The Influence of the Type of Sulphate Bond and Degree of Sulphation of Glycosaminoglycans on their Interaction with Lysosomal Enzymes

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Significant differences occur between the interaction of several sulphated glycosaminoglycans with a particular lysosomal protein, leading to inhibition in the case of lysosomal enzymes. The order of strength of inhibition at pH4 was: heparin > chondroitin 4-sulphate = chondroitin 6-sulphate > dermatan sulphate.

Previous papers have demonstrated that all of the leucocytic lysosomal enzymes studied are inhibited *in vitro* to a different degree by sulphated glycosaminoglycans in a time- and temperature-independent and pH-dependent manner (Avila & Convit, 1973, 1976). Additional experiments revealed strong differences in the physicochemical characteristics of the interaction existing between several sulphated glycosaminoglycans with regard to the same protein.

As Gelman & Blackwell (1974) have proposed that the interaction between glycosaminoglycans and cationic polypeptides depends on (a) the length of the polypeptide side chain, (b) the position of the carboxyl groups, (c) the glycosidic linkages, (d) the position of the sulphate groups (a stronger interaction occurs when the sulphate is on the C-6 side chain, as in heparin, than when directly on the galactose ring, as in chondroitin 4-sulphate) and (e) the degree of sulphation, I decided to study, using pure glycosaminoglycan preparations, the influence of factors (d) and (e) on the glycosaminoglycan-lysosomal enzyme interaction *in vitro*.

Materials and Methods

All materials used were obtained as stated previously (Avila & Convit, 1975, 1976). Chondroitin 4-sulphate (from whale cartilage, lot 4702; mol.wt. 50000), chondroitin 6-sulphate (from shark cartilage, lot S5601; mol.wt. 64000) and dermatan sulphate (from pig skin, lot S4201; mol.wt. 70000) were from Seikagaku Kogyo Co., Tokyo, Japan. All three preparations have a degree of sulphation of about 1.0 sulphate group/disaccharide unit (compared with 2.6 in heparin, including 1.05 *N*-sulphate groups) and were checked for purity by the electrophoretic technique of Taniguchi *et al.* (1974). Molecular weights were estimated by elution volume from a Sephadex G-200 column (Wasteson, 1971).

The standard conditions for the study of the

influence of sulphated glycosaminoglycan on enzyme activities were exactly as reported previously (Avila & Convit, 1976).

Results and Discussion

Table 1 shows that, when assayed at pH4.0, the various sulphated glycosaminoglycans tested strongly inhibited the 14 acid hydrolases measured, as reported previously (Avila & Convit, 1973, 1975). However, of particular interest were the strong differences existing in the degree of inhibition obtained, under the same experimental conditions, for a given sulphated glycosaminoglycan on the same lysosomal enzyme. These characteristic responses can group the several leucocytic lysosomal enzymes as follows: (1) activities that are more strongly inhibited by chondroitin 6-sulphate than by chondroitin 4-sulphate (α -mannosidase, α -glucosidase, β -galactosidase, *N*-acetyl- β -glucosaminidase and *N*-acetyl- β -galactosaminidase); (2) activities similarly inhibited in the presence of chondroitin 4- or 6-sulphate (β -glucuronidase, acid deoxyribonuclease, cathepsin B and α -galactosidase); (3) activities that are more strongly inhibited by chondroitin 4-sulphate than by chondroitin 6-sulphate (cathepsin C, α -fucosidase, β -glucuronidase and β -glucosidase); (4) activities very strongly inhibited by heparin (α -galactosidase, β -galactosidase, α -glucosidase, cathepsin B, *N*-acetyl- β -glucosaminidase and *N*-acetyl- β -galactosaminidase); (5) activities equally inhibited by chondroitin 4- or 6-sulphate and by dermatan sulphate (α -galactosidase, cathepsin B and acid deoxyribonuclease); (6) activities similarly inhibited by heparin and chondroitin 4-sulphate (α -mannosidase and β -glucuronidase); (7) activity poorly inhibited by all sulphated glycosaminoglycans tested (acid β -glycerolphosphatase).

Table 1 also shows that in all cases the degree of inhibition obtained at pH4.0 was much less at

Table 1. *Comparative study of the degree of glycosaminoglycan-induced inhibition of leucocytic lysosomal enzyme activities*

Highly purified lysosomal fractions obtained from human polymorphonuclear leucocytes and containing about 45–90 µg of total protein/ml were preincubated for 15 min at 37°C in 50 mM-sodium acetate/acetic acid buffer in the pH range 3.6–5.8 at a glycosaminoglycan/lysosomal-protein ratio of about 2:1. The enzymic reactions were always initiated by adding the respective substrate to the incubation mixture. Incubations were in all cases for 6 h. Values obtained only at pH 4.0 and 5.0 are shown and are expressed as percentages of the activity obtained with the same preparation treated as described, except that exogenous glycosaminoglycan was absent, and they are means for three different experiments, carried out with separate lysosomal preparations.

Enzyme	pH	...	Activity (% of control) in the presence of:							
			Heparin		Chondroitin 4-sulphate		Dermatan sulphate		Chondroitin 6-sulphate	
			4.0	5.0	4.0	5.0	4.0	5.0	4.0	5.0
β -Glucuronidase (EC 3.2.1.31)			21	72	16	99	21	94	33	112
α -Mannosidase (EC 3.2.1.24)			30	106	36	103	57	101	18	107
Acid β -glycerophosphatase (EC 3.1.3.2)			91	108	76	114	74	117	76	97
α -Glucosidase (EC 3.2.1.20)			7	93	45	112	29	91	11	105
β -Galactosidase (EC 3.2.1.23)			5	70	39	103	29	119	14	89
<i>N</i> -Acetyl- β -glucosaminidase (EC 3.2.1.30)			5	64	48	84	32	98	27	109
Cathepsin C (EC 3.4.14.1)			31	50	20	70	22	74	29	81
α -Galactosidase (EC 3.2.1.22)			6	40	22	106	28	112	27	91
Cathepsin B (EC 3.4.22.1)			10	66	20	80	27	97	20	82
α -Fucosidase (EC 3.2.1.51)			68	70	41	90	52	80	77	92
α -Arabinase (EC 3.2.1.-)			45	90	14	82	15	87	22	108
β -Glucosidase (EC 3.2.1.21)			46	86	10	86	37	87	37	100
<i>N</i> -Acetyl- β -galactosaminidase (EC 3.2.1.53)			2	47	20	77	46	81	12	90
Deoxyribonuclease II (EC 3.1.4.6)			13	62	23	90	28	82	20	93

pH 5.0, with a general tendency for enzyme activities to reach control values at about this latter pH. However, it is noteworthy that at pH 5.0, with very few exceptions, the enzymes studied remained inhibited in the presence of heparin, as noted previously (Avila & Convit, 1975). This is in opposition to the results obtained in the presence of the three other sulphated glycosaminoglycans, which showed no difference between them with regard to the same acid hydrolase.

It is then evident that, for the glycosaminoglycan-lysosomal-enzyme interaction, heparin (with *O*-sulphate and *N*-sulphate bonds) is more active than the other sulphated glycosaminoglycans (with only *O*-sulphate bonds), that chondroitin 4-sulphate and chondroitin 6-sulphate are equally active and that dermatan sulphate (with 4-*O*-sulphate groups) is less inhibitory than chondroitin 4-sulphate. These results do not fit the scheme of strength of interaction proposed for cationic polypeptides by Gelman & Blackwell (1973, 1974). It seems that, rather than the position of the *O*-sulphate groups in the mucopolysaccharide molecule, the important factors controlling the glycosaminoglycan-lysosomal-enzyme interaction are the degree of sulphation per disaccharide unit, the type of sulphate bond involved (*N*-sulphate groups interacting more strongly) and perhaps the sulphate-group distribution along the glycosamino-

glycan molecule, heterogeneity having been confirmed by Suzuki (1960) and Robinson & Dorfman (1969).

Our present results, however, do not discard any additional role of the carboxyl groups in the glycosaminoglycan-lysosomal-enzyme interaction, although previous results have already shown a weak inhibitory effect of the carboxyl groups of the hyaluronic acid on this interaction (Avila & Convit, 1975).

All these results put together, plus the strong inhibition obtained with the chondroitin 4-sulphate preparation, give further evidence for a role for this leucocytic lysosomal glycosaminoglycan as a regulator molecule of intragranular digestive activity, as suggested previously (Avila & Convit, 1975, 1976; Avila, 1977). On the other hand, the different degrees of inhibition obtained for a given enzyme in the presence of specific sulphated glycosaminoglycans (e.g. *N*-acetyl- β -galactosaminidase activity) could explain the variable residual activities found for several lysosomal enzymes in mucopolysaccharidoses (Van Hoof & Hers, 1968; Van Hoof, 1972; Kint *et al.*, 1973).

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