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Resistance of *O*-Acetylated Gonococcal Peptidoglycan to Human Peptidoglycan-Degrading Enzymes

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Two naturally occurring forms of gonococcal peptidoglycan (PG) were tested for their susceptibility to human PG hydrolases. Purified ^{3}H-labeled PG substituted extensively with *O*-acetyl derivatives (*O*-PG; from *Neisseria gonorrhoeae* FA19) and ^{14}C-labeled *O*-acetyl-deficient PG (non-*O*-PG; from *N. gonorrhoeae* RD_5) were mixed together and treated with either normal human sera (NHS) or with lysozyme purified from human polymorphonuclear leukocytes (PMN-LZ). The initial rate of hydrolysis of *O*-PG by NHS or by PMN-LZ was two- to fourfold less than that of its non-*O*-PG counterpart in the same tube. When the reactions were allowed to go to completion, NHS solubilized both PGs completely, whereas PMN-LZ solubilized all of the non-*O*-PG and left ca. 60% of the *O*-PG insoluble. The PMN-LZ-soluble fraction of *O*-PG consisted largely of glycosidically linked fragments with molecular weights greater than ca. 10^4, whereas the corresponding non-*O*-PG was degraded to lower-molecular-weight fragments, exclusively. At completion, NHS hydrolyzed both PGs to fragments whose size was equal to or smaller than that of the free disaccharide unit of PG, suggesting that human sera contain a peptide-splitting (amidase) activity and a glycosidase activity, in addition to that of the well-known muramidase. NHS also promoted the release of high-molecular-weight PG fragments from intact gonococci. The persistence of human hydrolase-resistant PG in the form of soluble macromolecular fragments may potentiate the biological effects of gonococcal PG in vivo.

Peptidoglycan (PG) is commonly recognized for its role in the maintenance of bacterial integrity (8). However, given the opportunity to interact with host tissues, PG fragments are also potent biological effectors that have a propensity to influence inflammatory and immune responses (5, 27). Previous studies from our laboratory (22, 26, 31, 32) argue that *Neisseria gonorrhoeae* represents a unique model organism in that PG-host interactions during natural infection might be particularly direct and extensive. Accordingly, we are testing the hypothesis that gonococcal PG, released from the bacteria by the action of endogenous autolysins or host-derived PG-degrading enzymes, e.g., human muramidase (lysozyme), contributes to the host response to gonococcal infections. As a part of the test of this hypothesis, we have been testing purified PG fragments (including those forms that conceivably gain access to host tissues in vivo) for their ability to initiate biological reactions associated with the modulation of inflammatory and immune responses. Indeed, collectively, gonococcal PG fragments have been found to possess diverse biological activities, including (i) intrinsic toxicity for human fallopian tubes in organ culture (25), (ii) consumption of human complement (19, 25), (iii) modulation of blastogenesis of murine splenocytes and human peripheral blood lymphocytes (R. A. Bennett, R. S. Rosenthal, and C. E. Wilde III, Abstr. Annu. Meet. Am. Soc. Microbiol. 1981, 040, p. 49), and (iv) arthritogenicity in a rat arthritis model (R. Rosenthal, R. Fouts, and K. Phadke, unpublished data).

The structural requirements of these gonococcal PG-mediated biological activities have not been completely defined. However, the last three activities appear to depend on high-molecular-weight glycosidically linked fragments. Similar structural features are also necessary for the optimal expression of many of the biological activities mediated by PG derived from gram-positive bacteria, e.g., complement activation (12, 35), pyrogenicity (1, 18), and arthritogenicity (6, 14, 15).

The requirement of certain PG-mediated biological activities for intact glycan chains takes on added interest in light of recent observations that most strains of gonococci possess PG in which the glycan chains are substituted extensively with *O*-acetyl derivatives (2, 23). This modification confers resistance on the PG to

degradation by some commercial PG hydrolases, e.g., hen egg white lysozyme (HEW-LZ), but not by others, e.g., Chalaropsis B lysozyme (2, 23). Although the presence of HEW-LZ-resistant *O*-acetylated PG is well documented in certain other gram-negative bacteria, e.g., *Proteus* sp. (17), gonococci appear to be unusual in that there is considerable variation in the extent of *O*-acetylation and HEW-LZ resistance between PGs isolated from the two prototype strains used to date (23).

We reasoned that if extensively *O*-acetylated gonococcal PG (*O*-PG) is intrinsically resistant to biodegradation by human PG hydrolases (as it is to HEW-LZ), then this might promote the persistence of PG in vivo in the high-molecular-weight biologically active forms. Accordingly, we examined the ability of human PG hydrolases present in serum or derived from polymorphonuclear leukocytes to degrade gonococcal *O*-PG and *O*-acetyl-deficient PG (non-*O*-PG).

MATERIALS AND METHODS

Bacteria. *N. gonorrhoeae* RD_5 (obtained from F. E. Young, Rochester, N.Y.), FA19 (from P. F. Sparling, Chapel Hill, N.C.), and F62 (from D. S. Kellogg, Atlanta, Ga.) were used. Strain RD_5 was the source of non-*O*-PG. The extent of *O*-acetylation of RD_5 PG, although detectable, is less than one third that of the PG (*O*-PG) of FA19 (23). The PG of F62 is *O*-acetylated to an extent similar to that of FA19 (Swim and Rosenthal, unpublished data).

The gonococci were maintained by serial passage on a clear solid medium (CTM) (34), and colonies were typed by the criteria of Swanson (34). The bacteria used in all experiments were nonpiliated and transparent according to this scheme. To radiolabel PG, we routinely cultured the gonococci as described previously (24, 26) at 37°C in a liquid medium ($LGCB^+$; pH 7.3) containing 0.4% (wt/vol) pyruvate as the carbon source and also containing either D-[6-^{3}H]glucosamine ($GlcNH_2$) or D-[1-^{14}C]$GlcNH_2$ (ICN Pharmaceuticals, Inc., Irvine, Calif.). In some experiments, a chemically defined medium (WSJM; 36), containing pyruvate in lieu of glucose and lacking sodium acetate, was employed.

Preparation of PG. Purified, intact (insoluble) PG, containing label added as [^{3}H]- or [^{14}C]$GlcNH_2$ in both amino sugars of the glycan backbone, was prepared by a trichloroacetic acid (TCA)-sodium dodecyl sulfate (SDS) extraction procedure (13, 26). The conditions for SDS treatment were 4% SDS in 0.05 M sodium acetate buffer (pH 5.1) at 96°C for 1 h (2, 23). To further reduce the level of non-PG amino acids in intact PG, we treated washed SDS-insoluble PG for 16 h with 50 μg of proteinase K (Boehringer Mannheim Corp. [New York, N.Y.] lot no. 1391436 or E. Merck AG [Darmstadt, West Germany] lot no. 790682) per ml in 0.05 M Tris-hydrochloride (pH 7.4), containing 0.2% SDS. We used proteinase K rather than pronase (as used previously by us; 19) because each of two different lots of pronase (Calbiochem, La Jolla, Calif.) eliminated the HEW-LZ resistance of FA19 PG. Furthermore, even some lots of proteinase K obtained from Boehringer Mannheim eliminated the HEW-LZ resistance of FA19 PG. These latter preparations may have contained a contaminating activity that removed the *O*-acetyl derivatives.

PG hydrolysis assays. Four sources of PG hydrolase activity were used: (i) HEW-LZ (Boehringer Mannheim), (ii) Chalaropsis B muramidase (Miles Laboratories, Inc., Elkhart, Ind.), (iii) individual normal human sera (NHS) or, in some experiments, an NHS pool, a mixture of equal volumes of four individual NHS, and (iv) purified human leukemic polymorphonuclear lysozyme (PMN-LZ), kindly provided by R. Rest, Tucson, Ariz. The PMN-LZ was purified from granule extracts of neutrophils obtained from a single donor suffering from chronic myelocytic leukemia by the method of Rest and Pretzer (20) for purifying lysosomal proteases. Fractions containing purified PMN-LZ were collected from a separate individual peak from the gel filtration step using Sephadex G-100 (20).

For experiments to determine the relative digestibility of non-*O*-PG and *O*-PG, assay tubes typically contained (i) a mixture of 12 μg each of intact ^{3}H-labeled FA19 *O*-PG and ^{14}C-labeled RD_5 non-*O*-PG, (ii) a source of PG hydrolase activity at the concentration indicated, and (iii) an appropriate buffer (see below). The two PGs were routinely mixed together in the same tube because this should allow a more direct comparison of the rates of digestion by a given source of enzyme. However, for any given PG, the rates of digestion obtained in these dual-labeled experiments were similar to those obtained when only a single label was employed. The buffer used for the PG digestion assays employing the NHS pool or HEW-LZ was 0.05 M phosphate buffer, pH 7.4; the same phosphate buffer plus 0.01% Triton X-100 was used for PMN-LZ, both in tubes used for diluting the enzyme and in the reaction tubes; 0.05 M sodium acetate buffer (pH 4.7) was used in the assays for Chalaropsis B muramidase activity. After preliminary experiments were performed to establish an appropriate enzyme concentration that would allow the determination of the kinetics of the reactions, the following final concentrations were employed in the standard assay: NHS (1:100 dilution), PMN-LZ (0.033 U/ml), HEW-LZ (0.5 μg/ml), and Chalaropsis B enzyme (2.0 U/ml). The reaction mixtures were incubated at 37°C; and at intervals, samples were pipetted into ice-cold 10% TCA, allowed to stand at 4°C for at least 30 min, and filtered over GF/A filters; and TCA-insoluble (macromolecular) radioactivity was measured by liquid scintillation counting (see below). In some experiments, the degree of digestion was assessed by determining the distribution of the ^{3}H and ^{14}C disintegrations per minute present in the supernatant and the pellet after centrifugation (36,000 × *g*, 45 min).

Bactericidal reaction. [^{3}H]- or [^{14}C]$GlcNH_2$-labeled strains F62 (serum sensitive; 4) and FA19 (serum resistant; 4) were cultured at 37°C to mid-exponential phase in WSJM. The cells were washed twice in WSJM and added at a final concentration of ca. 10^7 CFU/ml to 50-ml flasks, to which freshly frozen (−70°C) and thawed NHS pool (final dilution in WSJM, 1:4) or WSJM alone (control) was also added. The reaction mixtures (final volume, 2.0 ml) were incubated at 37°C. Immediately before the addition of NHS or WSJM alone (0 h) and at 1-h intervals thereafter, samples were serially diluted in WSJM and inocu-

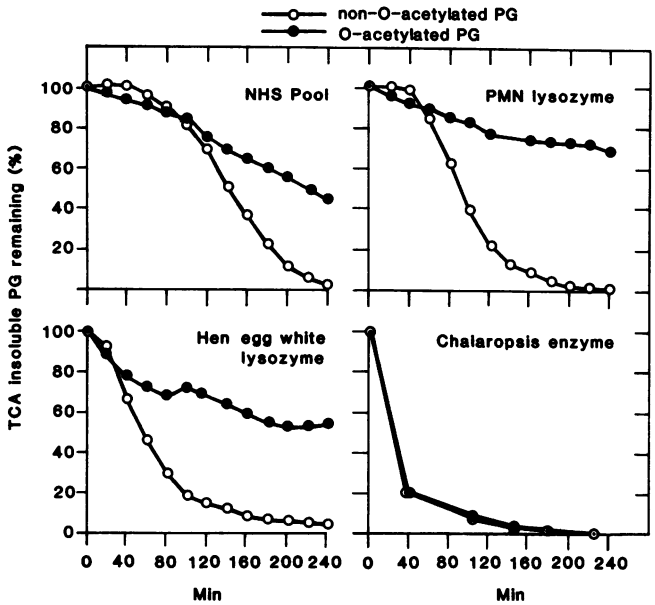

FIG. 1. Hydrolysis of ^{3}H-labeled *O*-PG and ^{14}C-labeled non-*O*-PG by the NHS pool (1:100), PMN-LZ (0.033 U/ml), HEW-LZ (0.5 μg/ml), and Chalaropsis B muramidase (2.0 U/ml). The assay mixtures contained both ^{3}H- and ^{14}C-labeled PG, each added at a concentration of 12 μg/ml.

lated onto CTM to determine the colony-forming units per milliliter. After 1, 2, or 3 h of incubation, the cultures were centrifuged (27,000 × *g*, 10 min), and the soluble radiolabeled material in the supernatants was filtered through a 0.45-μm membrane, heated at 75°C for 30 min to prevent further PG hydrolysis, and analyzed to detect the PG fragments (see below).

Analysis of soluble PG. We performed the characterization of the PG fragments by gel chromatography, using connected columns of G-50 and G-25, and by paper chromatography and electrophoresis in various solvents as previously described (31). The identification by paper chromatography of free radiolabeled amino sugars in acid hydrolysates has also been described before (31).

Radioactivity determination. To prepare samples for scintillation counting, we dissolved aqueous samples in Scintisol (Isolab Inc., Akron, Ohio), and samples, dried on paper, were dissovled in NCS-toluene cocktail as previously described (22). Radioactivity was determined with a model 3255 scintillation spectrometer (Packard Instrument Co., Downers Grove, Ill.) interfaced with an Apple II microcomputer. The data were corrected for quench and for the overlap of ^{14}C into ^{3}H channels by calibration curves determined by external standardization, and radioactivity was reported as disintegrations per minute.

RESULTS

Initial rates of digestion of *O*-PG and non-*O*-PG by PG hydrolases. The initial rates of hydrolysis of *O*-PG by NHS and human PMN-LZ (and by HEW-LZ) were considerably less than the corresponding rates of hydrolysis of non-*O*-PG in the same tubes (Fig. 1). Chalaropsis B lysozyme, on the other hand, hydrolyzed both PGs at similar rates. The data in Fig. 1 represent a single experiment employing each of the four sources of PG hydrolase activity, but the differential susceptibilities of *O*-PG and non-*O*-PG to both human enzymes and to HEW-LZ (Fig. 1) were quite reproducible in replicate experiments (n = 12 independent experiments performed on different days for the NHS pool and n = 3 for PMN-LZ and HEW-LZ). In these experiments, the initial rates of hydrolysis (measured as the slopes of the linear portions of the hydrolysis curves) of *O*-PG by each of these three enzymes were reliably only 25 to 50% as great as those of non-*O*-PG. For example, under the conditions of the standard hydrolysis assay, the mean rate of hydrolysis of non-*O*-PG by the NHS pool was 0.363 μg of PG per min, whereas the rate of hydrolysis of *O*-PG was 0.147 μg of PG per min ($P < 0.001$ by a two-tailed Student t test). *O*-PG was also relatively resistant to degradation by normal calf and rat serum and by PG hydrolase associated with washed, intact human peripheral blood lymphocytes (data not shown).

To make certain that the differential digestion of *O*-PG and non-*O*-PG by NHS was not due to some characteristic(s) peculiar to the particular NHS pool used, we performed the PG hydrolysis assay with individual NHS (n = 31) as the enzyme sources (Fig. 2). There was considerable variation among these sera in the individual rates of digestion of a given PG and in the

relative rate of digestion (defined as the ratio of the digestion rate of non-*O*-PG to that of *O*-PG). However, in 30 of the 31 sera tested, the rate of digestion of non-*O*-PG exceeded that of the *O*-PG in the same tube (Fig. 2). In 25 of these sera, the relative rate of digestion was greater than 1.5 ($P < 0.001$).

Extent of PG digestion by PG hydrolases. ^{14}C-labeled non-*O*-PG and ^{3}H-labeled *O*-PG were mixed together and treated with each of the four individual sources of PG hydrolase activity, and the reactions were allowed to go to completion, i.e., until the addition of fresh enzyme or continued incubation did not solubilize additional PG. At completion, all of the non-*O*-PG was rendered soluble, i.e., present in the supernatant after centrifugation, by both commercial muramidases and by both sources of human PG hydrolases (Table 1). However, when *O*-PG was treated with PMN-LZ (or HEW-LZ), more than half of the PG remained insoluble at the completion of the reaction, whereas NHS (like Chalaropsis enzyme) solubilized all of the *O*-PG. Replicate experiments ($n \geq 3$) performed identically to the representative assays shown in Table 1 confirmed these results. In these experiments, the percentage of *O*-PG that remained insoluble ranged from 40 to 65% when PMN-LZ was employed and from 25 to 55% when HEW-LZ was used, whereas in all cases, the treatment of *O*-PG with the NHS pool or Chalaropsis enzyme and the treatment of non-*O*-PG with any of the four enzyme preparations left less than 2% of the respective PG insoluble. It is of interest that, at completion, the NHS pool (in contrast to PMN-LZ) was able to solubilize all of the *O*-acetylated preparation (Table 1). This suggests that human serum contains PG hydrolase activity with substrate specificities in addition to those of PMN-LZ, i.e., serum hydrolases can cleave linkages in PG that are inaccessible to PMN-LZ.

Analysis of soluble PG. To characterize the PG fragments solubilized by human-serum- and PMN-derived PG hydrolases, the reactions were allowed to go to completion, residual insoluble PG (if any) was removed by centrifugation, and soluble PG fragments were fractionated by gel filtration (Fig. 3). The distribution of PG fragments solubilized by PMN-LZ (Fig. 3A) was markedly similar to the distribution of the fragments released by HEW-LZ (Fig. 3B). After treatment with either enzyme, virtually all of the non-*O*-PG was smaller in size than presumed PG hexamers (with a molecular weight of ca. 6,000; $Kd = 0.15$), but the distribution of soluble *O*-PG was shifted in favor of high-molecular-weight oligomers (Fig. 3A and B), and in fact, a considerable amount was present in the void volume (molecular weight, $>10^4$). It is apparent, therefore, that PMN-LZ leaves the majority of *O*-PG (and a minor amount of non-*O*-PG) as incompletely digested, glycosidically linked oligomers. In support of this contention is the finding that when the hydrolysis of all glycosidic linkages in either PG was accomplished by Chalaropsis enzyme as described before (23, 26), PG fragments no greater in size than tetramers ($K_d = 0.25$; Fig. 3E) were produced.

Because at the completion of the reaction, NHS was able eventually to solubilize all of the *O*-PG (Table 1), albeit at a slower rate than non-*O*-PG (Fig. 1), it was not surprising that NHS ultimately degraded both PGs to low-molecular-weight fragments, exclusively (Fig. 3D). Unexpectedly, however, these NHS-soluble products had an apparent molecular weight even lower than that of the free disaccharide *N*-acetylglucosaminyl-*N*-acetylmuramic acid (Fig. 3D). This suggested that NHS contains, in addition to the well-known muramidase activity, a peptide-splitting (and probably an additional glycosidase) activity capable of degrading PG. At intermediate times of incubation, i.e., before completion, NHS digestion of *O*-PG yielded fragments of higher molecular weight than corresponding fragments derived from non-*O*-PG (Fig. 3C).

Release of PG from viable gonococci subjected to NHS treatment. $GlcNH_2$-labeled strains F62 and FA19 were subjected to the serum-dependent bactericidal reaction. In agreement with others (4), we found that F62 was serum sensitive (viability was reduced by more than 99% during h 1 of incubation) and FA19 was serum resistant (viability remained essentially constant or actually increased during the incubation period). The gel filtration patterns (Fig. 4) of the soluble PGs released from the serum-sensitive and -resistant gonococci during this reaction (1 to 3 h of incubation) were similar in that each showed liberated low-molecular-weight PG fragments, but a substantial amount of the label was present as higher oligomers ($K_d = 0$ to 0.25). The evidence that these high-molecular-weight sub-

TABLE 1. Extent of digestion of *O*-PG and non-*O*-PG by PG hydrolases

Enzyme source	Concn	% Insoluble PG[a] remaining at completion from:	
		O-PG	Non-*O*-PG
NHS pool (diluted)	1:100	1.1	<1
PMN-LZ (U/ml)	0.033	62.4	<1
HEW-LZ (μg/ml)	0.5	51.6	<1
Chalaropsis enzyme (U/ml)	4.4	<1	<1

[a] Present in the pellet after centrifugation at 36,000 × *g* for 45 min.

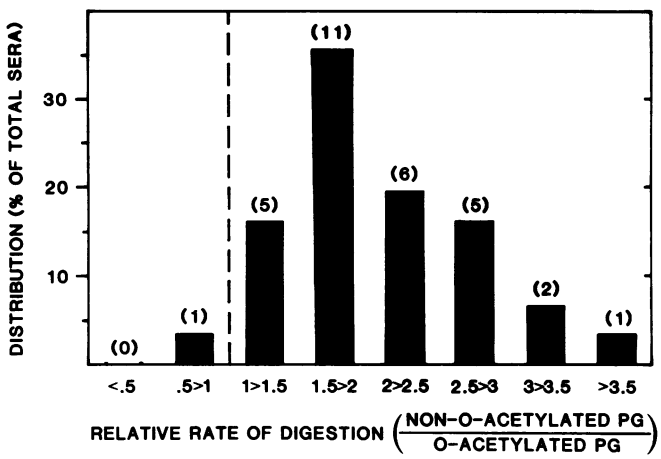

FIG. 2. Relative rate of digestion of non-*O*-PG and *O*-PG by individual NHS ($n = 31$). The rate of digestion of a given PG was determined from the slope of the linear portion of the hydrolysis curves (Fig. 1). The dashed line indicates the position of the theoretical mode if the digestion rates had been equivalent. The numbers in parentheses are the number of sera comprising the designated group.

stances were, indeed, PG was based on their content of radiolabeled $GlcNH_2$ and muramic acid almost exclusively (Fig. 5).

The presence of the free disaccharide, monomer, and dimer fragments in the supernatants of NHS-treated gonococci was not unexpected since FA19 and F62 (similar to other gonococci) are known to turn over macromolecular PG during growth in a liquid medium, e.g., WSJM, and to release these low-molecular-weight PG products into the environment (22, 31). High-molecular-weight PG fragments, however, were not released by gonococci in medium alone (data not shown), as reported previously (22, 31). It was of particular interest, therefore, that NHS promoted the release of high-molecular-weight glycosidically linked PG oligomers and did so from both serum-sensitive and -resistant strains.

DISCUSSION

This study demonstrates that the extensively *O*-acetylated PG, native to most strains of gonococci, is intrinsically resistant to the PG-degrading enzymes present in human serum or derived from human PMN. With respect to PMN-LZ, this resistance is absolute in the sense that at the completion of the reaction, the majority of *O*-PG remains insoluble as defined by its presence in the pellet after ultra-centrifugation. The remainder of the *O*-PG, although soluble by this criterion, exists almost exclusively as incompletely degraded glycan polymers of various chain lengths. This heterogeneity in solubility and in size of the final PMN-LZ digestion products probably reflects heterogeneity in the local distribution of *O*-acetyl derivatives within the native PG matrix. Therefore, in chemical terms, discrimination of PMN-LZ-derived fragments solely on the basis of insoluble versus soluble is probably a bit arbitrary. With respect to serum PG hydrolases, the resistance of *O*-PG is relative in the sense that *O*-PG is hydrolyzed more slowly than non-*O*-PG; at completion, both PGs are ultimately degraded to fragments equal to or smaller than free disaccharide. However, at intermediate times before the complete digestion of both PGs, NHS-soluble fragments derived from *O*-PG had a greater average chain length than did their *O*-acetyl-deficient counterparts.

The differing abilities of NHS and PMN-LZ to degrade *O*-PG and the very small size of the final NHS-soluble products indicate that human serum contains multiple enzymatic activities capable of degrading PG, i.e., peptide-splitting (amidase) and glucosaminidase activity, in addition to the well-known muramidase activity. Very recently, Ladesic et al. (16) described such an amidase activity in human and mouse plasma and, thus, we confirm their findings.

The presence of multiple PG hydrolases in serum (and possibly tears and other secretions as well) raises another issue. Historically, assays for lysozyme (muramidase) activity have often employed the measurement of bacterial lysis (typically that of *Micrococcus lysodeikticus*) as an indicator of enzyme activity. For purified preparations of muramidase, this seems quite useful as commonly practiced and as generally recommended by manufacturers (Biochemica catalogue, Boehringer Mannheim Corp.). However, for complex biological fluids, e.g., serum, such assays might not actually be measuring muramidase-mediated lysis, specifically, but rather the cumulative lytic effect of

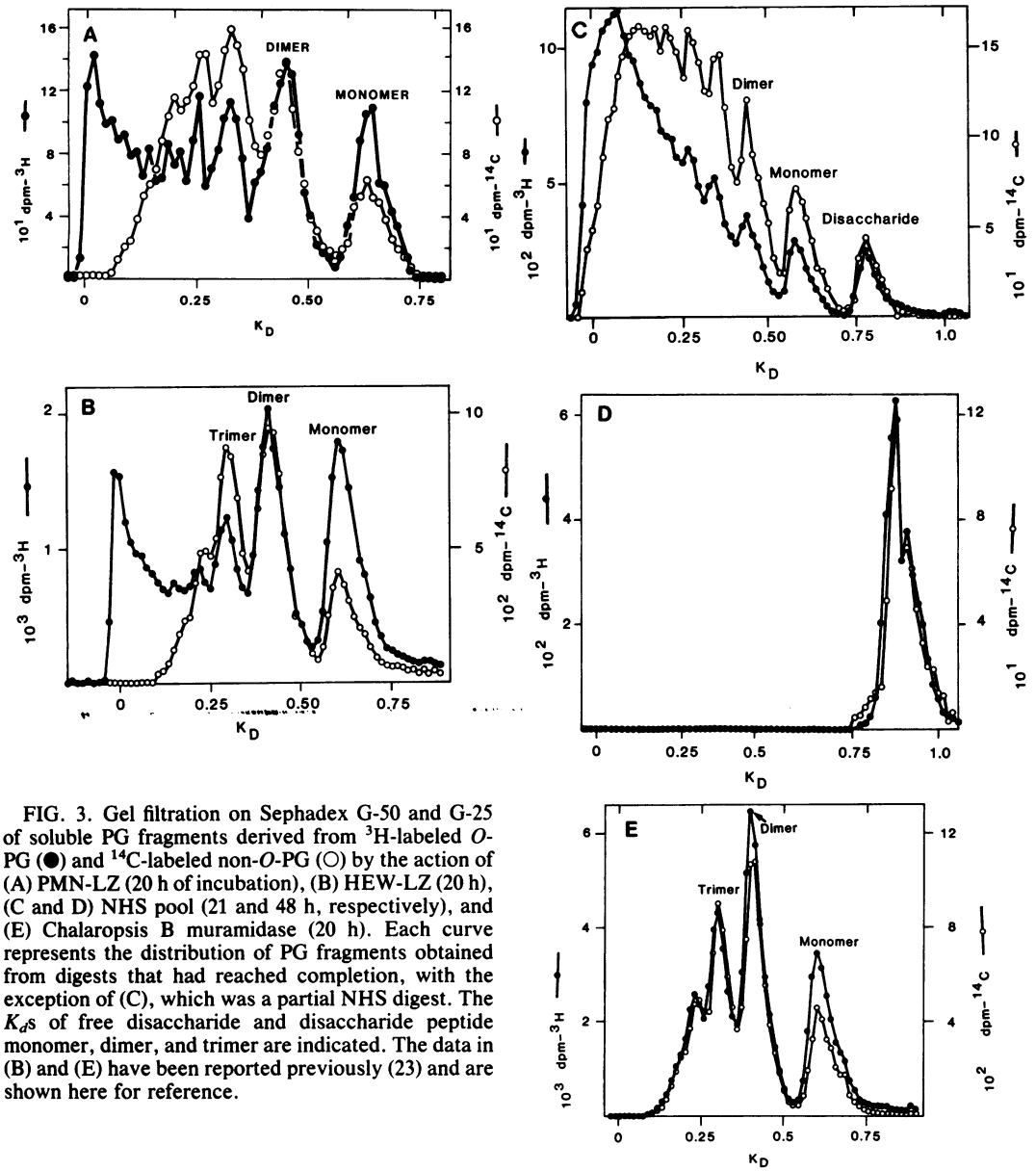

FIG. 3. Gel filtration on Sephadex G-50 and G-25 of soluble PG fragments derived from ^{3}H-labeled *O*-PG (●) and ^{14}C-labeled non-*O*-PG (○) by the action of (A) PMN-LZ (20 h of incubation), (B) HEW-LZ (20 h), (C and D) NHS pool (21 and 48 h, respectively), and (E) Chalaropsis B muramidase (20 h). Each curve represents the distribution of PG fragments obtained from digests that had reached completion, with the exception of (C), which was a partial NHS digest. The K_ds of free disaccharide and disaccharide peptide monomer, dimer, and trimer are indicated. The data in (B) and (E) have been reported previously (23) and are shown here for reference.

several PG hydrolases. Thus, unless it can be demonstrated that other PG hydrolases in such a mixture do not influence the lysis of target cells, we suggest that such assays be cautiously interpreted as to the specificity of the enzymes involved.

It is of interest that NHS stimulated the release of PG fragments from intact gonococci that were subjected to the serum bactericidal reaction (Fig. 4). Under the conditions of this experiment (3 h of incubation), the soluble PG fragments resulting from this reaction were of high molecular weight; although based on other data (Fig. 3D), even *O*-PG fragments would, presumably, eventually be completely degraded. In any event, this simple in vitro test is probably a fairly realistic simulator of at least some conditions experienced by gonococci in vivo. Thus, with our commitment to examine PG-host interactions in the context of forms of PG that are achievable during natural infections, the serum-dependent release of oligomeric *O*-PG fragments from intact bacteria takes on added interest. The mechanism of PG release under these conditions

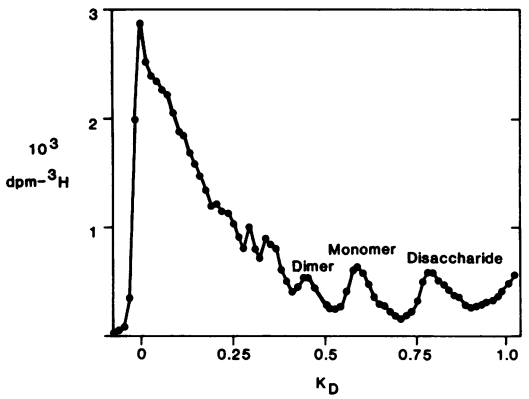

FIG. 4. Gel filtration on Sephadex G-50 and G-25 of [^{3}H]GlcNH$_2$-labeled material released from NHS-treated strain F62. The K_ds of low-molecular-weight PG standards are indicated.

is currently unclear. One possibility is that serum hydrolases somehow penetrate the gonococcal outer membrane and, thereby, directly attack the PG substrate. Alternatively, a component(s) of NHS might indirectly stimulate release by triggering native gonococcal autolytic activity.

The optimal expression of many of the biological activities of PG (including gonococcal PG) requires glycan-linked oligomers of the repeating disaccharide peptide subunit (15, 18, 19, 35). By promoting the persistence of these high-molecular-weight fragments, hydrolase-resistant *O*-PG might potentiate the pathobiological consequences of gonococcal PG-host interactions in vivo. Few studies have addressed the biological consequences of PG persistence in the context of forms of the molecule that are likely to gain direct access to host domains. However, studies by Schwab and co-workers over the years (7, 9, 10, 27–30, 33), employing a rat model in which arthritis is elicited by group A streptococcal cell walls, provide an important perspective. This model was designed to examine inflammatory injury due to streptococci with emphasis on some of the late noninfectious sequellae (for which there is some epidemiological evidence of PG involvement; 3, 21). Yet, lessons learned from this system might be applicable to other agents, e.g., gonococci and staphylococci, that (i) are associated with particularly extensive acute or chronic inflammation and (ii) possess hydrolase-resistant PG that is likely to persist in human tissues. In early studies with the streptococcal system (11, 30), it was found that group A cell walls (similar to gonococcal *O*-PG) were resistant to HEW-LZ and to lysosomal enzymes derived from human phagocytes. The structural basis of the resistance of streptococcal PG, however, related to its covalent attachment to the group-specific polysaccharide and to numerous unsubstituted amino groups (11, 30). When the lysozyme-resistant streptococcal walls were injected into appropriate experimental animals, the PG was not degraded; rather, it persisted in certain tissues, including macrophages and synovial tissue, and ultimately induced inflammatory joint disease (7, 9, 29, 33). Recently, it was shown (10) that the severity of arthritis in this model correlated with the molecular weight of the PG-polysaccharide complex employed, as well as with the chemical structure. Fragments on the order of 5×10^6 daltons caused the most severe acute inflammation but little chronic disease, whereas fragments of ca. 5×10^7 daltons caused only moderate acute disease but severe chronic erosive joint lesions (10). The mechanisms of PG-induced arthritis in this model are

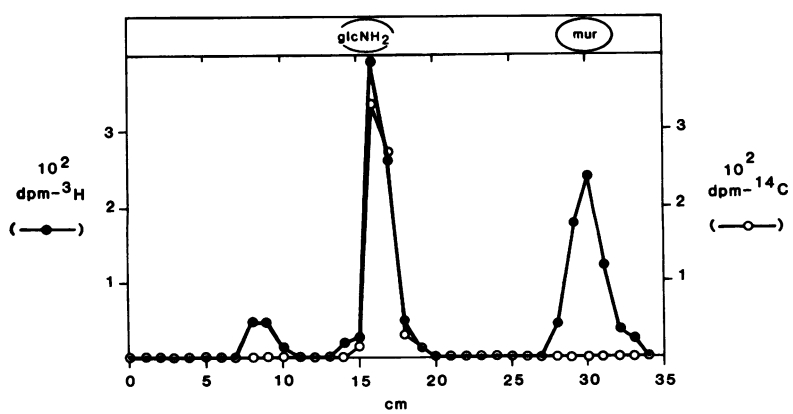

FIG. 5. Paper chromatography in butanol-acetic acid-water (5:1:2) of acid-hydrolyzed ^{3}H-oligomers (K_d = 0 to 0.25; Fig. 4) released by strain F62 during killing by NHS and of [^{14}C]GlcNH$_2$ (internal standard). The positions of the unlabeled standards, GlcNH$_2$, and muramic acid (mur) were detected with ninhydrin.

still not completely understood. However, the destructive process itself probably involves the activation of complement by both antibody-dependent and -independent mechanisms and accompanying mediators of type III immunological injury (12, 28).

A final point relates to the observed variation among individual human sera in the absolute and relative rates of hydrolysis of *O*-PG and non-*O*-PG (Fig. 2). This variation probably reflects differences in the kinds or amounts of PG hydrolase present in these sera. If PG does influence the inflammatory response to gonococci, as we suggest (19, 25), then these data suggest a host factor (conceivably of genetic origin) that could govern individual susceptibility to *O*-PG-mediated events. Thus, high levels of PG hydrolase activity in serum or secretions might diminish the extent of the host reaction, whereas low levels might predispose to a particularly extensive (potentially pathological) inflammatory response.

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LITERATURE CITED

1. **Atkins, E., and S. I. Morse.** 1967. Studies in staphylococcal fever. VI. Response induced by cell walls and various fractions of staphylococci and their products. Yale J. Biol. Med. **39**:297–311.
2. **Blundell, J. K., G. J. Smith, and H. R. Perkins.** 1980. The peptidoglycan of *Neisseria gonorrhoeae*: *O*-acetyl groups and lysozyme sensitivity. FEMS Microbiol. Lett. **9**:259–261.
3. **Braun, D. G., and S. E. Holm.** 1970. Streptococcal antigroup A precipitins in sera from patients with rheumatic arthritis and acute glomerulonephritis. Int. Arch. Allergy **37**:216–224.
4. **Cannon, J. G., T. J. Lee, L. F. Guymon, and P. F. Sparling.** 1981. Genetics of serum resistance in *Neisseria gonorrhoeae*: the *sac-1* genetic locus. Infect. Immun. **32**:547–552.
5. **Chedid, L., F. Audibert, and A. G. Johnson.** 1978. Biological activities of muramyl dipeptide, a synthetic glycopeptide analogous to bacterial immunoregulating agents. Prog. Allergy **25**:63–105.
6. **Chetty, C., D. G. Klapper, and J. H. Schwab.** 1982. Soluble peptidoglycan-polysaccharide fragments of the bacterial cell wall induce acute inflammation. Infect. Immun. **38**:1010–1019.
7. **Dalldorf, F. G., W. J. Cromartie, S. K. Anderle, R. L. Clark, and J. H. Schwab.** 1980. The relation of experimental arthritis to the distribution of streptococcal cell wall fragments. Am. J. Pathol. **100**:383–402.
8. **Daneo-Moore, L., and G. D. Shockman.** 1977. The bacterial surface in growth and division, p. 597–715. *In* G. Poste and G. L. Nicolson (ed.), Cell surface reviews, vol. 4. Elsevier/North-Holland Publishing Co., Amsterdam.
9. **Eisenberg, R., A. Fox, J. J. Greenblatt, S. K. Anderle, W. J. Cromartie, and J. H. Schwab.** 1982. Measurement of bacterial cell wall in tissues by solid-phase radioimmunoassay: correlation of distribution and persistence with experimental arthritis in rats. Infect. Immun. **38**:127–135.
10. **Fox, A., R. R. Brown, S. K. Anderle, C. Chetty, W. J. Cromartie, H. Gooder, and J. H. Schwab.** 1982. Arthropathic properties related to the molecular weight of peptidoglycan-polysaccharide polymers of streptococcal cell walls. Infect. Immun. **35**:1003–1010.
11. **Glick, A. O., J. M. Ranhand, and R. M. Cole.** 1972. Degradation of group A streptococcal cell walls by egg-white lysozyme and human lysosomal enzymes. Infect. Immun. **6**:403–413.
12. **Greenblatt, J., R. J. Boackle, and J. H. Schwab.** 1978. Activation of the alternate complement pathway by peptidoglycan from streptococcal cell wall. Infect. Immun. **19**:296–303.
13. **Hebeler, B. H., and F. E. Young.** 1976. Chemical composition and turnover of peptidoglycan in *Neisseria gonorrhoeae*. J. Bacteriol. **126**:1180–1185.
14. **Kohashi, O., C. M. Pearson, Y. Watanabe, and S. Kotani.** 1977. Preparation of arthritogenic and non-arthritogenic bacterial cell walls. Infect. Immun. **16**:861–866.
15. **Kohashi, O., C. M. Pearson, Y. Watanabe, S. Kotani, and T. Koga.** 1976. Structural requirements for arthritogenicity of peptidoglycans from *Staphylococcus aureus* and *Lactobacillus plantarum* and analogous synthetic compounds. J. Immunol. **116**:1635–1639.
16. **Ladesic, B., J. Tomasic, S. Kveder, and I. Hrsak.** 1981. The metabolic fate of ^{14}C-labeled immunoadjuvant peptidoglycan monomer. II. In vitro studies. Biochim. Biophys. Acta **678**:12–17.
17. **Martin, J. P., J. Fleck, M. Mock, and J. M. Ghuysen.** 1973. The wall peptidoglycans of *Neisseria perflava, Moraxella glucidolytica, Pseudomonas alcaligenes*, and *Proteus vulgaris* P18. Eur. J. Biochem. **38**:301–306.
18. **Oken, M. M., P. K. Peterson, and B. J. Wilkinson.** 1981. Endogenous pyrogen production by human blood monocytes stimulated by staphylococcal cell wall components. Infect. Immun. **31**:208–213.
19. **Petersen, B. H., and R. S. Rosenthal.** 1982. Complement consumption by gonococcal peptidoglycan. Infect. Immun. **35**:442–448.
20. **Rest, R., and E. Pretzer.** 1981. Degradation of gonococcal outer membrane proteins by human neutrophil lysosomal proteases. Infect. Immun. **34**:62–68.
21. **Rolicka, M., and B. F. Massell.** 1973. Antipeptidoglycan in rheumatic fever: agreement with carditis. Proc. Soc. Exp. Biol. Med. **144**:892–895.
22. **Rosenthal, R. S.** 1979. Release of soluble peptidoglycan from growing gonococci: hexaminidase and amidase activities. Infect. Immun. **24**:869–878.
23. **Rosenthal, R. S., J. K. Blundell, and H. R. Perkins.** 1982. Strain-related differences in lysozyme sensitivity and extent of *O*-acetylation of gonococcal peptidoglycan. Infect. Immun. **37**:826–829.
24. **Rosenthal, R. S., R. S. Fulbright, M. E. Eads, and W. D. Sawyer.** 1977. Ethylenediaminetetraacetic acid-sensitive antiphagocytic activity of *Neisseria gonorrhoeae*. Infect. Immun. **15**:817–827.
25. **Rosenthal, R. S., R. K. Sinha, B. H. Petersen, M. A. Melly, and Z. A. McGee.** 1981. Chemical and biological properties of gonococcal peptidoglycan, p. 7–11. *In* D. Danielsson and S. Normark (ed.), Genetics and immunobiology of pathogenic neisseria. Norrlands-trycki i Umea Ab, Umea, Sweden.
26. **Rosenthal, R. S., R. M. Wright, and R. K. Sinha.** 1980. Extent of peptide cross-linking in the peptidoglycan of *Neisseria gonorrhoeae*. Infect. Immun. **28**:867–875.
27. **Schwab, J.** 1979. Acute and chronic inflammation induced by bacterial cell wall structures, p. 209–214. *In* D. Schlessinger (ed.), Microbiology—1979. American Society for Microbiology, Washington, D.C.
28. **Schwab, J. H., J. B. Allen, S. K. Anderle, F. Dalldorf, R. A. Eisenberg, and W. J. Cromartie.** 1982. Relationship of complement to experimental arthritis induced in rats with streptococcal cell walls. Immunology **46**:83–88.

29. **Schwab, J. H., W. J. Cromartie, S. H. Ohanian, and J. G. Craddock.** 1967. Association of experimental chronic arthritis with the persistence of group A streptococcal cell walls in the articular tissue. J. Bacteriol. **95**:1728–1735.
30. **Schwab, J. H., and S. H. Ohanian.** 1967. Degradation of streptococcal cell wall antigens in vivo. J. Bacteriol. **94**:1346–1352.
31. **Sinha, R. K., and R. S. Rosenthal.** 1980. Release of soluble peptidoglycan from growing gonococci: demonstration of anhydro-muramyl-containing fragments. Infect. Immun. **29**:914–925.
32. **Sinha, R. K., and R. S. Rosenthal.** 1981. Effect of penicillin G on release of peptidoglycan fragments by *Neisseria gonorrhoeae*: characterization of extracellular products. Antimicrob. Agents Chemother. **20**:98–103.
33. **Smialowicz, R. J., and J. H. Schwab.** 1977. Cytotoxicity of rat macrophages activated by persistent or biodegradable bacterial cell walls. Infect. Immun. **17**:599–606.
34. **Swanson, J.** 1978. Studies on gonococcus infection. XII. Colony color and opacity variants of gonococci. Infect. Immun. **19**:320–331.
35. **Wilkinson, B. J., Y. Kim, and P. K. Peterson.** 1981. Factors affecting complement activation by *Staphylococcus aureus* cell walls, their components, and mutants altered in teichoic acid. Infect. Immun. **32**:216–224.
36. **Wong, P. T., R. K. Shockley, and R. H. Johnston.** 1980. WSJM, a simple chemically defined medium for growth of *Neisseria gonorrhoeae*. J. Clin. Microbiol. **11**:363–369.