Journal of Chromatography, 181 (1980) 108—114
Biomedical Applications

CHROMBIO. 425

Note

Differentiation of the polysaccharide side-chains of glycoproteins by a fingerprinting technique: heterogeneity of human gastric mucin

E. MOCZAR

Laboratoire de Biochimie du Tissu Conjonctif (GR CNRS No. 40), Faculté de Médecine, 8 Rue du Général Sarrail, 94010 Créteil (France)

and

D. WALDRON-EDWARD

Department of Surgery and Gastrointestinal Research Laboratory, McGill University, Donner Building, Montréal, Québec H3A 1A4 (Canada)

(Received June 12th, 1979)

Mucin glycoproteins, secreted into the gastrointestinal tract, differ in their cellular source, and are known to differ in their structure and composition. As biosynthesis is also controlled by genetic factors, differences are also found (from individual to individual) in glycoproteins secreted by the same cell type; the blood group inhibitory activity of the glycoproteins secreted by the gastric mucosa offers a well-known illustration of these differences. In order to study their physiological significance, a rapid method to scan the structural differences has been developed, based on the fact that the mucins are characterized by an O-glycoside linkage of the polysaccharide side-chain to the protein backbone. In alkali, the sugar chains are released by β-elimination, but are then subject to further uncontrolled degradation by base-catalysed "peeling" reactions [1—3]. Under suitably controlled conditions, however, a series of reduced oligosaccharides, as well as hexitols [3] and unsaturated alditols can be identified.

Recently, a two-dimensional chromatographic method was developed for the separation of oligosaccharides bearing acidic groups [4, 5]. The method has now been extended to permit the radioactive labelling of the neutral non-reducing oligosaccharides as well, in the products of the alkaline hydrolysis. These fingerprint techniques have been applied to the study of the polysaccha-

ride side-chains of human glycoproteins, obtained from gastric aspirates of A, B and O blood group donors. The results indicate that there are several differences, both in structure and in chain length, not only in terminal prosthetic groups but also in the inner core region of the carbohydrate moiety of these glycoproteins.

MATERIALS AND METHODS

The gastric juice was neutralised in vivo with phosphate buffer. Human mucins were separated by filtration and extensive washing from other soluble glycoproteins, as described elsewhere [6], from gastric juice aspirated from normal fasted subjects. Blood group and secretor status were determined in each case. Samples of lyophilized mucins from four or five donors of each blood group were pooled for analysis.

Partial alkaline degradation of the mucins was carried out as described previously [4]. Ten milligrams of mucin were stirred for six days at room temperature in 1 ml of 0.33 *M* KBH_4 in 1% sodium hydroxide. The reaction mixture was neutralized with Amberlite CG-120 (H^+). Fingerprinting of the alkaline hydrolysis products was carried out on 20 cm × 20 cm silica-coated thin-layer sheets (Schleicher & Schüll, Dassel, G.F.R., Selecta 1500). Oligosaccharide mixtures containing 0.2—1 μg of carbohydrate constituents were applied. The electrophoretic step was carried out in acetic acid—pyridine—water (10:1:89, v/v; pH 3.8) buffer at 10—20 V/cm for 2—3 h. The dried plates were chromatographed perpendicular to the electrophoretic migration with either solvent A, *n*-propanol—nitromethane—acetic acid—water (7:2:2:2, v/v), or solvent B, ethanol—nitromethane—acetic acid—water (5:3:3:3, v/v), by the ascending method until the solvent reached the upper edge of the sheet. The carbohydrate constituents were detected by sulphuric acid char or by the orcinol—sulphuric acid reagent [5, 7].

[^{14}C] Cyanohydrin formation

The reduced and neutralized alkaline degradation products of the mucins containing 0.1—0.2 μg of hexoses, as determined by the orcinol reaction, were dissolved in 50 μl of water, and 50 μg of galactose oxidase (Sigma, St Louis, Mo., U.S.A.) in 125 μl of phosphate-buffered saline (PBS) pH 7.0 were added. The mixture was incubated for 3 h at 37°. Forty microcuries of $K^{14}CN$ (specific activity 57 mCi/m*M*) in 25 μl of water were then added. The mixture was allowed to stand overnight at room temperature, then evaporated to dryness in a vacuum desiccator over KOH after the addition of one drop of 10% aqueous acetic acid. The residue was dissolved in 0.1 *M* acetic acid and evaporated again to dryness in vacuo. This operation was repeated three times to remove traces of radioactive HCN. The final residue was dissolved in 50 μl of water; 1—0.5 μl were deposited for fingerprinting and 1 μl was counted in a scintillation counter. Control experiments in which (a) the alkaline degradation product, or (b) the galactose oxidase was omitted, were performed with every series to detect radioactive artefacts. No artefacts, however, were detected between the starting point and the migration level of the disaccharides in the chromatographic step, although several non-specific radioactive spots were found in the

monosaccharide region of the control experiments. The fingerprints of the labelled oligosaccharides were exposed for 2—3 days to Kodak Kodirex films and developed with Kodak LX 14 developer. Samples of known oligosaccharides were applied to the chromatograms as markers.

Exclusion chromatography of the alkaline degradation products of the mucins was carried out on Sephadex G-25F columns (1 cm × 90 cm) eluted with 0.1 *M* acetic acid. The hexose level of the effluent was continuously monitored by the orcinol—sulphuric acid reagent in a Technicon device. The experiments were carried out in triplicate and the means of the quantities in each of the areas were compared (Fig. 1).

RESULTS

The fractionation of the alkaline degradation products on Sephadex G-25 columns indicate that the distribution of molecular weights of these products from donors of each group is similar, but differs in relative proportions (Fig. 1, Table I). Thus the ratio of the low-molecular-weight oligosaccharides to the higher molecular products, (peaks 3 + 4)/(peaks 1 + 2), is 0.55, 0.6 and 0.72 in the mucins of A, O, and B secretors, respectively. This finding, in itself inconclusive, suggests, however, that considerable differences must exist between the sugar chains of these glycoproteins.

Fingerprinting of the alkaline degradation products also shows marked differences between the glycoproteins of the A, B and O secretor groups (Fig. 2a and b). A satisfactory two-dimensional separation of the neutral and acidic reduced oligosaccharides liberated during the peeling reaction was obtained by this technique. The non-degraded macromolecular carbohydrate components exhibit electrophoretic mobility but are not displaced by the solvents used in the chromatography. The acidic oligosaccharides obtained from B and O secretor groups seem to be similar (Fig. 2). The separation of the neutral oligosac-

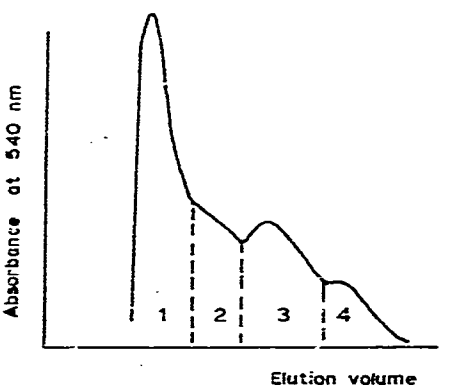

Fig. 1. Elution profile of the reduced alkaline degradation product (2 mg) on a Sephadex G-25F (1 cm × 90 cm) column. Eluent: 0.1 *N* acetic acid. The sugar content of the eluate was continuously monitored by the orcinol—sulphuric acid reagent using a Technicon device. The peak areas were compared by cutting out and weighing the region corresponding to the eluted fractions 1, 2, 3, and 4 (results, see Table I).

TABLE I

PERCENTAGE OF TOTAL HEXOSE FOUND IN EACH FRACTION ELUTED BY EXCLUSION CHROMATOGRAPHY OF THE ALKALINE DEGRADATION PRODUCTS OF GASTRIC MUCINS OBTAINED FROM A, B, AND O BLOOD GROUP DONORS *

Results are given as mean ± S.E.M. ($n = 4$).

Fraction	Percentage total carbohydrate (hexose)					
eluted**	Group O		Group A		Group B	
1	41.3 ± 1.8	1***	46.1 ± 2.5	1***	40.5 ± 1.2	1***
2	21.7 ± 1.0	0.51	18.6 ± 0.9	0.40	17.6 ± 1.0	0.43
3	26.7 ± 1.0	0.64	25.0 ± 1.1	0.56	31.0 ± 1.0	0.76
4	10.8 ± 0.9	0.26	9.4 ± 1.0	0.20	10.9 ± 1.1	0.27

* See Fig. 1.
** See Fig. 2.
*** Means values related to fraction 1.

charides is better when using solvent A for the chromatography step than solvent B.

The radioactive labelling reaction is based on cyanohydrin formation of the KCN with the aldehyde groups obtained by the action of the galactose or galactosamine of the sugar chain [8]. The cyanohydrin formation of the aldehydes proceeds quantitatively over several hours [10]. The chromatographic separation of the radioactive cyanohydrin derivatives of the oligosaccharides (Fig. 3) is superior to the separation of the parent oligosaccharides (Fig. 2a and b). This increased sensitivity can be used to differentiate products at both high and low concentration by varying the amounts applied. Thus, in Fig. 3, the neutral components are overloaded, whereas there are sufficient quantities of the radiolabelled minor components of acidic oligosaccharides to enable detection and confirmation of the results obtained in the fingerprinting of the parent oligosaccharides (Fig. 2). The similarity of the acidic oligosaccharides from B and O secretors is also confirmed by the autoradiograms (Fig. 3).

DISCUSSION

Gastric gel mucin comprises two-thirds by weight of the total non-dialysable solids in gastric juice, derived for the most part from epithelial and mucous neck cells of the gastric mucosa. Analysis has revealed fucose, galactose, N-acetylglucosamine and N-acetylgalactosamine in both fractions while the acidic fraction also contains sialic acid and sulphate. The polysaccharides are attached to a polypeptide core by O-glycoside linkages [9]. Thus, the alkaline degradation products of these mucins should be comparable to those obtained from the blood group substances [2, 3].

The fingerprints of the oligosaccharide from the alkaline borohydride degradation products and from their cyanohydrin derivative indicate that the reaction mixtures obtained from the A, B, and O secretor groups contain different

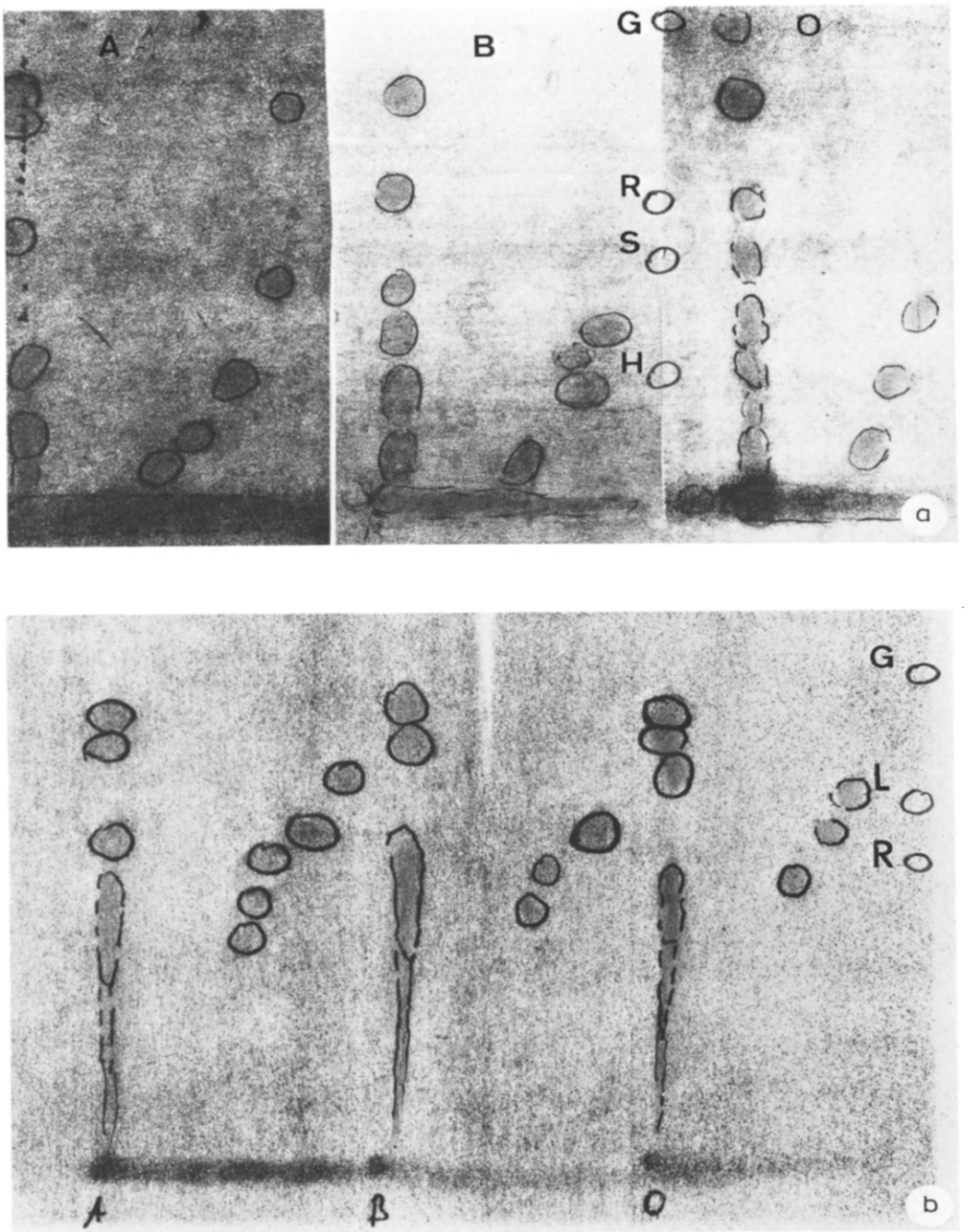

Fig. 2. Fingerprints of the alkaline degradation products on silica thin layers (Schleicher & Schüll, Selecta 1500, 20 cm × 20 cm) of the insoluble gastric mucins obtained from A, B, and O secretor groups. Ordinates: (a) chromatography in solvent A, and (b) in solvent B. Abcissae: electrophoresis in pyridine—acetate (pH 3.5). Detection: orcinol—sulphuric acid. S = Stachyrose, R = raffinose, L = lactose, G = galactose, H = glucosyl-galactosyl-hydroxylysine.

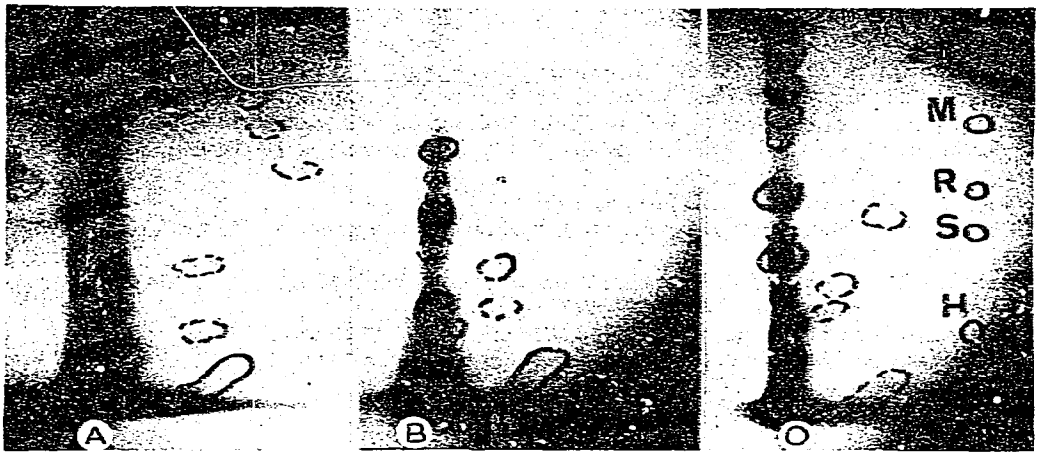

Fig. 3. Autoradiograms of the fingerprints of the cyanohydrin derivatives of the alkaline degradation products of the insoluble gastric mucins, obtained from A, B, and O secretor groups. Abcissae: electrophoresis (see Fig. 2). Ordinate: chromatography in solvent A. Abbreviations as in Fig. 2; M = maltose.

oligosaccharides. This finding implies that the major chain of these three types of mucin has to be different.

The glycoproteins used in these experiments have intense blood group activity, inhibiting the agglutination of erythrocytes by the appropriate antisera at dilutions as low as 0.1 μg/ml. A major terminal group of the polysaccharide side-chains must correspond therefore with the known structure of the blood group substances of the individual prosthetic groups. As the difference between the fingerprints involves several oligosaccharides it seems that not only the end-groups of the polysaccharides chains but also some interior sugar sequences have to be different. The results of the gel filtration experiments indicate also a different degree of fragmentation of the carbohydrate chains, implying structural differences in the interior of the carbohydrate fraction.

The fact that only slight differences were found in the overall sugar composition of the three glycoprotein complexes [6] studied, indicates that the fingerprinting of the oligosaccharides obtained by alkaline degradation of the mucins is required as a much more sensitive method for the detection of structural differences between the carbohydrate chains of these substances. However, more work is needed using oligosaccharides of precisely known structure before more exact conclusions can be drawn from the chromatographic data on the variations of the inner core region of the polysaccharide side-chains of these mucins.

ACKNOWLEDGEMENT

This work was supported in part by Grant MA 4058 from the Medical Research Council of Canada to D.W.-E., by the CNRS, GR No. 40, and by the University of Paris-Val de Marne, Créteil, France, to E.M.

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