Structural and serological studies of the O-specific polysaccharide of the bacterium *Proteus mirabilis* O10 containing L-altruronic acid, a new component of O-antigens

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Abstract **An acidic O-specific polysaccharide from the lipopolysaccharide of *Proteus mirabilis* O10 contains 2-acetamido-2-deoxy-D-glucose, 2-acetamido-2-deoxy-D-galactose, D-galacturonic acid, and L-altruronic acid, the last-named sugar having not been found hitherto in O-antigens. Structure of a branched tetrasaccharide repeating unit of the polysaccharide was established by ^{1}H and ^{13}C NMR spectroscopy, including two-dimensional COSY and rotating-frame NOE spectroscopy. The lateral L-altruronic acid residue plays the immunodominant role in manifestation of the O10 specificity of *Proteus*, whereas a disaccharide fragment of the main chain in common with the O-specific polysaccharide of *P. mirabilis* O43 provides the one-way serological cross-reactivity between anti-O10 serum and O43-antigen.**

Key words: Lipopolysaccharide serological specificity; Bacterial polysaccharide structure; D-Galacturonic acid; L-Altruronic acid; *Proteus mirabilis*

1. Introduction

Proteus mirabilis is a human opportunistic pathogen causing urinary tract infections, mainly in children and old patients [1]. The outer membrane lipopolysaccharide (LPS) is the major heat-stable T-independent surface antigen [2]. According to the Kauffmann-Perch serological classification based on the specificity of the O-polysaccharide chain of LPS (O-antigen), strains of *P. mirabilis* and *P. vulgaris* are divided into 49 O-serogroups [3,4], and 11 additional O-serogroups have been proposed [5].

Structural and serological studies showed that most LPSs have acidic O-specific polysaccharide chains containing D-glucuronic or D-galacturonic acids or their amides with L-amino acids, as well as sugar ethers with lactic acid (glycolactilic acids), acetals with pyruvic acid, and phosphodiester groups [6–10].

We now report the structure of the O-specific polysaccharide of *P. mirabilis* serogroup O10 which, in addition to D-galacturonic acid, contains L-altruronic acid, and the role of this new component of O-antigens in manifestation of the *Proteus* O10 specificity.

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2. Materials and methods

2.1. Bacteria, growth, isolation of lipopolysaccharides and O-specific polysaccharides

Strains of *P. mirabilis* O10 (PrK 19/57 and PrK 20/57) were from the Czech National Collection of Type Cultures (Institute of Epidemiology and Microbiology, Prague). The bacterium was grown on a nutrient broth (Warsaw Laboratory of Sera and Vaccines) supplemented with 1% glucose; at the end of the exponential phase bacterial cells were separated by centrifugation, washed with distilled water, and lyophilized.

LPSs were isolated by extraction of cells with hot aqueous phenol [11] and purified by treatment with ribonuclease and deoxyribonuclease and by ultracentrifugation [12] as described. LPSs were degraded with 1% HOAc at 100°C, and O-specific polysaccharides isolated by gel chromatography on Sephadex G-50 as described earlier [9]. Alkali-treated LPSs were prepared by saponification of LPSs with 0.25 M NaOH as described [10].

2.2. Chemical methods

Hydrolysis of the polysaccharide was carried out with 2 M CF_3CO_2H at 121°C for 2 h. Amino sugars were detected using a Biotronik LC-2000 amino acid analyzer, a column of an Ostion LG AN B cation-exchange resin, and the standard 0.35 M sodium citrate buffer (pH 5.28) at 80°C. Neutral sugars and uronic acids were identified using a Biotronik LC-2000 sugar analyzer, a column of a Dionex A×9-11 anion-exchange resin, and 0.5 M sodium borate buffer (pH 8.0) at 65°C or 0.04 M sodium phosphate buffer (pH 2.4) at 70°C, respectively [13].

Absolute configurations of monosaccharides were determined by a modified method [14,15], using GLC of acetylated (*S*)-2-butyl glycosides derived from the polysaccharide after acid hydrolysis.

For carboxyl reduction, the polysaccharide was methanolized with 1 M HCl in methanol at 80°C for 16 h; after evaporation, an excess of $LiBH_4$ in 70% aqueous *t*-butanol was added at 4°C, the mixture was kept for 16 h at 20°C, acidified with concentrated HOAc, boric acid was removed by evaporation three times with methanol, the residue was hydrolyzed with 2 M CF_3CO_2H at 121°C for 2 h and studied using a sugar analyzer as described above and, after borohydride reduction and acetylation, by GLC on a Hewlett-Packard 5890 instrument equipped with a capillary columns of Ultra 2 and SPB-5 stationary phases.

Oxidation of the polysaccharide with 0.1 M $NaIO_4$ followed by reduction with an excess of $NaBH_4$ was carried out as described [10].

2.3. NMR spectroscopy

NMR spectra were run with Bruker WM-250 (^{1}H) and Bruker AM-300 (^{13}C) spectrometers for solutions in D_2O at 45 and 70°C, respectively, with acetone (δ_H 2.225, δ_C 31.45) as internal standard. A rotating-frame NOE (ROESY) experiment was performed on a modified Bruker WM-250 spectrometer using the proposed pulse sequence [16] and a mixing time of 0.23 s; HDO signal was suppressed by irradiation during 1 s. An H-detected $^{1}H,^{13}C$ heteronuclear multi-quantum coherence (HMQC) experiment was carried out on a Bruker AM-300 spectrometer equipped with a BSV-3 generator as described [17].

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2.4. Antisera and serological techniques

Rabbit anti-O sera were prepared by immunization of New Zealand rabbits via the marginal ear vein injection with heat-killed (100°C, 2 h) bacterial suspensions (1.5×10^{10} CFU/ml) in doses of 250, 500 and 1000 μl over 3 weeks [18].

Quantitative precipitation [19], passive hemolysis and inhibition of passive hemolysis [10] were performed as described.

For ELISA, MaxiSorp (Nunc) plates were coated with LPS (500 ng per well) in phosphate buffered saline (PBS) (pH 7.2); after incubation for 3 h at 37°C with gentle shaking and then for 16 h at 4°C, the plates were washed 6 times with PBS, blocked with 10% skim milk in PBS, incubated for 1 h at 37°C with diluted rabbit antiserum, and then the color reaction was developed with peroxidase-conjugated goat anti-rabbit IgG (Sigma) using 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) as substrate. Absorption was measured at 405 nm using an EL 312e reader (Bio-Tek Instruments). The last serum dilution giving OD ≥ 0.2 was taken as the titer.

For inhibition of ELISA, the antiserum dilution giving OD = 1.00 in reaction with the homologous LPS was used. The last dose of inhibitor which causes reduction of absorption to OD = 0.5 was taken as the minimal inhibitory dose.

Human sera of blood donors were from the Municipal Health Care Group (Lodz). Peroxidase-conjugated sheep anti-human IgGAM was used as the second antibody to test the reactivity of human sera with LPS in ELISA.

3. Results

3.1. Determination of sugar composition of the O-specific polysaccharide

Full acid hydrolysis of the polysaccharide from *P. mirabilis* O10 strain PrK 19/57 afforded amino sugars and uronic acids. The amino sugars were identified using an amino acid analyzer as 2-amino-2-deoxyglucose and 2-amino-2-deoxygalactose in the ratio 1:1. Analysis using a sugar analyzer revealed two uronic acids, one of which had the same retention time as galacturonic acid and the other eluted near glucuronic acid. Neutral sugars were absent from the hydrolysate.

For identification of the second uronic acid, the polysaccharide was methanolized, and methyl(methyl uronosidoates) obtained were carboxyl-reduced with lithium borohydride by a modified method [20]. Subsequent acid hydrolysis and analysis of the products using a sugar analyzer revealed galactose, which was derived by reduction of galacturonic acid, and a hexose having the same retention time as altrose. Analysis of alditol acetates derived from the hydrolysate by GLC on two different stationary phases and by GLC-MS resulted in identification of acetylated galactitol, altritol, and a 1,6-anhydrohexopyranose in the ratio 1:0.43:0.55, as well as methyl 2-amino-2-deoxyhexosides, which were stable towards acid hydrolysis due to the presence of the free amino group.

The 1,6-anhydrohexopyranose was identified on the basis of the identity of the electron impact and chemical ionization mass spectra of the fully acetylated derivative with those of 1,6-anhydrotalopyranose and was, thus, 1,6-anhydroaltropyranose which, in addition to altrose, was derived by acid hydrolysis of methyl altroside formed by reduction of altruronic acid. The existence of an equilibrium between altrose and 1,6-anhydroaltropyranose in acidic media [21] and formation of 1,6-anhydroaltropyranose upon hydrolysis of an altrose-containing polysaccharide [22] have been described.

Determination of absolute configurations by a modified method [14,15] showed that galacturonic acid, 2-amino-2-deoxyglucose, and 2-amino-2-deoxygalactose have the D configuration. The L configuration of altruronic acid was determined by analysis of NOE and glycosylation effects on the ^{13}C chemical shifts in the polysaccharide (see below).

The ^{13}C NMR spectrum of the polysaccharide (Fig. 1) contained signals for four anomeric carbons in the region δ 96.2–104.3, two carbons linked to nitrogen (C2 of 2-amino-2-deoxyhexoses) at δ 50.5 and δ 54.0, two unsubstituted hydroxymethyl groups (C6 of 2-amino-2-deoxyhexoses) at δ 61.7 and 61.9 (data of the attached-proton test [23]), two carboxyl groups (C6 of uronic acids) in the region δ 173.7–176.1, 14 other sugar ring carbons in the region 68.5–82.6, and two *N*-acetyl groups at 23.4 and 23.7 (CH_3) and in the region δ 173.7–176.1 (CO).

Accordingly, the ^1H NMR spectrum of the polysaccharide contained, among others, signals for four anomeric protons at δ 4.77–5.40 and two *N*-acetyl groups at δ 2.02.

These data suggested that the polysaccharide has a tetrasaccharide repeating unit containing one residue each of 2-acetamido-2-deoxy-D-glucose (D-GlcNAc), 2-acetamido-2-deoxy-D-galactose (D-GalNAc), D-galacturonic acid (D-GalA), and L-altruronic acid (L-AltA).

3.2. Elucidation of structure of the O-specific polysaccharide

The ^1H NMR spectrum of the polysaccharide was assigned using selective spin-decoupling, two-dimensional COSY, and H,H-relayed COSY (Table 1). Signals for Gal*p*NAc were distinguished by the coupling constant values $J_{2,3}$ 10, $J_{3,4}$ 3, and $J_{4,5} < 1.5$ Hz, and those for Glc*p*NAc by the values $J_{3,4} \approx J_{4,5}$ 9 Hz [24] (the value $J_{2,3}$ for GlcNAc was not determined owing to the coincidence of the H2 and H3 signals at δ 4.05). The Gal*p*A residue was characterized by the same coupling constant values as GalNAc but distinguished by the absence of H6 protons and a higher-field position of the H2 signal (δ 4.04 for GalA as compared with δ 4.58 for GalNAc; the downfield displacement of the latter is caused by a deshielding effect of the *N*-acetyl group). The coupling constant values $J_{1,2} < 4$ Hz

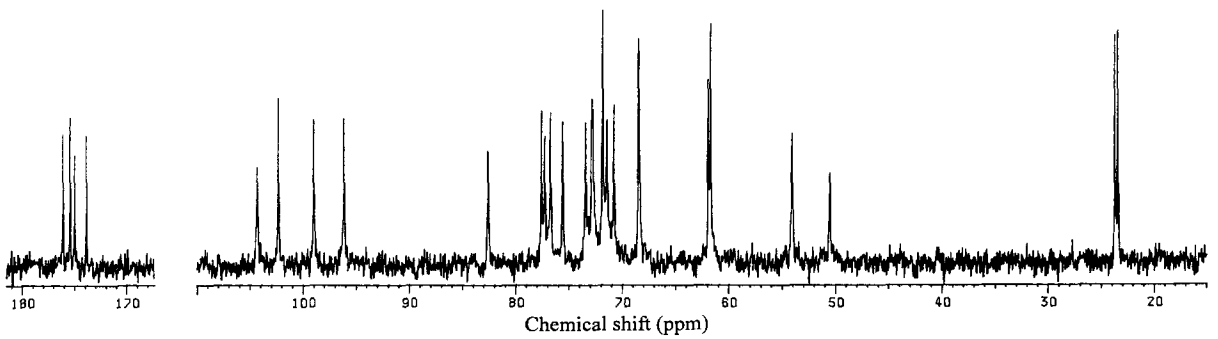


Fig. 1. ^{13}C NMR spectrum of the O-specific polysaccharide of *P. mirabilis* PrK 19/57.

Table 1

Data of ^{1}H NMR spectrum of the O-specific polysaccharide of *P. mirabilis* O10

Residue	Chemical shift (δ, ppm; *J*, Hz)							
	H1	H2	H3	H4	H5	H6a	H6b	CH_3CON
→3)-α-D-Gal*p*A-(1→	5.40	4.04	4.06	4.52	4.46			
	$J_{1,2}$ 3	$J_{2,3}$ 10	$J_{3,4}$ 3	$J_{4,5}$ <1.5				
→3)-α-D-Glc*p*NAc-(1→	5.00	4.05	4.05	3.81	4.34	3.94	3.70	2.02
	$J_{1,2}$ 3		$J_{3,4}$ 9	$J_{4,5}$ 9	$J_{5,6a}$ 3	$J_{6a,6b}$ 11	$J_{5,6b}$ 6	
→4)-α-D-Gal*p*NAc-(1→	5.17	4.58	4.21	4.35	4.32	3.83	3.69	2.02
3 ↑	$J_{1,2}$ 4	$J_{2,3}$ 10	$J_{3,4}$ 3	$J_{4,5}$ <1.5	$J_{5,6a}$ 3	$J_{6a,6b}$ 11	$J_{5,6b}$ 7	
α-L-Alt*p*A-(1→	4.77	3.53	3.63	4.38	4.52			
	$J_{1,2}$ 6	$J_{2,3}$ 8	$J_{3,4}$ 2.5	$J_{4,5}$ 2				

showed that all three sugar residues with the *gluco* and *galacto* configuration are linked by the α-glycosidic linkage [24].

The coupling constant values $J_{1,2}$ 6, $J_{2,3}$ 8, $J_{3,4}$ 2.5, and $J_{4,5}$ 2 Hz determined for the fourth sugar residue (Alt*p*A) demonstrated the axial (or close to axial) orientation of H1-H3 and the equatorial orientation of H4. These values were close to the corresponding values (7.8, 9.8, 3.4, and 1.0 Hz) for β-galactose, which has the same orientation of H1–H4, but different from those (< 1, 3.5, 4.4, and 10 Hz [24]) for α-altrose having the equatorial H1–H3 and the axial H4. Therefore, unlike free L-altrose, L-altruronic acid in the polysaccharide has a conformation close to $^4C_1$. Marked differences in the coupling constant values for AltA in the polysaccharide studied from those determined for AltA in a capsular polysaccharide from *Aerococcus viridans* var. *homari* ($J_{1,2}$ 7.5, $J_{2,3}$ 4.5, $J_{3,4}$ 4.4, and $J_{4,5}$ 5.3 Hz) [22] may be due to a higher contribution to the conformational equilibrium of a conformer (or conformers) B (boat) in the latter polysaccharide. The coupling constant value $J_{1,2}$ 6 Hz showed that the orientation of H1 and H2 of AltA in the polysaccharide studied is close to

Table 2

Data of ^{13}C NMR spectrum of the O-specific polysaccharide of *P. mirabilis* O10

Residue	Chemical shift (δ, ppm)							
	C1	C2	C3	C4	C5	C6	*C*H_3CON	CH_3*C*ON
→3)-α-D-Gal*p*A-(1→	102.3	68.5	75.6	68.5	72.8	175.0[a]		
→3)-α-D-Glc*p*NAc-(1→	99.0	54.0	82.6	71.8	72.8	61.9	23.4[b]	175.4[a]
→4)-α-D-Gal*p*NAc-(1→	96.2	50.5	77.3	76.7	73.4	61.7	23.7[b]	176.1[a]
3 ↑								
α-L-Alt*p*A-(1→	104.3	71.4	71.8	70.8	77.6	173.7[a]		

[a,b]Assignment could be interchanged.

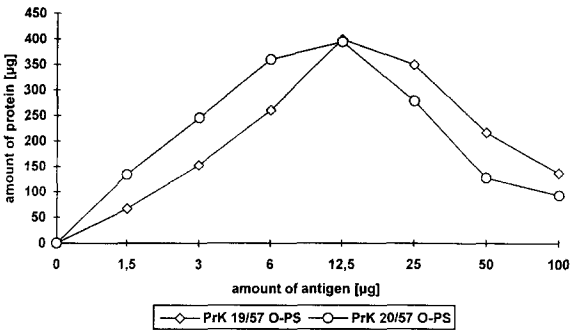


Fig. 2. Quantitative precipitation of O-anti-*P. mirabilis* PrK 20/57 serum with O-specific polysaccharides (O-PS) of *P. mirabilis* PrK 19/57 and PrK 20/57.

Table 3
Inhibition with *P. mirabilis* O10 antigens of ELISA and passive hemolysis in the homologous system anti-*P. mirabilis* PrK 20/57 serum/*P. mirabilis* PrK 20/57 LPS

Inhibitor	Minimal inhibitory dose (ng)	
	ELISA	Passive hemolysis
PrK 19/57 LPS	9.5	19
PrK 20/57 LPS	9.5	9.5
PrK 19/57 LPS-OH	39	19
PrK 20/57 LPS-OH	9.5	9.5
PrK 19/57 O-PS	39	9.5
PrK 20/57 O-PS	19	9.5
PrK 20/57 O-PS*	> 10 000	nt

LPS-OH, alkali-treated LPS; O-PS, O-specific polysaccharide; O-PS*, Smith-degraded O-specific polysaccharide; nt, not tested.

trans-diaxial, and, therefore, taking into account the $^4C_1$ conformation, it was concluded that AltA is linked by the α-glycosidic linkage.

Assignment of the ^{13}C NMR spectrum of the polysaccharide (Table 2) was performed using heteronuclear $^{13}C,^1H$ COSY and H-detected $^1H,^{13}C$ HMQC. The coupling constant values $^1J_{C1,H1}$ 170–174 Hz, which were determined from the gated-decoupling spectrum of the polysaccharide for GlcNAc, GalNAc, and GalA, indicated the equatorial orientation of H1 [25] and, thus, confirmed the α-configuration of these three sugar residues. AltA was characterized by a smaller coupling constant value $^1J_{C1,H1}$ 165 Hz that was consistent with the pyranose form of AltA and the axial orientation of H1 in the predominant $^4C_1$ conformer, that is, with its α-configuration [25].

A ROESY experiment with the polysaccharide revealed interresidue connectivities between the following protons: H1 GalA/H3 GlcNAc at δ 5.40/4.05; H1 GlcNAc/H4 and H6b GalNAc at δ 5.00/4.35 and δ 5.00/3.69, respectively; H1 GalNAc/H3 and H4 GalA at δ 5.17/4.06 and δ 5.17/4.52, respectively; and H1 AltA/H3 GalNAc at δ 4.77/4.21. These data suggested that the polysaccharide is branched with GalNAc at the branching point and AltA attached as a side chain and revealed the sequence of the sugar residues shown below.

In addition, intraresidue connectivities were observed for H1/H2 of the α-linked sugar residues at δ 5.00/4.05 (GlcNAc), 5.17/4.58 (GalNAc), 5.40/4.04 (GalA), and H1/H2 and H1/H3 of AltA at δ 4.77/3.53 and 4.77/3.63, respectively. These connectivities for AltA, together with the absence of any connectivity between axial H1 AltA and H5 AltA having the equatorial orientation in the $^4C_1$ conformer confirmed again the conformation of AltA and the α-configuration of its glycosidic linkage.

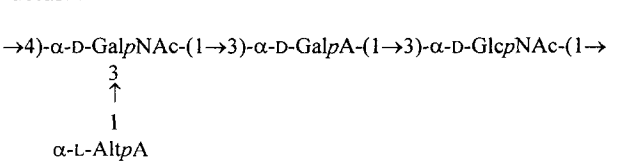
Substitution pattern of the polysaccharide could also be revealed by the ROESY data. Thus, a connectivity H1 GalA/H3 GlcNAc demonstrated unambiguously substitution of GlcNAc at position 3. Connectivities H1 AltA/H3 GalNAc and H1 GlcNAc/H4,H6b GalNAc showed that GalNAc is substituted at positions 3 and 4 since substitution at position 6 is excluded on the basis of the ^{13}C NMR data (see above) and the fact that no NOE connectivity could be observed between H1′ and H4 in 1,6-linked disaccharides but between H1′ and H6 in 1,4-linked disaccharides [26]. Similarly, substitution of GalA at position 3 followed from connectivities H1 GalNAc/H3,H4 GalA that is typical of a number of α-1,3-linked disaccharides with the *galacto* configuration of the glycosylated pyranose and the same absolute configuration of the constituent monosaccharide [26], while no NOE on H3 could be observed in any 1,4-linked disaccharide of this type.

The absence of any connectivity between H1 AltA and H4 GalNAc indicated different absolute configurations of these two monosaccharides since, in the case of their same absolute configuration, a NOE on H4 of GalNAc in the disaccharide fragment α-Alt*p*A-(1→3)-Gal*p*NAc would be observed not less intense than that on H3 [26] as, for instance, is the case in the disaccharide fragment α-D-Gal*p*NAc-(1→3)-D-Gal*p*A (see above). An additional substitution of GalNAc at position 4 does not break this rule [27]. Therefore, since GalNAc has the D configuration, AltA has the L configuration.

Analysis of the glycosylation effects on the ^{13}C chemical shifts in the disaccharide fragment α-Alt*p*A-(1→3)-Gal*p*NAc led to the same conclusion. Thus, the effect on C1 of AltA at δ 104.3 was ~7 ppm, which, in accordance with the known regularity [28], demonstrated different absolute configurations of AltA and GalNAc (the effect would not exceed 4 ppm in the case of their same absolute configuration). Again, an additional substitution of GalNAc at position 4 should not have any significant influence on the chemical shift of the C1 signal of AltA [29].

The ^{13}C NMR data for the polysaccharide (Table 2) confirmed independently also the positions of substitution of the sugar residues. In particular, these followed from downfield displacements by 5–11 ppm of the signals for C3 of GlcNAc and GalA, C3 and C4 of GalNAc in the spectrum of the polysaccharide, as compared with their positions in the spectra of the corresponding unsubstituted monosaccharides [30].

Therefore, the data obtained suggested that the repeating unit of the specific polysaccharide studied has the following structure:



Proteus mirabilis O10

The ^{13}C NMR spectra of the polysaccharides from both *P. mirabilis* O10 strains studied (PrK 19/57 and PrK 20/57) were identical and, hence, they have the same structure.

3.3. Serological studies

In accordance with the structural identity of the O-antigens of both strains studied, the corresponding O-antisera showed the same or similar serological reactivity in all tests used and, therefore, only results obtained with rabbit anti-PrK 20/57 serum are presented.

In ELISA the antiserum reacted with the homologous and PrK 19/57 LPSs to the titers 1:1 024 000 and 1:2 048 000, respectively, and in passive hemolysis test with both alkali-treated LPSs to the titer 1:128 000. In quantitative precipitation test (Fig. 2) the O-specific polysaccharides from strains PrK 19/57 and PrK 20/57 bound the same amount of antibodies: 12.5 μg antigen precipitated 400 μg of protein in the equivalence zone. LPSs, alkali-treated LPSs and O-specific polysaccharides from both strains were efficient inhibitors of ELISA and passive hemolysis in the homologous system (Table 3).

Periodate oxidation of the PrK 20/57 O-specific polysaccharide followed by borohydride reduction (Smith degradation) which destroyed the lateral AltA residue but did not affect the main chain of the polysaccharide resulted in complete loss of inhibitory activity (Table 3).

Cross-reactivity of anti-PrK 20/57 serum with proteinase K-treated cells of 32 other *P. mirabilis* O-serogroups was tested in dot-blot (data not shown). The antiserum cross-reacted with *P. mirabilis* O43 only. This result was confirmed by ELISA and passive hemolysis with LPS isolated from *P. mirabilis* O43: the titers were 1:2000 and 1:1280, respectively. Rabbit anti-O43 serum [31] did not cross-react with *P. mirabilis* O10 LPSs.

Frequency of occurrence in normal human sera of antibodies reacting with LPS of *P. mirabilis* PrK 20/57 was tested in ELISA. Of 105 samples of sera tested, 74 samples (about 70%) reacted to a titer in the range 1:100–1:800, most of them to the titer 1:200.

4. Discussion

In accordance with their position in the same *P. mirabilis* O10 serogroup, both strains studied produce LPSs having structurally identical O-specific polysaccharide chains and their O-antigens exhibit the same or similar serological reactivity.

As most other *Proteus* O-antigens, the O-specific polysaccharides studied are acidic. Moreover, they have a tetrasaccharide repeating unit containing two acidic components: D-galacturonic and L-altruronic acids. While D-galacturonic acid is one of the most common sugar components of *Proteus* O-antigens (e.g. [6,9,31]), L-altruronic acid has been found in this genus, as in any other Gram-negative bacterium, for the first time [32,33]. To the best of our knowledge, the only known bacterial polymer containing L-altruronic acid is the capsular polysaccharide of *Aerococcus viridans* var. *homari* [22].

Being a lateral sugar substituent in the polysaccharide, L-altruronic acid plays the immunodominant role in manifestation of the O10 specificity of *Proteus*, as shown by the complete loss of serological activity of the polysaccharide after selective degradation of this monosaccharide. However, although the O10 specificity is mainly defined by a unique component (L-AltA), the presence of a minor immunoglobulin fraction was found in anti-O10 serum which cross-reacts with the O-antigen of *P. mirabilis* O43 lacking L-altruronic acid [31]. Comparison of the structures of the O-specific polysaccharides of *P. mirabilis* O10 (established in this work) and *P. mirabilis* O43 (established earlier [31] and shown below) revealed the presence of a disaccharide fragment in common, namely, α-D-Gal*p*A-(1→3)-α-D-Glc*p*NAc, which accounts evidently for the weak cross-reaction observed. As for anti-O43 serum, this was reported to recognize a wider epitope containing at least three sugar residues [31] and does not cross-react with *P. mirabilis* O10 LPS.

→4)-α-D-Glc*p*-(1→4)-α-D-Gal*p*A-(1→3)-α-D-Gal*p*A-(1→3)-α-D-Glc*p*NAc-(1→

Proteus mirabilis O43 [31]

Interestingly, the *P. mirabilis* O10 and O43 polysaccharides share another peculiar feature: their tetrasaccharide repeating units contain two uronic acids with unsubstituted carboxyl groups. Such relatively high concentration of the negative charge is uncommon since no more than one acidic function per three sugar residues are present in acidic O-antigens of other bacteria, including those of other *Proteus* O-serogroups [32]. Such highly charged O-specific polysaccharide chains of LPSs may play a protective role in nonencapsulated bacteria.

Strains of *P. mirabilis* O10 are among *Proteus* stains most often isolated from patients' feces and urine [4]. Our finding that *P. mirabilis* O10-reacting antibodies occur in normal human sera rather frequently is consistent with the epidemiological data. It should be mentioned also that even a higher level of antibodies reacting with LPS of *P. mirabilis* O13 was detected in normal human sera (the reaction titers 1:400–1:800 were observed for 67% of the sera tested vs. 12% in the case of *P. mirabilis* O10, authors' unpublished data). This may indicate that these *P. mirabilis* O-serogroups are common in normal human microflora. However, it cannot be excluded that antibodies against structurally conserved epitopes in the core part of enterobacterial LPS are responsible for the reactivity described, and different reaction titers for various *Proteus* O-serogroups associated with different degree of accessibility of the core epitopes on LPS.

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