CELL RECOGNITION AND ADHESIVENESS: A POSSIBLE BIOLOGICAL ROLE FOR THE SULFATED MUCOPOLYSACCHARIDES

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SUMMARY: The sulfated mucopolysaccharide composition of different neonate, adult and tumoral tissues is reported. It is shown that each tissue has a characteristic composition with respect to the relative amount, type and molecular size of chondroitin sulfate A/C, chondroitin sulfate B and heparitin sulfate. Neonate and tumor tissues contain large amounts of chondrotin sulfate A/C which is nearly absent in most adult and normal tissues respectively. Based on these and other results a possible role for the sulfated mucopolysaccharides in cell recognition and adhesiveness is proposed.

INTRODUCTION: We have recently reported that rat tissues have characteristic sulfated mucopolysaccharide composition differing in relative amount, type and molecular size of chondroitin sulfate A/C, chondroitin sulfate B and heparitin sulfate (1). It was also recently shown that these compounds are present in variable amounts and types in all 22 species of invertebrates studied belonging to representative phyla of the animal kingdom (2). On the other hand no SMPS[1] could be found in various species of bacteria, protozoa and fungi analysed. These together with other data led to the suggestion that the SMPS might be involved in the process of cell differentiation, conferring on the cells some of their particular properties such as recognition and adhesiveness. If this hypothesis were correct one would expect differences in SMPS composition during development, differentiation and neoplastic transformation. Also, since the recognition process must be specific for each tissue one would also expect to

[1] Abbreviations used are: SMPS, sulfated mucopolysaccharides; ChS A/C, chondroitin sulfate A and/or C; ChS B, chondroitin sulfate B; HTS, heparitin sulfate; HEP, heparin.

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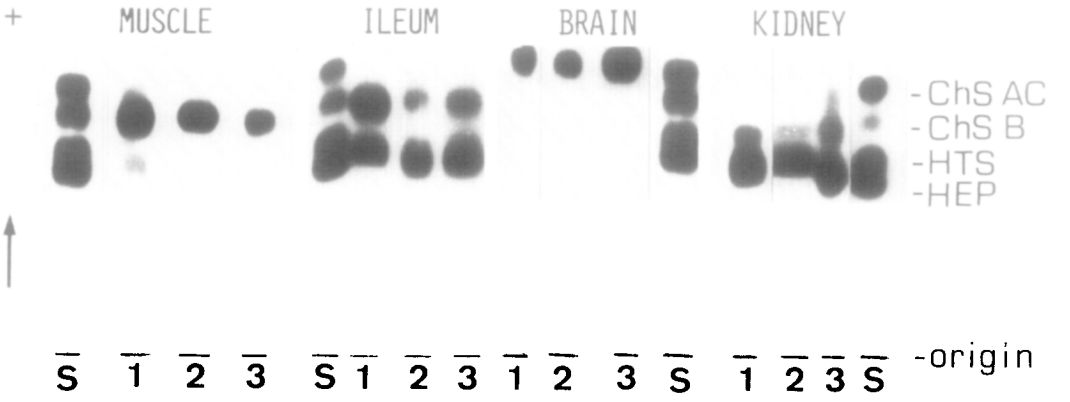

Fig. 1 - Agarose gel electrophoresis of sulfated mucopolysaccharides from selected tissues of some mammalian species.

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 tissue extracts from: 1, guinea-pig; 2, rabbit; 3, human.

find SMPS in all tissues as well as differences in SMPS composition between different tissues. The present communication reports these findings.

MATERIALS AND METHODS: Most of the materials and methods used have been fully described in several recent publications from this laboratory (1-5). Extraction of SMPS from mammalian tissues was made essentially as previously described (1). Slices from human biopsies (tumors and adjacent normal tissues) were removed for histological examination prior to the extraction of SMPS. For these histological studies the materials were stained with hematoxylin-eosin, and with alcian blue for the location of the SMPS.

RESULTS: Sulfated mucopolysaccharide composition of mammalian tissues - The SMPS extracted from muscle, ileum, brain and kidney of guinea pigs, rabbits and humans is shown in Fig. 1. Each tissue contains similar types of SMPS irrespective of the species

TABLE I

Sulfated mucopolysaccharide composition of some mammalian tissues

TISSUE	MAMMAL	TOTAL SMPS (µg/g dry tissue)	SULFATED MUCOPOLYSACCHARIDES (%)		
			ChS AC	ChS B	HTS
Brain	guinea pig	192	83*	<2	17
	rabbit	143	79*	<2	21
	human	385	86*	<2	14
Muscle	guinea pig	572	3	78	20
	rabbit	650	6	78	15
	human	316	14	61	24
Ileum	guinea pig	680	16	29	51
	rabbit	481	10	37	52
	human	299	5	50	45
Kidney	guinea pig	406	<2	22	78
	rabbit	121	12	24	62
	human	150	15	26	59

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

analysed. Also each tissue has a characteristic SMPS composition differing from others in the relative amount and type of chondroitin sulfate A/C, heparitin sulfate and chondroitin sulfate B (Table I). Differences in molecular weight were observed depending upon the tissue and/or species of origin. For example, the average molecular weights of liver heparitin sulfates obtained from guinea-pig, dog, hog, and human were respectively 16,500, 29,500, 11,000 and 5,500. Also the average molecular weights of heparitin sulfates obtained from kidney, lung, ileum, and aorta of humans were respectively 10,500, 13,000, 18,000, and 47,000.

TABLE II

CHANGES OF SULFATED MUCOPOLYSACCHARIDE COMPOSITION OF RAT TISSUES ACCORDING TO AGE

TISSUE	AGE (days)	TOTAL SMPS (µg/g dry tissue)	SULFATED MUCOPOLYSACCHARIDES (%)		
			ChS AC	ChS B	HTS
Liver	1	320	41	36	23
	10	-	11	43	40
	25	-	<2	47	53
	150	225	<2	48	52
Kidney	0	520	23	27	51
	10	-	7	15	78
	25	-	<2	11	89
	150	400	<2	11	89
Brain	0	560	71*	<2	29
	10	-	75	<2	25
	25	-	90	<2	10
	150	565	93**	<2	7

* 70% of 4-sulfated disaccharide and 30% of 6-sulfated disaccharide was formed after degradation with chondroitinase AC.
** Only 4-sulfated disaccharide was detected after degradation with chondroitinase AC.

Other tissues analyzed did also show a characteristic SMPS composition similar to those found in rat tissues (1).

Sulfated mucopolysaccharide composition of tissues from rats of different ages - The SMPS composition of some rat tissues at different ages is shown in Table II. The tissues from 1 day-old rats contain chondroitin sulfate AC (besides other SMPS) which disappears after the first 25 days. Also changes in the relative proportions of chondroitin sulfate B and heparitin sulfate occur in this period. The SMPS composition of the tissues after the first 25 days remained constant up 150 days. Similar changes were observed for

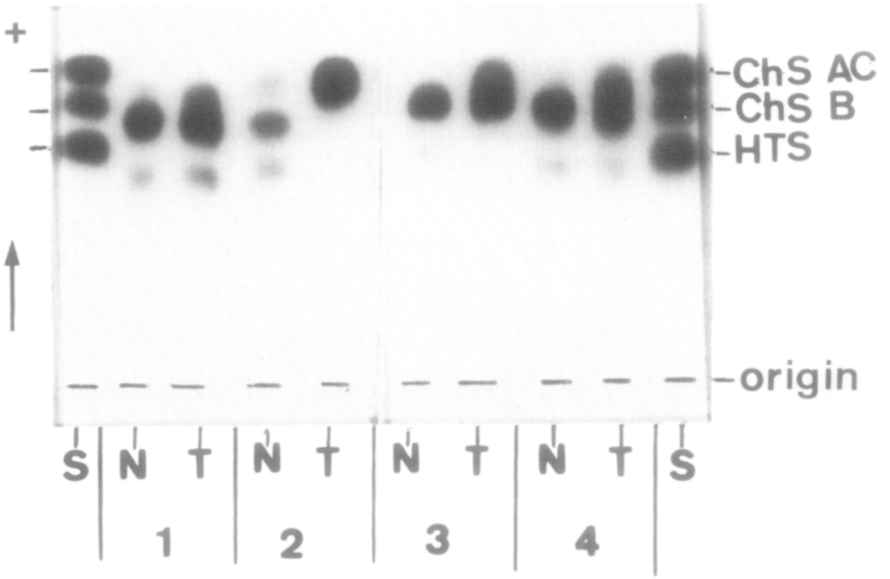

Fig. 2 - Agarose gel electrophoresis of sulfated mucopolysaccharides from some tumors.

The experiment was performed as described in Fig. 1, except that SMPS from different tumors were used.
1, Adenocarcinoma from gastric mucosa; 2, Pleomorphic adenoma from salivary gland; 3, uterine Leiomyoma; 4, Epidermoid carcinoma from Larynx. N, normal adjacent tissue; T, tumoral tissue.

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all the other rat tissues as well as guinea-pig and rabbit tissues (not shown).

Sulfated mucopolysaccharide composition of tumoral tissues - The SMPS composition of four types of solid human tumors compared with the normal adjacent tissues is shown in Fig. 2 and Table III. The most striking observation was the presence of high amounts of chondroitin sulfate A/C in tumors when compared with the apparently normal adjacent tissues. Degradation of this SMPS with chondroitinase AC and quantitation of the disaccharides formed has shown that chondroitin sulfate C accounts for 70% of the total chondroitin sulfate. Changes in the relative proportions of the other SMPS were also observed. Histological examination of the tumor tissues has shown that chondroitin sulfate A/C is present in the intercellular space.

TABLE III

SULFATED MUCOPOLYSACCHARIDES OF TUMORAL TISSUES

TISSUE	TOTAL SMPS (µg/g dry tissue)	SULFATED MUCOPOLYSACCHARIDES (%)		
		ChS AC	ChS B	HTS
Gastric mucosa:				
Adenocarcinoma	5,333	50	41	9
Normal *	3,410	32	51	17
Salivary gland:				
Pleomorphic adenoma	15,296	98	<2	2
Normal	1,488	25	47	29
Uterus:				
Leiomyoma	9,360	74	22	4
Normal	2,105	17	73	10
Larynx:				
Epidermoid carcinoma	3,221	45	43	2
Normal	2,410	11	70	19

* Adjacent "normal" tissue.

Identification of sulfated mucopolysaccharides - The identification of SMPS, reported in the present communication, was based on their electrophoretic migration, precipitation with cetyltrimethylammonium bromide in the agarose slides, characteristic metachromatic color with toluidine blue, susceptibility to enzymatic degradation with specific mucopolysaccharidases as well as by the type of degradation products formed by the action of the enzymes. This has already been described in detail elsewhere (1-5). Small amounts of heparin were present in a few of the mammalian tissues analyzed and will be reported in another communication. No keratosulfate was detected in the mammalian tissues studied.

DISCUSSION: The data reported in this communication as well as in the previous ones (1,2) shows that the SMPS meet most if not all the requirements for a role in the process of cell recognition and/or adhesiveness, as previously suggested (1,2). They appear to be present in all the tissue-organized life forms and show structural differences depending on the tissue or organism of origin. This would confer specificity to the cells. Furthermore SMPS changes occur during differentiation and dedifferentiation.

A role has recently been proposed for heparitin sulfate in cell differentiation (6) based among other data on the conspicuous presence of this compound in the outer cell coats (7) together with the fact that Ca^{++} ions are present also in the outer cell-layer in high amounts (8) and are effective ligands for SMPS. Changes in heparitin sulfate concentration in virus transformation (9) and cell division (10) led to the suggestion that heparitin sulfate would produce negative effects on cell growth by partially blocking the transport of nutrients and ions across the plasma membrane (6). Some changes in concentration of heparitin sulfate were also observed between most of the neonate and adult tissues in the present studies. Nevertheless no correlation could be drawn between the relative amount of heparitin sulfate and mitotic activity, thus casting some doubts on the suggested role for heparitin sulfate (6). For instance, a decrease of this compound was observed in brain when neonate and adult tissues were compared. Also no significant changes in the absolute concentration of heparitin sulfate (calculated from the data shown in Table III) were observed in three out of the four tumors analyzed when compared with the normal adjacent tissues. The results shown in the present paper, on the other hand, suggest that the type and relative proportions of heparitin sulfate and chondroitin sulfate B are the determinants for cell recognition and adhesion, possibly also using Ca^{++} as ligands. An interesting correlation could be observed between neonate and tumor tissues regarding the relative amounts of chondroitin sulfate A/C. Both tissues contain higher amounts of this chondroitin when compared with the respective adult tissues and the normal tissues adjacent to tumors, and both are in an exponential phase of growth. This together with the growth promoting activity of chondroitin sulfate C (11) suggests that this compound might play a role in the stimulation of cell division. It is altogether possible that the recognition/adhesion phenomena might be also related with the

regulation of cell division, as it has been suggested (12). If the SMPS are indeed involved in both phenomena, the stimulation of cell division by chondroitin sulfate AC could be explained as a disruption of the recognition sites by competition with Ca^{++} and the specific SMPS located in the outer cell coat. This is supported by the finding that in tumors chondroitin sulfate AC is present in the intercellular space.

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