Some time ago we reported the oxidation of 2,3-unsaturated hexopyranosides and 2-C-methylene glycosides with hydrogen peroxide in the presence of molybdenum trioxide catalyst to give the corresponding anomeric hydroperoxides 1-5.\(^1\) Relatively stable hydroperoxides 1-5 were used for enantioselective oxidation of prochiral alcohols and sulfides in the presence of Ti(OiPr)\(_4\) with stereoselectivities varied from about 10 to 50 e.e.\(^2\) They have, however, several significant drawbacks such as the accessibility, relatively low asymmetric induction and they cannot be regenerated to be used again after reoxidation.\(^2\)

Epoxidation of electrophilic olefins with anomeric hydroperoxides in the presence of a base in principle does not remove drawbacks of the reagents mentioned above.\(^3\)

Oxidation of readily available 2-deoxysugars or their methyl glycosides with 50 % hydrogen peroxide in dioxane in the presence of sulfuric acid\(^4\) providing corresponding hydroperoxides 6-12 in 48-75 % yields, which display similar properties to those 1-5.\(^4\)

Hydroperoxides 6-12 as chiral oxidants were tested using 2-methyl-1,4-naphtoquinone, chalcone and dibenzoylethylene in the presence of bases to afford corresponding epoxides with e.e up to 90%. After epoxidation, hemiacetals can be regenerated and reoxidized again to corresponding hydroperoxides.\(^4\)

PEG-bound Oligosaccharides as Biomolecular Recognition Structures for the Biomaterial Research

Birgit Sauerzapfe, Peter Gasteier, Karel Krenek, Warren W. Wakarchuk, Vladimir Kren, Martin Möller, and Lothar Elling

1Laboratory for Biomaterials, Institute of Biotechnology and Helmholtz Institute for Biomedical Engineering, RWTH Aachen University, Aachen, Germany
2DWI/ ITMC RWTH Aachen University, Aachen, Germany
3Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
4Laboratory for Glycosyltransferases & Neuroglycomics, Institute for Biological Sciences, NRC, Ottawa, Canada

The targeting of cells to biomaterial surfaces can be achieved by functionalization of the interface with specified biomolecular recognition structures. The aim is the production of biohybrid systems with nano- and micro-structured surfaces to control the molecular mechanism of the cell. The cell surface is very “sweet” and is surrounded by a thick layer of sugars. These sugar chains are arranged like antennas which act as recognition structures and information carriers. One important glycan structure is poly-N-acetyllactosamine (poly-LacNAc) that fulfils the major function during inter- and intracellular communication events. This sugar structure has been identified as important ligand for galectin-mediated cell adhesion to extra-cellular matrix glycoproteins (laminin, fibronectin). Therefore poly-LacNAc shall be synthesized by chemo-enzymatic methods, coupled to specialized surfaces and characterized for its usage in the biomaterial research.

The first step of our synthesis strategy comprised the chemical synthesis of β-glycosides of GlcNAc to facilitate a controlled coupling onto chemically functionalized surfaces. The best result we could reach with the product GlcNAc-tBoc which can be easily attached to amino-reactive surfaces. In a further step the glycoside was converted as acceptor substrate by the human enzyme β-1,4-galactosyltransferase 1 for the production of LacNAc in a biocatalytical one-pot-reaction. The enzymatic synthesis of poly-LacNAc structures was accomplished by the combination of His6-propeptide-β4GalT-1 with β3-GlcNAcT from Helicobacter pylori. Kinetic data and conditions for an optimal conversion of the hydrophobic substrates were determined. The functionalised LacNAc and poly-LacNAc structures could be easily purified by solid phase extraction. A chemical deprotection step yielded the amino-terminating sugar structures which were subsequently coupled to specialised PEG-star surfaces or functionalised microtiter plates. The functionalized surfaces were characterized by lectin-binding assays. An artificial extracellular matrix could be build up via the galectin His6CGL2 with collagen IV, fibronectin and laminin. Work is in progress to utilize these biofunctionalized surfaces for targeted cell adhesion and differentiation.

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Differences in mode of action of microbial endoxylanases

Mária Vršanská, Katarína Kolenová, Vladimír Puchart and Peter Biely

Institute of Chemistry, Slovak Academy of Sciences, SK 845 38 Bratislava, Slovakia
chemmvsa@savba.sk

Endo-β-1,4-xylanases (EC 3.2.1.8, EXs) are glycoside hydrolases (GHs) that catalyze the degradation of xylan, the main component of plant hemicelluloses. Microbial xylanases have been classified mainly into two GH families, family 10 and 11. Recent studies indicated a much greater heterogeneity of these enzymes and on the basis of amino acid sequence similarities EXs have been also classified in GH families 5, 7, 8 and 43 [1].

Mode of action of endo-β-1,4-xylanases of three glycoside hydrolase families, GH10, GH11 and GH5 was examined on 4-O-methyl-D-glucuronoxylan and on series of defined aldouronic acids. The structure of products of hydrolysis was determined by combination of MS and enzymatic treatment.

EXs of GH10 liberated from glucuronoxylan aldotetraouronic acid (MeGlcA³Xyl3) and EXs of GH11 aldopentaouronic acid (MeGlcA³Xyl4) as the shortest acidic fragments [2]. Depending on distribution of MeGlcA residues on the glucuronoxylan main chain, EX of GH5 from Erwinia chrysanthemi (XynA) generated series of shorter and longer aldouronic acids of backbone polymerization degree 3-14, in which the MeGlcA is linked exclusively to the second xylopyranosyl residue from the reducing end.

Aldotetraouronic acid MeGlcA³Xyl3 and aldopentaouronic acid MeGlcA³Xyl4 were resistant to the action of EXs of GH10 and GH11. EX of GH5 attacked aldotetraouronic and aldopentaouronic acids at the first glycosidic linkage from the reducing end [3].

The main differences in the mode of action of EXs of the above three families were observed with aldohexaouronic acid MeGlcA³Xyl4. The hydrolysis of this substrate by EXs of GH10 afforded Xyl2 and MeGlcA³Xyl3. The degradation of aldohexaouronic acid by EXs of GH11 did not correspond to simple hydrolysis and involved a glycosyl transfer reaction. EX of GH5 attacked the substrate at the reducing end to give xylose and MeGlcA³Xyl4.

RAMAN OPTICAL ACTIVITY OF CARBOHYDRATES AND GLYCOPROTEINS

Vladimír Kopecký Jr., Tomáš Pazderka, Vladimír Baumruk

Institute of Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 5, Prague 2, CZ-12116, Czech Republic; tel.: +420 221 911 472; fax: +420 224 922 797 e-mail: kopecky@karlov.mff.cuni.cz

Raman optical activity (ROA) – discovered by L. D. Barron et al. in 1973, which measures vibrational optical activity by means of a small difference in the intensity of Raman scattering from chiral molecules in right and left circularly polarized incident light or, equivalently, as the intensity of a small circularly polarized component in the scattered light is a powerful technique for the study of biomolecules in aqueous solutions [1]. Because of its sensitivity to the chiral elements of biomolecular structure, ROA provides new information about solution structure and dynamics complementary to that supplied by conventional spectroscopic techniques (e.g. circular dichroism, Raman scattering, infrared spectroscopy).

Proteins and carbohydrates are both excellent samples for ROA, giving rich and informative band structures over a wide range of vibrational spectrum. Raman and ROA spectra can provide information about the secondary structure content, type of the protein fold (based on spectral similarity with reference database) and in some cases of amino acid side chain conformation.

Carbohydrates in aqueous solutions are highly favorable samples for ROA because their highly coupled normal modes generate strong ROA bands with characteristic patterns (so called “fingerprint” areas) of the various structural types that are usually much easier to interpret than normal Raman band patterns [2]. ROA spectra of monosaccharides contain information on sugar ring conformation, relative disposition of OH groups around the ring, the absolute configuration and axial or equatorial orientation of groups attached to the anomeric carbon. Different rotational conformers of the exocyclic CH₂OH group can also be recognized. Di- and oligosaccharides can provide wealth of information about the C–O–C glycosidic linkage. Polysaccharides contain information about their rigidity, disordering or extended ordering of the structure.

Subsequently, taking in account above mentioned, uniqueness of ROA spectra of glycoproteins (especially in comparison with an ordinary Raman spectroscopy) lies in the ability to study and obtain information about the structures and dynamics of both the polypeptide and carbohydrate moiety and how they modulate each other's stability and behavior [3]. However, ROA has the potential to provide structural information on glycoproteins well beyond the capabilities of other spectroscopic techniques, especially on crucial peripheral oligosaccharide segments. A lot of work has to be done in this field.

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References
Explorations of heparin-oligosaccharides-proteins interactions by NMR and molecular modeling

M. Hricovini

Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia
Tel: +4212 5941 0323, Fax: +4212 5941 0222, hricovini@savba.sk

Some carbohydrates are known for their ability to induce important biochemical processes through their interactions with proteins. Polysaccharide heparin is known not only for its anticoagulant and antithrombotic activities but this glycosaminoglycan is involved in several other processes such as cell growth and differentiation, viral infections, cell-cell interactions, etc. The knowledge of three-dimensional (3D) structures of heparin-protein or heparin-oligosaccharides-protein complexes has therefore the potential to assist in the design of new carbohydrate-based therapeutic agents.

Different NMR parameters, chemical shifts, coupling constants and NOE’s have been used to determine 3D structures of heparin-oligosaccharides in the complexes with antithrombin and growth factors (FGF-1 and FGF-2). NMR data indicated that formations of the protein-carbohydrate complexes were accompanied by variation of the 3D oligosaccharide structures. This includes conformational changes at the glycosidic linkages as well as pseudorotation of the iduronic acid residues. Conformational analysis of heparin-pentasaccharide (GlcN,6-SO3α1-4GlcAβ1-4GlcN,3,6-SO3α1-4IdoA2-SO3α1-GlcN,6SO3OMe, AGA*IA M), which reproduces structure of the specific binding sequence of heparin for antithrombin III, revealed changes at the A-G and I-AM linkages during the binding with protein. Evidence was also found that the protein drives the conformation of the 2-O-sulphated iduronic acid residue towards the skewed $^{\text{2S0}}$ form. Furthermore, considerable influences of the sulphate groups have also been observed in complexes of heparin-tetrasaccharides with FGF-1 and FGF-2 proteins.

In order to have reliable heparin-oligosaccharide structures, necessary for analysis of their complexes with proteins, theoretical DFT calculations were performed at the B3LYP/6−311++G** level. NMR scalar spin-spin coupling constants were then computed from the optimized geometries. Among other coupling constants, $^3J_{H-H}$ values showed strong dependences upon 3D structures of the studied carbohydrates and varied up to 10 Hz. More than 70 different $^3J_{H-H}$ values allowed determination of the relationship between 3D structure of sulfated saccharides and three-bond coupling constants.

References

Molecular modelling as a glycobiocchemist’s best friend -
Structure-functional and computational analysis of lectin-saccharide interaction
on the way to protein engineering

Jan Adam¹, Martina Pokorná¹, Nikola Kostlánová¹, Ondřej Šulák¹, Zdeněk Krž¹, Anne Imberty², Jaroslav Koča¹ and Michaela Wimmerová¹,³

¹National Centre for Biomolecular Research and ³ Institute of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno ²CERMAV-CNRS, 601 rue de la Chimie, BP 53, 38041 Grenoble, France

The biochemistry of saccharides and protein-saccharide interactions is quite a vibrant field of research recently. Saccharides, thanks to their wide variability of conformational alternatives offer a great capacity for information encoding, and are able to serve as potent recognition agents. Lectins, on the other hand, are proteins capable of binding saccharide structures with different affinity and specificity. Therefore, the lectin-saccharide system of cellular recognition is widely employed in living organisms, being it in blood groups determination, bio-adhesion and virulence processes. Understanding the principles of this interaction would open the door to possibilities of targeted mutagenesis and subsequent protein engineering of lectins.

The protein engineering approach is a very valued tool in many branches of bio-science, a process of designing proteins with precisely defined and desired properties. However, this approach can be quite demanding for both time and budget.

The current possibilities offered by computational chemistry allow to perform sophisticated simulations and modelling of complicated, yet essential processes - the biomolecular interactions. Studies of several bacterial lectins from opportunistic human pathogens or phytopathogens were performed. Mutagenesis of PA-IIL lectin from Pseudomonas aeruginosa revealed the crucial importance of the amino acid composition of the binding loop for binding preferences [1]. In addition to in vitro mutagenesis of PA-IIL lectin, in silico mutants were created as well, and the resulting structures were used for docking experiments using different docking softwares (DOCK, AutoDock)[2]. The computational results were correlated with the experimental data. The aim of the project is to develop a reliable method of precognition of future lectin-saccharide interactions – not only in the case of PA-IIL, but a general, widely applicable method.

Another approach was applied to lectin RS20L from phytopathogen Ralstonia solanacearum, where docking experiments could help to characterized two independent binding sites and calculations can provide important hints for the direction of experimental research. By combination of the computational possibilities, the molecular biology and advanced structure-functional methods (isothermal titration microcalorimetry, surface plasmon resonance, differential scanning calorimetry), it is possible to gain a considerable amount of knowledge of the principles behind the interactions, in turn allowing to define mutations leading to desired properties. Combined with a reliable computational precognitive method, the way to fast and effective protein engineering is open.

References:

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Stereoselective synthesis of C-(1→3)-linked disaccharides as non-hydrolysable epitopes of glycoconjugates.

Ladislav Kniežo

Department of Chemistry of Natural Compounds, Institute of Chemical Technology, Prague, Technická 5, 166 28 Prague 6, Czech Republic.
E-mail: ladislav.kniezo@vscht.cz

The main problem with carbohydrate-based therapeutics is their instability to enzymatic cleavage by a large number of extra- and intracellular glycosidases and chemical hydrolysis. One way to increase the stability of the anomeric linkage is to replace the glycosidic oxygen atom with a methylene group to furnish e.g. a C-disaccharide.

We present here the stereoselective synthesis of new types of C-(1→3)-linked disaccharides and an approach to the study of their conformational behaviors. In our synthesis protected α-D-pyranosylacetaldehydes 1 of different configurations (D-gluco, D-galacto, and D-manno) was stereoselectively converted into corresponding D- or L-glucal derivatives 2 or 3. Further, the α-D-pyranosylacetaldehydes 1 could be easily epimerized to the thermodynamically more stable β-D-pyranosylacetaldehydes 4 and those afforded by the same reaction protocol derivatives 5 or 6. The glucal moiety in the obtained compounds can be easily converted into C-disaccharides such as 7 or may be submitted to stereoselective acetaminoglycosylation and afford C-disaccharides containing aminosugar moieties such as 8. Many of the structures obtainable in this way are mimetics of natural (1→3)-disaccharide structures found as end groups in Core-B structures of a number of proteoglycans and may be used in synthesis of enzyme non-hydrolysable disaccharide-based vaccines or in synthesis of other types of therapeutics, e.g. where the C-(1→3)-disaccharide structures are linked to the surface of dendrimers. In the C-(1→3)-disaccharides 9 or 10 bearing at the reducing end deoxyhexopyranoses with D or with L configurations (precursors of 2 or 3) we studied the conformational preferences around C-glycosidic as well as around C-aglyconic bonds.

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Synthesis and some biological properties of a non-ionogenic, pentakis-\textit{O}-tetradecylated lipid A mimic

M. Baráth,\textsuperscript{a} M. Petrušová,\textsuperscript{a} L. Novotná,\textsuperscript{b} J. Svoboda,\textsuperscript{b} A. Fišerová,\textsuperscript{b} V. Křen,\textsuperscript{b} L. Petruša\textsuperscript{a}

\textsuperscript{a}Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84538 Bratislava, Slovakia; \textsuperscript{b}Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ-14220 Prague, Czech Republic
Tel: +421-2-594 10 232
E-mail: chemlpet@savba.sk

Lipid A is a phospholipodisaccharidic unit of lipopolysaccharides (LPS, endotoxin), which constitute the exterior monolayer of the outer membrane in Gram-negative bacteria\textsuperscript{1}. A typical representative is \textit{E. coli} lipid A (1). It is fully responsible for the endotoxic activity of LPS. The syndromes most commonly connected with endotoxin are severe sepsis and septic shock, which are systemic complications of many diseases. On the other hand, lipid A is a highly potent stimulator of immune system\textsuperscript{2}. As there does not exist any effective treatment of the lethal sepsis to date, a search for an effective drug against this disease is inevitably needed. For the purpose, many natural and synthetic \textit{E. coli} lipid A mimics, which antagonize and more or less copy the structure of the natural endotoxin, have been designed\textsuperscript{3-5}.

The contribution introduces an expeditious synthesis and some biological properties of a non-ionogenic mimic of lipid A (2), containing five ethereally linked, linear C\textsubscript{14} hydrophobic chains on the O-2, O-3, O-4, O-2' and O-3' positions of its methyl α-gentiobioside skeleton. The mimic antagonizes the fluorochrome-labeled LPS internalization, LPS-mediated stimulation of cytotoxicity and cytokine production, and CD86 antigen expression by dendritic cells.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chemical Structures}
\caption{Chemical structures of lipid A (1) and the non-ionogenic lipid A mimic (2).}
\end{figure}

Acknowledgments: The work was supported by the APVT-51-039802 and VEGA-2/6129/26 grants.

References:
PORPHYRINS CONTAINING GLYCOSYLATED STEREOID: SYNTHESIS AND SELF-ASSEMBLY AND ELECTROCHEMICAL STUDIES

KAREL ZELENKA\textsuperscript{a}, TOMÁŠ TRNKA\textsuperscript{a}, PAVEL DRAŠAR\textsuperscript{b,c}

\textsuperscript{a}Department of Organic Chemistry, Charles University, Hlavova 2030, 128 40 Prague 2, Czech Republic
\textsuperscript{b}Institute of Organic Chemistry and Biochemistry, Flemingovo náměstí 2, 166 10 Prague 6, Czech Republic
\textsuperscript{c}Institute of Chemical Technology, 166 28 Prague 6, Czech Republic

The aim of this work was the syntheses and study of new porphyrins containing various number of glycosylated-steroid moieties. The idea was based on combination of three natural/synthetic motives with many interesting properties. In recent works, porphyrins substituted in \textit{meso}-positions by various glycosylated moieties were studied as new PDT sensitizers\textsuperscript{1} and used for self-assembly sol-gel systems\textsuperscript{2}. Also porphyrin receptors containing steroid units were prepared and used in electrochemistry\textsuperscript{3}, molecular recognition of saccharides\textsuperscript{4} and biologically important anions\textsuperscript{5}.

The authors aimed to demonstrate the possibility of combining known receptor molecules with carbohydrates that can serve as anchors, chiral selectors, or polarity modifiers, depending on their level and kind of protection. The method reported earlier\textsuperscript{6} was extended by \textit{A}\textsubscript{4} (I), \textit{trans-A}\textsubscript{2}B\textsubscript{2} (II) and A\textsubscript{3}B (III) types of porphyrin derivatives which were prepared. Their solvent driven aggregation and polarographic and voltammetric behaviour was studied. The synthetic details and some properties of prepared porphyrins are discussed.

![Porphyrin Structures](image)

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New synthetic approaches to C-D-galactofuranosyl compounds

M. Vojtech, M. Petrušová, L. Petruš

Institute of Chemistry, Slovak Academy of Sciences, 84538 Bratislava, Slovakia
Tel: +421-2-594 10 272
E-mail: chemmvot@savba.sk

D-Galactose occurs in mammalian glycoproteins and glycolipids only in the pyranose forms. Glyconjugates1 of several bacteria (e.g. Mycobacterium tuberculosis), protozoa and fungi contain D-galactofuranose units in furanose and pyranose forms. These residues appear to contribute to the pathogenicity of many of these organisms. Therefore, D-galactofuranose is very attractive for medical and scientific interests. Furanoid derivatives of D-galactose can be obtained by benzoylation at higher temperature3 or by the Fischer glycosidation2. Deamination of suitable 2-amino-2-deoxyaldoses gives C-D-galactofuranosyl compounds.4

We propose a new synthetic approach to C-D-galactofuranosyl compounds by kinetically controlled five-membered ring closure reaction of suitable derivatives. First, we achieved synthesis of model C-D-arabinofuranosyl compounds by acid-catalysed deacetylation of per-O-acetylated 1,2-dideoxy-1-nitro-D-arabino-hex-1-enitol, spontaneously followed by the Nef-type methanolysis of intermediate arabinofuranosymethylnitronic acids.5 Under the same conditions (a), 1,2-dideoxy-1-nitro-D-galacto-hept-1-enitol peracetate (1) behaved similarly and gave an anomeric mixture of D-galactofuranosymethanal dimethyl acetals (2).

We also tried to stop the transformation in the stage of C-D-galactofuranosylimethyl-nitronic acids, the tautomeric forms of C-D-galactofuranosynitromethanes. This was achieved by employing a triethylamine-catalysed deacetylation6 of 1 (b), and an anomeric mixture of major D-galactofuranosynitromethanes (3) and minor D-galactopyranosynitromethanes (4) was obtained.

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Large propeptides of fungal β-N-acetylhexosaminidases as novel enzyme regulators: the role of propeptide N- and O-glycosylation in the reconstitution of enzymatic activity and in the initial interaction of the propeptid with the enzyme

Karel Bezouška§,‡, Ondřej Plíhal,‡, Jan Sklenář,‡, Kateřina Hofbauerová,‡, Petr Novák,‡, Petr Man,‡, Petr Pompach,‡, Daniel Kavan§,‡, Helena Ryšlavá,§, Lenka Weignerová,‡, Andrea Charvátová-Pišvejcová,§, Vladimír Křen,‡

§Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, 14220 Praha 4, Czech Republic, ‡Department of Biochemistry, Faculty of Science, Charles University, Hlavova 8, 12840 Praha 2, Czech Republic, Institute of Physics, Faculty of Mathematics and Physics, Charles University, Ke Karlovu 5, 12116 Praha 2, Czech Republic.

AUTHOR EMAIL ADDRESS: bezouska@biomed.cas.cz

Filamentous fungi produce and secrete β-N-acetylhexosaminidases, Hex, as important components of the binary chitinolytic systems involved in the formation of septa and hyphenation. Enzyme reconstitution experiments published previously indicate that Hex can occur in the form of two molecular species containing either one or two molecules of the propeptide noncovalently associated with the enzyme dimer. Here we describe a novel mechanism for the regulation of activity of Hex based on the association of their catalytic subunits with the large N-terminal propeptides in vivo. We show that the enzyme precursor is processed early in the biosynthesis shortly after the addition of N-glycans through the action of a dibasic peptidase cleaving both before and after the dibasic sequence. The processing site for this unique dibasic peptidase different from kexins is conserved among the β-N-acetylhexosaminidases from filamentous fungi, and inhibition of the dibasic peptidase abrogates enzyme folding and activation. Binding of the released propeptide to the catalytic subunit of Hex is essential for its activation. Examination of the kinetics of Hex activation and dimerization in vitro allowed us to understand the unusually high efficiency of the assembly of this enzyme. We also report that the fungus is able to regulate actively the concentration of the processed propeptide in endoplasmic reticulum, and thus the specific activity of the produced Hex. This novel regulatory mechanism enables to control the catalytic activity and architecture of the secreted enzyme according to the needs of the producing cell at various stages of its growth cycle.

Despite all the knowledge on the role of the propeptide in Hex, the details of its initial interaction with the catalytic subunit were not known. We have employed sequence alignment, deglycosylation experiments, expression studies, enzyme reconstitution experiments, and enzyme folding / denaturation to study elements that are critically involved in these interactions. The propeptides of fungal Hex seem to be composed of evolutionarily conserved N- and C-terminal peptides of approximately 20 amino acids connected by 40 amino acid long loop, all of which seem to be essential for proper folding and activation of the enzyme. Interestingly, only amino acids seem to be insufficient for efficient reconstitution since the deglycosylated propeptides isolated from E.coli expressions have negligible reconstitution activities. Only propeptides containing the short glycosylation “markers” in the form of single GlcNAc or Man residues bound to Asn or Ser/Thr, respectively, positioned at exact amino acids within the sequence can reconstitute the activity efficiently. Our results will be discussed in the frame of a general role of protein glycosylation in protein folding and subunit association.

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β-N-Acetylhexosaminidase from *Talaromyces flavus* can accept highly modified substrates: New ways to enzymatic synthesis of glycomimetics

K. Slámová\textsuperscript{a,b}, P. Bojarová\textsuperscript{a}, R. Gažák\textsuperscript{b}, M. Macková\textsuperscript{b}, V. Křen\textsuperscript{a}

\textsuperscript{a}Institute of Microbiology, Centre of Biocatalysis and Biotransformation, Vídeňská 1083, 142 20, Prague, Czech Republic; \textsuperscript{b}Institute of Chemical Technology, Technická 5, 160 00, Prague, Czech Republic

E-mail: kristynaslamova@atlas.cz

β-N-Acetylhexosaminidase (EC 3.2.1.52) from *Talaromyces flavus* CCF 2686 was found to have very broad substrate specificity, accepting non-natural, modified glycosides. This enzyme has also exhibited substantial synthetic capacity. β-N-acetylhexosaminidase is known to have wobbling specificity – its natural substrates are both pNP-GlcNAc and pNP-GalNAc with the ratio of pNP-GalNAc/pNP-GlcNAc activity being 1.4. Production of extracellular β-N-acetylhexosaminidase was improved 5× by the use of mineral medium and GlcNAc as an inducer. The enzyme was purified to homogeneity (SDS-PAGE) in three steps – cation-exchange chromatography (Fractogel EMD SO\textsubscript{3}\textsuperscript{−}), gel filtration (Superdex 200) and chromatofocusing (Mono P). It is a dimer with native molecular weight 130 kDa, composed of two 65 kDa subunits. Recently, we have demonstrated that this enzyme is able to cleave and transfer 2-acetamido-2-deoxy-β-D-galacto-hexodialdo-1,5-pyranosyl moiety from p-nitrophenyl glycoside as a glycosyl donor, which opens the way (after aldehyde oxidation) to the synthesis of 2-acetamido-2-deoxy-β-D-galactopyranosyluronates (β-GalNAcA). Our preliminary data indicate that this enzyme is also able to cleave 6-sulphated GlcNAc moiety and also 2-acetamido-2-deoxy-β-D-glucopyranosyluronyl moiety. Transglycosylation reactions with these substrates are presently under investigation in order to synthesize new unnatural glycosides with C-6 modified moieties.

![Chemical structures](image-url)

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Polysaccharide deacetylases of carbohydrate esterase family 4 – unique metalloenzymes

Vladimír Puchart, Mária Mastihubová and Peter Biely

Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, SK-845 38 Bratislava 45, Slovakia; Phone: +421 2 59410229, fax: +421 2 59410222, e-mail: chempuk@savba.sk

Positional specificity of two CE4 enzymes, NodB-like domain of a multidomain xylanase U from Clostridium thermocellum (CtAxe) as well as acetylxylan esterase from Streptomyces lividans (SlAxe) was investigated. Of three monoacetates of 4-nitrophenyl β-D-xylopyranoside the acetylxylan esterases showed a clear preference for the 2-acetate. Both enzymes were inhibited by EDTA and activated by bimetal cations. The highest activation was observed in the presence of Co²⁺. Acetylated methyl β-D-xylopyranosides were deacetylated approximately equally in positions 2 and 3 suggesting that the enzymes bind the xylosides with the small methyl aglycone also in the opposite orientation. Nevertheless, both positions 2 and 3 of methyl β-D-xylopyranoside were deacetylated much faster in the presence of the activating metal ion. In contrast, replacement of the hydroxyl group at either of these positions with fluorine or hydrogen, as well as acetylation of both positions, abolished the enzyme activity, regardless the absence or the presence of Co²⁺. Thus, the presence of the free vicinal hydroxyl group seems to be a prerequisite not only for an efficient deacetylation of position 2 or 3, but also for the activation of the enzyme with cobalt ion. The demonstrated involvement of the vicinal hydroxyl groups in the deacetylation is in accord with 3-D structures of SlAxe and CtAxe [1] as well as of other CE4 metal-dependent GlcNAc- and MurNAc-deacetylases [2-4]. Determinants of substrate specificity of CE4 acetylxylan esterases will be discussed with that of CE4 chitin deacetylases and peptidoglycan deacetylases. The structure and specificity of the CE4 N-deacetylases will be compared with those of another metal-dependent N-deacetylases acting on GlcNAc moieties classified in other CE families.

New carbohydrate mimetics protecting CD69⁺ killer cells from apoptosis

Markéta Vančurová¹², Petr Pompach¹², Daniel Kavan¹², Monika Nálezková³, Lukáš Žídek³, Zdeněk Cimburek², Ondřej Hovorka² a Karel Bezouška¹²

¹Department of Biochemistry, Faculty of Science, Charles University Prague, Hlavova 8, 12840 Praha 2; ²Institute of Microbiology, Academy of Science of Czech Republic, Vídeňská 1083, 14220 Praha 4, ³National Centre for Research of Biomolecules, Masaryk University Brno

Programmed cell death (apoptosis) represent a physiological mechanism by which an organism removes senescent, stressed or improperly differentiated cells without the damage to the entire body. The use of this mechanism in antitumor immunity remains complicated since the malignant cells are not only very resistant towards apoptosis, but are even able to initiate the apoptosis of the bystanding cells. In relation to the antitumor activities of the healthy immune system, the ability of tumor cells to initiate the apoptosis of killer lymphocytes attacking them has attracted considerable attention.

Activated killer lymphocytes express on their surface receptor CD69 that has been long considered to be one of the major recognition and activation receptor of killer lymphocytes. However, surprisingly, the results with mice containing CD69⁻ deletions have shown that these mice were considerably more resistant towards experimental tumors than their normal counterparts. In our laboratory we have decided to undertake a molecular analysis of this immunological phenomenon. We have found that under conditions in vitro it seems possible to completely suppress the tumor-mediated apoptosis of CD69⁺ killer lymphocytes by both antibodies against the receptor as well as by its soluble form or by its high affinity ligands. The short lecture will primarily discuss the carbohydrate mimetics based on sialylTn disaccharide. We shall discuss the details of the reactivities of these mimetics with defined dimeric CD69 receptor, and its possible used in the combined therapies against clinically important tumors, especially melanomas.

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GlcNAc-DIMERS AGGREGATE NK-CELL RECEPTORS: GLYCOMIMETICS AS A “MOLECULAR RULER”

K. Křenek, P. Krist, M. Navrátil, K. Hofbauerová, K. Bezouška, V. Křen

Institute of Microbiology, Academy of Sciences of Czech Republic, Vídeňská 1083, 142 20 Prague 4, Czech Republic; e-mail: krenek@biomed.cas.cz, phone: +420-29644-2360, fax: +420-29644-2509

Derivatives of 2-acetamido-2-deoxy-β-D-hexopyranoses have a high affinity towards activation receptors of rat and human, lymphocytes and natural killer cells (NKR-P1 and CD69 receptors). After testing activity of monosaccharidic ligands we decided to synthesize polyvalent ligands based on glycoconjugates and glycodendrimers, which exhibit substantially higher activities than the low molecular ligands. Synthesis of these compounds is predetermined by the fact that the activation receptor of NK-cells has multiple activation sites.

The main aim of this work is to find relationship between the geometrical structure of NK-cell activation receptors and the distance of sugar units in the presented GlcNAc dimmers. Influence of the aliphatic spacer length (i.e. distance of sugars) will be compared with different biological properties of these compounds (binding properties, precipitation and aggregation of discussed receptors). Data obtained so far indicate a critical distance between two binding sites that has a fundamental importance for a rationale construction of GlcNAc-based glycomimetics for CD69 and NKR-P1 receptors.

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Lectin BclA: Characterization of binding properties

Lenka Malinovská1, Emilie Lameignere2, Margita Sláviková1, Annabelle Varrot2, Anne Imberty2, Michaela Wimmerová1

1National Centre for Biomolecular Research and Institute of Biochemistry, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic, email: 63581@mail.muni.cz
2CERMAV-CNRS, 601 rue de la Chimie, BP 53, 38041 Grenoble, France

Lectins are important in both symbiotic and pathogen interaction between some microorganisms and hosts because microbial lectins may play an essential role in mediating adhesion to cells and mucosal surfaces of the host. Adhesion protects pathogens from natural cleansing mechanisms (airflow in the respiratory tract, urine flow in the urinary tract), provides pathogens with better access to sources of nutrition and supports the penetration of pathogens into the tissues.

Lectins can play a crucial role also in an infection caused by a gram-negative bacterium Burkholderia cenocepacia. This bacterium is ubiquitous in the environment and may evoke a number of diseases in plants. Likewise, B. cenocepacia is an opportunistic pathogen that can infect immunocompromised humans, especially individuals suffering from cystic fibrosis.

B. cenocepacia genom contains three lectin-like sequences that are homologues of lectin PA-IIL from Pseudomonas aeruginosa [1], another gram negative bacterium associated with cystic fibrosis. P. aeruginosa is the most widespread infectious agens between cystic fibrosis patients whereas B. cenocepacia is the most dangerous. One of PA-IIL homologues, named BclA, was cloned and prepared in recombination form. Lectin binding properties were studied by several methods, including enzyme linked lectin assay, surface plasmon resonance, isothermal titration calorimetry and microarray.

PA-IIL is a tetramer (each of 11.7 kDa subunits consists of 114 amino acids) with an unusual binding mode [2] where a carbohydrate moiety is bound via two calcium ions. In contrast to PA-IIL, BclA is only 28 kDa dimer displaying similar binding mode. PA-IIL recognises preferentially L-fucose but can bind L-galactose, D-arabinose D-fructose and D-mannose as well [3]. In contrast, according to ELLA, calorimetry and microarray data, BclA binds only to mannose and mannosylated derivatives. No significant affinity to fucose was detected. Curiously, inhibition effect of free L-fucose was measured by SPR. As in all other experiments biotinylated fucose was used, BclA can probably bind only free L-fucose or short fucosylated derivates. This assumption is supported by a fact that methyl-α-L-fucoside displays one order lower inhibition activity than free sugar L-fucose.

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Novel microbial carbohydrate esterase involved in plant cell wall digestion

Silvia Špániková, Peter Biely

Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, chemspan@savba.sk, tel. +4212 59410 229, fax. +4212 59410 222

There are several types of covalent linkages between lignin and hemicelluloses in plant cell walls. One of them involves the ester linkages between 4-O-methyl-D-glucuronic acid or D-glucuronic acid residues of glucuronoxylans and hydroxyl groups of lignin alcohols [1]. During growth of the wood-rotting fungus Schizopyllum commune ATCC 38548 on 1,5 % cellulose, the fungus produced an esterase hydrolyzing various alkyl and aryl esters of 4-O-methyl-D-glucuronic acid. None of these compounds was attacked by other known carbohydrate esterases, such as acetylxylan esterases, feruloyl esterases and pectin methylesterases. The enzyme is called glucuronoyl esterase [2]. Fractionation of extracellular proteins of the fungus Schizophyllum commune resulted in purification of a glucuronoyl esterase that allowed determination of its internal amino acid sequence. Based on the sequence, a homologous gene in Phanerochaete chrysosporium was found. The gene sequence of the enzyme from Phanerochaete chrysosporium did not show any similarity to gene sequences of known enzymes, supporting the fact that we have discovered a novel type of carbohydrate esterase. The biotechnological potential of the new esterase will be investigated.

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References

β-Glucans are widely used as soluble fibre in human diet. They are linear or branched polysaccharides formed from β(1→3), β(1→4) and β(1→6) bound glucose units. Linear β-glucans with repeating fragments of β(1→3) and β(1→4) oligosaccharides are contained in cereals (higher contents are in oat and some varieties of barley). Moreover branched fungal β-glucans contain β(1→6) glycosidic linkage and they are common in yeast or mushroom cell walls.

Polysaccharides and their derivatives can be distinguished by thermal analysis according to the mechanism of their decomposition [1]. Thermogravimetry (TG) and differential scanning calorimetry (DSC) are commonly used thermal methods. TG monitors mass changes of sample depending on constant or increasing temperature. DSC measures the heat effects, caused by chemical or physical changes, as a function of temperature or time while the substance is heated at a uniform rate.

DSC measurement of β-glucans in inert atmosphere led to observation of both endothermic and exothermic events. DSC curves usually had several peaks, the first of which was caused by evaporation of water and the others were related to decomposition of β-glucans. Endothermic peaks may result from subsequent fragmentation of polysaccharide chains with formation of char and volatile products, whereas exothermic peaks may correspond to cross-linking reactions occurring during the thermal degradation and following thermal degradation of a new cross-linked material [2]. TG analysis proved significant differences in thermal stability and diverse speed of β-glucans decomposition. Results obtained confirmed that DSC and TG can be useful tools for discrimination of β-glucans because the mechanism of thermal decomposition depends on source, molecular mass and structural properties of these polysaccharides.

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Literature


Solid aggregates of porphyrin macrocycles on chitosan scaffold: preparation and characterisation

Andriy Synytsya a, Alla Synytsya b, Petra Blafková a, Martin Maryška c, Jana Ederová d, Jiří Špěvaček e, Jana Čopíková a, Karel Volka b, Vladimír Král b

a Department of Carbohydrate Chemistry and Technology, Technická 5, 166 28 Prague 6, Czech Republic. Tel.: 220 443 116, fax: 220 445 130, e-mail: sinicaa@vscht.cz
b Department of Analytical Chemistry, c Department of Glass and Ceramics, d Central Laboratories, Institute of Chemical Technology in Prague, Technická 5, 166 28 Prague 6, Czech Republic
e Institute of Macromolecular Chemistry of the Academy of Science, Heyrovského nám. 2, 162 06 Prague 6, Czech Republic

Interaction of meso-tetrakis(4-sulphonatophenyl)porphyrine (TPPS 4) with chitosan has been studied previously in aqueous solutions [1]. It has been found that this polysaccharide induce and support self-aggregation of TPPS 4 at appropriate concentrations and pH. The mode of aggregation was strongly dependent on pH: stacking (H-type) aggregates predominated at neutral and weak acidic conditions (pH 4.8–6.8) and tilted (J-type) aggregates at pH 2.5. CD titration experiments confirmed the formation of optically active macrocycles in the presence of chitosan.

An addition of powder porphyrin to chitosan solution (pH ~7 and 2) led to the rapid formation of coloured precipitates assigned respectively as chitosan/TPPS 4 complexes 1 and 2. Spectroscopic methods (vis-NIR, FT-IR, Raman and 13C CP-MAS NMR) confirmed that macrocycles are highly aggregated in both these complexes. H- and J-aggregates of TPPS 4 were indicated in 1 and 2, respectively. Elemental analysis and solid state NMR showed that 1 contained more chitosan (8.3–11.1 units per macrocycle) than 2 (5.8–7.5 units per macrocycle). DSC and TG experiments confirmed that both complexes are less thermostable than initial chitosan owing to specific ways of thermal degradation. Light microscopy indicated fibrous structure of 1 and lamellar structure of 2. SEM analysis showed that both complexes consist of spherical nanoparticles of similar shape and size (~20–50 nm). Structural difference between 1 and 2 could be explained by dissimilar ways of self-assembling of supramolecular structures. Possible models of TPPS 4/chitosan arrangement were proposed.


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Alpha-L-fucosidase from the bacterial strain *Paenibacillus thiaminolyticus*

Eva Benešová, Petra Lipovová, Blanka Králová  
Department of Biochemistry and Microbiology, ICT, Prague  
Technická 5, Praha 6, 166 28, CZ  
Tel: +420 224 353 028, Fax: +420 224 355 167  
e-mail: Eva.Benesova@vscht.cz

Alpha-L-fucosidases is an enzyme of the class of hydrolases, which is in many organisms involved in the metabolism of fucose-containing glycoconjugates (glycoproteins and glycolipids) and oligosaccharides. Deficiency of this exoglycosidase, that removes L-fucosyl residues from the non-reducing ends of sugar chains, causes an immedicable disease, called fucosidosis. As many of fucose-containing glycoconjugates are essential in many important processes of human body, as e.g. inflammation, cell-cell interaction, ligand-receptor interaction, signal transduction or growth regulation, the discovery of simple synthesis of fucose-containing molecules, is an aim of many current researches. These molecules could be used in therapies of some diseases including cancer and in medical treatment after transplantations. One solution could be an application of alpha-L-fucosidases with the ability to catalyze transglycosylation reaction.

In this work it was detected, that the mesophilic bacterial strain *Paenibacillus thiaminolyticus* is an excellent producer of alpha-L-fucosidase. Basic characteristics as e.g. $I_{\text{optimum}}$, $pH_{\text{optimum}}$, $K_m$, $V_{\text{lim}}$ or the stability were measured in the cytosolic extract after the disintegration of bacterial cells. After a purification procedure was established and during this process two different isoenzymes (named fuciso1 and fuciso2) were detected. Both isoenzymes were purified separately and their ability to catalyze transglycosylation reaction was tested. The amino acid sequence of N-terminus of fuciso1 was determined.

At present time the possibility of immobilization of fuciso1 and fuciso2 on different materials and the usage of these immobilized enzymes in catalysis of transglycosylation reactions is being tested. Sequences of both genes, which encode fuciso1 and fuciso2, will be determined in the future research.

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Synthesis of Regioisomers of Monosubstituted β-Cyclodextrin Derivatives as Modifiers of Porous Silicon Surface for Chemosensor Applications

Antonín Beran, Iva Tišlerová and Jindřich Jindřich

Charles University in Prague, Faculty of Science, Department of Organic Chemistry, Hlavova 2030, 128 40 Prague 2, email: jindrich@natur.cuni.cz

Cyclodextrins (CDs) are cyclic oligosacharides with truncated cone shape of the molecule. Their OH groups are situated outside and C-H bonds inside the CD ring. These properties are the reason for a different polarity of CD cavity and ring surface and are also the reason for their complexation abilities. Due to the ability of CD to form inclusion complexes with organic substances they can be used as modifiers of photoluminescence response of porous silicon.

The aim of this work was to prepare a set of β-CD derivatives bearing regioselectively connected spacers of different lengths containing terminal double bond, which will allow modification of porous silicon surface by hydrosilylation reaction. Target compounds are based on peracetylated 2-1-O-, 3-1-O- and 6-1-O- carboxymethyl monoderivatives of β-CD. Linear amines of chain lengths 3 and 10 carbons with terminal double bond were used as spacers.

![Scheme - Reaction sequence for preparation of 6-1-O- isomer](image)

References:

Acknowledgement:
This work was supported by grants GAUK 424/2004/B-CH/PrF and MSM 0021620857.
SYNTHESIS OF GlcNAc-TS MIMETIC PART OF THE POTENTIAL TRANSITION STATE ANALOG INHIBITORS OF GLYCOSYLTRANSFERASES

Balázs Csúsz, Ján Hirsch, Miroslav Koóš and Igor Tvaroška

Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia

Glycosyltransferases (GT’s) belong to the enzymes that are involved in the biosynthesis of N- and O-linked complex oligosaccharides of glycoproteins. The reactions catalyzed by these enzymes form new glycosidic linkages. Modifications in the oligosaccharide chains accompany many physiological and pathological cell processes [1], and may lead to serious disorders in animals as well as in humans [2].

This contribution, aimed at developing and greater understanding of the transition state (TS) analog inhibitors of the GT’s, introduces the synthesis of two precursors – namely benzyl 2-thio-6-O-β-D-fructofuranoside 1-phosphate (2) and benzyl 2-thio-6-O-β-D-fructofuranoside 1-(N,N-diisopropyl)phosphoramidate (3)– for the planned TS analog inhibitor 1.

Precursors 2 and 3 were prepared in one pot synthesis from benzyl 2-thio-3,4-di-O-acetyl-6-O-terc-butyldimethylsilyl-β-D-fructofuranoside - which is readily available from benzyl 2-thio-β-D-fructofuranoside (in 4 steps) – by treatment with bis(2-cyano-ethyl) phosphorochloridite [3] and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite [4], respectively, in the present of Hüning’s base, followed by oxidation of P[III] to P[V] by tert-butylhydroperoxide and subsequent deprotection of one or two 2-cyanoethyl protecting groups by mixture of satd. NH₃-MeOH and MeOH afforded the required 2 and 3, obtained in good yield.

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SYNTHESIS OF DIMER DERIVATES OF CYCLODEXTRINS BY METATHESIS OF MONOALLYL DERIVATES

MARTIN DIVIŠ, JINDŘICH JINDŘICH

Charles University, Faculty of Science, Department of Organic and Nuclear Chemistry, Hlavova 8, 128 43, Praha 2, e-mail: jindrich@natur.cuni.cz

Cyclodextrins (CDs) and their derivatives have good complexation abilities due to the rigid, cone-shaped cavity formed by α-1,4-linked D-glucopyranose units. Our research is focused on a preparation of a set of exactly defined monosubstituted derivatives of CDs for subsequent utilization in organic synthesis 1,2. The preparation of monosubstituted derivatives of CD which has easily transformable allyl group is the first and the main step. These monoderivatives can undergo olefin metathesis 3 using Grubbs catalyst 1st generation 4.

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Epoxide migration and pseudo-epoxide migration of 1,6:2,3- and 1,6:3,4-dianhydro-β-D-hexopyranoses

Martina Džoganová, Miloš Buděšínský, Tomáš Trnka, Miloslav Černý
IOCHB CAS, Flemingovo nám. 2, 16610, Prague 6, Czech Republic; tel. 220183370, dzoganova@gmail.com

Epoxide migration\(^1\), also called Payne rearrangement is a well-known phenomenon relating to \(\alpha\)-hydroxy epoxides which, under favorable steric conditions, undergo isomerization in alkaline solution at room temperature to give an equilibrium mixture of two isomeric \(\alpha\)-hydroxy epoxides. Furthermore, in the presence of hydroxide or other nucleophiles, in situ opening of the equilibrating species may be observed.

Epoxides have long been considered important in the chemistry of carbohydrates, and epoxide migration in the context of carbohydrate has been discussed.\(^2\)

We have investigated migration of 1,6:2,3-dianhydro- and 1,6:3,4-dianhydro-β-D-hexopyranoses since these epoxides are readily available, and therefore valuable starting material for organic synthesis. Isomerisation was effected by treatment with aqueous sodium hydroxide or sodium iodide in acetone. The composition of the resulting equilibrium mixtures of isomeric epoxides is in great agreement with findings that have already been published.\(^1\)

We have also synthesised various iodo derivatives of 1,6-anhydro-β-D-hexopyranoses as potential intermediates for pseudo-epoxide migration, by cleavage of epoxide ring of dianhydrohexopyranoses with hydrogen iodide in dioxane.

NMR was used for following the reaction mechanism of epoxide and pseudo-epoxide migrations and analysis of the composition of reaction mixtures. Obtained experimental data were compared with theoretical calculations.\(^3\) Chair-boat equilibration of 1,6-anhydro-3-deoxy-3-halogeno-β-D-glucopyranoses will also be discussed.

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STUDIES ON HYDROLYSIS KINETICS OF ACETAL LINKERS ON GLUCAN DERIVATIVES

Pavol Farkaš*, Slavomír Bystrický

Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia

*corresponding author’s e-mail chempalo@savba.sk

(1→3)-β-D-Glucan is polysaccharide that acts as a nonspecific immune system stimulant.[1] Recently, we have reported the preparation and characterization of functional derivatives of carboxymethylglucan (CMG) and glucuronoglucan (OXG) containing spacers of different length with dimethylacetale group, ready to bind any amino group.[2]

Further, we studied the hydrolysis kinetics of these derivatives. Reactions were carried out at various temperatures and at various pH values. We tested mineral and carboxylic acids, respectively as well as DOWEX cation resin. Pseudo-first order rate konstants, k₁, were evaluated, and second order rate konstants k₂ were calculated, k₁=k₂[H⁺]. Observed hydrolysis rate konstants (k₂) are well comparable with derivatives with different degree of substitution of glucans with linkers. Derivatives of OXG are hydrolysing faster compared to analogical derivatives of CMG. There was no hydrolytic degradation of polysacharide chain at optimal conditions. Nowadays these derivatives are used as an antigens carriers for preparation of artificial vaccine.

Synthesis of $3^1$-O-Alkylderivates of $\beta$-Cyclodextrin Using Wittig Reaction

PETR HEZKY$^a$, JINDŘICH JINDŘICH$^b$ and MICHAL KUSÁK$^b$

$^a$Institute of Organic Chemistry and Biochemistry, Academy of Science of the Czech Republic, Flemingovo n. 2, 166 10, Praha 6, Czech Republic, e-mail: hezky@uochb.cas.cz; $^b$Department of Organic and Nuclear Chemistry, Charles University, Hlavova 8, 128 40, Praha 2, Czech Republic, e-mail: jindrich@natur.cuni.cz

We have recently discovered a generally applicable method for regioselective monosubstitution of $\beta$-cyclodextrin at the $3^1$-O position$^1$. The method is based on the reaction of cinnamyl bromide with $\beta$-cyclodextrin which gives predominantly the $3^1$-O-cinnamylderivative. After acetylation, the cinnamyl group of the remaining $\beta$-cyclodextrin hydroxyl groups can be cleaved by ozonolysis. The formylmethylgroup formed by this reaction can be used for subsequent derivatization by Wittig reagents. We have demonstrated the utility of this procedure through the synthesis of a series of $3^1$-O-alkenyl derivates of $\beta$-cyclodextrin.

Reference

Microwave-assisted transformation of reducing saccharides.

Zuzana Hricoviniová

Institute of Chemistry, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia
Tel: +421 2 5941 0282, Fax +421-2-5941-0222, E-mail: chemhric@savba.sk

Mo(VI)-catalyzed interconversions of reducing sugars significantly simplified synthesis of various saccharides.1 The typical feature of these interconversions is both high stereoselectivity and a good yield. Despite beneficial attributes of the above-mentioned reactions new methodologies were further examined in order to optimize the reaction conditions. The knowledge of structure of Mo(VI) complexes and mechanism of isomerization of aldose/epi-aldose1 and 2-ketose/2-C-(hydroxymethyl)branched aldose2 lead to the idea that microwave irradiation might have a considerable effect on Mo(VI)-catalyzed saccharide transformations. Present investigation has been thus devoted to examine the effect of microwave irradiation on the Bílik reaction in which Mo(VI) complexes play a crucial role in isomerization of reducing sugars.

The effect of microwave irradiation resulted in the reaction rate enhancement up to 200 times. The yields were found similar, or even better, compared to the conventional synthetic methods.3 Analogous results were obtained in the mutual interconversion of 2-C-(hydroxymethyl)branched aldoses and 2-ketoses. The analysis of the equilibrium reaction mixtures showed high yields (60-80%) of products that could be achieved within minutes. The present data indicate that microwave heating has been shown to be a very useful method for preparation of such compounds.

References

A bacterial retaining glycosyltransferase LgtC catalyzes a transfer of a donor sugar to an acceptor without involving any catalytic base or nucleophile. To understand the mechanism of the reaction we analyzed structural properties of a donor substrate (UDP-Gal) and a fluorinated donor analog – a reversible inhibitor (UDP-FGαl) bound at the active site of LgtC using density functional theory methods (MPW1PW91 and B3LYP). The calculations indicate that the unique mechanism could be based on an indirect activation of a scissile glycosidic bond of the donor in the Michaelis complex by a His78-Lys250-Mn catalytic machinery of LgtC, which distorts the donor geometry toward an oxocarbenium ion-like transition state.
INVESTIGATION OF BINDING SPECIFICITY OF NK CELL RECEPTORS USING MASS SPECTROMETRY, INHIBITION ASSAYS, AND OLIGOSACCHARIDE ARRAYS

Daniel Kavan\textsuperscript{1,2}, Karel Bezouška\textsuperscript{1,2}, Hynek Mrázek\textsuperscript{1,2}, Luboslav Mihók\textsuperscript{1}, Petr Pompach\textsuperscript{2}, Janna Neumann\textsuperscript{3}, Petr Novák\textsuperscript{2} and Vladimír Havlíček\textsuperscript{2}

\textsuperscript{1}Department of Biochemistry, Faculty of Science, Charles University in Prague, Hlavova 8, CZ-12840 Prague 2, Czech Republic,
\textsuperscript{2}Institute of Microbiology, Centre of Biocatalysis and Biotransformation, Academy of Sciences of the Czech Republic, Videnska 1083, 14220 Prague, Czech Republic,
\textsuperscript{3}Institute of Organic Chemistry University of Hamburg, Martin-Luther-King Platz 6, D-20146 Hamburg, Germany.

Effector activities of natural killer (NK) cells are controlled by signals transmitted from activating and inhibitory receptors of both immunoglobulin and C-type lectin families. In our laboratory we investigate molecular mechanisms of activation of rat NK cells and human leukocytes through the rat NKR-P1A receptor and CD69 antigens, respectively. Our long term goal is to characterize the ligands for these receptors, to construct efficient mimetics for these ligands, and to use these compounds as antitumor and immunostimulatory compounds. In order to express the receptor proteins for ligand identification experiments we amplify several DNA fragments coding for various portions of the extracellular part of these receptors, and express the corresponding proteins using the bacterial expression systems. Proteins precipitated into the inclusion bodies are refolded \textit{in vitro}, and purified to homogeneity as shown by SDS PAGE. The proteins refold as noncovalent dimers of about 30kDa. The stability and solubility of the produced proteins was tested by thermal denaturation experiments, IR and Raman spectroscopy, and two-dimensional NMR spectroscopy. The proteins are stable at 37°C for at least one week without any signs of biochemical degradation, and can be heated up to 65°C without any loss of activity or 3D structure. Mass spectrometry, in particular FT-MS (Brucker Apex-Qe) in combination with chemical crosslinking was employed to check both the natural “crosslinking” by disulfide bridges as well as the results of the chemical cross-linking of the protein. The recombinant proteins and their mutants were used in inhibition as well as direct binding experiments in order to reveal their ligand binding specificities. While both receptors were shown to bind \textit{N}-acetylhexosamine monosaccharides, the affinity of these sugars for NKR-P1A receptor was much higher. Moreover, in order to explore the binding specificities of both proteins further, we developed techniques for their fluorescent labeling which allows us to measure their simultaneous binding using a standard microplate reader device (Tecan Safire\textsuperscript{2}). The results obtained using the oligosaccharide array technology support our previous findings concerning the fine specificities of both receptors for complex carbohydrate ligands, and confirm a good correlation between the data obtained using modern oligosaccharide arrays with those measured using a more classical approach.

Glycosyl Azides as New Donors for Glycosidases

V. Křen, P. Bojarová-Fialová, L. Petrásková

Laboratory of Biotransformation, Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, CZ 14220 Prague, Czech Republic; kren@biomed.cas.cz

Glycosidases are well suited for selective synthesis of glycosides due to their versatility, robustness and availability. However, traditional aryl glycosides are unable to cope with demands on solubility, ready and cheap synthesis. Therefore, new alternative donors for glycosidase-catalyzed synthesis are being sought for. This work aims to present glycosyl azides as a full-value option to other popular donors for transglycosylations.

Six well soluble glycosyl azides 1-4 (α-Gal, β-Gal, β-Glc, α-Man, β-GlcNAc and β-GalNAc) were prepared and tested for their hydrolysis and transglycosylation by a large panel (over 60 enzymes) of glycosidases from our Library of glycosidases. Cleavage of glycosyl azides generally proceeded somehow slowly than of p-nitrophenyl glycosides. β-N-Acetylhexosaminidases cleaved the gluco-substrate, however, they did not accept the galacto-analogue. This is a unique example of a change to GlcNAc-ase/GalNAc-ase activity ratio caused by aglycon that was explained by molecular modeling [1].

Glycosyl nitriles (e.g., 5) were prepared and used also as glycosyl donors for glycosidases [2]. Broad array of various glycosidases were tested to compare the reactions with common p-nitrophenyl glycosides and azides. Large series of new glycosides was prepared. Glycosyl azides proved to be good glycosyl donors for tested glycosidases of various specificities, with transglycosylation yields comparable to commonly used substrates. Their high water solubility and facile synthesis predestine them for broader use in enzymatic glycosylations.

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Phanerochaete chrysosporium Glucuronoyl Esterases:
Gene Expression in Different Fungi

Miroslava Křupalová¹, Silvia Špániková¹, Ronald P. de Vries² and Peter Biely¹

¹Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38, Bratislava, Slovakia
Phone: +421-2-59410229, Fax: +421-2-59410222, chemmkru@savba.sk
²Microbiology, Faculty of Biology, Utrecht University, Utrecht, Netherlands

Glucuronoyl esterase (GE) is a novel carbohydrate esterase hydrolyzing esters of 4-O-methyl-D-glucuronic acid and probably also these types of linkages between lignin and hemicellulose in plant cell walls [1, 2]. The first isolated and characterized GE is a product of wood-rooting fungus Schizophyllum commune [1].

The partial internal amino acid sequence of the S. commune GE was used for the search of homologous regions in genes of microorganisms with sequenced genomes. The homologous sequences have been found in internal sequences of functionally unidentified proteins of several filamentous fungi including two proteins of Phanerochaete chrysosporium. In this work we show that the fungus P. chrysosporium produces glucuronoyl esterase during growth on sugar beet pulp. Subsequently two genes coding for GEs from this strain were isolated, partially characterized and successfully expressed in Aspergillus vedensis, Schizophyllum commune and Picnoporus cinnabarinus.

The evidence for the occurrence of the unique glucuronoyl esterase gene(s) in genomes of a series of fungi, distinct from genes of other types of carbohydrate esterases (acetylxylan esterases, feruloyl esterases, pectinmethyl esterases) [3] leads to emergence of a new family of carbohydrate esterases with so far unknown biotechnological and biocatalytic potential.

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Sugar chains protect proteins against radicals generated from xenobiotics. A novel role for carbohydrate moieties in glycoproteins

Zuzana Kubínková1,2 Marie Stiborová1, Václav Martínek1, Jan Sklenář1,2, Miroslav Šulc1,2, Eva Frei3 & Karel Bezouška1,2

1Department of Biochemistry, Faculty of Science, Charles University Prague, Albertov 2030, CZ-12840 Prague 2, Czech Republic; 2Institute of Microbiology, Academy of Sciences of Czech Republic, CZ-14220 Prague 4, Czech Republic.
3Division of Molecular Toxicology, German Cancer Research Center, D-69120 Heidelberg, Germany

Glycosylation, one of the most common and abundant posttranslational modifications of proteins, greatly influences the properties of proteins. Today we know that there is no single unifying function for carbohydrates present in glycoproteins1. Carbohydrate chains in proteins protect against proteolysis2, facilitate protein folding3, and target proteins to specific cellular destinations4. Despite the numerous functions that have been suggested for protein glycosylation, for some highly glycosylated proteins, e.g. α1-acid glycoprotein, the function is only poorly understood5. Here we suggest a novel role for protein glycosylation, namely the protection of the polypeptide by the carbohydrate moieties from the attack of radicals generated in living cells.

The yeast *Candida albicans* is an opportunistic pathogen, part of the human commensal flora that causes infections in immunocompromised individuals with a high morbidity and mortality levels. A long-acting, effective and safe vaccine that protects against *C. albicans* and other important *Candida* species should significantly reduce the incidence of various forms of candidiasis by these etiologic agents. Recognition of yeasts by host cells is connected with yeast cell wall components. Approximately 80 to 90% of the cell wall of *C. albicans* is carbohydrate. Three basic constituents represent the major polysaccharides of the cell wall: glucan, chitin and polymers of mannose (mannan) covalently associated with proteins (mannoproteins) [1]. Mannan is a major virulent and protective factor of *C. albicans*. It is known, that mannan determines the antigenic specificity of *Candida* species. *C. albicans* mannan contains a backbone consisting of $\alpha$-1→6-linked D-mannopyranose units and many branches composed of $\alpha$-1→2; $\alpha$-1→3 and/or $\beta$-1→2-linked mannopyranose units. Presumably the $\beta$-1→2-linked mannopyranose units can be regarded as the epitopes dominating serotype A specificity. The function of many manno-oligosaccharides on modulation of immune system remains unknown. In this study, we used for immunization two synthetically prepared manno-oligosaccharides conjugated to protein carrier. Prepared conjugates were immunogenic in rabbits and re-injection elicited increase of specific serum antibodies levels. Based on these results, these newly synthesized manno-oligosaccharides could be considered to have an important role in anti-mannan serologic response.

Reference:

Acknowledgement
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STUDY OF ENZYMATIC ACYLATION OF METHYL α-D-GLUCOPYRANOSIDE BY PHENOLIC ACIDS AND THEIR NON-PHENOLIC ANALOGUES

Mária Mastihubová and Vladimír Mastihuba

Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia, e-mail: chemvrma@savba.sk, tel.: +421-2-59410246, fax.: +421-2-59410222

The functionalisation of unprotected sugars by classic acylation procedures affords mixtures of polysubstituted derivatives. This problem may be avoided by introduction of biocatalytic procedures possessing high degree of regioselectivity in the acylation of polyhydroxylated substrates. Feruloyl esterases [E.C. 3.1.1.73] (FAEs) represent a group of carboxylic acid esterases able to hydrolyse an ester bond between p-hydroxycinnamic acids and saccharides present in plant cell walls [1]. Recently, classification of feruloyl esterases into four sub-classes (A-D) was proposed based on similarities in hydrolysis activity profiles against esters of partly substituted hydroxycinnamic acids, their dimers, and protein sequence identities of the enzymes [2].

Previously, a scale of commercial enzyme preparations exhibiting feruloyl esterase activity has been tested in our laboratory for catalysis of trans-feruloylation reactions in organic solvents [3]. Lipolase 100T (lipase from Thermomyces lanuginosus) was found as the best feruloyl esterase preparation stable in acetonitrile. This contribution presents our results on investigation of the reactivity of different vinyl esters of several phenolic acids (4-hydroxybenzoic, vanillic, syringic, gallic, p-hydroxyfenylacetic, p-hydroxyfenyl-3-propionic, p-cumaric, ferulic, sinapic, caffeic) and their non-phenolic analogues (benzoic, 3,4,5-trimethoxybenzoic, 2-thiophene carboxylic, cinnamic 3,4-dimethoxycinnamic, 3,4,5-trimethoxycinnamic, 2-furyl-3-acrylic) as donors for enzymatic acylations. Methyl α-D-glucopyranoside was the acceptor of our choice for this study. The regioselectivity and yields of studied acylation strongly depend on donor structure. The reactivity decreased in the order: p-hydroxyfenylpropanoic acid > derivatives and analogues of non-phenolic cinnamic acids = derivatives and analogues of benzoic acids > derivatives of p-hydroxycinnamic acids > p-hydroxyfenylacetic acid > derivatives of p-hydroxybenzoic acid. The yields (79-12%) were affected by degree of hydroxylation on aromatic ring or position of hydroxyl on aromatic ring. When activated esters of phenolic acids served as acyl donors, the acylation occurred exclusively at primary position of methyl α-D-glucopyranoside. On the contrary, some amounts (below 10%) of 2,6-diacylated products were isolated from reaction mixtures with non-phenolic donors.

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References

Induction of extracellular glycosidases of capsular strain of the yeast
Cryptococcus laurentii

Csilla Mészárosová, J. Dudíková, N. Kolarova

Slovak Academy of Sciences, Institute of Chemistry, Dúbravská cesta 9, 845 38 Bratislava, Slovakia, emai: chemcila@savba.sk, fax:+421 2 59410222

During the past few decades, increasing attention has focused on pathogenic fungi both as fascinating research subjects and as the agents of serious illness in diverse patient populations. The patogenecity of each microorganism is influenced by various factors. In Cryptococcus, one of the most important virulence factors is the capsule and its extracellular soluble components. The high virulence of Cryptococcus strains is caused mainly by their ability to produce and secrete massive amounts of capsular material into body fluids.

While studies of capsule synthesis have progressed (1) capsule degradation remains poorly understood. Although enzymes from soil microbes can degrade capsule (2), no cryptococcal or human enzymes with this activity have been found.

To understanding of capsule release mechanism in Cryptococcus could be useful for preparation of immunomodulating agents.

The induction of glycosidase by variant carbon sources present in growth medium was investigated. We found that both $\alpha$-galactosidase and $\alpha$-glucosidase were induced by lactose, while the $\alpha$-mannosidase was induced by saccharose. At present we studied involvement of these glycosidase in the mechanism of capsule release.

References:
REGIOSELECTIVE ENZYMATIC FERULOYLATION OF REDUCING MONOSACCHARIDES

Zlatica Morávková and Mária Mastihubová

Institute of Chemistry, Center for Glycomics, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia, e-mail: chemoraz@savba.sk, tel.: +421-2-59410246, fax.: +421-2-59410222

Hydroxycinnamic acids are common components of plant cell walls as parts of lignin-carbohydrate complexes [1]. They are covalently linked to the polysaccharides through an ester linkage. Dimers of ferulic acid cross-link cell wall polysaccharides and ferulic acid have the potential to cross-link polysaccharides and lignin [2]. Feruloyl esterases [E.C. 3.1.1.73] (FAEs) represent a group of enzymes able to hydrolyse ester bonds between hydroxycinnamic acids and sugars present in plant cell walls [3]. Ferulic acid as the most abundant \(p\)-hydroxycinnamic acid has widespread industrial potential by virtue of its antioxidant properties and ability in oxidative reactions. Such natural antioxidant starts to find use in food, cosmetic, pharmaceutical and material industry. Ability of \(p\)-hydroxycinnamic acids to dimerise or polymerise by action of oxidases has initiated research in the domain of preparation different polyaromatic or polyphenolic materials [4].

Actually, our laboratory cooperates on a project dealing with preparation and grafting of phenolics derivatised by mono- and oligoaccharides into lignin to increase number of centers for non-covalent interactions between lignin and polysaccharides [5]. The esterification of ferulic acid by saccharides improves its solubility in water and opens wide possibilities to modulate its interaction with hydrophilic and hydrophobic materials. Recently, our group published results on tests of a scale of commercial enzyme preparations exhibiting feruloyl esterase activity for catalysis of feruloylation of different glycosides in organic solvents [6]. Lipolase 100T (lipase from Thermomyces lanuginosus) was found as the best enzyme preparation possessing feruloyl esterase activity. In this contribution, we present our results from enzymatic feruloylation of reducing monosaccharides (D-glucose, D-galactose, D-mannose, D-glucosamine and L-arabinose) by Lipolase 100T using vinyl ferulate as the donor. The insolubility of reducing monosaccharides in organic solvents had provoked search for another convenient aprotic polar organic solvent or introduction of a more polar cosolvent in amounts not affecting the enzyme activity. Therefore D-hexopyranoses (gluco-, galacto- and manno-) were preparatively feruloylated in mixture 5% DMF-CH\(_3\)CN exclusively on primary position in satisfactory yields.

Acknowledgement

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References

**D-Glucosamine - Bile Acid Conjugates**

**Nováková Z., Tomanová J., Drašar P.**

*Vysoká škola chemicko-technologická v Praze, Ústav chemie přírodních látek, Technická 5, 166 28 Praha 6*

Bile acids are produced in the liver by the oxidation of cholesterol, and are stored in gallbladder and secreted into the intestine in the form of salts. They act as surfactants, lipid emulsifiers and possess assisting ability to their absorption and digestion. Their structure consists of steroid skeleton with 5β anelation and at least one α-oriented hydroxy-group.

The surfactant and self-assembling capability is bound to their two different aspects - hydrophilic and hydrophobic side, which is combined with electron rich side chain.

Our aim is to prepare conjugates of D-glucosamine with chosen bile acids - connected by the amide bond. Substances prepared will be tested on the expected gelation abilities. Both parts of the conjugate do represent required properties (rather long lipophilic steroidal part and polar aminosugar moiety, both with controllable polarity and both with capability to act as hydrogen bond donors as well acceptors).

![Chemical structures](image)

R' = OH R=OH  cholic acid

R' = H R=H  lithocholic acid

R' = OCHO R=OCHO  cholic acid amide

R' = H R=H  lithocholic acid amide

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SORPTION OF PCP BY CELL WALL β-D-GLUCAN ISOLATED FROM SACCHAROMYCES CEREVISIAE

Martin Pajtinka¹*, Grigorij Kogan¹, Zuzana Sejáková², Katarína Dercová²

¹Institute of Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 845 38 Bratislava, Slovakia
²Department of Biochemical Technology, Institute of Biotechnology and Food Sciences, Faculty of Chemical and Food Technology, Slovak University of Technology, Radlinského 9, 812 37 Bratislava, Slovakia
*corresponding author’s e-mail chemmapa@savba.sk

Contamination with polychlorinated biphenyls (PCBs) represents serious environmental risk due to their widespread occurrence, toxicity, and recalcitrance. Pentachlorophenol (PCP) can be used as a model compound of PCBs for investigation of their possible elimination mechanisms. PCP sorption-desorption investigation was performed using insoluble yeast glucan isolated from the cell walls of Saccharomyces cerevisiae (β-D-glucan). For a comparison, another fungal polysaccharide from the mycelium of filamentous fungus Aspergillus niger (chitin-glucan complex, CG) and its carboxymethylated derivative (CM-CG) were used as well. Microcrystalline cellulose (MC) was used as another glucan matrix for comparison with the yeast β-D-glucan.

β-D-Glucan revealed significantly better sorption ability towards PCP than CG and CM-CG. The sorption capacity of the prepared yeast β-D-glucan matrix (2 g/100 ml H₂O) reached 99 % PCP extracted from the 100 mg/l water solution. Even reduced amounts of glucan demonstrated very high sorption capacity: 0.5 g/100 ml suspension bound almost 90 % PCP. Due to the very high sorption capacity of β-D-glucan, more sorption experiments were performed with lower mass of glucan sorbent. The sparing amounts of polysaccharides were 0.25 g and 0.1 g per 100 ml H₂O.

In the sorption-desorption experiments, when the sorbent with the bound PCP was eluted with water (pH 4.0), the lowest desorption (i.e. the highest retention) parameters were shown by CG. Desorption revealed by CG was in a range 0.25-2.1 % of the sorbed PCP, whereas β-D-glucan and CM-CG had desorption values between 0.23-2.29 % and 1.19-4.89 %, respectively. The sorption data obtained with MC were up to 28 % lower than those observed for yeast glucan at the same conditions. This significant difference can be explained by the fact that while cellulose is a linear molecule, yeast β-D-glucan adopts triple helical conformation, which might more tightly enclose the PCP molecule. Additional studies to investigate the interactions between various polysaccharides and PCP and the effect of pH and temperature on the sorption-desorption processes are needed to fully understand their sorptive characteristics. The data obtained indicate that yeast cell wall material could be used at the decontamination processing.

Additionally, sorption capacities of the crude material - lyophilized and dried cells of Saccharomyces cerevisiae - were determined. Yeast cell walls could present a potential more economical alternative of PCBs sorbent for decontamination of the polluted areas.

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The binding properties of the H5N1 influenza virus neuraminidase

Michal Raab, Igor Tvaroška

Institute of Chemistry, Slovak Academy of Sciences, Dúbravska cesta 9, 845 38 Bratislava, Slovakia

Phone: +421 2 5941 0203; e-mail: chemmrrsa@savba.sk

Neuraminidases encompass a group of enzymes that cleave the terminal sialic acid of the host cell oligosaccharide chain. In the case of influenza virus, this process is crucial in the infection cycle, enabling the replicated virions to spread from the infected cell. The molecular mechanism of the hydrolysis is still unclear, as well as the conformational behaviour of the sialic acid glycoconjugate during the reaction. To investigate the conformation changes in the first step of binding of the ligand, we have performed docking studies on several known neuraminidase inhibitors and several isomers of natural substrate – sialyllactose. Furthermore, a 39-molecular dynamics simulation has been performed to investigate the dynamical properties of the favored conformation.
PREPARATION OF MONO-O-SUBSTITUTED α-CYCLODEXTRINS

MICHAL ŘEZANKA, IVA RUDOVSKÁ, and JINDŘICH JINDŘICH

Charles University in Prague, Faculty of Science, Department of Organic Chemistry, Hlavova 2030, Prague 2, 128 43, Czech Republic
e-mail: rezanka@natur.cuni.cz; tel. +420221951575

Cyclodextrins\(^1\) (CDs) and their derivatives have good complexation abilities due to the rigid, cone-shaped cavity formed by α-1,4-linked D-glucopyranose units. Our research is focused on a preparation of a set of exactly defined monosubstituted derivatives of α-CD for subsequent utilization in organic synthesis.

The preparation of monosubstituted derivatives of CD which has easily transformable functional group\(^2\) – e.g. cinnamyl or allyl group – is the first and the main step. These groups can be transformed to the formylmethyl group by ozonolysis. The formylmethyl group can be oxidized to the carboxymethyl group (Scheme 1).

Position of the cinnamyl or the allyl group of α-CD derivatives was determined on peracetates by 2D NMR techniques.

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REFERENCES
Simple carbasugars from *Streptomyces lincolnensis* DSM 40355

Petr Sedmera, Petr Halada, and Stanislav Pospíšil

Institute of Microbiology, Academy of Sciences of the Czech Republic, Vídeňská 1083, CZ 14220
Prague 4, Czech Republic

Valienol (1) and two new deoxocarbasugars 2 and 3 were isolated from the fermentation broth of *Streptomyces lincolnensis* DSM 40355, the producer of lincomycin\(^1\,^2\) and rancinamycins\(^3\). Their structures were elucidated by NMR and MS.

GLYCALS AND THEIR USE IN PREPARATION OF 2-DEOXYGLYCOSIDES OF LUPANE TRITERPENOIDS

P. SPACILLOVA\textsuperscript{a}, J. SAREK\textsuperscript{a}, I. RUDOVSKA\textsuperscript{a}, M. HAJDUCH\textsuperscript{b}, X. SVOBODOVA\textsuperscript{c}

\textsuperscript{a}Department of Organic and Nuclear Chemistry, Faculty of Science, Charles University in Prague, Hlavova 8, 128 43, Prague; e-mail: pavla.spacilova@gmail.com; \\
\textsuperscript{b}Laboratory of Experimental Medicine, Faculty of Medicine and Faculty Hospital, Palacky University, Olomouc, Puskinova 6, 775 20, Olomouc, \\
\textsuperscript{c}I.Q.A., a.s. Jindricha Plachty 16, 150 00, Prague 5

Betulinines, triterpenoid derivatives prepared in our research group, show many interesting biological effects\textsuperscript{1}, mainly cytotoxic (IC50<10 μmol/l). Previously discovered active derivatives however have mostly failed to show optimal pharmacological properties. In particular difficulties with oral availability and solubility in water based solvent system was observed. We therefore decided to study derivatisation of selected compounds with the purpose of enhancing their pharmacological properties.

Glycosides are typical examples of natural prodrug. Furthermore it is known from literature\textsuperscript{2}, that application of 2-deoxyglucose together with conventional cytostatics (paclitaxel, adriamycin) leads to increase of cytostatic effect. According to this fact, it should be possible to observe the enhancement of cytotoxicity after 2-deoxyglycosylation of the derivative.

In this project acetylated 2-deoxyglucosides and 2-deoxygalactosides were synthesized by addition of corresponding acetylated glycals to triterpenoid hydroxyderivatives – hydroxyketone I a methyl ester II. Free 2-deoxyglycosides were synthesized by Zemplén’s deacetylation of acetylated 2-deoxyglycosides.

Cytotoxic activity of new compounds was tested against CEM cell line and gave promising results. Free 2-deoxyglycosides have shown markedly better solubility in water based solvent systems.

\begin{center}
\includegraphics{structures.png}
\end{center}

Structures of the new compounds were confirmed by spectral data.

LITERATURE
Synthesized glycosyl donor units were designed for stepwise synthesis of oligosaccharides which will allow further easy regioselective functionalization. Mannopyranoside 3 was prepared from D-glucose according to the procedure found in literature. \( p \)-methoxybenzyl group was used in position 3 instead of the benzyl group. Yields of these reactions were comparable to the published ones. However, during the step, besides the formation of substance 4, the methoxybenzyl group at \( O-3 \) position was split off. Yield of this reaction was at most only one third of the expected value. Some other possible reaction conditions will be tested in the future. Substances 5 and 7 will be further used for the preparation of glycosyl donors 6 and 8. They will be used in series of glycosylation reactions which were already successfully carried out using similar substances.

References:
A potential transition-state analog for glycosyltransferases: DFT study of conformational behavior

L. Sihelnikova, I. Tvaroska

Institute of Chemistry, Slovak Academy of Sciences, Dubravská cesta 9, 845 38 Bratislava Slovak republic
E-mail: chemsihl@savba.sk

Transition-state analogs of glycosyltransferases represent a group of potential inhibitors of these enzymes and are, therefore, valued tools for drug discovery. In this study, using knowledge of main structural features of the transition state for inverting glycosyltransferases, [2-cyclohexylsulfanyl-3,4,5-trihydroxy-tetrahydro-furan-2yl]methyl sulfate anion (Fig.1) was designed as a new scaffold representing a possible transition-state analog. Then conformational space available for this structure was monitored by means of three two-dimensional (Φ, Ψ) potential-energy surfaces, corresponding to the values of ω of 60°, -60°, and 180°. The optimizations of the geometries with the fixed dihedral angles Φ, Ψ, and ω were performed using the B3LYP density functional method with the 6-31+G* basis set within the framework of the Jaguar program [1]. The geometries of all local minima identified on the energy maps were then fully optimized with no constraints on the Φ, Ψ, and ω torsion angles at the 6-311++G** level. This led to 8 conformers. The influence of solvent on their stability was also evaluated. A superposition of calculated conformers with the predicted transition state structure showed that the preferred conformers in solution nicely mimic structural features of the transition state.


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Galactosylation of Nucleoside Analogues by Cold-Active β-Galactosidase

V. Spiwok*¹, J. Riedl¹, H. Dvořáková², I. Votruba³, A. Holý³, B. Králová¹

¹Department of Biochemistry and Microbiology and, ²Central Laboratories, Institute of Chemical Technology, Prague, Technická 5, 166 28 Prague 6, Czech Republic, ³Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Flemingovo nám. 2, 166 10 Prague 6, Czech Republic

*e-mail: spiwokv@vscht.cz

Cold-active β-galactosidase from Antarctic bacterium *Arthrobacter* sp. C2-2 is capable to catalyze transglycosylation reactions during hydrolysis of lactose. In order to test its synthetic potential we galactosylated two nucleoside analogues: 9-(S)-(2,3-dihydroxypropyl)adenine (DHPA) and 9-(2-hydroxyethyl)adenine (HEA). The enzyme was capable to synthesize monogalactosides of DHPA and HEA with yields of 40 % and 33 %, respectively. Also production of di- and putative trigalactosylated products was detected. Regioselectivity of galactosylation of DHPA was assessed by NMR spectroscopy. ¹H-¹H COSY and ¹H-¹³C HMBC experiments (in DMSO-d₆) proved that the sole monogalactosylated product is DHPA galactosylated in 3-hydroxyl position of 2,3-dihydroxypropyl moieties. These results illustrate a great synthetic potential of this enzyme.

This work was supported by the Academy of Sciences of the Czech Republic (GAAV KJB 500500512) and the Ministry of Education, Youth and Sports (MSM 6046137305).
GALECTIN-LIKE PROTEIN FROM RALSTONIA SOLANACEARUM

ONDŘEJ ŠULÁK, NIKOLA KOSTLÁNOVÁ, EDWARD P. MITCHELL, ANNE IMBERTY and MICHAELA WIMMEROVÁ

National Centre for Biomolecular Research & Institute of Biochemistry, Faculty of Science, Masaryk University, Kotlarska 2, 611 37 Brno, Czech Republic
ESRF Experiment Division, BP220, F-38043 Grenoble cedex, France
CEPMA-CNRS, BP 53, F-38041 Grenoble cedex 09, France

Ralstonia solanacearum is a Gram-negative bacterial pathogen that causes several diseases in a wide range of plants (tomatoes, peppers, eggplant, banana etc.) [1]. Lectins are carbohydrate-binding proteins or glycoproteins, which are highly specific for their sugar moieties in cell walls or membranes and thereby change the physiology of the membrane. Lectins are known for their ability to agglutinate erythrocytes in vitro.

Plant and animal pathogens use these protein-carbohydrate interactions in their strategy for host recognition and invasion. Since there are not any known treatments of resistant plant species, identifying target molecules for the bacterium in host plants could help to develop a strategy against its infection.

As far as we know, the R. solanacearum bacterium has been producing three soluble lectins, RSL (9.9 kDa) [2], RS-IIIL (11.6 kDa) [3] and newly discovered RS20L (20 kDa)

This contribution structurally and functionally describes RS20L, which has no sequence similarity to any known amino acid sequence. Although resolution of crystal structure showed high structural similarity to animal galectins, it doesn’t display sugar specificity to D-galactose.

References:

This work has been supported by Ministry of Education, Youth and Sports of the Czech Republic (LC06030) and Grant Agency (204/03/H016) of the Czech Republic.
Analysis of lipopolysaccharide (LPS) antigens of *Vibrio cholerae* O135 and O34 isolated from Slovak rivers

J. VRÁBLOVÁ\textsuperscript{a}, S. BYSTRICKÝ\textsuperscript{a}, M. SEMAN\textsuperscript{b}

\textsuperscript{a}Institute of Chemistry, Department for Immunochemistry of Glycoconjugates, Slovak Academy of Sciences, 845 38 Bratislava, Slovakia

\textsuperscript{b}Institute of Cell Biology, Faculty of Natural Sciences, Comenius University, 842 15 Bratislava, Slovakia
e-mail: chemjura@savba.sk

Cholera is a diarrheal disease caused by a gram-negative bacterium *Vibrio cholerae*. *Vibrio* exists naturally as an inhabitant of surface waters often as a part of a zooplankton. Transmission to humans become from contaminated water and food. Severe cholera disease symptoms are associated with serotypes O1 and O139. However, cholera-like diseases are also caused by other non-O1 serotypes.

More than 200 serotypes of non-O1 have been reported by now. As all gram-negative bacteria, their cell-wall contains characteristic lipopolysaccharide (LPS) structures -endotoxines. LPS is composed of three parts: lipid A, oligosaccharide core and O-specific polysaccharide. Lipid A is linked via core oligosaccharides to the O-specific polysaccharide. Lipid A and core oligosaccharides are conservative parts of the molecule. The O-polysaccharide, which contain mono- or oligosaccharide repeating units with enormous structural variation, determine the serological specificity of the bacterium and have a serum vibriocidal activity.

We have used two serotypes of non-O1 *Vibrio cholerae*: O135 and O34. There were chosen 4 isolates of each serotype and used for cultivation and analysis. Proportion of saccharides, proteins and nucleic acids were determined in raw LPS s by the spectrophotometry. Molecular weights of the elements (lipid A, polysaccharide core, O-specific chain as an antigen) were analysed by SDS-PAGE and HPLC. After purification of LPS s by a gel chromatography and HIC, samples were divided according to their molecular weights and hydrophobic interactions. After following dialysis and acid hydrolysis, the highly purified O-specific polysaccharide chain (O-antigen) was obtained, which underwent H-NMR spectral characterisation.

The work provides a confirmation of diversity of analysed serotypes on the basis of LPS variability, of the degree of coincidence with determined serotypes and of informational database about one important element of immunogen or antigen complex of *Vibrio cholerae*. The study of LPSs allows for research of defined glycoconjugates (O-antigen bound to a vehicle) Therefore, it allows to contribute to progress in creating a new stable, safe and mainly enough immunogen vaccine.

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Studies on the inducibility, properties and synthetic capability of fungal $\alpha$-N-acetylgalactosaminidase

L. Weignerová$^1$, E. Kupčíková$^1$, T. Filipí$^1$, D. Manglová$^1$, K. Bezouška$^2$ and V. Křen$^1$

lenka@wcontact.cz

$^1$Academy of Sciences of the Czech Republic, Institute of Microbiology, Center for Biocatalysis and Biotransformation, Vídeňská 1083, CZ 142 20, Prague 4

$^2$Academy of Sciences of the Czech Republic, Institute of Microbiology, Laboratory of Protein Architecture, Vídeňská 1083, CZ 142 20, Prague 4

In the large screening study performed with the aim to obtain a good producer of $\alpha$-N-acetylgalactosaminidase activity a library of filamentous fungi (42 strains), series of inducers and cultivation conditions were tested. Enzyme production of the best producer Aspergillus niger CCIM K2 was optimized and scaled up. $\alpha$-N-Acetylgalactosaminidase was purified to apparent homogeneity by cation exchange and gel filtration chromatography. $\alpha$-N-Acetylgalactosaminidase was biochemical characterized: native molecular weight was estimated by gel filtration to be approximately 440 kDa, the enzyme was optimally active at pH 1.5 and 55 °C with $\alpha$-nitrophenyl 2-acetamido-2-deoxy-$\alpha$-D-galactopyranoside and enzyme was found to be a retaining-type glycosidase. $\alpha$-N-Acetylgalactosaminidase was N-deglycosylated and N-terminal sequenced. $\alpha$-N-Acetylgalactosaminidase from A. niger CCIM K2 can be used for synthesis of $\alpha$-GalNAc-containing glycoconjugates using reverse hydrolysis approach.

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Stereoselective synthesis of 3-deoxy-3-(α-D-glucopyranosylmethyl)-D- and L-fagomine

Lukáš Werner, Ladislav Kniežo

Department of Chemistry of Natural Compounds, Institute of Chemical Technology, Prague, Technická 5, 166 28 Prague 6, Czech Republic
E-mail: lukas.werner@vscht.cz

The iminosaccharide D-fagomine 1 and some of its derivatives occur naturally, e.g. it has been found\(^1\) that the leaves and roots of the legume Xanthocersis zambesiaca contain not only fagomine 1 and its stereoisomers 3-epi-fagomine and 3,4-di-epi-fagomine but also glucosides of fagomine, i.e. 3-O-(β-D-glucopyranosyl)-fagomine, together with small amounts of 4-O-(β-D-glucopyranosyl)-fagomine. In order to gain more information on the role of glucosylated fagomine in Nature, we set out to synthesize some of their C-glycosides. In these compounds the glycosidic bond is replaced by the C-C bond, which mimics the glycosidic bond well but cannot undergo enzymatic hydrolysis and thus cannot liberate the iminosugar under in vivo conditions. Here we report stereoselective synthesis of 3-α-C-glucosides of D- and L-fagomine 2 and 3 from the corresponding C-disaccharides\(^2\). Ethyl glycoside of perbenzylated C-disaccharide 4 was converted via the corresponding thioglycoside 5 and reducing C-disaccharide 6, into substituted alditol 7 which, upon oxidation and double reductive amination, stereoselectively afforded pure perbenzylated D-fagomine C-glucoside 8. In the same manner, another diastereoisomer of starting C-disaccharide was stereoselectively converted into perbenzylated L-fagomine C-glucoside 9.

References:

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Organizing Committee

Jitka Moravcová
Department of Chemistry of Natural Compounds
Institute of Chemical Technology, Prague
Technická 5, 166 28 Prague, Czech Republic
e-mail: Jitka.Moravcova@vscht.cz

Vladimír Křen
Laboratory of Biotransformations
Institute of Microbiology
Vídeňská 1083, Prague, Czech Republic
e-mail: kren@biomed.cas.cz

Tomáš Trnka
Department of Organic Chemistry
Charles University
Hlavova 8, 128 40 Prague, Czech Republic
e-mail: trnka@natur.cuni.cz

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