ISSN 0006-2979, Biochemistry (Moscow), 2006, Vol. 71, No. 9, pp. 937-954. © Pleiades Publishing, Inc., 2006.
Published in Russian in Biokhimiya, 2006, Vol. 71, No. 9, pp. 1155-1174.

REVIEW

Bacterial Capsular Antigens. Structural Patterns of Capsular Antigens

Yu. S. Ovodov

Institute of Physiology, Komi Science Center, Urals Branch of the Russian Academy of Sciences, ul. Pervomaiskaya 50, 167982 Syktyvkar, Russia; fax: (8212) 241-001; E-mail: ovoys@physiol.komisc.ru

Received January 10, 2006
Revision received April 5, 2006

Abstract—Structural patterns of bacterial capsular antigens including capsular polysaccharides and exoglycans are given in this review. In addition, the immunological activity of capsular antigens and their role in type specificity of bacteria are discussed.

DOI: 10.1134/S000629790609001X

Key words: bacterial capsular antigens, capsular polysaccharides, bacterial exoglycans, structure, immunological activity, type specificity

Polysaccharides of the bacterial capsule and mucus of intercellular space (capsular polysaccharides, exoglycans, or exopolysaccharides) belong to a group of bacterial antigens termed "capsular antigens" ("K-antigens"). An enormous number of publications that peaked in the 1980s elucidate their structural patterns. A great number of monographs and reviews summarize these results (see, e.g., reviews [1-9]).

Microorganisms that penetrate into macroorganisms are heterologous for the latter. Some microorganisms penetrating into humans cause diseases and are known as pathogenic microorganisms [4]. The term "pathogenicity" means ability for a microorganism to cause a certain disease with expressed symptoms. Each microorganism in taxonomic relations is included in a certain family in accordance with its morphological signs as a member of some genus and species. Separate cultures of the species belong to various strains. Pathogenicity is an important indication of bacterial species in taxonomy. Many species of bacteria are pathogenic for humans and animals. However, different strains of single species can possess different levels of pathogenicity corresponding to the virulence of the strain. Thus, virulence is an indication of the strain, dividing strains into virulent and avirulent ones. Virulence of a strain is characterized by invasivity (ability for reproduction in the host) and by toxigenicity (capacity for production of toxins injuring the host tissues and causing symptoms of disease). Microbes can pass from one macroorganism to another, and penetration of a pathogenic agent into a host is called infection. The more virulent a pathogenic strain, the lower dose of the pathogenic microbe required for infection of the host [1, 3, 4].

Many microorganisms produce a rigid hydrated layer around the cell wall, the so-called capsule. In addition, all microbial cells produce specific mucus into intercellular space where intercellular interactions take place. The capsule defends the microbial cell from the action of unfavorable factors of the environment, functions as a regulator of the water regime of bacteria, and prevents dehydration of the microbial cell [1, 4].

Bacteria, being heterologous for a host, cause immune response in the host, especially in the case of pathogenic bacteria, which cause not only symptoms of disease during infection but also a protective reaction of the host [4]. Heterologous compounds stimulating immunity are called antigens. Immune response is a very complicated process that results in formation of specific proteins called antibodies; these interact with antigen and form antigen–antibody complexes rendering harmless the antigen and removing it from the host.

Antigens are usually localized on the surface of bacterial cells, namely, in the cell wall, capsule, and intercellular slime. Antigens are biopolymers, in particular polysaccharides and glycoconjugates (bioglycans) [4] playing important roles.

Bioglycans involved in the cell wall include lipopolysaccharides and lipopolysaccharide–protein complexes, so-called O-somatic antigens. Capsular poly-

saccharides are the basis of the bacterial capsule, and intercellular slime contains mainly polysaccharides termed exocellular polysaccharides, namely exoglycans or exopolysaccharides. This group of the various polysaccharide antigens is united in the capsular antigens (K-antigens) [1, 4] because capsular polysaccharides and exoglycans are similar to each other in relation to structural features and properties. The present review concerns capsular antigens.

All the capsular antigens possess sufficiently high immunogenicity in relation to immunoreactive animals and humans, providing a high titer of antibodies as a result of immunization. The homologous antibodies obtained are characterized by high specificity and interact with certain regions of the macromolecule of the corresponding capsular antigen, the so-called immunodeterminants, immunodominant group, or epitopes [4]. The presence of the capsular antigen immunodeterminants can be used to make additional immunological (serological) differentiation of bacteria into separate groups (serotypes, serovars), which differ in composition of immunodeterminant groups. Therefore, the capsular antigens determine type specificity of bacteria. The capsular antigens of a single type bear the same immunodeterminants and similarly interact with homologous antibodies. The K-antigens of different types fail to interact with heterologous antibodies, which are formed on immunization with bacteria or capsular antigens of various serotypes. So-called cross-reactions demonstrate a partial structural relationship of the capsular antigens of different types and can be used for structural studies of capsular antigens. They are observed in the presence of some common epitopes in the capsular antigens of different serotypes [1-9].

The capsular polysaccharides and exoglycans play an important role in the interaction of phytopathogens with plants (see, e.g., review [10]). Many of them possess rheological properties that produce highly viscous solutions and are gel-forming biopolymers. They are widely used in various branches of industry, in particular food, perfumery, pharmaceutical, and other industries (see, e.g., [11]).

This part of the review reveals structural patterns of the most studied capsular antigens of the most widespread Gram-positive and Gram-negative bacteria, which cause severe diseases and simultaneously possess high immunogenic activity to be used as vaccine components in prophylaxis and curing of these diseases [1, 3, 4].

CAPSULAR ANTIGENS OF GRAM-POSITIVE BACTERIA

Capsular antigens of pneumococci. The pneumococcal capsular polysaccharides of *Streptococcus pneumoniae* (*Diplococcus pneumoniae*), which are Gram-negative bacteria causing various forms of pneumonia, were the first ones studied in detail. Particularly significant contributions in studies of the type-specific pneumococcal capsular antigens have been made by the classical research of the American investigator M. Heidelberger, German scientist V. F. Gobel, Canadians C. T. Bishop, J. K. N. Jones, M. B. Perry, and H. J. Jennings, a Swedish group headed by B. Lindberg, the representatives of the Russian carbohydrate school headed by N. K. Kochetkov, and others.

There are numerous reviews devoted to immunology of pneumococcal capsular polysaccharides [3, 6, 12, 13].

The composition of pneumococcal capsular antigens is very diverse. In addition to the following common monosaccharides, i.e., glucose, galactose, rhamnose, and glucuronic acid, various glycosamines and, in particular, N-acetyl derivatives of D-glucosamine, D-galactosamine, D-mannosamine, D-fucosamine, 2,4-diamino-2,4,6-trideoxy-D-galactose occur in their sugar chains. Some capsular antigens show structural similarity with teichoic acids and contain residues of ribitol phosphate and glycerol phosphate, and pyruvate residues are included in many.

Pneumococci are divided into many types (more than 80). The repeating unit of the capsular antigen of pneumococci type 3 has the simplest structure, the dimer cellobiouronic acid [14]:

$$...\rightarrow 3)\beta\text{-D-GlcA}p(1\rightarrow 4)\text{-}\beta\text{-D-Glc}p(1\rightarrow ...$$

The structure of the capsular antigen of pneumococci type 8 is more complicated, containing residues of D-glucose and D-galactose in addition to cellobiouronic acid [15]:

$$...\rightarrow 4)\beta\text{-D-Glc}p\text{A}(1\rightarrow 4)\beta\text{-D-Glc}p(1\rightarrow 4)\alpha\text{-}$$

$$\text{-D-Glc}p(1\rightarrow 4)\alpha\text{-D-Gal}p(1\rightarrow ...$$

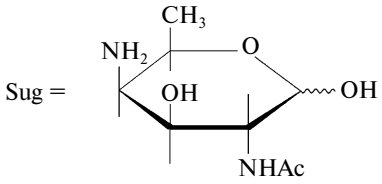
Antigens of both types are immunogens and produce specific antibodies homologous to capsular antigens during immunization of humans and animals. Note that antibodies against capsular antigens of both types recognize cellobiouronic acid in any polysaccharides containing its residues. Thus, e.g., partially oxidized cellulose containing residues of cellobiouronic acid shows pronounced cross-reaction with antibodies against both types of pneumococci. These data indicate that the cellobiouronic acid residue is a common epitope of the capsular antigens of pneumococci types 3 and 8.

This statement was confirmed by one of the effective methods of elucidation of determinant groups of polysaccharide antigens, namely, inhibition of serological reactions (precipitation, hemagglutination, etc.) of polysaccharides showing cross-reactions. Cellobiouronic acid is an effective inhibitor of reaction with antibodies against the capsular antigens of pneumococci types 3 and 8.

The unusual sugar 4-amino-2-acetamido-2,4,6-trideoxy-α-D-galactose (Sug) is a constituent of the repeating unit of the capsular antigen of pneumococci type 1 having the following structure [16]:

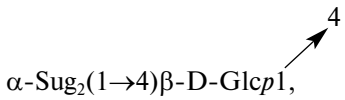
...→3)α-Sug(1→4)α-D-Gal*p*A(1→3)α-D-Gal*p*A(1→...,

where

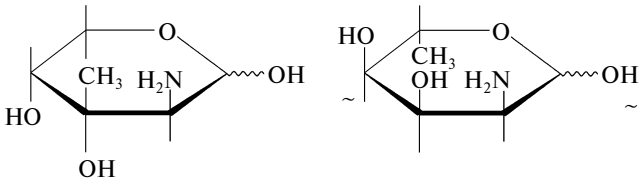


In addition, polysaccharide contains a non-stoichiometric number of O-acetyl groups, which occur in positions 2 and 3 of the α-D-galacturonic acid residues as shown recently [17].

The repeating unit of the capsular polysaccharide of pneumococci type 5 contained the residues of two unusual sugars [1]:



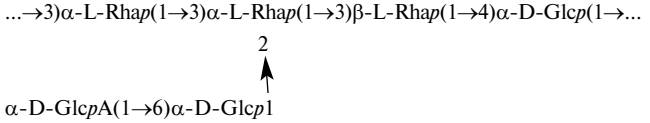
where Sug_1 and Sug_2 are 2-amino-2,6-dideoxy-L-galactose (1) and 2-amino-2,6-dideoxy-L-talose (2) or their N-acetyl derivatives:



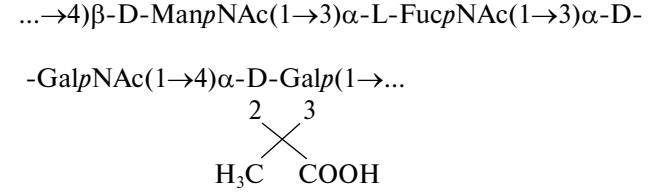
1 2

The capsular antigen of pneumococci type 1 belongs to the group of immunomodulatory zwitterionic polysaccharides containing both carboxyl groups and amino groups. In addition, the capsular polysaccharides of various strains of the pathogenic microorganism *Bacteroides fragilis* [9] cause abscesses and intraperitoneal sepsis [17], which can be fatal for humans, belong to this group. All these polysaccharides possess similar biological properties, although they substantially differ in chemical structural features. However, they are characterized by a high density of positive and negative charges due to simultaneous occurrence of amino and carboxyl groups, respectively [18]. These charges play a substantial role in immunological functions of these polysaccharides as confirmed by some investigations [9, 17, 19-21] where a chemical transformation of charged groups into neutral ones (e.g., acetylation of amino groups or reduction of carboxyls into hydroxymethyl groups) leads to a loss of physiological activity. Zwitterionic capsular polysaccharides interact with $CD4^+$ T-lymphocytes and stimulate them to function as a defense against the above bacteria [9].

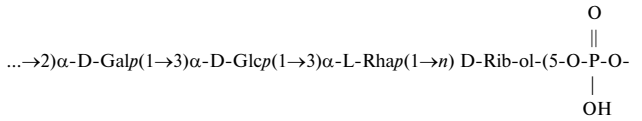
The capsular antigen of pneumococcus type 2 has a branched hexasaccharide unit as follows [22]:



The repeating unit of the capsular antigen of pneumococcus type 4 has great diversity including glycosamines and occurrence of the pyruvate residue [23]:



A residue of ribitol 5-phosphate is involved in the composition of the closely related capsular polysaccharides of pneumococci types 6A and 6B (*S. pneumoniae* (*n* = 3) [24] and *S. pneumoniae* (*n* = 4) [25], respectively):



The structural patterns of the capsular polysaccharides isolated from some other pneumococcal types are listed in Table 1. The sugar residues without special indications in Table 1 are of D-series and are in pyranose form.

As can be seen from the abovementioned data and Table 1, the structural patterns of the capsular polysaccharides of various types in one group are sufficiently similar each other (e.g., the structures of the capsular polysaccharides types 6A and 6B or types 9A, 9L, 9N, 9V). Some polysaccharides having the structure of teichoic acid type contain residues of glycerol phosphate or ribitol phosphate, and the polysaccharide of pneumococcus type 17F only differs in containing the arabinitol phosphate residue [31]. In some cases, a phosphate group is connected with sugar residues localized in the sugar chain of the repeating unit. An availability of the O-acetyl groups and pyruvate residue sets up additional specific regions of the antigen macromolecule similar to a branching of carbohydrate chain, which can be important in immunochemical behavior of similar polysaccharides.

Table 1. Chemical structures of some pneumococcal capsular polysaccharides

Type	Structural features	References
9A	→4)α-GlcA(1→3)α-Gal(1→3)β-ManNAc(1→4)α-Glc(1→4)β-Glc(1→	[26]
9L	→4)α-GlcNAc(1→4)α-GalA(1→3)α-Gal(1→3)β-ManNAc(1→4)β-Glc(1→	[27]
9N	→4)α-GlcA(1→3)α-Gal(1→3)β-ManNAc(1→4)α-Glc(1→4)β-Glc(1→	[28]
9V	→4)α-GlcA(1→3)α-Gal(1→3)β-ManNAc(1→4)β-Glc(1→4)α-Glc(1→ | OAc | OAc	[29]
14	→4β-Glc(1→6)β-GlcNAc(1→3)β-Gal1→ 4 β-Gal1 ↗	[30]
17F	→3)β-L-Rhaβ-(1→4)-D-Glcβ-(1→3)α-D-Galβ-(1→3)-L-Rha(2OAc)β-(1→4)α-L-Rha-(1→2)-D-Ara-ol1→PO_4^- 4 β-D-Gal*p*1 ↗	[31]
19F	→4)β-ManNAc(1→4)α-Glc(1→2)α-L-Rha*p*(1-PO_4^-→	[32, 33]
23F	→4)β-Glc(1→4)β-Gal(1→4)α-L-Rha(1→ | O | Gro2-O–P = O | OH 2 ↑ α-L-Rha1	[34, 35]

Note: Gro is the glycerol residue, Ara-ol is arabinitol.

The side chains play a similar role due to arising of additional terminal sugar residues. The single homopolysaccharide among pneumococcal antigens is β-D-glucan representing the capsular antigen of pneumococci type 37 and having the following structural pattern [36]:

...→3)β-D-Glc*p*(1→3)β-D-Glc1→...
6
↗
β-D-Glc(1→2)β-D-Glc1

Thus, the capsular polysaccharides of various types of pneumococci *S. pneumoniae* are characterized by diversity and features of chemical structural patterns.

Capsular antigens of streptococci. The capsular polysaccharides of streptococci groups A and C contain hyaluronic acid [1]:

→4)β-D-Glc*p*A(1→3)β-D-Glc*p*NAc(1→

Earlier, streptococci group B was divided into four serotypes: Ia, Ib, II, and III. In 1985, some strains group B not identified previously were related to serologically different type IV [37]. At present, nine serotypes of streptococcus group B are known as follows: Ia, Ib, II-VIII [38]. However, serotype III is the causative factor in the majority of cases of meningitis caused by streptococcus group B [39].

In the 1930s, R. C. Lancefield [40] first isolated from each serotype of streptococci group B two antigens: a group antigen common for all serotypes and type-specific capsular polysaccharides defined for each serotype. The type-specific polysaccharides were isolated by extraction of whole cells with hydrochloric acid, and they possessed similar sugar composition. Such extraction gives rise to immunologically incomplete antigen. The complete antigens, which are isolated by extraction of the whole micro-organisms with neutral or buffered solutions, contain additionally terminal residues of sialic acid

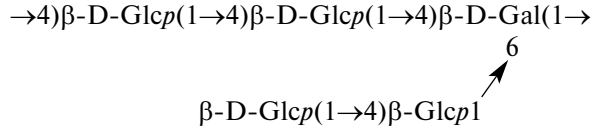
[40]. The presence of these residues is a substantial factor to immu-nologic activity of the complete capsular antigens [39].

Accordingly to structural features, the antigens of streptococci group B are an interesting group of closely related polysaccharides and bear a common terminal residue of sialic acid at the side carbohydrate chain. The structural patterns of streptococcal group B antigens are given in Table 2.

The phenomenon that the incomplete antigen of streptococci type III (deprived the sialic acid residue) has identical structure with the capsular antigen of pneumococci *S. pneumoniae* type 14 causes similarity of serological activity of the two antigens [3].

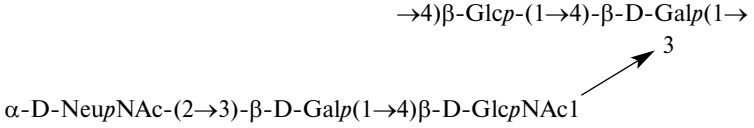
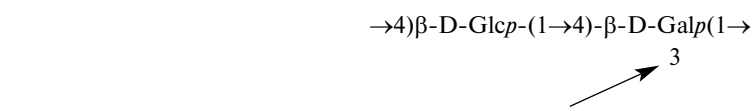
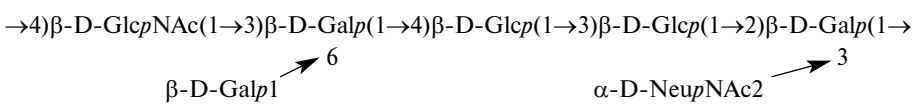
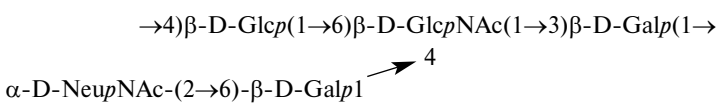
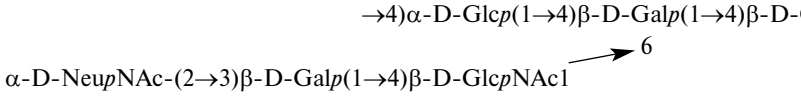
All the polysaccharides of streptococci group B possess branching structural features. In the complete antigens of types Ia, Ib, III, and IV, the terminal residues of β-D-galactopyranose are disguised by the sialic acid residues which occupy the terminal position in the genuine antigens and are of great serological importance for streptococci group B due to structural homology of the side chains with sugar chains of some important glycoproteins of human serum [3]: the terminal disaccharide α-D-Neu*p*NAc(2→3)β-D-Gal*p* of antigens of streptococci types Ia, Ib, and IV is identical to the terminal disaccharide of the human blood group substances of M and N groups, and the unit 6-O-(N-acetyl-α-D-neuraminyl)-β-D-galactopyranose of the streptococcal polysaccharide type III appeared to be a part of the human serotransferrin structure.

The type-specific polysaccharide of streptococci group D (*Streptococcus faecalis*) has the following repeating unit [43]:



Capsular antigens of other Gram-positive bacteria. In this connection, it is necessary to pay attention to mycobacteria, especially *Mycobacterium tuberculosis*, as

Table 2. Chemical structures of capsular polysaccharides of streptococci group B

Type	Structural features	References
Ia		[3]
Ib		[3]
II		[3]
III		[38, 41]
IV		[42]

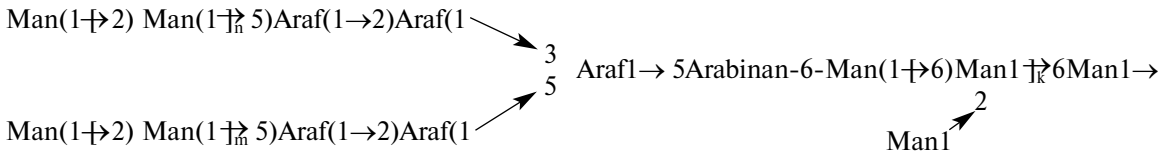
Note: The sialic acid residue represents N-acetyl-D-neuraminic acid (Neu*p*NAc).

causative factor of tuberculosis. Interest in investigation of tuberculous bacilli does not wane not only due to the importance of this microorganisms as a pathogen, but also in connection with elucidation of factors responsible for its development in the human body and due to the ability of mycobacteria to arrest the protective immune mechanisms in the host [44].

In spite of numerous works devoted to chemical characterization and biological activity of the cell components of tuberculous bacilli, it can be concluded that these compounds together or separately fail to provide a complete picture of virulence or its lack for some strains of the tuberculous microbe. It is interesting to note the presence of the mycobacterial capsule developing intracellularly, which is undoubtedly a part of the defense mechanism of the mycobacterial pathogen in relation to phagocytosis of the affected organism. In addition, components on the surface of the bacterial cell restrict access of macrophages to the actively growing bacterial cell. The main polysaccharides exported by the bacterial cell into the intercellular space also have immunosuppressive action. Three types of such polysaccharides were obtained from *M. tuberculosis* as follows: D-glucan, D-arabino-D-mannan, and D-mannan [45]. All they are neutral ones without acyl substituents.

Elucidation of their chemical structure demonstrated that D-glucan made up to 90% of all the polysaccharides contained the repeating unit and consisted of five or six →4)α-D-Glc*p*(1→ residues and one such unit substituted with β-D-Glc*p* residue in position 6.

One of the possible structures of arabinomannan is presented below. (All the sugars are in D-conformation.)



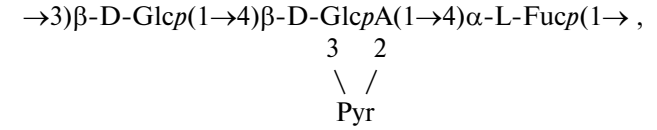
The structure of arabinan fragment included in the macromolecule failed to be elucidated completely; however, a substantial part of this region consist of 1,5-linked L-arabinose residues [45].

The third polysaccharide, mannan, appeared to represent a part of the arabinomannan core and consisted of 1,6-linked D-mannopyranose residues as a linear sugar chain with branching as some single D-mannose residues attached by 1,2-linkage.

Glucan of the tuberculous microbe plays a defensive role; it represents a disguising agent for the microorganism, and as a result, the host immune system fails to recognize *M. tuberculosis* as a heterologous factor. This phenomenon is called molecular mimicry of the bacterial pathogen [3]. In a similar way, the oligosaccharide moieties α-D-Man*p*(1→2)α-D-Man*p* forming segments of arabinomannan and mannan molecules on the outer surface coincide with fragments of the structure of animal tissue glycoproteins and therefore arabinomannan and mannan appeared to promote lack of recognition of tuberculous bacilli by the host cells. Moreover, immunosuppressive action of arabinomannan and mannan in relation to human lymphocytes [46] undoubtedly intensifies pathogenesis of *M. tuberculosis* and restricts the host immune response [45]. However, a direct correlation between the tuberculous microbe virulence and amounts of produced exoglycans failed to be found, requiring further study of the influence of exoglycan on phagocytosis [47].

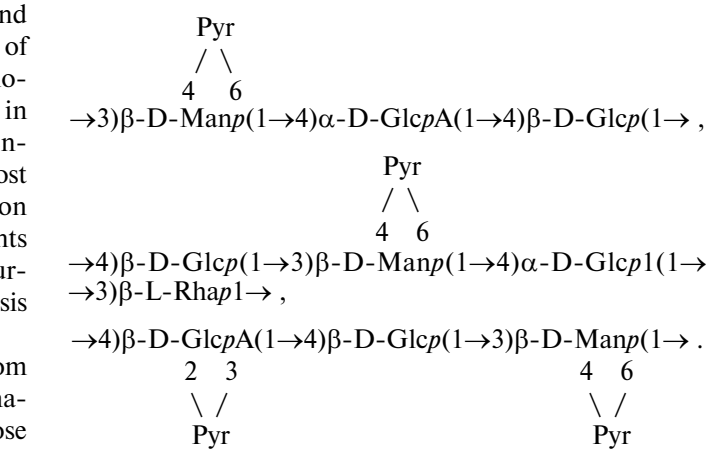
D-Arabino-D-mannans have been isolated from other mycobacteria. The backbone of these polysaccharides also consists of α-1,6-linked D-mannopyranose residues and the side chains are formed by α-1,2-linked D-mannopyranose residues and α-1,5-linked D-arabinofuranose residues [48]. Glucan consisting of 22 D-glucose residues was obtained from *M. smegmatis* [49].

Exoglycan produced by *M. convolunum* 240 was shown to possess the following structural features of the repeating unit [50]:

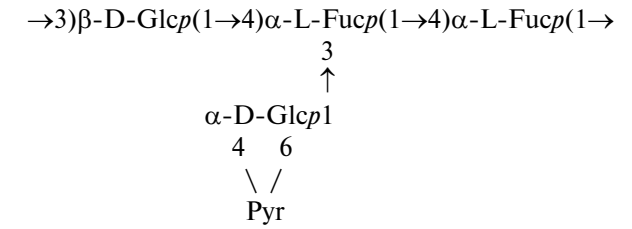


where Pyr is the pyruvate residue. (It must be noted that pyruvate forms cyclic ketal but not ester.)

Exoglycans of *M. lacticolum* [51], *M. album* B-88 [52], and *M. salivarum* M76 [53] are characterized by a unique structure as follows:



Exoglycans of *Corynebacterium insidiosum* [54] proved to have the following unique structure:



The macromolecular structural patterns and the presence of the pyruvate residue in these heteropolysaccharides indicated their relationship with the capsular antigens of pneumococci.

Staphylococcus aureus is known to be a causative factor of bacteremias, abscesses, septic arthritis, endocarditis, osteomyelitis, and infectious injuries treated with great difficulties due to a high resistance of the microorganism to antibiotics [55]. The virulence factors are the capsular polysaccharides of serotypes 5 and 8 in the first place. Their structural features have been elucidated recently [56]:

→4)β-D-ManNAcA(1→4)α-L-FucNAc(3OAc)-

-(1→3)β-D-FucNAc(1→ ,

→3)β-D-ManNAcA(4OAc)-(1→3)α-L-

-FucNAc(1→3)α-D-FucNAc(1→.

CAPSULAR ANTIGENS OF GRAM-NEGATIVE ENTEROBACTERIA

Many Gram-negative bacteria form a capsule containing capsular polysaccharides or produced exoglycans. They all represent the capsular bacterial antigens and play an important role in immunological behavior of these microorganisms. More studied are the capsular antigens of numerous representatives of the vast enterobacterial family (Enterobacteriaceae) [1, 3, 5], which are Gram-negative bacteria and include various species of pathogenic and nonpathogenic microorganisms. The well-known bacterium *Escherichia coli* and the numerous representatives of the genus *Salmonella* caused salmonellosis, typhoid fever, and other severe infectious diseases. The genus *Yersinia* is included in this family contains the species *Yersinia pestis*, which is the causative factor of plague.

Structural studies of enterobacterial capsular polysaccharides and exoglycans started to intensify since the end of the 1950s when powerful methods of structural studies of carbohydrate chains of polysaccharides were developed. The great contribution in structural studies of capsular antigens of enterobacteria of the Swedish school headed by B. Lindberg and Canadian investigators headed by G. G. S. Dutton must be especially noted. The capsular antigens of *E. coli* have been comprehensively studied by K. Jann and B. Jann who are representatives of the famous school of immunochemists (Freiburg, Germany) headed by O. Westphal and O. Luderitz.

Capsular antigens of *E. coli* [1]. On the basis of serological properties, all the capsular antigens of *E. coli* have been divided into three groups: A, B, and L [3]. The absence of substantial differences between these groups from the chemical point of view was observed: all these capsular antigens represent capsular polysaccharides with regular structures. However, during investigation of interrelations between antigenic structure and serologic (immunological) activity, an additional separation of the capsular antigens into chemotypes was made. Microorganisms of certain species possessing identical structural regions in their antigens, especially having identical determinant groups (epitopes) and showing cross-reactions with homologous antisera, are involved in a certain chemotype. On the basis of chemical differences in the structural patterns of various capsular antigens of *E. coli*, more than 100 chemotypes such as K1, K2, etc. have been isolated and identified. However, in spite of numerous investigations, the full structures of the capsular polysaccharides were elucidated for a comparatively small number of chemotypes only. In many cases the structural pattern was clarified partially.

All the capsular antigens are defined by a high serologic activity inducing a high titer of the specific antibodies on immunization of animals.

The great structural diversity of the capsular antigens of *E. coli* must be noted. In this connection, cross-reactions between antigens are observed comparatively rarely.

The K1-antigen so-called colominic acid, which is produced by several strains of *E. coli*, differs in structural features and has the following structure of the repeating unit [57]:

→8)α-D-Neu*p*NAc(2→.

Colominic acid can contain O-acetyl groups, but some strains produce colominic acid deprived of O-acetyl groups. If acetyl groups are present, they are distributed randomly between positions 7 and 9 of N-acetyl neuraminic acid.

The K92-antigen composed of the alternate 2,8- and 2,9-linked residues of N-acetyl neuraminic acid is structurally similar to K1-antigen [58]:

→8)α-D-Neu*p*NAc(2→9)α-D-Neu*p*NAc(2→.

Together with the linear K-antigens of *E. coli*, the branching capsular polysaccharides usually having one side chain per repeating unit have been isolated and char-

acterized. The sugar chains of K-antigens of *E. coli* contain the rarer monosaccharides in addition to the common sugar residues. Many *E. coli* K-antigens contain O-acetyl groups, and sometimes pyruvate residues play a substrate role in immunological activity of K-antigens as epitope constituents. The structural patterns of some chemotypes of K-antigens are given in Table 3 to demonstrate their diversity and features.

The pathogenic coli-bacteria cause abenteric diseases such as pyelonephritis, diarrhea, septicemia, and

Table 3. Structures of some K-antigens of *E. coli**

Chemotype	Structural features	References
K4	→3)β-Glc(1→4)β-GalNAc(1→ 3 β-Fruf1↗	[59]
K5	→4)β-GlcA(1→4)α-GlcNAc(1→	[60]
K12, K82	→3)α-L-Rha(1→2)α-L-Rha(1→5)β-KDO(2→ 7/8 | OAc	[61]
K23	→3)β-Ribf(1→7)β-KDO(2→	[62]
K27	→4)β-D-Glc*p*(1→4)α-D-Glc*p*A(1→3)α-L-Fuc*p*(1→ 3 α-D-Gal*p*1↗	[63]
K33	OAc ↓ 2 →3)α-D-Glc*p*(1→4)β-D-Glc*p*A(1→4)α-L-Fuc*p*(1→ 2,3 3 V ↑ Pyr(S) α-D-Gal*p*1	[64]
K52	AcO O ⋮ ‖ →3)α-D-Gal*p*(1–O–P–O→ 2 | β-D-Fruf2↗ OH	[65, 66]
K57	→2)β-Ribf(1→4)β-Gal(1→3)α-GlcNAc(1→4)α-GalA(1→	[67]
K100	→3)β-Ribf(1→2)Rib-ol(5-O-PO_3H	[68]

Note: Rib-ol, ribitol; KDO, 2-keto-3-deoxy-D-*manno*-octonate.

* Sugar residues without special indications belong to D-series and are present in pyranose form.

meningitis [8]. More than 13 various serologic groups [69] are present among enterotoxic *E. coli*. The pathogenic *E. coli* appeared to be invasive or enteropathogenic. Both types of pathogens bind primarily with epithelial cells. The enteropathogenic bacteria are received on the outer surface of the epithelial plasmatic membrane and concentrated on the outside of the epithelium. Their pathogenic action is caused by protein exotoxins (enterotoxins) which upset the metabolism processes in the host. Invasive coli-bacteria penetrate through the epithelial plasmatic membrane into tissues of organs and the bloodstream. They possess a powerful destructive action on a complement and phagocytes due to a high negative charge caused by capsular polysaccharides. This is a decisive step in infection because antibodies against the capsular antigens are formed substantially later. An action of the capsular polysaccharides is intensified by endotoxins (lipopolysaccharide protein complexes) and other components of cells invasive for *E. coli*. Overcoming nonspecific immune defense of the host with the capsular polysaccharide is a basis for invasive coli-bacteria infection [62]. Immune defense of animals becomes effective only after forming specific antibodies, which neutralize the protective action of *E. coli* capsule for the microorganism. In this case, a masked effect of K-antigens of some pathogenic *E. coli* due to their structural similarity to host substances must be taken into account. The polysaccharides of *E. coli* types K1 and K5 are the most typical in this relation as can be seen on a comparison of the polysaccharide structures (see above) with the structural patterns of the following ganglioside and the intermediates of heparin biosynthesis, respectively [1, 3, 70]:

α-NeuNAc(2→3)β-Gal(1→3)β-GalNAc(1→4)β-

-Gal(1→4)β-Glc→CERAMIDE
3
↖

α-NeuNAc(2→8)α-NeuNAc2,

(trisialoganglioside)

→4)α-GlcNAc(1→4)β-GlcA(1→4)α-GlcNAc(1→4)β-

-GlcA→$(Gal)_2$-Xyl-PROTEIN.

(intermediate of heparin biosynthesis)

K-Antigens of *Klebsiella*. The structure and properties of the capsular antigens of the genus *Klebsiella* as another representative of Enterobacteriaceae have been intensely studied [1]. Bacteria of this genus produce a powerful capsule, which determines their pathogenicity and virulence. *Klebsiella* bacteria deprived of capsule are devoid of pathogenicity and subjected immediately to phagocytosis when they penetrate into the animal body.

Three species of *Klebsiella* are of great importance in pathogenicity of humans as follows: *K. pneumoniae* is causative factor of pneumonia; *K. ozaenae* affects the mucous membrane of nose and the upper respiratory tracts causing severe *coryza foetida*; *K. rhinoscleromatis* are bacteria of rhinoscleroma which cause a chronic granulomatous process on skin, especially, on mucosa of the upper respiratory tracts with generation of nodules (rhinoscleromas).

All the diseases caused by *Klebsiella* are severe, chronic, and subjected to treatment with great difficulties using complex methods. Weak, unstable immunity is induced during illness, which explains the chronic character of these diseases [1, 3, 4].

Studies of the capsular antigens have demonstrated that *Klebsiella* bacteria can be divided into more than 80 serological types. Qualitative analysis of the sugar composition suggests that all the capsular antigens of *Klebsiella* represent acidic polysaccharide due to the presence in their composition of the residues of glycuronic acids, mainly glucuronic acid or residues of pyruvic acid (pyruvate). However, the absence of residues of amino sugars and phosphate group in the composition of the capsular polysaccharides of *Klebsiella* has been noted [1, 4].

The full structures of the capsular polysaccharide have been determined for most types of *Klebsiella*. This provides a more precise definition to the bacterial specification of all species and strains of the genus *Klebsiella* on the basis of the structural patterns of their K-antigens [1].

The first structure was established in 1966 for the capsular polysaccharide of *Klebsiella* type K54 (the sugar chain contains O-acetyl groups) [71]:

→6)β-D-Glc*p*(1→4)-α-D-Glc*p*A(1→3)-α-L-Fuc*p*(1→
4
↖
β-D-Glc*p*1

The structural patterns of some capsular polysaccharide have been defined more precisely due to development of modern structural methods, especially NMR spectroscopy. In particular, the more exact structure of the polysaccharide of *Klebsiella* type 52 was suggested to have the hexasaccharide repeating unit as follows [72]:

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

The structural patterns of the capsular polysaccharides of some other *Klebsiella* types are listed in Table 4. As seen from the table, most structures of *Klebsiella* K-antigens were established from the end of the 1970s to the beginning of the 1980s, especially due to research of the

Table 4. Structures of K-antigens of *Klebsiella* genus

Type	Structural features	References
1	2	3
K1	→4)β-D-Glc*p*A(1→4)α-L-Fuc*p*(1→3)β-D-Glc*p*(1→ 2 3 H_3C COOH	[73]
K5	→4)β-D-Glc*p*A(1→4)β-D-Glc*p*(1→3)β-D-Man*p*(1→ 2 2 3 OAc S H_3C COOH	[74]
K7	→3)β-D-Glc*p*A(1→2)α-D-Man*p*(1→2)α-D-Man*p*(1→3)β-D-Glc*p*(1→3)β-D-Gal*p*-(1→ 3 4 6 α-D-Gal*p*1 H_3C COOH	[75]
K11	→3)β-D-Glc*p*(1→3)β-D-Glc*p*(1→3)α-D-Gal*p*(1→ 4 4,6(R)Pyr-α-D-Gal*p*1	[76]
K13	→4)α-D-Glc*p*(1→3)β-D-Glc*p*(1→4)β-D-Man*p*(1→ 3 4,6(S)Pyr-β-D-Gal*p*(1→4)α-D-Glc*p*A1	[77]
K18	→3)α-L-Rha*p*(1→3)β-D-Gal*p*(1→4)α-D-Glc*p*(1→ 3 α-D-Glc*p*(1→4)β-D-Glc*p*A(1→2)α-L-Rha*p*1	[78]
K23	→3)β-D-Glc*p*(1→3)α-L-Rha*p*(1→ 2 β-D-Glc*p*A(1→6)α-D-Glc*p*1	[78]
K27	β-D-Glc*p*1 3 →3)β-D-Glc*p*(1→3)α/β-D-Gal*p*(1→3)β/α-D-Gal*p*(1→6)β-D-Glc*p*(1→ 4 6 2 H_3C COOH β-D-Glc*p*A1	[79]

Table 4. (Contd.)

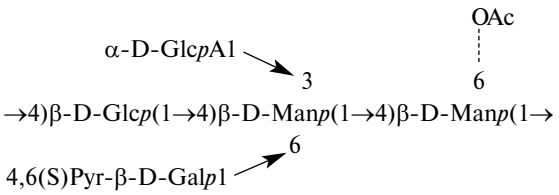
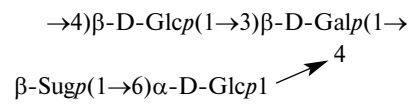
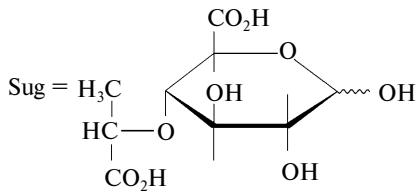
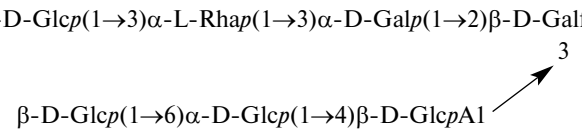
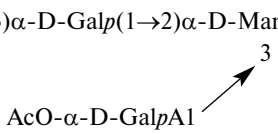
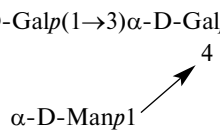
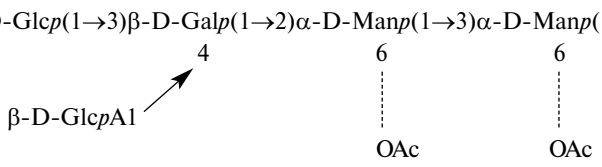
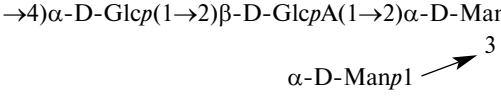
1	2	3
K30 K33		[80, 81]
K37	 	[30]
K41		[82]
K49		[1]
K57		[83]
K59		[84]
K62		[85]

Table 4. (Contd.)

1	2	3
K72	→3)β-D-Glc*p*(1→3)α-L-Rha*p*(1→2)α-L-Rha*p*(1→3)α-L-Rha*p*(1→ 3 4 H_3C COOH	[86]
K73	→3)β-L-Rha*p*(1→4)β-D-Gal*p*(1→4)β-D-Glc*p*(1→ 3 β-D-Glc*p*A1	[87]
K83	→3)β-D-Gal*p*(1→4)α-L-Rha*p*(1→ 3 α-D-Glc*p*A(1→3)α-D-Gal*p*1	[88]

Swedish carbohydrate school headed by B. Lindberg and the Canadian school headed by G. G. S. Dutton.

Consideration of structures of the *Klebsiella* capsular polysaccharides shows that all they possess regular structural features and represent as a rule linear or infrequently branching (not more than one side chain) carbohydrate chains consisting usually of the common sugar residues. As a rule, with some exceptions, the sugar residues are present in sugar chains in pyranose form. The rare sugars are comparatively infrequent in K-antigens of *Klebsiella*. However, in addition to O-acetyl groups, the residue of pyruvate, attached to positions 4 and 6 (more rarely 2 and 3) of the different sugar residues, especially D-galactose, is included rather often in the capsular polysaccharides of numerous types of *Klebsiella*.

K-Antigens of other enterobacteria. At present, the structural patterns of K-antigens of a great number of very different representatives of the Enterobacteriaceae family are elucidated. As a rule, they all are closely related in the structure of sugar chain with polysaccharides of *E. coli* and *Klebsiella* although, undoubtedly, each K-antigen is defined by a feature of chemical structure and is connected directly with immunological behavior. For example, the structural pattern of the capsular antigen of *Proteus mirabilis*, elucidated by Canadian researchers [89], can be considered. This microorganism is of great interest for physicians and scientists because it is involved in infections of the urinary tract and intensifies infectious urolithic disease. Capsules typical for the pathogenic *Proteus* strains are powerful virulence factors. Intensification of crystal growth in the urinary tract and formation of stones in the bladder are connected with the capsules. The capsular polysaccharide produced by the *P. mirabilis* capsule has the following structure:

...→3)β-D-Glc*p*NAc(1→4)α-L-Fuc*p*NAc(1→...
3
α-D-Glc*p*A1

A feature of this polysaccharide is the occurrence of the amino sugar residues as follows: N-acetyl-D-glucosamine and N-acetyl-L-fucosamine together with D-glucuronic acid residues usually occur in the capsular antigens. The simultaneous presence of such different sugar residues in the polysaccharide molecule is suggested to promote formation of stones in the urinary tract [89].

A series of pathogenic Gram-negative bacteria uses molecular mimicry of host molecules in order to prevent the immune response. Thus, *E. coli* types K4 and K5 produce capsular polysaccharides related to chondroitin and heparin, respectively [59, 60]. The microorganism *Pasteurella multocida* type A (the main choleric antigen of birds and a causative factor of bovine fever) pathogenic for humans and animals produce hyaluronan as a capsule [90] and types D and F causing rhinitis of pigs and cholera of birds, respectively, induce formation of capsular polysaccharides structurally related to heparin and chondroitin and having molecular mass of approximately 100-300 kD exceeding by an order of magnitude the size of similar polysaccharides of the living tissues. The capsular glycosaminoglycans are lowly immunogenic virulent factors that intensify pathogenicity of the corresponding microorganisms [91].

Clostridium perfringens is a very toxic, anaerobic microorganism causing necrosis of muscle tissue (gas gangrene) and involved in such infections as septicemia, alimentary toxoinfections, and uterine diseases. The spe-

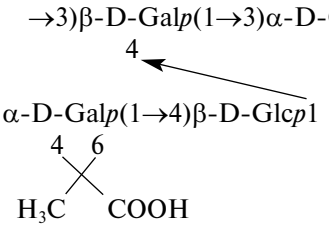
cific antigens of this bacterial species are the capsular heteropolysaccharides [92-98]. The sugar composition of the capsular polysaccharides has been elucidated only for a few of the numerous serotypes of *C. perfringens*. Strains associated with food poisons and named as serotypes of Hobbs [91] are insufficiently studied, and the primary structure [92] has been determined for only one of them, namely serotype Hobbs 5 as follows (all the sugar residues belong to D-series and are in pyranose form):

→4)β-Glc(1→3)β-GalNAc(1→4)β-Glc(1→3)β-

-GlcNAc(1→2)α-Gal(1→3)β-Man(1→

The heteropolysaccharide has a hexasaccharide repeating unit and differs considerably from the capsular polysaccharides of other bacterial species [7].

In conclusion, it must be mentioned concerning isolation and elucidation of the structure of the acidic exoglycan from the marine organism *Enterobacter agglomerans* [99] in connection with the fact that the capsular polysaccharides and exoglycans of nonpathogenic microorganisms and, in particular, marine bacteria may be used in the future as constituents of vaccines against diseases caused by such pathogenic microorganisms which produce K-antigens related structurally to the capsular polysaccharides and exoglycans of nonpathogenic bacteria. The determined structure of *E. agglomerans* polysaccharide is typical for K-antigens of the enterobacterial surface [99]:



The acidic capsular polysaccharides have been isolated from some marine bacteria of the genus *Alteromonas* (*A. haloplanktis* KMM156 [100], *Alteromonas* sp. 4MC17 [101], *A. macleodii* 2MMb [102], *A. nigrifaciens* [103]) and their structural patterns have been elucidated.

The capsular polysaccharide was isolated also from the marine bacterium *Alteromonas* sp. CMM15S [104]. It is defined by uniqueness in relation to its structural features—the repeating unit consists of acylated glycosamines only and possesses the following linear structure:

→3)α-D-Gal*p*NAc(1→4)α-L-Gal*p*ANAc(1→3)α-

-D-Qui*p*NAc4NAc(1→3)β-D-Qui*p*4NAlaAc(1→,

where Qui*p*NAc4NAc represents di-N-acetyl-D-bacillosamine, and Qui*p*4NAlaAc is 4-(N-acetyl-D-alanyl)-amino-4,6-dideoxy-D-glucose.

It is interesting to note that the latter sugar residue is unique for capsular polysaccharides.

Thus, the rare amino sugar residues are included in the repeating unit of the marine bacterium capsular polysaccharide.

K-ANTIGENS OF OTHER GRAM-NEGATIVE BACTERIA

Capsular antigens of *Haemophilus*. Structures of the type-specific capsular polysaccharides of *H. influenzae* were determined at the end of the 1970s and beginning of the 1980s [1, 3]. The microorganism is divided into six types (**a**-**f**), each of them producing specific K-antigen. Four types (**a**, **b**, **c**, and **f**) contain polysaccharides structurally similar to teichonic acids, and the capsular polysaccharides of types **d** and **e** are deprived of phosphate group and defined by a high content of glycosamines (Table 5).

As can be seen from Table 5, the type-specific polysaccharides of *H. influenzae* can be divided into two groups depending on acidic constituents: the first group contains the phosphate residue and the second one comprises residues of N-acetyl-D-mannosaminouronic acid (types **d** and **e**). Polysaccharide type **b** became the first one with elucidated structure of the repeating unit [106]. Most capsular *H. influenzae* polysaccharides consist of disaccharide repeating units.

K-Antigen isolated from *H. pleuropneumoniae* serotype 5 [111] was found to possess an unusual structural pattern for the capsular polysaccharides of *Haemophilus* genus. *Haemophilus* (*Actinobacillus*) *pleuropneumoniae* is etiologic factor of pleuropneumonia of pigs as the main respiratory disease of these animals, which is widely distributed in the World, especially in western Canada and USA. The main factor of virulence of this microorganism is represented by the capsular polysaccharide. The structure of its repeating unit is as follows (the residue with asterisk being 2-keto-3-deoxy-D-*manno*-octonate):

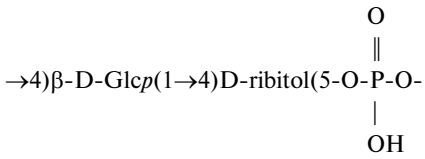
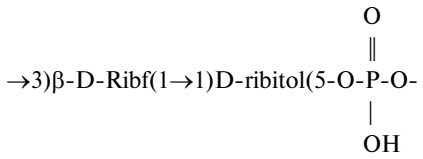
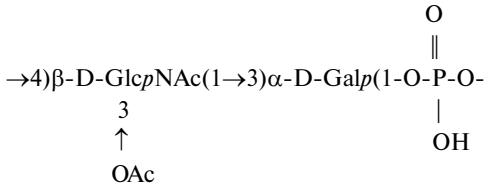
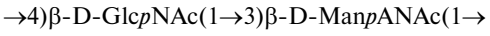
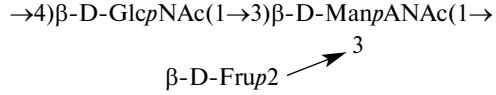
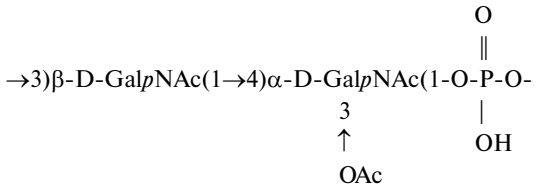
→6)α-D-Glc*p*NAc(1→5)β-D-KDO*p*(2→.

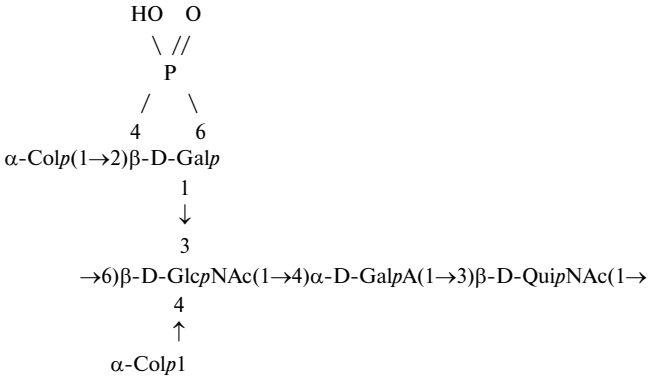
The capsular polysaccharides of some other *H. pleuropneumoniae* serotypes (they are 10 in all) also have a structural relationship with O-specific polysaccharides (see, e.g., [112, 113]).

K-Antigens of choleric vibrion. The capsular polysaccharide containing determinants of O-specific lipopolysaccharide was isolated from *Vibrio cholerae* OI39 [114], the causative factor of cholera, which is a very severe disease of the gastrointestinal tract.

The repeating unit of this polysaccharide is a branching hexasaccharide with unusual sugar residues of N-acetyl-D-quinovosamine (QuiNAc) and immunodominant colitose (Col is 3,6-dideoxy-L-galactose) typical for O-specific chains of some lipopolysaccharides:

Table 5. Structures of the capsular polysaccharides of *H. influenzae*

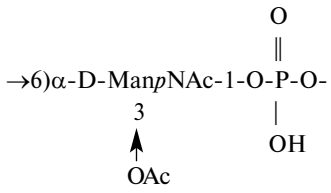
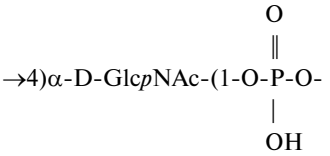
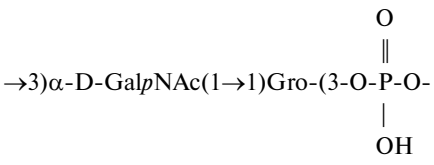
Type	Structural features	References
a		[105]
b		[106]
c		[107]
d		[108]
e		[109]
f		[110]



The strain *V. cholerae* 0139 produces polysaccharide capsule in contrast to common strains. This microorganism type is the etiologic factor of cholera in India and Bangladesh. O-Antigens of some enterobacteria (in particular, *Salmonella greenside* and *E. coli* O55) have similar structural patterns of O-specific polysaccharide and show cross-reactions with *V. cholerae* O139 due to occurrence of the common epitope as disaccharide of the side chain: α-Col*p*(l→2)β-D-Gal*p* attached by 1,3-linkage to the β-D-Glc*p*NAc residue of the backbone.

K-Antigens of *Neisseria* genus. The structures of various *N. meningitidis* serotypes have been elucidated by the

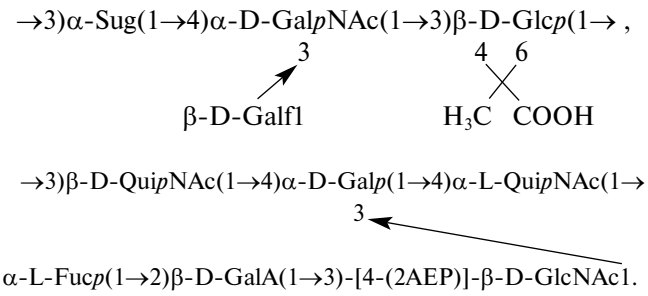
Table 6. Structures of the capsular polysaccharides of *N. meningitidis*

Type	Structural features	References
A		[115]
B	→8)α-D-Neu*p*Ac(2→	[116]
C	→9)α-D-Neu*p*Ac(2→ AcO -7/8	[116]
29e	→3)α-D-Gal*p*NAc(1→7)β-D-KDO*p*(2→ AcO-7/8	[116]
W-135	→6)α-D-Gal*p*(1→4)α-D-Neu*p*Ac(2→	[116]
X		[115]
Y	→4)α-D-Neu*p*Ac(2→4)α-D-Neu*p*Ac(2→ contains O-acetyl groups	[116]
Z		[117]

Canadian group of researchers headed by H. Jennings [3]. The structural patterns are listed in Table 6. As can be seen from this table, polysaccharides of three types (A, X, Z) are structurally related to teichonic acids and comprise the phosphate residue. It is interesting to note the occurrence of residues of N-acetyl-D-neuraminic acid in some capsular polysaccharides of *N. meningitidis* (types B, C, W-135, and Y), the capsular antigen of type B represents colominic acid, the capsular polysaccharide of *E. coli* type K1.

K-Antigens of *Bacteroides fragilis*. The capsular polysaccharide complex of *B. fragilis* possesses unusual biological properties and comprises two polysaccharides with unique chemical structure [118]. The obligate anaerobic Gram-negative rods of *B. fragilis* are very often detected in the humans with such infections as intraperitoneal sepsis or bacteremia. The polysaccharide complex of this microorganism intensifies arising intraperitoneal abscess, and can prevent induction of abscess on systematic introduction during intraperitoneal sepsis. These properties of the polysaccharide complex are connected with T-cell immune mechanism. Both polysaccharides A and B included in the complex are defined by the occurrence of

positively charged amino groups and negatively charged carboxyl and phosphate groups in the repeating unit. The structural features of polysaccharides A (where Sug is 4-amino-2-acetamido-2,4,6-trideoxy-D-galactose) and B (where 2AEP is 2-aminoethyl phosphate), respectively, are shown below:



Namely, the occurrence of opposite charged groups in the capsular polysaccharides is needed for development of intraperitoneal abscesses as well as for defense against induction of the similar abscesses.

The bacterial capsular polysaccharide antigens (K-antigens) have attracted great attention of specialists in carbohydrate chemistry, immunochemistry, immunology, and medicine beginning in the 1920s [1-12, 62, 70]. This is connected with the phenomenon that capsular polysaccharides, on one hand, determine virulence of microorganisms and symptoms of various diseases caused by the different Gram-positive and Gram-negative bacteria possessing capsule and producing polysaccharides in the microbial medium and, on the other hand, they represent antigens or haptens as the basis of immunogens inducing immunity to the pathogenic microorganisms noted above.

The complicated structure of the capsular polysaccharides very often called for the development and application of new methods of structural analysis of sugar chains. Studies of structures of most capsular polysaccharides are of great interest for specialists in bioorganic chemistry and chemistry of polysaccharides. High physiological activity and, first of all, immunomodulatory action became a subject for researchers in the field of molecular immunology. Considerably fewer investigations have been devoted to elucidation of the dependence of physiologic activity of the capsular polysaccharides on their chemical structure. This very complicated problem remains relevant for bioorganic chemistry and molecular immunology.

Author expresses his sincere thanks to Corresponding Member of the Russian Academy of Sciences, Prof. V. E. Vaskovsky (Pacific Institute of Bioorganic Chemistry, Far Eastern Branch of the Russian Academy of Sciences, Vladivostok) for assistance in selection of references, and to Yu. A. Ovchinnikova, engineer of the Institute of Physiology, Komi Science Center, Urals Branch of the Russian Academy of Sciences (Syktyvkar), for substantial assistance in preparation of the article for publication.

This work was supported by grants as follows: Leading Scientific Schools (NSh-5796.2006.4), Russian Foundation for Basic Research (No. 06-04-48079), Programs of Presidium of the Russian Academy of Sciences "Molecular and Cellular Biology" and "Basic Sciences for Medicine".

REFERENCES

1. Kenne, L., and Lindberg, B. (1983) *Bacterial Polysaccharides*, in *The Polysaccharides* (Aspinall, G. O., ed.) Vol. 2, Academic Press, N. Y., pp. 314-344.
2. Scheerson, R., Robins, J. E., Chu, C., Sutton, A., Schiffman, G., and Vann, W. E. (1983) *Progr. Allergy*, **33**, 144-158.
3. Jennings, H. J. (1983) *Adv. Carbohydr. Chem. Biochem.*, **41**, 155-208.
4. Ovodov, Yu. S. (1997) *The Chemical Basis of Immunity* [in Russian], Syktyvkar University Publishers, Syktyvkar.
5. Lee, C.-J. (1987) *Mol. Immunol.*, **24**, 1005-1019.
6. Van Damm, J. E. G., Fleer, A., and Snippe, H. (1990) *Antonie van Leeuwenhoek*, **58**, 1-47.
7. Roberts, I. S. (1996) *Annu. Rev. Microbiol.*, **50**, 285-315.
8. Weintraub, A. (2003) *Carbohydr. Res.*, **338**, 2539-2547.
9. Tzianabos, A., Wang, J. Y., and Kasper, D. L. (2003) *Carbohydr. Res.*, **338**, 2531-2538.
10. Leigh, J. A., and Coplin, D. L. (1992) *Annu. Rev. Microbiol.*, **46**, 307-346.
11. Atkins, E. (1987) in *Industrial Polysaccharides. Progress in Biotechnology* (Yalpani, M., ed.) Elsevier Science Publishers, Amsterdam, pp. 177-198.
12. Fedson, D. S., Musher, D. M., and Eskola, J. (1999) in *Vaccines* (Plotkin, S. A., and Orenstein, W. A., eds.) Saunders, Philadelphia, pp. 553-607.
13. Moreau, M., and Schulz, D. (2000) *J. Carbohydr. Chem.*, **19**, 419-434.
14. Reeves, R. E., and Goebel, W. F. (1941) *J. Biol. Chem.*, **139**, 511-519.
15. Jones, J. K. N., and Perry, M. B. (1957) *J. Am. Chem. Soc.*, **79**, 2787-2793.
16. Lindberg, B., Lindqvist, B., Lonngren, J., and Powell, D. A. (1980) *Carbohydr. Res.*, **78**, 111-117.
17. Stroop, C. J. M., Xu, O., Retzlaff, M., Abeygunawardana, C., and Bush, C. A. (2002) *Carbohydr. Res.*, **337**, 335-344.
18. Polk, B. J., and Kasper, D. L. (1977) *Ann. Int. Med.*, **86**, 567-571.
19. Tzianabos, A. O., Finberg, R. W., Wang, Y., Chan, M., Onderdonk, A. B., Jennings, H. J., and Kasper, D. L. (2000) *J. Biol. Chem.*, **275**, 6731-6740.
20. Tzianabos, A. O., Onderdonk, A. B., Rosner, B., Cisneros, R. L., and Kasper, D. L. (1993) *Science*, **262**, 416-419.
21. Tzianabos, A. O., Wang, J. Y., and Lee, J. C. (2001) *Proc. Natl. Acad. Sci. USA*, **98**, 9365-9370.
22. Kenne, L., Lindberg, B., and Svensson, S. (1975) *Carbohydr. Res.*, **40**, 69-75.
23. Jansson, P.-E., Lindberg, B., and Lindquist, U. (1981) *Carbohydr. Res.*, **95**, 73-80.

24. Rebers, P. A., and Heidelberger, M. (1961) *J. Am. Chem. Soc.*, **83**, 3056-3059.
25. Kenne, L., Lindberg, B., and Madden, J. K. (1979) *Carbohydr. Res.*, **73**, 175-182.
26. Bennet, L. G., and Bishop, C. T. (1980) *Can. J. Chem.*, **58**, 2724-2793.
27. Richards, J. G., and Perry, M. B. (1984) *Can. J. Biochem. Cell Biol.*, **62**, 666-677.
28. Rossell, K. G., and Jennings, H. J. (1983) *Can. J. Biochem. Cell Biol.*, **61**, 1102-1107.
29. Perry, M. B., Daoust, V., and Carlo, D. J. (1981) *Can. J. Biochem.*, **59**, 529-533.
30. Lindberg, B., Lonngren, J., and Powell, D. A. (1977) *Carbohydr. Res.*, **58**, 177-186.
31. Jones, C., Aguilera, B., van Boom, J. H., and Buchanan, J. G. (2002) *Carbohydr. Res.*, **337**, 2353-2358.
32. Lee, C.-J., and Fraser, B. A. (1980) *J. Biol. Chem.*, **255**, 6847-6853.
33. Ohno, H., Yadomae, T. Y., and Miyazaki, T. (1980) *Carbohydr. Res.*, **80**, 297-304.
34. Perry, M. B., Daoust, V., and Lowe, R. (1977) *Abst. WHO 3rd Int. Conf. Immunity, Immun. Cerebrospinal Meningitis*, Marburg.
35. Richards, J. C., and Perry, M. B. (1988) *Biochem. Cell Biol.*, **66**, 758-771.
36. Knecht, J. C., Schiffman, G., and Austrian, R. (1970) *J. Exp. Med.*, **132**, 475-487.
37. Jelinkova, J., and Motlova, J. (1985) *J. Clin. Microbiol.*, **21**, 361-365.
38. Gonzalez-Outeirino, J., Kadirvelraj, R., and Woods, R. J. (2005) *Carbohydr. Res.*, **340**, 1097-1106.
39. Baker, C. J., and Barrett, F. E. (1974) *J. Am. Med. Assoc.*, **230**, 1158-1160.
40. Tai, J. Y., Gotschlich, E. G., and Lancefield, R. C. (1979) *J. Exp. Med.*, **149**, 58-66.
41. Kasper, D. L., Baker, C. J., Baltimore, R. S., Grabb, J. H., Schiffinan, G., and Jennings, H. J. (1979) *J. Exp. Med.*, **149**, 327-339.
42. Di Fabio, J. L., Michon, F., Brisson, J.-R., Jennings, H. J., Wessels, M. R., Benedi, V.-J., and Kasper, D. L. (1989) *Can. J. Chem.*, **67**, 877-882.
43. Pazur, J. H., and Forsberg, L. S. (1978) *Carbohydr. Res.*, **60**, 167-178.
44. Brennan, P. J., Hunter, S. W., McNeil, M., Chatterjee, D., and Daffe, M. (1990) in *Microbial Determinants of Virulence and Host Response* (Ayoub, E. M., Cassel, G. H., Branche, W. C., and Henry, T. J., eds.) American Society for Microbiology, Washington, DC, pp. 55-74.
45. Lemassu, A., and Daffe, M. (1994) *Biochem. J.*, **297**, 351-357.
46. Ellner, J. J., and Daniel, T. M. (1979) *Clin. Exp. Immunol.*, **35**, 250-257.
47. Ortalo-Magne, A., Anderssen, A. B., and Daffe, M. (1996) *Microbiology*, **142**, 927-935.
48. Misaki, A., Azuma, I., and Yamamura, Y. (1977) *J. Biochem.* (Tokyo), **82**, 1759-1770.
49. Forsberg, L. S., Dell, A., Walton, D. J., and Ballou, C. E. (1982) *J. Biol. Chem.*, **257**, 3555-3563.
50. Zubkov, V. A., Nazarenko, E. L., and Botvinko, I. V. (1993) *Russ. J. Bioorg. Chem.*, **19**, 427-432.
51. Kochetkov, N. K., Sviridov, A. F., Arifkhodzhaev, K. A., Chizhov, O. S., and Shashkov, A. S. (1979) *Carbohydr. Res.*, **71**, 193-203.
52. Shashkov, A. S., Sviridov, A. F., Arifkhodzhaev, K. A., and Chizhov, O. S. (1982) *Bioorg. Khim.*, **8**, 1252-1255.
53. Sviridov, A. F., Shashkov, A. S., Kochetkov, N. K., Botvinko, I. V., and Egorov, N. S. (1982) *Bioorg. Khim.*, **8**, 1242-1251.
54. Gorin, P. A. J., Spencer, J. F. T., Lindberg, B., and Lindh, F. (1981) *Carbohydr. Res.*, **79**, 313-315.
55. Pfaller, M. A., Jones, R. N., Doern, G. N., and Kugler, K. (1998) *Antimicrob. Agents Chemother.*, **42**, 1762-1770.
56. Jones, C. (2005) *Carbohydr. Res.*, **340**, 1097-1106.
57. McGuire, E. J., and Binkley, S. B. (1964) *Biochemistry*, **16**, 247-251.
58. Egan, W., Liu, T.-Y., Dorow, D., Cohen, J. S., Robbins, J. D., Gotschlich, E. G., and Robbins, J. B. (1977) *Biochemistry*, **16**, 3687-3692.
59. Rodriguez, M.-L, Jann, B., and Jann, K. (1988) *Eur. J. Biochem.*, **177**, 117-124.
60. Vann, W. F., Schmidt, M. A., Jann, B., and Jann, K. (1981) *Eur. J. Biochem.*, **116**, 359-364.
61. Schmidt, M. A., Jann, B., and Jann, K. (1982) *FEMS Microbiol. Lett.*, **14**, 69-74.
62. Jann, K. (1983) in *Bacterial Lipopolysaccharides* (Anderson, L., and Unger, F. M., eds.) American Chemical Society, Washington, D.C., pp. 171-191.
63. Jann, B., Shashkov, A. S., Kochanowski, H., and Jann, K. (1995) *Carbohydr. Res.*, **277**, 353-358.
64. Leslie, M. R., Parolis, H., Parolis, L. A. S., and Petersen, B. O. (1998) *Carbohydr. Res.*, **309**, 95-101.
65. Jann, K., Jann, B., and Beyart, O. (1985) *Eur. J. Biochem.*, **147**, 601-609.
66. Oscarson, S., and Sehgelmeble, F. W. (2005) *Tetrahedron: Asymmetry*, **16**, 121-125.
67. Parolis, H., Parolis, L. A. S., Stanley, S. M. R., and Dutton, G. G. S. (1990) *Carbohydr. Res.*, **200**, 320-328.
68. Egan, W., Tsui, F. R., Schneerson, R., and Robbins, J. B. (1984) *J. Biol. Chem.*, **259**, 811-813.
69. Nataro, J. P., and Kaper, J. B. (1998) *Clin. Microbiol. Rev.*, **11**, 142-201.
70. Jennings, H. J. (1990) *Curr. Top. Microbiol. Immunol.*, **150**, 97-127.
71. Conrad, H. E., Bamburg, J. R., Epley, J. D., and Kindt, T. J. (1966) *Biochemistry*, **5**, 2808-2817.
72. Stenutz, R., Erbing, B., Widmalm, G., Jansson, P.-E., and Nimmich, W. (1997) *Carbohydr. Res.*, **302**, 79-84.
73. Erbing, C., Kenne, L., Lindberg, B., Lonngren, J., and Sutherland, I. W. (1976) *Carbohydr. Res.*, **50**, 115-120.
74. Dutton, G. G. S., and Yang, M. T. (1973) *Can. J. Chem.*, **51**, 1826-1832.
75. Kjellberg, A., Widmalm, G., Jansson, P.-E., and Nimmich, W. (1995) *Carbohydr. Res.*, **273**, 53-62.
76. Thurow, H., Choy, Y.-M., Frank, N., Niemann, H., and Stirm, S. (1975) *Carbohydr. Res.*, **41**, 241-255.
77. Niemann, H., Frank, N., and Stirm, S. (1977) *Carbohydr. Res.*, **59**, 165-177.
78. Dutton, G. G. S., Mackie, K. L., and Yang, M.-T. (1978) *Carbohydr. Res.*, **65**, 251-263.
79. Churms, S. C., Merrifield, E. H., and Stephen, A. M. (1980) *Carbohydr. Res.*, **81**, 49-58.
80. Lindberg, B., Lindh, F., Lonnngren, J., and Nimmich, W. (1979) *Carbohydr. Res.*, **70**, 135-144.
81. Lindberg, B., Lindh, F., Lonngren, J., and Sutherland, I. W. (1979) *Carbohydr. Res.*, **76**, 281-284.

82. Joseleau, J.-P., Lapeyre, M., Vignon, M., and Dutton, G. G. S. (1978) *Carbohydr. Res.*, **67**, 197-212.
83. Kamerling, J. P., Lindberg, B., Lonngren, J., and Nimmich, W. (1975) *Acta. Chem. Scand.*, **B29**, 593-598.
84. Lindberg, B., Lonngren, J., Ruden, U., and Nimmich, W. (1975) *Carbohydr. Res.*, **42**, 83-93.
85. Dutton, G. G. S., and Yang, M.-T. (1977) *Carbohydr. Res.*, **59**, 179-192.
86. Choy, Y.-M., and Dutton, G. G. S. (1974) *Can. J. Chem.*, **52**, 684-687.
87. Batavyal, L., and Roy, N. (1981) *Carbohydr. Res.*, **98**, 105-113.
88. Lindberg, B., and Nimmich, W. (1976) *Carbohydr. Res.*, **48**, 81-84.
89. Perry, M. B., Bundle, D. R., Daoust, V., and Carlo, D. J. (1982) *Mol. Immunol.*, **19**, 235-246.
90. DeAngelis, P. L., Gunay, N. S., Toida, T., Mao, W.-J., and Linhardt, R. J. (2002) *Carbohydr. Res.*, **337**, 1547-1552.
91. DeAngelis, P. L. (2002) *Glycobiology*, **12**, 9R-16R.
92. Baine, H., and Cherniak, R. (1971) *Biochemistry*, **10**, 2948-2952.
93. Cherniak, R., and Henderson, B. G. (1972) *Infect. Immun.*, **6**, 32-37.
94. Lee, L., and Cherniak, R. (1974) *Infect. Immun.*, **9**, 318-322.
95. Hughes, J. A., Turnbull, P. C. B., and Stringer, M. F. (1976) *J. Med. Microbiol.*, **9**, 475-485.
96. Cherniak, R., and Frederick, H. M. (1977) *Infect. Immun.*, **15**, 765-771.
97. Hobbs, B. C. (1965) *J. Appl. Bacteriol.*, **28**, 74-82.
98. Kalelkar, S., Glushka, J., van Halbeek, H., Morris, L. C., and Cherniak, R. (1997) *Carbohydr. Res.*, **299**, 119-128.
99. Okutani, K., and Kobayashi, H. (1991) *Nippon Suisan Gakkaishi.*, **57**, 1949-1956.
100. Gorshkova, R. P., Nazarenko, E. L., Zubkov, V. A., Ivanova, E. P., Ovodov, Yu. S., and Knirel', Yu. A. (1993) *Bioorg. Khim.*, **19**, 327-336.
101. Nazarenko, E. L., Gorshkova, R. P., Zubkov, V. A., Shashkov, A. S., Ivanova, E. P., and Ovodov, Yu. S. (1993) *Bioorg. Khim.*, **19**, 733-739.
102. Nazarenko, E. L., Zubkov, V. A., Shashkov, A. S., Knirel', Yu. A., Gorshkova, R. P., Ivanova, E. P., and Ovodov, Yu. S. (1993) *Bioorg. Khim.*, **19**, 740-751.
103. Gorshkova, R. R., Nazarenko, E. L., Zubkov, V. A., Shashkov, A. S., Knirel, Yu. A., Paramonov, N. A., Meshkov, S. V., and Ivanova, E. P. (1997) *Carbohydr. Res.*, **299**, 69-76.
104. Zubkov, V. A., Nazarenko, E. L., Gorshkova, R. P., Ivanova, E. P., Ovodov, Yu. S., Shashkov, A. S., Knirel', Yu. A., and Paramonov, N. A. (1995) *Carbohydr. Res.*, **275**, 147-154.
105. Branefors-Helander, P., Erbing, C., Kenne, L., and Lindberg, B. (1977) *Carbohydr. Res.*, **56**, 117-122.
106. Crisel, R. M., Baker, R. S., and Dorman, D. E. (1975) *J. Biol. Chem.*, **250**, 4926-4930.
107. Egan, W., Tsui, F.-R., Climenson, P. A., and Schneerson, R. (1980) *Carbohydr. Res.*, **80**, 305-316.
108. Branefors-Helander, P., Kenne, L., Lindberg, B., Pettersson, K., and Unger, P. (1981) *Carbohydr. Res.*, **88**, 77-84.
109. Tsui, F.-R., Schneerson, R., and Egan, W. (1981) *Carbohydr. Res.*, **88**, 85-92.
110. Branefors-Helander, P., Kenne, L., and Lindgvist, B. (1980) *Carbohydr. Res.*, **79**, 308-312.
111. Altman, E., Busson, J.-R., and Perry, M. B. (1987) *Eur. J. Biochem.*, **170**, 185-192.
112. Altman, E., Busson, J.-R., and Perry, M. B. (1986) *Biochem. Cell Biol.*, **64**, 707-716.
113. Altman, E., Busson, J.-R., and Perry, M. B. (1987) *Biochem. Cell Biol.*, **65**, 414-422.
114. Knirel, Yu. A., Paredes, L., Jansson, P.-E., Weintraub, A., Widmalm, G., and Albert, M. J. (1995) *Eur. J. Biochem.*, **232**, 391-396.
115. Bundle, D. R., Smith, I. C. P., and Jennings, H. J. (1974) *J. Biol. Chem.*, **249**, 2275-2281.
116. Bhattacharjee, A. K., Jennings, H. J., Kenny, C. P., Martin, A., and Smith, I. C. P. (1975) *J. Biol. Chem.*, **250**, 1926-1932.
117. Jennings, H. J., Resell, K.-G., and Kenny, C. P. (1979) *Can. J. Chem.*, **57**, 2902-2907.
118. Tzianabos, A. O., Kasper, D. L., and Onderdonk, A. B. (1995) *Clin. Infect. Dis.*, **20**, Suppl. 2, S132-S140.