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Methylation – an uncommon modification of glycans*

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Abstract

A methyl group on a sugar residue is a rarely reported event. Until now this kind of modification has been found in the kingdom of animals only in worms and molluscs, whereas it is more frequently present in some species of bacteria, fungi, algae and plants, but not in mammals. The monosaccharides involved as well as the positions of the methyl groups on the sugar vary with the species. Methylation seems to play a role in some recognition events but details are still unknown. This review summarises the current knowledge on methylation of sugars in all kinds of organism.

Keywords

glycan; methylation; sugar

Introduction

Methylation is a modification which has been often found on proteins (linked to amino-, carboxyl- or sulphydryl-groups), DNA and RNA. It is highly important for modulation and regulation of processes, developmental changes, cell signalling and aging. Changes in the methylation pattern (hypermethylation as well as undermethylation) are related to several diseases (Schulz 1998).

On proteins the methylation is known as a regulator in various cellular processes influencing protein-protein interactions, stability, localisation and enzyme activity. Much is known about methylamines, mainly methylated arginine or lysine, especially of histones but also of some other proteins (Peters and Schübeler, 2005). Also carboxy groups of amino acids may form methyl esters. Those methylations play significant roles in cell recognition processes. On structural proteins methyl thioesters with unknown function have been identified (Branscombe Miranda et al., 2004; Zhang et al., 2012). Epigenetic mechanisms are regulated by DNA methylation causing a closed chromatin state with repressed, inactive genes. This modification plays an important role in imprinting, X-inactivation, oncogenesis, inflammatory and immunological processes (Salozhin et al., 2005; Barnes, 2011; Garaud et al., 2011; Poetsch et al., 2011).

Methylation of the “third estate” of building macromolecules, the carbohydrates, occurs in some bacteria, fungi, plants, worms and molluscs. It has not been found in mammals. It is an additional option to modulate the structure of glycan molecules, however, a clear determination of the function is still missing. As summarised in this review, the study of methylation of sugars is primarily restricted to structural phenomenology.

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Bacteria

In bacteria methylated structures have been identified for the first time. Up to now various sugars containing differently linked, sometimes multiple, methyl groups have been identified which are important constituents of bacterial glycans, mainly lipopolysaccharides. In the early studies 2-*O*-Me-L-Rha has been found as constituent of scopamycin A, a metabolite of a strain of *Streptomyces aureofaciens* (McAlpine et al., 1971) and as part of the antibiotic aranciamycin (Keller-Schierlein et al.,1970). Surprisingly also its mirror image, 2-*O*-Me-DRha, is synthesized by *Mycobacterium tuberculosis* (Demareau-Ginsburg and Lederer, 1963). Due to the medical relevance of this organism, the methylation abilities of mycobacteria have been intensively studied. They display frequently 3-*O*-Me-Rha, but also Rha with di- or trimethylation or methylated talose (reviewed by Schorey and Sweet, 2008). Besides 3-*O*-Me-Rha, 3,4-di-*O*-Me-Rha, 3-*O*-Me- and 2,3-di-*O*-deoxy talose furthermore 4-*O*-Me-GlcA, 2,4-di or 3,4-di-Me-GlcA are present in *Mycobacterium habana* strain TMC5135 (Khoo et al., 1996). Recently also 4-*O*-Me-Rha has been found in serotype 13 glycopeptidolipid from *Mycobacterium intracellulare* (Naka et al., 2011). The biological significance of these methylated sugars in *Mycobacteria* is still not clear. There are some hints pointing to a role in the regulation of the fatty acid metabolism but final proof is still missing (Jackson and Brennan, 2009).

The second group of bacteria which is well investigated for their methylation potential is Rhizobia. They are gram negative symbiotic soil bacteria which are able to fix nitrogen in interaction with their plant host. *Rhizobium etli* CE3, a symbiont of legumes, displays 2-*O*-Me-Fuc, 3-*O*-Me-6-deoxy-L-talose and polymethylated fucose (2,3,4-tri-*O*-Me-L-Fuc) on the lipopolysaccharide which may facilitate symbiotic interactions in terms of nodules development and nitrogenase activity (Forsberg et al., 2000; Noel et al., 2004). Also in *Bradyrhizobium* Sp. strain ORS285 2-*O*-Me-Fuc may play a role in the molecular dialogue between the photosynthetic bacterial symbiont and its tropical aquatic legume host (*Aeschynomene spp.*) (Renier et al., 2011). In *Bradyrhizobium japonicum* strain USDA110 2-*O*-Me-Fuc has been found in the lipooligosaccharide nodulation signal (Sanjuan et al., 1992). In another strain, JS314, a disaccharide containing 4-*O*-Me-Man has been identified (Carlson and Krishnaiah, 1992). Mesorhizobia, often symbionts of wild herbs and tree legumes, display 3-*O*-Me-Rha and the very rare 4-*O*-Me-GlcNAc residue (Fig. 1 A) (Zdorovenko et al., 2009). Recently 3-*O*-Me-Fuc has been found in *Mesorhizobium huakuii* strain S-52 capping as a terminal sugar an O-chain (Turska-Szewczuk and Russa, 2011). As part of the capsular polysaccharide from *Sinorhizobium fredii* HWG35 a dimethylated Gal residue (2,4-di-*O*-Me-Gal) has been identified (Rodriguez-Carvajal et al., 2005). While the sugar composition of the capsular polysaccharide (repeating units of one neutral and one acidic hexose) seems to influence the ability of this microorganism to form nitrogen-fixing nodules with Asiatic and/or American soybean cultivars, the methylation in particular does not seem to be of functional relevance for this process.

Another soil inhabitant, the recently newly described gram positive actinomycete *Cryptosporangium mongoliense*, shows also traces of 3-*O*-Me-Rha, but no other methylated sugars (Ara et al., 2011).

The plant pathogen *Pseudomonas syringae* pv. phaseolicola, a phytopathogen, contains 3-*O*Me-Rha within the *O*-polysaccharide of the lipopolysaccharide (Zdorovenko et al., 2001), whereas *Erwinia carotovora* ssp. *carotovora* GSPB 436 , another phytopathogenic bacterium, terminates the polymer main chain of the O-polysaccharide with 4-*O*-Me-Man (Senchenkova et al., 2003).

The presence of a methyl phosphate group at O-3 of α-mannopyranose, serves also as the signal for termination of the chain elongation in *Klebsiella pneumoniae* O3, *Hafnia alvei* PCM 1223 and *Escherichia coli* O9/O9a LPS (Kubler-Kielb et al., 2011).

The human pathogen *Bacillus anthracis* and related organisms are not easy to detect. Their methylated sugars (3-*O*-Me Rha, 2-*O*-Me-Rha, 2-*O*-Me-Fuc) on the surface of the spores help as an identification signal, which distinguishes them clearly from mammalian sugars. Here the methylated sugars are important targets for biodetection technology and enable a rapid detection (Fox et al., 2003).

Novel glycolipids were found as an old heritage, presumable from cyantobacteria, in the sediments of an Antarctic lake (Ace Lake). Docosanyl 3-*O*-Me-Rha and 3-*O*-Me-Xyl were identified as the methylated sugars (Sinninghe Damsté et al., 2001).

Fungi

The first description of methylation in fungi was in the early eighties, when the N-glycosylation pathway of the yeast- and mycelial-form cells of the dimorphic fungus *Mucor rouxii* was investigated. It could be shown that the high mannosidic structures contained 3-*O*-Me-Man residues, especially in the mycelia cells (Lederkremer and Parodi,1984). Those structures are resistant to α-mannosidase digest, but do not interfere in the cleavage by endoglycosidase H.

In extracellular polysaccharides from mould species (Mucor, Rhizopus, Rhizomucor, Absidia, Syncephalastrum, Thamnidium) 2-*O*-Me-Man residues were found to be involved in forming an immunodominant carbohydrate (De Ruiter et al., 1994).

A water-soluble extracellular polysaccharide from the edible basidomycete fungus *Pleurotus ostreatoroseus* Sing, which is known in the Orient for its medicinal properties, was identified to be a partially 3-O-methylated 1,4-linked α-D-galactopyranan (Rosado et al., 2002). While the fruiting bodies of *Hericium erinaceus,* a traditional Chinese medicinal fungus, contain a fucogalactan with 3-*O*-Me-Rha residues terminating the polymer main chain (Zhang et al., 2006), the fruiting bodies of *Phellinus igniarius*, another fungus used in Chinese medicine, contain a neutral polysaccharide with 3-*O*-Me-Gal as a constituent (Yang et al., 2007). So far, no hints are given on the medical properties or the immunological behaviour of the methylated glycans of these fungi.

Amoebae

Lysosomal enzymes isolated from the slime mould *Dictyostelium discoideum* display one or two methyl phosphate groups on high mannosidic N-glycans which contain 6 or 7 mannose residues (Gabel et al., 1984). It is speculated that the corresponding pathway for the biosynthesis of phosphorylated high mannosidic N-glycans must therefore be different to the one in higher organisms.

Algae and Plants

Methylation of sugars in plants is a rather frequent modification. Several sugars have been found to be involved, with Gal in algae and GalA in higher plants as the most common ones. Also the linkage of the methyl group is variable. Due to the availability of reasonable amounts of material, most of the studies could be performed using NMR-techniques.

Extracellular proteoglycan produced by *Rhodella grisea* (unicellular red alga) contains a high amount of 3-*O*-Me-Xyl (26 %) and traces of 4-*O*-Me-Xyl and 2,3-di-*O*-Me-Rha or Fuc

(Capek et al., 2008). Recently, 6-O-methylation on non-terminal Man residues was determined on N-glycans of the red microalga *Porphyridium sp.* (Levy-Ontman et al., 2011). Especially red seaweed is quite well investigated. Galactans from *Cryptonemia* species contain 2-*O*-, 4-*O*- and 6-*O*-Me-Gal residues in their galactans besides other unusual forms of Gal (3,6-anhydro-D- and L-Gal and 3,6-anhydro-2-*O*-Me-L-Gal as a minor constituent). Furthermore the galactans are to some extend sulfated (Zibetti et al., 2009). Sulfated polysaccharides containing similar methylated Gal residues (2-*O*-Me-L-Gal and 6-*O*-Me-DGal) were also isolated from *Georgiella confluens* and *Bostrychia montagnei* (Kolender and Matulewicz, 2002; Duarte et al., 2002). The main acidic polysaccharides from *Jania rubens* carry besides the rare occurring sulfated or methoxylated 3,6-anhydro-L-Gal, also some minor fractions with 3-*O*-Me-D-Gal, 3-*O*-Me-L-Gal and 2,3-di-*O*-Me-D-Gal (Navarro and Stortz, 2008). The neutral galacto-glucurono-xylo-glyccan of *Apophloea lyallii* is built up by repeating units of [-α-D-2-*O*-Me-Gal-β1,4-D-GlcA-α1,3-D-Xyl-1,4-] (Fig. 1 B). In a minor fraction 3-*O*-Me-Gal was found (Watt et al., 2002).

Regarding mosses and ferns there is not much information. Just *Selaginella apoda* L., a lycophyte known as meadow spike moss, has been found to contain in 3-*O*-Me-Gal in its primary cell walls (Popper et al., 2001).

Although occurring in algae quite frequently, methylated Gal is a rare compound in higher plants. It has been found just as 3-*O*-Me-Gal in polysaccharides of *Acanthus ebracteatus*, a mangrove, and *Salvia officinalis L.*, an herb. Both are used as medical plants in traditional medicine (Capek and Hříbalová, 2004; Hokputsa et al., 2004; Capek 2008).

Most other studies revealed methylated uronic acids in the polysaccharides of higher plants. 4-*O*-Me-GlcA is a constituent of xylans which have been found in the aqueous suspension of sugar beet pulp (*Beta vulgaris*), alkaline extracts of the pericarp of pricky pear seeds of *Opuntia ficus-indica* and *Tamarix austromongolica*, a fast growing tree used for prevention of wind erosion and control of desertification (Dinand and Vignon, 2001; Habibi et al., 2002; Sun et al., 2011).

In most of the investigated higher plants 6-*O*-Me-GlcA is the only or at least main methylated sugar present. It has been detected in the hot aqueous extract of the dietary fiber of green winter melon *Benincasa hispida* (Das et al., 2009a); the immunoenhancing heteropolysaccharide isolated from unripe green fruits of the aubergine (egg plant) *Solenum melongena* (Ojha et al., 2009); the polysaccharide of unripe green tomato (*Lycopersicon esculentum*) (Chandra et al., 2009); pectic polysaccharide from pods of green bean (*Phaseolus vulgaris* L.) (Patra et al., 2012); the corm of *Amorphophallus campanulatus*, where the GalA is in addition acylated on C4 (Das et al., 2009b) and the polysaccharide from the fruits of *Lagenaria siceraria* (Fig. 1 C), the calabash, a melon used for food but also as a container for liquids in earlier times. It is also known for its healthy effects, where the GalA is in addition O-acetylated on C3 (Ghosh et al., 2009). 6-*O*-Me-GalA was found together with 2-*O*-Me-Xyl in a xylan isolated from the stem of the same plant (Ghosh et al., 2008) and in a hot water extract of stems of *Amaranthus gangeticus* L., which is used mainly as an ornamental and vegetable plant but also in medical treatment (Sarkar et al., 2009). In a heteropolysaccharide extracted from the leaves of *Catharanthus rosea*, another plant with medical potential, originated from Madagaskar, 6-*O*-Me-GalA was found together with 6-*O*Me-Glc (Patra et al., 2010).

Another kind of sugar composition was determined for the pectic polysaccharide rhamnogalacturonan II from red wine. It is a nonasaccharide containing a backbone from made from Rha, Ara, Gal and apiose with branching of Ara, Rha and 2-*O*-Me-Fuc which is

O-acetylated in some cases. A similar structure, a heptasaccharide also with 2-*O*-Me-Fuc was presented for *Arabidopsis thaliana* (Glushka et al., 2003).

Worms

Caenorhabditis elegans, the model worm, a non-parasitic soil nematode, is well investigated for its glycosylation pattern and the related modifications. It displays a rather complex glycan pattern including modifications with phosphorylcholine and a small amount of terminal linked 2-*O*-Me-Fuc in N- as well as in O-glycans (Guérardel et al., 2001; Haslam et al., 2002; Paschinger et al., 2008). To date, other sugars have not been found to be methylated in this species.

Another nematode, *Toxocara canis*, a parasite of dogs and other canid animals all over the world, contains two O-linked trisaccharide structures on the excretory-secretory antigens composed of terminal 2-*O*-Me-Fuc, a Gal residue, which is 4-O-methylated in some cases and a GalNAc linked to the protein. In *Toxocara cati*, a close relative of *T. canis*, the same trisaccharide is present in a dimethylated version (Khoo et al., 1991). Both structures are highly antigenic.

The annelid *Alvinella pompejana*, found strictly around deep-sea hydrothermal vents, lives in directly secreted tubes build from organo-mineral material with a carbohydrate part containing 2-*O*-Me-L-Fuc, 3-*O*-Me-L-Fuc and 2,4-di-*O*-Me-L-Fuc (Talmont and Fournet, 1991). No information is given on the position of these sugars within the glycan chain.

The platyhelminth *Dugesia japonica* displays 3-*O*-Me-Man in terminal position on N-glycan cores or terminating $Man_5GlcNAc_2$-structures. A fucose residue, extended by a Gal residue and in some cases with a further methyl hexose, linked to the innermost GlcNAc is present in some of the glycans and seems not to influence the methylation status (Fig. 1 D) (Paschinger et al., 2011; Natsuka et al., 2011).

More studies are necessary on nematodes and other worms to make a statement whether different kinds of methylation patterns (methylated Fuc and/or methylated hexoses) are characteristic features for different phyla of worms.

Molluscs

Methylation in snails is a frequent modification in N- as well as in O-glycans in most of the investigated species. So far the methyl groups have been found mainly linked to hexoses: Man and Gal, but also reports on methylated GlcNAc and GlcA are available. For the first time methylation in gastropods (3-*O*-Me-Man, 3-*O*-Me-Gal) was reported in 1977 in the hemocyanin, the oxygen-carrier of arthoropods and molluscs, of *Helix pomatia* (Hall et al., 1977). Later analysis of the hemocyanin of *Lymnea stagnalis* revealed as the lowest molecular mass N-glycan a core made from two GlcNAc and three Man residues containing one Xyl linked β1,2 to the β-Man and 3-O-bound methyl groups linked to the two terminal Man residues (van Kuik et al., 1986). Terminal 3-*O*-Me-Gal residues on larger structures were confirmed for *Lymnea stagnalis* and found on a diphosphonopentaosylceramide of the sea hare *Aplysia kurodai* (van Kuik et al., 1987, Araki et al., 1986). The hemocyanins of *Rapana thomasiana* and of the functional unit RvH1 of *Rapana venosa* also were found to contain 3-*O*-Me-Gal, the latter together with some terminal 3-*O*-Me-GlcNAc residues (Stoeva et al., 1995; Dolashka-Angelova et al., 2003).

Analysis of other molluscs revealed more variations: Tridacnin, a lectin isolated from the marine clam *Hippopus hippopus*, contains terminal 6-*O*-Me-Man and terminal dimethylated Man residues (Puanglarp et al., 1995). A novel glycosphingolipid from spermatozoa of the

fresh water bivalve *Hyriopsis schlegelii* was identified carrying 4-*O*-Me-GlcA on its glycan (Hori et al., 1983).

At that time all methylated sugars described in molluscs were determined in terminal position of the glycans. In the mid-nineties, due to the increasing technical opportunities, it became possible to identify also minor compounds of the glycan spectrum which are present in very low amounts. Again, the hemocyanin of *Helix pomatia* was under investigation using 500/600-MHz ^{1}H-NMR spectroscopy. With the advanced technology 21 more N-glycan structures could be determined in detail. Those showed a high degree of methylated Gal residues, mainly 3-*O*-Gal, and also methylation of Gal on the C4 was shown. In addition for the first time in molluscs, some of the methylated Gal residues were found not to be in terminal position of the glycan, but were elongated by methylated Gal residues (Lommerse et al.,1997).

Further studies on gastropods revealed mostly similar patterns. The hemocyanin of *Haliotis tuberculata* was found to contain 3-*O*-Me-Man and 3-*O*-Me-Gal and the major *Biomphalaria glabrata* shell matrix protein was shown to display a core structure terminated by two 3-*O*-Me-Man residues (Marxen et al., 2003; Idakieva et al., 2004). In a later study the N-glycan spectrum of hemolymph glycoproteins of the latter organism was analysed showing a broad pattern of different structures containing 3-*O*-Me-Man and 3-*O*-Me-Gal as methylated constituents. While 3-*O*-Me-Man was determined as terminal sugar of some antennae a methylated hexose elongated by an amino sugar and one hexose is not further determined (Lehr et al., 2007). In agreement with the data of Lommerse et al., 1997 it can be speculated that the 3-*O*-Me-Man is a terminating signal for further elongation whereas the 3-*O*-Me-Gal may be further elongated. An elongation of methylated sugars seems to be a very rare occasion in gastropods, as these two reports are so far the only published data. However, we also had some hints on internal methylated sugars in large glycans of *Arion lusitanicus* but the amounts were too low to determine the structures in detail.

In the course of our own studies we analysed the N-glycosylation patterns of whole tissue extracts derived from Arion lusitanicus, Limax maximus, Cepaea hortensis, Planorbarius corneus, Arianta arbustorum and *Achatina fulica* and found very frequently terminal located 3-*O*-Me-Man residues on high mannosidic and paucimannosidic structures and to a lesser extend methylated Gal on larger glycans (Fig. 1 E and F) (Gutternigg et al., 2004; Gutternigg et al., 2007). The O-glycosylation patterns of the same species and additionally of *Biomphalaria glabrata*, *Clea helena* and *Helix pomatia* revealed a high amount of methylated Gal and some methylated Man (Stepan et al., unpublished results). The methylated Man was identified as exclusively 3-O-methylated whereas the galactose occurs 3-O- as well as 4-O-methylated (Stepan et al., 2010). The distribution of the two types of methylated Gal residues varies within the different species. While 3-*O*-Me-Man occurs in every investigated snail. 3-*O*-Me-Gal is absent in *Achatina fulica* and *Arion lusitanicus*; 4-*O*-Me-Gal is missing in *Arianta arbustorum* and *Biomphalaria glabrata*. It is not possible to correlate the kind and amount of methylation with the occurrence of the snail (water or land living species) or with appearance (shell carrying or slug).

The N-glycan structure of the egg extracellular coat of the mollusc bivalve *Unio elongatulus* was determined as $Glc_1Man_9GlcNAc_2$, containing no methyl group (Di Patrizi et al., 2001) and the major egg glycolipoproteins from the perivitellin fluid of the apple snail *Pomacea canaliculata* also do not display methyl groups (Dreon et al., 2004). This correlates with our own observations where we did not find any methylation in eggs of *Arion lusitanicus* (Gutternigg et al., 2004). This may be due to different expression levels of methyltransferase(s) in different developmental stages. More studies are needed to confirm this theory.

Methyltransferases

Knowing so many glycan structures modified by methyl groups it is rather surprising that most of the corresponding methyltransferases are still unknown. There are only few data on methyltransferases acting on sugar residues. All these data have been generated from bacterial sources, mainly mycobacterial strains. For *Mycobacterium smegmatis* a methyltransferase (*mtf1*) was identified which methylates Rha at the C3 position. This methylation is a requirement for any further methylation of the glycopeptidolipid (Weisman and Ballou,1984; Patterson et al., 2000). A second putative gene (*mtf2*) located in the same gene cluster was identified to methylate the fatty acid of the glycopeptidolipid rather than the sugars (Jeevarajah et al., 2002). Furthermore also the two genes responsible for the methylation of Rha in C4 and C2 position, *rmt4* and *rmt2* respectively, have been identified and their sequentially action – first 4-O-methylation, then 2-O-methylation, both after the initial 3-O-methylation – was shown (Jeevarajah et al., 2004). One more bacterial methyltransferase transferring a methyl group to the C2 position of a galacturonosyl residue of a teichuronic type polyssaccharide was identified in *Rhizobium meliloti* (Ruiz and Ugalde,1998).

It is not completely clear if the methylation is always performed as the final modification step after the glycan has been biosynthesized (directly from S-adenosylmethionine as donor), or if there is also the option that a glycosyltransferase (in the case of *mycobacteria* the rhanmosyltransferase) is able to transfer the already methylated sugar residue (from a nucleotide methyl sugar) onto the growing oligosaccharide chain (Miyamoto et al., 2006; Schorey and Sweet, 2008).

Function

Structural analysis of glycans and their modifications is carried out by a broad range of analytical methods, including gas chromatography with different derivatisation protocols, liquid-chromatography with or without prior labelling often followed by mass spectrometry and several NMR techniques. (For more information on methodical details see Geyer and Geyer 1994; Gerwig and Vliegenthart 2000; Mechref et al. 2009, North et al. 2009; Stepan et al. 2011). These methods are often challenging when the material is limited and the glycans are only semi-purified, but the analysis of the function of a small modification of the glycan is even more difficult.

In some of the above mentioned studies of bacteria, the glycosylation abilities are correlated with cell signaling, recognition or adhesion function. The mycobacterial glycopeptidolipids are involved in pathogenicity, the immunological response to infection and colony morphology. The immune response of a host via Toll-like receptor 2 is influenced by the glycopeptidolipid profile of *Mycobacterium intracellulare* and *M. avium*, for sure by a modification by acetylation, but there are also some hints that methylation on Rha may play a role for the effect (Sweet at al., 2008; Naka et al., 2011).

It is obvious that in plants Gal (for algae) and GalA (for higher plants) are the primary sugars decorated with methyl groups. Even when many of the investigated plants are known for some medical effect no correlation with this modification of the glycan has been documented so far. Perhaps methylation may occur also in many other plants but they are not investigated in detail as they do not show medical relevance.

In fungi (diverse mould species) the 2-*O*-Me-Man residues decorated glycan reacts with rabbit IgG antibodies. After removal of the terminal 2-*O*-Me-Man residues by an exo-α-D-mannosidase prepared from *Trichoderma harzianum*, the antigenicity is abolished (De Ruiter

et al., 1994). Other investigated fungi are known for their medical usability, but no correlation of this attribute to methylation of glycan chains is given.

Glycosylation plays an important role in the communication between helminth parasites such as *Schistosomes* and their snail host. For example imitating the immunogenic surface coat of the parasite by synthetic sugar-albumin conjugates a down-regulation of extracellular-signal regulated kinase activities, protein kinase C activities and phagocytosis can be detected (Plows et al., 2005). Methylated sugars have not been used for such studies so far. Comparison of the N-glycosylation patterns of glycoproteins derived from the hemolymph of *Biomphalaria glabrata* strains which are different susceptible to *Schistosoma mansoni* infection, showed that Xyl and terminal Fuc residues are responsible for the cross reactivity with schistosomal glycconjugates. The methyl hexoses, present on snail glycans, seem not to be important for the antigenicity of the structures (Lehr et al., 2010).

Glycan chains of the functional unit RtH2-e from *Rapana thomasiana* hemocyanin, some of them contain terminal 3-*O*-Me-Gal, are involved in the antigenicity of this protein (Siddiqui et al., 2007). These experiments were carried out with a mixture of glycans of *Rapana*, so the influence of methylation cannot be determined. But immunological cross reaction between α-macroglobulin and hemocyanin, both occurring in the hemolymph of *Helix pomatia*, seems to be related to the carbohydrate content of these two glycoproteins, especially to Xyl and 3-*O*Me-Gal (Siddiqui et al., 2009). This is the only demonstration that a methylated structure is involved in an antigenic recognition event.

Conclusion

Methylation of sugar residues is a rare modification. As far as we know, mammals do not carry this modification on their glycans, but many other organisms display methylated structures which are heterogenous in terms of involved sugars as well as in terms of the linkage of the methyl group. Methylation on hexose residues is rather frequent but the decorated sugar may also be a Fuc, Rha, Xyl, amino sugar or uronic acid.

The type of methylation pattern correlates to some extend with the species. For typical examples see Fig. 1. Whereas in bacteria and plants the methylated sugars occur often within the oligosaccharide chain, in all other organisms the methylated sugars are mainly in terminal position. Especially 3-*O*-Me-Man is terminally located and seems to be a stop signal for a further elongation of the glycan chain. This can be seen in plants as well as in gastropods. Some methylated monosaccharides are very frequent in some species, others are rare in all phyla. In molluscs, Man and Gal are the typically methylated residues. In plants Gal, together with uronic acids (GalA and GlcA), are very frequent; however also Xyl and Fuc are present. Bacteria show the whole spectrum of methylated sugar residues, even when methylated hexoses (Man, Gal and Glc) are only minor compounds (Tab.1). For all other organisms the data are too limited to make a statement on frequency.

No methylation has been found so far in arthropods. However, it has to be kept in mind that only a few species are well investigated: those insects which are used as expression systems, mainly lepidoptera, some hymenoptera species (wasp, bee, hornet) which are of medical relevance due to their venom and *Drosophila melanogaster*, the model insect. All other insects and other arthropods still wait for the elucidation of their biochemical and molecular biological properties. Let us see which surprises are hidden there!

Also the determination and characterisation of methyltransferases as well as detailed investigation of the function of the methylation on sugars is still an open field for further investigation. Even when the current data are rather limited and do not allow valid predictions, a function of such a modification, which alters the chemical behaviour of a

sugar rendering it more hydrophobic, is very likely. The frequently occurring “methyl sugar”, fucose (a deoxyhexose) is indeed an important structural feature in many recognition events.

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Abbreviations

Ara	arabinose
Fuc	fucose
Gal	galactose
GalA	galacturonic acid
Glc	glucose
GlcA	glucuroonic acid
GlcNAc	N-acetylglucosamine
Hex	hexose
Man	mannose
Me	methyl
Rha	rhamnose
Tal	talose
Xyl	xylose

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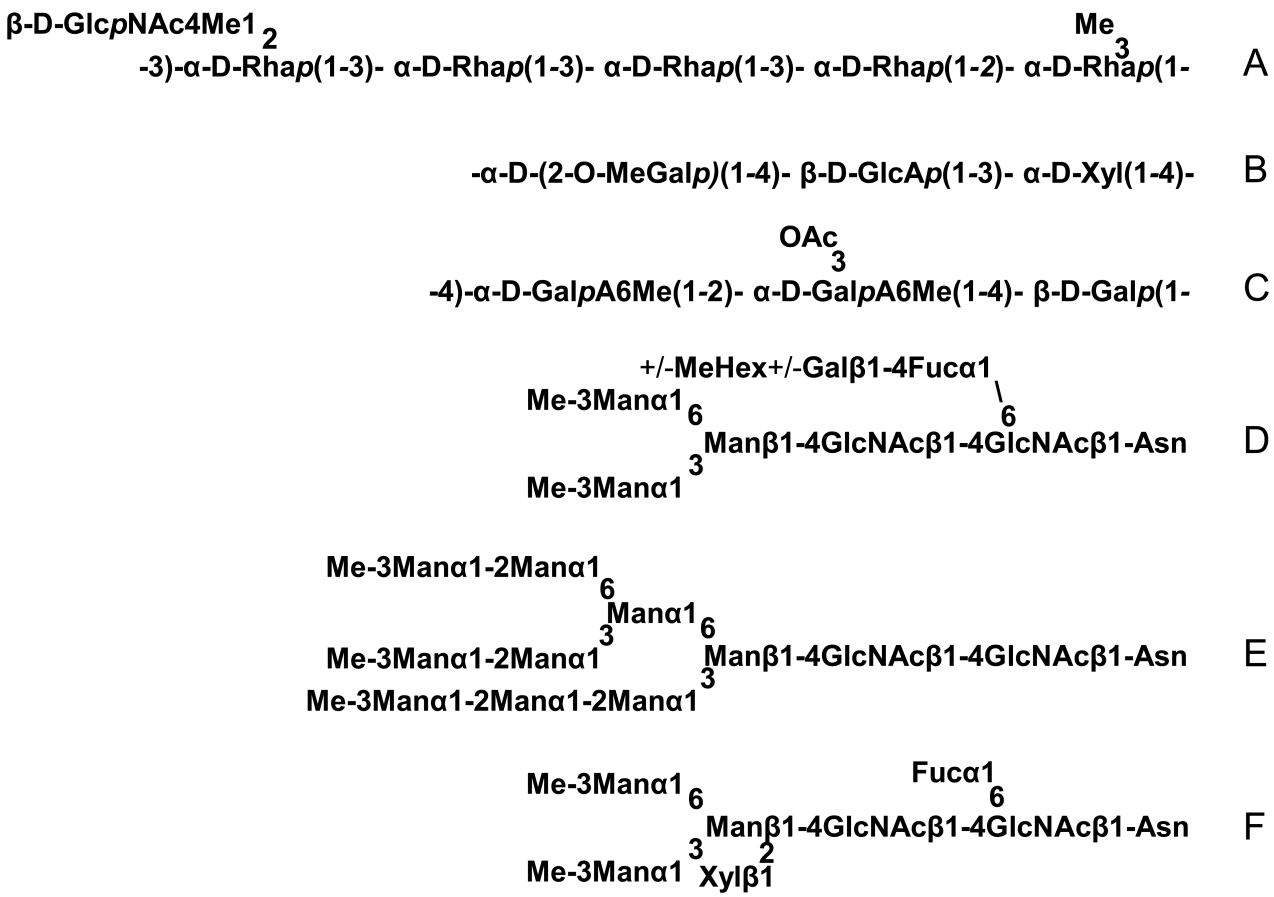

Fig 1. Typical methylated glycan structure.

(A) O-polysaccharide repeating unit of *Mesorhizobium loti* (Zdorovenko et al., 2009); (B) Polysaccharide repeating unit from the red seaweed *Apophloea lyallii* (Watt et al., 2002); (C) Polysaccharide repeating unit isolated from fruiting bodies of *Lagernaria siceraria* (Ghosh et al., 2009); (D) N-glycan from *Dugesia japonica* (Paschinger et al., 2011); (E) and (F) N-glycans from *Arion lusitanicus* (Gutternigg et al., 2004).

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Table 1

Methylated sugars analysed in detail so far

Sugar	Linkage	Bacteria	Algae and Plant	Other organism
Mannose	2-*O*-Me			Diverse mould species: De Ruiter et al., 1994;
	3-*O*-Me			*Mucor rouxii*: Lederkremer and Parodi, 1984; Platyhelminth: Paschinger et al., 2011; Natsuka et al., 2011; Gastropod: Hall et al., 1977; v. Kuik et al., 1986; Marxen et al., 2003; Gutternigg et al., 2004; Idakieva et al., 2004; Gutternigg et al., 2007; Lehr et al., 2007; Stepan et al., 2010;
	4-*O*-Me	*Bradyrhizobium japonicum*: Carlson and Krishnaiah, 1992; *Erwinia carotovora:* Senchenkova et al., 2003;		
	6-*O*-Me		Red microalga: Levy-Ontman et al., 2011;	Mollusc: Puanglarp et al., 1995;
	Di/poly-methylated			Mollusc: Puanglarp et al., 1995;
Galactose	2-*O*-Me		Seaweed: Duarte et al., 2002; Kolender and Matulewicz, 2002; Watt et al., 2002; Zibetti et al., 2009;	
	3-*O*-Me		Seaweed: Watt et al., 2002; Navarro and Stortz, 2008; Plant (lycophyte): Popper et al., 2001; Higher plants: Hokputsa et al., 2004; Capek and Hříbalová, 2004; Capek, 2008;	Fungi: Rosado et al., 2002: Yang et al., 2007; Gastropod: Hall et al., 1977; Stoeva et al., 1984; Araki et al., 1986; v. Kuik et al., 1987; Lommerse et al., 1997; Dolashka-Angelova et al., 2003; Idakieva et al., 2004; Gutternigg et al., 2007; Stepan et al., 2010;
	4-*O*-Me		Seaweed: Zibetti et al., 2009;	Nematode: Khoo et al., 1991; Gastropod: Lommerse et al., 1997; Stepan et al., 2010;
	6-*O*-Me		Seaweed: Duarte et al., 2002; Kolender and Matulewicz, 2002; Zibetti et al., 2009;	
	Di/poly-methylated	*Sinorhizobium fredii:* Rodriguez-Carvajal et al., 2005;	Seaweed: Navarro and Stortz, 2008;	
Glucose	6-*O*-Me		Plant: Patra et al., 2010;	
Xylose	2-*O*-Me		Plant: Ghosh et al., 2008;	
	3-*O*-Me	Sediments (cyanobacteria?): Sinninghe Damsté et al., 2001;	Red algae: Capak et al., 2008;	
	4-*O*-Me		Red algae: Capak et al., 2008;	
Fucose	2-*O*-Me	*Rhizobium etli:* Forsberg et al. 2000; Noel et al., 2004; Bradyrhizobia: Sanjuan et al., 1992; Renier et al., 2011;	Plants: Glushka et al., 2003;	Annelid: Talmont and Fournet,1991; Nematode: Khoo et al 1991; Guéradel et al 2001; Haslam et al. 2002; Paschinger et al. 2008;

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Sugar	Linkage	Bacteria	Algae and Plant	Other organism
		Bacillus anthracis and related organisms: Fox et al., 2003;		
	3-*O*-Me	*Mesorhizobium huakuii*: Turska-Szweczuk and Russa, 2011;		Annelid: Talmont and Fournet, 1991;
	Di/poly-methyated	*Rhizobium etl:* Forsberg et al., 2000;	Red algae: Capak et al., 2008;	Annelid: Talmont and Fournet, 1991;
Rhamnose	2-*O*-Me	*Bacillus anthracis* and related organisms: Fox et al., 2003; *Streptomyces:* Keller-Schierlein et al., 1970; McAlpine et al., 1971; *Mycobacterium tuberculosis:* Demareau-Ginsburg and Lederer, 1963;		
	3-*O*-Me	*Bacillus anthracis* and related organisms: Fox et al., 2003; *Mycobacteria:* Khoo et al., 1996; Schorey and Sweet, 2008; *Mesorhizobium:* Zdorovenko et al., 2009; *Pseudomonas:* Zdorovenko et al., 2001; *Cryptosporangium mongoliense:* Ara et al., 2011; Sediments (cyanobacteria?): Sinninghe Damsté et al., 2001;		Fungi *(Hericium erinaceus):* Zhang et al., 2006;
	4-*O*-Me	*Mycobacterium intracellulare:* Naka et al., 2011;		
	Di/poly-methylated	*Mycobacteria:* Khoo et al., 1996;	Red algae: Capak et al., 2008;	
GlcNAc	3-*O*-Me			Gastropod: Dolashka-Angelova et al., 2003;
	4-*O*-Me	Mesorhizobia: Zdorovenko et al., 2009;		
Glucuronic acid	4-*O*-Me	*Mycobacterium habana:* Khoo et al., 1996;	Plant: Dinand and Vignon, 2001; Habibi et al., 2002; Sun et al., 2011;	Mollusc: Hori et al., 1983;
	Di/Poly-methylated	*Mycobacterium habana:* Khoo et al., 1996;		
Galacturonic acid	6-*O*-Me		Plant: Sarkar et al., 2011; Patra et al., 2010; Patra et al., 2012; Ojha et al., 2009; Ghosh et al., 2008; Ghosh et al., 2009; Das et al., 2009a; Das et al., 2009b; Chandra et al., 2009 Patra et al., 2010;	
(deoxy) Talose	3-*O*-Me	*Mycobacterium:* Khoo et al., 1996; Schorey and Sweet, 2008; *Rhizobium etli:* Forsberg et al., 2000;		