Carbohydrate chains from human bronchial mucus glycoproteins: a wide spectrum of oligosaccharide structures

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Bronchial mucus is secreted as a gelatinous layer on the surface of the human respiratory tract and is an important component of the mucociliary system which continuously moves inhaled particles towards the upper part of the respiratory tract. It contains highly viscous mucus glycoproteins, or mucins, synthesized by goblet cells of the bronchial epithelium and mucous cells of the submucosal glands (Lamb & Reid, 1968; Spicer *et al.*, 1973). These macromolecules are responsible, to a large extent, for the rheological properties of the respiratory mucus, the efficiency of the mucociliary clearance and the defence of the underlying mucosa.

Hypersecretion of mucins occurs in different chronic bronchial diseases such as chronic bronchitis or cystic fibrosis. The secreted mucins are glycoproteins which may contain several hundreds of carbohydrate chains *O*-glycosidically linked to the peptide backbone. These mucins may have blood-group determinants and may contain acid residues such as *N*-acetylneuraminic acid and sulphate. In fact they correspond to a broad population of molecules from neutral mucins, almost devoid of acidic characters, to mainly sialylated molecules and mainly sulphated mucins.

Average carbohydrate chain length may be estimated from the chemical composition: it is in the range of five to six sugars for the mainly sialylated mucins which may correspond to adult normal mucins (Lafitte *et al.*, 1977), to more than 10 sugars for the mainly sulphated mucins (Lamblin *et al.*, 1979).

In order to determine if structural mucin abnormalities may occur in pathological conditions, such as cystic fibrosis, acidic mucins with an average carbohydrate chain length of 10 sugars have been treated with alkaline borohydride. This treatment produces a mixture of reduced oligosaccharides and glycopeptides, the carbohydrate moieties of which correspond to neutral chains having from one to more than 10 sugars, to sialylated chains which may be short and to sulphated chains which are longer (Roussel *et al.*, 1975).

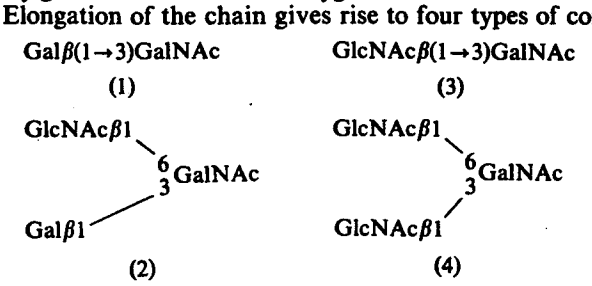
Limited fractions of small reduced oligosaccharides containing either neutral chains with an average length of four sugars or sialylated chains, with the same range of length, have been purified from acidic mucins secreted by patients suffering from chronic bronchitis or from cystic fibrosis (Lamblin *et al.*, 1980; Van Halbeek *et al.*, 1982).

These fractions have been subfractionated using ion-exchange chromatography on DAX4 column, paper chromatography and/or high-performance liquid chromatography (Boersma *et al.*, 1981; Lamblin *et al.*, 1983). The complete structures of 26 oligosaccharides have been determined.

The structures of three short oligosaccharides have been determined by combining periodate oxidation, methylation analysis and glycosidase treatment (Lamblin *et al.*, 1980). The structures of 11 additional neutral oligosaccharides have been determined by combining methylation analysis and 500 MHz ^{1}H-n.m.r. spectroscopy (Van Halbeek *et al.*, 1982). More recently, another seven neutral and five sialylated oligosaccharides have been identified merely by a combination of carbohydrate analysis and 500 MHz ^{1}H-n.m.r. spectroscopy (G. Lamblin *et al.*, 1984*a*,*b*).

The oligosaccharide chains of bronchial mucins are joined to the peptide via an *O*-glycosidic linkage between *N*-acetylgalactosamine and the oxygen of serine or threonine.

Elongation of the chain gives rise to four types of core:



These types of core have been found before in other mucins, type 3 and 4 being the less common (Hounsell & Feizi, 1982).

Fourteen oligosaccharides, five with a type-2 core and the

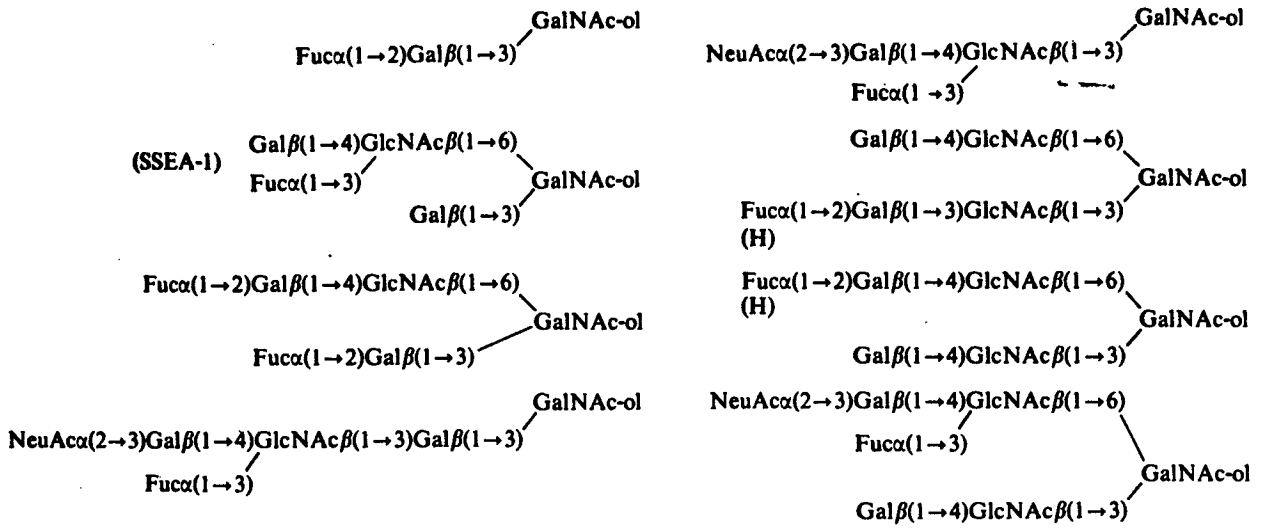


Fig. 1. *The more complete neutral and sialylated bronchial oligosaccharides*

others with a type-4 core, have an upper branch made of *N*-acetyl-lactosamine unit linked β(1→6) to the *N*-acetyl-galactosaminitol residues. Some of them contain this simple sequence which might be responsible for antigen I activity; the upper branch of others may be substituted by fucosyl residues linked α(1→2) to galactose, giving a blood group H determinant or linked α(1→3) to a penultimate *N*-acetyl-glucosamine generating a SSEA-1 determinant (Hounsell *et al.*, 1981) (Fig. 1).

Eight oligosaccharides have a lower branch made of a *N*-acetyl-lactosamine unit (type-2 sequence), a structure made of galactose β(1→3) linked to *N*-acetylglucosamine (type-1 sequence) being present in the lower branch of four oligosaccharides. Fucosyl residues may occur on these lower branches α(1→2) linked to galactose and α(1→3) linked to the penultimate *N*-acetylglucosamine as in the upper branches (Fig. 1).

Sialic acid has been found to be linked α(2→3) to galactose in the five sialylated oligosaccharides isolated as yet (Fig. 1). However, it should be pointed out that they correspond to a minor part of the total sialylated oligosaccharides.

Until now, most of the sialylated oligosaccharides isolated from other mucins had sialic acid linked to the central GalNAc (Carlson, 1968; Slomiany *et al.*, 1980; Van Halbeek *et al.*, 1981; Berger *et al.*, 1982). Such a linkage might also occur in bronchial mucins secreted by goblet cells since these cells are labelled by limulin which is a lectin specific for sialic acid linked to *N*-acetylgalactosamine (Mazzuca *et al.*, 1977).

These preliminary data about the heterogeneity of carbohydrate chains of human bronchial mucins raise several questions.

(1) How wide is this heterogeneity? Twenty-six oligosaccharide structures have already been determined from two fractions of relatively small oligosaccharides. When one realizes that bronchial acid mucins usually have an average carbohydrate chain length of about 10 sugars, it is quite conceivable that several hundreds of different carbohydrate structures might occur in bronchial mucins.

(2) What is the origin of such an oligosaccharide diversity? Does it correspond to incomplete biosynthesis or partial degradation of longer chains? Heterogeneity has been observed in acid mucins from patients with chronic bronchitis or with cystic fibrosis. It is not known whether it is more profound than in normal mucins and whether structural abnormalities occur in certain diseases. Mucin-synthesizing cells might secrete different varieties of molecules. In other animal mucins, a carbohydrate heterogeneity has also been established (Slomiany *et al.*, 1980).

(3) Is there a function for such a diversity? If it occurs normally to a certain extent in individual secretions, it is possible that these carbohydrate structures might represent recognition sites for various inhaled bacteria and trap them allowing their clearance with the mucus escalator.

To answer these questions adequately is a challenge for future research.

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