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Structure of the O-polysaccharide and serological studies of the lipopolysaccharide of *Proteus penneri* 60 classified into a new *Proteus* serogroup O70

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Abstract

An alkali-treated lipopolysaccharide of *Proteus penneri* strain 60 was studied by chemical analyses and ^{1}H, ^{13}C and ^{31}P NMR spectroscopy, and the following structure of the linear pentasaccharide–phosphate repeating unit of the O-polysaccharide was established:

6)-α-D-Gal*p*-(1 → 3)-α-L-Fuc*p*NAc-(1 → 3)-α-D-Glc*p*NAc-(1 → 3)-β-D-Qui*p*4NAc-(1 → 6)-α-D-Glcp-1-*P*-(O →

Rabbit polyclonal O-antiserum against *P. penneri* 60 reacted with both core and O-polysaccharide moieties of the homologous LPS. Based on the unique O-polysaccharide structure and serological data, we propose to classify *P. penneri* 60 into a new, separate *Proteus* serogroup O70. A weak cross-reactivity of *P. penneri* 60 O-antiserum with the lipopolysaccharide of *Proteus vulgaris* O8, O15 and O19 was observed and discussed in view of the chemical structures of the O-polysaccharides.

Keywords: Proteus penneri; Lipopolysaccharide; O-antigen; Bacterial polysaccharide structure; O-serogroup; 4-Acetamido-4,6-dideoxy-D-glucose

1. Introduction

Gram-negative opportunistic rod-shaped bacteria from genus *Proteus* are a common cause of urinary tract infections in individuals with structural abnormal urinary tracts or long-term urinary catheters [1]. They cause a significant bacteriuria mainly in bladder but also have propensity for kidney. *Proteus* infections are perhaps most noted for their association with a formation of debilitating kidney and bladder stones [2]. In the genus *Proteus*, there are four clinically important species: *Proteus mirabilis*, *Proteus vulgaris*, *Proteus penneri* and *Proteus hauseri* [3]. Many virulence factors of these organisms have been identified, including several types of fimbriae, hemolysins, flagella, invasiveness, swarming growth, enzymes, including proteases and ureases, capsular polysaccharide and lipopolysaccharide (LPS, endotoxin) [4,5].

The serological specificity of *Proteus* strains is defined by the chemical structure of the O-polysaccharide chain of the LPS (O-antigen). Based on the O-antigens, strains of two species, *P. mirabilis* and *P. vulgaris*, have been classified first into 49 O-serogroups

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[6] and later into 11 additional serogroups [7]. About 15 further O-serogroups have been proposed for the third medically important species, *P. penneri* [8,9]. O-polysaccharides of the most *P. penneri* strains studied so far are acidic due to the presence of uronic acids and various acidic non-sugar components, such as amino acids, lactic and pyruvic acids and phosphate groups [8–12]. Now we report on the structure of another acidic phosphorylated O-polysaccharide isolated from *P. penneri* strain 60. Based on the unique chemical structure of the O-polysaccharide and only a weak serological cross-reactivity of O-antiserum against *P. penneri* 60 with *Proteus* LPS, strain *P. penneri* 60 was classified into a new, separate *Proteus* serogroup O70.

2. Materials and methods

2.1. Bacterial strains and growth

Proteus penneri 60 was isolated from urine of a woman with bacteriuria in a Hospital in Łódz (Poland). *P. vulgaris* O8 (17/57), O15 (30/57) and O19 (37/57) were from the Czech National Collection of Type Cultures (CNCTC, Institute of Epidemiology and Microbiology, Prague, the Czech Republic).

The bacteria were grown on nutrient broth (The Warsaw Laboratory of Sera and Vaccines, Poland) supplemented with 1% glucose. Dry bacterial mass was obtained from aerated culture as described previously [13].

2.2. Isolation and degradation of lipopolysaccharide

LPS was isolated from dried bacterial cells of *P. penneri* 60 by extraction with hot aqueous phenol [14] and purified by treatment with cold (4 °C) aqueous 50% CCl_3CO_2H followed by dialysis of the supernatant. The yield of the LPS was 4.6% of dried bacterial mass (w/w).

Mild acid hydrolysis of the LPS (100 mg) was performed with aqueous 2% HOAc at 100 °C until precipitation of lipid A. The precipitate was removed by centrifugation (13,000*g*, 20 min), and the supernatant fractionated by gel permeation chromatography (GPC) on a column (56 × 2.6 cm) of resin Sephadex G-50 (S) (Pharmacia, Sweden) in 0.05 M pyridinium acetate buffer (pH 4.5), with monitoring using a Knauer differential refractometer (Germany). The yield of the resultant oligosaccharide was 17% of the LPS weight.

Mild alkaline O-deacylation of the LPS (75 mg) was performed with aqueous 12.5% ammonia (37 °C, 16 h). The precipitate was removed by centrifugation (13,000*g*, 20 min), and the supernatant was fractionated by GPC on a column (80 × 2.5 cm) of TSK HW-40 in aqueous 1% HOAc. The yield of the alkali-treated LPS (LPS-OH) was 40% of the LPS (w/w).

2.3. Rabbit antiserum and serological assays

Polyclonal O-antiserum was obtained by immunisation of rabbits with heat-inactivated bacteria of *P. penneri* 60 according to the published procedure [15]. SDS–PAGE using 12% acrylamide, immunobloting, absorption, enzyme immunosorbent assay (EIA) using LPS and passive immunohemolysis (PIH) using alkali-treated LPS as antigen, as well as inhibition experiments were performed as described in detail previously [16].

2.4. Sugar analysis

The LPS-OH was dephosphorylated with aqueous 48% HF (7 °C, 16 h) followed by hydrolysis with 2 M CF_3CO_2H (120 °C, 2 h), monosaccharides were reduced with 0.25 M $NaBH_4$ in 1 M aqueous ammonia (25 °C, 1 h), acetylated with a 1:1 (v/v) mixture of pyridine and acetic anhydride (120 °C, 30 min) and analysed by GLC. The absolute configurations of the monosaccharides were determined by GLC of the acetylated (+)-2-butyl glycosides [17,18]. As the reference for FucNAc the polysaccharide of *P. vulgaris* O8 containing L-FucNAc was used. GLC was performed using a Hewlett–Packard 5890 Series II instrument equipped with an HP-1 fused silica column (0.20 mm × 25 m) and a temperature program of 170–180 °C at 1 °C min^{-1} followed by a program of 180–230 °C at 7 °C min^{-1}.

2.5. Methylation analysis

Methylation of the LPS-OH (2 mg) was performed with CH_3I in dimethylsulfoxide in the presence of sodium methylsulfinylmethanide [19]. Partially methylated monosaccharides were derived by hydrolysis under the same conditions as in sugar analysis, converted into the alditol acetates and analysed by GLC–MS on a TermoQuest Finnigan mass spectrometer model Trace GC 2000 equipped with an EC-1 column (0.32 mm × 30 m), using a temperature gradient of 150 (2 min) to 250 °C at 10 °C min^{-1}.

2.6. NMR spectroscopy

^1H, ^{13}C, and ^{31}P NMR spectra were recorded with a Bruker DRX-500 spectrometer in D_2O at 20 °C using internal acetone (δ_H 2.225, δ_C 31.45) or external 85% aqueous H_3PO_4 (δ_P0) as references. One- and two-dimensional experiments were performed using standard pulse sequences and data obtained were processed using Bruker software XWINNMR 2.6.