Aplicaciones de la Glicobiología en la salud humana. III Simposio Cubano de Carbohidratos

José A Cremata

Departamento de Química de Carbohidratos. Divisón de Química-Física.
Centro de Ingeniería Genética y Biotecnología, AP 6162, La Habana 10600, Cuba.
E-mail: jose.cremata@cigb.edu.cu

Hace poco tiempo relativamente se introdujo el término "Glicobiología" para describir la disciplina que estudia el complejo papel que desempeñan los carbohidratos en los diferentes glicoconjugados presentes en todos los organismos. En 1988, el profesor Raymond Dwek, actual director del Instituto de Glicobiología de la Universidad de Oxford, en una entrevista concedida a un programa matinal de la televisión británica, definió por primera vez este vocablo que une estrechamente la biología y la química de los carbohidratos, y que, en la actualidad, ha despertado gran interés en la comunidad científica internacional.

El desarrollo de vacunas humanas es un tema que particularmente acapara la atención de muchos investigadores en todo el mundo. La eficacia de las vacunas en los grupos poblacionales y edades de mayor riesgo, las enfermedades emergentes y los problemas relacionados con los costos de los programas de vacunación a nivel mundial, hacen de este tema un punto de creciente interés de las industrias biotecnológica y biofarmacéutica.

Entre los aspectos a los que mayor atención se le prestó en el III Simposio Cubano de Carbohidratos, celebrado en La Habana los días 17 y 18 de abril del 2001 se encuentran la síntesis organoenzimática de glicoconjugados a partir de oligosacáridos sintéticos y proteínas portadoras que estimulan las respuestas de memoria o las dependientes de células T del sistema inmunitario. Se discutieron casos como la síntesis de las unidades repetitivas de los serotipos de *Streptococcus pneumoniae* 6B, 14 y 3 conjugadas a proteínas portadoras como el toxoide tetánico y CRM197 (reporte "Towards Oligosaccharide-Protein Conjugate Vaccines and Diagnostics"); y la síntesis química de un fragmento nonasacarídico del determinante antigénico del serotipo 19F de *S. pneumoniae* y de un disacárido del lipopolisacárido de *Bordetella pertusis* (reporte "Synthesis of Spacer-Containing Fragments of Streptococcus and Bordetella Cell-Surface Carbohydrates"). También se abordó ampliamente la química de los brazos espaciadores en la conjugación de oligosacáridos sintéticos (reportes "Towards Oligosaccharide-Protein Conjugate Vaccines and Diagnostics" y "Synthesis of Spacer-Containing Fragments of Streptococcus and Bordetella Cell-Surface Carbohydrates").

En relación con las estrategias de desarrollo de vacunas conjugadas, se ha logrado obtener una vacuna glicoconjugada contra el *Haemophilus influenzae* tipo b (Hib) que es económicamente efectiva a partir de la purificación del polisacárido capsular del Hib. En este sentido se han propuesto algunas vías de conjugación económicamente factibles y de fácil escalado fundamentalmente para su producción en países en vías de desarrollo con pocos recursos financieros. Un ejemplo lo constituyen los resultados de la colaboración entre RIVM y BioFarma de Indonesia (reporte "Development of a Cost-Effective Glycoconjugate Vaccine Against *Haemophilus influenzae* Type B"). El Laboratorio de Estructura Molecular del Instituto Nacional de Estándares Biológicos y Control del Reino Unido, propuso criterios de evaluación para la descripción de los métodos de análisis aceptados para la caracterización de vacunas conjugadas. Este laboratorio tuvo en cuenta la consideración actual de que los bioensayos no permiten predecir de forma adecuada las posibilidades de protección con estos glicoconjugados cuando se utilizan como vacunas, al igual que la tendencia reciente a sustituir los bioensayos por técnicas de caracterización química y bioquímica. Entre las técnicas de caracterización se han empleado RMN, espectrometría de masas, dicroísmo circular y espectroscopia de fluorescencia (reporte "Spectroscopic Studies of the Structure and Stability of Glycoconjugate Vaccines"). Estos aspectos se discutieron recientemente en el Encuentro Internacional sobre el Control de Vacunas Conjugadas auspiciado por la OPS [1].

En cuanto a la química analítica, se mencionaron trabajos relacionados con los métodos de perfilado para la caracterización del patrón de glicosidación de proteínas mediante el la utilización del ácido 4-aminobenzoico como reactivo fluorescente. Este reactivo permite separar con gran resolución las estructuras asialocomplejas en columnas de HPLC de fase normal (TSK Amide-80), y su combinación con intercambio iónico para separar complejos sialilados (reporte "HPLC Profiling of *N*-glycans on Glycoproteins"). La utilización de métodos de perfilado ha mostrado las ventajas de este tipo de metodología en el análisis de la variación del patrón de glicosidación de la IgG sérica en procesos autoinmunitarios. Estos métodos han sugerido que la medición de las diantenas no galactosidadas es un parámetro indicativo de la tendencia al aumento de esta glicoforma, tal como se ha demostrado rotundamente en la literatura científica especializada durante los últimos 10-15 años. No obstante, no es recomendable utilizar estos métodos como medida absoluta en el diagnóstico y seguimiento de algunas enfermedades, en particular la artritis reumatoide (reporte "Serum IgG Glycosylation Pattern in Autoimmune Diseases: Is this a Good Marker?").

Recientemente ha tomado auge el papel de los sialoglicoepítopos involucrados en procesos inflamatorios y de adhesión celular. De aquí que la síntesis de inhibidores potentes de las sialiltransferasas pueda tener hoy en día aplicación terapéutica importantes en procesos inflamatorios y metastásicos fundamentalmente. La síntesis química de este tipo de inhibidores (reporte "Design of New Glycosyltransferase Inhibitors"), al igual que la obtención de glicomiméticos

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de agregados multivalentes que contienen terminales glicosídicas mediante catálisis con metales de transición (reporte "Recent Developments in Organo-Metallic Chemistry Toward the Syntheses of Glycomimetics"), son aspectos de gran relevancia en los que se trabaja en la actualidad.

El tema de las enfermedades congénitas debidas a alteraciones en la vía de glicosidación de proteínas merece mención aparte [2]. Se destacan los resultados obtenidos en las investigaciones del Dr. Hudson Freeze, quien dirige el Programa de Glicobiología del Instituto Burnham, La Jolla, California. Conocidos como alteraciones congénitas de la glicosidación *(*CDG, del inglés *congenital disorders of glycosylation)*, han sido clasificados en dos grupos fundamentales y, a su vez, en diferentes tipos. En el caso del tipo Ib, la deficiencia de la enzima fosfomanosa-isomerasa —transforma la fructosa-6-fosfato en manosa-6-fosfato— impide que el donante adecuado esté disponible para la síntesis del oligosacárido precursor. A partir del conocimiento de una vía alternativa de síntesis de manosa-6-fosfato, la adición de manosa en la dieta de estos enfermos (tipo Ib) ha provocado una mejora sustancial de los pacientes. La incidencia de este tipo de enfermedad no es elevada; sin embargo, la búsqueda de soluciones a otras variantes de CDG garantizaría una mejora en la calidad de vida de aquellos niños que nacen con esta deficiencia (reporte "Congenital Disorders of Glycosylation: Seize the Opportunity").

1. Encuentro Internacional sobre el Control de Calidad de Vacunas Conjugadas (OMS/OPS). Mayo 2-5, 2001. La Habana, Cuba.
2. Alper J. Saving lives with sugars. Science 2001;291:2339–40.

Towards Oligosaccharide-Protein Conjugate Vaccines and Diagnostics

JP Kamerling[1], MJL Thijssen[1], J Niggemann[1], D Michalik[1], DJ Lefeber[1], JAF Joosten[1], KM Halkes[1], HJ Vermeer[1], K Ágoston[2], J Kerékgyárto[2], JFG Vliegenthart[1]

Streptococcus pneumoniae is a Gram-positive bacterium that can cause pneumonia, otitis media and meningitis in humans. To date, up to 90 different serotypes of this bacterium have been identified by their capsular polysaccharide. The commercially available current polyvalent vaccine is constituted of the purified capsular polysaccharides of 23 serotypes of *S. pneumoniae.* Nevertheless, this vaccine is poorly immunogenic in persons of high risk such as children under the age of two years and elderly persons. Since a polysaccharide-induced immune response does not evoke an immunological memory, only short-term protection is provided. Moreover, the induction of tolerance is a severe problem. These disadvantages, especially the absence of long-term protection, may be overcome by the use of oligosaccharide-conjugate based vaccines. In the framework of our studies towards oligosaccharide-conjugate based vaccines against *S. pneumoniae* we have focused on the organic/enzymatic synthesis of spacer-containing fragments of the repeating units of the capsular polysaccharides of the *S. pneumoniae* serotypes 6B, 14 and 3. The various fragments were conjugated with protein carriers *(e.g.* tetanus toxoid and CRM197), and the obtained neoglycoproteins were subjected to immunological studies, including immunizations of mice.

Human schistosomiasis is one of the major parasitic diseases, second only to malaria in generating morbidity and suffering in tropical zones world-wide. One of the species, infectious to humans is *Schistosoma mansoni.* The life cycle of the parasite is partly enacted in man and partly in fresh-water snails. Because for cure of the infection chemotherapy is available, early diagnosis is important. In the context of developing more advanced diagnostics based on the carbohydrate part of *Schistosoma* antigens we have focused on the organic synthesis of spacer-containing fragments of the carbohydrate moieties of the gut-associated circulating anodic antigen of the worm stage and of the glycocalyx of the cercarial stage. The various fragments were conjugated with bovine serum albumin, and the obtained neoglycoproteins were tested for their interaction with panels of antibodies (ELISA and BIAcore studies).

[1]Bijvoet Center, Department of Bio-Organic Chemistry. Utrecht University, PO Box 80.075, 3508 TB Utrecht, The Netherlands. Tel: +31 30 2533479; Fax: +31 30 2540980; E-mail: kame@boc.chem.uu.nl
[2]Institute of Biochemistry, Faculty of Sciences, University of Debrecen, PO Box 55, H-4010 Debrecen, Hungary.

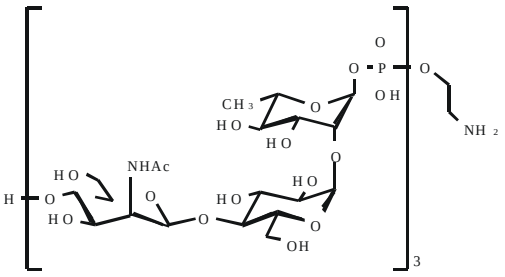
Synthesis of Spacer-Containing Fragments of *Streptococcus* and *Bordetella* Cell-Surface Carbohydrates

M Nilsson[1], T Norberg[2]

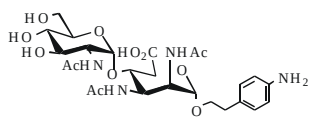
Two examples of chemical synthesis of bacterial surface carbohydrate structures will be presented: synthesis of a nonasaccharide fragment of the capsular polysaccharide from *Streptococcus pneumoniae* 19F, and synthesis of a disaccharide fragment from the lipopolysaccharide of *Bordetella pertussis.* Both structures were synthesized starting from from thioglycoside monosaccharide building blocks. In the case of the Pertussis disaccharide, a 2,3-diazidomannose monomer derivative was first synthesized by a 9-step sequence from glucose, and was then used as acceptor in a glycosylation reaction with a glucosamine derivative. In the case of the *Streptococcus* nonasaccharide, a trisaccharide building block was was first constructed, this was then oligomerized in a stepwise manner using solution H-phosphonate chemistry.

The prepared oligosaccharides have been conjugated to suitable proteins and the conjugates are currently being subjected to biological tests.

[1]Medivir AB, Lunastigen 7, SE-141 44 Huddinge, Sweden (magnus.nilsson@medivir.se).
[2]Department of Chemistry, Swedish University of Agricultural Sciences. PO Box 7015, S-750 07 Uppsala, Sweden (thomas.norberg@kemi.slu.se).



Streptococcus pneumoniae 19F nonasaccharide



Bordetella pertussis disaccharide

*D*evelopment of a Cost-Effective Glycoconjugate Vaccine Against *Haemophilus influenzae* Type B

M Beurret[1], JG Kreeftenberg[2]

Following the introduction of safe and efficacious glycoconjugate vaccines in the last decade, the eradication of meningitis and other systemic infections caused by *Haemophilus influenzae* type b (Hib) is now almost complete in the industrialized nations. To achieve access of the less economically endowed areas to these recent medical progresses, it is possible to apply publicly known glycoconjugation procedures for simple and rapid scale up and production of cost-effective vaccines.

An important aspect of this approach is to directly involve the local vaccine producers of these areas. Such a partnership has been initiated between RIVM (Bilthoven, The Netherlands) and Bio Farma (Bandung, Indonesia), involving departments of both institutes at all levels of research, scale up and, soon, production. The procedures used for the chemical modification of the Hib polysaccharide and its conjugation to a carrier protein, as well as for the purification of the glycoconjugate vaccine thus obtained, will be discussed.

[1]National Institute of Public Health and the Environment (RIVM), Laboratory for Vaccine Research.
[2]Bureau of International Cooperation. PO Box 1, 3720 BA Bilthoven, The Netherlands. Tel: +31 (30) 274 39 86; Fax: +31 (30) 274 44 29; E-mail: Michel.Beurret@rivm.nl

*S*pectroscopic Studies of the Structure and Stability of Glycoconjugate Vaccines

C Jones, X Lemercinier, CK Gee, DT Crane, S Austin

As biological assays are poorly predictive of the ability of glycoconjugate vaccines to protect infants, quality control of these products is heavily dependent on physicochemical approaches. We have developed a range of spectroscopic techniques to characterise the structure and stability of both the saccharide and carrier protein moieties of commercial glycoconjugate vaccines, and their components.

This talk will provide an overview of the techniques we have used. NMR spectroscopy has been used to characterise the polysaccharide, activated polysaccharide and saccharide chains in the conjugate, and to observe depolymerisation of the chains. CRM197, a common carrier protein, has been analysed by electrospray mass spectrometry (ESMS), circular dichroism (CD) and fluorescence spectroscopy, both in its native form and in the glycoconjugate. Fluorescence and CD have been used to monitor denaturation of the carrier in the conjugate when heated, and how the presence of saccharide chains modulates the behaviour of the carrier protein. The location of the saccharide chains have been determined by coupled HPLC-ESMS of Asp-N-derived peptides and glycopeptides.

Laboratory for Molecular Structure, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Herts EN6 3QG, UK. Tel. +44 1707 654753; Fax: +44 1707 646730; E-mail: cjones@nibsc.ac.uk

*H*PLC Profiling of N-Glycans on Glycoproteins

CT Yuen, CK Gee, C Jones

Monitoring of protein glycosylation such as of the *N*-linked types becomes increasingly important and necessary as more recombinant glycoprotein therapeutics, for example glycoprotein hormones and cytokines are produced. There are many approaches and strategies but as they all have their pros and cons, it becomes a matter of equipment availability as well as personal preference and expertise. The general strategy is: a) release of oligosaccharides either by enzymes or chemicals; b) tagging of oligosaccharides with a chromophore/fluorophore to increase detection sensitivity and c) profiling and identification of the released oligosaccharides.

In this presentation, we will describe an HPLC-based procedure to profile *N*-linked glycans obtained from a variety of commercially available glycoproteins to demonstrate the methods. This involves the release of *N*-glycans by PNGase F, derivatization with 4-aminobenzoic acid (4ABA), profiling using anion exchange and normal phase HPLC and identification of oligosaccharide components by mass spectrometry.

N-Glycans released from as little as 1 μg glycoprotein and labeled with 4ABA were easily profiled on a normal phase HPLC column (TSK Amide-80). When an anion exchange separation was included, 4ABA labeled *N*-glycans could be profiled and compared according to their charges. Some unresolved *N*-glycan components when using only normal phase column alone could then be separated. Low picomolar quantity of 4ABA derivatives can be detected with confidence on the system.

Conclusion: Since only common reagents and conventional HPLC system are used, the whole process is easy to perform, control and adapt for most general laboratories. Complementary techniques such as capillary electrophoresis, MS, LC-MS or enzyme microsequencing can also be applied to the derivatised oligosaccharide fractions if desired.

Laboratory of Molecular Structure, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire EN6 3QG, UK. Tel: +44 (0)1707 654753; Fax: +44 (0)1707 646730; E-mail: cyuen@nibsc.ac.uk

*S*erum IgG Glycosylation Pattern in Autoimmune Diseases: Is this a Good Marker?

JA Cremata, R Montesino

IgG consists of two heavy and two light polypeptide chains linked together by disulfide bridges. According to its biological functions the IgG molecule can be divided into the antigen binding fragment (Fab) and the region responsible for the effector functions (Fc). In the Fc region, specifically the two CH2 domains contain conserved glycosylation sites at Asn-297, to which complex biantennary oligosaccharides are attached. Normal serum IgG shows heterogeneous oligosaccharide profiles in their outer arms. The asialo complex oligosaccharides can be grouped mainly into three sets depending on whether they contain 0, 1 or 2 galactose residues (G0, G1 and G2 respectively). Hypogalactosylation of IgG has been shown to affect

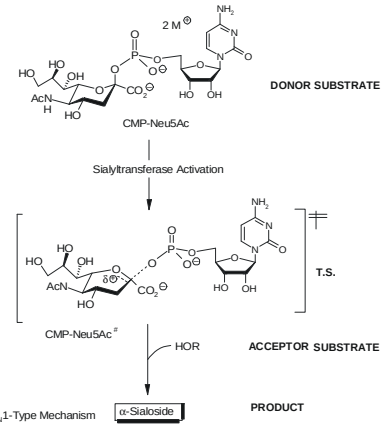
some of the effector functions including binding to complement component C1q. Recently, it has been suggested that the orientation of the oligosaccharide chains affects the ability to activate complement. Healthy people show G1 > G0 as the characteristic glycosylation pattern. Glycosylation profile of IgG in autoimmune diseases has been already described. Decreased levels of galactosylation (G0 > G1) have been noted in a restricted range of diseases, in which rheumatoid arthritis (RA) is included. It is characterized by the presence of a rheumatoid factor (autoantibodies against the Fc region of IgG whose structure has already been reported) while psoriatic arthritis (PsA) is not associated to autoantibodies. Association between glycosylation changes and disease has been postulated. The purpose of the present study is to look at the oligosaccharide profiles of IgG present in RA and PsA patients compared with healthy control. While most of the samples corresponding to RA patients showed glycosylation profiles where the G0 structure predominate, there are some in which normal patterns were observed. But this is not the case for PsA where better correlation with increased G0 population in serum IgG is apparent. This result suggests the necessity to be extremely careful when using glycosylation pattern as a possible diagnostic marker in RA disease.

GlycoLab, Department of Carbohydrate Chemistry. Center for Genetic Engineering and Biotechnology. PO Box 6162, Havana, Cuba. E-mail: jose.cremata@cigb.edu.cu

Design of New Glycosyltransferase Inhibitors

RR Schmidt

Sialic acid containing epitopes are involved in important biological processes, such as cell adhesion and inflammation. There is also a correlation between the sialyl content of glycoconjugates and the malignancy of tumor cells. Recently, an interesting correlation between α(2-6)-sialylation of *N*-acetyllactosamine and B lymphocyte activation and immune function was reported, which could find medicinal application. Therefore, in order to study the influence of sialyl residues in biological systems, it is highly desirable to develop efficient inhibitors acting on sialyltransferases (Figure). Similar observations hold for other sugar residues.



Scheme. Sialyl transfer: S_N1-Type Mechanism

Based on our previous studies on glycosyltransferase inhibitors, the synthesis of efficient substrate and transition state analogous inhibitors of glycosyltransferases and especially of sialyltransferases has been undertaken. Particularly rewarding were mimetics of the presumed transition state of the glycosyl donors, i.e. of nucleoside-phosphate sugars. The basic considerations are exhibited in the Figure. Our recent results, particularly on sialyltransferase inhibition, will be discussed.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft. I am particularly indebted to my collaborators, who will be individually mentioned.

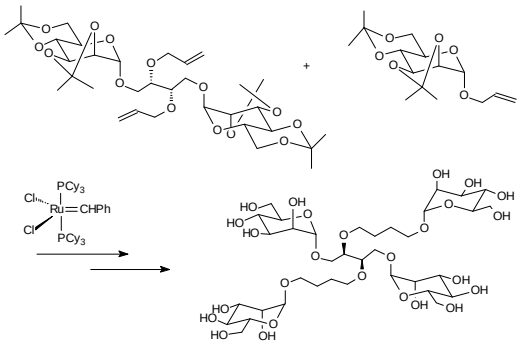
Fachbereich Chemie, Universität Konstanz, M 725, 78457 Konstanz, Germany. Phone: +49-7531-88 2538; Fax: +49-7531-88 3135; E-mail: Richard.Schmidt@uni-Konstanz.de

Recent Developments in Organo-Metallic Chemistry Toward the Syntheses of Glycomimetics

R Roy, R Dominique, B Liu

Carbohydrate-containing clusters of various valencies were synthesized using transition metal-catalyzed reactions. Thus, olefin self-metathesis with Grubb's catalyst [Cl2Ru(PCy3)2=CHPh], were used successfully from either O- and C-alkenyl glycosides to generate a wide range of clusters and their precursors. Furthermore, palladium catalyzed cross coupling between 2-propynyl and 4-iodophenyl glycosides afforded novel "sugar-rods" useful in studying multivalent carbohydrate-protein interactions. Finally, cyclotrimerization of terminal as well as symmetrical alkyne derivatives with dicobalt octacarbonyl allowed easy access to trimers and "molecular-asterisks", respectively. In the Figure presented below, mannoside clusters designed to inhibit bacterial infections by *E. coli* strains were prepared by a sequence of reactions involving olefin self-metathesis followed by catalytic asymmetric dihydroxylation using Sharpless reagents.

Department of Chemistry, University of Ottawa, Ottawa, ON, Canada K1N 6N5. Tel: (613)-562-5800, ext. 6055; Fax: (613)-562-5170; E-mail: rroy@science.uottawa.ca



Congenital Disorders of Glycosylation: Seize the Opportunity

H Freeze

The functions of protein glycosylation are so diverse that one review stated that "all the theories are correct" while noting that none is always true. Predicting

the outcome of altered glycosylation at the molecular, cellular, or organismic level is a difficult chore. N-linked glycosylation is the best-characterized biosynthetic pathway, partly because mutants in many of the steps are available in yeast, mammalian cells or mice. Recently, human mutations in this pathway have been identified and show the importance of glycosylation for our species. The rapid development of this field in the past 5 years owes its success to sharp-eyed clinicians and the availability of biochemical and molecular assays in several model systems. The clinical presentations of the patients with these congenital disorders of glycosylation (CDG) are extremely broad, but often involve tissues where the demand for glycosylation is high or cell growth is rapid. Mental and psychomotor retardation is common but not universal, while intestinal problems, growth retardation, and decreased levels of liver-derived glycoproteins often affect the patients. A few of the disorders are treatable with simple monosaccharide dietary therapy. Given the high frequency of mutations in some of the genes that cause CDG, it is quite possible that these disorders are much more common than currently appreciated. Clinical awareness of these disorders is the first step toward identifying the patients. Glycobiologists and Glycochemists can help by educating academic physicians about this field. Physicians have not explored glycosylation as a root cause of disease. The unique language, complicated biosynthetic pathways, and heterogeneity present significant educational challenges. Even the very best medical education programs seldom include more than a glance at glycosylation. Now that specific glycan function has credibility, we have an opportunity to reach out to the medical community. We should seize that chance.

Glycobiology and Carbohydrate Chemistry Program. The Burnham Institute, 10901 North Torrey Pines Road, La Jolla, CA 92037, USA. Tel: 858-646-3142; Fax: 858-646-3193; E-mail: hudson@burnham.org

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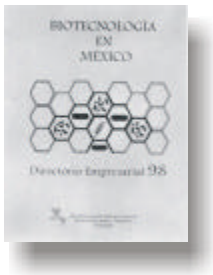

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