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Structure of a new ribitol teichoic acid-like O-polysaccharide of a serologically separate *Proteus vulgaris* strain, TG 276-1, classified into a new *Proteus* serogroup O53

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Abstract—An unusual ribitol teichoic acid-like O-polysaccharide was isolated by mild acid degradation of the lipopolysaccharide from a previously non-classified *Proteus vulgaris* strain TG 276-1. Structural studies using chemical analyses and 2D ^{1}H and ^{13}C NMR spectroscopy showed that the polysaccharide is a zwitterionic polymer with a repeating unit containing 2-acetamido-4-amino-2,4,6-trideoxy-D-galactose (D-FucNAc4N) and two D-ribitol phosphate (D-Rib-ol-5-*P*) residues and having the following structure:

→3)-β-D-Fuc*p*NAc4N-(1→1)-D-Rib-ol-5-*P*-(O→1)-D-Rib-ol-5-*P*-(O→,

where the non-glycosylated ribitol residue is randomly mono-O-acetylated. Based on the unique O-polysaccharide structure and the finding that the strain studied is serologically separate among *Proteus* bacteria, we propose to classify *P. vulgaris* strain TG 276-1 into a new *Proteus* serogroup, O53.

Keywords: *Proteus vulgaris*; Ribitol phosphate; Serological classification; Deamination; NMR spectroscopy

1. Introduction

Gram-negative bacteria of the genus *Proteus* from the family *Enterobacteriaceae* are opportunistic pathogens that cause urinary tract infections, the most frequently diagnosed kidney and urological disorders. These bacteria are seen predominantly in hospitalized patients, who may have received antibiotics or instrumentation, including catheterization, of the urinary tract.[1] In the genus *Proteus,* there are four clinically important named species: *P. mirabilis*, *P. vulgaris*, *P. penneri* and *P. hauseri* as well as three unnamed genomospecies 4, 5 and 6.[2,3] Virulence factors and properties of *Proteus* sp. that mediate the infectious process are swarming phenomenon, adherence due to the fimbriae or glycocalyx, flagella, hemolysins, invasiveness, enzymes, including proteases and ureases, capsular polysaccharide, and outer membrane lipopolysaccharide (LPS, endotoxin).[4]

As in other Gram-negative bacteria, the O-specificity of *Proteus* is defined by the structure of the carbohydrate moiety (O-polysaccharide, O-antigen) of the LPS. Based on the O-antigens, strains of two species, *P. mirabilis* and *P. vulgaris*, have been classified into up to 60 O-serogroups.[5–7] Recently, we have established

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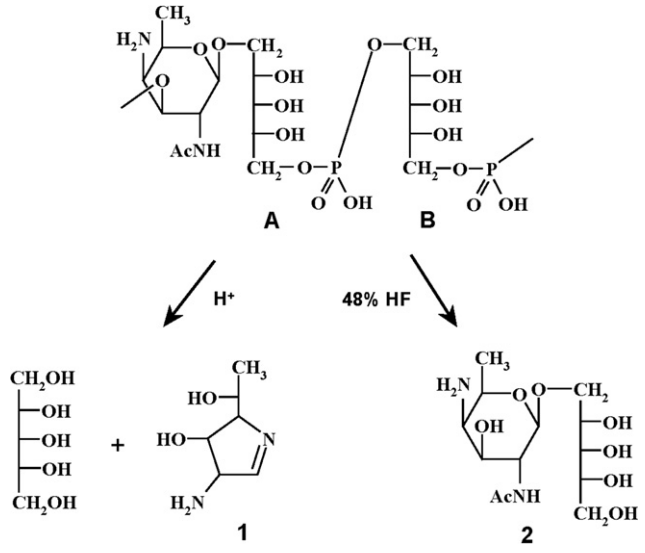
the O-polysaccharide structures of most classified and a number of non-classified strains of these and other *Proteus* species and, based on structural and serological data, proposed some 15 more O-serogroups.[8–13] Now we report on a new O-polysaccharide structure of a previously non-classified serologically separate *P. vulgaris* strain, which is a candidate for a further new *Proteus* O-serogroup.

2. Results and discussion

2.1. Elucidation of the O-polysaccharide structure

The O-polysaccharide was obtained by mild acid hydrolysis of the LPS, isolated from dried bacterial cells of *P. vulgaris* TG 276-1 by the phenol–water procedure,[14] followed by GPC on Sephadex G-50. Full acid hydrolysis of the polysaccharide resulted in ribitol, which was identified by GLC after acetylation. Further studies showed that the polysaccharide also contains 2-acetamido-4-amino-2,4,6-trideoxygalactose (FucNAc4N), but no FucN4N could be detected in the hydrolysate owing to full destruction of this acid-labile monosaccharide.[15] Instead, an amino compound with a high retention time on a cation-exchange resin was detected in amino acid analysis, which was isolated by cation-exchange chromatography. Its MALDI-TOF mass spectrum showed a pseudomolecular $[M+H]^+$ ion peak at m/z 145.15, which may belong to a FucN4N degradation product **1** (calculated molecular mass 144.09 Da), shown in Scheme 1. In addition, there were mass peaks in the region m/z 317–393 for putative products of polymerization of **1** or/and other primary degradation product(s).



Scheme 1. Structure and degradations of the O-polysaccharide of *P. vulgaris* TG 276-1 (O53) and its degradation products (**1**, **2**).

The ^{13}C NMR spectrum of the O-polysaccharide (Fig. 1A) indicated an irregularity, most likely, owing to non-stoichiometric O-acetylation as there were signals for *O*-acetyl groups at δ 21.7–21.8 (CH_3) and 174.5 (CO). Indeed, the spectrum of the O-deacetylated polysaccharide (Fig. 1B) showed a pattern typical of a regular polymer. It contains signals for one mono-N-acetylated 6-deoxydiaminohexose (FucN4N), including those for an anomeric carbon (C-1) at δ 102.4, CH_3–C group (C-6) at δ 16.8, two nitrogen-bearing carbons at δ 52.4 and 55.2 and an *N*-acetyl group at δ 23.6 (CH_3) and 176.2 (CO). In addition, there were 10 more signals, from which four signals at δ 67.7–68.0 and 71.9 belonged to OCH_2–C groups (data of a DEPT-135 experiment). Taking into account the GLC analysis data (see above), these signals could be assigned to C-1 and C-5 of two ribitol residues. The ^1H NMR spectrum showed, inter alia, signals for an anomeric proton (H-1) at δ 4.64 and CH_3–C group (H-6) at δ 1.36 of FucN4N as well as one *N*-acetyl group at δ 2.08. The ^{31}P NMR spectrum contained two signals for monophosphate groups at δ −0.29 and 1.59.

The ^1H and ^{13}C NMR spectra of the O-deacetylated polysaccharide were assigned (Table 1) using 2D ^1H, ^1H COSY, TOCSY and ^1H, ^{13}C HSQC spectroscopy. The monosaccharide was identified as 2,4-diamino-2,4,6-trideoxy-β-galactose (β-FucN4N) based on ^1H, ^1H coupling constants characteristic of the β-*galacto* configuration ($J_{1,2}$ 8.5, $J_{2,3}$ 10.5, $J_{3,4}$ 3.2, $J_{4,5}$ <2, $J_{5,6}$ 6.6 Hz) and correlations in the HSQC spectrum of the protons at the nitrogen-bearing carbons (H-2 and H-4) to the corresponding carbons (C-2 and C-4) at δ 52.4 and 55.2. The NMR data also confirmed the presence of two pentitol (ribitol) residues. Asymmetric chemical shift patterns of both residues excluded the occurrence of 1,5-poly(ribitol phosphate) as a separate chain or a block(s), which would have a symmetric pattern (compare published data[16] shown in Table 1).

The 2D ROESY spectrum of the O-deacetylated polysaccharide showed a correlation between H-1 of FucN4N and H-1a,1b of one of the ribitol residues (unit **A**) at δ 4.64/3.85 and 4.64/3.96. This finding indicated glycosylation of unit **A** at position 1, which was confirmed by a downfield displacement, due to an α-effect of glycosylation, of the C-1 signal from δ 62.8 in non-substituted ribitol[16] to δ 71.9 in unit **A**.

The C-5 signal of unit **A** as well as C-1 and C-5 signals of the other ribitol residue (unit **B**) were also shifted downfield but to a lesser degree (to δ 67.7–68.0) due to phosphorylation (Table 1). A similar shift from δ 68.9 (Table 1) to 73.3 was observed also for the C-3 signal of FucN4N, thus suggesting the presence of a phosphate group at position 3 of the sugar moiety. Accordingly, the H-3 signal of FucN4N at δ 4.50 stood apart from other signals in the low-field region of the ^1H NMR spectrum and was additionally split by coupling to phos-