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Preliminary communication

Separation of a complex mixture of oligosaccharides by HPLC on bonded-primary amine packing using a linear-gradient solvent system

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One of the most convenient methods for separation of neutral monosaccharides or low mol.-wt. oligosaccharides involves high-performance, liquid chromatography (h.p.l.c.) on bonded-primary amine packing[1–4]. Few separation methods have been reported for higher mol.-wt. oligosaccharides[5–8], and they essentially concern D-glucose polymers. Partial separation of a complex mixture of oligosaccharides containing neutral sugars and 2-acetamido-2-deoxyhexoses have been reported[9,10], but these methods, using an isocratic elution, did not separate isomeric oligosaccharides. In the present work, we report a new technique of oligosaccharide separation using a linear-gradient solvent system.

A fraction of oligosaccharides was obtained by alkaline borohydride degradation of human, bronchial-mucus glycoproteins, followed by fractionation by chromatography on Dowex AG 1-X2 ion-exchange resin according to their acidity, and by gel filtration according to their molecular size. The fraction (Ic in ref. 11) contained a mixture of low mol.-wt., neutral, and reduced carbohydrate chains. Its composition was: 10.8% of L-fucose, 25.9% of D-galactose, 20.1% of 2-acetamido-2-deoxy-D-glucose, and 20.5% of 2-acetamido-2-deoxy-D-galactitol, corresponding to a mixture of oligosaccharides having an average carbohydrate-chain length of 4.3 sugars. The mixture was fractionated by h.p.l.c.

The chromatograph consisted of two pumps from Waters Associates Inc. (Milford, MA 01257), Model 6000A, with a solvent programmer from Waters Associates Inc., model 660, coupled to a Uvicord detector, LKB, model S2138. The universal injector was from Waters Associates, model U6K. and the chromatograms (Fig. 1) were recorded with an LKB model 2250 recorder. The chromatograph was equipped with a column (25 × 0.46 cm i.d., E. Merck) of 5 μ Lichrosorb-NH_2. The elution was performed with a linear gradient of 17:3 to 3:2 (v/v) acetonitrile–water, for 90 min at room temperature at a flow rate of 1 mL/min. Water was first de-ionized and then treated with the Milli-q system (Millipore Corp., Bedford, MA 01730). All solvents were degassed by sonication. Although nonspecific for carbohydrate, the wavelength of detection was 206 nm. The sample of oligosaccharides (100 μg in 10 μL of water) was in-

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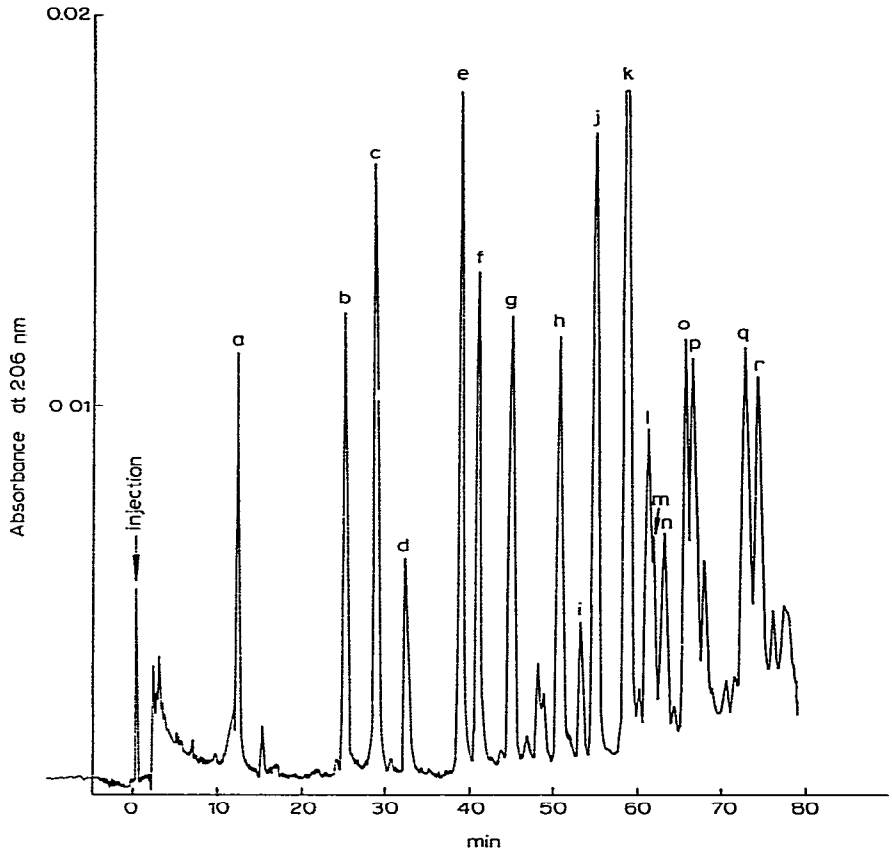

Fig. 1. Elution pattern of Fraction Ic (100 μg) on 5 μ Lichrosorb-NH_2.

jected through the loop injector and separated into 19 fractions.

The carbohydrate composition of the major and well-separated fractions, relative to the content of 2-acetamido-2-deoxy-D-galactitol taken as 1, is reported in Table I and indicates oligosaccharides having from two to five sugars. The chemical composition of minor or badly-separated fractions (Fractions i, l, m, p, q, and r) is not reported. This method allowed the separation of trisaccharide isomers (e, f, and g). Resolution and retention times of the various oligosaccharides depend on the concentration of water in the mobile phase and on the number of sugar residues of each oligosaccharide. Until now, most of the oligosaccharide separations were performed with isocratic elution and did not allow the separation of isomeric oligosaccharides. Moreover, the present conditions of gradient elution may be modified to optimize the separation according to the number of sugar residues. It has been postulated previously[12] that hydrogen bonding is the main factor involved in the separation of sugars on bonded-primary amine packing.

TABLE I

MOLECULAR COMPOSITION OF HUMAN BRONCHIAL OLIGOSACCHARIDES [a]

Oligosaccharides	Carbohydrate components [b]			
	L-Fuc	D-Gal	D-GlcNAc	D-GalNAc-ol
a				1
b			0.9	1
c		1		1
d	0.9	0.9		1
e		1.2	0.9	1
f		1	0.9	1
g		1	0.9	1
h	1	1.2	0.9	1
j		1.2	1.8	1
k		2	0.9	1
n	1	1.8	1.2	1
o	0.2	2	1.7	1

[a] Separated by h.p.l.c. on a 5 μ Lichrosorb-NH_2 column eluted with a linear-gradient solvent system.
[b] Determined by gas–liquid chromatography; 2-acetamido-2-deoxy-D-galactitol (D-GalNAc-ol) was taken as 1.

The separation of isomeric oligosaccharides suggests that the total number of hydroxyl groups, as well as their configuration determine the retention time of each oligosaccharide.

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