Carbohydrate Research, 118 (1983) C1–C4
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Preliminary communication

Fractionation of oligosaccharides containing sialic acid by liquid chromatography on amino silicagel

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(Received December 1st, 1982; accepted for publication in revised form, March 2nd, 1983)

Recently, liquid chromatography under elevated pressure (50–100 MPa) (h.p.l.c.) on alkylamine-modified silicas (specially on 5-μm packing material) has been recognized as a convenient method of separation of high-molecular-weight oligosaccharides containing neutral sugars and 2-acetamido-2-deoxyhexoses[1–6]. The mechanism by which the sugars are retained and are eluted from these solid phases has been extensively reported and reviewed[7,8].

We have reported[5] earlier the separation of neutral oligosaccharides from human bronchial-mucus performed with a linear gradient of 17:3 to 3:2 (v/v) acetonitrile–water. The alkylamine-bonded column may function as a weak anion-exchanger, and this could explain the retention on the column of compounds containing an organic acid. H.p.l.c. procedures utilizing an anion-exchange system have recently been developed to perform the separation of sialyloligosaccharides. Various elution procedures, such as isocratic elution[2] with 11:9 (v/v) acetonitrile–sodium acetate buffer, pH 5.8, and linear-gradient elution[9,10] with 4:1 to 2:3 (v/v) acetonitrile–phosphate buffer, pH 5.2, or 25 to 500mM phosphate buffer[11], pH 4.0, have been described for the elution of acidic oligosaccharides. We report now the resolution of sialyloligosaccharides on an amine column by means of a linear gradient of acetonitrile–water–ammonium hydrogencarbonate (pH 7.5). This mobile phase suppressed the ionization of the primary-amine packing and could be removed by evaporation. This method is, therefore, convenient for isolating, from a complex mixture, pure acidic oligosaccharides.

In the present work, sialyloligosaccharides from bonnet monkey cervical-mucus were separated by h.p.l.c. into seven well-separated fractions (Fig. 1A), the molecular composition of which is shown in Table I, and human bronchial sialyloligosaccharides into eleven less well-separated fractions (Fig. 1B); the latter fractions did not always correspond to molecular proportions (Table I). This result is explained by the very high heterogeneity found in bronchial oligosaccharides[12] and shows the limits of this h.p.l.c. technique for very heterogeneous mixtures of sialyloligosaccharides.

0008-6215/83/$03.00

TABLE I

MOLECULAR COMPOSITION[a] OF SIALYLOLIGOSACCHARIDES FRACTIONATED BY LIQUID CHROMATOGRAPHY

Mucin subfraction	*Carbohydrate component*					
	L-*Fuc*	D-*Gal*	D-*GlcNAc*	D-*GalNAc*	*Neu.Ac*	D-*GalNAcol*
Cervical						
1					0.8	1
2		1			0.8	1
3	0.7	0.9			0.7	1
4[b]						
5	0.7	2.1	0.9	1.1	1	1
6	0.6	1.6	0.8	1.4	0.9	1
7	0.5	3.4	1	1.2	1	1
Bronchial						
1	0.7	1	1		0.8	1
2		0.6	1.2		0.6	1
3	0.9	2.1	1.1		1.3	1
4[c]						
5	0.1	1	2.2		0.5	1
6	0.1	0.5	1		0.4	1
7	0.1	0.5	0.5		0.7	1
8	0.5	1.6	1.3		0.7	1
9		0.3	1.5		0.3	1
10		0.6	0.8		0.6	1
11	0.3	0.8	0.8		0.9	1

[a] Relative to 2-acetamido-2-deoxy-D-galactitol taken as 1. [b] An insufficient amount of this subfraction was obtained to allow determination of the molecular composition. [c] This subfraction was contaminated with noncarbohydrate material.

EXPERIMENTAL

Materials. – The reduced sialyloligosaccharides used in this work were obtained from bonnet monkey, mid-cycle cervical-mucus glycoproteins[13] and from bronchial-mucus glycoproteins[14].

Liquid chromatography. – The chromatograph was equipped with two pumps from Waters Associates Inc. (Milford, MA 01257, U.S.A.), model 6000A; a solvent programmer from Waters Associates Inc., model 660, coupled to a Uvicord detector LKB, model S 2138; a universal injector from Waters Associates, model U6K; an LKB model 2250 recorder; and 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 4:1 to 1:1 acetonitrile–water containing 2.5mM ammonium hydrogencarbonate for 70 min at room temperature, and 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, U.S.A.). All solvents were degassed by sonication. Although nonspecific for carbohydrate, the wavelength of detection was 206 nm, and 100 μg of oligosaccharides in water (5 μL) were injected through the loop injector.

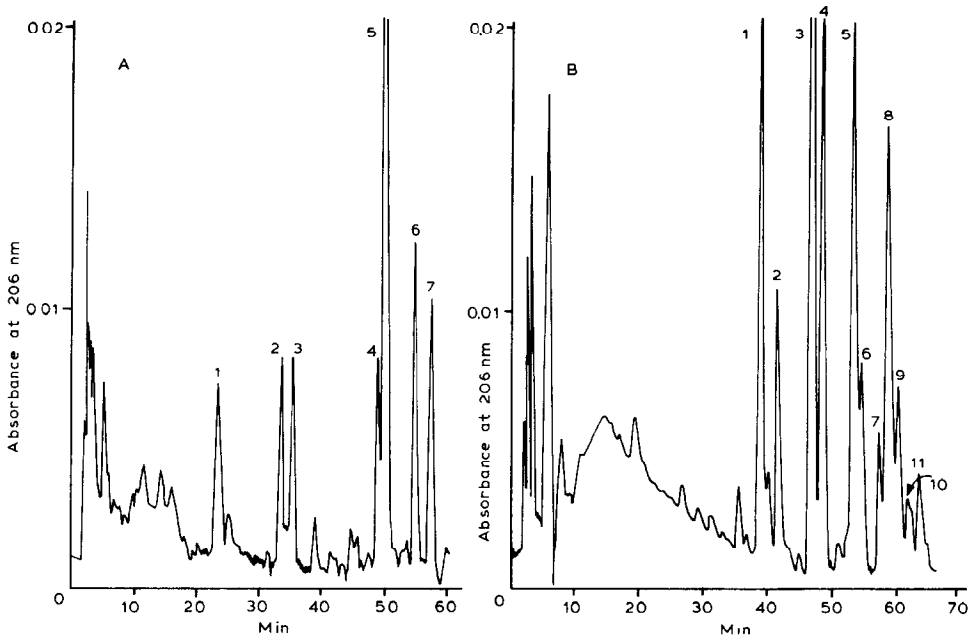

Fig. 1. Separation of sialyloligosaccharides in liquid chromatography under elevated pressure: (A) Bonnet monkey cervical-mucus oligosaccharides. (B) Human bronchial-mucus oligosaccharides. The chromatography was performed as described in the Experimental section.

Elution profiles of both oligosaccharide subfractions are given in Fig. 1, and the molecular composition of the major well-separated fractions relative to the content of 2-acetamido-2-deoxy-D-galactitol (taken as 1) is reported in Table I.

ACKNOWLEDGMENTS

This work was supported by a grant from l'Association Française de Lutte contre la Mucoviscidose, by la Fondation pour la Recherche Médicale Française and by contract INSERM No. 803025. The authors thank Bénédicte Gillet for her technical assistance and Catherine Masson for typing the manuscript.

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