Krátke ústne oznámenia

SMALL CHANGES IN AGLYCON STRUCTURE MAY INFLUENCE THE OUTCOME OF GLYCOSYLATION

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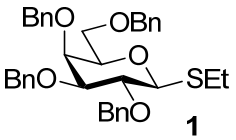
Can small changes in aglycon structure of a glycosyl acceptor cause significant changes in outcome of glycosylation? To answer this question we glycosylated lactose derived diols **2** and **3** with thioglycoside **1** under identical conditions. Oligosaccharide fraction was isolated by gel chromatography on Bio-Beads Sx3 column in toluene and analyzed by HPLC and 2D NMR spectroscopy.

We found that *four* oligosaccharides were present in oligosaccharide fraction in the case of glycosyl acceptor **2** with benzyl aglycon, the two major components being 1-3'-linked trisaccharide and 1-3',1-4'-linked-tetrasaccharide. To our surprise only *two* compounds were found in oligosaccharide fraction when seemingly similar lactoside **3** with only one extra methylene group in aglycon was used as the glycosyl acceptor.

We also prepared a series of monohydroxy glycosyl acceptors **4** with various aglycons and glycosylated them under identical conditions. The results obtained will be presented.



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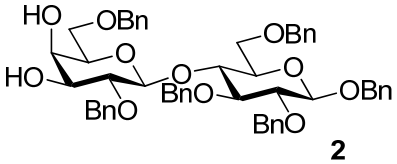

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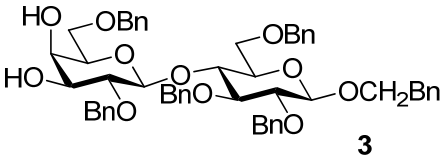

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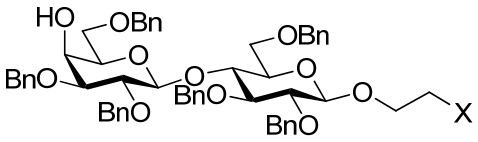

4 X = N_3; NHCOR

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STRUCTURAL DIFFERENCES IN THE ACTIVE SITES OF GOLGI ALPHA MANNOSIDASE II AND ALPHA LYSOSOMAL MANNOSIDASE: INSIGHTS FROM HOMOLOGY MODELING, DOCKING AND PKA CALCULATIONS

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Several methods of computational chemistry were used to understand differences in an enzymatic selectivity of Golgi alpha-mannosidase II (GM) and lysosomal alpha-mannosidase (LM). The 3D structures of human GM as well as human and drosophila LM were built using sequence alignment and homology modeling. Then, terminal fragments of natural substrates were docked into the actives sites of the enzymes using the Glide program of the Schrödinger suite. The obtained docked poses of substrates were analyze to reveal factors governing the substrate specificities of GM versus LM. To shed more light on enzymatic specificity of mannosidases, the pK_a values of ionizing groups of amino acid residues of the enzymes were also calculated at the empirical level using the PropKa program. The main interest was focused on residues in the active sites, especially on pKa values of aspartic acid residues that directly interact with substrates, natural, and synthetic reversible inhibitors.

The calculations predict almost identical 3D geometry of the active sites in a vicinity of the zinc ion cofactor, but different ionizing states of the aspartic acid residues at pH optima of both enzyme types, namely 7 in GM versus 5 in LM. Active-site residues responsible for the selectivity of GM as well as a strategy for the structure-based design of selective inhibitors of human GM were proposed.

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PREDICTION OF CHIROPTICAL PROPERTIES OF CARBOHYDRATES

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Prediction of various structural, physical, chemical and spectral properties using computational chemistry methods is a very useful tool in organic synthesis and conformational analysis and is becoming more or less standard at present time. However, determination of an absolute configuration and prediction of chiroptical properties[1] of chiral molecules is still a challenging task. As a majority of naturally occurring compounds are chiral, possible applications are very vast and a lot of attention is given to the development of reliable methods for the prediction of chiroptical properties.
Optical rotation and vibrational circular dichroism (VCD) were chosen as examples of chiroptical properties and experimental measurements and *ab intio* and DFT calculations were performed for a series of small, conformationally rigid carbohydrates, namely bicyclic derivatives of methyl tetrofuranosides.
It is shown that optical rotation depends, besides other, on the conformation of a molecule and hence it is a sensitive tool for the conformational analysis, provided a suitable method, basis set and, of course, a solvent representation is used for the predictions. As the resulting optical rotation is very sensitive to the orientation of exocyclic groups, a particular attention is paid to geometry optimizations and energy calculations of the conformers involved.

This work was supported by the Ministry of Education of the Czech Republic, project MSM6046137305, and Grant Agency of the Academy of Sciences, project IAA400550702.

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FREE ENERGY MODELLING OF CARBOHYDRATE CONFORMATIONS USING METADYNAMICS

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Conformational changes in carbohydrate molecules, such as ring puckering or oligosaccharide bending, are often associated with relatively high free energy barriers. Therefore modelling of these processes can be computationally demanding. Metadynamics is a recently introduced molecular modelling technique, which provides much more efficient exploration of the conformational space compared to usual molecular dynamics simulation. Moreover, it quantitatively evaluates the free energy surface of the studied system. We present the results of application of this method in modelling of monosaccharide ring puckering and in rotation of Ramachandran dihedrals of disaccharides. The influence of water environment is illustrated by the comparison of free energy surfaces of the studied molecules in vacuum and in explicitly modelled water environment.

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POLYSACCHARIDE MICROARRAYS FOR TRANSGLYCOSYLASE SCREENING

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Transglycosylases, in general, are enzymes that disproportionate glycan molecules by cleaving their main chains and ligating the fragments containing newly formed reducing ends to free non-reducing ends of other poly- or oligosaccharide molecules, usually of the same type [1]. In the in vitro transglycosylase assays, radioactively or fluorescently labelled oligosaccharides are used as glycosyl acceptors.
So far, the best studied transglycosylase is plant xyloglucan endotransglycosylase (XET, EC 2.4.1.207) catalyzing both xyloglucan endotransglucosylation (XET-activity) and to a smaller extent also its hydrolysis (XEH-activity). Another transglycosylase that has been partially characterized to date is plant mannan endotransglycosylase/hydrolase (MTH) having the specificity for β-mannans [2]. Transglycosylases for other matrix polysaccharides presumably exist but have not been discovered yet [3].
The objective of our work was to devise a method for simultaneous detection or screening of multiple transglycosylase activities in plant extracts. For this purpose, we prepared a glycochip carrying an array of different polysaccharide substrates attached covalently ad/or non-covalently to a solid support. The glycochip is incubated in reaction mixtures containing diverse fluorescently labelled oligosaccharides acting as acceptor substrates, crude plant extract and succinate buffer at pH 5.5. The reaction is stopped by washing off the reaction mixture with polar solvent mixture and the fluorescence is scanned/quantified using Typhoon Phosphoimager. Transglycosylation is revealed on glycochip as bright spots indicating incorporation of the labelled oligosaccharide into the polysaccharide fixed on the solid support. Control incubations were done with boiled plant extracts.
Using the described glycochip with crude extracts from germinating nasturtium (*Tropaeolum majus*) seeds and *Arabidopis* seedlings, we detected some new hetero-transglycosylations, e. g. the incorporation of sulforhodamine-labelled xyloglucan oligosaccharides (XGOS-SR) into galactomannan, 4-O-methyl glucuronoxylan and/or arabinoxylan.

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TRANSGLYCOSYLATION – A UNIVERSAL MECHANSM IN THE FORMATION OF PLANT AND FUNGAL CELL WALLS

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Plant and fungal cell walls (CW) are composite structures consisting of insoluble polysaccharide skeleton glued together with an amorphous polysaccharide- and protein-polysaccharide matrix. Their formation is a complex and well organized process. Individual components are synthesized by the so-called Leloir pathway from glycosyl donors NDP-sugars by enzymes (glycosyltransferases) localized either in the plasma membrane (e. g. cellulose synthase in plants and β-1,3-glucan and chitin synthases in fungi) or within cisternae of the endoplasmic reticulum and Golgi apparatus and transported by exocytosis into the cell exterior. In the final stage of cell wall formation, the individual wall components become covalently cross-linked *in situ* by enzymes called transglycosylases. Transglycosylases cleave in an endo- fashion polysaccharide chains and transfer their fragments by newly created reducing ends to other polysaccharide molecules of the same (homotransglycosylases) or different type (heterotransglycosylases). The transglycosylases are involved in restructuring of the cell walls during morphogenesis, incorporation of new material into the wall structure during growth and cell wall loosening.
Until recently, the only proven plant transglycosylase was xyloglucan endotransglycosylase (XET). The enzyme cuts and rejoins molecules of xyloglucan in the plant primary cell walls. Studies on substrate specificity have shown that some isoenzymes of XET are non-specific and catalyze the so called hetero-transfer between molecules of soluble cellulose derivatives and xyloglucan or β-1,3-glucan. Other transglycosylases recently discovered in plants are mannan transglycosylase/hydrolase, MTH (1) and mixed-linkage β-1,3/β-1,4-glucan:xyloglucan transglycosylase, MXE (2). High-throughput screening using a polysaccharide glycochip introduced recently in our laboratory has revealed existence of a broad spectrum of plant endo-transglycosylases (3).
The cell wall of the yeast *Saccharomyces cerevisiae* contains covalent complexes consisting of β-1,3-glucan, β-1,6-glucan and chitin. Two β-glucanases with transglycosylation activities have been described in yeast so far. Gas1 is an endo-β-1,3-glucanase capable of cleaving large laminarioligosaccharides and transfer their fragments to other laminarioligosaccharide molecules under formation of β-1,3-bond (4). The second enzyme is Bgl2 which *in vitro* can cleave β-1,3-glucan chains and attach the fragments to a nonreducing end through a β-1,6-linkage (5). Both enzymes in order to catalyze the glycosyl transfer require relatively high (mM) concentrations of oligosaccharide donors and acceptors. Recently, we have discovered that proteins Crh1 and Crh2 are in fact transglycosylases transferring portions of nascent chitin to β-1,3-linked side chains of β-1,6-glucan in the yeast cell walls (6).

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MOLECULAR REGULATION OF PROTEIN SIALYLATION IS CHANGED IN THE NEOPLASTIC TRANSFORMED THYROID GLAND TISSUE

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Introduction: Thyroid gland tumors belong to the frequent endocrine diseases. The changes of the glycosylation of cellular structures are an important factor, often found during the neoplastic transformation. Sialic acid, usually located in the terminal position of the oligosaccharide chains, can be used as a marker of this process. The present work evaluated the association between the neoplastic process and the molecular changes involved in sialylation process in the cells of thyroid gland.

Methods: The cases of malignant papillary and follicular carcinomas and benign follicular adenomas were analyzed. The total RNA was isolated from archival formalin-fixed in paraffin embedded material and analyzed by real-time RT-PCR using the specific primers for sialyltransferase 1, 7 (responsible for alpha-2,6 linkage of sialic acid in terminal position), sialyltransferase 4A, 4B, 4C and 9 (responsible for alpha-2,3 linkage of sialic acid in terminal position). The results were correlated with the quantitative evaluation of protein sialylation by lectin histochemistry.

Results: When compared to the parenchymatous goiter, a significant increase of m-RNA for sialyltransferase 4C and sialyltransferase 4B was detected in the well differentiated follicular respectively papillary carcinoma of the thyroid gland. The sialyltransferase 1, 7 and 9 did not show any significant changes in the expression. These results correlated with the finding of strong luminal sialic acid positivity detected in well differentiated carcinomas especially in the alpha-2,3 linkage. Follicular cells in normal thyroid gland, adenomas and benign goiter showed weak or no expression of sialic acid.

Conclusion: The data showed only weak membrane-bound sialic acid positivity in benign adenomas and goiters in comparison with malignant papillary and follicular carcinomas, with alpha 2,3-linked sialic acid predomination. Increased membrane sialic acid expression may be the result of increased sialyltransferase 4C or 4B transcription, respectively in the transformed tissue. This fact may be an important diagnostic aid, especially in respect to aspiration cytology evaluation and the differential diagnostics of thyroid gland neoplastic lesions.

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TWO GLUCURONOYL ESTERASES OF *PHANEROCHAETE CHRYSOSPORIUM*

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Glucuronoyl esterase, a recently discovered carbohydrate esterase, hydrolyses alkyl and arylalkyl esters of D-glucuronic and 4-*O*-methyl-D-glucuronic acid. The filamentous basidiomycete *Phanerochaete chrysosporium* contains two genes, *ge1* and *ge2*, that encode glucuronoyl esterase. We heterologously expressed both genes in *Schizophyllum commune*, *Pycnoporus cinnabarinus* and *Aspergillus vadensis* and demonstrated that the proteins encoded by these genes exhibited glucuronoyl esterase activity. Comparison of GE1 and GE2 amino acid sequences showed that they are 52% identical. They differ mainly in that GE1 contains a carbohydrate-binding domain and a proline/serine rich linker, and GE2 does not. The putative catalytic domains of GE1 and GE2 are even more identical (65%). Sequence analysis demonstrated differences in promoter region of *ge1* and *ge2* which suggest that their expression could be subject to different type of regulation. We also showed that not all fungal genomes contain glucuronoyl esterase homologues and that their presence is not assigned to a specific fungal clade. Comparison of identified amino acid sequences of putative glucuronoyl esterases with sequences of acetylxylan esterases, feruloyl esterases and pectin methyl esterases confirmed that glucuronoyl esterases form a separate family of carbohydrate esterases (CE15) postulated to be involved in the cleavage of the ester linkages between hemicellulose and lignin. These enzymes are interesting candidates for biotechnological applications.

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TRANSFORMATION OF *THERMOBIFIDA FUSCA* ß-D-MANNOSIDASE TO MANNOSYNTHASE BY SITE-DIRECTED MUTAGENESIS

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Oligosaccharides comprising β-mannopyranoside bond have a potential as prebiotics and anti-adhesives (1) and it can be found in the core trisaccharide of N-linked glycoproteins. The β-mannopyranoside linkage is undoubtedly the most difficult glycosidic bond to create by conventional chemical synthesis.

The ß-D-mannosidase, isolated from *T. fusca* (2), hydrolyses ß-1,4-mannosyl linkage with a retaining mechanism.

The enzyme belongs to glycosyl hydrolase 2 family with a putative E530 and E443 nucleophile and acid/base, respectively. By site-directed mutagenesis, we have replaced E530 with serine residue creating E530S ß-D-mannosidase mutant. This mutant showed no hydrolytic activity on *para*-nitrophenyl-ß-D-mannopyranoside (*p*NP-Man), providing an evidence of E530 to be a catalytic nucleophile. The E530S mutant was capable of transferring α-D-mannosyl fluoride as donor to acceptors with different pyranose rings creating novel ß-mannosyl links. Structural determination of the mannosythase reactions products were carried out by 1D and 2D ^{1}H, $^{1}H/^{13}C$ NMR and ESI/MS spectroscopy. The E530S mannosidase mutant behaves as ß-D-mannosynthase producing di-, tri- and tetrasaccharides with different interglycosidic bonds and it exhibits significant affinity towards beta-glycoside acceptors (Table 1). Surprisingly, the E530S mutant showed hydrolitic activity on *p*NP-β-D-glycuronides.

Acceptor	**Transferase activity/Oligosaccharide Yield** Peak area % (A_{300})				**Conversion %**	**Hydrolase activity** Peak area % (A_{400})
	di-	**di-**	**tri-**	**tri-**		
*p*NP-β-D-glucopyranoside	21,8	35,5	0,6	1,8	**59,7**	0
*p*NP-β-D-xylopyranoside	21,3	6,5	0,4	11,2	**39,4**	0
*p*NP-β-D-mannopyranoside	25	-	3,2	0,2	**28,4**	0
*p*NP-α-D-mannopyranoside	7,5	-	-	-	**7,5**	0
*p*NP-N-acetyl-β-D-glucosaminide	1,5	4,5	0,2	-	**6,2**	0
*p*NP-β-D-glucuronide	-	-	-	-	-	+

Table 1. *T. fusca* E530S catalysed transmannosylations using α-ManF as donor

The engineered mannosynthase is a promising canditate in regio- and stereospecific oligosaccharide synthesis.

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ELECTROSPRAY ION TRAP FRAGMENTATION OF SYNTHETIC ANALOGS OF THE TETRASACCHARIDE SIDE CHAIN OF THE *BACILLUS ANTHRACIS* EXOSPORIUM MAJOR GLYCOPROTEIN.

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Bacillus anthracis is the causative agent of anthrax, a fatal desease in humans and other mammals, caused by gram-positive rod-shaped aerobic soil bacterium. Because of possible use of some form of the bacterium as a biological weapon, there is a wordwide effort to develop a powerful vaccine for the desease. *B. Anthracis* is an endospores-forming pathogen; when vegetative cells are deprived of their essential nutrients. Therefore, a strategy toward the vaccine for antrax could be based on targeting spores with neutralizing antibodies that are specific for a component on their surface. Such is, for example, the tetrasaccharide β-Ant-(1->3)-α-L-Rhap-(1->3)-α-L-Rhap-(1->2)-L-Rhap (Ant stands for 4,6-dideoxy-2-O-methyl-4-(3-hydroxy-3-methylbutamido)-D-glucose, antrose) which was discovered to be the oligosaccharide side chain of a collagen-like region of the major glycoprotein of the *B. Anthracis* exosporium. With the aim to prepare synthetic tetrasaccharide models with aglycon allowing conjugation to suitable carriers, the methyl 6-hydroxyhexanoyl glycosides of saccharides related to tha said tetrasaccharide were prepared. To find useful methodology for structure elucidation of compounds of this class, we studied mass spectral fragmentation of synthetic model compounds, and here we describe their positive and negative mode electrospray multistage ion trap mass spectrometry (ESI MS^n).

The ESI MS^n spectra of α- and β-rhamnosides I and II exhibit qualitatively the same fragmentation pathways. Main pathway in ESI MS/MS fragmentation of rhamnosyl dimers III and IV is shortening of the chain. The next compounds V and VI are α and β anomers of trimers of the same type. As expected, the ESI MS/MS spectra of both anomers are qualitatively identical. The main pathway of the fragmentation of $[M+Na]^+$ adducts at *m/z* 607 is shortening of the oligomeric chain, giving rise to dimeric and monomeric sodium adducts. 4,6-Dideoxy-2-O-methyl-4-(3-hydroxy-3-methylbutamido)-D-glucose (anthrose) containing terasaccharides differ from D-rhamnooligosaccharides derivatives by the presence of 4-(3-hydroxy-3-methylbutamido)- substituent. Since anthrose is the end end unit of the the oligosaccharide side chain of a collagen-like region of the major glycoprotein of the *B. Anthracis* exosporium this sugar serve as a marker in the identification of the bacteria. The primary phenomenon involved in the ESI method is the attack of $[M+Na]^+$ adducts by the sodium cations. The of sodium cations to the molecule of the methyl 6-hydroxyhexanoyl glycoside of antrose has been studied by quantum chemical calculations.

Characteristic of the ESI MS fragmentation in negative mode is production of deprotonated molecular ions and fragments. The $[M-H]^-$ ions are formed after expulsion of a proton from molecules. Compared to ESI/IT MS/MS positive spectra, the spectra obtained by negative mode are simpler and easier to interpret.

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