SULFATION OF HEPARAN SULFATE IN ISOLATED RAT INTESTINAL EPITHELIAL CELLS

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1. Introduction

Heparan sulfate, a polysaccharide structurally related to heparin [1], is a ubiquitous constituant of cell surfaces [2–4]. Consequently, heparan sulfate has been implicated in several specific cellular functions such as growth control, cell–cell contact and binding of lipoprotein lipase and lipoproteins [5].

Structure–function relationships have been established for polysaccharides [6,7]; thus, as in heparin, *N*-sulfate groups of heparan sulfate may be required for selective binding of plasma components, functional implications of such binding have been proposed for lipoprotein lipase [8,9]. The fact that the epithelial cells of the intestine proliferate rapidly [10] induced us to study the sulfation process of heparan sulfate chains in isolated rat intestinal epithelial cells.

Here we demonstrate the presence of heparan sulfate in these isolated cells and show the high metabolic activity of this polysaccharide. In addition, the results underline the high degree of sulfation of its chains; this is likely to be a basis for the biological function displayed by heparan sulfate.

2. Material and methods

Sprague-Dawley male rats (200–250 g body wt) were injected intraperitoneally with 1 mCi sodium [^{35}S]sulfate (spec. act. 763 mCi/mmol, New England Nuclear Corp.), and 2 h later decapitated. Intestinal epithelial cells from the jejuno-ileum were isolated by hand shaking in a dispersing solution containing EDTA (2.5 mM) as in [11]. The isolated cells were suspended in a Krebs-Ringer phosphate buffer (pH 7.4) and then sonicated. Labelled macromolecular material was obtained after proteolytic digestion with pronase (Kaken Chemical Co., Tokyo) by alternate precipitations with cetylpyridinium chloride and ethanol according to [12]. The uronic acid content was estimated by the carbazole reaction [13] as modified [14]. The hexosamine content was determined by the colorimetric method in [15]. The neutral sugar content was determined by the orcinol method as in [16].

Electrophoresis of glycosaminoglycans was carried out on cellulose acetate strips (cellogel Sebia) in 0.1 M pyridine formate buffer (pH 3.0) [17]. Ion-exchange chromatography was performed on DEAE-cellulose column as in [18]. ^{35}S-Labelled samples were mixed with standards (hyaluronic acid, chondroitin sulfate and heparin) and applied to a DE-52 column (1 × 5 cm) which was then eluted with a LiCl gradient (0.05–3 M in 0.05 M sodium acetate buffer (pH 4.0)).

Enzymic degradation of glycosaminoglycans [19] was carried out with chondroitinase ABC [20] or with heparitinase [21]. The hexosamine moiety of each glycosaminoglycan was identified after chromatography on Kodak K 511V plaque as in [22]. Deaminative cleavage of heparan sulfate with $NaNO_2$ was performed as in [23]; degradation products were resolved by gel chromatography on a Sephadex G-25 column (1 × 100 cm) [24].

3. Results and discussion

Macromolecular constituants of isolated epithelial cells were separated, by electrophoresis on cellulose

Abbreviations: GlcNAc, *N*-acetylated-D-glucosamine; $GlcNSO_3$, *N*-sulfated-D-glucosamine; HexN, hexosamine; HexUA, glycuronic acid; aMan, D-anhydromannose

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Table 1
Heparan sulfate content of isolated intestinal epithelial cells and its contribution to macromolecular radioactivity

Fraction	μg	μg (%)	cpm	cpm (%)	Spec. act.[a]
Total macromolecular material	522.5	100	205 125	100	39 260
Glycopeptides	471	90	170 250	83	36 145
Heparan sulfate	28.5	5.5	22 565	11	79 170
Dermatan sulfate	23	4.4	12 310	6	53 510

[a] Specific activities are expressed in cpm/100 μg each fraction

After in vivo incorporation of sodium [^{35}S]sulfate, intestinal epithelial cells from the jejuno-ileum were isolated according to [11]. Macromolecular material was obtained after proteolytic digestion with pronase by alternate precipitations with cetylpyridinium chloride and ethanol. Electrophoresis was performed on cellulose acetate strips in 0.1 M pyridine formate buffer (pH 3.0). The radioactivity of each fraction was determined by counting of the corresponding stained area [12]

acetate strips, into 3 fractions (table 1). Glycopeptides constituted the bulk of the macromolecular material [25,26]. Glycosaminoglycans were identified as heparan sulfate and dermatan sulfate on the basis of electrophoretic criteria (the same electrophoretic migration as that of standard glycosaminoglycans) and of analytical criteria (specific hydrolyses of heparan sulfate and dermatan sulfate by heparitinase and chondroitinase ABC, respectively) as generally employed [24,27]. It was also verified that the only hexosamine found in heparan sulfate was glucosamine and that in dermatan sulfate was galactosamine.

Chemical composition of these 3 components is shown in table 2. In the glycopeptide fraction, the neutral sugar/amino sugar molar ratio is comparable to that generally observed [28]. The hexosamine and uronic acid contents of heparan sulfate and dermatan sulfate (table 2) agree with the known structures of these polysaccharides [29].

After administration of sodium [^{35}S]sulfate, the glycopeptides and the glycosaminoglycans label significantly. Because of the presence of a large group of glycopeptides (90% of the fraction), the incorporation detected into the glycosaminoglycans, in terms of total ^{35}S-radioactivity is low. Approximately 83% of the incorporated radioactivity is present in the glycopeptide fraction and 17% in the glycosaminoglycan fraction (table 2). Nevertheless, in terms of specific radioactivities, the higher ones were obtained for the glycosaminoglycan fractions. Uptake of radioactivity is found to occur to a variable degree according to the type of glycosaminoglycan chain; a higher specific radioactivity is obtained for heparan sulfate chains than for dermatan sulfate chains; the preferential labeling of heparan sulfate could be taken as an index of the existence of topographically different pools of metabolic precursors as suggested in [30]. It is interesting to underline that the higher metabolic activity

Table 2
Chemical composition of macromolecular material from isolated intestinal epithelial cells

Glycopeptides				Heparan sulfate		Dermatan sulfate	
Neutral sugars		Amino sugars		HexN	HexUA	HexN	HexUA
(mg)	(μM)	(mg)	(μM)	(%)		(%)	
20.5	119	8.9	49	28	33	29	31

For details, see legend of table 1. The number of mg and μmol neutral and amino sugars are given per 100 mg total proteins. Hexosamine and uronic acid content of heparan sulfate and dermatan sulfate are expressed as percentage of dry weight

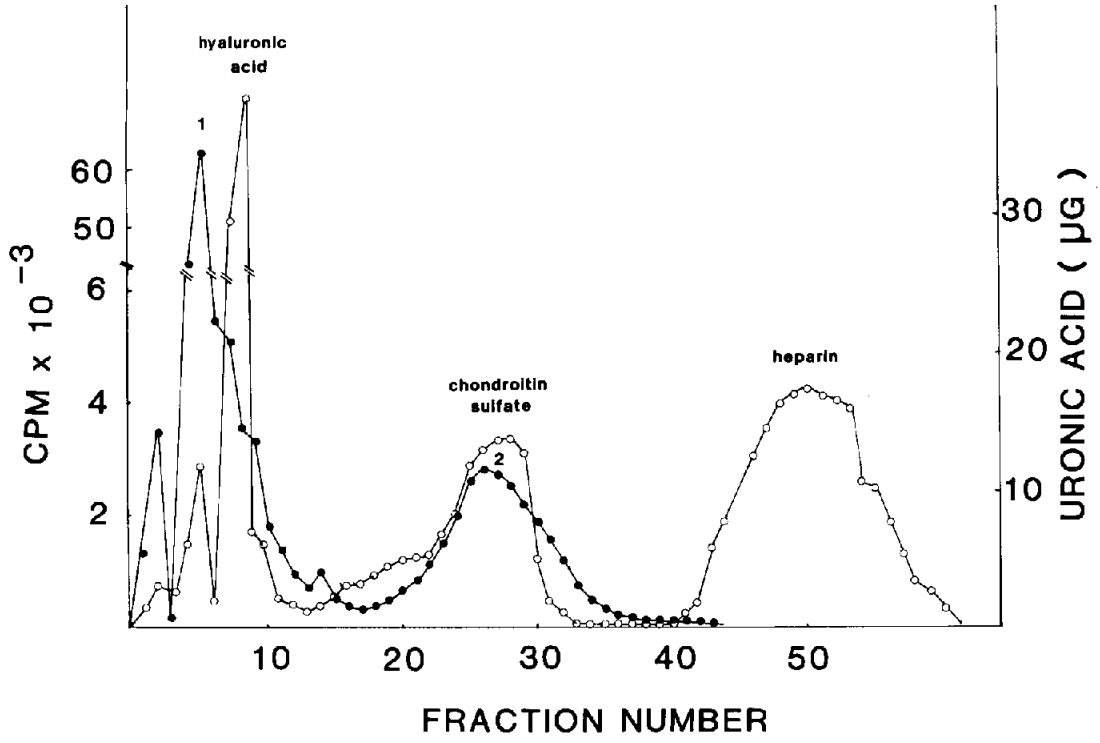

Fig.1. DEAE-cellulose chromatography of radioactively labelled heparan sulfate from isolated intestinal epithelial cells prepared from rats pre-injected with sodium [^{35}S]sulfate. Pronase-resistant labelled macromolecular material was degraded with chondroitinase ABC. After dialysis, the medium was mixed with standard preparations of hyaluronic acid (HA), chondroitin sulfate (CS) and heparin (Hep), and was then applied to a DE-52 ion-exchange column (1 × 5 cm) which was eluted with a linear gradient of 0.05–3 M LiCl in 0.05 M sodium acetate buffer (pH 4.0). Effluent fractions of 2.5 ml were collected and analysed for radioactivity (•–•) and for uronic acid (○–○). The radioactivity obtained was seeding to: (1) glycopeptides; (2) heparan sulfate.

is observed for the cell-surface localized heparan sulfate. Our results suggest the particular metabolic role of heparan sulfate.

Heparan sulfate was isolated from the other macromolecular material after chondroitinase ABC digestion and dialysis to remove degradation products of dermatan sulfate, by ion-exchange chromatography on DEAE-cellulose column [4] (fig.1). The ^{35}S-labelled material emerged as two sets of peaks: the first eluting before standard hyaluronic acid and is shown to consist of a large group of glycopeptides; the second has an elution position in the same area as the standard chondroitin sulfate [31]. The ^{35}S-labelled material of this peak is related to heparan sulfate. As described above, the identity of heparan sulfate is confirmed by its resistance to digestion with either chondroitinase ABC and AC or hyaluronidase. In contrast, it was sensitive to heparitinase and was quantitatively degraded to oligosaccharides by nitrous acid indicating the presence of sulfaminogroups [23,32] (fig.2).

The heparan sulfate fractions were further characterized with regard to fragmentation profiles obtained after deaminative cleavage. In accordance with the chemical analyses, the heparan sulfate showed an increasing degree of fragmentation with increasing *N*-sulfate content [33]. Gel chromatography of heparan sulfate degradation products obtained under deaminative conditions shows no void volume peak (no block of hexUA-GlcNAc), but reveals two peaks of oligosaccharides in the retarded fractions (fig.2): the most retarded one had the same elution position as the disaccharide standard, 2-acetamido-2-deoxy-3-*O*-(β-D-gluco-4-ene-pyranosyluronic acid)-D-galactose. Analysis of the hexosamine and uronic acid content of this peak show a molar ratio of hexosamine to uronic acid approximately zero and confirm that it represents a disaccharide peak [24,34]. If the consecutive peaks differ by a disaccharide unit, the less

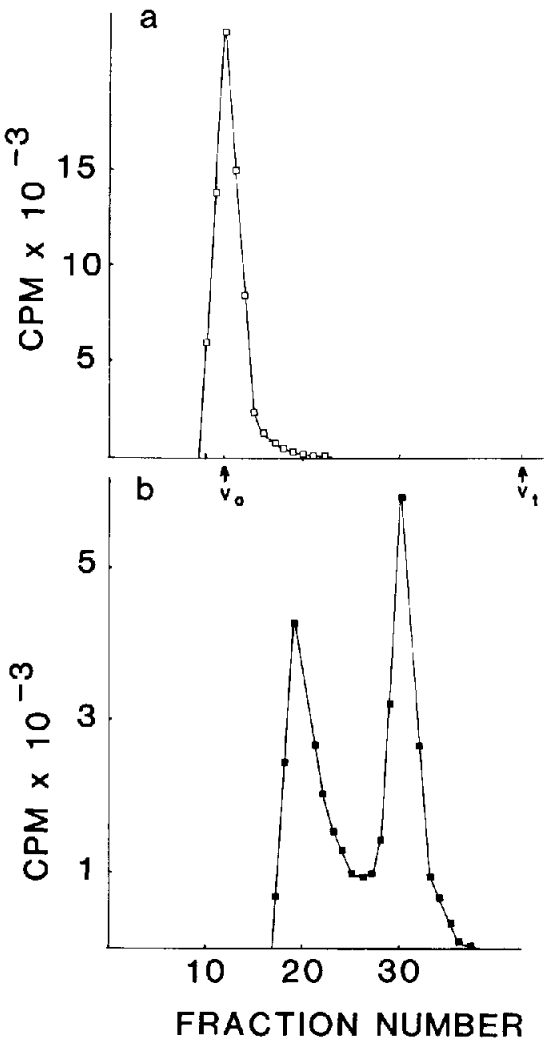

Fig.2. Analysis of *N*-sulfate distribution in heparan [^{35}S]sulfate from isolated intestinal epithelial cells. Heparan [^{35}S]sulfate isolated after gel chromatography on DE-52 as in fig.1 was subjected to gel filtration on Sephadex G-25 (1 × 100 cm) before (a) or after treatment with $NaNO_2$ (b). Columns were eluted with 0.2 M NaCl at 2 ml/h. Fractions of 2 ml were collected and analysed for radioactivity. V_0 and V_t of the column are indicated by arrows.

retarded one would represent a tetrasaccharide peak; this assumption was confirmed by the determination of the molar ratio of hexosamine to uronic acid of this peak which was found to be 0.5. Determination of radioactivities of disaccharides and tetrasaccharides show that the radioactivity of the former is greater than that of the latter, respectively; in this case, the ratio of radioactivities is near to two.

Thus, deaminative degradation by nitrous acid of heparan sulfate chains of our fractions yielded two categories of fragments: one of tetrasaccharide fragments (hexUA–GlcNAc–hexUA–aMan) and the other of disaccharides fragments (hexUA–aMan). No larger sized oligosaccharides were found in the degradation products. These results indicate that after sulfation, most of the glucosamine residues within heparan sulfate chains are sulfated and corresponding glucosaminidic linkages are cleaved by treatment with nitrous acid.

These data demonstrate an active sulfation of heparan sulfate of isolated intestinal epithelial cells.

The distribution of sulfate-substituted glucosamine residues within the isolated heparan sulfate was examined by treating the preparation with nitrous acid. The results obtained underline that heparan sulfate of our fractions is 'over-sulfated'. In heparan sulfate of isolated epithelial cells, consecutive repeats of hexUA–$GlcNSO_3$ may be present to a considerable extent. This is consistent with the biological effects of cell-surface heparan sulfate; effects requiring specific sequences within the polysaccharide chain involving most likely *N*-sulfate groups, as identified in the antithrombin binding site of heparin [35].

Investigations currently in progress will enable us to specify the relationship between the structure of 'over-sulfated' heparan sulfate of isolated intestinal epithelial cells and the characteristics of its interaction with plasma proteins and fibronectin.

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