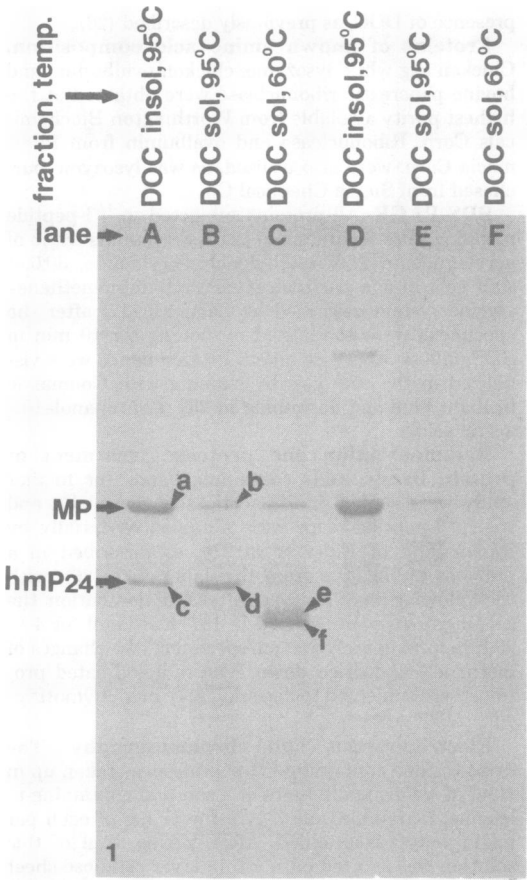

FIG. 1. *Outer membranes prepared from strain F62 opaque colony forms (lanes A through C) and transparent colony forms (lanes D through F). The outer membrane material was incubated with DOC and separated into pellet (DOC-insoluble) fractions by ultracentrifugation. These fractions were subjected to SDS-PAGE after solubilization at 95°C (boiling) or 60°C in SDS, as indicated. Note the occurrence of an MP having the same apparent molecular weight in both opaque and transparent forms, in both DOC-insoluble and DOC-soluble fractions (bands a and b), and in all preparations regardless of temperature of solubilization. The opaque colony form membranes were solubilized at 95°C (lanes A and B), and in these membranes there was a 24,000-dalton minor protein band (bands c and d). This $hmP24_{OP}$ band was absent from transparent colony form membranes (lanes D through F). In specimens of DOC-soluble material from opaque colony forms, solubilization at 60°C yielded two bands at approximately 22,000 (band e) and 21,000 daltons (band f) in place of the band at 24,000 daltons (bands c and d). Both MP (bands a and b) and hmP (bands c through f) were examined by ^{125}I-peptide mapping, as shown in Fig. 2a through f. The letter designations used for the protein bands in the SDS-PAGE gel of this figure correspond to the ^{125}I-peptide pattern letter designations in Fig. 2a through f.*

and was much more prominent in the DOC-insoluble or pellet samples (Fig. 1, lanes A and D) for both opaque and transparent colony-derived material. The MP34 band did not vary in migration characteristics with different conditions of heating during solubilization of the membranes in SDS (Fig. 1, lanes A through F). MP34 appeared to have the same subunit molecular weight for opaque and transparent colony forms, as demonstrated previously (25, 27). The opaque colony outer membranes contained an hmP band of 24,000 apparent molecular weight in specimens boiled in SDS (Fig. 1, lanes A and B). This 24,000-dalton hmP (hmP24) band was either absent or greatly reduced in staining intensity in transparent colony outer membranes (Fig. 1, lanes D through F) and appeared to be associated with colony opacity in this strain. This 24,000-dalton hmP_{OP} ($hmP24_{OP}$) was more prominent than MP in the DOC-insoluble material (Fig. 1, lane B), whereas the converse was true in the DOC-insoluble fraction (Fig. 1, lane A). The hmP24 band was seen only in material that had been solubilized in SDS by boiling; outer membrane material solubilized at 60°C had two other, lower-molecular-weight bands which were not present in boiled specimens (Fig. 1, lane C).

^{125}I-peptide maps of major and minor outlet membrane proteins of strain F62. MP and hmP bands (a through f) were excised from the gel shown in Fig. 1 and yielded the ^{125}I-peptide maps shown in Fig. 2. The MP34 bands in the DOC-soluble and DOC-insoluble fractions had identical ^{125}I-peptide patterns (Fig. 2a and b). MP34 and hmP24 had distinctly different ^{125}I-peptide patterns, as discussed in detail below. No difference in radioiodinated peptides was seen when the single 24,000-dalton band in 95°C-treated DOC-soluble proteins (Fig. 2d) was compared with the double band of smaller molecular weight found in similar material solubilized at 60°C (Fig. 2d through f). One difference in ^{125}I-peptide patterns was found in comparisons of hmP24 bands in DOC-insoluble and DOC-soluble fractions, both of which were solubilized by boiling (see legend to Fig. 2).

^{125}I-peptide maps of hmP_{OP}'s of different gonococcal strains. Cell wall outer membranes were obtained from opaque colony forms of several gonococcal strains and subjected to SDS-PAGE. MP bands of these strains varied in apparent molecular weight from 34,000 to 32,000. The most prominent hmP_{OP}'s exhibited differing molecular weights (from 26,000 to 24,000); additional minor bands were seen in some of the outer membrane preparations (e.g., strains 10677-2, 120176-1, and 120176-2), but these were not studied here. For each strain the

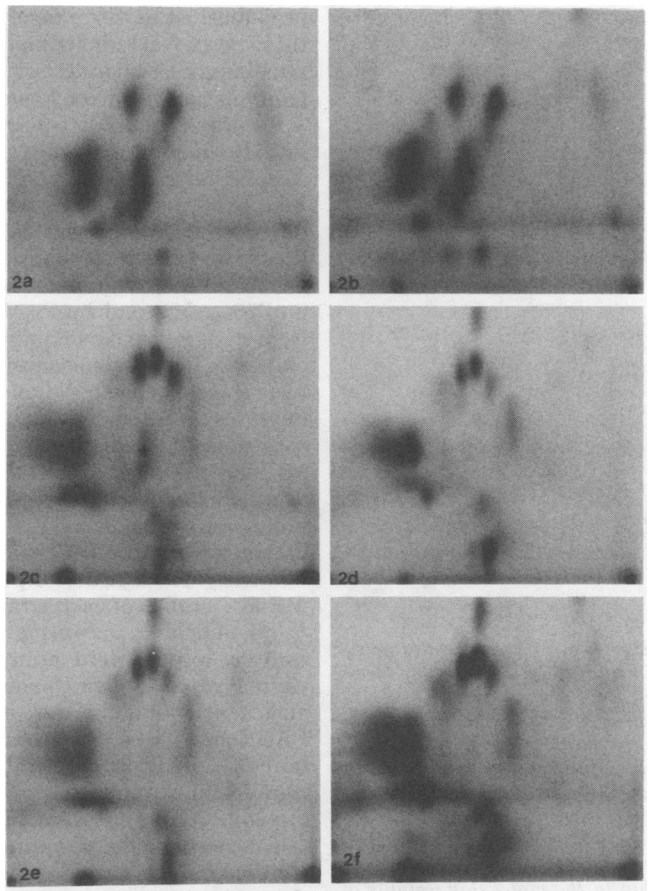

FIG. 2. *^{125}I-peptide maps of proteins shown in gels of Fig. 1. These maps include both MPs (a and b) and hmP's (c through f). The MP34 bands in DOC-insoluble fractions (a) and DOC-soluble fractions (b) are interpreted as being identical, although there is greater intensity in general for all radioiodinated moieties in (b). The hmP24 bands in the DOC-insoluble fraction (c) and the DOC-soluble fraction (d) are different in their ^{125}I-peptide patterns by virtue of a prominent radioiodinated spot (marked with a star) that is found only in the DOC-insoluble preparation. The hmP24 band in the DOC-soluble fraction solubilized for SDS-PAGE at 95°C (d) is identical in its ^{125}I-peptide map to minor proteins of 22,000 (f) and 21,000 (e) apparent molecular weights in DOC-soluble material solubilized at 60°C. Directions of electrophoretic and chromatographic separations were as shown in Fig. 4f.*

predominant hmP_{OP} band was excised (Fig. 3) and subjected to ^{125}I-peptide analysis; the resultant autoradiographic map patterns for five strains studied are shown in Fig. 4. There was a moderate degree of apparent structural similarity among all of the hmP_{OP}'s studied regardless of their subunit molecular weights. In each hmP_{OP} ^{125}I-peptide map, approximately 17 to 19 radio-emitting spots were identified after a 1-day exposure; 10 of these radioiodinated moieties were present in every hmP_{OP} examined. These conclusions are summarized in Fig. 4f and were based on ^{125}I-peptide mapping of hmP_{OP}'s from 14 different gonococcal strains.

^{125}I-peptide maps of two hmP_{OP}'s in the same outer membrane preparation. More than one hmP was found in some opaque colony forms, as previously noted (18, 24). This was the situation for strain KH7189, in which hmP26 and hmP24 bands were present in the outer membranes of the opaque colony forms (Fig. 5a). These hmP_{OP} bands were excised from the gel and were subjected to ^{125}I-peptide mapping along with the MP band (Fig. 5b through d). The MP34 ^{125}I-peptide pattern of strain KH7189 (Fig. 5b) was essentially identical to the MP34 pattern of strain F62 (Fig. 2a), as well as the MP34 and MP33 patterns of other gonococcal strains previously studied (26). ^{125}I-peptide maps of hmP26 and hmP24 from strain KH7189 differed from one another with respect to the relative positions of several relatively clearly de-

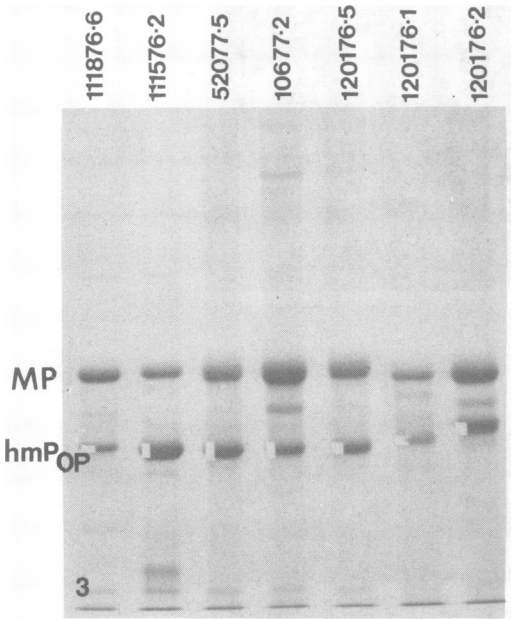

FIG. 3. *SDS-PAGE profiles of several outer membrane proteins used in this study. These outer membranes are all derived from opaque colony forms of the strains noted. Each preparation has, in addition to an MP band, a prominent minor protein band (hmP$_{OP}$) that is unique to the opaque colony form in each strain. The apparent molecular weights of these hmP$_{OP}$'s vary from 24,000 (strains 111876-6, 111576-2, 52077-5, 10677-2, and 120176-5) to 25,000 (strain 120176-1) or 26,000 (strain 120176-2). Note the apparent inverse relationship in the molecular weights of the MP and hmP$_{OP}$ moieties in these preparations. The actual portion excised from each band and utilized for ^{125}I-peptide mapping can be seen. The radioiodinated peptide maps for these and other hmP$_{OP}$'s are shown in Fig. 4 and 5.*

fined radioiodinated spots (Fig. 5); this difference suggested that the hmP26 and hmP24 bands represent slightly different proteins. The hmP26$_{OP}$ and hmP24$_{OP}$ in outer membranes from opaque colonies of strain F62 (data not shown) exhibited ^{125}I-peptide patterns identical to those found for strain KH7189.

^{125}I-peptide maps of hmP$_{LA}$. Two hmP bands of 29,000 and 28,000 daltons were quantitatively increased in the outer membranes of strain MS11 gonococci that exhibited high levels of leukocyte association (LA+) (27) and were subjected to ^{125}I-peptide mapping (Fig. 6). These hmP29$_{LA}$ and hmP28$_{LA}$ bands were identical to one another by radioiodinated peptide pattern analysis and had ^{125}I-peptide patterns similar to those of the hmP$_{OP}$'s described above. However, hmP28$_{LA}$ and hmP29$_{LA}$ had two radioiodinated species that had not been seen previously in ^{125}I-peptide maps of MP, hmP$_{OP}$, or gonococcal pilus (unpublished data) preparations. Except for these two novel radioiodinated species, all of the remaining ^{125}I-peptides of hmP$_{LA}$'s could be found in hmP$_{OP}$'s from one or more strains.

Similarities in ^{125}I-peptide maps of MP and hmP$_{OP}$. Strains F62 and 120176-2 were selected for a comparison of the ^{125}I-peptides in their MPs and hmP$_{OP}$'s since these two proteins differ in their apparent molecular weights in these two strains, as follows: strain F62, MP34 and hmP24$_{OP}$; strain 120176-2, MP32 and hmP26$_{OP}$. MP and hmP$_{OP}$ bands were excised from gels, and chymotryptic ^{125}I-peptides were obtained; the specimens were then subjected to two-dimensional separation individually and as mixtures of MP and hmP$_{OP}$. The results (Fig. 7) suggested ^{125}I-peptide homology between the MP and the hmP$_{OP}$ of each strain for at least four radioiodinated moieties; three of these four radio-emitting spots have been found in all MPs previously examined. There is also questionable homology for a fifth ^{125}I-peptide between the MP and hmP$_{OP}$ of each strain.

^{125}I-peptide mapping of proteins of known amino acid composition. Hen egg white lysozyme, bovine pancreatic ribonuclease, and hen ovalbumin were subjected to SDS-PAGE and ^{125}I-peptide mapping in parallel with hmP26$_{OP}$ of gonococcal strain 111576-2. The autoradiograms were exposed for 20 h, a time usually appropriate for resolving ^{125}I-peptide maps of gonococcal outer membrane proteins; the resulting radioiodinated peptide patterns are shown in Fig. 8a through d. In both egg white lysozyme and ribonuclease preparations, a few very heavily radio-emitting spots were present, along with a number of less intensely labeled moieties (Fig. 8a and b); in these "overexposed" autoradiograms, the total number of emitting spots exceeded the number expected on the basis of the number of tyrosine residues in each protein (egg white lysozyme, three tyrosines; ribonuclease, six tyrosines) (3). However, when the length of exposure of autoradiograms was decreased to 3 h, only the most heavily emitting spots were visible, and the number of such spots closely approximated the number of tyrosine residues in each protein (Fig. 8a* and 8b*). For ovalbumin the 20-h exposure (Fig. 8c) yielded radio-emitting spots roughly equivalent in intensity to those of the accompanying hmP26$_{OP}$; approximately 11 dominant ^{125}I-labeled moieties were visualized for ovalbumin, which contains approximately nine tyrosine residues per protein molecule (1). From these observations it is clear that the total number of radioiodinated spots visualized by this ^{125}I-peptide mapping method did not correspond to either the number of tyrosines in a protein or the number of tyrosine

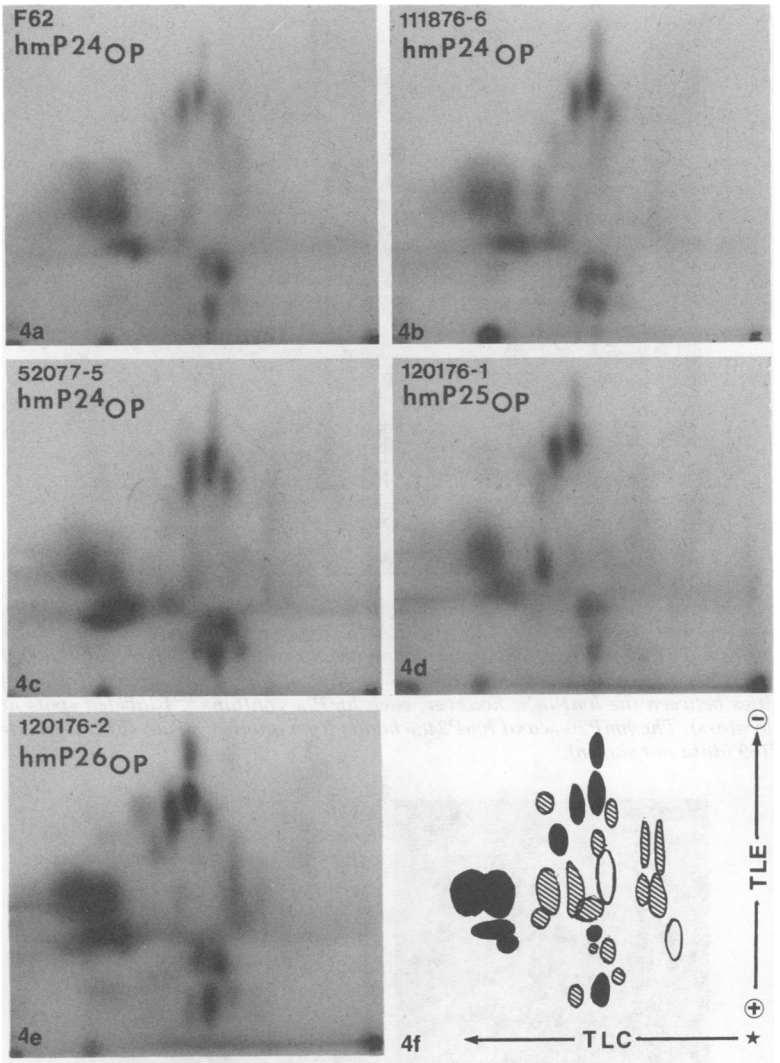

FIG. 4. *^{125}I-peptide maps for hmP$_{OP}$'s from five gonococcal strains. These maps are quite similar with regard to several radioiodinated spots. Those radio-emitting spots that are clearly defined in the hmP$_{OP}$ ^{125}I-peptide maps were numbered, and the distribution of these spots in the maps examined is summarized in (f). The ^{125}I-labeled moieties found in every hmP$_{OP}$ examined to date are shown in (f) (solid areas), as are the directions of electrophoretic (TLE) and chromatographic (TLC) separations. Those radiolabeled spots that appear in the hmP$_{OP}$ of only one strain are noted (open areas), as are the spots that are found in the hmP$_{OP}$'s of many but not all strains (cross-hatched areas). There is considerable variation in the intensities of various radiolabeled moieties in different autoradiograms. Variation is also seen in the extent of migration in the electrophoretic separation. This latter variation can be resolved by referring to the positions of the arginine and leucine standards which were included in each peptide map.*

plus histidine residues; however, the number of spots that most heavily emitted in the ^{125}I-peptide map of a given protein roughly corresponded to the number of spots expected on the basis of the tyrosine content of the protein. By comparing the ^{125}I-peptide maps for proteins of known structures with the map of strain 111576-2 hmP24$_{OP}$, I estimate that this hmP26 outer membrane protein has 10 to 15 tyrosine residues per molecule.

DISCUSSION

Gonococci from opaque colonies have been shown previously to have one or more surface proteins that are absent or quantitatively dimin-

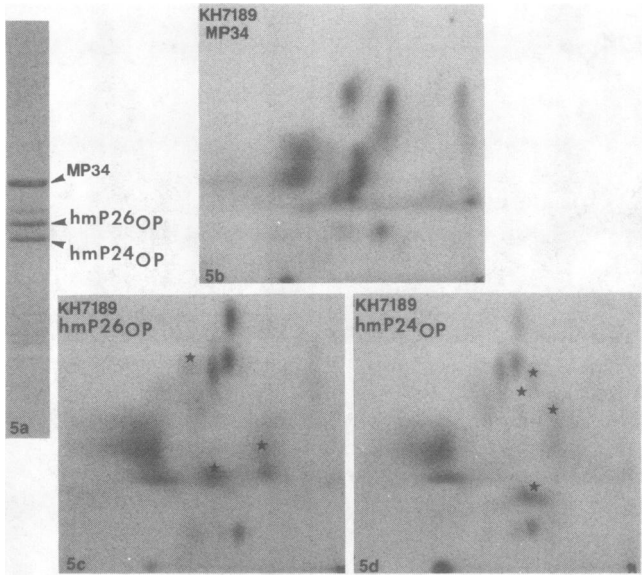

FIG. 5. *Outer membranes were prepared from opaque colony forms of strain KH7189 gonococci, and the proteins of these membranes were separated by SDS-PAGE (a) after Coomassie brilliant blue staining. These opaque colony forms have two hmP$_{OP}$'s, with apparent molecular weights of 24,000 and 26,000. The ^{125}I-peptide maps of these hmP24$_{OP}$ and hmP26$_{OP}$ bands, as well as that of MP34, are shown (b through d). Note the overall similarities between the hmP$_{OP}$'s; however, each hmP$_{OP}$ contains ^{125}I-labeled spots not found in the other (marked by stars). The hmP26$_{OP}$ and hmP24$_{OP}$ bands from another strain (F62) were identical to those shown for KH7189 (data not shown).*

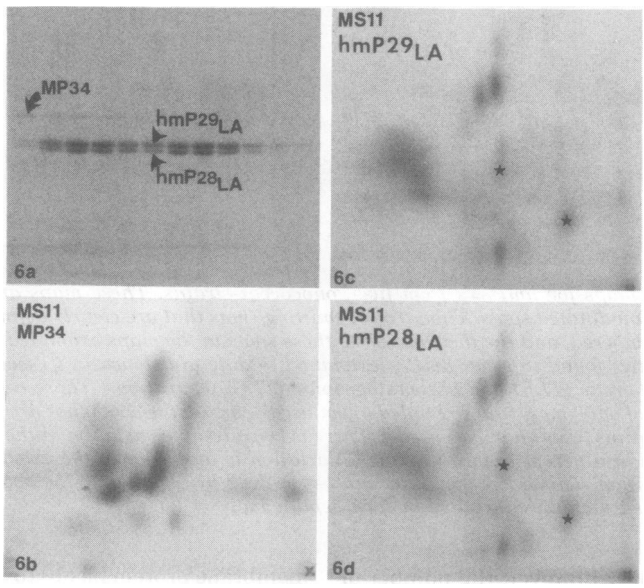

FIG. 6. *Fractions from chromatographic separation of proteins of MS11 leukocyte association (LA+) gonococcal outer membranes were subjected to SDS-PAGE; MP34 and the minor proteins hmP29$_{LA}$ and hmP28$_{LA}$ predominate in different fractions, as shown in Fig. 8a. These bands were excised from the gel and subjected to ^{125}I-peptide analysis; the resulting ^{125}I-peptide maps for MP34 (b), hmP29$_{LA}$ (c), and hmP28$_{LA}$ (d) are shown. The ^{125}I-peptide patterns of hmP29$_{LA}$ and hmP28$_{LA}$ are in general quite similar to those of the hmP$_{OP}$'s of both MS11 and other strains (Fig. 4). The two hmP$_{LA}$'s are identical to one another. In both hmP29$_{LA}$ and hmP28$_{LA}$, note the presence of two ^{125}I-labeled moieties (marked by stars) which have not been observed in any other gonococcal proteins examined.*

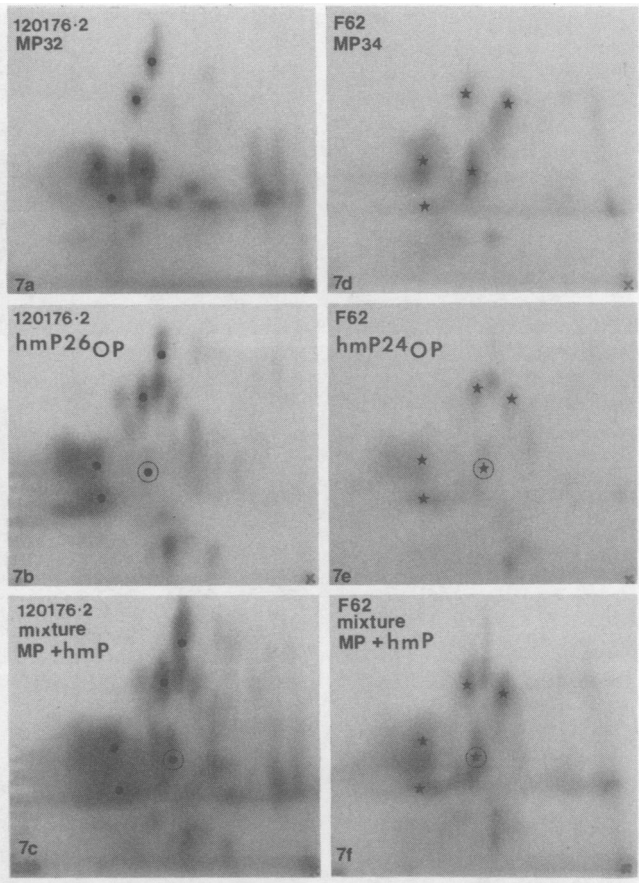

FIG. 7. *Comparison of MP and hmP$_{OP}$ bands from two strains, 120176-2 and F62. The bands were compared by carrying out ^{125}I-peptide mapping on each band (a, b, d, and e) and then subjecting a mixture of MP and hmP$_{OP}$ specimens from each strain to two-dimensional separation (c and f). On the basis of comparing the individual ^{125}I-peptide autoradiograms and by virtue of apparent comigration of ^{125}I-labeled spots in the MP plus hmP$_{OP}$ mixtures, a number of radioiodinated moieties appear to be the same for both MP and hmP$_{OP}$ (marked by solid dots for 120176-2 and by stars for F62). For each strain, a homologous ^{125}I-labeled spot is one which was found only in hmP$_{OP}$ bands of DOC-insoluble material (see legend to Fig. 2). One ^{125}I-peptide with apparent homology in MP and hmP$_{OP}$ for each of the two strains is not found in hmP$_{OP}$ of DOC-soluble material (circled dots and circled stars). The other ^{125}I-peptides noted as homologous between MP and hmP$_{OP}$ are common to all hmP$_{OP}$'s and MPs examined.*

ished in isogenic organisms from transparent colonies (24). These hmP's are readily labeled by reacting lactoperoxidase and ^{125}I with whole, intact gonococci and are highly susceptible to removal from whole organisms by tryptic digestion (23–25, 27). In the present study the opaque colony-associated proteins were found to reside in the cell wall outer membrane, to vary in molecular weight from strain to strain, and to increase in apparent molecular weight with boiling in SDS before SDS-PAGE; the ^{125}I-peptide patterns of these different heat-modifiable molecular weight forms were identical, suggesting that no change in primary peptide structure occurred during boiling in SDS.

The hmP$_{OP}$'s were more readily solubilized from outer membranes by DOC than MPs, as previously noted (9, 16, 25). When cell wall outer membranes containing MP34 and hmP24$_{OP}$ moieties were incubated in DOC and subjected to ultracentrifugation, the MP and hmP bands were found in both pellet and supernatant fractions; the MP band predominated in the pellet or DOC-insoluble fractions, whereas the hmP$_{OP}$ was more abundant in the supernatant material. ^{125}I-peptide maps of the MP34 bands from these two DOC-separated fractions revealed no difference in the radioiodinated species visualized by autoradiography. The hmP24$_{OP}$ bands from DOC-soluble and DOC-insoluble material were questionably different in their ^{125}I-peptide patterns in that the 24,000-dalton band of DOC-

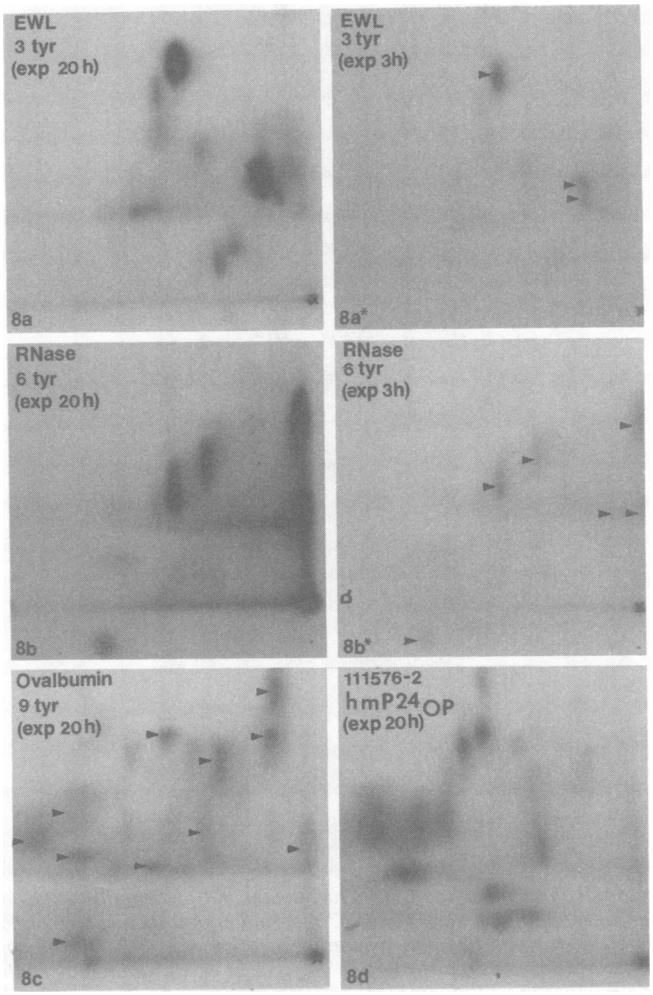

FIG. 8. *Egg white lysozyme (EWL), ribonuclease (RNase), and ovalbumin were subjected to SDS-PAGE, and the appropriate bands were excised for ^{125}I-peptide mapping along with an hmP24$_{OP}$ from strain 111575-2. Autoradiograms of the ^{125}I-peptides for egg white lysozyme were exposed for 20 or 3 h (a and a*). Similarly, exposed autoradiograms for ribonuclease were also obtained (b and b*). The dominant ^{125}I-labeled moieties in the 3-h-exposed autoradiograms for egg white lysozyme and ribonuclease, as well as those for ovalbumin (20-h exposure), are marked with arrowheads, and the numbers of tyrosyl (tyr) residues per molecule are indicated.*

insoluble material contained one radiolabeled spot not found in the DOC-soluble fraction.

Comparative ^{125}I-peptide mapping of hmP$_{OP}$'s from a number of strains shows an apparent high degree of structural homology among the proteins. This degree of similarity exists even for hmP$_{OP}$'s of different apparent molecular weights. Approximately one-half of the ^{125}I-emitting moieties visualized in the chymotryptic digests were seen in all of the opacity-associated proteins examined. This degree of homology appears to be somewhat greater than that observed for MPs of different strains. The apparent structural homology in hmP$_{OP}$'s is of interest because these minor proteins seem to have extensive exposure on the gonococcal exterior. One would expect that a combination of structural homology and surface location would lead to significant cross-reactivity among opaque colony forms from different strains with regard to antibody-mediated reactions; however, it is extremely presumptive to translate findings from this ^{125}I-peptide study into immunological terms. It is not entirely clear that the ^{125}I-peptide method utilized yields information only about peptide structure, nor can it be stated that all peptide constituents of hmP$_{OP}$'s are visualized by this method. A number of identical or very similar

^{125}I-peptides are found in both hmP_{OP}'s and MPs. Three such ^{125}I-labeled spots are seen in all MPs and in all hmP's examined to date. One ^{125}I-peptide common to all hmP's is found only in MP34s and MP33s; another radioiodinated moiety typical of hmP's is present only in hmP32's and not in MP34s or MP33s. This finding has interesting possible implications. Hildebrandt et al. (11) have found that susceptibilities of gonococci to killing by pooled normal human sera correlate with the apparent molecular weights of the MPs of the outer membranes. In that study the serum-resistant strains had lower-molecular-weight MPs, whereas the MPs of the serum-sensitive organisms were of higher molecular weight. The strains used by Hildebrandt et al. were obtained. By SDS-PAGE in my laboratory, the serum-resistant strains had MP32s, whereas the serum-sensitive strains had MP34s. ^{125}I-peptide mapping of the MP34 of strain KH7189 and the MP32 of strain KH6305 showed that they are identical to other gonococcal MPs of similar apparent molecular weight. Studies in my laboratory have shown a correlation between susceptibility to killing by pooled human serum and colony darkness/opacity for several strains and suggest that opacity-associated proteins can be targets for antibody plus complement in bactericidal reactions (12). hmP_{OP}'s and MPs appear to have several homologous ^{125}I-peptides, which suggest that a degree of primary structure homology exists for these proteins. It is interesting to speculate whether the same peptide sequences would be "visible" to antibody and/or complement regardless of whether they are found in MP or hmP moieties on the gonococcal surface. If these homologous sequences of ^{125}I-peptides were exposed on the surface of a gonococcal cell wall as either MP or hmP, the organism might be liable to killing by antibody plus complement. Other MPs, as well as hmP's, might lack suitable targets for antibody plus complement, and these serum-resistant proteins could endow the organism with some serum resistance. Varying combinations of these serum-sensitive and serum-resistant MPs and hmP's would create gonococci whose overall susceptibility to serum antibodies would reflect the properties of one or more surface proteins. Both the structure of MP (11) and the structure of hmP (12) could be influential in determining susceptibility and resistance to killing by serum.

More than one outer membrane hmP is sometimes found associated with colony opacity as in strain KH7189 of the present study; these hmP_{OP}'s have different apparent molecular weights (24,000 and 26,000), and their ^{125}I-peptide patterns are slightly different. These ^{125}I-peptide differences have also been found for $hmP26_{OP}$ and $hmP24_{OP}$ bands of opaque colony forms of strain F62 (unpublished data). Differences in structure, as revealed by ^{125}I-peptide mapping, have also been found in comparisons of hmP_{LA} and hmP_{OP} bands in the same outer membrane preparations. One of these differences concerns two ^{125}I-labeled spots that are present in both $hmP28_{LA}$ moieties but not in any of the hmP_{OP}'s or other outer membrane or pilus proteins that have been examined by ^{125}I-peptide mapping; it will be of interest to determine whether these novel hmP_{LA} ^{125}I-peptides are related to the biological activities of the minor proteins whose presence correlates with high levels of interaction between gonococci and human peripheral blood neutrophils in vitro.

The lack of strict correspondence between the number of ^{125}I-peptides visualized by the technique used and the number of tyrosyl residues in protein molecules was not unexpected. Chloramine T-catalyzed radioiodination of proteins produces monoiodo and diiodo derivatives of both tyrosine and histidine, along with oxidation of methionine and cystine (7). In addition, there is possible limited access of chymotrypsin to some susceptible sequences because of incomplete unfolding of the protein molecule under the conditions used. These variables limit rigorous interpretation of the ^{125}I-peptide maps generated in this study with regard to number of specific amino acid residues; however, identical patterns are reproducibly found for any given protein, and gonococcal membrane proteins that are otherwise similar consistently show a marked degree of apparent structural homology by ^{125}I-peptide mapping. Although the origins and compositions of the radioiodinated moieties seen with this ^{125}I-peptide mapping method are unclear, the migration characteristics of the radioiodinated moieties are undoubtedly reflections of the structures of the proteins.

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