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Note

Identification of 2-amino-2-deoxyglucose residues in the peptidoglucan of *Streptococcus pneumoniae*

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The cell wall of Gram-positive bacteria contains[1] a teichoic acid (TA) and a peptidoglycan (PG). In pneumococci, the TA is a choline-containing ribitol TA[2–4], and the PG is a lysine-containing PG in which the peptide units are primarily in the forms of dimers and trimers[2]. The TA is covalently linked to a PG unit by *N*-acetylmuramic acid 6-phosphate and involves ~20% of the muramic acid residues in the glycan moiety[5,6]. Pneumococcal autolysin (*N*-acetylmuramyl-L-alanine amidase[7,8]) interacts[9] with TA, and its activity is controlled by the lipoteichoic acid (LTA) of *S. pneumoniae*[10]. The pneumococcus cell is sensitive to sodium deoxycholate (Na-DOC) and is lysed by the action of autolysin, which is induced with Na-DOC[2,5]. Thus, DOC-induced lysis is a convenient tool to study cell-wall structure, since the lysate reflects the structure of the teichoic acid–peptidoglycan complex, except for the peptide substitutions. Some physiological studies of the pneumococcal cell surface have been carried out, but the structure of the pneumococcal TA-PG complex has not been fully elucidated. We now report the occurrence in the *S. pneumoniae* (IID 559) peptidoglycan of 2-amino-2-deoxyglucose residues that are not *N*-acetylated.

2-Amino-2-deoxyglucose residues were detected and identified by colorimetry and g.l.c. in association with the deamination procedure used to study the structure of heparin and heparan sulfate[11,12].

Pneumococcal peptidoglycan fragments (HF-GP) were prepared by degradation[4] with hydrogen fluoride of the cell-wall fragments prepared by Na-DOC-induced cell lysis. Quantification of 2-amino-2-deoxyhexose by the indole–HCl method[13] and of the total 2-acetamido- and 2-amino-2-deoxyhexose by the Morgan–Elson method[14] showed that ~43% of the 2-amino-2-deoxyhexose residues were not *N*-acetylated.

Reaction with nitrous acid converts 2-amino-2-deoxyglucosyl residues into 2,5-anhydromannose. As shown in Fig. 1, HF-GP was degraded by nitrous acid, indicating the presence of hexosamine residues. To identify the hexosamine, the

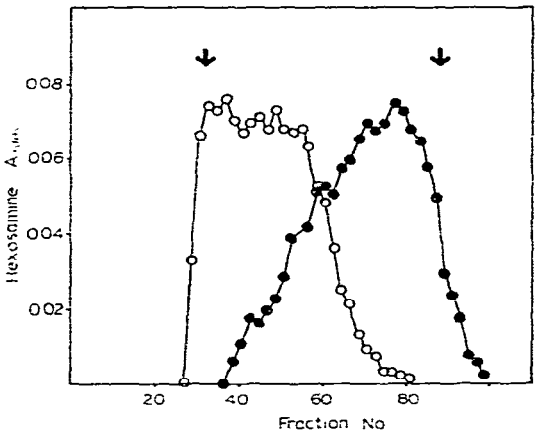

Fig. 1. Elution with water of HF-GP and its nitrous acid-degradation product from a column (1.5 × 90 cm) of Sephadex G-75. Fractions (1.6 mL) were assayed for total hexosamine after hydrolysis (4M HCl, 110°, 2 h): —○—, HF-GP; —●—, NO_2-degraded HF-GP. Arrows indicate void and bed volumes.

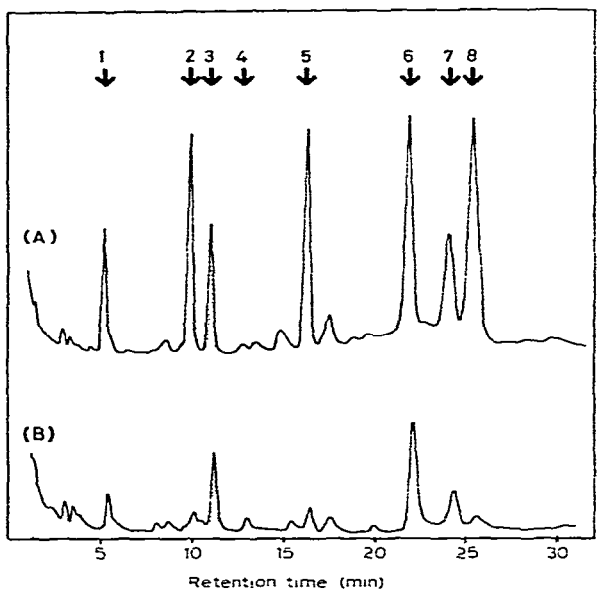

Fig. 2. G.l.c. of the alditol acetates derived from an acid hydrolysate of the nitrous acid-degradation products, using a Shimadzu instrument with a glass column (0.3 × 200 cm), a liquid phase of 3% silicone OV-225, and a temperature programme 170→250° at 4°/min from the time of injection: A, cell-wall fragments; B, HF-GP; 1, 2,5-anhydroribitol; 2, ribitol; 3, 2,5-anhydromannitol; 4, 2,5-anhydrotalitol; 5, glucitol; 6, "muramicitol", 7, 2-amino-2-deoxyglucitol; 8, 2-amino-2-deoxy-galactitol.

degraded HF-GP was hydrolysed and the products were analysed as acetates by g.l.c. (Fig. 2). In order to rule out the occurrence of *N*-deacetylation during degradation with HF, the same procedure was performed on the solubilised cell-wall fragments. 2,5-Anhydromannitol was detected after hydrolysis and reduction of the deamination products of both HF-GP and the cell-wall fragments, and the amount of 2-amino-2-deoxyglucitol formed was less than that of the "muramicitol" (Fig. 2; arrows 7 and 6, respectively).

Thus, it is concluded that a large proportion of the 2-amino-2-deoxyglucosyl residues in the peptidoglycan of *S. pneumoniae* are not *N*-acetylated. Other strains of *S. pneumoniae* (R36a, ATCC 27336) contain such 2-amino-2-deoxyglucosyl residues (unpublished results) in their cell walls, which suggests that they participate in the resistance of the bacterium to lysozyme digestion[15].

EXPERIMENTAL

Preparation of solubilised cell-wall fragments. — *Streptococcus pneumoniae* IID 559 cultured in CD medium[16] (24 L) and grown at the late exponential phase was lysed[5] by adding Na-DOC to 0.1%. The resulting solution was dialysed against tap water and then distilled water. The non-dialysable fraction was concentrated to ~0.5 L and adjusted to pH 4.0, in order to remove the DOC, proteins, and nucleic acids[5]. The resulting supernatant solution (500 mL) was defatted with chloroform–methanol (1:1, 1.1 L)[17,18]. Almost all of the hexosamine-containing macromolecules (teichoic acid and peptidoglycan) were present in the upper phase of the mixture, which was concentrated and lyophilised.

Preparation of HF-GP. — The teichoic acid-free, peptidoglycan fragment (HF-GP) was prepared by treatment[4] of the cell-wall fragments (200 mg) with 46% aqueous hydrogen fluoride (2 mL) for 72 h at 4°. HF was removed *in vacuo* over NaOH. The resulting material was dissolved in a small volume of water and eluted with water from columns (75 × 2 cm) of Sephadex G-25 and G-50 in series. The fraction (35 mg) eluted in the void volume, which contained muramic acid and glucosamine as major components of the hydrolysate, was isolated by lyophilisation. The second fraction, which contained teichoic acid constituents as the major components, was not examined further.

Treatment of HF-GP with nitrous acid[19]. — To a solution of HF-GP (5 mg) in water (0.5 mL) were added $NaNO_2$ (25 mg) and M acetate buffer (pH 3.6, 0.25 mL). After 20 min at room temperature, the mixture was neutralised with saturated, aqueous $NaHCO_3$ and then reduced with $NaBH_4$. After decomposition of excess of $NaBH_4$ with acetic acid, the neutralised material was applied to a column of Sephadex G-75.

Identification of 2,5-anhydromannose. — HF-GP (1 mg) was treated with nitrous acid and reduced with $NaBH_4$ as described above, and the mixture was desalted by passage through a column of Sephadex G-15. The resulting material was hydrolysed with 4M HCl for 30 min at 110°, the hydrolysate was concentrated, and the residue was reduced with $NaBH_4$. After decomposition of excess of $NaBH_4$ with

acetic acid and removal of the boric acid by evaporation with methanol, the resulting material was treated with acetic anhydride–sodium acetate for 2 h at 140°. The alditol acetate derivatives were subjected to g.l.c. Since deamination at pH 3.6 and 1.6 gave the same ratios of 2,5-anhydromannitol and 2-amino-2-deoxyglucitol, deamination of HF-GP was complete.

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