COMMENTARY

Intestinal candyfloss

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In this issue of the *Biochemical Journal*, Robbe et al. have employed a state-of-the-art MS technique to re-examine the sweet and sticky sugar covering of human intestinal mucosa. The availability of highly sensitive MS methods has been instrumental in determining the complex structures of mucin oligosaccharides, and in demonstrating that there are variations in structure along the sections of the gut. In contrast to previous studies, these results described by Robbe et al. show some correlation with the activities of enzymes synthesizing mucin oligosaccharides in the human colon. Many questions still remain to be answered: for example,

regarding the regulation of the relative amounts of sugar chains in individual mucins, and their roles in the homeostasis of the intestinal mucosa. Structural studies are a solid basis for understanding the functions of sugar chains, and the mechanisms and significance of changes during the development of intestinal disease.

Key words: colon, glycoprotein biosynthesis, intestinal mucosa, mucin, oligosaccharide structure.

COLONIC MUCIN STRUCTURES – TRUE OR FALSE?

By using MS to analyse the structure of human intestinal mucin carbohydrate chains (O-glycans), in this issue of the *Biochemical Journal* Robbe et al. [1] have shown that even minor structures can be determined out of a mixture of complex oligosaccharides. This very difficult structural work made use of state-of-the-art nano-ESI-Q-TOF MS/MS (nano-electrospray ionization quadrupole time-of-flight tandem MS) technology.

Previous structural studies were carried out by staining methods, or after tedious fractionation of mucin oligosaccharides, by MS, gas chromatography, NMR and chemical or enzymic methods to determine the monosaccharide composition, antigenicity, size and acidity, as well as the linkages and anomeric configurations in the sugar sequences. This represented lengthy and tedious work, and required relatively large amounts of start-up material, but some of the minor structures may have escaped detection, and it would be an enormous task to repeat these procedures for various regions in the intestinal tract. The novel nanospray MS/MS technology is of very high sensitivity and, because of the analyses of secondary ions, allows the determination of individual structures in a mixture of mucin oligosaccharides. Thus the results of Robbe et al. [1] may more accurately reflect the true population of structures in the original mucins from various regions of the intestines. By choosing nanospray MS/MS as the only analytical method, there are still some inherent risks, since it is difficult to determine the branching patterns and attachment points between sugars of isomeric O-glycans, and the precise identification of anomeric linkages between sugars (α or β) requires additional NMR, immunochemical or enzymic methods.

The intestinal mucosa is covered by a protective layer of sweet fuzzy proteins (mucins) resembling candyfloss. Since the report by Podolsky [2], it had been thought that these sugar chains were structurally based only on one core structure (core 3). However, this is in contrast to the presence of the enzymes

synthesizing four major core structures (1 to 4) in colonic mucosa [3]. Thus the finding by Robbe et al. [1] of core 1 to 4 structures in colonic mucins shows more correlation with the enzymic studies than previous investigations. Small amounts of O-glycans with core 5 have also been found in the adult mucins, as well in adenocarcinoma and in meconium; thus core 5 may be primarily an oncofetal structure. O-Glycans with core 6 have previously been described in human fetal gastrointestinal mucin and other mucins, but were not detected by Robbe et al. [1]. It is thus possible that core 6 arose from degradation of core 2.

Interestingly, sialic acid was found only α 2-3-linked to Gal (galactose). These structural findings suggest that mucin-producing cells do not express the enzyme that synthesizes sialyl(α 2-6)-Gal linkages. Sialic acids in the colon are rich in O-acetyl groups which prevent recognition by antibodies and bacterial neuraminidases, and thus have a protective role. Since O-glycans were removed by β -elimination in the study by Robbe et al. [1], these O-acetyl groups were not detected, but have previously been demonstrated by a different method. There appears to be little preference of terminal structures for a specific core structure. The possible exception is the Sd^a antigen, which has only been found on core 3, but perhaps because this is the most abundant core in colonic mucins.

PROPOSED FUNCTIONS OF THE COMPLEX RANGE OF COLONIC MUCIN OLIGOSACCHARIDES

Secreted mucins found in the intestinal mucus gel protect the epithelium via hundreds of different O-linked oligosaccharides. At least ten different mucin peptides are expressed in the gastrointestinal tract in a tissue-specific fashion along sections of the intestines and along colonic crypts, which implies functional differences between mucins. Their gene expression is altered in inflammatory bowel disease and in gastrointestinal cancers, and may reflect regional lymph node metastases. Alterations of the

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peptide backbone are accompanied by qualitative and quantitative structural changes of the O-glycan chains [3–9].

The tissue- and disease-specific variations of O-glycans may be due to the different expression of glycosyltransferases, but may also arise from the influence of different peptide backbones on the assembly of O-glycans [4]. Although this remains to be proven, we expect that different mucin peptides have characteristic numbers of and attachment sites for O-glycans, and that the O-glycan structures and their functions at these various sites may differ.

A number of immune functions have been attributed to O-glycans and mucins. The main function of intestinal mucins in the mucus gel is thought to be lubrication and protection of the underlying epithelium. However, lubrication could probably be achieved with a much smaller range and with simpler O-glycan structures than those of colonic mucins. Both rat and human colonic mucins have mainly core-3-based O-glycans, but it is not yet clear if these glycans have a functional advantage. Conformational studies suggest that the overall presentation of terminal structures may depend on the core structures of O-glycans; thus core-3-based determinants may present themselves in a functionally important way.

Intestinal candyfloss represents a niche for microbes. For example, bacteria are known to adhere to mucins, and specifically to partial oligosaccharide sequences found in mucins. Gut bacteria also have sulphatases and glycosidases that cleave terminal sugars of mucins, thus promoting an erosion of the mucus gel and contributing to structural heterogeneity. The terminal residues of O-glycans can function in the prevention of such degradation processes. The biological significance of the great diversity in mucin O-glycan structures may be to provide optimal protection against a variety of noxious agents and microbes in the intestines. Multiple attachment sites for commensal bacteria colonizing the gut may prevent selection for a few pathogenic ones. Thus, in the deleterious environment of the colon, the variety of complex structures appears to be crucial for homeostasis of the gut mucosa.

DOES MUCIN BIOSYNTHESIS IN THE COLONIC MUCOSA CORRESPOND TO MUCIN STRUCTURES?

The enzymes synthesizing O-glycan core structures 1 and 2 occur in many human cells; however, the remaining core structures of colonic mucins (3, 4 and 5) appear to be typical for mucins synthesized in goblet cells and other mucin-secreting cell types. The enzymes synthesizing core structures 1 to 5, and those extending and terminating O-glycan chains have been demonstrated in human colonic mucosa [3,10,11]. The control of O-glycan biosynthesis involves many complex factors and is still not well understood. The extensive heterogeneity of O-glycan structures is thought to arise from the competition of a number of different enzymes for the same glycoprotein substrate structure. Changes in the relative amounts of oligosaccharides in disease thus arise from alterations in the relative activities of these competing enzymes.

The localization of biosynthetic enzymes within the assembly line in the Golgi, as well as the membrane environment, are crucial factors for the control of glycoprotein biosynthesis. Because sugars are added individually, new substrates are continuously created and have to be co-localized with the respective enzymes. If the normal assembly line of enzymes and their substrates is disturbed, premature synthesis of terminal structures and truncation of chains may occur. The great structural heterogeneity of colonic mucins could thus also be explained by a broad distribution of early-acting enzymes within the Golgi which partly

prevents intermediate-acting enzymes from encountering their substrates.

Although most colonic mucin O-glycans are based on the core 3 structure, *in vitro* assays of biosynthetic enzymes in colonic mucosa showed that the activity synthesizing core 3, core 3 β 3-GlcNAc (*N*-acetyl-D-glucosamine) transferase, is relatively low. One would therefore expect to have mainly core 1 and core 2 structures instead of core 3 structures. It is possible that as yet unknown factors activate core 3 β 3-GlcNAc transferase *in vivo*, and that these factors are no longer present in the *in vitro* assays. The enzyme may exist in a protein–membrane complex *in vivo* that results in much higher activity. Thus the intracellular organization of the biosynthetic assembly line *in vivo* can be highly efficient, but appears to be disrupted upon homogenization and solubilization of cells *in vitro*. It is possible that core 3 β 3-GlcNAc transferase is localized to early Golgi compartments, or is closely associated with the first enzyme in the pathway, polypeptide GalNAc (*N*-acetylgalactosamine) transferase; this would prevent other core structures from being formed, resulting in mainly core 3 structures.

On the basis of *in vitro* assays we expect that core 3 structures are quantitatively converted to core 4. How can we explain the low amount of core 4 in colonic mucins? The enzyme synthesizing core 4 (from core 3) may be placed in compartments where it cannot utilize unmodified core 3 as a substrate, and encounters elongated core 3 structures which can no longer be converted to core 4. Perhaps the sialyltransferase that adds sialic acid in α 2-6 linkage to the GalNAc residue of core 3 is highly active early in the pathway, thus preventing the action of core 4 β 6-GlcNAc transferase. The lack of correlation between the relative amounts of O-glycans in colonic mucins and the enzyme activities synthesizing these structures in colonic mucosa requires more answers, mainly regarding the control of biosynthetic pathways.

Future investigations should aim at elucidating the intracellular organization of the glycosyltransferase assembly line that regulates highly efficient mucin glycosylation. Furthermore, the basis for the regio-specific and disease-related differences in O-glycan structures, and the functional implications of these differences, need to be clarified. What are the attachment sites in the mucin backbone for the individual chains on the mucin peptide? Are the different mucin peptides indeed differently glycosylated? Does this lead to different bacterial colonization? How does the sweet and sticky candyfloss support the absorptive functions of the intestines? Reliable and sensitive structural analysis of glycans is a solid foundation to lead to answers to these questions.

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