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Minireview

Surface polysaccharides from *Acinetobacter baumannii*: Structures and syntheses

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ABSTRACT

The emergence of multidrug-resistance *Acinetobacter baumannii* requires novel approaches for prevention, treatment and diagnosis. The structures of surface polysaccharides from *A. baumannii* are valuable tools to understand pathogenesis, virulence and immunogenicity. The synthesis of bacterial mono- or polysaccharides may result in novel probes to become important therapeutic options in the fight against *A. baumannii*. This report exemplifies the relevance of glycochemistry for the development of new antibiotics.

1. Introduction

Acinetobacter are generating increasing interest within the scientific community and more particularly in related fields of the biomedical sciences. As a consequence, the number of research papers found in PubMed under the subject "*Acinetobacter*" has increased almost every year over the last two decades. Why does a Gram-negative bacterium motivate such an impressive wave of scientific activities? The reason lies in the significant roles of *Acinetobacter* in the colonization and infection of patients in hospitals around the globe, along with the emergence of multiresistant strains.[1,2] Of particular interest, *Acinetobacter baumannii* (Gram-negative coccobacilli) is the main species within its genus implicated in the cause of nosocomial infections[3,4] and has emerged as a highly antibiotic-resistant pathogen[5–8] with strains resistant to carbapenem,[9–12] colistin,[13–15] tigecycline[16] as well as all clinically used antibiotics.[17–21] *A. baumannii* infects patients receiving mechanical ventilations,[22] military personal with war-related injuries,[23] and patients in long-termed care facilities.[24,25] The type of infections produced by this pathogen includes pneumonia, bacteremia, endocarditis, skin infections, urinary tract infections and meningitis.[26–29]

Since the mid-1980s, the taxonomy of the genus *Acinetobacter* has undergone extensive revision. To date, this genus comprises 38 species, of which 27 have been assigned species names, based on DNA/DNA hybridization[30–32] (previous attempts aimed at typing *Acinetobacter* strains included biotyping, phage typing, serotyping, and characterization of outer membrane proteins).[33–37] *A. baumannii* is the main species responsible for nosocomial infections[4] and routine clinical diagnostic laboratories often have difficulties in differentiating this species from other *Acinetobacter* using well-validated identification methods.[38,39] Thus, capsule (K-antigen) and/or O-antigen serotyping may be helpful for the identification of strains belonging to the medically relevant *A. baumannii* species. A great amount of research has been accomplished by the group of Pantophlet toward the generation of monoclonal antibodies (mAbs) to identify *Acinetobacter* strains.[40–42] These useful components can also serve as epidemiological tracking tools to assess the prevalence of *Acinetobacter* serotypes in clinical environments.[43]

Confusion over the phenotypic nature of *A. baumannii* has been raised in the literature. For a long time, this bacterium was considered to possess a smooth (S)-type LPS architecture, i.e. lipid A linked to a core oligosaccharide region (inner and outer domains) and the latter linked to an O-polysaccharide chain (O-antigen). Recent studies indicate the absence of gene encoding a ligase required to link carbohydrate polymer to the lipooligosaccharide.[44] These findings suggest that *A. baumannii* LPS is rough (R)-type and a capsule is produced. The composition of these surface polysaccharides has tremendous impact over pathogenesis, virulence and immunogenicity.[45]

The elucidation of the polysaccharide chemical structure of *A. baumannii* generated interest in understanding the biosynthetic pathways of those crucial components. Specifically, *A. baumannii* possesses a unique molecular machinery that regulates the transfer of multiple Kdo residues by glycosyltransferases. The distinctiveness of Kdo transferases for the LPS biosynthesis seems unusual and

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studies at the enzymatic level could help to better understand the unique diversity of the genus *Acinetobacter*.[46]

Bacterial surface polysaccharides can elicit antibodies during infection. Thus, antibodies can serve as markers or can be of diagnostic value for clinical samples. Similarly, using surface polysaccharides, the development of a prophylactic vaccine is a promising strategy to reduce infections from this pathogen. Recently, the groups of McConnell[47] and Bonomo[48] brilliantly highlighted considerations for the development of a vaccine against *A. baumannii*, and discussed new approaches to circumvent the burden caused by this pathogen. Consequently, the disclosure of the K-antigen and/or O-antigen could allow the design of better immunogenic tools in order to study humoral responses to *A. baumannii*.

This review will shed some lights over the phenotypic nature of *A. baumannii*, by collecting all the information related to its surface polysaccharides. Furthermore, the synthesis of these virulence determinants will also be covered and a special emphasis will be directed toward formation of ketosidic bonds. Two excellent reviews have been published partially on this subject by Pantophlet[49] and Knirel.[50] A comprehensive analysis of the chemical structures of *A. baumannii* surface polysaccharides is required and may benefit the glycochemical and medical sciences.

2. Surface polysaccharides

The O-antigen is the most variable structure of the LPS component, therefore allowing the possibility to develop efficient O-serotyping schemes. Elucidation of the chemical structure of those crucial components may prove useful for further research toward antibody specificity. As mentioned above, Hall and co-workers recently discovered that *Acinetobacter* spp. lack O-antigen gene cluster, therefore suggesting that only a capsule is produced. A large amount of the surface polysaccharides isolated so far were initially designated as O-antigenic components. *Even though many of the structures reported so far belong to CPS and not the O-antigen, in this section they will be classified as reported in their original papers.*

First of all, Haseley and Wilkinson have reported in 1995 the isolation of O-polysaccharides of *A. baumannii* serovar O2.[51] Further studies suggested that this serovar belongs to genomic specie 13TU strain 108.[52] At present, 26 different polymeric saccharides have been characterized, as presented in Table 1. Of general consideration, aminoglycosides with a D-configuration are typically expressed onto all strains of *A. baumannii* surfaces. In addition, the repeating unit of surface polysaccharides of serovar O5, O24, and of strains 24, 34, MG1, LAC-4, 1053, AB5075 and D36 are composed exclusively of aminoglycosides. *N*-Acetyl glucosamine (Glc*p*NAc) and *N*-acetyl galactosamine (Gal*p*NAc) are the most frequent glycosides isolated although some unusual aminoglycosides have been found in some strains. For example, 3-aminoquinovose (3-amino-3,6-dideoxy-D-glucose, Qui3*N*R) and 4-aminoquinovose (4-acetamido-4,6-dideoxy-D-glucose, Qui4*N*Ac) have been identified in serovar O12/O23 and in strain 9, respectively. Correspondingly, strains 24, MG1 and AB5075 possess surface polysaccharides bearing 2,4-diamino-2,4,6-trideoxy-D-glucose (Qui*N*Ac4*N*R). Furthermore, other surface amino sugars, belonging to the higher monosaccharide family, were isolated from serovar O24, strains LAC-4, A74, ACICU, RBH4, LUH5550 and D36. In the case of serovar O24, legionaminic acid (Leg) was isolated from the polymeric structure whereas 8-epilegionaminic acid (8eLeg) was found in strain LAC-4. As for strain A74, ACICU, RBH4 and LUH5550, di-*N*-acylpseudaminic acid (Pse5Ac7NR) was isolated from the bacteria capsule. Lastly, a novel non-2-ulosonic acid have been recovered for the first time from biological source (strain D36), and was designated 5,7-di-N-acetyl-acinetaminic acid (Aci5Ac7Ac).[53]

All of the saccharides showed in Table 1 possess D-configurations, except for most of the FucNAc and Rha moieties, along with the L-Gal*p*NAcA moiety isolated in strain MG1. A really similar polymer was isolated from the O-antigen of the LPS of *A. baumannii* strain 24. They only differ in the absolute configuration of the Gal*p*NAcA residue and the acetylation pattern at O-6 of the Glc*p*NAc. Also, the polymer of strain 24 possesses the same uncommon disaccharide [→4)-α-D-Gal*p*NAcA-(1→3)-β-D-Qui*p*NAc4NAc-(1→] than the polymer isolated in the LPS of *A. haemolyticus* strain ATCC 17906.[80] These structural similarities are not exclusive to the polysaccharides isolated from those two spp. Here are other important structural similarities between *A. baumannii* strains, *Acinetobacter* spp. and other bacteria:

(1) Serovar O12 and O23 have the exact same polysaccharide found on their surfaces. The same applies for strains A74 and ACICU. Polysaccharides found in strains SMAL, ATCC 17961 and ATCC 17978 have the exact same carbohydrate backbone, with the only difference that the 4OH of the Glc*p*NAc3NAcA moiety is acetylated for strain ATCC 17978.
(2) The initially reported polymeric O-antigen isolated from the LPS of serovar O16 was also found in the surface polysaccharide of serovar O11 and O12. The similarities in their surface compositions seem to provide scope for cross-reaction between some of these serovars.[59]
(3) A minor polymer containing Rha and GlcNAc is proposed to be isolated in serovar O18 or even the presence of a small portion of the polymer isolated from serogroup O10.
(4) The same linear tetrasaccharide found in serovar O7 can be found in serovar O10 [→3)-α-D-Glc*p*NAc-(1→2)-α-L-Rha*p*-(1→2)-α-L-Rha*p*-(1→3)-α-L-Rha*p*-(1→]. This surface polysaccharide is not exclusive to *Acinetobacter*, similar polymer also occurs in other bacteria, including *Serratia marcescens*,[81] *Pseudomonas solanacearum*,[82] and *Escherichia coli*.[83]
(5) Serovar O11 and strain D46 have the exact same polysaccharide found on their surfaces. Partial structural similarities can be observed for a surface tetrasaccharide found in serovar O11 [→3)-α-D-Gal*p*NAc-(1→4)-β-D-Gal*p*NAc-(1→3)-α-D-Gal*p*-(1→6)-β-D-Gal*p*-(1→] where the α-D-Gal*p*NAc residues in *Acinetobacter* 13TU strain 108 is linked at the 3-position of the other β-D-Gal*p*NAc residue.[66]
(6) Both polymers isolated in serovar O24 and strain LAC-4 have a similar trisaccharide containing higher monosaccharide residues: Leg (D-glycero-D-galacto isomer) and 8eLeg (L-glycero-D-galacto isomer) respectively. The linear polysaccharide for serovar O24 is [→3)-α-L-Fuc*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→4)-β-Leg*p*5AR7Ac-(2→] whereas it is [→3)-α-L-Fuc*p*NAc-(1→3)-α-D-Glc*p*NAc-(1→8)-α-8eLeg*p*5Ac7Ac-(2→] for strain LAC-4.[a] The residue 8eLeg can also be found in other bacterial glycopolymers,[84] more significantly the α-D-Glc*p*NAc-(1→8)-α-8eLeg*p*5Ac7Ac disaccharide can be found in *Providencia stuartii* O20.[85]
(7) Structure of the CPS of *A. baumannii* NIPH146 shares the same trisaccharide unit [→6)-β-D-Glc*p*-(1→3)-β-D-Gal*p*NAc-(1→4)-β-D-Gal*p*NAc-(1→4)-α-D-Gal-(1→] than polysaccharides found in strain SMAL, ATCC 17961 and ATCC 17978.

Besides the points discussed above, is there discernible trends concerning structural similarities of surface polysaccharides between *A. baumannii* strains? Most of the elucidated structures (19 of the current 26) are branched, although serovar O24, strains 24, MG1, LAC-4, RBH4, 1053 and AB5075 remain linear. Some of the isolated surface polysaccharides possess non-carbohydrate substituents, which in turn could influence the tertiary conformation of the antigen. The relative antigenicity of a given polysaccharide is also influenced by its secondary or tertiary conformations.[49] In addition, similar non-carbohydrate components have been elucidated in the

[a]For Leg*p*5R7Ac, R is about 50% acetyl and 50% *S*-3-hydroxybutyryl.