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

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Peptidoglycan from *Neisseria gonorrhoeae* RD$_5$ 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* RD$_5$ (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 RD$_5$ 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, RD$_5$ 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 [³H]glucosamine-labeled FA19 and PG from [¹⁴C]glucosamine-labeled RD$_5$ 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 RD$_5$ 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 RD$_5$ 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 RD$_5$ PG (11), in which it was documented that virtually no muramidase-sensitive glycosidic linkages were left intact; i.e., digestion was complete. Whereas RD$_5$ PG yielded exclusively low-molecular-
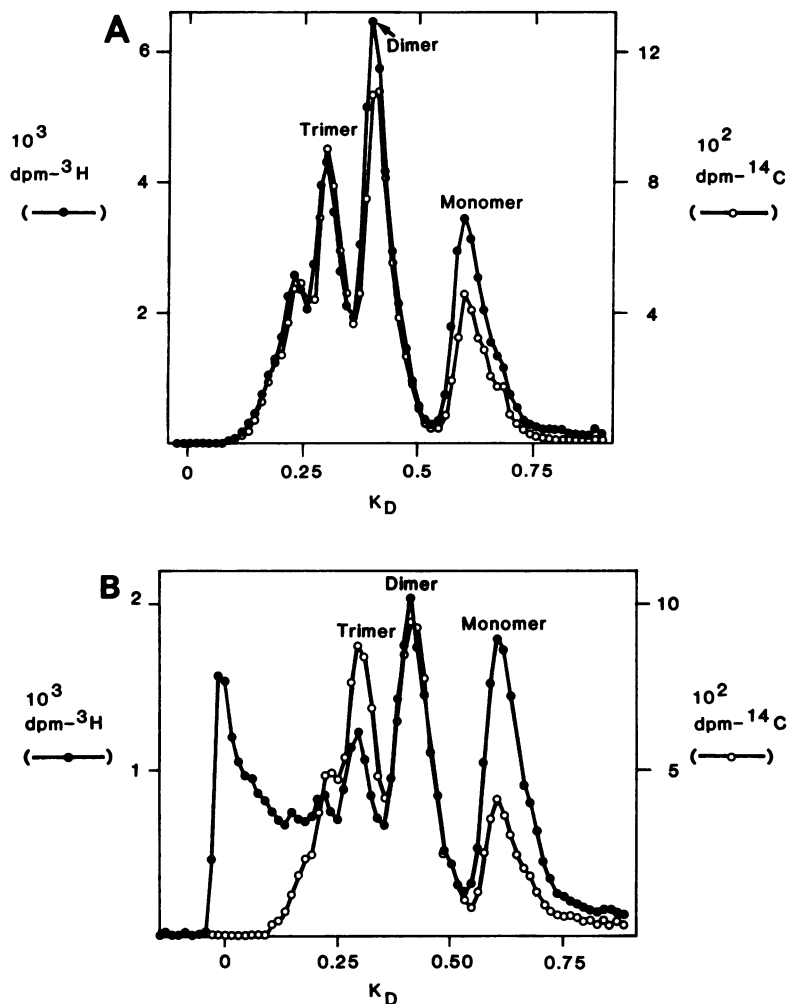
FIG. 1. Gel filtration on connected columns of Sephadex G-50 and G-25 of [³H]glucosamine-labeled PG from strain FA19 (●) and [¹⁴C]glucosamine-labeled PG from strain RD₅ (O) that had been treated extensively with either *Chalaropsis* B muramidase (A) or HEW lysozyme (B). The two PG preparations were mixed prior to treatment with an individual enzyme. After incubation, soluble PG was obtained by ultracentrifugation and applied to the columns. Peaks identified as disaccharide peptide monomer (molecular weight, about 1,000), dimer, and trimer are indicated.

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 RD₅ 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.
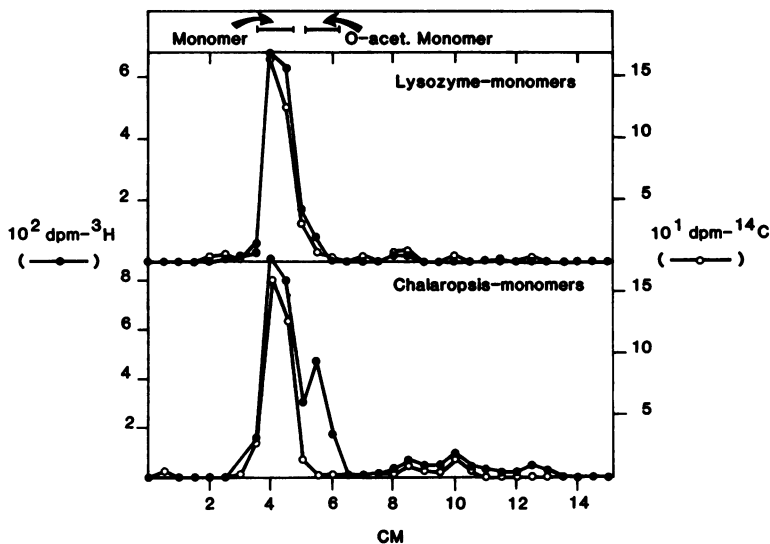
FIG. 2. Thin-layer chromatography on silica gel-coated plastic sheets in isobutyric acid–1 M ammonia (5:3, vol/vol) of disaccharide peptide monomers obtained from HEW lysozyme (top) or *Chalaropsis* muramidase (bottom) digests of FA19 ^{3}H-labeled PG (●) and RD$_5$ ^{14}C-labeled PG (○). Samples were chromatographed three times before cutting into strips and determining radioactivity. Standards of radiolabeled gonococcal PG monomer and O-acetylated monomer are indicated.

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).

TABLE 1. Degree of O-acetylation of peptidoglycan fractions in *Chalaropsis* muramidase digests

Gonococcal strain	% O-Acetylation[a]		
	Monomer	Dimer	Monomer + dimer
RD$_5$	12	22	18
FA19	56	51	53
FA102	47	43	45
FA140	57	43	48

[a] Percentage of O-acetylation represents the molar proportion of O-acetyl groups divided by the total disaccharide units present in the fraction, × 100.

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