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Colony Opacity and Protein II Compositions of Gonococci

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Changes in the presence of outer membrane protein II (P.II) constituents of gonococci were demonstrated by selecting opacity variants and defining the ^{125}I-labeled bands of parental and variant organisms. In general, colony opacity phenotype was a convenient, reliable guide for obtaining variants that differed from their parents by the apparent single-step gain or loss of one P.II constituent. Within a given strain (three strains were studied), particular P.II species were associated with particular opacity phenotypes. This was well demonstrated in strain JS3, in which five different P.II constituents were identified and compared. Four of these five P.II moieties were consistently associated with a characteristic degree of colony opacity; presence of the fifth P.II (P.IIa) did not correlate with a discernible increase in opacity when present either alone or in combination with other opacity-associated P.II moieties. The electrophoretic migration characteristics for each of the five P.II constituents of this strain differed with regard to apparent molecular weight and the effects of temperature and 2-mercaptoethanol. The high prevalence of colony opacity variants indicates that gonococcal populations are capable of presenting a variety of surface components to their external environment.

Gonococci propagated on clear, solid medium form colonies that exhibit diversity in size, edge morphology, and opacity characteristics. Small diameters and sharp edges typify colonies containing piliated gonococci, whereas large colonies with indistinct borders contain nonpiliated organisms (20). Both large and small colonies exhibit variation in their coloration and optical opacity (14). Colorless, transparent colonies generally contain organisms whose outer membranes are devoid of heat-modifiable proteins, whereas opaque-colony gonococci exhibit one or more of these particular outer membrane proteins (4, 10, 15) that constitute the protein II (P.II) family (18, 19).

A number of biological characteristics have been correlated with colony opacity. These include virulence in chicken embryos (13), adherence to diverse eucaryotic cell types (6, 11, 21), susceptibility to killing or growth inhibition by exogenous proteases (1, 14), and susceptibility to killing by pooled human sera (2, 7). The majority of those studies have compared opaque versus transparent colonies within one or more strains in their biological assay schemes.

Recently, the phenomena of colonial opacity and outer membrane P.II composition were studied again in an attempt to explain confusing results obtained with immunoprecipitation experiments. The observations from that study are the topic of the present paper, which makes four main points: (i) gonococci within any given strain can exhibit the presence of one or more P.IIs; (ii) addition or loss of each P.II is a single-step phenomenon that is independent of the presence or absence of other P.IIs on the same organism; (iii) several degrees of opacity are found within a given strain, and these correlate with both number and kind of P.II constituents present; and (iv) different P.II constituents exhibit apparent diversity in properties such as their influence on colony opacity, heat-modification characteristics, etc. Taken together, these findings suggest that all opaque gonococci are not equal. Several kinds and various combinations of P.IIs can exist among opaque colony preparations from a single strain and from different strains.

MATERIALS AND METHODS

Gonococci. Strains JS1 (F62), JS2 (10677-2), and JS3 (120176-2) were grown on the modified, clear typing medium previously described (18) at 36°C in a 5% CO_2 atmosphere. Single colonies were selected and passaged daily.

Microscopy. A Bausch & Lomb Stereozoom 7 microscope was used with the diffusing and polished surfaces of a substage reflector for observing colony color and opacity characteristics, respectively. Other models of stereo microscopes tried (American Optical, Wild, Zeiss, and Leitz) seemed inferior for discerning the color and opacity characteristics of gonococcal colonies. Photographs of colonies on solid medium

were obtained with a Canon 35-mm camera attached to this microscope and were taken after 21 to 22 h of growth since the differentiation of opacity variants seemed clearest then with the particular medium and growth conditions used.

Radioiodination of gonococci. Gonococci were removed from solid medium plates with Dacron swabs and suspended to an optical density at 540 nm of 0.6 in phosphate-buffered saline (PBS) of the following composition: 136 mM NaCl–2 mM KCl–8 mM Na_2HPO_4–1.4 mM KH_2PO_4–1 mM $CaCl_2$–1 mM $MgCl_2$ (pH 7.2). A sample (1.5 ml) was centrifuged, and the pellet was suspended with blending in a Vortex mixer in 40 μl of PBS. A portion (5 μl) of 10^{-5} M KI was added, and, after mixing, the suspension was transferred to a screw-top glass vial previously coated with 10 μg of 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril (Iodogen; Pierce Chemical Co., Rockford, Ill.). [^{125}I]Na was added as 50 μCi in 5 μl. After agitation, the vial was incubated at room temperature for 6 min. The contents of the vial were transferred to a 1.5-ml Microfuge tube containing 1 ml of ice-cold PBS. Centrifugation was followed by two washes in cold PBS and dilution of the final pellet of radioiodinated gonococci in 100 μl of PBS. The gonococci were either used immediately or frozen at −30°C and used 2 days to 2 weeks later.

SDS-PAGE. The preparation of ^{125}I-labeled gonococci for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) involved adding 4 μl of the suspension described above to 16 μl of solubilizing solution of the following composition: 4% sodium lauryl sulfate (British Drug House)–8% 2-mercaptoethanol (2-ME)–20% glycerol–1.25 mM Tris-hydrochloride (pH 6.8). For some experiments described below, 2-ME was omitted from this mixture. After being mixed, the entire sample was boiled for 5 min or incubated in a water bath at the desired temperature (25, 40, or 60°C). A brief centrifugation was followed by the loading of the entire contents to a 12.5% polyacrylamide slab gel with the composition described by Laemmli (9), which was electrophoresed at 40 mA constant current in the Tris-glycine buffer of Laemmli. For two-dimensional SDS-PAGE analysis, the ^{125}I-labeled gonococci were solubilized under one set of conditions (e.g., in the presence of 2-ME for 15 min at 60°C) and subjected to the first-dimension electrophoresis. Through reference to previous gels, desired portions (2 mm by 2 to 4 cm) of this first gel were selected, excised, loaded into sealed plastic bags with about 200 μl of solubilizing solution of the desired composition (either with or without 2-ME), and submerged in a water bath at the desired temperature (usually 100°C for 5 min). This gel was then placed on top of a stacking gel and electrophoresed through stacking and separating gels that were compositionally identical to those used for the first dimension. Autoradiograms of wet or dried gels were obtained by exposing Kodak X-Omat AR or TL film at room temperature overnight.

Trypsin treatment of ^{125}I-labeled gonococci. Portions (4 μl) of PBS suspensions of ^{125}I-labeled gonococci were added to equal volumes of PBS alone or PBS containing either 0.8 or 0.08 μg of trypsin (Grade III, Sigma Chemical Co., St. Louis, Mo.). After incubation at room temperature for 20 min, 12 μl of solubilizing solution (with 2-ME) was added, the tube was immediately boiled for 5 min, and SDS-PAGE was performed.

Estimation of the prevalence of opacity variants. Single nonpiliated colonies were selected after 18 to 20 h of incubation and were lifted from the medium surface with a small fragment of filter paper. This colony-bearing paper was immersed in 1 ml of proteose-peptone saline (Proteose-Peptone no.3, Difco Laboratories, Detroit, Mich.), and serial 10-fold dilutions were made in the same menstruum. Samples (0.1 ml) of the appropriate dilutions were pipetted onto a plate and spread with a glass rod. After 21 to 24 h, the total number of colonies and the colonies of each phenotype were counted.

RESULTS

General comments. A rather simple strategy was used. Single transparent (nonopaque) (O^-) colonies containing gonococci that lacked any detectable P.II were selected and passaged. The vast majority of colonies in the resultant culture population resembled the parental colony in having O^- characteristics; however, there were occasional variants which displayed either intermediate (O^+) or marked (O^{++}) degrees of opacity. When each of these opaque variants was passaged, the progeny resembled their respective parental colonies in opacity phenotype, except for occasional variants with greater or lesser opacity. Each of these steps was followed by transferring desired colonies, radioiodinating the gonococci in the resultant population, and subjecting the whole bacterial lysates to SDS-PAGE and autoradiography. In this manner, the presence or absence of P.II bands in variant progeny versus those in parental cultures could be determined. Acquisition and loss of colony opacity and P.II constituents have been followed for both piliated and nonpiliated organisms of these three strains, and the same principles seem to apply regardless of piliation phenotype. All the studies depicted below, with the exception of those in which the piliated organisms shown in Fig. 1 were used, were done with nonpiliated gonococci because of technical convenience. Nearly all the experiments described below involved selections of variants which were more opaque than their parents. Many similar observations have been made with selection of less opaque variants, but those studies are not shown.

Opacity variants in strains JS1 and JS2. In each of two strains (JS1 and JS2), three opacity forms were regularly present. In otherwise O^- cultures, both O^+ and O^{++} colonies were regularly found. On subculture, the O^+ and O^{++} phenotypes were maintained in over 95% of the progeny. Both O^- and O^{++} variants were present in progeny of O^+ colonies; since these O^{++} colonies arose from O^+ parental stock, they were designated $O^{++(+)}$. On repeated passage of

$O^{++(+)}$ colonies, only O^+ variants were found in addition to the parental phenotype. In cultures of O^{++} colony forms selected within O^- populations (designated hereafter $O^{++(-)}$), only O^- variants were seen. The morphologies of these colony opacity phenotypes and of the characteristic "contaminants" in populations of each phenotype are shown in Fig. 1.

The SDS-PAGE profiles visualized by autoradiography after radioiodination of whole organisms from one of these two strains and the morphological features of colony opacity variants are also depicted in Fig. 1. The O^- gonococci contained two main ^{125}I-labeled constituents, protein I (P.I) and protein III (P.III); no P.II constituents were demonstrated. In the O^+ colony phenotypes of both strains, a single P.II (IIβ in JS1 and IIδ in JS2) was present in addition to P.I and P.III. Likewise, for $O^{++(-)}$ preparations, only a single P.II (IIα in JS1 and IIγ in JS2) was visible, but this P.II (IIα or IIγ) had a different apparent subunit molecular weight than the P.II (IIβ or IIγ) seen in the O^+ colony forms of the respective strain. In both strains, the $O^{++(-)}$ P.II was of smaller apparent size than the P.II of O^+ colonies. Both of these P.IIs (IIα and IIβ in JS1; IIγ and IIδ in JS2) were present in $O^{++(+)}$ organisms.

Prevalence of colony opacity variants. The occurrences of particular opacity variants in progeny populations from single parental colonies were assessed in several experiments, one of which is depicted in Table 1. The majority of these estimates were done with strain JS1; similar occurrences of variants were found in one experiment each with strains JS2 and JS3. In progeny of strain JS1 O^- ($P.II^-$) colonies, O^+ ($P.IIα^+$) and O^{++} ($P.IIβ^+$) variants were similarly prevalent. A similar prevalence was found for O^{++} colonies arising from a passage of an O^+ colony ($IIβ^+ \rightarrow IIα^+,β^+$). P.II constitution changes were seen more commonly when variant progeny that were less opaque than their parents were scored ($O^+ \rightarrow O^-$, $O^{++(-)} \rightarrow O^-$, and $O^{++(+)} \rightarrow O^+$). I suspect that this was partly due to the method used for single-colony selection. Any colonies with foci visibly darker or lighter than the remainder of the colony were not used because they yield large numbers of variant progeny. Dark, more opaque foci are readily seen in transparent colonies; light, less opaque foci in opaque colonies are less easily seen. This difference may have contributed to the high apparent prevalance of more transparent progeny due to the inadvertent selection of colonies that contained large numbers of less opaque variants.

Strain JS3 opacity variants. For the two strains described above, correlations between colony opacity phenotype and P.II constituents were straightforward. In strain JS3, however, more complicated relationships were found. (i) Five distinct P.IIs were regularly found in this strain. (ii) One particular P.II (IIa) was not associated with colony opacity. (iii) Two P.IIs (IIb and IIc) were difficult to differentiate by one-dimensional SDS-PAGE, especially when they coexisted. (iv) Different P.IIs exhibited retarded migration in SDS-PAGE at different temperatures.

When a single O^- colony of strain JS3 was passaged, four distinctly different opacity variant forms were regularly found. Each of these four variants (O^{1+}, O^{2+}, O^{3+}, and O^{4+}) was selectively passaged, and the progeny were subjected to ^{125}I labeling, SDS-PAGE, and autoradiography (Fig. 2). In the experiment shown in Fig. 2, the selection of only two O^- colonies (Fig. 2, B and C) and the screening of their progeny provided all four opacity variants; each opaque variant (Fig. 2, B1 to B5 and C1 to C5) bore a single P.II. A different P.II was found for gonococci of each different opacity phenotype.

A fifth P.II was discovered accidentally since organisms bearing this protein were not distinguished by any discernible increase in their colonial opacity. Organisms bearing only this P.II (IIa) displayed no noticeable opacity; opaque colony-forming organisms that bore P.IIa and one or more additional P.II constituents exhibited opacity similar to that of organisms lacking P.IIa but having the other P.II moieties. P.IIa was first found on O^- gonococci that had been fortuitously selected from a population of O^- organisms lacking P.IIa. O^- $P.IIa^+$ gonococci were used for the selection of opaque variants exhibiting one or more additional P.II constituents (Fig. 3).

Comparisons of some properties of the five P.II species found in strain JS3 are shown in Fig. 4–7 and in Table 2. Each of the P.II constituents except P.IIa was associated with colonies of a slightly different opacity phenotype, as mentioned above (Fig. 4). All of these proteins were heat modifiable (Fig. 4–6). Conversion of each P.II from its fast to slow migrating form occurred in a characteristic temperature range for each protein (Table 2 and Fig. 5). P.IIa was present partially in its slower migrating form after solubilization of the gonococcus at only 25°C, whereas P.IId was incompletely converted to the slow form even after the gonococci were boiled in SDS for 5 min (Fig. 5 and 6). P.IIb, P.IIc, and P.IIe exhibited heat modifiability that was intermediate between these extremes. The heat-modifiability characteristics of these five P.II constituents are shown in Fig. 4 and 6. Incomplete conversion of P.IId to a slower migrating form is seen in Fig. 6 and is particularly clear in the two-dimensional gel analysis.

The electrophoretic migrations of four of these

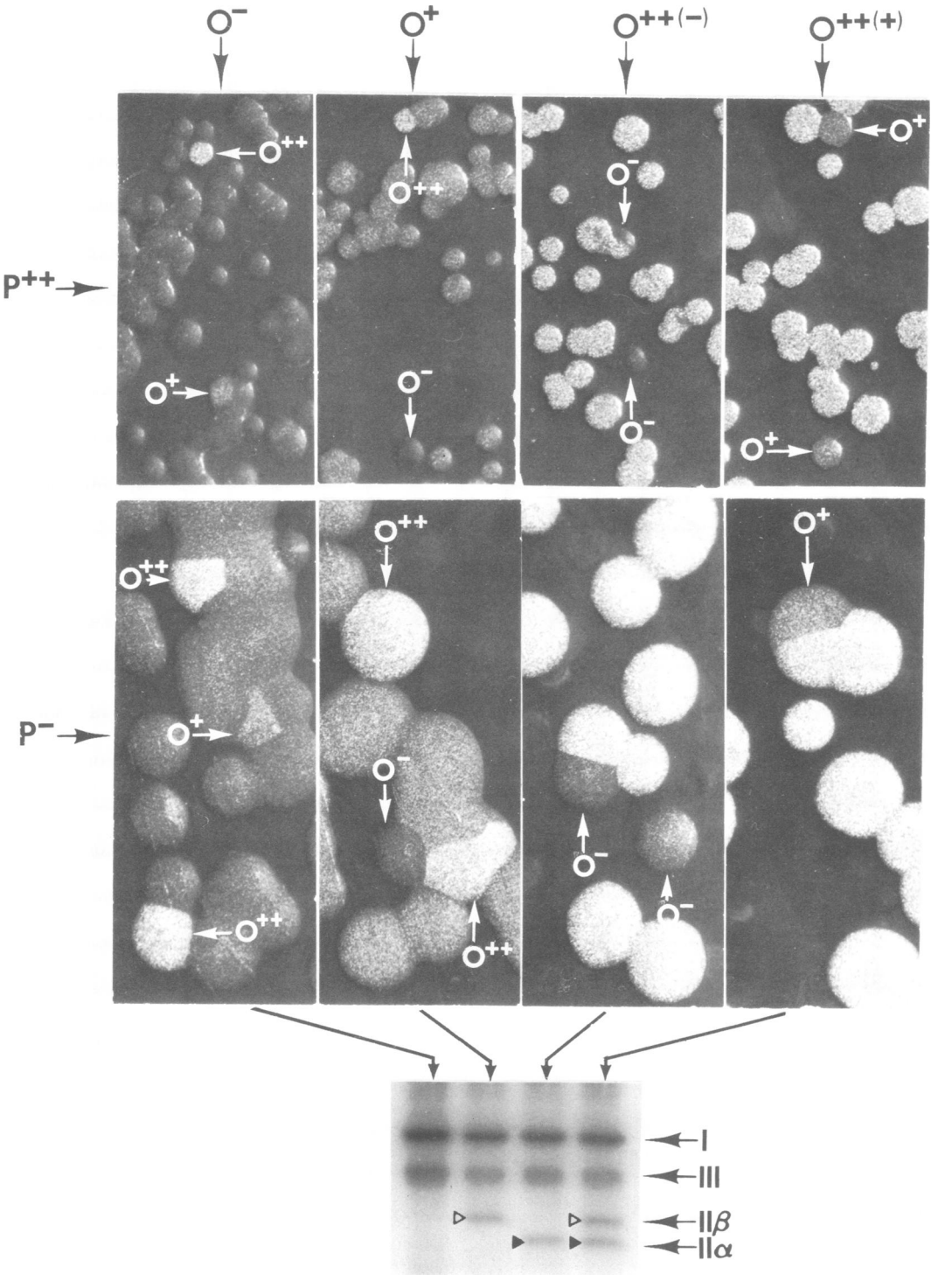
O⁻
O⁺
O⁺⁺(−)
O⁺⁺(+)
P⁺⁺
P⁻
O⁺⁺
O⁺
O⁻
I
III
IIβ
IIα

FIG. 1. Colony morphology and SDS-PAGE profiles of strain JS1 opacity variants. Heavily piliated (P^{++}) and nonpiliated (P^{-}) gonococcal colonies with four distinct opacity phenotypes are shown in the top two panels. Portions of solid medium cultures with overall transparent (O^{-}), opaque (O^{+}), and very opaque ($O^{++(-)}$ and $O^{++(+)}$) phenotypes are shown. The areas shown have been selected to display the typical variants present in each phenotype population. These variants have colonial opacities as indicated on the micrographs. Note that in the O^{-} populations, both O^{+} and O^{++} variants are present, whereas in O^{+} cultures, the variants are O^{-} or O^{++}. In very opaque cultures derived from O^{-} parents ($O^{++(-)}$), the sole variant phenotype is O^{-}. In very opaque populations derived from O^{+} parental forms ($O^{++(+)}$), only O^{+} variants are present on repeated passage. In the bottom panel is the central portion of an SDS-PAGE slab gel of strain JS1 organisms of opacity phenotypes shown in the micrographs. The O^{-} organisms have only P.I and P.III as dominant ^{125}I-labeled constituents. Both opacity forms derived from O^{-} stock (O^{+} and $O^{++(-)}$) bear a single P.II (IIα or IIβ), but these differ in their apparent molecular weights in these specimens, which were solubilized at 60°C for 30 min. The $O^{++(+)}$ organisms have both P.IIα and P.IIβ, apparently having expressed the faster migrating P.IIα in addition to the slower migrating P.IIβ found in their O^{+} parents. On the basis of degree of opacity, $O^{++(-)}$ and $O^{++(+)}$ colonies cannot be consistently differentiated; however, the opacity characteristics of their less opaque variants (O^{-} in $O^{++(-)}$ and O^{+} in $O^{++(+)}$) are reliable indicators of the lineage and phenotype of the opaque colony culture. The transitions depicted here were typical for strains JS1 and JS2 and can be summarized as follows: O^{-} ($P.II^{-}$) → O^{+} ($P.II\beta^{+}$ in JS1 and $P.II\delta^{+}$ in JS2); $O^{++(-)}$ (P.IIα in JS1 and $P.II\gamma^{+}$ in JS2) → $O^{++(+)}$ ($P.II\alpha^{+},\beta^{+}$ in JS1 and $P.II\gamma^{+},\delta^{+}$ in JS2).

five P.II constituents were also influenced by the presence or absence of 2-ME in the solubilizing mixture (Fig. 7). Only P.IIa exhibited no dependence on 2-ME for its migration characteristics; it was found in a slow migrating form after boiling with or without 2-ME. The major portion of P.IIe was in a slow migrating form without 2-ME, and the major portion of P.IIb remained in a fast migrating form in the absence of 2-ME; conversion of P.IIc and P.IId was intermediate since about half of each of these proteins were in either slow or fast migrating forms after solubilization in SDS devoid of 2-ME. These conclusions are very rough, since P.III partially obscures the slow migrating forms of P.IIc and P.IId in specimens solubilized without 2-ME, and this precluded scanning, quantifying peak areas, etc., in a one-dimensional gel. Two-dimensional SDS-PAGE for gonococci solubilized without 2-ME (first dimension) and with 2-ME (second dimension) clearly showed the differences described above among these P.II species in their behaviors after exposure to 2-ME (Fig. 7).

TABLE 1. Occurrence of opacity variants among gonococci in single colonies of strain JS1

Parental colony and variant colony opacity (and P.II) characteristics	Prevalence of variants (%)[a]
O^{-} (II^{-}) → O^{+} ($II\beta^{+}$)	0.39 ± 0.13
O^{-} (II^{-}) → O^{++} ($II\alpha^{+}$)	0.31 ± 0.15
O^{+} ($II\beta^{+}$) → O^{++} ($II\alpha^{+},\beta^{+}$)	0.37 ± 0.26
O^{+} ($II\beta^{+}$) → O^{-} (II^{-})	3.1 ± 1.9
$O^{++(-)}$ ($II\alpha^{+}$) → O^{-} (II^{-})	0.92 ± 0.52
$O^{++(+)}$ ($II\alpha^{+},\beta^{+}$) → O^{+} ($II\beta^{+}$)	0.93 ± 0.45

[a] Values from observations on progeny from five colonies selected for each opacity phenotype (mean ± standard deviation).

After incubation of whole, ^{125}I-labeled gonococci with trypsin, all of the P.II constituents of strain JS3 were susceptible to hydrolysis (Fig. 8).

The SDS-PAGE profiles of P.II-containing variants of strains JS1, JS2, and JS3 were compared in a single-dimension gel; specimens solubilized in the presence of 2-ME at 60°C for 30 min and at 100°C for 5 min were used (Fig. 9). P.IIa of strain JS3 was very similar in the apparent size of its slow migrating form to P.IIα of strain JS1 and P.IIγ of strain JS2 after solubilization at 100°C. P.IIβ of JS1 and P.IIc of JS3 were quite similar in the apparent size of their slow migrating forms, and P.IIδ of JS2 resembled P.IId of JS3 in migration after boiling.

DISCUSSION

During growth in vitro, gonococci exhibit frequent changes in the surface-exposed, heat-

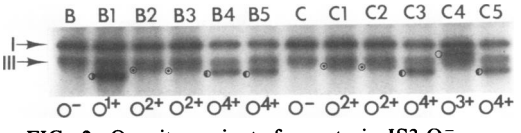

FIG. 2. Opacity variants from strain JS3 O^{-} gonococci lacking P.II. Two O^{-} colonies (B and C) were passaged, and opaque variants within each population were selected. These opaque variants (B1 to B5 and C1 to C5) displayed the opacity phenotype noted below the autoradiogram. Each contained one and only one P.II, designated as follows: ◑, P.IIb; ◐, P.IIc; ⊙, PIId; ○, P.IIe. All specimens in this SDS-PAGE autoradiograph were solubilized by boiling for 5 min. The transitions which are shown are summarized as follows (P.II constitutions of progeny and parent are given along with lane designations within parentheses): II^{-} → IIb^{+} (B → B1; C → C3 and C5); II^{-} → IIc^{+} (B → B4 and B5); II^{-} → IId^{+} (B → B2 and B3; C → C1 and C2); II^{-} → IIe^{+} (C → C4).

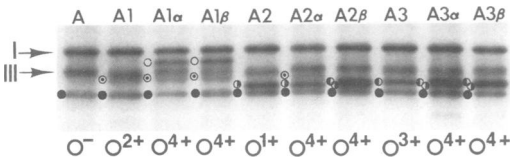

FIG. 3. Opacity variants from strain JS3 O⁻ gonococci containing P.IIa. The SDS-PAGE autoradiogram in this figure depicts an experiment in which a single O⁻ colony (A) of gonococci bearing P.IIa (●) was passaged, and three opaque variants (A1, A2, and A3) were selected. Each of these has gained a single P.II in addition to P.IIa. Passage of colonies A1, A2, and A3 resulted in the appearances of more variants exhibiting enhanced opacity (A1α and A1β from A1, etc.). In each of these very opaque variants, another P.II was seen in addition to the two found in their parents. Symbols are as in the legend to Fig. 2. Note that this simple experiment yielded variants containing all of the five P.II constituents found to date in this strain. The transitions depicted by SDS-PAGE profiles are as follows: $IIa^+ \rightarrow IIa^+,b^+$ (A → A2); $IIa^+,b^+ \rightarrow IIa^+,b^+,d^+$ (A2 → A2α); $IIa^+,b^+ \rightarrow IIa^+,b^+,c^+$ (A2 → A2β); $IIa^+ \rightarrow IIa^+,c^+$ (A → A3); $IIa^+,c^+ \rightarrow IIa^+,b^+,c^+$ (A3 → A3α, A3β); $IIa^+ \rightarrow IIa^+,d^+$ (A → A1); $IIa^+,d^+ \rightarrow IIa^+,c^+,d^+$ (A1 → A1α, A1β).

modifiable P.II constituents of their outer membranes. The occurrence of an opacity variant bearing a particular P.II seems to occur about once per 100 to 10,000 organisms; this corresponds roughly to previously published colonial opacity transition change rates of approximately 10^{-3} to 10^{-4} per cell per generation (12). A given gonococcal strain can exhibit one or several different P.II constituents (10, 15), and these appear to vary in occurrence independent of the presence of other P.IIs, pili, or particular P.I species. In the first two strains studied (JS1 and JS2), variations in opacity were clearly correlated with the presence or absence of one or both of two distinct P.II constituents. It was fortuitous that these initially studied organisms had such simple P.II compositions, because it allowed the deciphering of the basic relationships between P.II composition and colonial opacity, one-step gain or loss of P.II moieties, etc. However, recent studies with additional strains have shown that the observations on strain JS3 are probably more typical for gonococci in general than are the observations on strains JS1 and JS2. In strain JS3, five different P.IIs were found. Although some of these showed clear correlations with particular degrees of colonial opacity, one P.II (IIa) was not correlated with a particular opacity phenotype. When P.IIa was the sole P.II constituent, colonies were transparent; when P.IIa coexisted with another P.II, the colonies had degrees of opacity predictable from the presence of the additional P.II. I would guess that nearly every strain of gonococci would, for example, display one or more p.II constituents whose presence was not accompanied by a noticeable degree of colony opacity, as was found for P.IIa in strain JS3.

The significance of P.IIs in regard to their influence on pathogenicity or virulence of these organisms is obscure, but this is true for other gonococcal constituents also. Piliation is commonly regarded as the sine qua none for virulence of gonococci. But, as I have pointed out before, about the same kind and amount of evidence implicates colonial opacity-associated P.IIs in gonococcal virulence as implicates gonococcal pili (17). Recent studies have documented striking differences in the colonial opacity phenotypes and P.II compositions of gonococci isolated from males versus females (7, 8), from different portions of the genital tract of an individual female (3), from the same women during serial cultures (7), from women of different ages, etc. (3, 7, 8). These findings suggest that colonial opacity and P.II compositions confer properties on gonococci which, through interaction with host components, are selected for or against. Two previous studies have documented the simultaneous presence of two to six

TABLE 2. Comparisons of P.II constituents of strain JS3

P.II constituent	Apparent molecular wt[a]		Colony opacity phenotype	Modification of apparent molecular wt by 2-ME[b]	Modification of apparent molecular wt by temp (°C)[c]
	Fast, unmodified	Slow, modified			
IIa	26	29	O^-	−	<25, 60
IIb	26.8	30.2	$O^{-/+}$ (O^{1+})	+	100, 100
IIc	27	30.5	O^{++} (O^{4+})	−/+	100, 100
IId	26.5	31	O^+ (O^{2+})	+	100, >100
IIe	28.5	33	$O^{+/++}$ (O^{3+})	−/+	60, 100

[a] In kilodaltons; weights were determined through reference to Bio-Rad low-molecular-weight markers.

[b] −, entirely present in slow form in presence or absence of 2-ME; −/+, majority seen in slow form in absence of 2-ME and remainder converted to slow form in presence of 2-ME; +, very little present in slow form in absence of 2-ME and all converted to slow form in presence of 2-ME.

[c] The first number denotes the lowest temperature at which conversion to the slower migrating form was seen, and the second number gives the temperature at which this conversion seemed complete.

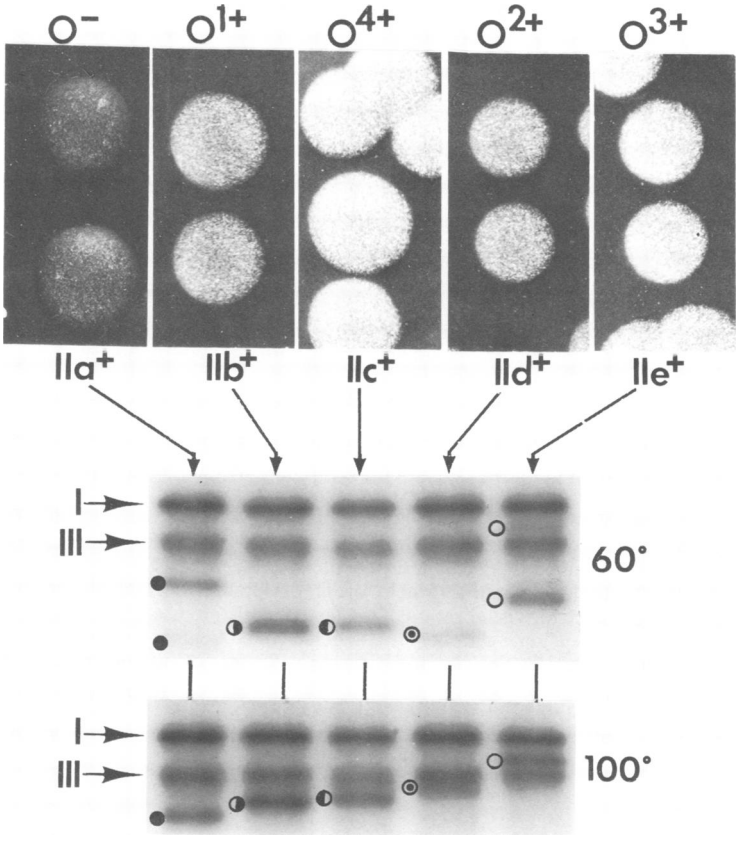

FIG. 4. Strain JS3 variants that exhibit a single P.II. Colony opacity is shown and noted above each micrograph for gonococci in strain JS3 that have the P.II constituent indicated below the micrograph. The autoradiograms of ^{125}I-labeled gonococci with opacity phenotypes corresponding to those shown in the micrographs are also shown after solubilization at either 60°C for 30 min or 100° for 5 min. At 60°C, most of the heat-modifiable proteins (IIb, IIc, IId, and IIe) are in their fast migrating forms, whereas the bulk of IIa has been converted to its slow migrating form; a small portion of IIe is also in its slow form after solubilization at 60°C. After solubilization at 100°C, the heat-modifiable proteins are completely converted to their slow migrating forms, except for a small amount of IId (not shown). Note the lack of complete correspondence in the relative apparent weights of these P.II moieties at the two different solubilization temperatures. Symbols are as in the legend to Fig. 2.

P.II constituents in a seemingly homogenous population of gonococci (10, 15). Each of the several P.II constituents of strain P9 influences the biological reactivities of organisms bearing these outer membrane proteins (11). A number of comparisons have been made of opaque versus transparent gonococci, including susceptibilities to killing by serum (2, 7) or proteolytic enzymes (15), attachment avidities to tissue culture and other epithelial cells or tissues (6, 21), virulence in chicken embryos (13), and others; it is not known which properties of opacity variants causally relate to a particular biological reactivity. Additional comparisons of colony variants will be made in the future, and, in anticipation of these, the present study makes the following points. (i) All opaque gonococci

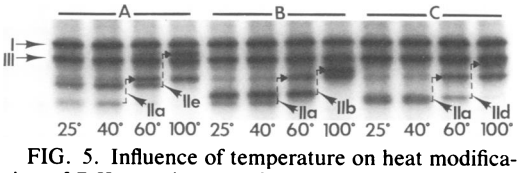

FIG. 5. Influence of temperature on heat modification of P.II constituents of strain JS3. Three opacity variants (A, B, and C) of strain JS3, each of which had P.IIa and one additional P.II, were solubilized at 25°C for 30 min, at 40°C for 30 min, at 60°C for 30 min, and at 100°C for 5 min as noted. In each preparation, P.IIa exhibited nearly complete conversion to its low migrating form in the 60°C-solubilized specimens. Conversion of P.IIe to its slow migrating form commenced at 60°C and was complete at 100°C. P.IIb showed beginning and completed conversion at 100°C. P.IId was incompletely converted to its slow migrating form even after boiling.

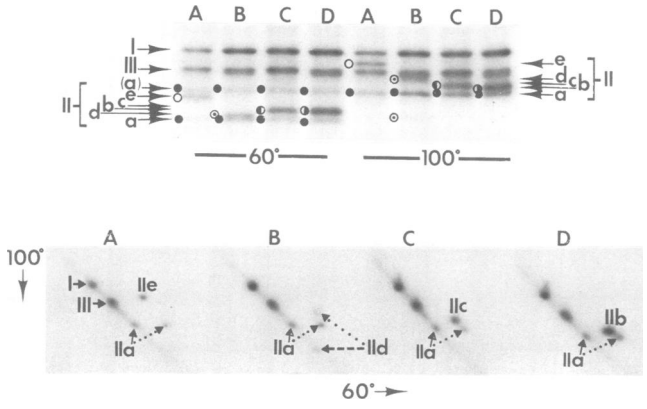

FIG. 6. One- and two-dimensional SDS-PAGE analyses of heat-modification of strain JS3 P.II constituents. Variants of strain JS3 (A to D) were chosen to contain P.IIa and one additional P.II. After labeling with ^{125}I, these variants were solubilized for SDS-PAGE in the presence of 2-ME at either 60°C for 15 min or 100°C for 5 min (top panel). For two-dimensional analysis, the same preparations were first solubilized at 60°C for 15 min and then subjected to SDS-PAGE, and the gel strips were excised and boiled for 5 min. Note that the time of solubilization at 60°C was shorter than that used in the gel depicted in Fig. 5. This was done so that P.IIa would be only partially converted to its slow migrating form by the first solubilization, and the conversion of this protein could serve as an internal reference for visualization of the heat modifications of P.IIe, P.IId, P.IIc, and P.IIb. In the autoradiogram of the two-dimensional gel, those ^{125}I-labeled constituents whose migration characteristics do not change in 60 versus 100°C solubilization lie on the diagonal. Those constituents that do show a shift at 100 versus 60°C migrate more slowly in the second dimension and, hence, are found above the diagonal. In this gel, P.Is and P.IIIs exhibit no modification after solubilization, whereas each of the P.II constituents does show heat modification. Note the incomplete conversion of P.IId to its slow form even after boiling (B, top and bottom panels).

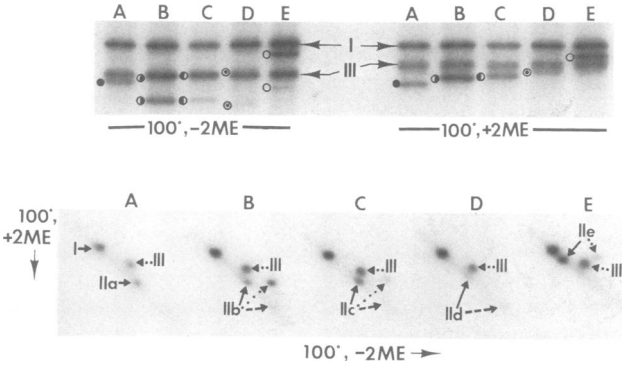

FIG. 7. Influence 2-ME on migration of P.II constituents of strain JS3. Five variants of strain JS3 that each contained a single P.II were subjected to SDS-PAGE after being boiled for 5 min in the absence or presence of 2-ME (top panel). P.I exhibited no change in apparent molecular weight in the absence versus presence of 2-ME, whereas P.III increased in apparent size upon solubilization in this reducing agent. These characteristics of P.I and P.III are also seen well in the two-dimensional gel analysis (bottom panel) in which specimens were solubilized at 100°C in the absence of 2-ME, electrophoresed, cut out, solubilized in 2-ME-containing solution, and reelectrophoresed. The P.I spot remains on the diagonal, whereas P.III is seen above this diagonal, indicating its shift in migration after exposure to 2-ME. P.II constituents exhibited different susceptibilities to change in migration characteristics in the presence of 2-ME. P.IIa was completely converted to its slow migrating form even in the absence of 2-ME; P.IIe was almost completely in its slow form under the same conditions; and P.IIb, P.IIc, and P.IId all had partial conversion without 2-ME. In the two-dimensional gel, a minor portion of each of P.IIb, P.IIc, and P.IId were still in the fast migrating form after the gel strips were boiled in 2-ME; it is not clear whether this results from a failure of the gel strips to reach the same temperatures as are obtained in liquid suspensions boiled during solubilization. Symbols are as given in the legend to Fig. 2.

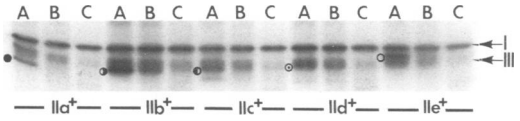

FIG. 8. Hydrolysis of strain JS3 P.II constituents by trypsin. Gonococci bearing different P.II constituents (IIa$^+$, IIb$^+$, etc.) were radioiodinated, and three equal portions of each were incubated with buffer alone (lanes A), or with trypsin at final concentrations of 10 μg/ml (lanes B) or 100 μg/ml (lanes C) for 15 min at room temperature. These reaction mixtures were then subjected to SDS-PAGE. A central portion of the resultant autoradiogram is shown. Note the lack of hydrolysis of P.I by trypsin in this strain and the striking loss of each P.II at the higher trypsin concentration. Symbols are as given in the legend to Fig. 2.

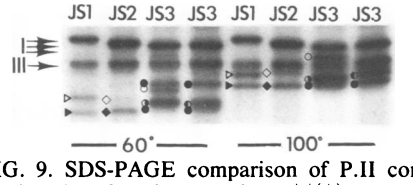

FIG. 9. SDS-PAGE comparison of P.II constituents of strains JS1, JS2, and JS3. O$^{++(+)}$ colony forms of strains JS1 and JS2 are shown along with two variants of strain JS3 that contain four of the five P.II forms found in this strain; the analysis was performed after ^{125}I labeling and solubilization at either 60°C for 30 min or 100°C for 5 min, both in the presence of 2-ME. Note the differences in apparent subunit molecular weights of the P.I constituents and the identical apparent subunit sizes of the P.III moieties in these three strains. Neither P.I nor P.III moieties differed in apparent size in 60- versus 100°C-solubilized specimens. Symbols used to denote P.II constituents are as follows: ▶, JS1 P.IIα; ▷, JS1 P.IIβ; ◆, JS2 P.IIγ; ◇, JS2 P.IIδ; ●, JS3 P.IIa; ◑, JS3 P.IIb; ◐, JS3 P.IIc; ○, JS3 P.IIe. Note the similarities in apparent size for JS1 P.IIα, JS2 P.IIγ, and JS3 P.IIa in the specimens solubilized by boiling; these same proteins are not identical in apparent size in the specimens solubilized at 60°C.

are not equal in the number or in the kinds of P.IIs they possess. (ii) Any time one deals with more than 10^3 gonococci, one probably has a mixture of different colony opacity forms. (iii) P.IIs vary considerably in their electrophoretic behavior as mirrored by their differences in both heat modifiability and 2-ME susceptibility. (iv) Careful stereo microscopic observations on opacity phenotypes yield reliable clues as to which P.IIs are present; however, these visual assessments are inadequate in defining some differences that can occur in P.II composition. (v) The presence or absence of one particular P.II showed no correlation with colonial opacity in one strain studied. (vi) The presence of some P.IIs is difficult to discern by SDS-PAGE in single-dimension gels of specimens subjected to only one protocol for solubilization before electrophoresis.

Previous studies using ^{125}I-labeled peptide mapping to compare SDS-PAGE-separated P.IIs suggest that all members of this outer membrane protein family exhibit considerable structural similarity (5, 15). In spite of these overall similarities, more recent studies have shown that surface-exposed, radioiodinatable residues of P.IIs on whole gonococci differ considerably from one P.II species to another, even within a single strain (5). Radioimmunoprecipitation techniques utilizing rabbit antisera raised against whole gonococci demonstrate considerable antigenic/immunogenic uniqueness among different P.IIs (unpublished data). These observations suggest that the facade of the gonococcus is markedly influenced by its P.II constituents.

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