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STRUCTURAL DIFFERENCES OF DERMATAN SULFATES FROM DIFFERENT ORIGINS*

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ABSTRACT

The dermatan sulfates from hog, rat, rabbit, and beef liver, hog, rat, beef, and dog spleen, and hog skin were isolated and submitted to structural analysis. All of them migrated as single bands, close to the standard position for dermatan sulfate in agarose-gel electrophoresis. In polyacrylamide gel, however, each dermatan sulfate showed a characteristic electrophoretic migration-pattern: one, two, or three polydisperse bands, corresponding to different molecular weights, were obtained for the dermatan sulfates according to their origins. Chemical analysis showed that all of the dermatan sulfates here described are hybrid polymers composed of D-glucuronic and L-iduronic acid-containing disaccharide units. The relative position of these units in the polymer chains and the presence of 6-sulfated disaccharides were determined with the aid of chondroitinases B and AC from *Flavobacterium heparinum*. These studies show that each dermatan sulfate has a unique structure as regards the molecular weight, the presence of 6-sulfated disaccharide units, and also the relative amount and position of glucuronic and iduronic acid residues in the chains. These findings suggests a tissue- and species-specificity for the dermatan sulfates.

INTRODUCTION

Dermatan sulfate was first isolated from pig skin by Meyer and Chaffee[1]. This glycosaminoglycan is a stereoisomer of chondroitin 4-sulfate, differing at C-5 of the hexuronic acid moieties; D-glucuronic acid only was detected in acid hydrolyzates of chondroitin 4-sulfate, whereas both L-iduronic acid and small proportions of D-glucuronic acid appeared in the hydrolyzates of dermatan sulfate preparations[2]. Structural studies on dermatan sulfates extracted from skin[3–9], gastric

*Abbreviations: CS: chondroitin sulfate; Ch4S: chondroitin 4-sulfate or chondroitin sulfate A; Ch6S: chondroitin 6-sulfate or chondroitin sulfate C; DS: dermatan sulfate or chondroitin sulfate B; HS: heparan sulfate or heparitin sulfate, GAG: glycosaminoglycan(s) "mucopolysaccharides(s)"; ΔDi4S: 2-acetamido-2-deoxy-3-*O*-α-L-*threo*-hex-4-enopyranosyluronic acid)-4-*O*-sulfo-D-galactose; ΔDi6S: 2-acetamido-2-deoxy-3-*O*-α-L-*threo*-hex-4-enopyranosyluronic acid)-6-*O*-sulfo-D-galactose; ΔDiOS: 2-acetamido-2-deoxy-3-*O*-α-L-*threo*-hex-4-enopyranosyluronic acid)-D-galactose.

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mucosa[10], aorta[11], umbilical cord[11], lung[12], and fibrous cartilage[13] have shown that all of them are hybrid polymers. At least two types of disaccharide unit are represented in the dermatan sulfate molecules: Type A, 2-acetamido-2-deoxy-3-*O*-(β-D-glucopyranosyluronic acid)-4-*O*-sulfo-D-galactose, and Type B, 2-acetamido-2-deoxy-3-*O*-(α-L-idopyranosyluronic acid)-4-*O*-sulfo-D-galactose.

A chondroitinase B, which is specific for 4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-L-iduronic linkages[14] and a chondroitinase AC, which is specific for 4-*O*-(2-acetamido-2-deoxy-β-D-glycopyranosyl)-D-glucuronic acid linkages[2,15], were isolated from *Flavobacterium heparinum*. Enzymic analysis with these chondroitinases have shown that, in the pig-skin dermatan sulfate, the glucuronic acid-containing disaccharides are segregated in a specific region of the molecule, forming an oligosaccharide of molecular weight 3,000, and not alternated throughout the molecule[14]. The same oligosaccharide was also formed from human-skin dermatan sulfate[16].

Studies on the distribution of glycosaminoglycans have shown that each animal tissue has a characteristic glycosaminoglycan composition and that dermatan sulfate is present in almost all mammalian tissues[17,18]. It is possible, however, that each dermatan sulfate from each specific tissue has a unique structure as regards the amount and relative position of the different types of disaccharide units.

This paper describes the structure of twelve different preparations of dermatan sulfate isolated from hog, rat, rabbit, beef, and dog spleen, hog, rat, rabbit, and beef liver, and hog skin, by different extraction procedures. Commercial pig-skin dermatan sulfate was used as standard in all of the experiments.

EXPERIMENTAL

Materials. — The tissues from adult rat, rabbit, hog, beef, and dog were obtained immediately after death. The hog skin was shaved, cut into small pieces, defatted and dried with acetone, powdered, and stored at −20°. The fresh livers and spleens were blended with acetone in an Omni Mixer Homogeneizer (DuPont Instruments/Sorvall, Newton, CT), dried, and stored at −20°. Chondroitin 4-sulfate (from whale cartilage), chondroitin 6-sulfate (from shark cartilage), dermatan sulfate (from pig skin), chondroitinase AC II (EC 4.2.2.5) and chondroitinase ABC (EC 4.2.2.4) were purchased from Miles Laboratories (Elkhart, IN, U.S.A.). Chondroitinase AC and chondroitinase B from *Flavobacterium heparinum* were prepared as previously described[14,19]. 1,3-Diaminopropane was purchased from Aldrich Chemical Co. (Milwaukee, WI, U.S.A.). Trypsin (Type II-crude) from porcine pancreas, papain from *Papaya* latex, and deoxyribonuclease I (crude) from bovine pancreas, were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Superase from Chas. Pfizer & Co. (New York, NY, U.S.A.).

Extraction of sulfated glycosaminoglycans. — A. Potassium acetate method. The glycosaminoglycans were extracted from the dry tissues by the method of Scott

et al.[20], as modified by Toledo and Dietrich[17]. The acetone powder from the tissues was digested with trypsin and then with deoxyribonuclease. After these incubations, anhydrous potassium acetate was added to achieve a 2M concentration and the pH was adjusted to 5.7. After 2 h of incubation at 60°, Celite was added to the mixture, which was filtered at 60°. The filtrate was collected and kept overnight at 4°. The precipitate formed in the cold was collected by centrifugation and 2 volumes of ethanol were added to the supernatant. It has been shown that only heparin precipitates with potassium acetate in the cold, and that all of the other sulfated glycosaminoglycans remain in the heparin-free supernatant[17,21]. The precipitate formed after the addition of ethanol was also collected by centrifugation, dried, and analyzed for sulfated glycosaminoglycans.

B. Alkaline hydrolysis method. Alternatively, the dermatan sulfate was extracted from hog skin by alkaline hydrolysis of the tissue as described by Meyer and Chaffee[1] and purified according to Schiller *et al.*[22].

C. Sequential extraction of glycosaminoglycans from hog liver. The dermatan sulfate was selectively extracted from hog liver by a modification of the potassium acetate method as follows: the dry tissue (1 g) was digested with trypsin and deoxyribonuclease as already described[17,21]. After these incubations, anhydrous potassium acetate was added to the mixture to achieve a concentration of 0.5M, with shaking to ensure complete dissolution of the salt. The pH was adjusted to 4.5 and the suspension was maintained in an oven for 2 h at 60°. Celite was added (~100 mg/g of dry tissue) and the suspension was filtered at 60°. The filtrate was collected in a centrifuge tube and kept overnight at 4°. To the supernatant, clarified by centrifugation in the cold, 2 volumes of ethanol were added. After being kept overnight at 4°, the precipitate formed was collected by centrifugation, washed with 80% ethanol, and dried. It was then dissolved in 100–500 μL of water and analyzed for dermatan sulfate. The residue obtained after filtration at 60° was suspended in water (10 mL/g of initial dry tissue). Anhydrous potassium acetate was added to achieve a 2M concentration, the pH was adjusted to 5.7, and the remaining glycosaminoglycans were extracted according to the potassium acetate method already described. Glycosaminoglycan analysis of the fractions thus obtained showed that the dermatan sulfate was extracted from hog liver by 0.5M potassium acetate at pH 4.5. Heparan sulfate remained in the tissue residue free of dermatan sulfate, and was extracted in the second step by 2M potassium acetate at pH 5.7.

Purification of dermatan sulfate. — A. Agarose-gel electrophoresis. The glycosaminoglycans (150 mg) extracted from the mammalian tissues were purified and fractionated by large-scale agarose-gel electrophoresis in 0.05M 1,3-diaminopropane acetate buffer, pH 9.0, as already described[23]. The fractions containing dermatan sulfate were pooled and concentrated to 2–4 mL. Two volumes of ethanol were added and the precipitate formed after being kept overnight at 4° was collected by centrifugation, washed with 80% ethanol, and vacuum dried.

B. Precipitation with copper in alkaline medium. Alternatively, the hog-liver-dermatan sulfate was precipitated by Cu^{2+} in alkaline medium, as already described

by Cifonelli *et al.*[24], from the mixture of glycosaminoglycans extracted from the tissue by the potassium acetate method. To 1 mg of glycosaminoglycans dissolved in 1 mL of water, 0.8 mL of Benedict reagent[24] and 0.1 mL of 10M NaOH were added. After being kept for 15 min at room temperature with continuous agitation, the precipitate formed was collected by centrifugation, resuspended in 0.5 mL of water, and further purified by the addition of ethanol (2 volumes) in the presence of one drop of 2M NaCl. The precipitate formed after being kept overnight at 4° was collected by centrifugation, washed with 80% ethanol, dried, and analyzed for glycosaminoglycans. To assess the reproducibility and recovery of the method, one artificial mixture of standard dermatan, chondroitin, and heparan sulfates and ten preparations of hog-liver glycosaminoglycans were fractionated. The results showed a recovery of 85% for the dermatan sulfate and also showed that only dermatan sulfate precipitates with Cu^{2+} in alkaline medium. The other glycosaminoglycans remain in solution. Contamination in the dermatan sulfate fraction by other glycosaminoglycans was ~10%.

Identification, quantification, and characterization of glycosaminoglycans. — The glycosaminoglycans were identified and quantified by a combination of agarose-gel electrophoresis and enzymic degradation with specific mucopolysaccharides[25]. Four different buffer systems were used for agarose-gel electrophoresis: 0.06M sodium barbital buffer, pH 9.0 (ref. 26), 0.05M 1,3-diaminopropane acetate buffer, pH 9.0 (ref. 25), 0.05M sodium phosphate buffer, pH 8.0, and a discontinuous barium acetate–1,3-diaminopropane acetate electrophoresis system[27]. The glycosaminoglycan quantitations were performed by densitometry of the agarose-gel slabs after electrophoresis and staining by Toluidine Blue, as previously described[25]. The enzymic degradations with chondroitinases B and AC were performed as described by Michelacci and Dietrich[14]. The products formed were chromatographed in 5:3 (v/v) 2-methylpropanoic acid–M NH_3 and quantitated by densitometry after staining by silver nitrate[28] or, alternatively, made visible with the aid of a u.v. lamp. They were eluted from the paper with water and quantitated either by the absorption at 232 nm or from the hexosamine content after acid hydrolysis. The molecular weights of the glycosaminoglycans and their degradation products were determined by polyacrylamide electrophoresis in gel slabs[28]. Amino sugars were determined after acid hydrolysis (4M HCl for 6 h at 100°) by a modified Elson–Morgan reaction[29]. Uronic acids were determined by a modification of the carbazole method[30] and identified after acid hydrolysis (M HCl for 2 h at 100°) by paper electrophoresis as described by Kosaki and Yosizawa[31]. Total sulfate was measured after acid hydrolysis (8M HCl for 6 h at 100°) by two methods previously described[32,33].

RESULTS

Extraction and fractionation of glycosaminoglycans. — The glycosaminoglycans of hog, rat, and beef liver and spleen, rabbit liver, dog spleen, and hog skin

were extracted and separated from heparin by the "potassium acetate method". Fig. 1 shows the densitometric records of agarose-gel electrophoresis in two different buffer systems of the compounds thus obtained and also of the glycosaminoglycans extracted from hog skin by the "alkaline hydrolysis method". The relative amounts and yields of these compounds are presented in Table I. As already reported[17], mixtures of chondroitin, dermatan, and heparan sulfates were obtained. These glycosaminoglycans occur in most mammalian tissues as minor components. In order to establish a methodology for isolation of dermatan sulfate in high yields, and supposing that, once established the same method could be applied to any tissue, extraction conditions employing different concentrations of potassium acetate at different pH values were used for hog liver. Fig. 2 (IIa) shows that only dermatan sulfate is extracted by incubation of the trypsin-digested tissue with 0.5M KOAc at pH 4.5 for 2 h at 60°. The heparan sulfate remains in the tissue residue and can be extracted in a second step by 2M KOAc at pH 5.7 (Fig. 2-IIb). However, when this method was applied to other tissues, fractions containing mixtures of variable amounts of chondroitin, dermatan, and heparan sulfates were obtained. We were unable to find conditions that could be applied to any tissue to permit selective extraction of dermatan sulfate. In consequence, we were obliged to extract the glycosaminoglycans and then, in a second step, isolate the dermatan sulfate. This isolation was accomplished by large-scale gel electrophoresis[23] in 0.05M 1,3-diaminopropane acetate buffer, pH 9.0 (Fig. 2-Ia). Alternatively, the hog-liver dermatan sulfate (but not the dermatan sulfates extracted from other tissues) could be precipitated with Cu^{2+} in an alkaline medium, as described by Cifonelli *et al.*[24] (Fig. 2-Ib).

Electrophoretic migration and chemical analysis of dermatan sulfates. — The densitometric records of agarose- and polyacrylamide-gel electrophoresis of dermatan sulfates from different origins are shown in Fig. 3. Although small differences in the electrophoretic mobilities of different dermatan sulfates could be observed in agarose gel, all of them migrated as single bands, close to the standard position for dermatan sulfate. However, in polyacrylamide gel, each dermatan sulfate showed a characteristic migration pattern. All of them migrated as polydisperse bands, but whereas the hog-skin dermatan sulfate displayed only one band, the dermatan sulfates extracted from liver and spleen showed two or three bands each, corresponding to different molecular weights (Fig. 3 and Table II).

The dermatan sulfate from hog skin extracted either by the "potassium acetate method" or by the "alkaline hydrolysis method" showed the same migration behavior (Fig. 4).

The dermatan sulfate from hog liver isolated either by the "sequential extraction method" with 0.5M KOAc, pH 4.5, or by extraction with 2M KOAc, pH 5.7, and purification by Cu^{2+} in an alkaline medium, or by large-scale agarose-gel electrophoresis, also presented the same electrophoretic behavior. This result indicates that the appearance of polydisperse bands in polyacrylamide-gel electrophoresis is not a consequence of the extraction procedure (Fig. 4).

TABLE I

GLYCOSAMINOGLYCANS OBTAINED FROM DIFFERENT MAMMALIAN TISSUES

Tissue	*Extraction procedure*	*Glycosaminoglycans*						
		μg/g Dry tissue				*Percent amounts*		
		Total	*CS*	*DS*	*HS*	*CS*	*DS*	*HS*
Hog skin	Alkaline hydrolysis	253	2	48	205[a]		19	81[a]
Hog skin	Potassium acetate	760	2	669	91[b]		88	12[b]
Hog liver	Sequential extraction	105	2	64	41		61	39
Hog liver	Potassium acetate–copper precipitation	100	2	61	39		61	39
Hog liver	Potassium acetate–Agarose-gel fractionation	108	2	67	41		62	38
Hog spleen	Potassium acetate	771	185	370	216	24	48	28
Rat liver	Potassium acetate	267	16	88	163	6	32	62
Rat spleen	Potassium acetate	172	28	84	60	16	49	35
Rabbit liver	Potassium acetate	429	107	124	198	25	29	46
Dog spleen	Potassium acetate	354	42	142	170	12	40	48
Beef liver	Potassium acetate	850	2	425	425		50	50
Beef spleen	Potassium acetate	635	20	171	444	3	27	70

[a]This fraction contains a mixture of heparan sulfate (32 μg/g dry tissue) and heparin (173 μg/g of dry tissue). [b]This fractions contains a mixture of heparan sulfate (38 μg/g dry tissue) and hyaluronic acid (53 μg/g of dry tissue).

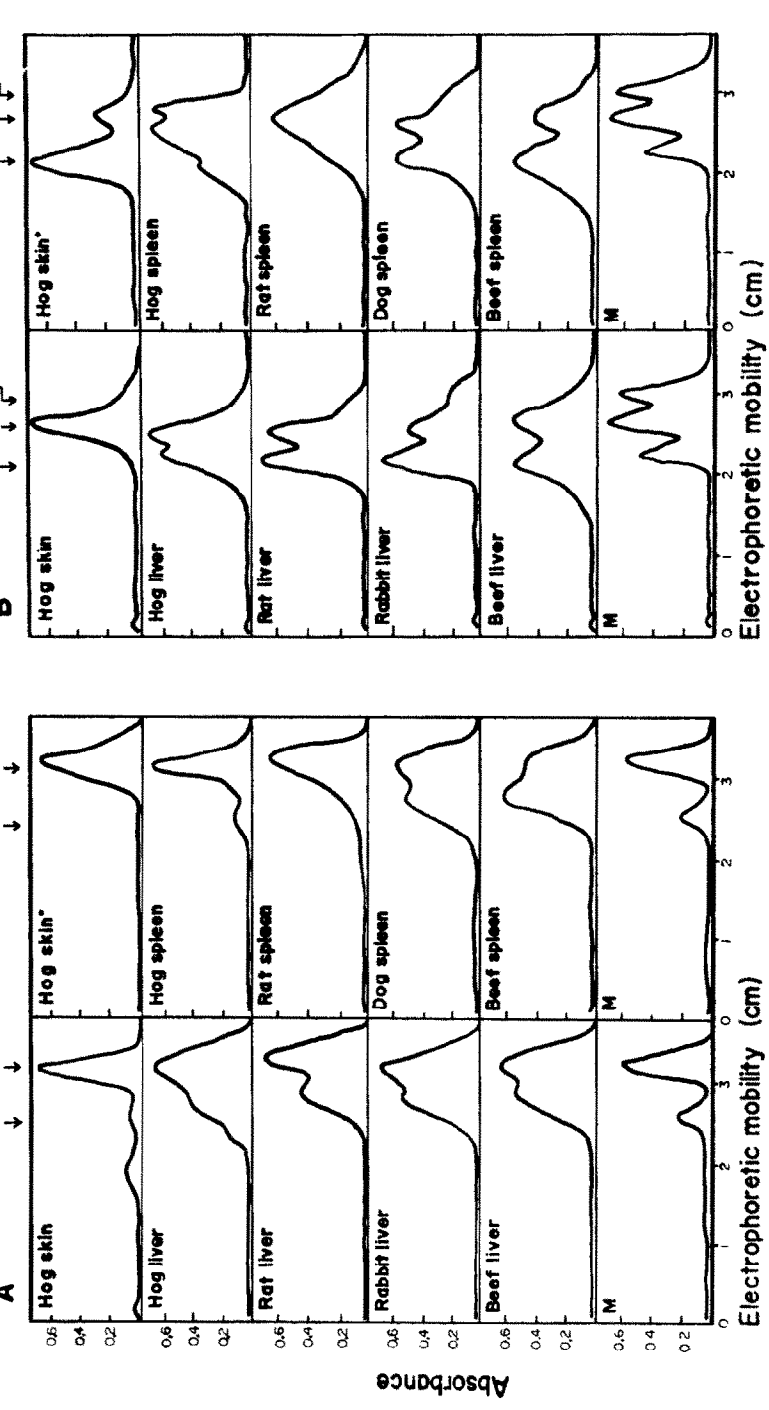

Fig. 1. Densitometric records of the agarose-gel electrophoresis of glycosaminoglycans extracted from some mammalian tissue. The glycosaminoglycans extracted from the tissues by the "potassium acetate method" or from hog skin by the "alkaline hydrolysis method" (Hog skin*) were applied to agarose-gel slabs (7.5 × 7.5 × 0.2 cm). The electrophoresis was performed in two different buffer systems: *A*, 0.05M sodium phosphate buffer pH 8.0; and *B*, 0.05M 1,3-diaminopropane acetate buffer, pH 9.0. M denotes a standard mixture containing heparan sulfate (HS), dermatan sulfate (DS), and chondroitin 4-sulfate (CS); Hep, heparin.

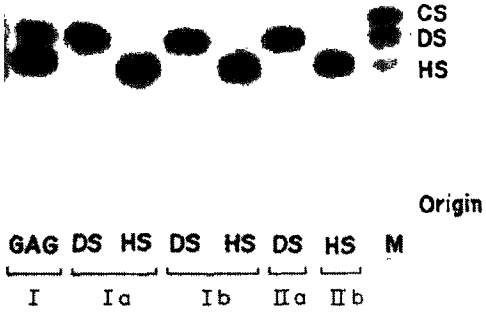

Fig. 2. Agarose-gel electrophoresis of the hog liver glycosaminoglycans prepared by different experimental procedures. Aliquots (10 μg) of the glycosaminoglycans were applied to agarose-gel slabs in 0.05M 1,3-diaminopropane acetate buffer, pH 9.0, and submitted to electrophoresis as already described. I: Glycosaminoglycans extracted by the "potassium acetate method" and fractionated by precipitation with copper in alkaline medium (Ia) or by large scale, agarose-gel electrophoresis (Ib). II: Glycosaminoglycans sequentially extracted by 0.5M potassium acetate at pH 4.5 (IIa) and then, in a second step, by 2M potassium acetate at pH 5.7 (IIb).

In order to test the possibility that the high-molecular-weight bands correspond to dermatan sulfate molecules still attached to peptide chains, treatments by proteolytic enzymes (trypsin, papain, and "Superase") or by sodium borohydride in alkaline medium[24] were performed. Again, no modification was observed in the electrophoretic migration of the polymers.

The results of chemical analysis are shown in Table II. 2-Amino-2-deoxy-D-galactose was the only hexosamine present. Iduronic and glucuronic acids were detected in all of the dermatan sulfates by paper electrophoresis after acid hydrolysis of the polymers.

Enzymic analysis of dermatan sulfates. — To assess the relative position of the glucuronic and iduronic acid residues in the dermatan sulfate chains, enzymic degradation with chondroitinases B and AC were performed. The products formed were detected by paper chromatography and by polyacrylamide-gel electrophoresis. In addition to unsaturated, 4-sulfated disaccharides and unsaturated tetrasaccharides, oligosaccharides having different molecular weights were obtained by the action of chondroitinase B. These compounds were prepared on a larger scale and again incubated with chondroitinase B and also with chondroitinase AC. None of the oligosaccharides were further degraded by chondroitinase B, but they were all degraded by chondroitinase AC, forming unsaturated 4-sulfated disaccharides (ΔDi4S) and variable amounts of unsaturated 6-sulfated disaccharides (ΔDi6S).

Polydisperse oligosaccharides having different molecular weights were also obtained by the action of chondroitinase AC upon the intact polymers, unequivocally showing that all of them are hybrid polymers containing glucuronic and

TABLE II

ANALYTICAL DATA FOR DERMATAN SULFATES OF DIFFERENT ORIGINS

Dermatan sulfate from	*Hexosamine* (μM)	*Uronic acid* (μM)	*Sulfate* (μM)	*Molecular weight* ($\times 10^{-3}$)	
				Modal value	*Range*
Hog skin (alkaline hydrolysis)	1 0	0.61	0 90	13.0	7.0–23.0
Hog skin (potassium acetate)	1.0	0.60	0.88	13.0	7.0–23.0
Hog liver (sequential extraction)	1.0	0.69	1 01	8.5	1.7–20.5
				34 0	20.5–45.0
Hog liver (potassium acetate)	1.0	0.68	1.11	8 5	1.7–20.5
				34 0	20.5–45.0
Hog spleen	1.0	0.97	1.10	2.5	1 1–4.8
				6.5	4 8–12.0
				23.0	12.0–34.0
Rat liver	1.0	0.64	1.02	6 5	2.5–10.0
				14.5	10.0–29 0
Rat spleen	1 0	0.84	1.66	7.0	2.8–15 5
				22.0	15.5–43.0
Rabbit liver	1.0	0.70	1 07	7.0	1.8–10.5
				20.5	10.5–39.5
Dog spleen	1.0	0 75	0.85	5.0	2.0–12.0
				30.5	12.0–43 0
Beef liver	1.0	0.76	1 14	3.0	2.0–4.5
				8 0	4.5–15.5
				34.0	15.5–58.0
Beef spleen	1.0	0.76	1 48	7 0	2.0–19.0
				34.0	19.0–52.0

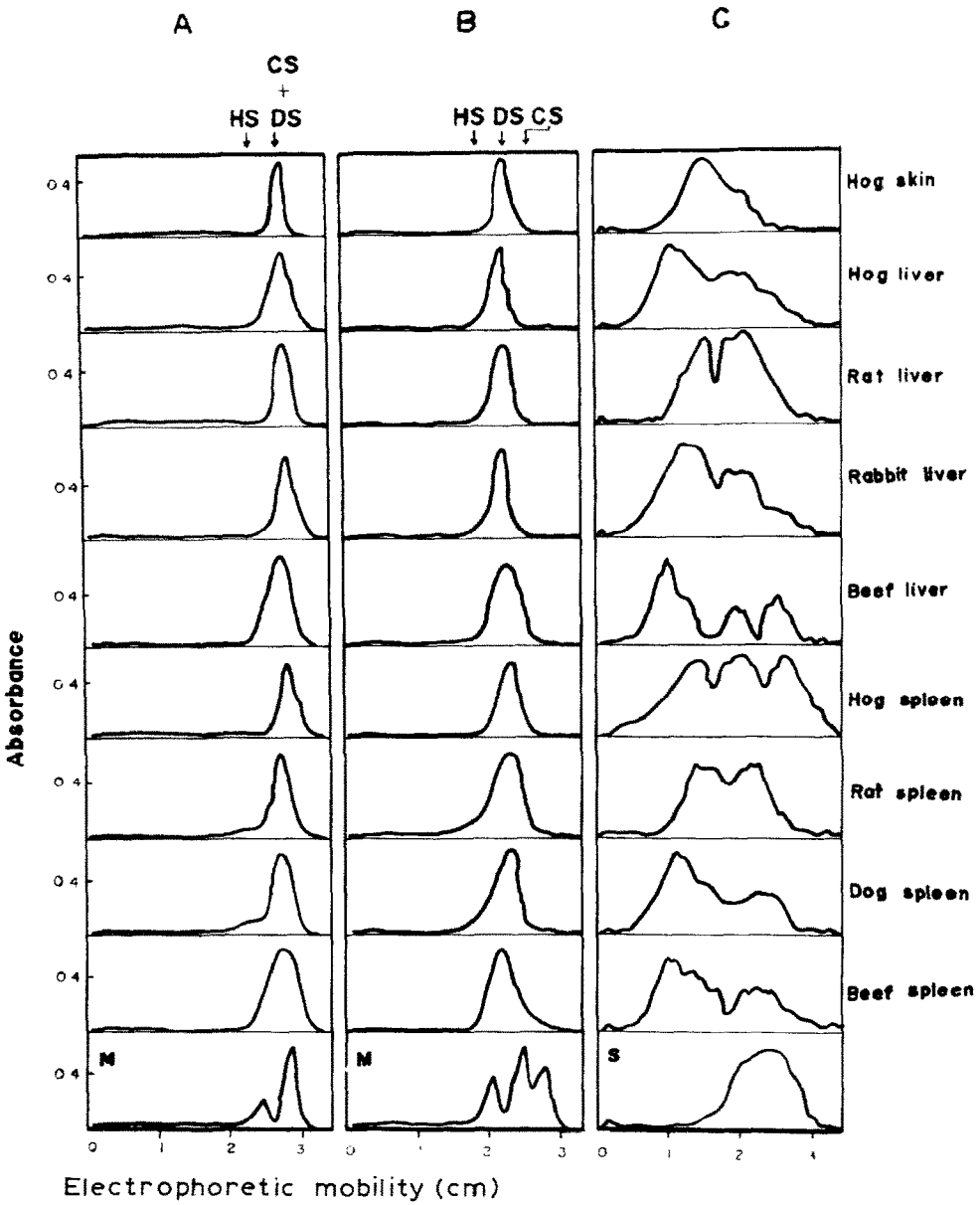

Fig. 3. Agarose- and polyacrylamide-gel electrophoretic migration of dermatan sulfates. Dermatan sulfates (5–10 μg) extracted from the tissues by the "potassium acetate method" and purified by large-scale agarose-gel electrophoresis were applied to agarose- (A and B) or polyacrylamide-gel slabs (C) in phosphate (A), 1,3-diaminopropane acetate (B), or barbital (C) buffer-systems. Electrophoresis was performed as already described.

TABLE III

DEGRADATION PRODUCTS OF THE DERMATAN SULFATES[a]

Dermatan sulfate from	*Degradation products formed by*							*Oligosaccharide resistant to*			
	Chondroitinase B			*Condroitinase AC*				*Chondroitinase B*		*Chondroitinase AC*	
	Oligo (%)	*Tetra (%)*	*ΔDi4S (%)*	*Oligo (%)*	*Tetra (%)*	*ΔDi4S (%)*	*ΔDi6S (%)*	*Modal (× 10^{-3})*	*Range*	*Modal*	*Range*
Hog skin[b]	40	5	55	55	5	40	0	3.0[c]		4.5	1.7–16.5
Hog liver[b]	40	12	48	50	11	33	6	4.5	2.4–7.0	5.0	3.0–29.0
Hog spleen	43	20	37	37	20	28	15	3.2	2.0–6.0	5.2	1.8–16.5
Rat liver	47	16	37	37	16	47	0	2.7[c]		3.0	2.0–20.0
Rat spleen	42	30	28	43	25	32	0	6.0	3.0–7.5	8.5	1.5–18.0
Rabbit liver	39	23	38	39	24	27	10	4.0	2.0–8.0	3.5	2.0–11.0
Dog spleen	38	33	29	33	37	30	0	4.0	1.0–6.0	7.2	2.0–15.5
Beef liver	40	20	40	48	21	23	18	5.6	1.0–6.0	6.3	3.0–15.5
Beef spleen	43	17	40	45	17	23	15	4.5	2.5–6.5	7.0	1.5–10.0

[a]The dermatan sulfates of different origins (1 mg) were incubated with 3×10^{-2} units of chondroitinase B or AC in 0.05M ethylenediamine acetate buffer pH 8.0 in a final volume of 100 μL for 18 h at 20° or 37°, respectively. After incubation, the mixtures were spotted onto Whatman no. 1 paper as 5-cm bands and chromatographed in 2-methylpropanoic acid–M ammonia (5:3, v/v) for 48 h. The products formed were located with the aid of a u.v. lamp and eluted with water. Hexosamine determinations were then performed on the eluates. The relative concentrations of the products are expressed in percent of total hexosamine present in the incubated dermatan sulfates. The molecular weight of the oligosaccharides were measured by polyacrylamide-gel electrophoresis. [b]Dermatan sulfates prepared by the "potassium acetate extraction procedure" and purified by "agarose gel electrophoresis". [c]Monodisperse oligosaccharides.

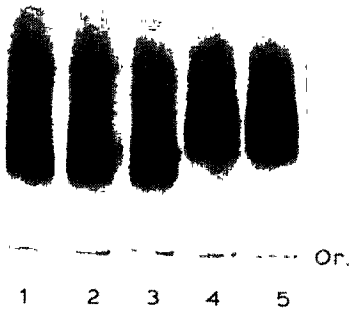

Fig. 4. Polyacrylamide-gel electrophoresis of hog liver and hog skin dermatan sulfates prepared by different methods. Aliquots (10 μg) of the dermatan sulfates were applied to a polyacrylamide-gel slab in 0.06M barbital buffer pH 8.6 and submitted to electrophoresis as already described. 1,2,3. Hog-liver dermatan sulfates extracted by the "potassium acetate method" (1,2) or by the "sequential extraction method" (3) and purified by precipitation with copper in alkaline medium (1) or by large-scale gel-electrophoresis (2). 4,5. Hog-skin dermatan sulfate extracted by the "potassium acetate method" (4) or by the "alkaline hydrolysis method" (5) and purified by large-scale agarose-gel electrophoresis.

iduronic acid residues in the same chain. These oligosaccharides, resistant to the action of chondroitinase AC, were totally degraded to unsaturated, 4-sulfated disaccharides by chondroitinase B. The molecular weights of these oligosaccharides, which constitute clusters of glucuronic or iduronic acid-containing disaccharide units in the intact polymers, are listed in Table III. These data show that not only the molecular weights of the polymers but also the size of the clusters vary from one to another type of dermatan sulfate.

The relative amounts of oligo-, tetra- and di-saccharides formed by the enzymes are also presented in Table III. Different amounts of oligo- and tetrasaccharides accumulated. The only disaccharide formed by chondroitinase B was ΔDi4S. Variable amounts of ΔDi6S were produced by the action of chondroitinase AC from dermatan sulfates of hog and beef liver and spleen, and from rabbit-liver.

DISCUSSION

A systematic study on the fine structure of dermatan sulfates was initiated. In order to investigate variations according to the tissue of origin, the animal species, and the preparation procedures, a total of twelve different preparations of dermatan sulfate was analyzed by chemical, physicochemical, and enzymic methods.

Dermatan sulfates extracted from hog skin by two different methods show the same structure, as do as hog-liver dermatan sulfates prepared by three different procedures. These results indicate that the extraction and purification methods used here did not cause structural changes in the dermatan sulfates.

On the other hand, dermatan sulfates of different origins show unique structures. Enzymic analysis with chondroitinases B and AC show that three types of disaccharide units may be present: type A: 2-acetamido-2-deoxy-3-*O*-(β-D-glucosyluronic acid)-4-*O*-sulfo-D-galactose; type B: 2-acetamido-2-deoxy-3-*O*-(α-L-idosyluronic acid)-4-*O*-sulfo-D-galactose; and type C: 2-acetamido-2-deoxy-3-*O*-(β-D-glucosyluronic acid)-6-*O*-sulfo-D-galactose.

All of the dermatan sulfates are hybrid polymers containing at least two different types of disaccharide unit. Nevertheless, the relative amount and position of these units, as well as the molecular weight of the polymers, is characteristic of each dermatan sulfate. The tetrasaccharides produced by the enzymes constitute the regions of the molecules where the glucuronic acid and iduronic acid-containing disaccharide units alternate along the chains. In contrast, the oligosaccharides correspond to the clusters containing disaccharide units of types A and C or type B. The size of these clusters, as well as the relative amounts of tetrasaccharides, vary from one dermatan sulfate to another. For instance, considering the hog dermatan sulfates here described concerning (*a*) their molecular weights (13,000 for skin, 8,500 and 34,000 for liver, and 2,500, 6,500, and 23,000 for spleen); (*b*) the regions where the glucuronic and iduronic acid-containing disaccharide units alternate along the polymeric chains, represented by the tetrasaccharides produced by the enzymes (5% for skin, 12% for liver, and 20% for spleen), and (*c*) the proportions of 6-sulfated disaccharides (0% for skin, 6% for liver, and 15% for spleen) as well as the relative amounts and size of the clusters, it becomes clear that the number and distribution of different disaccharide units along the molecules vary from one dermatan sulfate to another. Furthermore, considering the dermatan sulfates extracted from liver or spleen of different animal species, the same conclusion may be reached. These results indicate that the relative position of the disaccharide units is individual to each dermatan sulfate.

The dermatan sulfates from liver and spleen here described display two or three different modal molecular-weights. It is unlikely that one of these bands corresponds to a free dermatan sulfate and the other a dermatan sulfate-proteoglycan, as proteolytic enzymes or treatment with alkaline sodium borohydride did not cause any change in the migration patterns. It is possible that these molecular populations constitute self-aggregated molecules. In fact, the self association of dermatan sulfate molecules from pig skin[35,36], beef and human aorta[37], and of dermatan sulfate proteoglycans from bovine sclera[38,39] has already been demonstrated. Nevertheless, the possibility that they do correspond to different structures and are specific to different cell types or subcellular organelles has not been ruled out and is now under investigation.

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