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Glycosaminoglycans of the Fat Globule Membrane of Cow Milk

Dora Lis and Benito Monis

Primera Cátedra de Histología e Instituto de Biología Celular, Facultad de Ciencias Médicas, Universidad Nacional de Córdoba, Casilla Postal 220, 5,000 Córdoba, Argentina

Membranes of fat globules of cow milk contained 163 μg/100 mg (dry weight) of glycosaminoglycans (expressed as uronic acid); 62.5% of the uronic acids corresponded to hyaluronic acid, the remaining consisted of sulfated glycosaminoglycans (chondroitin-4-(-6) sulfates, and dermatan and heparan sulfates) with different degrees of sulfation.

Key words: glycosaminoglycans, glycocalyx, milk fat globule membrane, hyaluronic acid, chondroitin-sulfates, heparan sulfates

The membrane of the milk fat globule, like most cell surfaces [1], contains glycoproteins and glycolipids [2–6]. To date there are no data on the presence of glycosaminoglycans on it.

The present report deals with the isolation and characterization of these glycoconjugates from the fat globule membrane of cow milk.

METHODS

The membrane fraction was obtained from a pool of 10 liters of cow milk by churning and centrifugation [7]. Electron microscopic controls revealed a rather pure membrane preparation devoid of casein granules.

For glycosaminoglycan isolation, the membrane preparation was extracted with 19 volumes of chloroform-methanol (2:1, by volume), digested for 6 h with papain [8], treated with trichloroacetic acid (10% final concentration), neutralized with NaOH, and run through a Sephadex G-50 column (2.5 × 30 cm). The excluded material was dried, digested with ribonuclease [9], and rechromatographed on Sephadex G-50.

This crude preparation was fractionated on a DEAE-Sephadex A-25 column (0.25 × 20 cm) by stepwise elution [10]. All fractions were concentrated and salted out on Sephadex G-25.

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Uronic acids [11], hexosamines [12], sulfates [13], and proteins [14] were determined in duplicate. Monosaccharides were identified by paper chromatography using an ethyl acetate-pyridine-water (12:5:4, by volume) system and were stained with benzidine [15].

DEAE-Sephadex A-25 fractions were electrophoresed on cellulose acetate paper in 0.1 M barium acetate (pH 6.6) and 0.1 M HCl (pH 1.2) buffers [16, 17]. Electrophoretograms were stained with 1% Alcian blue (pH 2.5), 0.05% toluidine blue in 65% ethanol, or the periodic acid-Schiff reagent (PAS) [18].

Hyaluronic acid was further characterized by leech hyaluronidase digestion [19]. Chondroitin-4- and chondroitin-6-sulfates, and dermatan-sulfates were determined by the proportions of disaccharides obtained by digestion with chondroitinases AC and ABC [20].

RESULTS

The glycosaminoglycan preparation obtained from the fat globule membranes contained uronic acids, hexosamines, sulfates, and proteins (Table I). Paper chromatography revealed glucosamine, galactosamine, glucuronic acid, galactose, mannose, and glucose.

Chromatography on DEAE-Sephadex A-25 yielded five fractions. The first fraction, eluted with 0.1 M NaCl, which did not contain uronic acids, accounted for about 80% of the hexosamines and "proteins" of the crude preparation. Cellulose acetate electrophoresis [16] followed by PAS or Alcian blue staining indicated that glycopeptides were present in this fraction. The remaining four fractions contained all the uronic acids and sulfates of the crude preparation. The 0.5 M NaCl fraction contained 62.5% of the uronic acids (Table I). Electrophoresis revealed two spots. A major one migrating like hyaluronic acid was almost completely digested by leech hyaluronidase [19]. From the eluate of the second spot sulfates, hexosamines, and most "proteins" were recovered (Fig 1). Sulfated glycosaminoglycans, present in the three remaining fractions, were mostly concentrated in the 1.0 M NaCl fraction (Table I). Electrophoresis and enzymatic digestions demonstrated that these fractions contained mainly galactosaminoglycans with minor amounts

TABLE I. Composition of the Crude Glycosaminoglycan Preparation and DEAE-Sephadex A-25 Fractions From Membranes of the Fat Globule of Cow Milk

Component	Crude preparation (μg/100 mg dry weight)	DEAE-Sephadex fractions[a] (M NaCl) 0.1	0.5	1.0	1.5	2.0
Uronic acids	163	0	62.5	32.9	3.3	1.3
Hexosamines	1,434	78.1	14.1	3.8	1.6	2.4
Sulfates	153	0	17.5	32.5	42.5	7.5
Proteins	2,810	79.3	20.7	0	0	0
Δ di-4-S[b]	–	–	–	22.0	15.8	15.5
Δ di-6-S	–	–	–	5.8	8.1	9.0
Δ di-0-S	–	–	–	6.3	7.6	7.1

[a]Results are expressed as percentage of total glycosaminoglycan components.

[b]Disaccharides obtained by chondroitinase AC digestion.

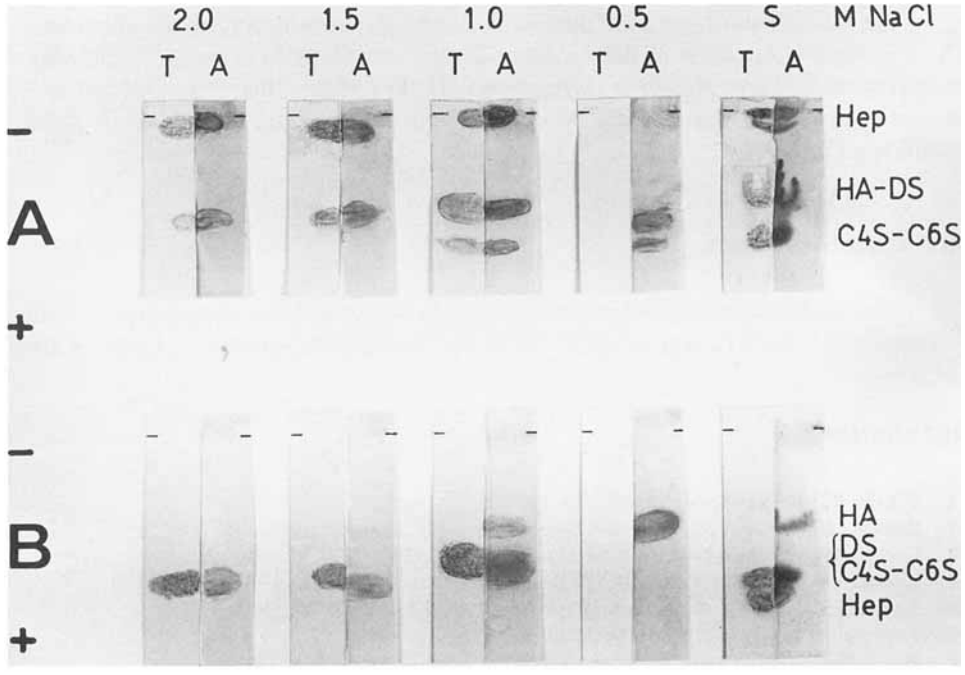

Fig 1. Electrophoresis on cellulose acetate of the DEAE-Sephadex A-25 fractions from membranes of cow milk fat globules: A) in barium acetate, pH 6.6; B) in HCl, pH 1.2. Strips were stained with Alcian blue, pH 2.5 (A) or toluidine blue (T). HA) hyaluronic acid; C4S) chondroitin-4-sulfate; C6S) chondroitin-6-sulfate; DS) dermatan sulfate; Hep) heparan.

of heparan sulfates, which differed in the degree of sulfation (Fig. 1, Table I). Chondroitinase AC digested 97.2% of the galactosaminoglycans, yielding 53.3% of the 4-sulfate-disaccharide (Δ di-4-S), 22.9% of the 6-sulfate-disaccharide (Δ di-6-S), and 21.0% of the nonsulfated disaccharide (Δ di-0-S). Disulfated disaccharides were also found in the chromatograms. Table I shows the percentage distribution of the chondroitinase AC digestion products in the different fractions. The remaining 2.8% of the galactosaminoglycans corresponded to dermatan sulfate.

DISCUSSION

Our data support the view that, in addition to the widespread distribution of glycosaminoglycans in the intercellular substances of connective tissues, these complex carbohydrates are also bound to the plasma membrane of epithelial cells of ectoblastic derivation, since it has been shown that the membrane of the milk fat globule originates mainly in the lumenal plasmalemma of the alveolar cells of the mammary gland [5, 21, 22]. Furthermore, the demonstration of glycosaminoglycans bound to the plasma membrane of certain cells of endodermal or mesodermal origin [23–28] would suggest that they are also a constituent of cell membranes as has been shown for other glycoconjugates [1, 29]. Like these, glycosaminoglycans would be located, because of their distinct hydrophilic properties, at the outer aspect of the cell surface [30] as an intrinsic component of the glycocalyx.

The functional properties of membrane-bound glycosaminoglycans are unknown. The polyanionic properties of these substances may contribute to maintain fat globules in suspension by preventing their aggregation [31]. In addition, they may function as carriers of cations, such as calcium, which have been demonstrated in the milk fat globule membrane [5, 6, 31].

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