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LIPOPOLYSACCHARIDE (LPS, ENDOTOXIN) OF *PROTEUS* BACTERIA – CHEMICAL STRUCTURE, SEROLOGICAL SPECIFICITY AND THE ROLE IN PATHOGENICITY

Abstract: Lipopolysaccharides (LPS) of Gram-negative bacteria are composed of three regions: O-specific chain (OPS), the core oligosaccharide and lipid A. All three regions of *Proteus* LPS were studied. The differences in the structure of OPS serve as the basis for the serological classification of *Proteus* strains. The serological classification scheme of these bacteria currently consists of 76 serogroups. The structural diversity of the core region is characteristic for *Proteus* sp. and distinguishes this genus from other bacteria. In this paper the results of structural, immunochemical and serological studies of all three regions of *Proteus* LPS, as well as a function of LPS as endotoxin and its role in the formation of urinary stones, swarming phenomenon and bacterial growth in biofilm are reported.

Key words: bacterial lipopolysaccharide, endotoxin, *Proteus*.

1. INTRODUCTION

The genus *Proteus* was described in 1885 by HAUSER and originally had two species *P. mirabilis* and *P. vulgaris*. It belongs to the *Enterobacteriaceae* family and currently consists of five species: *P. mirabilis*, *P. vulgaris*, *P. penneri*, *P. hauseri* and *P. myxofaciens*, a well as three unnamed *Proteus* genomospecies 4, 5 and 6. *Proteus myxofaciens* is the only *Proteus* species without any significance in the pathogenicity of humans, it has been isolated form living and death larvae of the gypsy moth *Porthetia dispar* (JANDA, ABBOT 2006). *Proteus* rods are widely distributed in the natural environment where they are involved in the decomposing of the organic matter of the animal origin, they are also present in the intestines of humans and animals. They are opportunistic bacterial pathogens which under favorable conditions cause urinary tract infections (UTI), wound infections, meningitis in neonates or infants

and rheumatoid arthritis (MOHR O'HARA et al. 2000; JANDA, ABBOT 2006). UTI caused by these bacteria usually take place in patients with urinary catheter in place or with structural and/or functional abnormalities in urinary tract, as well as after surgical intervention in the urogenital system. *Proteus* rods are also associated with nosocomial infections. *P. mirabilis* causes UTI with the highest frequency among all *Proteus* species. It causes complicated infections and infections in long catheterized patients. *Proteus* rods can cause hematogenous infections and ascending infections, however, the latter are more common for these microorganisms (WARREN 1996).

The most characteristic features of *Proteus* bacteria is their ability to swarm on solid surfaces (BELAS 1996). Swarming results from the bacterial transformation of peritrichously flagellated short rods called swimmer cells to elongated, multinucleated nonseptated forms with increased number of flagella termed as swarmer cells. Swimmer cells grow in liquid media, whereas swarmer cells are formed in solid media. Population of swarmer cells can migrate in coordinated way on the solid media and then disintegrate into short rods. The process of differentiation of swimmer cells to swarmer cells, their migration on solid media and disintegration to short rods, known as swarming phenomenon or swarming growth, is cyclical (RATHER 2005). Both morphologically and physiologically different cells – swimmer short rods and swarmer elongated cells play an important role in pathogenesis. Highly flagellated swarmer cells are thought to be crucial in ascending UTI infections, whereas short rods containing fimbriae are responsible for colonization of host mucosal surface (BELAS 1992).

Proteus rods have evolved multiple virulence factors which act in concert but not individually. These are fimbriae, flagella, enzymes (urease, hydrolyzing urea to CO_2 and NH_3; proteases degrading antibodies, tissue matrix proteins and proteins of complement system; deaminases hydrolyzing amino acids to α-keto-acids, playing a role of siderophores binding iron) and toxins – hemolysins as well as endotoxin – lipopolysaccharide (LPS) (COKER et al. 2000; MOBLEY 1996; RÓŻALSKI et al. 1997; RÓŻALSKI 2002).

2. LPS – GENERAL INFORMATION

Lipopolysaccharide of Gram-negative bacteria are composed of three genetically and structurally distinct regions: O-specific chain (O-antigen, O-specific polysaccharide), the core oligosaccharide and lipid A, which anchors the LPS molecule to the bacterial outer membrane (RAETZ, WHITFIELD 2002). All three regions of *Proteus* LPS have been studied (RÓŻALSKI 2002). LPS containing all these three regions is produced by smooth forms of bacteria. Rough strains or rough mutants of different classes (Ra-Re mutants) synthesize LPS containing

lipid A and core region or part of the core region (RÓŻALSKI et al. 2002). Lipopolysaccharide participates in the physiological function of outer membrane of bacterial cells and is essential for its growth and survival. It is also a target for interaction with antibacterial drugs and immune mechanisms of the host. LPS, released from the bacterial surface in infected macroorganism induces a spectrum of biological activities important in the pathogenesis, particularly in septic shock (ŁUKASIEWICZ, ŁUGOWSKI 2003).

The differences in the structure of O-antigens serve as the basis for the serological classification of *Proteus* strains. The serological classification scheme of KAUFMAN and PERCH includes 49 different *P. mirabilis* and *P. vulgaris* O-serogroups and 19 serologically distinct flagellar H-antigens (KAUFFMANN 1966). The chemical and serological studies performed in the Institute of Microbiology and Immunology, University of Lodz in collaboration with N. D. ZELINSKY Institute of Organic Chemistry, Russian Academy of Sciences in Moscow, Russia, have led to establishing additional O-serogroups, which were created to classify *P. penneri* and *P. hauseri* strains, as well as *P. myxofaciens*, *Proteus* genomospecies and some *P. mirabilis* and *P. vulgaris* strains unclassified before (KNIREL et al. 1993; KNIREL et al. 1999; RÓŻALSKI 2004). The serological classification scheme currently consists of 76 serogroups (DRZEWIECKA et al., 2004; DRZEWIECKA, SIDORCZYK 2005; ZABŁOTNI, 2006). In this review I report on the chemical structure of the O-antigens of *Proteus* bacilli and their serological specificity, as well as on the core and lipid A region of endotoxin and on selected biological roles of LPS of these bacteria.

3. O-SPECIFIC PART OF LPS

Proteus O-antigens are linear or branched polysaccharides built up of oligosaccharide repeating units. All polysaccharides with one exception of *P. vulgaris* O53 contain amino sugars either D-glucosamine (GlcN) or D-galactosamine (GalN). Acidic O-specific polysaccharides (OPS) represent the 90% of *Proteus* O-antigens. OPSs of these bacteria are acidic due the presence of uronic acids – D-glucuronic acid (GlcA), D-galacturonic acid (GalA), L-altruronic acid (L-AltA). Some OPSs are acidic also due to the presence of (R) or (*S*) lactic acid or less often malonic (Mal), pyruvic (Pyr) or succinic (Suc) acids, which are linked to the sugar residues. Hexuronic acids GlcA and GalA usually have a free carboxyl group, however, they are very often amidated with α-amino group of amino acids – L-alanine (L-Ala), L-lysine (L-Lys), L-serine (L-Ser) and L-threonine (L-Thr). Two OPSs of *P. mirabilis* O13 and *P. myxofaciens* O60 contain amides of GlcA and GalaA, respectively with N^{ε}-[(R)-1-carboxyethy]l-L--lysine (AlaLys). (DRZEWIECKA et al. 2004; RÓŻALSKI et al. 2002; Różalski 2004).

Table 1: The chemical structure of the O-specific polysaccharides (OPSs) of *Proteus* sp.

Strain/Serogroup	Chemical structure of OPS	Literature
P. vulgaris OX19 (O1)	α-L-Qui*p*NAc-(1→ 3 →4)-α-D-Gal*p*NAc-(1→4)-α-D-Gal*p*-1-*P*-(O→4)-α-L-Qui*p*NAc-(1→3)- -β-D-Glc*p*NAc-(1→	KNIREL et al. 1999
P. vulgaris OX2 (O2)	→2)-β-D-Glc*p*-(1→6)-α-D-Glc*p*NAc-(1→3)-α-L-Qui*p*NAc-(1→3)-β-D- Glc*p*NAc6Ac-(1→	KNIREL et al. 1999
P. mirabilis OXK (O3)	α-D-Gal*p*A6(L-Lys)-(1→ α-Glc*p*-(1→ 4 2 →6)-β-D-Gal*p*NAc-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Gal*p*NAc-(1→	KACA et al. 1987
P. vulgaris O4	→2)-β-D-Qui*p*4N[L-Ala(R-3HOBu)]-(1→3)-α-D-Gal*p*-(1→4)-β-D-Glc*p*A- -(1→3)-β-D-Glc*p*NAc-(1→	RÓŻALSKI et. al. 2002
P. vulgaris O8 *Proteus* genomo- -species 5	α-D-Galp-(1→ 3 →3)-β-D-Glc*p*A-(1→4)-α-L-Fuc*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→	RÓŻALSKI et. al. 2002
P. vulgaris O12	α-D-Glc*p*-(1→6)-α-D-Gal*p*NAc4Ac-(1→ 3 →6)-β-D-Glc*p*-(1→4)-α-L-Fuc*p*NAc-(1→3)-β-D-Glc*p*NAc- -(1→3)-Gro-1-*P*-(O→	RÓŻALSKI et. al. 2002
P. vulgaris O17	Etn*P*→ 6 →2)-β-D-Fuc*p*3N(*R*3HOBu)-(1→6)-α-D-Glc*p*-(1→4)-β-D-Glc*p*-(1→3)-α-D- Glc*p*NAc-(1→	TORZEWSKA et al. 2006
P. vulgaris O22	α-D-Qui*p*3NAc2,4Ac2→ 3 →2)-β-L-Rha*p*-(1→4)-α-L-Rha*p*-(1→4)-β-D-Glc*p*A-(1→3)-β-D -Glc*p*NAc-(1→	TOUKACH et al. 1999

P. mirabilis O27	β-D-Glc*p*NAc-(1→ Etn*P*→ 4 6 →3)-β-D-Glc*p*A6(L-Lys)-(1→3)-α-D-Gal*p*A6(L-Ala)-(1→3)-α-D-Glc*p*NAc-(1→	VINOGRADOV et al. 1989
P. mirabilis O28	→4)-α-D-Gal*p*A6(L-Lys)-(1→4)-α-D-Gal*p*-(1→3)-α-D-Gal*p*A6(L-Ser)4Ac- -(1→3)-β-D-Glc*p*NAc-(1→	RADZIEJEWSKA-LEBRECHT et al. 1995
P. vulgaris O32	→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→4)-β-D-Gal*p*A- (1→3)-β-D-Glc*p*NAc-(1	BARTODZIEJSKA et al. 1998
P. vulgaris O47	β-D-Glc*p*N-(1→ 4 →3)-β-D-Gal*p*NAc-(1→4)-α-D-Gal*p*NAc3Ac-(1→3)-(β-D-Gal*p*NAc-(1→	RÓŻALSKI et al. 2002
P. mirabilis O49	α-D-Quip4NSuc-(1→ 4 →2)-α-D-Gal*p*A-(1→3)-α-L-Rha*p*-(1→4)-α-D-Glc*p*-(1→2)-α-L -Rhap-(1→3)-β-D-Glc*p*NAc-(1→	KONDAKOVA et al. 2004
P. hauseri (O52)	→2)-α-D-Gal*p*4,6(R-Pyr)-(1→4)-β-D-Gal*p*-(1→3)-β-D-Glc*p*NAc-(1→	PEREPELOV et al. 2004
Proteus genomo- -species 4 (O56)	α-D-Glc*p*-(1→ 2 →4)-β-D-Qui*p*3NAc-(1→6)-β-D-Glc*p*NAc-(1→4)-β-D-Gal*p*A-(1→3)-α-D- -d-Gal*p*NAc-(1→	ZYCH et al. 2005
P. [redacted]iens (O60)	→6)-β-D-Glc*p*NAc-(1→3)-β-D-Glc*p*NAc-(1→4)-β-D-GlcpA6(D-AlaL-Lys)- -(1→6)-σ-D-Gal*p*NAc-(1→	SIDORCZYK et al. 2003
P. penneri O63	α-D-Gal*f*NAc-(1→4)-β-D-Glc*p*A-(1→ 4 →3)-α-D(-d-Gal*p*-(1→4)-β-D-Gal*p*-(1→3)-β-D- -Glc*p*NAc-(1→	DRZEWIECKA, SIDORCZYK 2005

Table 1: (cd.)

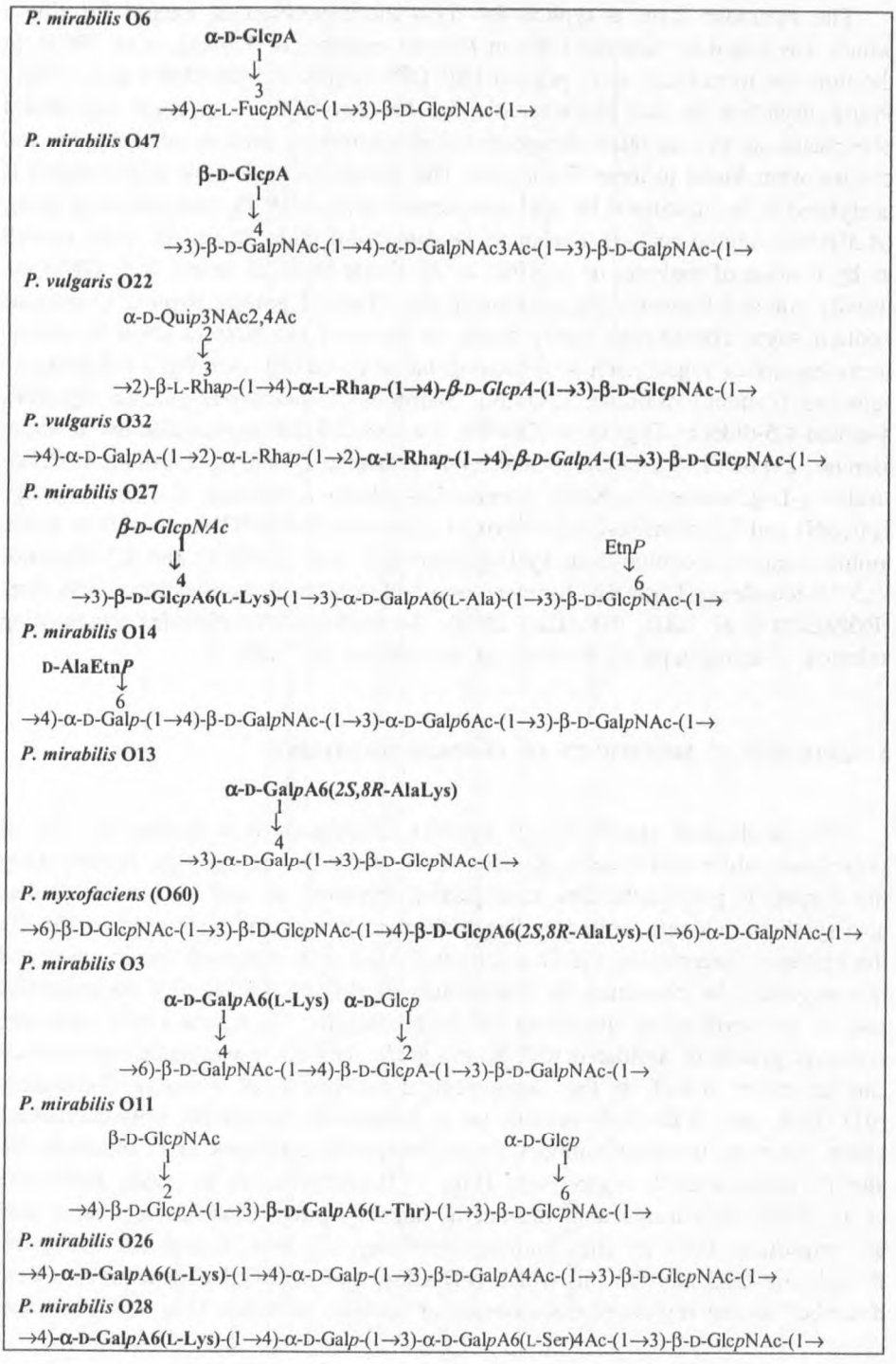
Strain/Serogroup	Chemical structure of OPS	Literature
P. penneri O66	β-L-Rha*p*NAc3NAc-(1→ 3 →4)-α-D-Glc*p*-(1→3)-α-L-6dTal*p*2Ac-(1→3)-β-D-Glc*p*NAc-(1→	DRZEWIECKA, SIDORCZYK 2005
Proteus genomo--species 6 (O69)	α-D-Glc*p*A3/4Ac-(1→ 4 →6)-β-D-Glc*p*N(L-Ala)3Ac-(1→4)-β-D-Glc*p*A-(1→3)-β-D--Glc*p*NAc6Ac-(1→	ZYCH et al. 2005
P. penneri O73	Etn*P*→ 6 →4)-Rib-ol-5-*P*-(O→4)-β-D-Glc*p*-(1→3)-β-D-Gal*p*-(1→3)-β-D-Gal*p*NAc-(1→	DRZEWIECKA, SIDORCZYK 2005

The pyranose form is typical for most monosaccharides, except for ribose which was found in furanose form in *Proteus* endotoxins (KNIREL et al. 1997). In the furanose form GalN in *P. penneri* O63 OPS is present (ARBATSKY et al. 1998). Phosphorylation is also characteristic for *Proteus* OPSs – glycerol and ribitol phosphates, as well as other phosphate-linked substituents such as ethanolamine and choline were found in these O-antigens. The amino group of most amino sugars is acetylated or is substituted by acyl components such as (*R*)-hydroxybutanoyl group (*R*-3HOBu), amino acids (L/D-alanine, D-alanine dipeptide, D-aspartic acid) as well as by residue of malonic or succinic acids. Sugar residues in ca. 35% OPSs are usually non-stoichiometrically substituted with O-acetyl groups. *Proteus* O-antigens contain sugar constituents rarely found in bacterial products or even in nature, including deoxy sugars such as 6-deoxy-L-talose (L-6dTal), 2-amino-2,6-dideoxy-L--glucose (L-quinovosamine, L-QuiN), 3-amino-3,6,-dideoxy-D-glucose (Qui3N), 4-amino-4,6-dideoxy-D-glucose (Qui4N), 2-amino-2,6-dideoxy-L-galactose (L-fucosamine, L-FucN), 3-amino-3,6-dideoxy-D-galactose (Fuc3N), 2,4-diamino-2,4,6--trideoxy-D-galactose (FucN4N), 2-amino-2,6-dideoxy-L-mannose (L-rhamnosamine, L-RhaN) and 2,3-diamino-2,3,6-trideoxy-L-mannose (L-RhaN3N), as well as acidic amino sugars – 2-amino-2-deoxy-D-galacturonic acid (GalNA) and 5,7-diamino--3,5,7,9-tetradeoxy-L-glycero-L-manno-non-2-ulosonic acid (pseudaminic acid, Pse) (RÓŻALSKI et al. 2002; RÓŻALSKI 2004). O-specific polysaccharides representing selected O-serogroups of *Proteus* sp. are shown in Table 1.

4. SEROLOGICAL SPECIFICITY OF *PROTEUS* O-ANTIGENS

The serological specificity of *Proteus* O-antigens was studied by use of polyclonal rabbit anti-O sera specific to the particular serogroups. In this study the O-specific polysaccharides, their partial structures, as well as in some studies also synthetic antigens corresponding to *Proteus* O-antigens were used to identify the epitopes determining the O-specificity. As it was expected uronic acids and hexosamines, the characteristic compounds of *Proteus* OPSs, play an important role in the serological specificity of these antigens. GlcA and GalA with free carboxyl groups or amidated with amino acids, as well *N*-acetylated amino-acids can be often found in the serological determinants of *Proteus* O-antigens. α-D-GlcA and β-D-GlcA present as a branch of O-specific polysaccharides chain, serve as immunodominant sugars in specific epitopes in *P. mirabilis* O6 and *P. vulgaris* O47, respectively (Fig. 1) (CEDZYNSKI et al. 1998; RÓŻALSKI et al. 2002). Hexuronic acid present in linear O-polysaccharides can also play an important role in the immunospecificity. It was found for OPSs of *P. vulgaris* O22 and O32 in which β-D-GlcA and α-D-GalA respectively, were described as the important components of specific epitopes. (Fig. 1) (TOUKACH

Fig. 1: Epitopes in O-specific polysaccharides recognized by homologous *Proteus* O-antisera (for literature see text). Epitopes are marked with the bold letters



et al. 1999; BARTODZIEJSKA et al. 1998). The immunodominat role of the lateral N-acetyl-D-glucosamine residue linked to the β-D-GlcA(-L-Lys) was noticed in the specificity of *P. mirabilis* O27 (VINOGRADOV et al. 1989). In some *Proteus* O-specific polysaccharides unusual compounds can also play immunodominant role, however, it is not a rule. Such a role is played by a unique component *N*-[(*R*)-1-carboxyethyl]ethanolamine phosphate linked to the Gal residue in *P. mirabilis* O14 OPS (PEREPELOV et al. 1999). The immunodominat position in OPSs of *P. mirabilis* O13 and *P. myxofaciens* O60 is occupied by AlaLys linked to the GalA and GlcA, respectively (SWIERZKO et al. 2001; SIDORCZYK et al. 2003).

The most common epitopes showed for *Proteus* O-antigens were uronic acids substituted by amino aids. The importance of α-D-GalA(-L-Lys) in the specificity of *P. mirabilis* O3 (KACA et al. 1987), O26 (SHASHKOV et al. 1996) and O28 (RADZIEJEWSKA-LEBRECHT et al. 1995), as well as α-D-GalA(-L-Thr) in the specificity of *P. mirabilis* O11 (Arbatsky et al. 2000) were found, however α-D-GalA(-L-Ser) in *P. mirabilis* O28 appeared to be out of importance in the specificity.

Polyclonal anti-O sera contain antibodies of different types of specificity. Usually, the major fraction of antibodies recognizes the main epitope which defines the group specificity, whereas the minor fractions can bind other epitopes in O-antigen or in core region of LPS. O-specific antibodies may cross react with LPS of strains belonging to the same species or genus but classified into other serogroups, as well as even with LPS of taxonomically different bacteria. Indeed, the characteristic feature of *Proteus* O-antisera is cross reactivity with heterologous lipopolysaccharides of the same genus and less often with LPS from other bacterial genera (RÓŻALSKI et al. 2002). Due to the common α-L-FucNAc-(1→3)-α/β-D-GlcNAc disaccharide such cross reactivity was observed in antigen – antibodies systems of LPS and heterologous antisera of *P. vulgaris* O8, O12, O39 and in *P. mirabilis* O6 (Fig. 2) (RÓŻALSKI 2004). The marked serological relationship showed LPS of *P. mirabilis* O7 and O49, containing D-Qui4N N-acetylated with malonic and succinic acid, respectively (KONDAKOVA et al. 2004). Close serological relatedness was also shown between *P. vulgaris* O22 and O32, due to the presence of similar trisaccharides fragments α-L-Rha-(1→4)-β-GlcA-(1→3)-β-D-GlcNAc and α-L-Rha-(1→4)-β-GalA-(1→3)-β-D-GlcNAc, respectively (TOUKACH et al. 1999; BARTODZIEJSKA et al. 1998). Comparison of the O-antigen structures of *P. vulgaris* O17 and *P. vulgaris* O45 revealed the presence of similar trisaccharide epitopes α-D-Glc*p*NAc-(1→2)-β-D-Fuc3N[*R*3HOBu]-(1→6)-α-D-Glc and β-D-Glc*p*NAc-(1→2)-β-D-Fuc3NAc-(1→6)-α-D-GlcNAc, respectively, which may account for the serological relatedness of these strains (TORZEWSKA et al. 2006).

Fig 2: Cross-reacting epitopes in OPSs from: *P. vulgaris* O8, O12, O39 and *P. mirabilis* O6; *P. mirabilis* O7 and O49; *P. vulgaris* O22 and O32; *P. vulgaris* O17 and O45 (for literature see text and Table 1). Epitopes are shown in bold type

***P. vulgaris* O8**

α-D-Galp
1
↓
3
→3)-β-D-Glc*p*A-(1→4)-**α-L-Fuc*p*NAc-(1→3)-α-D-Glc*p*NAc-**(1→

***P. vulgaris* O12**

α-D-Glc*p*-(1→6)-α-D-Gal*p*NAc4Ac
1
↓
3
→6)-β-D-Glc*p*-(1→4)-**α-L-Fuc*p*NAc-(1→3)-β-D-Glc*p*NAc-**(1→3)-Gro-1-*P*-(O→

***P. vulgaris* O39**

→8)-β-Pse*p*5Ac7Ac-(2→3)-**α-L-Fuc*p*NAc-(1→3)-α-D-Glc*p*NAc-**(1→

***P. mirabilis* O6**

α-D-Glc*p*A-
1
↓
3
→4)-**α-L-Fuc*p*NAc-(1→3)-β-D-Glc*p*NAc-**(1→

***P. mirabis* O7**

β-D-Qui*p*4NMal
1
↓
6
→2)-β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→3)-β-D-Glc*p*NAc-(1→

***P. mirabilis* O49**

α-D-Qui*p*4NSuc
1
↓
4
→2)-α-D-Gal*p*A-(1→3)-α-L-Rha*p*-(1→4)-α-D-Glc*p*-(1→2)-α-L-Rha*p*-(1→3)-β-D-Glc*p*NAc-(1→

***P. vulgaris* O22**

α-D-Qui*p*3NAc2,4Ac
2
↓
3
→2)-β-L-Rha*p*-(1→4)-**α-L-Rha*p*-(1→4)-β-D-Glc*p*A-(1→3)-β-D-Glc*p*NAc-**(1→

***P. vulgaris* O32**

→4)-α-D-Gal*p*A-(1→2)-α-L-Rha*p*-(1→2)-**α-L-Rha*p*-(1→4)-β-D-Gal*p*A-(1→3)-β-D-Glc*p*NAc-**(1→

***P. vulgaris* O17**

→2)-β-D-Fuc*p*3N(3HOBu)4Ac-(1→6)-α-D-Glc*p*3Ac-(1→4)-β-D-Glc*p*A-(1→3)-**α-D-Glc*p*NAc-(1→**

***P. vulgaris* O45**

→2)-β-Fuc*p*3NAc-(1→6)-α-Glc*p*NAc-(1→4)-α-Gal*p*NAc-(1→4)-α-Gal*p*A-(1→3)-**β-Glc*p*NAc-(1→**

Proteus O-antigens show also similarity with OPSs from bacteria of other genera. This fact can be reflected in the cross reactivity of *Proteus* O--antisera with heterologous LPS. For example, such cross reactivity was found between anti-*P. mirabilis* O13 serum, as well anti-*P. myxofaciens* serum and LPS from *Providencia alcalifaciens* O14 and O23. Serological studies revealed an important role of AlaLys for the specificity of these O-antigens (KOCHAROVA et al. 2003; TORZEWSKA et al. 2004a). The common compound D-Qui4N carrying N-linked N-acetyl-aspartic acid is responsible for antigenic relationship of O-antigens of *P. mirabilis* O38, as well as *P. alcalifaciens* O4 and O33 (KOCHAROVA et al. 2004; TORZEWSKA et al. 2004b). Strong structural and serological similarity was also found between *P. vulgaris* O21, *P. mirabilis* O48 and *Hafnia alvei* 744 and PCM 1194 (BARTODZIEJSKA et al. 2000).

P. mirabilis strains classified into OXK serogroup (O3) cross-react with sera directed to *Orientia tsutsugamushi*. Similar cross reactivity was shown between *P. vulgaris* OX19 (O1), as well as *P. vulgaris* OX2 (O2) and antibodies from patients with rickettsial infections. Strains belonging to these serogroups are commonly used in unspecific Weil-Felix test for serodiagnostics of rickettiosis. Structural and immunochemical studies revealed that the common epitopes which are recognized by these antibodies reside in *Proteus* OPSs, however, their exact structures remain unknown (RÓŻALSKI et al. 1997).

5. CORE REGION

The core region of *Proteus* lipopolysaccharides was studied in rough mutants or in smooth forms classified into different serogroups (RADZIEJEWSKA--LEBRECHT et al. 1989; VINOGRADOV et al. 1994; VINOGRADOV et al. 2002). The structural diversity of the core is characteristic for *Proteus* sp. and makes it different from *E. coli* and *Salmonella* (HOLST 1999; VINOGRADOV et al. 2002). The core region of *Proteus* strains is composed of two parts – inner part, common for several number of strains and second, outer part, which is characterized by a structural variability from strain to strain. The common part is not identical in all *Proteus* strains and is subdivided to three forms known as glycoforms I–III (Fig. 3). The outer part of core region (outer core) contains oligosaccharide characteristic for particular *Proteus* strains (Table 2) (VINOGRADOV et al. 2002).

Fig. 3: Chemical structure of the inner core region of Proteus LPS (for chemical structure of the outer core see Table 2). (VINOGRADOV et al. 2002, RÓŻALSKI 2004)

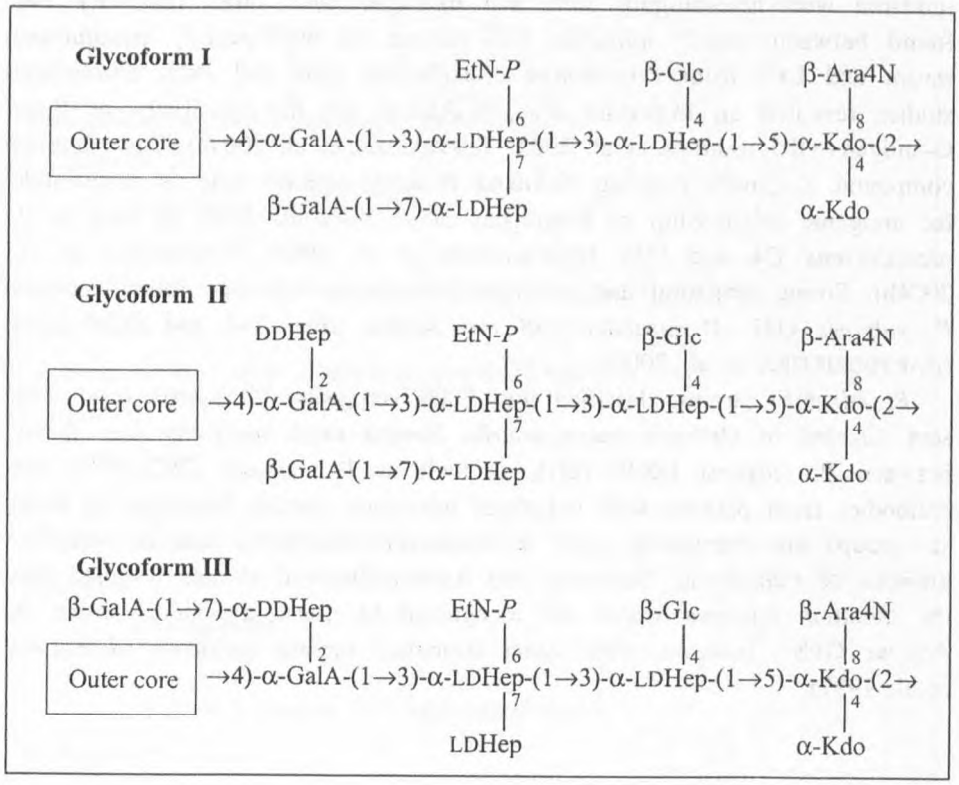


Table 2: The outer core oligosaccharides of selected *Proteus* strains (VINOGRADOV et al. 2002)

Proteus strains	Outer core structure
P. mirabilis O3; R110	α-DD-Hep-(1→6)-α-GlcN
P. mirabilis O6	β-Qui4NAlaAla-(1→3)-α-Gal-(1→6)-α-GlcNAc-(1→4)-α-GalN
P. mirabilis O27	β-Glc-(1→5)-(*1S*)-Gal*o*NAc-(1→4,6)-α-GalN
P. mirabilis O28, *P. penneri* 42	β-GalALys4PEtn-(1→3)-β-GlcNAc-(1→6)-GlcNGly
P. mirabilis O57	β-Qui4NAlaAla-(1→3)-α-GalNAc-(1→6)-α-Glc-(1→4)-α-GalN
P. vulgaris O2, O4, O8, OX19; *P. penneri* 2, 11, 17, 19, 107	β-Gal-(1→4)-(*1S*)-Gal*o*NAc-(1→4,6)-α-GalN

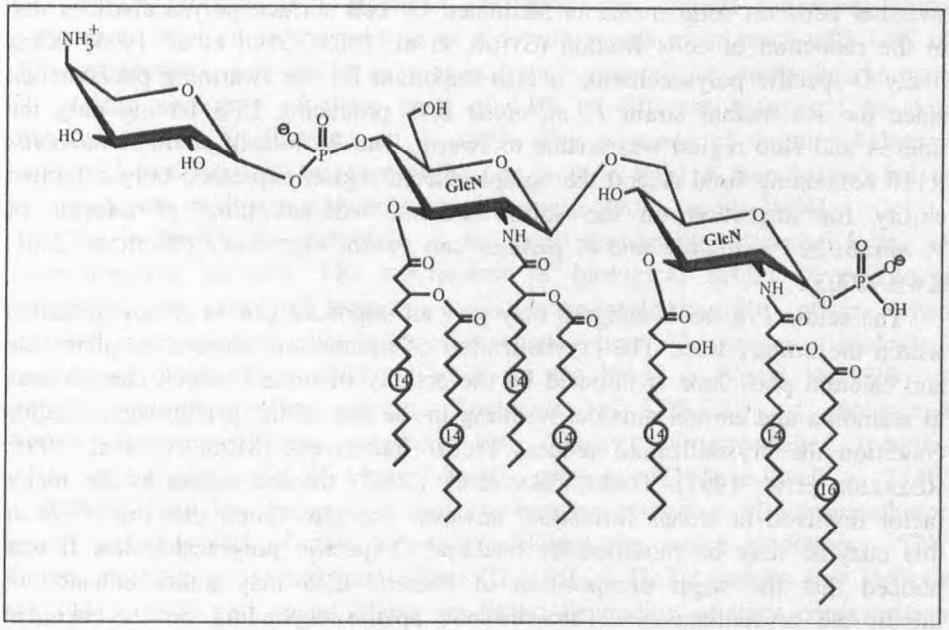
Table 2: (cont.)

Proteus strains	Outer core structure
P. vulgaris O25	β-Kdo-(2→3)¬ β-GlcNAc-(1→2)-β-Gal-(1→6)-α-GlcN
P. penneri 7, 14, 21	α-Glc-(1→4)-α-GalNAc-(1→2)-α-DD-Hep-(1→6)-α-GlcNGly
P. penneri 16, 18	α-FucNHb-(1→4)-α-Gal-(1→6)-β-Glc-(1→3)-α-GalN6*P*-Etn

6. LIPID A

Lipid A is a biological domain of endotoxin. Structurally it is a conservative region of LPS and in *Proteus* contains glucosamine disaccharide substituted with phosphate residues and fatty acids. *Proteus* lipid A differs form lipid A of *E. coli* and *Salmonella* in the presence of 4-amino-4-deoxy-arabino-L-arabinose, which quantitatively substitutes the ester-linked phosphate residue of glucosamine backbone (Fig. 4) (SIDORCZYK et al. 1983).

Fig. 4: Chemical structure of the lipid A of *P. mirabilis* (SIDORCZYK et al. 1983).



7. BIOLOGICAL ROLE OF *PROTEUS* LPS

Proteus LPS as an endotoxin and as a cell surface antigen is associated with broad spectrum of biological activities, and with interactions with bacterial or eukaryotic cells. O-specific polysaccharide chain is exposed outside bacteria, and with capsular polysaccharides are involved in glycocalyx formation. Glycocalyx forms bacterial capsule, and via lectins or cations binds bacterial cells and makes possible the adherence of bacterial populations to each other and to the epithelial cells of macroorganisms or artificial surface e.g. urological catheters (COSTERTON et al. 1978). These properties of glycocalyx enable bacteria to grow in the form of biofilm on a solid surface. Bacterial biofilm is defined as matrix-enclosed bacterial population adherent to the surfaces. Bacteria enclosed in glycocalyx capsule are protected against the action of antibodies and other antibacterial substances, as well as immune mechanisms (DONLAN, COSTERTON 2002). They also differ in expression of particular virulence factors and metabolic activity, in comparison to bacteria growing in liquid media (LEWIS 2005).

As it was mentioned above, *Proteus* bacteria under appropriate conditions undergo a differentiation of vegetative rod shape short cells into longer forms called swarmer cells. The population of swarmer cells is then able to migrate on solid surfaces termed swarming (BELAS 1996; RATHER 2005). It was shown that surface polysaccharides are important for this migration. The migration of swarmer cells on solid media is facilitated by cell surface polysaccharides due to the reduction of cells friction (STHAL et al. 1983; GYGI et al. 1995). Most likely O-specific polysaccharide is also important for the swarming phenomenon since the Re mutant strain *P. mirabilis* R45 producing LPS having only the lipid A and Kdo region was unable to swarm. The Ra mutant strain *P. mirabilis* R110 containing lipid A and the complete core region expressed only a limited ability for migration on the solid medium, whereas most of s-forms of *P. mirabilis*, *P. vulgaris* and *P. penneri* can swarm vigorously (BABICKA 2001; KWIL 2003).

The acidic *Proteus* O-antigens may play an important role in stones formation within the urinary tract. The crystallization of ammonium magnesium phosphate and calcium phosphate is initiated by the activity of urease, which cleaves urea to ammonia and carbon dioxide, resulting in the rise of the pH. In such alkaline condition the crystallization process occurs intensively (MOBLEY et al. 1996; RÓŻALSKI et al. 1997). TORZEWSKA et al. (2003) showed urease as the major factor involved in stones formation, however she also found that the effect of this enzyme may be modified by bacterial O-specific polysaccharides. It was noticed that the sugar composition of *Proteus* LPS may either enhance or inhibit the crystallization of struvite and apatite depending on its chemical

structure and ability to bind cations. OPS of *P. vulgaris* 12 has bound magnesium and calcium ions weakly, but increased the crystallization process, whereas OPSs of *P. vulgaris* O47 which are able to bind large amounts of these ions inhibited the process of crystallization (for OPS structure see Table 1). Most likely, the Mg^{2+} and Ca^{2+} weakly bound to the polysaccharides, and could be then easily released from the bacterial surface. This phenomenon causes local supersaturation of the solution and leads to the increase in crystallization and stone formation.

LPS of S-forms of Gram-negative bacteria contributes to their resistance against bactericidal action of the serum. One of the possible explanations for the bactericidal effect is the action of membrane attack complex (MAC) of activated complement. MAC is extremely hydrophobic and forms pores in bacterial membranes, which lead to the bacterial lyses and death. In S-forms of bacteria hydrophobic MAC cannot pass the hydrophilic barrier of a long chain of O-specific polysaccharides to gain their outer and inner membranes (KACA, UJAZDA 1998; MIELNIK et al. 2004). Biological studies of bactericidal activity of sera against *Proteus* strains confirmed this hypothesis. It was shown that *P. mirabilis* R-mutants synthesizing the LPS molecule without O-specific polysaccharide are sensitive to the serum action, whereas most of *P. mirabilis* S-forms, as well as around 50% of S-forms of *P. vulgaris* and *P. penneri* are resistant (BABICKA 2001; FUDAŁA 2003; KWIL 2003).

The biological role of the core region is not clear. It is immunogenic which was shown by use of R-mutants. The antibodies against the core region particularly directed to the heptose or Kdo subregions cross react with LPS of different bacteria, and can be used as endotoxin neutralizing antibodies because of a close similarity of these parts of LPS in different groups of bacteria (POLLACK 1999; DI PADOVA et al. 1999). The presence of 4-amino-4-deoxy--arabino-L-arabinose in the core region, as well in lipid A (see below) led to the resistance of *Proteus* bacteria to polymyxin (BOLL et al. 1994).

LPS is known as endotoxin – the most important virulence factor of Gram-negative bacteria. The mechanism of biological action of endotoxin is common to most of bacteria. It shows pathophysiological effects when released from bacterial cells to blood-vascular system. The centre of biological activity of endotoxin is lipid A, which in the blood is bound by LPB (lipopolysaccharide binding protein). Complexes of LPS-LPB are recognized by receptors on different eukaryotic cells (monocytes/macrophages, lymphocytes, endothelial cells of blood vessels) such as CD14, as well as TLR2 and TLR4 (tool like receptors 2 and 4) resulting activation of transmembrane signal and induction of cells to produce biologically active mediators – TNF (tumor necrotizing factor), interleukins (IL-1, IL-6, IL-8), oxygen free radicals (O_2^-, H_2O_2, NO) and others. These mediators depending of their concentration in macroorganisms elicit beneficial or most often detrimental effects e.g.

DIC (disseminated intravascular coagulation) and ARDS (acute respiratory distress syndrome), resulting in multiple organ system failure (MOSF) and shock syndrome (ŁUKASIEWICZ, ŁUGOWSKI 2003).

8. SUMMARY

Bacteria from the genus *Proteus* synthesized lipopolysaccharides which are built according to the common scheme characteristic for *Enterobacteriaceae* family, however, markedly different in detail structure and biological activity, when compared with other representatives of this family. It serves as the basis of the serological classification of these bacteria. Long term structural and immunochemical studies led us to show the molecular basis of this classification. Antigenically, *Proteus* is heterogenous because of structural differences in the O-specific part of LPS. Until now 76 serogroups have been describe for this genus. Acidic O-specific polysaccharide and structural diversity of the core region are the characteristic features of *Proteus* LPS. The acidic character of LPS of these bacilli has most likely very important biological consequence in the formation of bladder or kidneys stones during urinary tract infections. LPS as a endotoxin is also an important virulence factor playing an important pathophysiological role, particularly during sepsis. The future studies will most probably show the exact role of *Proteus* LPS on the particular stages of infections.

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