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Immunochemical Studies on the O-Antigens of *Proteus mirabilis* O23 and *Proteus vulgaris* O23

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Abstract. Analysis by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy demonstrated that the O-specific polysaccharides of *Proteus mirabili*s PrK 42/57 and *P. vulgari*s PrK 43/57 are structurally similar to that of *P. vulgari*s PrK 44/57 and different from the polysaccharide of *P. mirabili*s PrK 41/57 studied earlier. The lipopolysaccharides of these strains were tested using enzyme immunosorbent assay, passive hemolysis and Western blot with O-antisera against *P. mirabili*s 42/57 and *P. vulgari*s 43/57 and 44/57, as well as with cross-absorbed O-antisera. The chemical and serological data revealed the basis for combining the four strains into *Proteus* serogroup O23 and division of this serogroup to three subgroups, one for *P. vulgari*s 43/57 and 44/57 and two others for *P. mirabili*s 41/57 and 42/57.

Key words: *Proteus mirabilis*; lipopolysaccharide; O-specific polysaccharide; O-antigen; structure; serological reactivity.

Introduction

Bacteria of the genus *Proteus* are opportunistic pathogens which, under favorable conditions, cause urinary tract infections leading to severe complications, mainly formation of bladder and kidney stones[8]. The *Proteus* rods frequently cause infections in patients with a urinary catheter in place or with structural or functional abnormalities in the urinary tract. In the genus *Proteus* there are four clinically important species: *P. mirabilis*, *P. vulgaris*, *P. penneri* and *P. hauseri*[5]. A number of virulence factors of *Proteus* that mediate the infectious process have been described, including fimbriae, swarming phenomenon, urease, proteases, and lipopolysaccharide (LPS, endotoxin)[7, 8].

The serological specificity of *Proteus* strains is defined by the chemical structure of the O-specific polysaccharide chain (OPS, O-antigen) of the LPS. Based on the O-antigens, two species, *P. mirabilis* and *P. vulgaris*, have been classified into 60 serogroups, a few of which are divided into subgroups[4]. In particular, serogroup O23 is divided into four subgroups, represented by two *P. mirabilis* strains, PrK 41/57 (O23a: 1a, 1c, 1e) and PrK 42/57 (O23a, 23c, 23d: 2a, 2d, 2f), and two *P. vulgari*s strains, PrK 43/57 (O23a, 23c: 12a) and PrK 44/57 (O23a, 23c: 2a, 2d, 2f).

Abbreviations used: EIA – enzyme immunosorbent assay, GalA – galacturonic acid, LPS –lipopolysaccharide, LPSOH – alkali-treated lipopolysaccharide, NMR – nuclear magnetic resonance, OPS – O-specific polysaccharide, PHT – passive hemolysis test.

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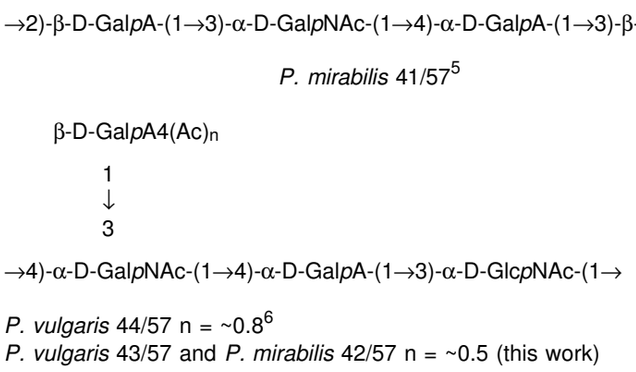

Fig 1. Structures of the OPS of *Proteus* O23 strains. The position of minor O-acetyl groups present in the OPS of *P. mirabilis* 41/57 has not been determined

The chemical structures of the OPS of *P. mirabilis* 41/57 and *P. vulgaris* 44/57 have been established previously[3, 6] and are shown in Fig. 1. Both OPS have tetrasaccharide repeating units with the same sugar composition but different structures, the OPS of *P. vulgaris* O23 being branched and that of *P. mirabilis* O23 linear. They also differ in the configuration of the glycosidic linkage of the GlcNAc residue and in the O-acetylation patterns. Taking into account these distinctions, we assumed that the antigenic determinants at the non-reducing ends of both OPS are identical and serve as the basis for classification of these strains into the same *Proteus* serogroup[6].

In this paper, we report structural analysis of the OPS of two other strains of *Proteus* O23, *P. mirabilis* 42/57 and *P. vulgaris* 43/57, as well as serological studies of the O-antigens of all serogroup O23 strains.

Materials and Methods

Bacterial strains, cultivation and isolation of LPS. *P. mirabilis* O23 and *P. vulgaris* O23 came from the Czech National Collection of Type Culture (Institute of Epidemiology and Microbiology, Prague). Bacteria were cultivated under aerobic conditions in nutrient broth (BTL, Łódź, Poland). The LPS were isolated from dried bacterial mass by hot phenol/water extraction[10] and purified by treatment with a cold 50% solution of trichloroacetic acid followed by separation of a precipitate by centrifugation and dialysis of the supernatant.

Preparation of alkali-treated LPS and OPS. Alkali-treated LPS (LPSOH) were prepared by treatment of the LPS with 0.25 M NaOMe in absolute MeOH at 37°C for 15 h[11], the product then dissolved in water and lyophilized. The OPS were prepared by mild acid degradation of the LPS with 2% HOAc at 100°C until precipitation of a lipid, followed by gel-permeation chromatography on a column (70 × 2.6 cm) of Sephadex G-50 using pyridinium acetate buffer pH 4.5 (4 ml pyridine and 10 ml acetic acid in 1 l water) and monitoring of elution with a Knauer differential refractometer.

NMR spectroscopy. The NMR spectra were recorded on a Bruker DRX-500 spectrometer for samples in 99.96% D_2O at 60°C, using standard Bruker software and the XWINNMR 2.1 program to acquire and process the data. Chemical shifts were determined with acetone as internal standard (δ_C 2.225, δ_C 31.45).

Serological techniques. O-antisera against *Proteus* strains were produced by immunization of New Zealand white rabbits with heat-killed bacteria (100°C, 2.5 h) as described previously[1]. Enzyme immunosorbent assay (EIA) and passive hemolysis test (PHT) were performed as reported earlier[1, 9]. The LPS and LPSOH were used as antigens in EIA and PHT, respectively. Absorption of antisera (1 ml, diluted 1:50 with phosphate-buffered saline) was carried out at 4°C for 1 h with packed sheep red blood cells (100 µl) coated with the respective alkali-treated LPS (200 µg LPS in 0.2 ml sheep red blood cells). The level of antibodies after absorption was determined using PHT. Sodium deoxycholate polyacrylamide gel electrophoresis of the LPS of *Proteus* O23 and Western blot with anti-O23 sera were performed as described previously[2].

Results and Discussion

The OPS of *P. mirabilis* 42/57 and *P. vulgaris* 43/57 were obtained by mild acid degradations of the corresponding LPS followed by gel-permeation chromatography on Sephadex G-50. Studies by ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectroscopy showed that the isolated OPS have practically the same structure, which is also similar to the structure of the OPS of *P. vulgaris* 44/57 shown in Fig. 1. The three OPS have the same carbohydrate backbone and differ only in the degree of O-acetylation of the lateral residue of β-galacturonic acid (β-GalA), which is ~80% in *P. vulgaris* 44/57[6] and ~50% in the two other strains studied (Fig. 1). The degree of O-acetylation of the OPS was determined by the ratio of the integral intensities of the signals in the ^{1}H and ^{13}C NMR spectra, particularly those for H2 of the O-acetylated and non-acetylated β-GalA residues in the ^{1}H NMR spectra, which lay apart from the other ^{1}H NMR signals at 3.35 and 3.46 ppm, respectively (Fig. 2).

The LPS of *Proteus* O23 strains were tested with O-antisera against *P. mirabilis* 42/57, *P. vulgaris* 43/57 and *P. vulgaris* 44/57 in Western blot, EIA and PHT.

In Western blot, all O-antisera recognized slowly migrating bands of both homologous and heterologous LPS, corresponding to high-molecular-mass LPS species with a large number of O-antigen repeating units (Fig. 3). In addition, anti-*P. vulgaris* 43/57 serum recognized fast migrating bands of the LPS of *P. mirabilis* 41/57, *P. vulgaris* 43/57 and *P. vulgaris* 44/57, which correspond to LPS species devoid of the O-antigen (Fig. 3B). Therefore, these three strains share an epitope(s) on the LPS core, whereas the LPS of *P. mirabilis* 42/57 has a different core-linked epitope.

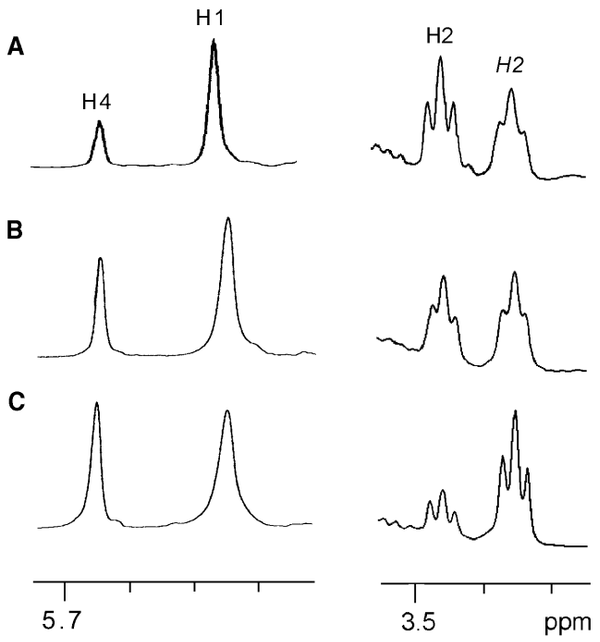

Fig. 2. Parts of the ^{1}H NMR spectra of the OPS of *P. mirabilis* 42/57 (**A**), *P. vulgaris* 43/57 (**B**) and 44/57 (**C**). Shown are signals for H2 of the O-acetylated (*H2*) and non-acetylated (H2) β-GalA residue, H4 of the non-acetylated β-GalA (H4) and H1 of α-GalA residue (H1)

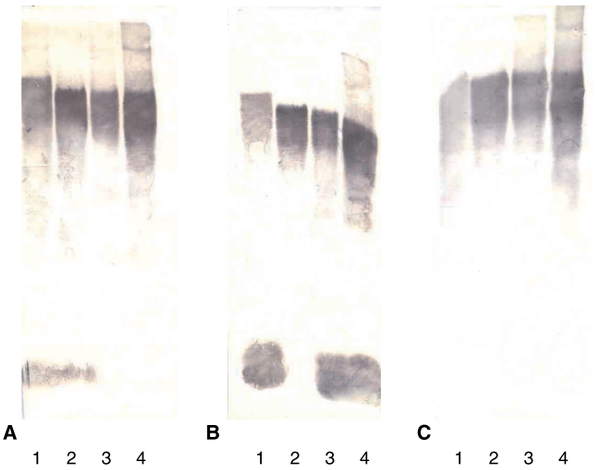

Fig. 3. Western blot of the LPS from *P. mirabilis* 41/57 (1), *P. mirabilis* 42/57 (2), *P. vulgaris* 43/57 (3) and *P. vulgaris* 44/57 (4) with anti-*P. mirabilis* 42/57 (**A**), anti-*P. vulgaris* 43/57 (**B**) and anti-*P. vulgaris* 44/57 (**C**) sera

In EIA and PHT, anti-*P. vulgaris* 43/57 and anti-*P. vulgaris* 44/57 sera reacted to the same titer with the LPS from both *P. vulgaris* 43/57 and 44/57 (Table 1). They also showed similar EIA binding curves (Fig. 4) and, hence, a difference in the degree of O-acetylation in the O-antigens of these two strains (see Fig. 1) has no importance for the serological reactivity.

Anti-*P. vulgaris* 43/57 and anti-*P. vulgaris* 44/57 sera also showed a cross-reactivity in EIA with the LPS of *P. mirabilis* 42/57, but it was weaker than the reactivity with the homologous LPS. Anti-*P. mirabilis* 42/57 serum also reacted more weakly with the LPS of *P. vulgaris* 43/57 and 44/57 than with the homologous LPS (Table 1). In PHT, differences in the cross-reactivity in both ways were insignificant (Table 1), which could be accounted for by the different presentations of the antigens in PHT compared with EIA. The observed cross-reactivity is consistent with the close structural similarity of the O-antigens of the three strains (Fig. 1). The difference in the reactivity revealed by EIA may

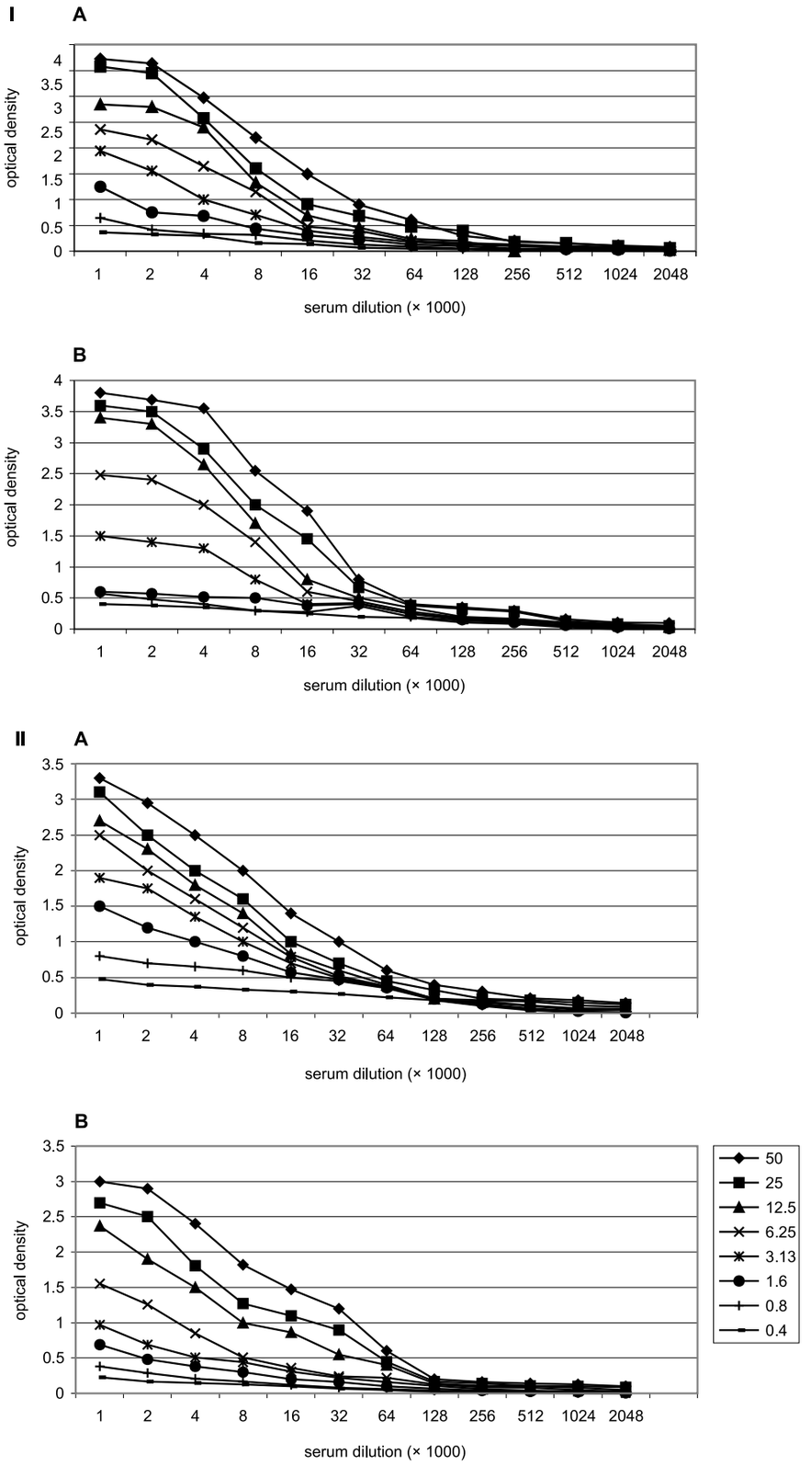

Fig. 4. EIA binding curves of polyclonal anti-*P. vulgaris* 43/57 (I) and anti-*P. vulgaris* 44/57 (II) sera to the LPS of *P. vulgaris* 43/57 (**A**) and *P. vulgaris* 44/57 (**B**)

Table 1. Reactivity of anti-*Proteus* O23 sera with *Proteus* O23 LPS (reciprocal titre)

Antigen from	O-antiserum against		
	P. mirabilis 42/57	*P. vulgaris* 43/57	*P. vulgaris* 44/57
PHT			
P. mirabilis 42/57	*12 800*	6 400	6 400
P. vulgaris 43/57	6 400	*6 400*	6 400
P. vulgaris 44/57	6 400	12 800	*12 800*
EIA			
P. mirabilis 42/57	*512 000*	32 000	32 000
P. vulgaris 43/57	64 000	*128 000*	128 000
P. vulgaris 44/57	64 000	128 000	*128 000*
P. mirabilis 41/57	64 000	8 000	8 000

LPSOH and LPS were used as antigen in PHT and EIA, respectively. Data of the reactions with the homologous antigen are italicised.

Table 2. Reactivity with LPSOH from homologous strain in PHT of anti-*Proteus* O23 sera absorbed with LPSOH from *Proteus* O23 LPS (reciprocal titre)

O-antiserum against	O-antiserum against		
	P. mirabilis 42/57	*P. vulgaris* 43/57	*P. vulgaris* 44/57
Control	10 240	5 120	10 240
P. mirabilis 42/57	*10*	5 120	5 120
P. vulgaris 43/57	5 120	*40*	160
P. vulgaris 44/57	5 120	10	*10*

Data of the reactions of O-antisera absorbed with the homologous antigen are italicised.
Sheep red blood cells were used as a control.

be substantiated by the occurrence of different LPS core epitopes in *P. vulgaris* 43/57 and 44/57 on one hand and *P. mirabilis* 42/57 on the other, as demonstrated by Western blot (Fig. 3B).

The reactivity of the LPS of *P. mirabilis* 41/57 in EIA with the O-antisera studied, especially with those against *P. vulgaris* 43/57 and 44/57, was significantly weaker compared with the homologous reactions. This finding is to be expected, taking into account the different structure of the O-antigen of *P. mirabilis* 41/57 (Fig. 1).

Studies with absorbed O-antisera essentially confirmed the EIA and PHT data (Table 2). The LPS of either *P. vulgaris* 43/57 or *P. vulgaris* 44/57 removed most antibodies from anti-*P. vulgaris* 43/57 and anti-*P. vulgaris* 44/57 sera. Absorption of anti-*P. mirabilis* 42/57 serum with the LPS of *P. vulgaris* 43/57 or 44/57 and, vice versa, absorption of anti-*P. vulgaris* 43/57 and anti-*P. vulgaris* 44/57 sera with the LPS of *P. mirabilis* 42/57 reduced the titer only insignificantly.

No reactivity was observed when anti-*P. vulgaris* O23 sera were tested in EIA with the LPS of a number of *P. mirabilis*, *P. vulgaris* and *P. penneri* strains containing in the OPS moiety various sugars in common with the LPS of *Proteus* O23. This finding and the cross-reactivity described above confirmed the expediency of combining *P. vulgaris* 43/57, 44/57, *P. mirabilis* 41/57 and 42/57, and only these strains, in one *Proteus* serogroup, O23. The epitope common to all serogroup O23 strains (epitope O23a), which is responsible for the cross-reactivity, has been suggested earlier to reside on the terminal non-reducing end of the O-specific polysaccharides[6]. Another epitope, which is shared by *P. mirabilis* 42/57, *P. vulgaris* 43/57 and 44/57, is evidently associated with the tetrasaccharide repeating units, having essentially the same structure in the three strains. This epitope seems to correspond to epitope O23c in the Kauffmann-Perch classification scheme (see Introduction). Finally, epitope 23d, characteristic for the LPS of *P. mirabilis* 42/57 only, may be located on the LPS core, which was demonstrated by Western blot to be serologically different in this strain from the core epitopes in the three other *Proteus* O23 strains.

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