COMPLEXES OF SULFOGYLCOSAMINOGLYCANS WITH HEXAMMINECOBALT (III)

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The hexamminecobalt (III) cation forms complexes with protein-chondroitin-4-sulfate and heparin in dilute solutions, interacting with these biopolymers with all three of its valencies. The conditions disturbing the results of determinations of the total content of anionic groups in sulfoglycosaminoglycans on the basis of the quantity of hexamminecobalt (III) bound with them were established.

KEY WORDS: sulfoglycosaminoglycans; polysaccharides; heparin; protein-chondroitin-4-sulfate; hexamminecobalt (III).

The total content of anionic groups in sulfoglycosaminoglycans can be determined [7] and these biopolymers separated quantitatively from other glycosaminoglycans [1, 5] with the aid of hexamminecobalt (III) chloride. In view of the considerable but insufficiently explored opportunities for the use of hexamminecobalt (III) chloride in the analytical and preparative chemistry of the glycosaminoglycans, a more detailed investigation is required of the correlation between the content of total anionic groups in different sulfoglycosaminoglycans and the quantity of hexamminecobalt (III) bound with them.

In this investigation complexes of hexamminecobalt (III) with various preparations of protein-chondroitin-4-sulfate (PC4S) and heparin were studied.

EXPERIMENTAL

PC4S was isolated from the cartilage of bovine tracheal rings [6]. Fractionation and purification of the various preparations of this biopolymer were not carried to the same degree of homogeneity, in accordance with the aims of the investigation. Individual fractions were isolated from a preparation of heparin (Spofa, Czechoslovakia). Nitrogen (micro-Kjeldahl), amino sugars [2], sulfate [3, 4], and hexuronic (a slightly modified carbazole method [9]) and sialic [11] acids in the preparations were estimated quantitatively.

Complexes of hexamminecobalt (III) and glycosaminoglycans were obtained by slow (with stirring) addition of a solution of hexamminecobalt (III) chloride containing double the amount of this salt (for the weight of glycosaminoglycan taken) to a dilute solution of the biopolymer. After 18-20 h at 20°C the complex was harvested by centrifuging (200 g, 4°C), washed with water to remove chloride ions, then with 80-96% ethanol (three times) and ether, and then dried in vacuo over $CaCl_2$ and paraffin, and then over P_2O_5 [6].

The content of hexamminecobalt (III) in the complexes was determined photometrically at 447 nm (SF-4A spectrophotometer). The solution of the complex (10 mg in 10 ml 2 M NaCl) was centrifuged (15,000 g, 4°C, 10 min) before photometry. A standard graph was plotted for a solution of hexamminecobalt (III) chloride over the range from 0.8 to 6.0 mmoles hexamminecobalt (III) in 1 ml 2 M NaCl [7].

All the anionic groups in the biopolymers tested are monovalent, so that A equivalents of anionic groups $\times 1^{-1}$ g glycosaminoglycan anion = $3M/(1 - \alpha)$, where M is the number of moles of hexamminecobalt (III) in 1 g of the complex and $\alpha = (3M \cdot m_i)/(n \cdot b)$ g of base displaced by hexamminecobalt (III) from 1 g of the original salt of the preparation (m_i is the atomic or molecular weight of the given base, n its valence, and b the weight of the sample of the preparation taken to prepare the complex, in grams). The content of

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TABLE 1. Results of Analysis of PC4S and Heparin Preparations

Glycosaminoglycan	Preparation	Results of analysis per gram glycosaminoglycan anion													
		nitrogen	amino sugar[1] (A)	sulfate (B)	glucuronic acid[2] (C)	sialic acid	B+C+D (E)	anion group total (F)	$\frac{B}{A}$	$\frac{C}{A}$	$\frac{E}{F}\times 100$	F−E	F−B	$\frac{F-B}{A}$
		mmoles						μeq				mmoles		
PC4S	I	3.43	1.36	1.36	1.49	0.04	2.89	3.09	1.00	1.09	94	0.20	—	—
	II	4.14	1.49	1.34	1.60	0.03	2.97	3.12	0.90	1.07	95	0.15	—	—
	III	3.76	1.50	1.30	1.53	0.04	2.83	3.00	0.87	1.02	95	0.13	—	—
	IV	2.54	1.38	1.40	1.78	0.04	3.22	3.24	1.01	1.29	99	0.02	—	—
	V	3.94	1.39	1.40	1.83	0.03	3.26	3.33	1.01	1.31	98	0.07	—	—
Heparin	IHP	1.04	1.41	4.19	—	—	—	5.64	2.97	—	—	—	1.45	1.03
	IIHP	1.20	1.37	4.51	—	—	—	5.94	3.29	—	—	—	1.43	1.04
	IIIHP	1.16	1.41	4.58	—	—	—	6.09	3.25	—	—	—	1.51	1.07
	IVHP	1.19	1.41	4.55	—	—	—	6.09	3.17	—	—	—	1.54	1.09
	VHP	1.23	1.32	5.93	—	—	—	6.57	4.04	—	—	—	1.24	0.94
	VIHP	1.17	1.32	5.17	—	—	—	6.60	3.92	—	—	—	1.43	1.08
	VIIHP	1.19	1.32	5.00	—	—	—	6.42	3.79	—	—	—	1.42	1.07
	VIIIHP	2.10	1.36	4.45	—	—	—	6.15	3.27	—	—	—	1.70	1.25
	IXHP	1.65	1.37	4.82	—	—	—	6.36	3.52	—	—	—	1.54	1.12

[1]N-acetylgalactosamine in PC4S and glucosamine in heparin.
[2]Hexuronic acids in heparin were not determined because the method is unsuitable for this particular biopolymer [8].

structural components in the preparations was calculated by the equation: B moles of component $\times 1^{-1}$g glycoglycan anion = $C/[(1-a)\cdot m_2]$ where C is the quantity of the given structural component in g/g of the initial salt of the preparation, and m_2 the molecular weight of the residue of this component in the biopolymer.

RESULTS AND DISCUSSION

The preparations of PC4S studied differed in their content of nitrogen, amino sugar, and glucuronic, sulfuric, and sialic acids and also in the total content of all anionic groups determined from the amount of hexamminecobalt (III) bound with them (Table 1). The ratio between the sulfate content to that of N-acetylgalactosamine was 1 or a little under (preparations II and III). The ratio between the content of glucuronic acid and this particular amino sugar in preparations I-III was 1 and much higher in preparations IV and V. The total content of anionic groups in preparations I-III was 0.13-0.20 mmole greater than the total of the values obtained by separate determinations of the residues of sulfuric, glucuronic, and sialic acids. In the preparations IV and V, however, it was practically equal to this total (Table 1, F-E). The content of carboxyl groups in preparations I-III was thus a little higher than that corresponding to their content of glucuronic and sialic acids. With a comparatively high nitrogen (and protein, correspondingly) content in these preparations, the total of the residues of sulfuric, glucuronic, and sialic acids accounts for 94-95% of the total content of anionic groups of the macromolecules in these preparations. When the nitrogen content was lower (in preparation IV) this total was practically 100% of their content. The difference observed was evidently due to the presence of a protein containing a high proportion of dicarboxylic amino acids [4], linked by covalent bonds with the polysaccharide component of the PC4S macromolecule.

In preparation V, in which the nitrogen content was relatively high, only 2% of the total content of anionic groups was accounted for by carboxyl groups of the protein component. This preparation was probably contaminated with a protein, linked by electrovalent bonds, replacing hexamminecobalt (III) during the formation of the complex. This conclusion is in agreement with the fact that the ratio between the contents of glucuronic acid and the amino sugar in preparation V was the same as in preparation IV, which contained only 2.54% of nitrogen.

It can be concluded from these results that 5-6% of the total content of anionic groups of PC4S may be accounted for by carboxyl groups of the protein components of this glycosaminoglycan, linked by covalent bonds.

For each residue of amino sugar in the heparin preparations IHP-IVHP there were three, and in the preparations VHP-VIIHP, there were four sulfuric acid residues. The first differed from the second in having a somewhat higher percentage of amino sugar and of hexuronic acids. The ratio of the difference between the total content of anionic groups and the sulfate content to the glucosamine content was practically equal to 1 in all the heparin preparations except VIIIHP and IXHP (Table 1). Since the repeating disaccharide

structures of heparin consist of glucosamine and hexuronic acid residues and they differ in the number of sulfate groups [10], the difference between the total content of all anionic groups and the sulfate content in chemically homogeneous fractions of this biopolymer corresponds to their content of hexuronic acids (Table 1, F-B). Consequently, the heparin preparations studied, except VIIIHP and IXHP, were chemically individual fractions and the difference mentioned above corresponded exactly to their content of hexuronic acids. The quantity of protein linked to polysaccharide by covalent bonds in the highly purified heparin fractions was very small and the binding of hexamminecobalt (III) by the carboxyl groups of the amino acids was beyond the limit of sensitivity of the method. In nonhomogeneous preparations of VIIIHP and IXHP the ratio of the difference between the total content of anionic groups and the sulfate content to the amino sugar content was greater than 1, a result evidently accounted for by the relatively high percentage of nitrogen (protein) in them and, correspondingly, by the additional binding of hexamminecobalt (III) by carboxyl groups of the amino acids.

In homogeneous preparations of PC4S and heparin the total number of equivalents of acid residues calculated from the results of separate determinations of these components thus accounted for 94-100% of the total content of anionic groups determined from the quantity of hexamminecobalt (III) bound with them. This proves that hexamminecobalt (III) reacts with sulfoglycosaminoglycans in very dilute solutions completely with all three valencies. The probability that a certain part of this base reacts with these polyanions with one or two valencies is extremely small because of the exceptionally large number of anionic groups arranged linearly and uniformly in the macromolecules of these biopolymers. The scale of these interactions is beyond the limits of sensitivity of the methods available for analysis of the glycosaminoglycans.

The most important sources of error in the determination of the total content of anionic groups in sulfoglycosaminoglycans with the aid of hexamminecobalt (III) chloride may be: the presence of a protein linked by electrovalent bonds in the preparation, inadequate dilution of the original solution of the biopolymer, and the possibility that the complex may decompose on drying. The use of hexamminecobalt (III) chloride for the determination of hexuronic acids from the difference between the total content of anionic groups and the sulfuric acid content provides sufficiently accurate values if these acids alone are contained in the glycosaminoglycans. If, however, the preparations contain other acids besides sulfuric and hexuronic, the total content of the anionic groups, itself an important characteristic of sulfoglycosaminoglycans, can be used as a control when the content of the individual acid residues in these biopolymers is determined separately. From the content of hexamminecobalt (III) in the complexes, it is easy to calculate (see the first two equations) the content of the base in the original salt of the sulfoglycosaminoglycan, and this eliminates the need for determining the ash content of the preparation.

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