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Characterization of the LPS of *Branhamella catarrhalis*: Identification of the Human P Blood-Group Galabiose Epitope

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During the last decade, *Branhamella catarrhalis* has come to be recognized as a cause of acute upper and lower respiratory tract infections¹. Recent chemical and immunological investigations have shed some light on the properties of the surface lipopolysaccharide (LPS) expressed by this pathogen^{2,3}. The LPS is comprised of a hydrophobic lipid A moiety and an oligosaccharide component but lacks a high-molecular-weight O-polysaccharide chain. The oligosaccharide component has been found to be composed of D-glucose, D-galactose, D-glucosamine and 3-deoxy-D-manno-octulosonic acid (Kdo): aldoheptose and phosphate, the usual components of the core oligosaccharides of enteric bacteria, are not components of this LPS. Although a comprehensive serotyping scheme for *B. catarrhalis* strains has not been described, at least three LPS types have been recently reported⁴. In order to understand the molecular basis for antigenic variation among *B. catarrhalis* strains we have undertaken a comparative analysis of the LPS oligosaccharide components from a reference strain and several clinical isolates. Here we report the complete structure of the reference strain using chemical and high resolution NMR techniques. The α -D-Gal_p-(1-4)- β -D-Gal disaccharide unit representing the human P blood-group galabiose epitope was identified in this strain. This epitope is also expressed by pathogenic strains of *Haemophilus influenzae* type B, and is implicated in virulence potential.

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¹H- and ¹³C-NMR Characterization of a Major Sulfated Glycolipid in an Extremely Halophilic Bacterium, Strain 172

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Extremely halophilic bacteria adapted to high osmolarity contain sulfated glycolipids as the major polar lipids. An unknown glycolipid (HALS) from strain 172¹ was assumed to be sulfated as well. On TLC, HALS migrated between S-TGD (sulfated tri-glycosyl diether-glycerol) and S-TeGD (sulfated tetra-glycosyl diether-glycerol) in an acidic solvent system, and a little slower than SM2 (sulfated gangliotriaosylceramide) in a neutral one. Methylation analysis and negative FAB/MS showed that the structure of HALS was Man α 1-2Glc α 1-1 (diether-glycerol), DGD-1, substituted by a sulfate at C-6 of Man, and a further possibility of substitution by an unknown group at C-2 of Man. In this study, we carried out ¹H- and ¹³C-NMR analysis of HALS, mono-sulfated DGD-1 (S-DGD-1) from strain R-4² and desulfated HALS, and characterized the substituents of HALS.

C-6 of Man in S-DGD-1 and HALS resonated at 4.9 ppm

downfield from that of desulfated HALS, due to substitution with a sulfate group. C-2 of Man in HALS was also downfield shifted by 4.9 ppm. The shift increments of H-2, H-6a and H-6b of HALS were compatible with H-3 of HSO₃-3GalCer and H-6a and H-6b of HSO₃-6GalCer, respectively. Densitometry on TLC stained with azure A and IR spectroscopy showed that HALS contained 2 mol sulfate groups. Results described above unanimously support that HALS is 2,3-diether-1-[2,6-(HSO₃)₂- α -mannosyl-1-2- α -glucosyl]-glycerol. This is the first case of a glycolipid with a bis-sulfated hexose.

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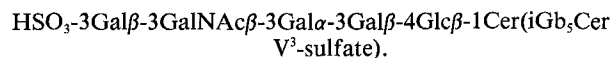
Structural Characterization of a Mono-Sulfated Isoglobopentaosylceramide, The Second Isogloblo-Series Sulfoglycosphingolipid, from Rat Kidney

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Rat kidney contains 6 sulfated glycolipid species which belong to the gala-series (SM4s), and the ganglio-series (GlcCer I³-sulfate, SM3, SM2, SB2 and SB1a). Recently, we found a novel sulfoglycosphingolipid based on the isogloblo-series core and established the complete structure to be iGb₄Cer IV³-sulfate (1). In the present study, we isolated from rat kidney a more complex sulfoglycolipid (Kc) that also belongs to the isogloblo-series.

Kc was purified by FPLC with DEAE-Toyopearl and HPLC with Iatrobeads. The structure was characterized by solvolysis, compositional analyses, ¹H-NMR, negative LSIMS, FT-IR and permethylation analyses. The two-dimensional DQF-COSY experiment evidenced the presence of a 3-O-sulfated Gal in the molecule. The interresidue signals observed in the NOE spectrum confirmed that Kc contains a Gal α 1-3Gal structure. The major ceramide consisted of 24:0/t18:0, deduced from the compositional analysis, ¹H-NMR and LSIMS. The characteristic fragment ions for a sulfate and sulfated oligosaccharides were observed in LSIMS and collision-induced dissociation linked scanning spectra. From the above results, the complete structure of Kc was proposed to be



The yield of iGb₄Cer V³-sulfate was 0.1 nmol/g wet tissue, which was about one third of that of iGb₄Cer IV³-sulfate. iGb₄Cer, a species specific glycolipid of rat, is not characteristic of rat tumor but found to be associated with some malignant neoplasms of rat. Therefore, it seems relevant to clarify how the expression of these two sulfoglycolipids of the isogloblo-series relates to the malignant alteration of rat kidney.

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