Complex carbohydrates - Structure and function with respect to the glycoconjugate composition of the cupula of the semicircular canals.

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The semicircular canal cupula has been described as a gelatinous partition of the ampulla which is displaced by the endolymph during physiological rotation. According to morphological studies the ampulla appears fixed around its perimeter to the ampullary walls and is assumed to behave as a kind of diaphragm undergoing maximal displacement just above the crista, and lesser displacement in regions closer to the apex [McLaren et al., 1979]. In most species the cupula is thought to form an ovoid structure stretched between the sensory epithelium and the walls and the roof of the membranous ampulla. In that it forms a complete partition of the ampulla lumen the cupula transforms shifts of the canal fluid to deflections of the sensory hair cells. The cupula itself is separated from the crista by the subcupular space which is bridged by the hair bundles of the sensory cells and by fine filaments. By the diaphragm-like displacement of the cupula shear forces are transmitted to the tips of the kinocilia [McLaren et al., 1979; Landolt et al., 1975; Rüscher & Thurm, 1989; Nagel et al., 1991].

To be able to fulfill these functions the cupula has to meet several biophysical requirements: (i) adhesiveness to the ampullary walls, (ii) cohesiveness and stability against shear forces generated by the movements of the endolymph, (iii) elasticity when acting as a diaphragm in the lumen of the ampulla, and (iv) it is at least very likely, that the cupula must have a distinct osmotic resistance avoiding swelling and shrinking at conditions of osmotic changes.

So far the cupula has been morphologically been described to consist of a densely fibrous zone in the upper part, and a subcupular space containing minute fibrillar elements anchoring the main portion of the cupula to the sensory epithelium and the interstitial cells [Vilstrup, 1950]. According to present information it is assumed that the fibrous network consists of different glycosylated proteins and that the meshwork is composed of mucopolysaccharides, glycoproteins and proteoglycans. Only few constituents have so far been structurally

identified including the glycoprotein otogelin [Cohen-Salmon et al., 1997].

According to these pieces of information, it has to be assumed that different types of complex carbohydrates are present in the cupula, although little is known about their exact molecular composition, in particular about the types of structure and the biological roles of complex carbohydrates of the cupula.

It is the aim of this review to briefly describe the structure and function of complex carbohydrates of glycoproteins and proteoglycans both in general and with particular respect to the potential roles sugar chains may play in the cupula, i.e. the molecular organization of these constituents, their biophysical properties, and their biological functions. A better knowledge of the complex carbohydrates of the cupula should contribute to the understanding of the molecular basis of the physiological function of the cupula and of pathological alterations.

General

While complex carbohydrates covalently linked to proteins and lipids were long considered to be biological compounds that lack any biological specificity serving merely as structural components, it has become evident within the last three decades that complex carbohydrates in particular those covalently linked to proteins and lipids form a highly diverse and complex class of biomolecules that are almost ubiquitous in any living organism and mediate a large number of biological functions ranging from purely structural roles to such complex processes as molecular "on/off" or "tuning" functions or cell-cell communication operative e.g. in the immune system or during embryonic development [selected monographs and reviews, Rademacher et al 1988; Kobata 1992; Varki 1993; Lis and Sharon 1993; Fukuda and Hindsgaul 1994; Montreuil et al 1995]. Moreover, inherited and acquired carbohydrate disorders have been discovered playing key roles in the aetiology and progression of various types of disease [selected references, von Figura and Hasilik 1986; Hakomori 1989; Etzioni et al 1992; Muramatsu 1993; van Dijk et al. 1994].

Complex carbohydrates are important components of cells where they are major constituents of subcellular organelles and of the plasma membrane linking cells to neighboring cells and to the extracellular matrix. A second major group of glycoconjugates are secreted either in soluble form to extracellular fluids and to the plasma, or as

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Table 1 Types of glycoconjugates in eukaryotic cells

Type	Glycans	Distribution
Glycoproteins	<i>N</i> - and <i>O</i> -linked mono-, di- and oligosaccharides	
plasma / serum glycoproteins		plasma / serum, extracellular fluids, effusions
membrane glycoproteins		cell surface membrane and intracellular membranes
matrix glycoproteins		connective tissues, extracellular matrix
glycoprotein hormones		plasma / serum
cytoplasmic / nucleoplasmic glycoproteins		cytoplasm, nucleus
Proteoglycans	glycosaminoglycans	connective tissue, extracellular matrix, cell surface membrane
Glycolipids	mono-, di-, oligosaccharides	
cerebrosides		
sulfatides		cell membranes
gangliosides		

insoluble molecules to the extracellular matrix and to the cellular mucus of epithelia (Tab. 1). This article will focus on complex carbohydrates of glycoproteins and proteoglycans.

Composition and Structure

Complex carbohydrates of glycoproteins and proteoglycans are characterized by a high degree of structural diversity. This characteristic is one of the molecular bases for their biological functions. The first source of structural diversity derives of the chemical nature of the oligosaccharide monomers, the monosaccharides which may be arranged in various compositions and sequences within an oligo- or polysaccharide. Oligo- and polysaccharides were for a long time regarded to be constituted by D-galactose, D-mannose, D-glucose, L-fucose, D-xylose, L-arabinofuranose, the amino sugars N-acetyl-D-glucosamin and N-acetyl-D-galactosamin, and sialic acids. Novel analytical methods have lead to the discovery of additional monosaccharides as components of complex carbohydrates [for review, Vliegthart and Montreuil, 1995]. Second, as monosaccharides usually have three or four free hydroxyl groups, the oligosaccharide monomers may be linked to each other at different positions. In addition to different linkages between sugars arranged in a linear sequence, this rises the possibility of branching. Third, since *O*-glycosidic linkages may be either in α or β position, each disaccharide can exist in two anomeric conformations. The structural diversity is even extended by a significant but limited motional flexibility of oligosaccharide conformation [Homans 1995]. As a result the various monosaccharides in complex carbohydrates at least theoretically may exhibit a much greater structural variability than proteins or nucleic acids.

Hence, similar to these two classes of biomolecules complex carbohydrates may encode biological information.

Although an extremely high number of carbohydrate structures is theoretically conceivable, certain restrictions have been observed which form the basis for several levels of classification. First, in glycoproteins mono-, di-, oligo-, or polysaccharides are covalently bound to the polypeptide by either *N*-linkage or *O*-linkage [Vliegthart and Montreuil 1995]. Proteoglycans are a subclass of glycoproteins distinguished from the other glycoproteins by the nature, quantity, and arrangement of their sugar side chains termed glycosaminoglycans. Glycosaminoglycans are much longer (100-200 monosaccharide residues) than glycans in other glycoproteins, and form long unbranched chains composed of characteristic disaccharide repeats. Glycosaminoglycans contain anionic residues such as sulfate and uronic acid giving them a highly negative overall charge. With respect to the basic disaccharide units four different types of glycosaminoglycans can be distinguished: (1) hyaluronan, (2) chondroitin and dermatan sulfate, (3) heparan sulfate and heparin, and (4) keratan sulfate that except for hyaluronan are all found attached to proteins. In the extracellular matrix of the tectorial membrane keratan sulfate and chondroitin 4- and 6-sulfate proteoglycans have been observed [Munyer & Schulte, 1994] that might also be present in the cupula. Although composed of repetitive motifs, glycosaminoglycans of a given proteoglycan exhibit marked heterogeneity within as well as between the individual oligosaccharide chains caused by sulfate substitution and epimerization [Kjellen and Lindahl 1991].

N-linked oligosaccharides have an N-acetylglucosaminyl-asparagine bond with the N-acetylglucosaminyl residue being the innermost sugar of a common pentasaccharide core unit Man α 1-6(Man α 1-

Fig. 1 Structures of *N*-linked and *O*-linked Oligosaccharides.

3)Man β 1-4GlcNAc β 1-4GlcNAc [Fig.1]. Different mono- and oligosaccharides may be attached to this core forming three different types of *N*-linked glycans [for ref., Kobata 1992; Vliegthart and Montreuil 1995]: Oligomannosyl or high mannose type glycans only having D-mannose residues linked α 1-3, α 1-6 or α 1-2 to the trimannosyl core, complex type glycans have outer antennae composed of N-acetyl-D-glucosamine, D-galactose, L-fucose, and sialic acid, and hybrid type glycans with outer branches of the complex type linked to the Man α 1-3 arm, and D-mannose residues linked to the Man α 1-6 arm of the trimannosyl core. *O*-linked glycans attached to serine or threonine residues can be constituted by a single monosaccharide GalNAc-Ser/Thr, disaccharides such as NeuAc α 2-6GalNAc-Ser/Thr, or larger oligosaccharides containing D-GalNAc, D-Gal, D-GlcNAc, L-fucose and sialic acid. [for review Schachter and Brockhausen 1992]. Apart from these 'classical' structural groups of *N*- and *O*-linked glycans several novel carbohydrate structures have been identified on glycoproteins such as glycosaminoglycans *N*-linked to asparagine on cell surface glycoproteins [Roux

et al. 1988]. In the cupula the presence of *N*-linked oligosaccharides has been demonstrated for the glycoprotein otogelin [Cohen-Salmon et al., 1997]. Very recent studies on the carbohydrate constituents of the cupula from salmon have revealed the presence of components of *N*-linked carbohydrate chains of the complex type including L-fucose, D-mannose, D-galactose and D-glucosamine, of sugars present in *O*-linked oligosaccharides and of xylose, i.e. of the monosaccharide linking glycosaminoglycan chains to the protein backbone of proteoglycans [Tauber et al., unpublished]. Moreover, a roughly quantitative estimation comes to the conclusion that sugars make up approximately one fifth of the molecular constituents of the cupula from salmon.

Biological functions

The biological functions of complex carbohydrates are mainly based on the following biophysical and structural features: *a.* the property to form an extremely high number of different oligomers, each of which having a very complex structure, *b.* the highly hydrophilic nature of the

monosaccharide constituents, and *c.* the three-dimensional size of the oligosaccharide side chains of glycoproteins and in particular of proteoglycans. In many glycoproteins the sugar chains are in total larger than the polypeptide they are linked to. By virtue of their size the sugar chains can form a tightly packed meshwork covering cellular or acellular surfaces or filling the interspace of a fibrillar network.

The biological importance of complex carbohydrates is also indicated by their widespread distribution in the organism and by the prevalence or even exclusive presence of distinct types of complex carbohydrates at certain histological and subcellular sites. Extracellular glycoproteins are present in the different tissues and compartments of the organism, in particular in connective tissues and in the extracellular matrix of parenchymal tissues. *O*-linked sialylated and sulfated oligosaccharides are the major components of mucus glycoproteins on epithelial surfaces [Shimizu & Shaw, 1993; Allan & Garner, 1980]. On the cellular level, glycoproteins and glycolipids are the predominant components of the cell surface constituting the glycocalyx that faces the extracellular microenvironment and is in contact to neighbouring cells, as well as of subcellular organells [Hart *et al.*, 1989].

The sugar chains of glycoproteins and proteoglycans have been shown to be biologically essential for different reasons [Varki, 1993; Jentoft, 1990]. First, by virtue of their structural diversity the glycans of glycoproteins are extremely well-suited to serving as recognition structures that interact specifically with different types of receptors having binding sites for distinct structural oligosaccharide epitopes [Ashwell & Harford, 1982]. Oligosaccharide-receptor interactions have been shown to be pivotal for numerous biological processes including intercellular recognition and adhesion [Bevilacqua & Nelson, 1993]. Sugar-specific receptors termed mammalian lectins have been shown to be exposed on the surface of various cell types. Moreover, carbohydrate binding sites may form distinct functional epitopes of non-receptor proteins such as the proteoglycans aggrecan, versican and neurocan [Drickamer and Taylor, 1993]. Second, the sugar chains of proteins have been shown to be crucially important for the biophysical properties of proteins. During biosynthesis of the proteins in the endoplasmic reticulum, sugars are responsible for the proper folding of the polypeptide chain [Helenius *et al.*, 1997]. In addition, oligosaccharides may stabilize the conformation of proteins even after the proteins have been properly folded and to maintain solubility once glycoproteins have been secreted into the extracellular fluid or matrix. Furthermore, there is evidence, that complex carbohydrates may protect glycoproteins from proteolytic cleavage by either masking proteolytic cleavage sites or by conferring intramolecular stability to proteins.

In a third type of function complex carbohydrates are essential structural components of the extracellular matrix. In mucin-like glycoproteins, and in proteoglycans sugars

such as sialic acid confer a rigid rod like structure upon the glycoprotein monomers. Moreover, they contribute to the binding of water molecules and ions such as calcium. In summary, they are essential for the 3-dimensional structure and for biophysical properties of the extracellular matrix.

Glycoproteins may also have barrier functions separating distinct biological compartments or in distinct glycoproteins may turn on and off or modulate specific functions for example in receptors or binding proteins. In addition sugar chains of mucopolysaccharides and proteoglycans have been shown to serve as specific binding sites for proteins of the extracellular matrix such as laminin or fibronectin, of the cell surface such as CD44 or of growth factors or chemokines. Glycoproteins, proteoglycans and mucopolysaccharides are, therefore, regarded essential for the formation and stability of the extracellular matrix linking the different matrix components together. It is very likely that complex carbohydrates present in the cupula of the semicircular canals mainly serve these latter functions. The identification and structural analysis of the carbohydrate components of the cupula will undoubtedly open new vistas into the molecular basis of cupula function.

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