Polish J. Chem., **73**, 895–907 (1999) **REVIEW ARTICLE**

Structure of the O-Antigenic Polysaccharides of *Proteus* Bacteria*

by Y.A. Knirel[1], W. Kaca[2,3], A. Rozalski[2] and Z. Sidorczyk[2]**

[1]*N.D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Leninsky Prospekt 47, Moscow 117913, Russia*
[2]*Institute of Microbiology and Immunology, University of Łódź, Banacha 12/16, 90-237 Łódź, Poland*
[3]*Center of Microbiology and Virology, Polish Academy of Sciences, Lodowa 106, 93-232 Łódź, Poland*

(Received December 23rd, 1998)

Data on the composition and structure of the O-specific polysaccharides (O-antigens) of the lipopolysaccharides of the genus Proteus are summarized and discussed as the molecular basis for serotyping of these medically important bacteria.

Key words: bacterial polysaccharide, O-antigen, structure, epitope specificity, *Proteus*

1. Introduction

Gram-negative bacteria of the genus *Proteus* from the family *Enterobacteriaceae* are common in human and animal intestines. However, under favourable conditions, they cause infections of wounds, burns, skin, eyes, ears, nose and throat, as well as intestinal and urinary tract infections. From them, most important are urinary tract infections, which can lead to severe complications, such as acute or chronic pyelonephritis and formation of bladder and kidney stones. *P. mirabilis* is the second after *Providencia stuartii* and the third after *Escherichia coli* and *Klebsiella pneumoniae* the most common cause of catheter-associated bacteriuria and complicated urinary tract infections, respectively [1]. Potential virulence factors and features of *Proteus* rods, mediating the infectious processes, are fimbriae, flagella, urease, proteases, hemolysins, invasiveness, capsular polysaccharide and lipopolysaccharide (LPS) [2].

LPS is an integral component of the outer membrane of the cell envelope, which is essential for bacterial survival. It represents the endotoxin which, after being released from bacterial cells, causes a broad spectrum of pathophysiological effects, which in severe cases may end in septic shock [3]. LPS serves also as the main surface antigen of *Proteus*, which is recognized by specific antibodies. It consists of three moieties, which are different in their structure and biosynthetic control: a lipid part termed lipid A, which anchors LPS into the outer membrane and is known as the endotoxic principle of LPS, an oligosaccharide core region, and an O-specific polysaccharide (O-chain, O-antigen), which defines the immunological O-specificity of the bacterium. *Proteus* strains are serologically heterogeneous, due to a high diversity in composition and structure of the O-antigen. Accordingly, strains of *P.*

* Dedicated to Prof. Aleksander Zamojski on the occasion of his 70th birthday.
**Correspondence to Dr. Y.A. Knirel; e-mail: knirel@ioc.ac.ru

mirabilis and *P. vulgaris* are classified into 60 O-serogroups [4,5]. Strains of *P. penneri* have not been completely classified yet.

Chemical, immunochemical and immunobiological studies have been undertaken, aiming to understand the immunospecificity of *Proteus* LPS and its potential role in the pathogenicity of the bacteria on the molecular level. In these studies, special attention was paid to the O-specific polysaccharide. The present review summarizes our present knowledge on the composition and structure of *Proteus* O-antigens. Serological classification and cross-reactivity of various *Proteus* strains are discussed in view of the chemical structures of the O-antigens. Most of these data were obtained in authors' own laboratories; part of them has been summarized previously [6].

2. Composition and structure of the O-antigens

O-Antigens of *Proteus* are branched or linear polysaccharides built up of oligosaccharide repeating units, varying from a trisaccharide to a hexasaccharide (Table 1). Remarkably, all polysaccharides contain amino sugars, most common being D-glucosamine (GlcN), which is present in almost all polysaccharides. Other common components are D-galactosamine (GalN, a component of 16 polysaccharides), D-glucose (11), D-galactose (16), L-rhamnose (LRha, 5), D-glucuronic acid (GlcA, 10) and D-galacturonic acid (GalA, 15). In addition, some rare monosaccharides were found, such as D-ribose (2), 6-deoxy-L-talose (L6dTal, 1), 2-amino-2,6-dideoxy-L-glucose (LQuiN, L-quinovosamine, 3), 2-amino-2,6-dideoxy-L-galactose (LFucN, L-fucosamine, 3), 3-amino-3,6-dideoxy-D-glucose (Qui3N, 2), 3-amino-3,6-dideoxy-D-galactose (Fuc3N, 1), 2,3-diamino-2,3,6-trideoxy-L-mannose (LRhaN3N, 1) and L-altruronic acid (LAltA, 1). Two of these sugars, LRhaN3N and LAltA, have not been reported as components of other bacterial LPS, whereas the 6-deoxyamino sugars and 6dTal found in a few LPS only [7].

Table 1. Structures of the O-specific polysaccharides of *Proteus*.

Serogroup (strain)	Structure of the repeating unit[a]	References
P. vulgaris		
O1 (OX19)	-4)LQuiNAc(α1-3)GlcNAc(β1-4)GalNAc(α1-4)Galα1-*P*-(O-LQuiNAc(α1-3)-]$_n$	[22,46]
O2 (OX2)[b]	-2)Glc(β1-6)GlcNAc(α1-3)LQuiNAc(α1-3)GlcNAc6Ac(β1-	[22,47]
O8	-3)GlcA(β1-4)LFucNAc(α1-3)GlcNAc(α1-Gal(α1-3)-]$_n$	[36]
O19	-4)GalNAc(α1-3)LFucNAc(α1-3)GlcNAc(β1-3)Gal(α1-	[48]
O22	-4)LRha(α1-4)GlcA(β1-3)GlcNAc(β1-3)LRha(β1-Qui3NAc2,4Ac(α1-2)-]$_n$	[49]
O25	-3)GlcNAc(β1-2)LRha(α1-2)Rib*f*(β1-4)GalNAc(β1-Glc3*R*Lac(α1-3)-]$_n$	[39]
O32[c]	-2)LRha(α1-2)LRha(α1-4)GalA(β1-3)GlcNAc(β1-4)GalA(α1-	[37]
O45	-4)GalNAc3Ac(α1-3)GalNAc(β1-3)GalNAc(β1-GlcA(β1-4)-]$_n$	[29]

Table 1 (continuation)

P. mirabilis		
O3 (OXK)	-3)GalNAc(β1-6)GalNAc(β1-4)GlcA(β1- GalA6Lys(α1-4)┘ Glc(α1-2)┘	[22,33]
O6	-3)GlcNAc(β1-4)LFucNAc(α1- GlcA(α1-3)┘	[28,50]
O10	-3)GlcNAc(α1-4)GalNAc(α1-3)GalA(α1- LAltA(α1-3)┘	[51,52]
O13	-3)GlcNAc(β1-3)Gal(α1- GalA6DAlaLys(α1-4)┘	[53]
O14a,14b	-4)GalNAc(β1-3)Gal6Ac(α1-3)GalNAc(β1-4)Gal(α1- DAlaEtn-*P*-(O-6)┘	[35]
O14a,14c	-6)Glc(β1-3)Gal(β1-3)GlcNAc(β1-3)Gal(α1- DAlaEtn-*P*-(O-6)┘	[35]
O23; O23,56[c]	-4)GalA(α1-3)GlcNAc(β1-2)GalA(β1-3)GalNAc(α1-	[28,54]
O24	-3)GlcNAc(β1-4)GalNAc(β1-4)GlcNAc(β1- Gal(β1-3)┘	[55]
O26[c]	-4)Gal(α1-3)GalA4Ac(β1-3)GlcNAc(β1-4)GalA6Lys(α1-	[30,38]
O27[d]	-3)GalA6Ala(α1-3)GlcNAc(β1-3)GlcA6Lys(β1- Etn-*P*-(O-6)┘ GlcNAc(β1-4)┘	[34,56]
O28	-4)Gal(α1-3)GalA4Ac6Ser(α1-3)GlcNAc(β1-4)GalA6Lys(α1-	[31,57]
O29	-3)GalNAc(β1-4)GalNAc(β1-4)GlcA(β1- GalNAc(α1-3)┘	[58]
O30[b]	-4)GlcA(β1-6)GalNAc(α1-6)GlcNAc(β1-3)GlcNAc4Ac(β1-	[30,59]
O43	-4)GalA(α1-3)GalA(α1-3)GlcNAc(α1-4)Glc(α1-	[60]
O57	-6)Gal(β1-3)GalNAc(β1-4)GalNAc(β1-3)Gal(α1- Glc(α1-6)┘ Gro1-*P*-(O-3)┘	[61]
P. penneri		
O61 (52)	-4)Gal(β1-3)GlcNAc(β1-4)GalNAc(β1-3)GalNAc(α1- GlcNAc(β1-3)┘	[23]
O62 (41)[b]	-2)Gal6Ac(α1-3)GlcNAc(β1-3)LRha(α1-2)LRha(α1- Glc(β1-3)GlcNAc4*S*Lac(β1-2)┘	[26,62]
O63 (22)	-4)GalA(β1-3)GlcNAc6(β1-3)Gal(α1- Gal*f*NAc(α1-4)GlcA(β1-4)┘	[24]
O64 (62)[b]	-6)GlcNAc3*S*Lac(β1-3)Gal(α1-3)GlcNAc6Ac(β1-	[16,17]
O64 (19)	-4)GlcNAc3*S*Lac(β1-3)Gal(α1-3)GlcNAc(β1-	[18]
O64 (71)	-4)GlcNAc(β1-3)Gal(α1-3)GlcNAc(β1-	[19]
O65 (34)	-4)Glc(β1-3)GalNAc(β1-4)GalNAc(β1-4)Gal(β1-	[25]
O66 (2)	-3)GlcNAc(β1-4)Glc(α1-3)L6dTal2Ac(α1- LRhaNAc3NAc(β1-3)┘	[27]
O67 (8)	-4)GalA(β1-3)GalNAc(α1-3)LFucNAc(α1-3)GlcNAc(β1- Glc(β1-6)┘ Glc(α1-4)┘ Etn-*P*-(O-6)┘	[63]
(11)[b,e]	-4)GlcNAc6Ac(β1-4)GalNAc(β1-3)LRha(β1- GalA3Ac6Thr(α1-3)┘	[32,57]
(14)	-2)Rib*f*(β1-4)Gal(β1-3)GlcNAc(β1-2)Qui3NDAlaAc(β1- 4)GalA6Ala(α1-	[41,42]
(15)[f]	-2)Gal4,6*R*Pyr(α1-4)Gal(β1-3)GlcNAc(β1-	[15]
(16)[e]	-6)Glc(α1-4)GlcA(β1-3)GlcNAc(α1-2)Fuc3N*R*3HOBu(β1- Glc(α1-2)┘	[64]

Table 1 (continuation)

(25)[b]	-4)GlcA(β1-3)GlcNAc6Ac(β1-6)GlcNAla3Ac(α1-GlcA3/4Ac(α1-4)—]$_{-}$	[65]
(26)	-6)GlcNAc(α1-3)LQuiNAc(α1-3)GlcNAc(α1-	[66]
(42)[g]	-2)Glc(β1-4)Glc(β1-3)Glc(β1-4)GalA(α1-	[67]

Abbreviations. 6dTal, 6-deoxytalose; AltA, altruronic acid; QuiN, 2-amino-2,6-dideoxyglucose (quinovosamine); FucN, 2-amino-2,6-dideoxygalactose (fucosamine); Qui3N, 3-amino-3,6-dideoxyglucose; Fuc3N, 3-amino-3,6-dideoxygalactose; RhaN3N, 2,3-diamino-2,3,6-trideoxymannose; 3HOBu, 3-hydroxybutyryl; Lac, 1-carboxyethyl ("lactyl"); Pyr, 1-carboxyethylidene ("pyruvyl"); Gro, glycerol; Etn, 2-aminoethanol; AlaEtn, 2-(1-carboxyethylamino)ethanol; AlaLys, N^{ε}-(1-carboxyethyl)lysine; P, phosphate. [a]If not stated otherwise, sugars have the D configuration and occur in the pyranose form, amino acids have the L configuration. [b]*O*-Acetylation is not stoichiometric. [c]In addition, minor OAc is present at unknown position. [d]Phosphorylation is not stoichiometric. [e]Previously, this structure was erroneously reported as belonging to *P. penneri* 12 [32]. [f]The same structure was reported for *P. vulgaris* ATCC 49990 [14]. [g]The same structure was reported for *P. mirabilis* mutant strain R14/S1959 [68].

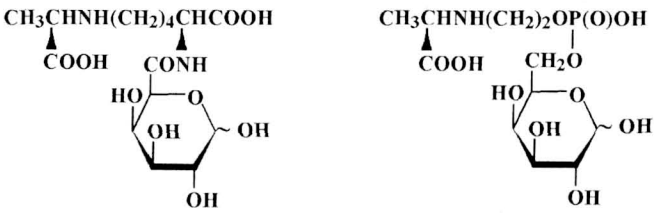
The pyranose form is characteristic for most monosaccharides, except for ribose, which is always present in the furanose form. Surprisingly, the O-antigen of *P. penneri* O63 contains GalN in the furanose form that has not been hitherto reported for this sugar in nature.

Hexuronic acids either have the free carboxyl group or are amidated with the α-amino group of amino acids. From them most common is L-lysine, which is attached to GlcA or GalA in four polysaccharides, but L-alanine, L-serine and L-threonine, all linked to GalA, occur as well. The O-antigen of *P. mirabilis* O13 includes a unique component, an amide of GalA with N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine (DAlaLys, **1**). This amino acid has been reported elsewhere as a component of a *Providencia alcalifaciens* O-antigen, where it amidates GlcA [8].

Some other polysaccharides are acidic due to the presence of a phosphate group. In *P. vulgaris* O1 and *P. mirabilis* D52, galactosyl phosphate or glycosyl-ribitol phosphate are present within the polysaccharide chain (the full structure of the latter has not been established yet [9]). Oligosaccharide-phosphate repeating units of this kind are not uncommon in teichoic acids of gram-positive bacteria and in capsular polysaccharides [10], but occur rarely in LPS of gram-negative bacteria (among a few other examples are O-antigens of *Hafnia alvei* [11]). In other phosphorylated polysaccharides of *Proteus*, the phosphate group (*P*) links various non-sugar substituents to a sugar in the main chain, such as glycerol to GalNAc, 2-aminoethanol (Etn) to GlcNAc, or 2-[(*R*)-1-carboxyethylamino]ethanol (DAlaEtn) to Gal (**2**). From them, Etn-*P* is typically found in the core region and lipid A of LPS, but not in the O-specific polysaccharide. DAlaEtn-*P*, a unique component of two polysaccharides of *P. mirabilis* O14, has not been found in other natural carbohydrates.

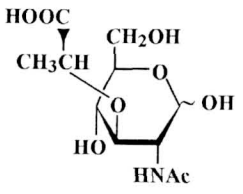
(*R*)- or (*S*)-Lactic acid is present in the polysaccharides of *P. vulgaris* O25, *P. penneri* O62 and O64, where it is connected by the ether linkage to Glc (at position 3) or to GlcNAc (at position 3 or 4), respectively. 2-Acetamido-3-[(*S*)-1-carboxyethyl]-2-deoxy-D-glucose (GlcNAc3*S*Lac, *N*-acetylisomuramic acid, **3**) is an isomer at the lactic acid residue of *N*-acetylmuramic acid, which is a widespread component of bacterial cell-wall peptidoglycan. An acidic component of the O-antigen of *P. penneri* 15 is pyruvic acid, which is attached by the acetal linkage to positions 4 and 6 of Gal (**4**).

Scheme 1

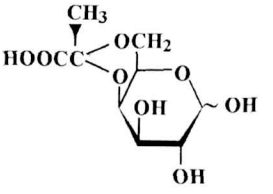


1

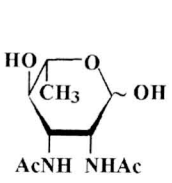
2



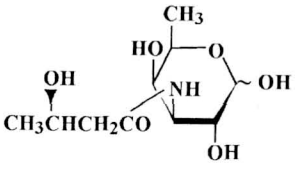
3



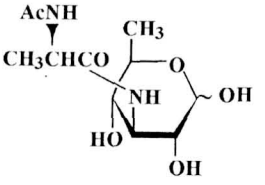
4



5



6



7

Thus, acidic polysaccharides represent the majority of the *Proteus* O-antigens (~80%). In some of them (*e.g.*, in *P. mirabilis* O23 and O43), half of the constituent monosaccharides are negatively charged. This feature is quite uncommon for O-antigens of other bacterial genera, which are either neutral or carry no more than one acidic group per three sugar residues [7]. A number of *Proteus* polysaccharides include both negatively and positively charged groups. For instance, the O-antigen of *P. mirabilis* O27 contains two carboxyl groups of lysine and alanine, a phosphate group, and two free amino groups of lysine (ε-amino group) and 2-aminoethanol.

A high concentration of charged groups on the surface polysaccharides may be important for adaptation of *Proteus* microorganisms to grow under different pH. This suggestion is favoured by a frequent occurrence in clinical isolates of *P. mirabilis* O3, O27 and O28 [4], all containing an amide of a hexuronic acid with lysine in the O-specific polysaccharide. The acidic character of the surface polysaccharides also enables the *Proteus* bacteria to bind, *via* electrostatic interaction, metal cations, Mg^{2+} and Ca^{2+}. This binding may enhance the formation of struvite and carbonate apatite stones in urinary tract under alkaline conditions in the presence of ammonia, one of the products of urea decomposition caused by *Proteus* bacteria [12]. Recently, a role

of an acidic polysaccharide, rich in GalA and GalN, was demonstrated for migration of multiflagellated *P. mirabilis* swarm cells by the reduction of surface friction [13], that may also be important in ascending urinary tract infections.

The amino groups of most amino sugars are *N*-acetylated to the corresponding acetamido derivatives, *e.g.*, LRhaNAc3NAc (**5**) in *P. penneri* O66. A few O-antigens include other acyl substituents, such as (*R*)-3-hydroxybutyryl group at Fuc3N (Fuc3N*R*3HOBu, **6**), *N*-acetyl-D-alanyl at Qui3N (Qui3NDAlaAc, **7**), and L-alanyl at GlcN (GlcNAla) in *P. penneri* strains 16, 14 and 26, respectively. In many O-antigens, sugar constituents carry an *O*-acetyl group (or groups), *O*-acetylation being as a rule non-stoichiometric; in some polysaccharides the degree of *O*-acetylation is so low that the exact location of the *O*-acetyl groups could be determined only tentatively, if at all.

Most *Proteus* O-antigens have a unique structure, but some of them possess marked similarity. Thus, LPS of *P. penneri* 15 and *P. vulgaris* O19 (ATCC 49990) have the identical repeating units of the O-antigens, whereas their core types are different [14,15]. The polysaccharides of *P. penneri* strains 19 and 62 from serogroup O64 have the same sugar composition and differ only in the mode of substitution of GlcNAc3*S*Lac (at position 4 or 6, respectively) and *O*-acetylation of the latter [16–18]. In *P. penneri* strain 71, which also belongs to serogroup O64, the polysaccharide has the same carbohydrate backbone as in *P. penneri* strain 19, but lacks the lactic acid residue and thus, unlike two other representatives of this serogroup, is neutral [19]. Some other polysaccharides have the same or similar trisaccharide fragments (*e.g.*, compare the O-antigens of *P. mirabilis* O3 and O29, *P. vulgaris* O19 and *P. penneri* O67, *P. mirabilis* O26 and *P. vulgaris* O32, *P. vulgaris* O22 and O32, Table 1) or even tetrasaccharide fragments (*P. mirabilis* O26 and O28). Often occurring in *Proteus* O-antigens are common disaccharide fragments, such as LFucNAc(α1-3)GlcNAc in *P. vulgaris* O8 and O19, *P. mirabilis* O6 and *P. penneri* O67, or LQuiNAc(α1-3)GlcNAc in *P. vulgaris* O1, O2 and *P. penneri* strain 26, *i.e.* in all polysaccharides that contain these 6-deoxyamino sugars. Such structural similarity of the O-antigens results often in serological cross-reactivity of *Proteus* strains (see Section 3).

For chemical studies, the O-specific polysaccharides were released from LPS by mild acid hydrolysis, which cleaves the linkage between the core region and lipid A. The main approach to structural elucidation of *Proteus* O-antigens was NMR spectroscopy at high field, including two-dimensional homonuclear correlation (COSY and TOCSY), H-detected $^{1}H,^{13}C$ heteronuclear correlation (HMQC or HSQC) and nuclear Overhauser effect spectroscopy (NOESY or ROESY). The NMR techniques allowed determination of both composition of the polysaccharides, including the relative absolute configurations of the constituent monosaccharides [20], and the full structure of the repeating unit. Most unusual non-sugar constituents were isolated in the free state (AlaEtn, AlaLys) or linking to the corresponding sugar (GalA6DAlaLys, Qui3NDAlaAc, GlcNAc4*S*Lac) by full acid hydrolysis or solvolysis with anhydrous hydrogen fluoride [21]. Then, they were identified by NMR spectroscopy, specific optical rotation and, after derivatization, by GLC/mass spectrometry. Free compounds were compared directly with synthetic authentic samples.

When necessary, oligosaccharide fragments were obtained from the polysaccharides by Smith degradation, partial acid hydrolysis or solvolysis with anhydrous hydrogen fluoride, and involved in NMR and mass spectrometric studies. Some O-antigens of *Proteus* are extremely labile towards acid hydrolysis and undergo depolymerization to the chemical repeating units under the mild acidic conditions that are used for delipidation of the LPS. Thus, the presence of the galactosyl phosphate linkage accounts for the high lability of the O-antigen of *P. vulgaris* O1 (**8**). Treatment of LPS of this strain with dilute acetic acid resulted in the complete cleavage of the glycosyl phosphate linkage to give a phosphorylated pentasaccharide (**9**) with a Gal residue at the reducing end [22].

-4)LQuiNAc(α1-3)GlcNAc(β1-4)GalNAc(α1-4)Galα1-*P*-(O-
LQuiNAc(α1-3)-]
8

P-(O-4)LQuiNAc(α1-3)GlcNAc(β1-4)GalNAc(α1-4)Gal
LQuiNAc(α1-3)-]
9

Interestingly, two structurally similar acidic O-antigens of *P. penneri* serogroup O64 differ significantly in their stability towards acids. While the polysaccharide of strain 62 with 6-substituted GlcNAc3*S*Lac is quite stable, the polysaccharide (**10**) of strain 19, where GlcNAc3*S*Lac is 4-substituted, undergoes depolymerization to a trisaccharide (**11**) that corresponds to the O-antigen repeating unit [18]. In this case the facilitation of the hydrolysis may be accounted for by a participation of the carboxyl group of the lactic acid in the neighborhood of the glycosidic linkage between GlcNAc and GlcNAc3*S*Lac.

-4)GlcNAc3*S*Lac(β1-3)Gal(α1-3)GlcNAc(β1-
10

GlcNAc3*S*Lac(β1-3)Gal(α1-3)GlcNAc
11

3. Epitope specificity of the O-antigens

Table 1 shows that each serologically different *Proteus* strain produces an O-specific polysaccharide of a unique structure. These data are in consistence with the serological classification of *P. vulgaris* and *P. mirabilis* strains [4,5] and serve as the basis for chemotyping of the bacteria. Moreover, together with new serological data [23–27], the chemical data allowed an extension of the classification scheme to add new serogroups O61 to O67. They consist of *P. penneri* strains only, which have not been formerly classified.

Serological epitopes, which are recognized by specific antibodies, are associated with various structural fragments of the O-antigens. Immunochemical studies, using

rabbit antibodies and homologous and heterologous LPS as well as released O-specific polysaccharides, chemically modified polysaccharides and synthetic antigens, revealed the molecular basis for the epitope specificity of a number of *Proteus* O-antigens. Most O-antibodies to the branched polysaccharides are directed against the lateral carbohydrate and non-carbohydrate substituents. The linear part of the polysaccharides is also involved in the reactivity, and extended epitopes, up to the whole repeating units, may be necessary for effective binding of antibodies.

Uronic acids, mainly GlcA and GalA, and their amides often serve as immunodominant sugars in the specific epitopes, as demonstrated using synthetic glycopolymers containing various uronic acids and their amides with amino acids. Examples are lateral α-linked and β-linked GlcA residues in the O-antigens of *P. mirabilis* O6 [28] and *P. vulgaris* O45 [29], respectively, and a residue of αGalA6Lys in the main chain of the polysaccharides of *P. mirabilis* O26 and O28 [30,31]. Based on the cross-reactivity of *P. penneri* 11 O-antiserum with LPS of *P. mirabilis* O3 (S1959), R14/S1959, O23 and O28 with known O-antigen structures, three kinds of epitopes were characterized in the O-antigen of *P. penneri* 11 [32]. They differ in the size, which increases from a single lateral residue of αGalA to αGalA6Thr and a disaccharide αGalA6Thr(1-3)βGalNAc. Disaccharide-associated epitopes, that include amides of GalA and GlcA with lysine, αGalA6Lys(1-4)βGalNAc and βGlcNAc(1-3)βGlcA6Lys, were revealed also in the O-antigens of *P. mirabilis* O3 (S1959, OXK) [21,33] and O27 [34], respectively.

A number of cross-reactions were observed between different *Proteus* strains in ELISA and other serological assays. In most cases the cross-reactivity could be substantiated by sharing of an epitope (or epitopes) by structurally similar, but not identical, polysaccharides. Thus, two strains of *P. mirabilis* O14 demonstrated strong two-way serological cross-reactivity, due to the presence in the O-antigens of a common antigenic epitope associated with a non-carbohydrate acidic substituent, 2-[(*R*)-1-carboxyethylamino]ethyl phosphate (DAlaEtn-*P*). Carbohydrate backbones of their polysaccharides are different and define unique epitopes in the two strains [35]. In contrast, the serological relatedness of two strains of *P. penneri* O64 is based on the presence of the same (in strains 19 and 71) or a very similar backbone (strain 62). Serological differences between these strains are related to the presence or absence of the (*S*)-lactic acid residue in GlcNAc or/and to the mode of substitution of GlcNAc3*S*Lac (at position 4 or 6) [18,19].

In most cross-reactive strains, common epitopes are associated with identical or similar oligosaccharide fragments, such as disaccharides in *P. mirabilis* O6 and *P. vulgaris* O8 [36], trisaccharides in *P. vulgaris* O22 and O32 [37], or tetrasaccharides in *P. mirabilis* O26 and O28 [30,38]. However, the O-specific polysaccharides of some other cross-reactive strains exhibit no structural similarity as, for instance, *P. penneri* O65 and O67 [25] or *P. penneri* strains 14, 15, 52 and *P. vulgaris* O19 [15]. Western immunoblotting with the corresponding LPS revealed that in these cases the cross-reactivity is due to the presence of antibodies directed against the core region of LPS. It can be suggested, that the core regions are similar or identical, but their structures have not been determined yet and the degree of their diversity cannot be estimated.

A number of *Proteus* O-antigens demonstrate structural similarities with other bacterial polysaccharides. Some of them, that contain at least a trisaccharide fragment in common, are shown below. A fragment shared by the O-antigen of *P. vulgaris* O25 (**12**) [39] and the capsular polysaccharide of *Alteromonas haloplanktis* KMM 156 (**13**) [40] is branched and includes a lateral acidic monosaccharide 3-*O*-[(*R*)-1-carboxyethyl]-D-glucose (Glc3*R*Lac). In two other pairs, **14** and **15**, **16** and **17**, the related polysaccharides are linear. Such similarity between the antigens of taxonomically remote bacteria have been observed not only for *Proteus,* but also for some other genera. It may result in misidentification of strains using serological methods. However, the O-antigens of *P. penneri* 14 (**14**) [41,42] and *Escherichia coli* O:114 (**15**) [43] exhibited only a weak cross-reactivity [41], that may be accounted for by different sizes of the repeating units (a pentasaccharide in the former and a tetrasaccharide in the latter polysaccharide). No data on the serological relationship between the other pairs of the related polysaccharides are available.

-3)GlcNAc(β1-2)LRha(α1-2)Rib*f*(β1-4)GalNAc(β1-
Glc3*R*Lac(α1-3)┘
12 (*P. vulgaris* O25 [39])

-4)GlcNAc(β1-2)LRha(α1-2)LRha(β1-
Glc3*R*Lac(α1-3)┘
13 (*Alteromonas haloplanktis* KMM 156 [40])

-2)Rib*f*(β1-4)Gal(β1-3)GlcNAc(β1-2)Qui3NAcDAla(β1-4)GalA6Ala(α1-
14 (*P. penneri* 14 [41,42])

-3)Rib*f*(β1-4)Gal(β1-3)GlcNAc(α1-4)Qui3NAcSer(β1-
15 (*Escherichia coli* O:114 [43])

-2)LRha(α1-2)LRha(α1-4)GalA(β1-3)GlcNAc(β1-4)GalA(α1-
16 (*P. vulgaris* O32 [37])

-2)LRha*p*(α1-2)LRha*p*3Ac(α1-4)Gal*p*A(β1-3)Gal*p*NAc(β1-
17 (*Shigella flexnerii* 6 biovar newcastle [44])

Well known is a serological cross-reactivity between *Proteus* OX group strains (*P. vulgaris* O1 and O2 and *P. mirabilis* O3) and human *Rickettsia* antibodies. This allowed the use of *Proteus* antigens for serodiagnostics of rickettsiosis, such as typhus, spotted fevers and tsutsugamushi (Weil-Felix test) [45]. The cross-reactivity is one-way only, since rabbit *Proteus* antibodies do not bind surface antigens of *Rickettsia.* Immunochemical studies showed that cross-reactive *Proteus* epitopes are located within the O-specific polysaccharides. The structures of the three *Proteus* O-antigens, which are involved in theWeil-Felix test, were established [22] and found to be unique among bacterial polysaccharides (Table 1). These findings mark an important step to the understanding of the Weil-Felix test on the molecular level, that require also determination of so far unknown structures of *Rickettsia* antigens.

4. Conclusions

Structures of 39 O-antigens of *Proteus* have been elucidated so far using chemical and NMR spectroscopic methods. They represent a peculiar group of polysaccharides which, in addition to monosaccharides widespread in nature, contain some rarely occurring sugars and non-sugar components, such as 2,3-diamino-2,3,6-trideoxy-L-mannose, L-altruronic acid, 2-[(*R*)-1-carboxyethylamino]ethanol and N^{ε}-[(*R*)-1-carboxyethyl]-L-lysine. Most of the polysaccharides are acidic, due to the presence of hexuronic acids, their amides with amino acids, phosphate groups, residues of lactic and pyruvic acid.

The acidic character of the O-antigens may contribute to the virulence of clinically important *Proteus* strains. *Proteus* LPS with chemically defined acidic and neutral O-specific polysaccharide are useful in studies of the mechanism of formation of bladder and kidney stones. They serve also as molecular markers for chemical taxonomy of *Proteus* species and their phylogenetic relationships to other bacteria.

The diversity of the O-antigen structures is reflected in the wide spectrum of the specificity of antibodies, produced by the immune system against *Proteus* bacteria. Therefore, the chemical data represent the molecular basis of the serological classification, proposed by Kauffmann and Perch for *P. mirabilis* and *P. vulgaris*. They are useful for the creation of a similar classification of *P. penneri*, based on the unique collection of strains of this species in the Institute of Microbiology and Immunology, University of Łódź, Poland. Characterization of particular epitopes, which are recognized by specific antibodies, demonstrated the implication of the O-antigens in the serological cross-reactivity, which is typical of many *Proteus* strains.

The O-antigens and O-antisera with defined epitope specificity can be used for serodiagnosis and epidemiological studies of *Proteus*. They can show the distribution of various *P. mirabilis, P. vulgaris* and *P. penneri* strains among clinical isolates and their affiliation to particular O-serogroups. It will be interesting also to investigate the potential protectivity of polyclonal antibodies against *Proteus* O-antigens from different serogroups to select those of them, which give cross-protection in *Proteus* infections.

Acknowledgments

Authors express gratitude to their Masters, Prof. N.K. Kochetkov and Prof. K. Kotelko. Authors thank Dr. U. Zähringer (Research Centre Borstel, Germany) for critical reading of the manuscript. This work was partially supported by RFBR grants 96-04-50460 and 96-15-97380 (for YK), and the Polish State Committee for Scientific Research: grants 4PO5A 078 14 (for WK), 4PO5A 140 14 (for AR) and 6PO4A 007 14 (for ZS).

REFERENCES

1. Warren J.W., in *Urinary tract infections. Molecular pathogenesis and clinical management*, (Mobley H.L.T. and Warren J.W. Eds, ASM Press, Washington, 1996), pp. 3–27.
2. a. Penner J.L., in *The prokaryotes*, (Ballows A., Trüper H.G., Harder W. and Schleifer H. Eds, Springer-Verlag KG, Berlin, 1992), vol. III, pp. 2849–2863; b. Rozalski A., Sidorczyk Z. and Kotelko K., *Microbiol. Mol. Biol. Rev.*, **61**, 65 (1997).

3. Rietschel E.T., Kirikae T., Schade F.U., Mamat U., Schmidt G., Loppnow H., Ulmer A.J., Zähringer U., Seydel U., Di Padova F., Schreier M. and Brade H., *FASEB J.*, **8**, 217 (1994).
4. Larsson P., *Methods Microbiol.*, **14**, 187 (1984).
5. a. Kauffmann, F., *The Bacteriology of Enterobacteriaceae* (Williams and Wilkins, Baltimore MD, 1966), pp. 333–360; b. Penner J.L. and Hennessy C., *J. Clin. Microbiol.*, **12**, 304 (1980).
6. Knirel Y.A., Vinogradov E.V., Shashkov A.S., Sidorczyk Z., Rozalski A., Radziejewska-Lebrecht J. and Kaca W., *J. Carbohydr. Chem.*, **12**, 379 (1993).
7. Knirel Y.A. and Kochetkov N.K., *Biochemistry (Moscow)*, **59**, 1325 (1994).
8. Kocharova N.A., Vinogradov E.V., Borisova S.A., Shashkov A.S. and Knirel Y.A., *Carbohydr. Res.*, **309**, 131 (1998).
9. Gmeiner J., *Eur. J. Biochem.*, **74**, 171 (1977).
10. Kenne, L. and Lindberg, B., in *The Polysaccharides*, (Aspinall G.O. Ed, Academic Press, NY, 1983), pp. 287–363.
11. a. Petersson C., Jachymek W., Klonowska A., Lugowski C., Niedziela T. and Kenne L., *Eur. J. Biochem.*, **245**, 668 (1997); b. Gamian A., Katzenellenbogen E., Romanowska E., Grosskurth H. and Dabrowski J., *Carbohydr. Res.*, **307**, 173 (1998); c. Katzenellenbogen E., Kocharova N.A., Zatonsky G.V., Bogulska M., Witkowska D., Shashkov A.S., Romanowska E. and Knirel Y.A., *J. Carbohydr. Chem.*, in press.
12. Dumanski A.J., Hedelin H., Edin-Liljegren A., Beauchemin D. and McLean R.J.C., *Infect. Immun.*, **62**, 2998 (1994).
13. Gygi D., Rahman M.M., Lai H.C., Carlson R., Guard-Petter J. and Huhges C., *Mol. Microbiol.*, **17**, 1167 (1995).
14. Perry M.B. and MacLean L.L., *Carbohydr. Res.*, **253**, 257 (1994).
15. Zych K., Kowalczyk M., Toukach F.V., Paramonov N.A., Shashkov A.S., Knirel Y.A. and Sidorczyk Z., *Arch. Immunol. Ther. Exp.*, **45**, 435 (1997).
16. Knirel Y.A., Paramonov N.A., Vinogradov E.V., Shashkov A.S., Kochetkov N.K., Sidorczyk Z. and Swierzko A., *Carbohydr. Res.*, **235**, C19 (1992).
17. Sidorczyk Z., Swierzko A., Zych K., Paramonov N.A., Vinogradov E.V., Shashkov A.S. and Knirel Y.A., *Arch. Immunol. Ther. Exp.*, **44**, 179 (1996).
18. Senchenkova S.N., Shashkov A.S., Knirel Y.A., Kochetkov N.K., Zych K. and Sidorczyk Z., *Carbohydr. Res.*, **293**, 71 (1996).
19. Sidorczyk Z., Zych K., Kocharova N.A., Shashkov A.S., Toukach F.V. and Knirel Y.A., in *Abstr. Pap. Vth Conference of the International Endotoxin Society*, (Santa Fe, 1998), Abstr. NM 54.
20. Shashkov A.S., Lipkind G.M., Knirel Y.A. and Kochetkov N.K., *Magn. Reson. Chem.*, **26**, 735 (1988).
21. Knirel Y.A., Vinogradov E.V. and Mort A.J., *Adv. Carbohydr. Chem. Biochem.*, **47**, 167 (1989).
22. Ziolkowski A., Shashkov A.S., Swierzko A., Senchenkova S.N., Toukach F.V., Cedzynski M., Amano K.-I., Kaca W. and Knirel Y.A., *FEBS Lett.*, **411**, 221 (1997).
23. Sidorczyk Z., Zych K., Swierzko A., Vinogradov E.V. and Knirel Y.A., *Eur. J. Biochem.*, **240**, 245 (1996).
24. Arbatsky N.P., Mamyan S.S., Shashkov A.S., Knirel Y.A., Kochetkov N.K., Zych K. and Sidorczyk Z., *Carbohydr. Res.*, **310**, 85 (1998).
25. Toukach F.V., Arbatsky N.P., Shashkov A.S., Knirel Y.A., Zych K. and Sidorczyk Z., *Carbohydr. Res.*, **312**, 97 (1998).
26. Zych K., Knirel Y.A., Paramonov N.A., Vinogradov E.V., Arbatsky N.P., Senchenkova S.N., Shashkov A.S. and Sidorczyk Z., *FEMS Immunol. Med. Microbiol.*, **21**, 1 (1998).
27. Shashkov A.S., Arbatsky N.P., Toukach F.V., Knirel Y.A., Moll H., Zähringer U., Zych K. and Sidorczyk Z., *Eur. J. Biochem.*, in press.
28. Cedzynski M., Swierzko A.S., Ziolkowski A., Rozalski A., Kaca W., Paramonov N.A., Vinogradov E.V. and Knirel Y.A., *Microbiol. Immunol.*, **42**, 7 (1998).
29. Bartodziejska B., Shashkov A.S., Torzewska A., Grachev A.V., Ziolkowski A., Paramonov N.A., Rozalski A. and Knirel Y.A., *Eur. J. Biochem.*, **259**, 212 (1999).
30. Shashkov A.S., Toukach F.V., Paramonov N.A., Ziolkowski A., Senchenkova S.N., Kaca W. and Knirel Y.A., *FEBS Lett.*, **386**, 247 (1996).
31. Radziejewska-Lebrecht J., Shashkov A.S., Grosskurth H., Bartodziejska B., Knirel Y.A., Vinogradov E.V., Rozalski A., Kaca W., Kononov L.O., Chernyak A.Y., Mayer H. and Kochetkov N.K., *Eur. J. Biochem.*, **230**, 705 (1995).
32. Sidorczyk Z., Swierzko A., Knirel Y.A., Vinogradov E.V., Chernyak A.Y., Kononov L.O., Cedzynski M., Rozalski A., Kaca W., Shashkov A.S. and Kochetkov N.K., *Eur. J. Biochem.*, **230**, 713 (1995).
33. Swierzko A., Cedzynski M., Knirel Y.A., Senchenkova S.N., Kocharova N.A., Shashkov A.S., Amano K.-I., Kyohno K. and Kaca W., *Biochemistry (Moscow)*, **62**, 21 (1997).

34. Vinogradov E.V., Krajewska-Pietrasik D., Kaca W., Shashkov A.S., Knirel Y.A. and Kochetkov N.K., *Eur. J. Biochem.*, **185**, 645 (1989).
35. Perepelov A.V., Ujazda E., Senchenkova S.N., Shashkov A.S., Kaca W. and Knirel Y.A., *Eur. J. Biochem.*, in press.
36. Perepelov A.V., Babicka D., Shashkov A.S., Arbatsky N.P., Senchenkova S.N., Rozalski A. and Knirel Y.A., *Carbohydr. Res.*, in press.
37. Bartodziejska B., Shashkov A.S., Babicka D., Grachev A.V., Torzewska A., Paramonov N.A., Chernyak A.Y., Rozalski A. and Knirel Y.A., *Eur. J. Biochem.*, **256**, 488 (1998).
38. Shashkov A.S., Toukach F.V., Paramonov N.A., Senchenkova S.N., Kaca W. and Knirel Y.A., *Biochemistry (Moscow)*, **61**, 15 (1996).
39. Knirel Y.A., Kaca W., Paramonov N.A., Cedzynski M., Vinogradov E.V., Ziolkowski A., Shashkov A.S. and Rozalski A., *Eur. J. Biochem.*, **247**, 951 (1997).
40. Gorshkova R.P., Nazarenko E.L., Zubkov V.A., Ivanova E.P., Ovodov Y.S., Shashkov A.S. and Knirel Y.A., *Bioorg. Khim.*, **19**, 327 (1993).
41. Sidorczyk Z., Swierzko A., Vinogradov E.V., Knirel Y.A. and Shashkov A.S., *Arch. Immunol. Ther. Exp.*, **42**, 209 (1994).
42. Vinogradov E.V., Shashkov A.S., Knirel Y.A., Kochetkov N.K., Sidorczyk Z. and Swierzko A., *Carbohydr. Res.*, **219**, C1 (1991).
43. Dmitriev B.A., L'vov V.L., Tochtamysheva N.V., Shashkov A.S., Kochetkov N.K., Jann B. and Jann K., *Eur. J. Biochem.*, **134**, 517 (1983).
44. Dmitriev B.A., Knirel Y.A., Sheremet O.K., Shashkov A.S., Kochetkov N.K. and Hofman I.L., *Eur. J. Biochem.*, **98**, 309 (1979).
45. Vinson J.W., in *Manual of Clinical Immunology*, (Rose R. and Friedman F. Eds, American Society for Microbiology, Washington, 1976), pp. 500–504.
46. Senchenkova S.N., Shashkov A.S., Toukach F.V., Ziolkowski A., Swierzko A., Amano K.-I., Kaca W., Knirel Y.A. and Kochetkov N.K., *Biochemistry (Moscow)*, **62**, 461 (1997).
47. Cedzynski M., Knirel Y.A., Amano K.-I., Swierzko A., Paramonov N.A., Senchenkova S.N. and Kaca W., *Biochemistry (Moscow)*, **62**, 15 (1997).
48. Vinogradov E.V., Kaca W., Knirel Y.A., Rozalski A. and Kochetkov N.K., *Eur. J. Biochem.*, **180**, 95 (1989).
49. Toukach F.V., Bartodziejska B., Senchenkova S.N., Wykrota M., Shashkov A.S., Rozalski A. and Knirel Y.A., *Carbohydr. Res.*, submitted for publication.
50. Beynon L.M., Dumanski A.J., McLean R.J.C., MacLean L.L., Richards J.C., Perry M.B., *J. Bacteriol.*, **174**, 2172 (1992).
51. Shashkov A.S., Senchenkova S.N., Toukach F.V., Ziolkowski A., Paramonov N.A., Kaca W., Knirel Y.A. and Kochetkov N.K., *Biochemistry (Moscow)*, **61**, 1100 (1996).
52. Swierzko A., Shashkov A.S., Senchenkova S.N., Toukach F.V., Ziolkowski A., Cedzynski M., Paramonov N.A., Kaca W. and Knirel Y.A., *FEBS Lett.*, **398**, 297 (1996).
53. Shashkov A.S., Toukach F.V., Senchenkova S.N., Ziolkowski A., Paramonov N.A., Kaca W., Knirel Y.A. and Kochetkov N.K., *Biochemistry (Moscow)*, **62**, 509 (1997).
54. Uhrín D., Chandan V. and Altman E., *Can. J. Chem.*, **73**, 1600 (1995).
55. Senchenkova S.N., Zatonsky G.V., Ujazda E., Shashkov A.S., Kaca W. and Knirel Y.A., *Biochemistry (Moscow)*, **62**, 1444 (1997).
56. Vinogradov E.V., Pietrasik D., Shashkov A.S., Knirel Y.A. and Kochetkov N.K., *Bioorg. Khim.*, **14**, 1282 (1988).
57. Vinogradov E.V., Knirel Y.A., Kochetkov N.K., Radziejewska-Lebrecht J. and Kaca W., *Bioorg. Khim.*, **19**, 1132 (1993).
58. Perepelov A.V., Shashkov A.S., Senchenkova S.N., Knirel Y.A., Literacka E. and Kaca W., *Biochemistry (Moscow)*, submitted for publication.
59. Shashkov A.S., Toukach F.V., Ziolkowski A., Paramonov N.A., Senchenkova S.N., Kaca W. and Knirel Y.A., *Biochemistry (Moscow)*, **61**, 575 (1996).
60. Cedzynski M., Knirel Y.A., Rozalski A., Shashkov A.S., Vinogradov E.V. and Kaca W., *Eur. J. Biochem.*, **232**, 558 (1995).
61. Uhrín D., Brisson J.-R., MacLean L.L., Richards J.C. and Perry M.B., *J. Biomolec. NMR*, **4**, 615 (1994).
62. Knirel Y.A., Paramonov N.A., Vinogradov E.V., Kochetkov N.K., Sidorczyk Z. and Zych K., *Carbohydr. Res.*, **259**, C1 (1994).
63. Sidorczyk Z., Swierzko A., Knirel Y.A., Shashkov A.S., Vinogradov E.V. and Kochetkov N.K., in *Abstr. Pap. VIIth European Symposium on Carbohydrates*, Cracow, 1993, Abstr. B 077.

64. Vinogradov E.V., Sidorczyk Z., Swierzko A., Rozalski A., Daeva E.D., Shashkov A.S., Knirel Y.A. and Kochetkov N.K., *Eur. J. Biochem.*, **197**, 93 (1991).
65. Arbatsky N.P., Shashkov A.S., Widmalm G., Knirel Y.A., Zych K. and Sidorczyk Z., *Carbohydr. Res.*, **298**, 229 (1997).
66. Shashkov A.S., Arbatsky N.P., Widmalm G., Knirel Y.A., Zych K. and Sidorczyk Z., *Eur. J. Biochem.*, **253**, 730 (1998).
67. Knirel Y.A., Shashkov A.S., Vinogradov E.V., Kochetkov N.K., Swierzko A. and Sidorczyk Z., *Carbohydr. Res.*, **275**, 201 (1995).
68. Bartodziejska B., Radziejewska-Lebrecht J., Lipinska M., Knirel Y.A., Kononov L.O., Chernyak A.Y., Mayer H. and Rozalski A., *FEMS Immunol. Med. Microbiol.*, **13**, 113 (1996).