Strain-Related Differences in Lysozyme Sensitivity and Extent of O-Acetylation of Gonococcal Peptidoglycan

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Received 18 February 1982/Accepted 6 April 1982

Peptidoglycan from Neisseria gonorrhoeae RD3 was completely degraded by hen egg white lysozyme and was not extensively O-acetylated. In contrast, peptidoglycans from gonococcal strains FA19 and FA102 (a penicillin-resistant mutant derived from FA19), were markedly resistant to digestion by hen egg white lysozyme and were extensively O-acetylated.

The peptidoglycan (PG) of Neisseria gonorrhoeae is of interest both as a target for β-lactam antibiotics (1, 3–5, 13, 16, 17) and as a possible modulator of the host reaction to gonococcal infections (6, 10, 12). The basic structure of gonococcal PG is similar to that of other gram-negative bacteria and contains approximately equimolar amounts of muramic acid, glucosamine, L-alanine, glutamic acid, meso-diaminopimelic acid, and D-alanine (6, 11, 17). The peptide cross-linking of separate glycan strands is of the direct type (chemotype I) involving the meso-diaminopimelic acid and D-alanine residues; the extent of the cross-linking is approximately 40% (1, 11), slightly higher than that of most other gram-negative organisms.

Recently, there have been some seemingly conflicting reports regarding the lysozyme sensitivity of gonococcal PG and the presence of O-acetyl derivatives on some of the amino sugar residues, a modification that is known to confer resistance of PG from other organisms to the degradative action of hen egg white (HEW) lysozyme (8). Although not specifically addressing the question of O-acetylation, two groups (6, 9) found that the PG of N. gonorrhoeae RD3 (a highly autolytic strain that also exhibits the highest rate of PG turnover among gonococci so far examined) was completely solubilized by HEW lysozyme. This indirectly implied that RD3 PG was devoid of (or deficient in) O-acetylated derivatives. In contrast, Blundell et al. (1, 2) found that the PG of several other strains of gonococci, including FA19, was not fully sensitive to HEW lysozyme and was extensively O-acetylated. These workers (2) suggested that prior failure to detect HEW lysozyme-resistant PG was due to the use of a harsh sodium dodecyl sulfate extraction at a slightly alkaline pH, conditions which would be likely to hydrolyze any O-acetyl groups present.

To resolve this discrepancy, we have now compared the extent of HEW lysozyme sensitivity and O-acetylation in PG from two strains, RD3 and FA19 (both nonpiliated and transparent [15]). PG from each strain was prepared in parallel with a modification of a prior purification scheme (6, 9) that reportedly does not remove O-acetyl groups from PG (2). PG from [1H]glucosamine-labeled FA19 and PG from [14C]glucosamine-labeled RD3 were isolated from trichloroacetic acid-precipitated gonococci by extraction with 4% sodium dodecyl sulfate-0.05 M sodium acetate buffer, pH 5.1 (96°C, 1 h). 3H- and 14C-labeled PGs (500 μg of each) were mixed together and treated exhaustively either with Chalaropsis B muramidase (a lysozyme which completely hydrolyzes PG independently of the presence of O-acetylated amino sugars) as before (11) or with HEW lysozyme (100 μg in 0.05 M phosphate buffer, pH 7.0, 37°C, 8 h). The degree of hydrolysis was assessed by determining the distribution of disintegrations per minute present in the supernatant and pellet after ultracentrifugation (36,000 × g, 45 min). Chalaropsis muramidase completely solubilized both of the preparations. However, whereas HEW lysozyme completely solubilized the RD3 14C-labeled PG, it solubilized only about 60% of the FA19 3H-labeled PG in the same tube.

Gel filtration of the Chalaropsis muramidase digests on connected columns of Sephadex G-50 and G-25 revealed a virtually identical pattern of soluble PG fragments for both RD3 14C-labeled PG and FA19 3H-labeled PG (Fig. 1A). The distribution of individual fragments (disaccharide peptide monomer, dimer, etc.) was similar to that reported previously for RD3 PG (11), in which it was documented that virtually no muramidase-sensitive glycosidic linkages were left intact; i.e., digestion was complete. Whereas RD3 PG yielded exclusively low-molecular-
weight fragments after HEW lysozyme treatment (Fig. 1B), a considerable proportion of the HEW lysozyme-soluble FA19 PG remained as high-molecular-weight oligomers, as was also observed with strain IL260 (1). Thus, based on the distribution of soluble and insoluble PG after ultracentrifugation, as well as on gel filtration profiles of the soluble products, FA19 PG (but not RD₃ PG) was markedly resistant to the hydrolytic action of HEW lysozyme, as also was that of the penicillin-resistant mutant FA102 (1) derived from FA19 (14).

Monomer fractions obtained from the Chalaropsis and HEW lysozyme digests were isolated and subjected to thin-layer chromatography on silica gel-coated plastic sheets in a mixture of isobutyric acid and 1 M ammonia (5:3, vol/vol) to separate O-acetylated and non-O-acetylated monomers (7). Only the monomer fraction of FA19 PG obtained by the action of Chalaropsis muramidase yielded detectable levels of the O-acetylated derivative (Fig. 2). It should be noted that by cutting the thin-layer sheets into strips and assaying for radioactivity in the scintillation counter, we could reliably detect the O-acetylated monomer only if it accounted for greater than approximately 10% of the disintegrations per minute in the non-O-acetylated monomer.
To obtain better resolution, $^{14}$C-labeled PG from each strain was digested with the Chalaropsis enzyme and the products were subjected to thin-layer chromatography, developed twice with solvent (7), and examined by autoradiography (1). The radioactive bands observed were cut out and assayed as before. The results, converted to the percentage of O-acetylation, are shown in Table 1. RD$_5$ PG did possess some O-acetylated monomers (12%), but this percentage was substantially less than the proportion of O-acetylated monomers from FA19 (56%) or from the mutants FA102 and FA140 derived from FA19 (17). In addition, the dimers from RD$_5$ were considerably less O-acetylated than the dimers obtained from the other strains.

The above data indicate that, contrary to previous supposition (2, 11), the PG of different strains of *N. gonorrhoeae* can differ markedly in sensitivity to HEW lysozyme, and this sensitivity correlates with the extent of O-acetylation. To our knowledge, this is the first demonstration of such a variation in the degree of O-acetylation within a single bacterial species. Strain RD$_5$, which has PG that is O-acetyl deficient, exhibits a particularly high rate of PG turnover (6, 9) and releases concurrently large amounts of soluble PG fragments into the medium (9). Further studies are needed to determine whether the extent of O-acetylation is correlated with the rate of turnover of gonococcal PG (or with other physiological parameters) and to determine the possible relevance of lysozyme-resistant PG in influencing the outcome of host-gonococcus interactions (1).

The work at the Indiana University School of Medicine was supported by Public Health Service grant RO1 AI 14826 from the National Institute of Allergy and Infectious Diseases, and the work at the University of Liverpool was supported by the Medical Research Council, London.

### LITERATURE CITED

