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Interaction with Lectins and Differential Wheat Germ Agglutinin Binding of Pyocin 103-Sensitive and -Resistant *Neisseria gonorrhoeae*

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Strains of *Neisseria gonorrhoeae* were treated with pyocin 611 131 (pyocin 103) from *Pseudomonas aeruginosa* PA103, and isogenic resistant variants were isolated. The interaction of pyocin-sensitive and isogenic pyocin-resistant strains with wheat germ agglutinin (WGA) agglutinated all pyocin-sensitive, but not pyocin-resistant, strains. Binding of WGA to three pyocin-sensitive strains and their isogenic pyocin-resistant variants was examined quantitatively by using fluorescein-conjugated lectin. Pyocin-resistant strains maximally bound one-third to one-eighth the quantity of WGA bound by isogenic-sensitive strains. Linear Scatchard plots revealed homogeneous WGA-binding sites on three pyocin-sensitive and one pyocin-resistant strains. Biphasic Scatchard plots, obtained with two pyocin-resistant strains, show that WGA-binding sites in these strains are heterogeneous. The number of WGA-binding sites for pyocin-sensitive organisms ranged from 8×10^5 to 1×10^6 sites per coccus and from 1×10^5 to 3×10^5 sites per coccus for pyocin-resistant strains. The apparent association constant for WGA binding to pyocin-sensitive strains ranged from 3×10^6 to 6×10^6 liters/mol and from 6×10^6 to 1×10^7 liters/mol for pyocin-resistant strains. Gonococcal lipopolysaccharide was shown to serve as the pyocin 103 receptor by inhibition of pyocin activity. Lipopolysaccharide from a pyocin 103-resistant strain was not able to inhibit pyocin 103 activity. Pyocin 103 resistance was correlated with a structural alteration involving *N*-acetylglucosamine residues in gonococcal lipopolysaccharide. Based on interactions with wheat germ, soybean, and ricin lectins, a model of lipopolysaccharide structure in *N. gonorrhoeae* is presented.

Bacteriocins are antimicrobial substances, produced by bacteria, which inhibit members of the same or closely related species (22). However, several bacteriocins (pyocins) produced by *Pseudomonas aeruginosa* have been shown to inhibit the growth of *Neisseria gonorrhoeae* (23, 25, 27). One such bacteriocin, pyocin 103 (pyocin 611 131), described by Morse et al. (18), has been classified as an R-type pyocin because of its resistance to tryptic digestion and its morphological similarity to a bacteriophage tail assembly. Electron microscopic studies (18) show that R-type pyocin 103 interacts directly with the cell surfaces of sensitive strains of *N. gonorrhoeae*, whereas microorganisms resistant to the inhibitory action of pyocin show no pyocin-cell surface interaction. Although surface receptors responsible for pyocin binding to sensitive gonococci were not identified, it was suggested (18) that the receptor may reside in a region of gonococcal lipopolysaccharide (LPS) structure. This suggestion is supported by findings with other R-type pyocins which show that LPS serves as the receptor in sensitive strains of *P. aeruginosa* (4, 7, 16). In addition, Sadoff et al. (23) have demonstrated that gonococcal LPS can act as a receptor for several pyocins, although the relationship between pyocins described by Sadoff et al. and pyocin 103 is unclear. Since nearly all strains of *N. gonorrhoeae* tested are found to be pyocin 103 sensitive (18; D. Stein, personal communication), the receptor would be expected to reside in a common core region of LPS structure.

Schaefer et al. (24) tested 165 strains of *N. gonorrhoeae* and found that 164 were agglutinated by wheat germ lectin (WGA). The interaction of 54 strains of *N. gonorrhoeae* with 14 different lectins was examined by Allen et al. (1) and was used to obtain structural information about gonococcal cell surface carbohydrates. All

54 gonococcal strains tested were agglutinated by WGA. Lectin-mediated agglutination of gonococcal strains was attributed to lectin interaction with cell envelope LPS (1, 5). Because all strains tested were agglutinated by WGA, it was suggested that this lectin binds to β-*N*-acetyl-D-glucosamine residues in a region of LPS common core structure (1).

In the present study, pyocin 103-resistant variants of *N. gonorrhoeae* and the pyocin 103-sensitive strains from which they were derived were examined for differences in either lectin-mediated agglutination or their ability to bind fluorescein-conjugated lectins. In addition, the ability of isolated, purified gonococcal LPS to inhibit pyocin 103 activity was studied.

MATERIALS AND METHODS

Organisms. Strains W102 and W103 of *N. gonorrhoeae* were obtained from the Centers for Disease Control, Atlanta, Ga., as part of their 500 strain series. Gonococcal strain F62 was obtained from P. F. Sparling, University of North Carolina, Chapel Hill. RUG40 (ATCC 19424) has been previously described (12). Each of these strains is sensitive to pyocin 103. Gonococcal strain JW31 and its pyocin-resistant variant JW31R were from the strain collection of S. A. Morse. All gonococcal strains were Kellogg type T4 (10) and opaque as described by Swanson (29).

Pyocin 103-resistant variants RUG40R-11, RUG40R-16, W102R, W103R, and F62R-2 were selected in the following way. Sensitive strains (10^8 colony-forming units) were spread on GCK agar, made by adding 1% (vol/vol) Kellogg supplement (28) to GC medium base (Difco Laboratories). In the center of each plate, 5 U (50 μl) of pyocin 103 was spotted. After incubation at 37°C in 5% CO_2, isolated colonies growing within the zone of inhibition were picked, subcultured, and retested for pyocin resistance. Strains were repeatedly exposed to pyocin to insure selection of resistant variants.

Pyocinogenic *P. aeruginosa* PA103 (ATCC 29260) was from the strain collection of S. A. Morse. Pyocin-sensitive *P. aeruginosa* P14 was obtained from M. Kageyama, Mitsubishi-Kasei Institute of Life Sciences, Tokyo, Japan.

Purification and assay of pyocin 103. The induction and partial purification of pyocin 103 were performed as previously described (27). Briefly, cultures of *P. aeruginosa* PA103 were treated with mitomycin C (final concentration, 1 μg/ml) and then centrifuged to remove cell debris. Ammonium sulfate was added to supernatant fluids to 70% saturation, and precipitated material was sedimented by centrifugation. Pelleted precipitate was dissolved in 0.5 M Tris buffer, pH 7.5, containing 0.5 M $MgCl_2$ and 0.5 M $MgSO_4$ and then dialyzed against the same buffer. For the present study, after dialysis, pyocin 103 was further purified by gel filtration to remove low-molecular-weight inhibitors of gonococcal growth and cellular proteins from *P. aeruginosa*. Gel filtration was done by using a column (1.2 by 500 cm) of Bio-Gel A-15m (Bio-Rad Laboratories) with a flow rate of 6 ml of 0.5 M Tris buffer per h. Pyocin 103 was obtained as a single peak of optical density at 280 nm ($OD_{280\ nm}$) in the void volume. Fractions showing pyocin activity were pooled and sterilized by filtration through a 0.45-μm filter (Nalgene Labware Div., Nalge/Sybron Corp.). The inhibitory activity of pyocin preparations was determined, and their volumes were adjusted to contain a standard activity of 100 U/ml. One unit of activity is the minimum amount of pyocin required to cause a 99% decrease in the viability of 2×10^8 cells of *P. aeruginosa* P14.

Growth media and culture conditions. Gonococcal strains were grown on GCK solid medium. Gonococci, used to prepare Formalin-fixed suspensions, were grown on a solid, chemically defined medium (CDM) described by Catlin (3). All strains cultivated on solid medium were grown for 18 h at 37°C in a 5% CO_2 atmosphere.

Bacteria used to prepare LPS were grown in liquid GCP medium containing the following (grams per liter): proteose peptone no. 3 (Difco), 15.0; soluble starch (Difco), 0.5; K_2HPO_4 4.0; KH_2PO_4 1.0; and NaCl, 5.0. Before the medium was autoclaved, it was adjusted to pH 7.2 by using 6 N NaOH. Before the GCP medium was inoculated with gonococci, 1% (vol/vol) Kellogg supplement and 0.042% (wt/vol) $NaHCO_3$ were added. Large volumes of *N. gonorrhoeae* were grown in GCP medium (11 liters), using a New Brunswick Scientific Co. Fermentor (model MF 114) inoculated with 1 liter of logarithmically growing cells in GCP medium. Cultures were grown for 8 h at 36°C, at which time growth was stopped by the addition of 0.25% (wt/vol) solid sodium azide. Killed cells were harvested by centrifugation (900 × *g*, for 45 min, at 4°C). Pellets were washed twice in 0.85% (wt/vol) saline, suspended in a minimal amount of deionized water, and lyophilized. Lyophilized powders were stored at −20°C until used for LPS preparation.

P. aeruginosa PA103 and P14 were grown in GCP medium, as modified by Stein et al. (27).

Lectins. *Triticum vulgaris* WGA was purified by affinity chromatography as described by Marchesi (15). Lentil (*Lens culinaris*) lectin was purchased from Calbiochem-Behring Corp. Concanavalin A (*Canavalia ensiformis*) was purchased from Pharmacia Fine Chemicals, Inc. Ricin (*Ricinus communis*, RCA-120) was purchased from Sigma Chemical Co. Lectins from *Dolichos biflorus*, *Arachis hypogaea* (peanut agglutinin), *Sophora japonica*, and *Limulus polyphemus* (limulin) were purchased from E-Y Laboratories Inc. Fluorescein-coupled wheat germ and limulin with $OD_{495\ nm}/OD_{280\ nm}$ ratios of 1.65 and 1.33, respectively, were obtained from E-Y Laboratories Inc.

Lectin solutions were prepared immediately before use in lectin buffer consisting of 0.1 M Tris (pH 7.3) containing 0.15 M NaCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 1 mM $MnCl_2$, and 0.025% (wt/vol) sodium azide.

Gonococcal suspensions used for agglutination and binding. Standardized suspensions of gonococci, used for lectin agglutination and binding assays, were prepared by growing gonococcal strains on CDM agar. Agar plates received 10 ml of lectin-Formalin buffer, made by adding 2 ml of formaldehyde solution (37%, wt/vol) to 98 ml of lectin buffer, and cells were gently suspended with a sterile cotton swab.

Cell suspensions from three separate plates were pooled and stored overnight in lectin-Formalin buffer at 4°C. Formalin-fixed bacteria were washed by centrifugation (900 × *g*, for 45 min at 4°C), twice in saline and once in lectin buffer. Washed, pelleted cells were resuspended and vigorously mixed with a Pasteur pipette to disaggregate any small clumps of organisms. The volume was adjusted with lectin buffer until the suspension showed an $OD^{1\,cm}_{650\,nm}$ of 1.00 (Beckman DU spectrophotometer). The number of bacteria in adjusted suspensions was determined by using a Petroff-Hauser counting chamber and phase-contrast microscopy. The number of cells in a suspension with an $OD^{1\,cm}_{650\,nm}$ of 1.00 was 2.3×10^9 to 2.9×10^9 cocci per ml. Samples of each suspension were also Gram stained; only preparations with intact diplococci and little or no cell debris were used for further study. Agglutinations of gonococcal strains were performed in microtiter U-plates as previously described (1). The smallest quantity (in micrograms) of lectin capable of producing agglutination was recorded as the minimal agglutinating dose.

LPS isolation. LPS was isolated from gonococcal strains JW31 and JW31R by a modification of the hot phenol-water extraction procedure of Westphal and Jann, as described by Johnston et al. (8). Acetone-precipitated crude LPS was dissolved in water, treated overnight with approximately 1,000 U each of RNase and DNase (Worthington Diagnostics), and subjected to a second hot phenol-water extraction. Final purification was achieved by repeated sedimentation in the ultracentrifuge until the $OD^{1\,cm}_{260\,nm}$ and $OD^{1\,cm}_{280\,nm}$ of the aqueous supernatant fluid were <0.05. The gelatinous pellet was suspended in distilled water, lyophilized to a dry powder, and stored at −20°C. 2-Keto-3-deoxyoctonate is a distinctive constituent of gonococcal LPS (20). Isolated LPS preparations were analyzed for their 2-keto-3-deoxyoctonate content by the method of Karkhanis et al. (9) and found to contain between 6.3 and 6.8% 2-keto-3-deoxyoctonate by weight. This value is in good agreement with that reported by Perry et al. (20) for purified gonococcal LPS. For the quantitation of LPS, samples of lyophilized powder were dried to a constant weight in vacuo at 23°C over P_2O_5. Stock LPS solutions were made by dissolving analytically weighed amounts of dried LPS in known volumes of distilled water.

Quantitative lectin binding. Fluorescein-coupled WGA (WGA-FITC) was used to quantitatively assay WGA binding to pyocin-sensitive and -resistant gonococcal strains. Samples of gonococcal suspensions having 3.5×10^8 cocci were incubated with increasing concentrations (0 to 300 μg/ml) of WGA-FITC. The final volume in all tubes was adjusted to 0.5 ml with lectin buffer, and the suspensions were incubated for 60 min at 23°C. Cells were then sedimented by centrifugation (15,600 × *g*, for 15 min, at 23°C), and supernatant fluids containing unbound WGA-FITC were saved. The pelleted cells were washed twice with 0.25 ml of lectin buffer. WGA-FITC specifically bound to gonococcal cells was eluted by suspending the washed pellets in 0.5 ml of 0.5 M *N*-acetyl-D-glucosamine (GlcNAc) for 60 min at 23°C. Cells were sedimented by centrifugation, and supernatant fluids containing eluted WGA-FITC were saved. The quantitative recovery of lectin ranged between 90 and 105% of the amount added.

Immediately after the elution of WGA-FITC from cells, supernatant fluids containing eluted or unbound WGA-FITC were assayed in a spectrophotofluorometer (Aminco-Bowman) with an excitation wavelength of 490 nm and a fluorescence emission wavelength of 510 nm. A calibration curve was prepared for each experiment by using the fluorescence emission of samples containing known amounts of WGA-FITC. The fluorescence emission of the saved supernatant fluids was measured, and the micrograms of WGA-FITC present in each sample were determined from the calibration curve.

Scatchard plots were constructed from quantitative binding data, as described by Knutson et al. (11).

Inhibition of pyocin by LPS. Purified LPS, isolated from strains JW31 and JW31R, was assayed for its ability to inhibit the bactericidal action of pyocin. Pyocin (100 U/ml), in samples of 0, 5, 10, 25, or 50 μl, was added to 0.5 ml of GCP medium containing 20 μg of JW31 or JW31R LPS per ml. Mixtures were incubated for 30 min at 37°C with shaking (New Brunswick Scientific Co., model G76). Inhibition by LPS of bactericidal activity was assayed by adding 2×10^8 colony-forming units of JW31 gonococci, contained in 0.5 ml of GCP medium, to pyocin-LPS mixtures. Cultures were incubated for an additional 30 min with shaking. The colony-forming units of JW31 gonococci remaining after 30 min were then determined on serial 10-fold dilutions of reaction mixtures. Samples of the dilutions were spread, in triplicate, on GCK agar. Colonies were counted after 48 h of incubation.

Agar diffusion. Agar diffusion was performed as previously described (1).

RESULTS

Agglutination of *N. gonorrhoeae* by lectins. Pyocin-sensitive variants and their matched resistant strains were assayed for lectin-mediated agglutination by nine different lectins. Figure 1 shows an agglutination assay obtained with WGA and several matched pairs of pyocin-sensitive and -resistant strains. Pyocin-sensitive strains (rows A, C, and F) were all strongly agglutinated by WGA, although prozones were evident in wells having high lectin doses (50 μg). Pyocin-resistant strains (rows B, D, E, and G) showed no agglutination by WGA over the range from 50 to 0.05 μg of lectin. Table 1 summarizes the results of agglutination assays for all matched pyocin-sensitive and -resistant pairs obtained with wheat germ, soybean, ricin, *S. japonica*, peanut, *D. biflorus*, limulin, concanavalin A, and lentil lectins. Although several differences in lectin-mediated agglutination between pyocin-sensitive and -resistant variants were found, the loss of WGA agglutinability was the only difference common to all pyocin-resistant strains. WGA has been shown to bind sialic acid residues (2, 17, 21) in addition to *N*-acetylglucosamine (6). However, the failure of limulin,

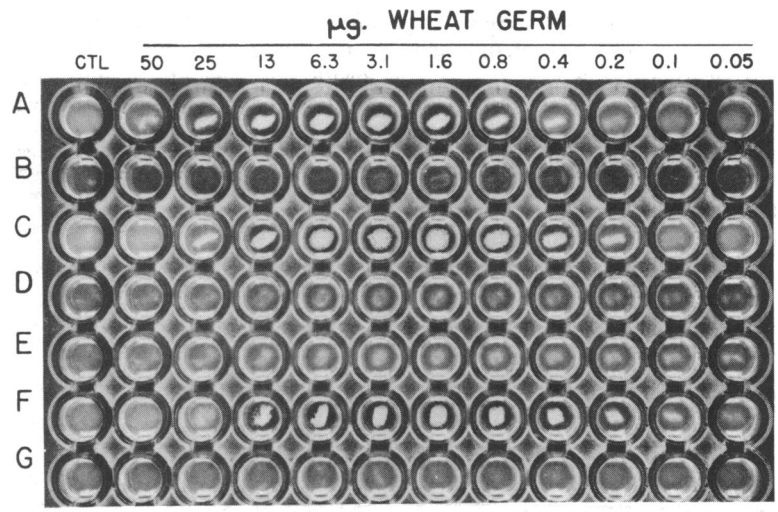

FIG. 1. *Agglutination assay of pyocin-sensitive and -resistant variants of N. gonorrhoeae by WGA. Controls (CTL), Not receiving WGA. Gonococcal strains in each row: A, JW31; B, JW31R; C, RUG40; D, RUG40R-11; E, RUG40R-16; F, F62; and G, F62R-2.*

TABLE 1. *Agglutination of isogenic pyocin-sensitive and -resistant strains of N. gonorrhoeae by lectins*

Strain	Minimal agglutinating dose (μg)						
	Wheat germ	Soybean	Ricin	*S. japonica*	Peanut	*D. biflorus*	Lim, Len, ConA[a]
JW31	0.8	0.05	0.1	3.1	0.4	NA[b]	NA
JW31R	NA	0.05	0.2	1.6	0.8	1.6	NA
RUG40	0.2	1.6	0.1	6.3	3.1	6.3	NA
RUG40R-11	NA	0.8	25.0	NA	NA	NA	NA
RUG40R-16	NA	0.2	25.0	13.0	NA	NA	NA
W102	0.2	0.1	0.4	3.1	0.8	NA	NA
W102R	NA	0.1	0.4	3.1	0.8	NA	NA
W103	0.1	0.2	0.2	3.1	0.8	NA	NA
W103R	NA	0.1	0.2	13.0	0.8	NA	NA
F62	0.05	0.05	0.05	0.05	0.2	3.1	NA
F62R-2	NA	0.05	0.2	0.2	0.2	NA	NA

[a] Lim, Limulin; Len, lentil; ConA, concanavalin A.
[b] NA, Not agglutinated by 50 μg of lectin.

a lectin specific for sialic acid (14), to agglutinate these strains suggests that sialic acid is not present.

Quantitative lectin binding. Binding of WGA to pyocin-sensitive and pyocin-resistant gonococci was determined by using fluorescein-conjugated lectin. Quantitative WGA-FITC binding by six strains is shown in Fig. 2. Although strains RUG40R-11, JW31R, and F62R-2 were not agglutinated by WGA (Table 1), they bound small, but significant, amounts of lectin. With sufficient lectin added to saturate all available binding sites, pyocin-sensitive RUG40, JW31, and F62 bound 2.8, 4.2, and 8.0 times more lectin, respectively, than their matched pyocin-resistant variants did.

Scatchard plots (Fig. 3A and B) revealed strain-dependent qualitative differences in WGA-FITC binding. For strains RUG40, JW31, F62 (Fig. 3A), and RUG40R-11 (Fig. 3B) the plots are linear over the entire range of lectin binding, and the slope for each strain is different. Strains JW31R and F62R-2 produced biphasic Scatchard plots (Fig. 3B). The number of binding sites per coccus and the apparent association constant (K_a) for WGA-FITC binding, determined from the Scatchard plots (11), are summarized in Table 2. Pyocin-resistant strains had

had a higher K_a for WGA-FITC binding, but had fewer binding sites per coccus than did their matched pyocin-sensitive strains.

The possibility that limulin lectin may have bound to test strains, although no agglutination had been observed, was examined. No limulin binding to strain JW31, JW31R, RUG40, RUG40R-11, F62, or F62R-2 was detected when cells were incubated with either 20 or 100 μg of fluorescein-coupled limulin.

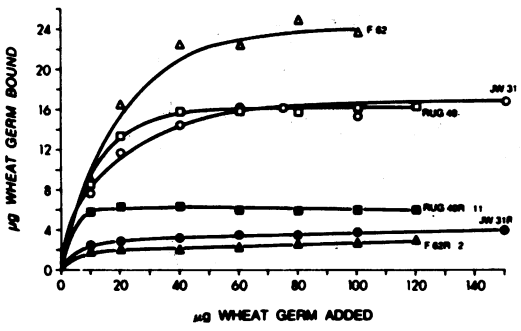

FIG. 2. *Wheat germ binding to gonococcal strains. Samples (3.5 × 10⁸ cocci) of pyocin-sensitive (RUG40, JW31, and F62) or pyocin-resistant (RUG40R-11, JW31R, and F62R-2) gonococci were incubated with WGA-FITC, and the amount of lectin bound was determined by fluorescence spectrophotometry.*

Inhibition of pyocin activity by LPS. Adsorption of pyocin to receptors of sensitive cells results in contraction and inactivation of pyocin

TABLE 2. *Apparent association constants and WGA-binding sites per coccus for N. gonorrhoeae*

Strain	K_a (10^6)[a]	Sites per coccus (10^5)[b]
JW31	2.8	8.5
JW31R	6.1	1.7
	1.0[c]	2.2[c]
RUG40	6.3	8.2
RUG40R-11	10.7	2.9
F62	5.3	12.0
F62R-2	7.0	1.1
	0.1[c]	2.3[c]

[a] Apparent association constant (liters per mole) determined from the slope of lines in Fig. 3.

[b] Determined by extrapolating lines in Fig. 3 to their intercept on the abscissa to obtain r = moles bound per 3.5×10^8 cocci at infinite free lectin concentration ($r/c = 0$). The value r multiplied by 6.02×10^{23} particles per mol and divided by 3.5×10^8 cocci yields the number of WGA-binding sites per coccus.

[c] Determined from the portion of the lines in Fig. 3 following the change in slope.

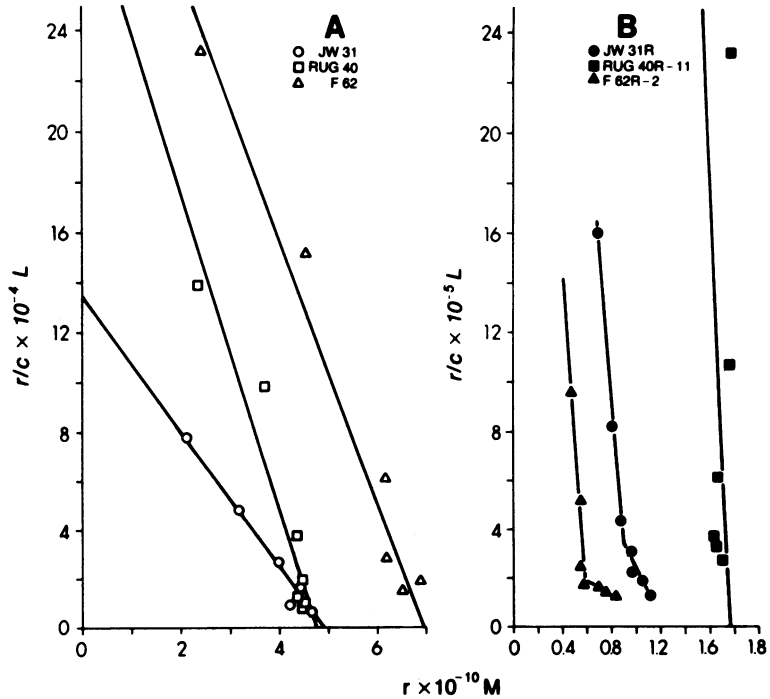

FIG. 3. *Scatchard plots of WGA-FITC binding to pyocin-sensitive (A) and pyocin-resistant (B) gonococci. The number of moles bound by 3.5 × 10⁸ cocci (r) and the concentration of free WGA-FITC in moles per liter (c) were determined from spectrophotofluorometric measurements, using a molecular weight of 36,000 for WGA-FITC.*

(18). The ability of phenol-extracted LPS to act as a receptor and to inactivate pyocin was determined by the bactericidal activity remaining after incubation of pyocin with 10 μg of JW31 LPS or JW31R LPS (Fig. 4). The addition of 5 U of pyocin to a culture of JW31 organisms caused a decrease of 10^4 in the number of colony-forming units (0.01% viable cells). Prior incubation of pyocin with LPS from strain JW31 completely inhibited its bactericidal activity. However, no decrease in bactericidal activity was observed after incubation with LPS from strain JW31R.

The LPS from strains JW31 and JW31R differed in their ability to precipitate with WGA in agar diffusion. Strain JW31 LPS gave a strong band of precipitation, whereas strain JW31R LPS gave a faint or barely detectable band.

DISCUSSION

Matched, isogenic pyocin 103-sensitive and -resistant strains were used for the isolation of LPS or for the examination of the interaction with lectins. Inhibition of pyocin 103 activity by purified LPS (Fig. 4) confirms the suggestion by Morse et al. (18) that LPS serves as the pyocin 103 receptor in sensitive strains of *N. gonorrhoeae*. It also extends the findings of Sadoff et al. (23) to include pyocin 103 among pyocins inhibited by gonococcal LPS. Moreover, the finding that purified LPS isolated from an isogenic pyocin-resistant strain fails to inhibit pyocin activity (Fig. 4) provides evidence that pyocin resistance is mediated by LPS structural alterations which affect pyocin binding. Clues to the nature of structural changes accompanying pyocin resistance may be inferred from a comparative examination of lectin interactions with cell surface carbohydrates of isogenic pyocin-sensitive and -resistant strains.

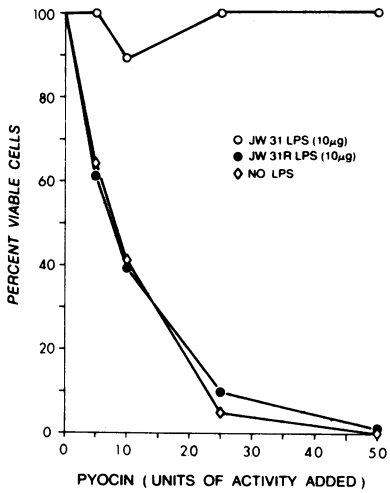

FIG. 4. *Inhibition of pyocin activity by LPS. Cultures of JW31 organisms (2 × 10^8 cells) received pyocin or pyocin pretreated with LPS isolated from strain JW31 or JW31R. The number of viable cells remaining after 30 min was determined as described in the text.*

A role involving GlcNAc in pyocin 103 binding is suggested by data presented in Table 1. WGA, a lectin specific for GlcNAc (6), agglutinated all pyocin-sensitive strains, but failed to agglutinate isogenic pyocin-resistant variants. No other lectin tested produced an agglutination pattern which could be correlated with pyocin 103 sensitivity. Because binding may occur in the absence of agglutination, the quantitative estimation of lectin binding by three matched pairs of isogenic pyocin-sensitive and -resistant strains was studied. Pyocin-resistant variants RUG40R-11, JW31R, and F62R-2 bound small, but significant, quantities of WGA-FITC compared with matched isogenic pyocin-sensitive strains (Fig. 2). The ability of excess free GlcNAc to quantitatively elute bound WGA-FITC from the surface of both pyocin-sensitive and -resistant strains demonstrates that WGA-FITC interaction with gonococci is ligand specific. Interaction with WGA may not be attributed uniquely to the occurrence of GlcNAc units because of its ability to interact with sialic acid (2, 17, 21). Sialic acid has been reported as a constituent of gonococcal LPS (30); however, the failure of limulin to show any detectable binding or to agglutinate any of the strains tested (Table 1) suggests that the WGA binding described in this study is not mediated by sialic acid residues.

Perry et al. (20) have provided chemical data for the linkages of some carbohydrate constituents of gonococcal LPS. Isolation of 3,4,6-tri-*O*-methyl-D-glucosamine and 3,4-di-*O*-methyl-D-glucosamine after exhaustive methylation of gonococcal LPS indicates that GlcNAc occurs both as nonreducing end groups and internal (1,6)-linked units. Both of these forms of GlcNAc have been shown to interact with WGA (6). Galactose (Gal) and GlcNAc were the only residues found to occupy nonreducing terminal positions (20).

Scatchard plots obtained with strains RUG40, JW31, F62 (Fig. 3A), and RUG40R-11 (Fig. 3B) are linear, indicating a single type of homogeneous WGA-FITC-binding site in each strain. However, biphasic Scatchard plots obtained with strains JW31R and F62R-2 (Fig. 3B) indicate at least two types of sites in each of these strains, with different binding affinities for WGA-FITC (Table 2). Spontaneous reversion from pyocin resistance to pyocin sensitivity for JW31R suggests that 1 to 2% of the JW31R

population may be pyocin-sensitive revertants. However, JW31R possesses 20% as many high-affinity binding sites as does JW31 (Table 2). Therefore, although a small number of pyocin-sensitive revertant cells could be present, they cannot account for the number of homogeneous, high-affinity, WGA-FITC-binding sites in pyocin-resistant strains. The number of high-affinity WGA-FITC-binding sites in pyocin-resistant strains was 65 to 91% less than that of isogenic pyocin-sensitive strains (Table 2). A reduced number of WGA-FITC-binding sites could be due to the presence of fewer LPS molecules on the surface of pyocin-resistant strains. Alternatively, reduction could result from LPS structural changes involving terminal GlcNAc, (1,6)-linked GlcNAc, or both. Comparable yields of LPS obtained per gram (dry weight) of JW31 and JW31R organisms suggest that similar amounts of LPS are present on both strains. Furthermore, a reduction of 80% in the total number of LPS molecules present on strain JW31R and a 91% reduction for strain F62R-2 would be expected to affect the ability of all lectins to agglutinate these strains. However, soybean, ricin, *S. japonica*, and peanut agglutinins show no significant differences in the minimal agglutinating dose for either JW31R or F62R-2 when compared with isogenic pyocin-sensitive parent strains (Table 1). Since agglutination by these lectins is unchanged, it is unlikely that pyocin-resistant strains JW31R and F62R-2 possess fewer LPS molecules on their surface than strains JW31 and F62 possess. The same argument may be advanced for RUG40R-11, based upon its undiminished soybean agglutinin reactivity. However, significant differences in the minimal agglutinating dose of ricin, *S. japonica*, and peanut agglutinin were found; thus, the possibility of a reduction in the total number of LPS molecules in the variant RUG40R-11 cannot be excluded.

That LPS structural alterations are responsible for the reduction in WGA-FITC-binding sites is supported by data in Table 2. Differences in K_a between pyocin-sensitive and matched pyocin-resistant strains indicate that WGA-FITC binds to pyocin-resistant variants at sites structurally different from those of pyocin-sensitive strains. Consistent with this finding are results of agar diffusion studies which reveal that purified LPS from strains JW31 and JW31R differ in their ability to precipitate with WGA. In addition, immunochemical studies currently in progress show that LPS from strains JW31 and JW31R is antigenically distinct. The loss of terminal GlcNAc or (1,6)-linked GlcNAc and the failure to *N*-acetylate glucosamine are structural changes which could bring about the reduction in the number of WGA-binding sites and could contribute to pyocin resistance by loss or disruption of the pyocin 103-binding site.

Whereas the carbohydrate composition of gonococcal LPS is known (19, 26, 30), the detailed structural organization of sugars remains to be established. Lectin data suggest a model for gonococcal LPS having a central carbohydrate backbone to which are attached several short branch structures. The carbohydrate backbone and branches may terminate in either GlcNAc, Gal, or GalNAc, whereas (1,6)-linked GlcNAc is restricted to an interior location (20). Binding of WGA to pyocin-sensitive gonococci would occur at branches terminating with GlcNAc, a location favorable to the formation of intercellular cross-links required for agglutination. The presence of multiple branches terminating in GlcNAc on each LPS backbone would effectively block WGA access to internal (1,6)-linked GlcNAc located in the LPS core. This would explain the homogeneity of WGA-FITC-binding sites in pyocin-sensitive strains despite the presence of alternate binding sites which become available in pyocin-resistant strains. Pyocin 103-resistant variants may lose GlcNAc residues from branch termini or lack an *N*-acetyl substituent. As a result, WGA gains access to and binds internal (1,6)-linked GlcNAc where it is less available to form the intercellular cross-links necessary for agglutination. A structural basis for the higher K_a of WGA-FITC binding to pyocin-resistant strains is not clear, although increased affinity could result from consecutive (1,6)-linked GlcNAc units or ionic stabilization of WGA-FITC binding or both (13).

This model predicts that binding of pyocin 103 is mediated by terminal nonreducing GlcNAc residues on the surface of *N. gonorrhoeae*. It is likely that pyocin 103 would form its tight association with the cell surface by interacting with multiple branches, terminating in GlcNAc, from adjacent LPS molecules. A requirement for multiple interactions in pyocin binding has been shown for other R-type pyocins (7), and receptor sites located on LPS carbohydrate branch structures have been suggested by Meadow and Wells (16) for some pyocins of *P. aeruginosa*.

The nature of the low-affinity binding site in strains JW31R and F62R-2 is not known. Weak interaction of WGA with GalNAc has been proposed by Peters et al. (21), due to structural similarities of GlcNAc and GalNAc pyranose rings at the equatorial C-2 acetamido and C-3 hydroxyl groups. Thus, low-affinity sites may arise from regions of very high GalNAc density.

Whereas interaction with lectins provides information about carbohydrate components of

gonococcal LPS, the definitive elucidation of LPS fine structure requires chemical data. Chemical and immunochemical studies in progress should furnish independent proof of the structural basis for differential lectin and pyocin reactivity in strains JW31 and JW31R.

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