CHARACTERISTIC DISTRIBUTION OF SULFATED MUCOPOLYSACCHARIDES IN DIFFERENT TISSUES AND IN THEIR RESPECTIVE MITOCHONDRIA

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SUMMARY: The distribution of sulfated mucopolysaccharides in different rat tissues and in their respective mitochondria is reported. It is shown that each tissue has a characteristic composition, differing from each other regarding the relative amount, type and molecular size of chondroitin sulfate A/C, chondroitin sulfate B, and heparitin sulfate. It is also shown that the mucopolysaccharide composition of the mitochondria is similar to the composition of the tissue of origin. The possible biological functions of these compounds are discussed in view of this characteristic distribution.

INTRODUCTION: It is now becoming evident that a variety of SMPS[1] are present in all mammalian tissues (1) as well as in stablized cell lines in culture (2-4). Although several studies on the distribution of these compounds in individual tissues have been reported (1,5-7) none has appeared comparing the relative distribution of the mucopolysaccharides in different tissues. It seemed to us that this comparative study could shed some light on the knowledge of the possible biological functions of these compounds. It also seemed important to analyse the SMPS composition of a specific subcellular particle from different tissues in order to verify whether its composition would follow one single pattern in different tissues or the pattern of the tissues of origin. The

1) Abbreviations used are: SMPS, sulfated mucopolysaccharides; $ChSO_4$ A/C, chondroitin sulfate A or C; $ChSO_4$ B, chondroitin sulfate B; Hep SO_4, heparitin sulfate.

recent development of new methods of identification of mucopolysaccharides in relatively impure tissue extracts has overcome the main technical difficulties for such systematic study (8,9).

The present communication reports the type and amount of SMPS extracted from rat tissues and from their respective mitochondria.

MATERIALS AND METHODS: Fractionation of subcellular particles - The methods used for the preparation of mitochondrial and microsomal fractions from rat liver, brain, and kidney were those described by Mahler and Cordes (10), Babitch (11), and Adam and Simpson (12) respectively. The kidney mitochondrial fraction was further purified by isopycnic centrifugation as described for liver mitochondria (10). Succinic dehydrogenase and glucose 6-phosphatase were used as the mitochondria and microsomes markers, respectively, in order to test the degree of purification of the mitochondrial fractions. The succinic dehydrogenase assay indicated that kidney, liver, and brain mitochondrial fractions were respectively 81%, 96% and 75% pure. The glucose 6-phosphatase activity indicated that liver and kidney mitochondrial preparations were slightly contaminated with microsomes.

Extraction of sulfated mucopolysaccharides - Whole tissues of adult albino rats (Wistar strain) and their respective mitochondrial fractions were ground with 10 volumes of acetone. After standing overnight at 4°C the mixture was centrifuged, washed once with acetone and dried under vacuum. The dried material (1 g) was suspended in 20 ml of 0.05M Tris-HCl buffer, pH 8.0. To this suspension 10 mg of trypsin were added. After incubation for 18 hours at 37°C under a layer of toluene, the pH of the solution was brought to 11 by the addition of NaOH and maintained for 8 hours at room temperature. The pH of the mixture was then brought to 6.0 with HCl and centrifuged. To the supernatant solution 2 vol. of alcohol was added. The precipitate formed after 24 hours at 5°C was collected by centrifugation. The precipitate obtained from whole tissue was dissolved in 5 ml of water, and ammonium sulfate added to 90% saturation. After 12 hours the solution was centrifuged and the supernatant dialyzed for 48 hours, dried, and dissolved in 100 µl of water. In some tissues (particularly liver and spleen) the samples were treated with RNAse and DNAse to remove nucleic acids that contaminated these preparations. The precipitates from the mitochondrial fractions were treated with DNAse and RNAse instead of the ammonium sulfate precipitation step, as follows: after the alcohol precipitation step the precipitate was incubated for 18 hours at 30°C in 1 ml of 0.05 M sodium citrate, pH 5.0, with a mixture of DNAse and RNAse (1 mg/g of dry tissue). After incubation the SMPS were precipitated with 2 volumes of alcohol, dried and resuspended in 100 µl of water/g of initial dried particles.

Identification and quantitation of sulfated mucopolysaccharides - The SMPS were identified by a combination of agarose gel electrophoresis and enzymatic degradation with specific mucopolysaccharidases as previously described (8,13).

The agarose gel electrophoresis was also performed in 0.05 M propanediamine-acetate buffer, pH 9.0, instead of barbital buffer (9). When this buffer was used the slides were stained with a solution of 0.01% toluidine blue containing 1% of acetic acid, 50%

of ethanol and 49% of water. The destaining solution contained 1% of acetic acid, 50% of ethanol, and 49% of water.
The quantitation of the mucopolysaccharides was performed in the agarose slides by densitometry after toluidine blue staining. The error of the method was in the order of ± 4.5%. The extinction coefficients of the mucopolysaccharides were calculated using standards of chondroitin sulfate A, chondroitin sulfate B (Miles Lab. Elkhart, Indiana) and heparitin sulfate B (14).
Six successive extractions from two different tissues have shown a 15% variation of the total SMPS extracted although no significant changes in the proportions of the compounds were observed.
Paper chromatography of the enzymatic degradation products were performed in isobutyric acid-1M NH_3, 5:3, v/v. The relative amounts of disaccharide products were quantitated after silver nitrate staining as previously described (10). Molecular weight determinations were performed in polyacrylamide gel electrophoresis (11) after fractionation of the individual mucopolysaccharides by large scale agarose gel electrophoresis in propanediamine-acetate buffer.

RESULTS: Mucopolysaccharide composition of rat tissues - The agarose gel electrophoresis of the crude SMPS extracted from several tissues is shown in Fig. 1. All the tissues examined contain

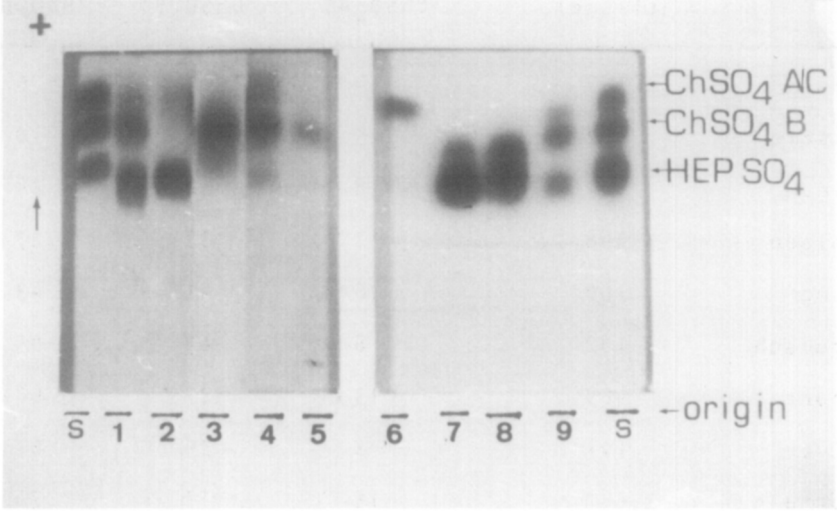

Figure 1. Agarose gel electrophoresis of sulfated mucopolysaccharides obtained from different tissues.

Aliquots of 5 µl were applied in 5 x 7.5 cm (0.2 cm, thick) agarose gel (0.9% agarose in 0.05 M Propanediamine-acetate, pH 9.0) at 1 cm from the negative electrode. The agarose gel was then subjected to electrophoresis for 1 hour at 120 V. The SMPS in the gel were fixed with cetavlon and stained with toluidine blue.
S-mixture of the standards. SMPS extracts from: 1, heart; 2, aorta; 3, spleen; 4, muscle; 5, ileum; 6, brain; 7, kidney; 8, liver; 9, lung.

at least two to three types of mucopolysaccharides. Nevertheless the proportion and type of mucopolysaccharides vary from one tissue to another. For instance heparitin sulfate is the major SMPS in aorta and kidney. Aorta has also small amounts of chondroitin sulfates AC and B while kidney has a small amount of chondroitin sulfate B but no chondroitin sulfate AC. The percent amounts and the types of SMPS found in the different tissues are given in Table I. Heparitin sulfate is present in all the tissues

TABLE I

Distribution of sulfated mucopolysaccharides in rat tissues

TISSUE	TOTAL SMPS (µg/g of dry tissue)	% OF SULFATED MUCOPOLYSACCHARIDES		
		$ChSO_4AC$	$ChSO_4B$	$HepSO_4$
Brain	168	94*	<2	6
Muscle	62	25	65	10
Ileum	187	8	68	24
Spleen	448	11	62	27
Lung	180	16**	41	43
Stomach	182	8	47	45
Heart	465	13	27	60
Liver	121	<2	39	61
Aorta	1,610	15	11	74
Kidney	650	<2	12	88

* Only 4- sulfated disaccharide was detected after degradation with chondroitinase AC.

** 87% of 4- sulfated disaccharide and 13% of 6- sulfated disaccharide were formed after degradation with chondroitinase AC.

examined varying from 6% in brain up to 88% in kidney. Chondroitin sulfate B is detected in all tissues but brain. Again the relative proportions vary substantially, from 11% in aorta to 68% in ileum. Chondroitin sulfate A and or AC were not detected in liver and kidney. The total amount of SMPS in the tissues also varies substantially, from 62 µg/g of dry tissue in muscle up to 1,610 µg/g in aorta.

Average molecular weight of the sulfated mucopolysaccharides from different tissues. According to the tissue of origin the average MW of chondroitin sulfate AC varies from 7,200 in spleen up to 9,500 in aorta, whereas the heparitin sulfate varies from 13,600 in liver up to 160,000 in heart (Table II). Some of the tissues contain two different MW heparitin sulfates (Table II). Differences in MW of chondroitin sulfate B extracted from the different tissues were also observed.

TABLE II

Average molecular weight of the sulfated mucopolysaccharides obtained from different tissues.

TISSUE	AVERAGE MOLECULAR WEIGHT		
	$ChSO_4AC$	$ChSO_4B$	$HepSO_4$
Muscle	9,000	13,500	50,000
Spleen	7,200	24,000	62,000 155,000
Heart	8,700	16,000	60,000 160,000
Liver	-	10,500	13,600
Aorta	9,500	-	39,000
Kidney	-	20,100	22,000

Mucopolysaccharide composition of mitochondria from different tissues. The agarose gel electrophoresis of the SMPS extracted from liver, kidney, and brain and from their respective mitochon-

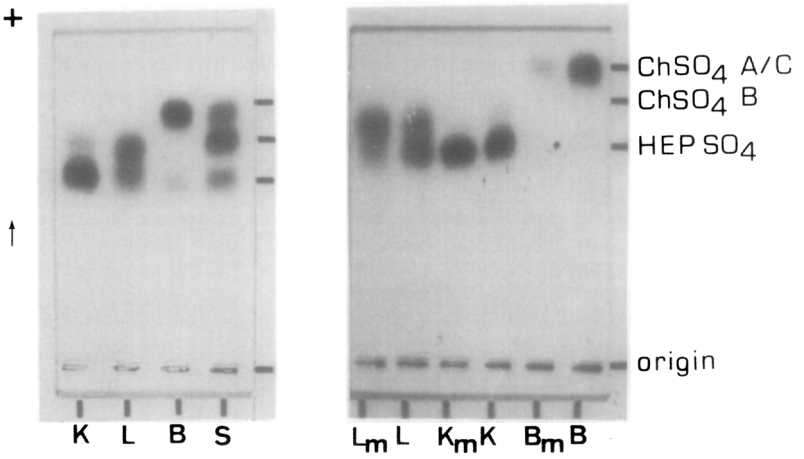

Figure 2. Agarose gel electrophoresis of sulfated mucopolysaccharides from mitochondria of different tissues.

Aliquots of 5 µl of extract (corresponding to about 50 mg of dry tissue) were applied in a 5 x 7.5 cm (0.2 cm - thick) agarose gel slab (0.9% agarose in 0.05 M propanediamine-acetate buffer pH 9.0) at 1 cm from the negative electrode. The agarose gel was then subjected to electrophoresis for 1 hour at 120 V. The SMPS in the gel were fixed with CETAVLON and stained with toluidine blue. S, standard mixture. SMPS extracts from: K, kidney; L, liver; B, brain; L_m, liver mitochondria; K_m, kidney mitochondria; B_m, brain mitochondria.

drial fractions is shown in Fig. 2. The SMPS compositions of the three mitochondrial fractions differ dramatically from one another. Brain mitochondria contain almost exclusively chondroitin sulfate A whereas kidney mitochondria contain heparitin sulfate, and liver mitochondria contain a mixture of chondroitin sulfate B and heparitin sulfate. The mucopolysaccharide composition of these mitochondrial preparations follows closely the composition of their tissues of origin. The percent amounts as well as the total amount of the SMPS in the mitochondrial fractions compared with their tissues of origin are shown in Table III. The percent amounts of the SMPS in mitochondria from kidney and brain are pratically the same as in their respective tissues of origin whereas in liver a comparatively higher amount of chondroitin sulfate B is found in the mitochondria. The amounts of SMPS in brain and kidney mitochondria are similar to the amounts of SMPS in their whole tissues whereas four times as much SMPS are present in liver mitochondria when compared with its respective tissue in a weight basis.

TABLE III

Mucopolysaccharide composition of mitochondria obtained from different tissues.

Tissue	Total SMPS (µg/g of dry tissue)	% of sulfated mucopolysaccharides		
		$ChSO_4A$	$ChSO_4B$	Hep SO_4
Brain				
whole tissue	156	96	< 2	4
mitochondria	120	93	< 2	7
Liver				
whole tissue	111	<2	42	58
mitochondria	580	<2	70	30
Kidney				
whole tissue	668	<2	11	89
mitochondria	510	<2	5	95

It is conceivable that the SMPS of the mitochondrial fractions would actually be derived from the microsomes that contaminate these preparations. In order to test this possibility the SMPS content of the microsomal fraction was analysed. It was found that the SMPS of liver and kidney mitochondria and microsomes were pratically the same in a weight basis thus excluding this possibility for liver and kidney mitochondrial preparations. In brain, the microsomal fraction contained a higher amount of SMPS than the mitochondrial preparation by weight of dried particles. The possibility that the SMPS of brain mitochondria would be derived from a hypothetical microsomal contamination was not excluded. If this were the case brain mitochondria would not contain SMPS at all.

A possible artefact in detecting SMPS in mitochondria would arise if complex formation of these compounds with the particles could occur. To test this, the mitochondrial fraction was extracted with phenol/water without proteolytic digestion. No SMPS was ex-

tracted under these conditions, thus indicating that the compounds are covalently bound to the particles. These combined experiments strongly suggest that the extracted SMPS are indeed derived from the mitochondria.

The finding that the SMPS composition of the mitochondria follows that of the whole tissue helps to discard the possibility that these compounds were derived from other structures present in the whole tissue such as blood vessels, lymphatics, etc. which could mask the true tissue SMPS composition shown in Table I.

Identification of the sulfated mucopolysaccharides. The identification of the SMPS from the tissues and from the mitochondrial fractions were based in the following results: Chondroitin sulfate A/C: Same electrophoretic migration in two buffer systems as the standard chondroitin sulfate A/C; degradation by chondroitinases AC and ABC but not by the heparinase and heparitinases; formation of 4- and 6- sulfated disaccharides after degradation with chondroitinase AC. Chondroitin sulfate B: Same electrophoretic migration in two buffer systems as the standard chondroitin sulfate B; degradation by chondroitinase ABC but not by chondroitinase AC, heparitinases and heparinase; formation of 4-sulfated disaccharide by the action of chondroitinase ABC. Heparitin sulfate: Same electrophoretic migration in two buffer systems as the standard heparitin sulfate; degradation by heparitinases but not by the chondroitinases AC and ABC; formation of glucosamine 2,6 disulfate and N-acetylglucosamine by the action of crude induced F. heparinum extracts.

Besides these properties the three types of SMPS isolated from the tissues and from the mitochondrial fractions were precipitated by cetyltrimethylammonium in the agarose gels and exhibited the characteristic metachromatic colour after toluidine blue staining.

Heparin and keratosulfate were not present in detectable amounts in the tissues and mitochondria examined.

DISCUSSION: The results presented in this communication indicate that SMPS are present in all the tissues and mitochondria examined. Furthermore, the tissues differ from each other regarding the relative amount, type, and molecular size of the SMPS. These results also confirm the data obtained by other authors for the SMPS content of some of the tissues individually examined, such as rat brain (5) and rat kidney (6).

Very little is known about the eventual function of these SMPS. Several workers have shown that heparitin sulfate is located at the cell surface (4,15,16). This, together with other observations led some authors to suggest that this compound is involved in binding, and/or transport of ions and positively charged proteins (7,17). The presence of SMPS in nuclear membranes led other authors to suggest that they might play a role in a variety of nuclear functions such as control of cationic environment, transport selectivity into the nuclei, regulation of nuclease activity, etc. (18). The present finding that all the tissues and mitochondria examined contain different SMPS speaks against a specific function, such as ion transport, regulation of enzyme activity, etc., unless the same specific function could be performed by different SMPS in different tissues and mitochondria.

Since qualitative and quantitative differences of SMPS were found among all the tissues examined and since the mitochondrial SMPS composition follows that of the tissue of origin, one is inclined to suggest that these compounds might be involved in the process of cellular differentiation and/or maturation. Thus the SMPS could confer to each type of cell some of its particular properties, such as cell adhesiveness and recognition, contact inhibition, morphological characteristics, etc. In accordance with these views are the recent findings that virus-transformed cells produce different heparitin sulfates (19); that differences in the type of SMPS were found between various hepatoma cells when compared with normal liver cells (16) and also that heparitin sulfate metabolism is possibly correlated with aggregation after cell division (20).

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