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Haemophilus influenzae type b (Hib) is a major cause of bacterial meningitis in children in the United States and many other countries. To prevent Hib disease, Hib polysaccharide (PS)-protein conjugate vaccines have been developed by a few manufacturers. Measurement of PS content in the conjugate vaccines is required to assure their quality and effectiveness. The PS content in the vaccines is currently measured by ribose, a component of the PS, using the colorimetric orcinol assay. This assay is not useful for a vaccine containing lactose, a sugar stabilizer, due to its interference. We have developed a simple HPLC method to quantitate the PS in the vaccines regardless of the presence of the sugar stabilizer. The PS in the vaccines was depolymerized in sodium hydroxide. The major depolymerized product (82%) was isolated and characterized by composition analysis and ^{31}P NMR to be a single repeating unit of the PS, ribitol-ribose-phosphate. The PS repeating unit in the alkali-treated vaccines was separated from the large amount of sugar stabilizer by high-performance anion-exchange chromatography using CarboPak PA-1 column (Dionex Corp.) and quantitated by pulsed amperometric detection. The procedure is simple and reproducible. This HPLC method can quantitate PS levels as low as 0.1 μg or at a PS concentration of one μg/ml, and the sensitivity is at least 30-fold higher than that of the orcinol assay. This HPLC method of quantitation may be applied to other bacterial PS or PS-conjugate vaccines after hydrolysis of PS to monosaccharides or oligosaccharides.

S15.27

Structural Determination of the Lipooligosaccharides of *Haemophilus ducreyi* and Possible Therapeutic Targets

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Haemophilus ducreyi, a Gram-negative bacterium that colonizes genital mucosae, is the pathogenic agent that causes chancroid, a genital ulcer disease. Among the major surface antigens presented on the outer membrane of this organism are lipooligosaccharides (LOS). The focus of our research is to investigate the roles played by LOS in adhesion to mucosal epithelial cells, evasion of the host response and the formation of genital ulcers.

We have determined the first complete structure of the major LOS from *H. ducreyi* strain 35000 as well as the partial structures from other strains, using various mass spectrometric and two-dimensional nuclear magnetic resonance techniques. All of the wild-type organisms we have studied show the presence of epitopes that mimic blood group antigens, presumably allowing these organisms to evade the host immune response. We have also found that many strains contain LOS that are sialylated on a terminal lactosamine. Sialic acid is believed to be crucial to the pathogenicity and virulence of *Haemophilus ducreyi,* since it is known for other bacteria that the removal of sialic acid leaves them vulnerable to lysis by human serum and attack by human neutrophils.

We hope that the kinds of structural information we are providing will lead to therapeutic strategies to fight this disease. For example we are in the process of isolating the sialyltransferase that effects the transfer of sialic acid to lactosamine. Knowledge of the amino acid sequence and three-dimensional conformation of this enzyme will permit the design of specific inhibitors. Other possible targets for rational drug design are the additional glycosyltransferases needed for LOS biosynthesis, some of which could be targeted so as to disrupt the synthesis of the oligosaccharide epitopes that are immunochemically similar to the host's blood group antigens.

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S15.28

Approaches to the Vectorisation of Drugs via Cyclodextrin Based Glycoconjugates

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The cyclodextrins (CDs) are a class of oligosaccharides widely studied for their ability to transport pharmaceutically active molecules. However their transport within a biologically active medium will be non-specific. In order to target sites, cells or micro-organisms it is necessary to couple biologically active recognition systems onto the host molecule.

We have coupled, via a Lemieux type spacer, a number of saccharide groups onto the primary face of β-CD, using amide couplings at both the carbohydrate and CD linkage points. Preliminary studies using recognition by the Cell Wall Lectin of Kluyveromyces Bulgaricus show reasonably strong binding for the β-galactosamide system and this molecule has been tested for its vectored transport of Itraconazole, a potent fungicide.

The use of a chemico-enzymatic approach has allowed us to prepare a spacer linked *N*-Ac-lactosamine system, and the molecular properties of this molecule and the parent *N*-Ac-glucosamine system will be presented.

S15.29

Glycosylation of Haemoglobin in Red Blood Cells of Spontaneously Hypertensive Rats and Normotensive Wistar-Kyoto Rats

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Epidemiologic and clinical research may suggest similar pathophysiology of coronary heart diseases, hypertension and diabetes. We postulate that the changes in insulin level in development of hypertension may be related to decreased glycosylation of blood proteins.

The glycosylation of haemoglobin in red blood cells was assayed in nine weeks old spontaneously hypertensive rats (SHR) and normotensive Wistar – Kyoto rats (WKY). These two groups had different systolic blood pressure (WKY: