A CHONDROITIN SULPHATE-PROTEIN IN KURLOFF CELLS FROM GUINEA PIG SPLEENS

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1. Introduction

Kurloff cell inclusion bodies have been identified in the lymphocyte population of both male and female guinea pigs [1] and have been shown to increase greatly in number during pregnancy or on administration of oestrogens. Histochemical methods employed by Marshall and Swettenham [2] showed that these inclusion bodies were mainly composed of sulphated polysaccharides, while further chemical analysis by Muir and Marshall [3] confirmed that this material was similar in chemical composition and electrophoretic mobility to chondroitin sulphate, was digested by testicular hyaluronidase but contained no detectable linked amino acids. Further investigation of this material was therefore undertaken, with particular emphasis on establishing the presence or absence of linked amino acids, in view of the possibility that Kurloff inclusion bodies were analogous to those in pathological conditions such as Hurler's syndrome.

2. Experimental

Adult male and female guinea pigs of mixed varieties were injected intramuscularly with 5 mg (0.5 ml) of an aqueous suspension of oestradiol monobenzoate (CIBA Laboratories, Horsham), followed by a second injection of 2 mg 21 days later. The animals were killed at 35 days, and the spleens removed and stored at -20°. Material was pulverized in a steel die after freezing in liquid nitrogen and the proteoglycans extracted by repeated homogenisation in 0.15 M sodium acetate, pH 6.8, at 4°. They were purified by precipitating twice with 9-aminoacridine hydrochloride and converting to the soluble sodium salt [4] with Dowex-50W 8% cross-linked (Na^+ form). Finally, the material was precipitated with ethanol containing sodium acetate, washed with ethanol, acetone and dried.

Nucleic acids were removed by redissolving in phosphate buffer and digesting with ribonuclease and deoxyribonuclease [5]. The material was then re-precipitated with cetyltrimethyl ammonium bromide (CTAB) on a cellulose column (38 × 2 cm) (Whatman Chromedia CF11), washed with 1% (w/v) CTAB containing 0.005 M sodium sulphate, and the purified proteoglycans eluted with 0.75 M magnesium chloride. Column chromatography was carried out using Sephadex G-200 (Pharmacia Ltd.) on a column 0.9 × 100 cm and 6% agarose (a gift from Dr. C.P.Tsiganos) on a column 0.9 × 45 cm. Samples were applied and eluted in water.

Solubility profiles of the proteoglycan-cetylpyridinium chloride (CPC) complexes and of the glycosaminoglycans (GAG) obtained after digestion with crystalline papain [5] (EC 3.4.4.10, British Drug Houses, Poole, Dorset) were determined using the method of Antonopoulos et al. [6]. The amounts of hexose [7], uronic acid [8], pentose [9], hexosamine [4] and amino acids [4] were determined in both the proteoglycan and free glycosaminoglycan. Normal and Kurloff-acetone dried spleens were digested with papain, the GAG isolated [4], and total amounts estimated [8]. The GAG were fractioned on Dowex-1 (Cl^- form) [10] and the molar ratios of GalN to GlcN determined in each fraction [11].

3. Results and discussion

The total GAG content based on dry weight of Kurloff spleens was four times that of control spleens. The major component of control spleens, which was eluted from Dowex −1 with 1.0 M NaCl, contained only GlcN and had an electrophoretic mobility similar to that of heparan sulphate from aorta. The other fraction eluted with 1.3 M NaCl contained both hexosamines (GalN to GlcN of 1:1.5). Kurloff spleen material on the other hand separated into 3 fractions, which were eluted with 1.0 M, 1.3 M and 1.5 M NaCl. Each fraction contained predominantly GalN with molar ratios of GalN to GlcN of 40:1, 150:1 and 80:1 respectively. Thus the increase in the GAG content of the spleens was due to an increase in chondroitin sulphate only.

After nuclease digestion and purification on a CTAB-cellulose column the isolated proteoglycans were eluted from Sephadex G-200 with the void volume, suggesting that the GAG were part of larger proteoglycans. Furthermore, the elution profile from 6% agarose, where a single component was again eluted with the void volume, showed that these compounds were of considerable size and were larger than some of the smaller proteoglycans of pig laryngeal [11] or articular cartilage [12] and larger than an intracellular chondroitin sulphate-protein of human granulocytes [13], which was partially retarded on Sephadex G-200. The intracellular material of cultured Hurler fibroblasts was also of smaller size since it was retarded on G-200 [14].

The solubility profile on elution from micro CPC-cellulose columns showed that two proteoglycan components were present (fig. 1), 41% of the material recovered being eluted in 0.5 M $MgCl_2$ and 55% in 0.6 M $MgCl_2$. After digesting with papain (fig. 2), three components were separated, being eluted in 0.4 M, 0.45 M, 0.5 M $MgCl_2$ and constituting 22%, 41% and 30% respectively of the material. The preponderance of material with a high critical electrolyte concentration is indicative of long GAG chains.

Amino acid analyses after hydrolysis [4,11] showed that serine was the major constituent, followed by glycine and glutamic acid in almost equimolar amounts (see table 1). Total protein was calculated to be 4% by weight of the proteoglycan, while hexosamine accounted for 37.5%, hexuronic

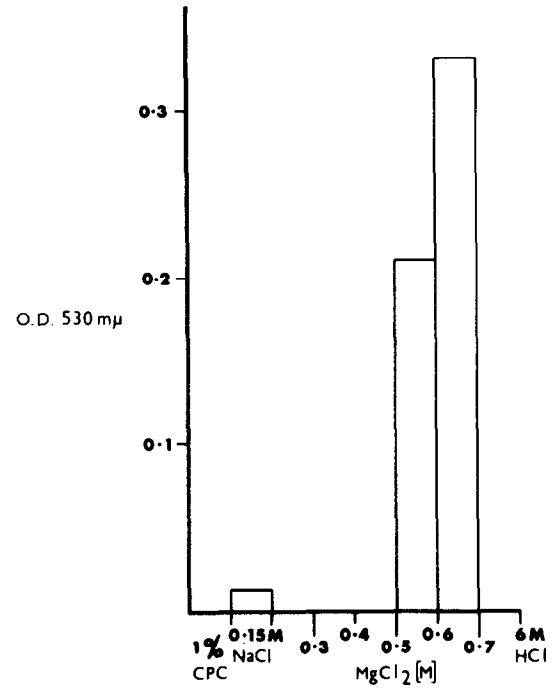

Fig. 1. Solubility profile of CPC complexes of purified proteoglycan from Kurloff cells, 100 μg applied to a CPC-cellulose column (see text).

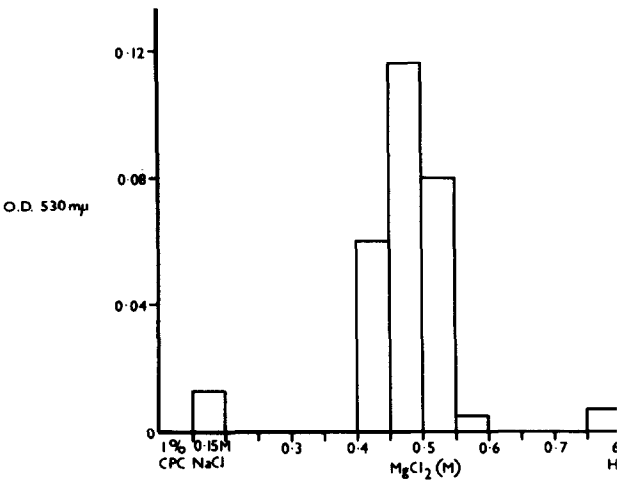

Fig. 2. Solubility profile of CPC complexes of glycosaminoglycans from Kurloff cells after digestion with papain; 50 μg applied to a CPC-cellulose column (see text).

Table 1
Analysis of the chondroitin sulphate-protein from Kurloff cells.

Amino acids* (μM/g)		Carbohydrate (% dry wt)	
Asp	19.2	Hexosamine	37.5%
Thr	18.1	Hexuronic acid	33.8%
Ser	37.1	Hexose	1.5%
Glu	33.2	Pentose	0.6%
Pro	14.0		
Gly	34.0		
Ala	18.7	*Molar ratios*	
Cys	11.7	Hexosamine: hexuronic acid	1.09:1
Val	4.8		
Ile	6.9		
Leu	15.7	Hexose: pentose	2.095:1
Thr	4.8		
Phe	7.3		
Lys	9.3		
His	14.5		
Arg	6.3		

* Not corrected for losses during hydrolysis.

acid 33.8%, hexose 1.5% and pentose 0.6%. The precise molar ratio of GalN to GlcN could not be determined because of the small amount of material available and the low proportion of GlcN, which was less than 1% of the total hexosamine present. This contrasts with extra-cellular chondroitin sulphate proteins of cartilage, which contain appreciable amounts of GlcN attributed to keratan sulphate, the sole exception being a fraction of higher mobility separated by electrophoresis [4], which like the Kurloff cell material contained less protein than other protein-polysaccharides. The molar ratios of serine, pentose and hexose were 1:1.07:2.2, agreeing closely with the theoretical ratio of 1:1:2 if each serine residue in the protein core were linked via xylose and two galactose residues to the chondroitin sulphate chain [15]. In this, the Kurloff cell material again resembles the cartilage fraction of higher electrophoretic mobility, where it was calculated that the majority of serine residues were linked to chondroitin sulphate chains [4].

From the molar ratios of hexosamine to xylose and to serine, the average length of chondroitin sulphate chains was calculated to be between 51 and 57 disaccharide units. This is significantly longer than the chains in either larger or smaller protein polysaccharides from pig laryngeal and articular cartilage, which were calculated to be 28 [11] and 20–26 [12] disaccharide units respectively.

These results together with those of Muir and Marshall [3] show that Kurloff material consists of a chondroitin sulphate-protein which, from the histological appearance of the cells [2,3], is assumed to be intracellular.

The origin and function of the Kurloff material is unknown. The chondroitin sulphate chains were not only longer than those in cartilage but the hexose content was only 1.5%, whereas that of extracellular protein polysaccharides from cartilage [11,12] contained appreciably more hexose than could be accounted for by that due to the linkage region and that due to keratan sulphate. It is possible that additional hexose may be necessary for export of chondroitin sulphate proteins from cells. The size of the proteoglycan compared with those of granulocytes and Hurler fibroblasts and the longer chondroitin sulphate chains suggest that the Kurloff inclusion bodies may not be lysosomes engorged with incompletely degraded compounds as has been suggested is the origin of the inclusions of Hurler cells [14,16].

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References

[1] J.C.G.Ledingham, J. Path. Bact. 50 (1940) 201.
[2] A.H.E.Marshall and K.V.Swettenham, J. Anat. 93 (1959) 348.
[3] H.Muir and A.H.E.Marshall, Nature (London) 191 (1961) 706.
[4] H.Muir and S.Jacobs, Biochem. J. 103 (1967) 367.
[5] I.Olsson and S.Gardell, Biochem. Biophys. Acta 141 (1967) 348.
[6] C.A.Antonopoulos, S.Gardell, J.A.Szirmai and E.R.De Tyssonsk, Biochim. Biophys. Acta 83 (1964) 1.
[7] W.E.Trevelyan and J.S.Harrison, Biochem. J. 50 (1952) 298.
[8] T.Bitter and H.Muir, Analyt. Biochem. 4 (1962) 330.
[9] C.P.Tsiganos and H.Muir, Analyt. Biochem. 17 (1966) 495.

[10] J.Knecht, J.A.Cifonelli and A.Dorfman, J. Biol. Chem. 242 (1967) 4652.
[11] C.P.Tsiganos and H.Muir, Biochem. J. 113 (1969), in press.
[12] K.Brandt and H.Muir, Biochem. J. (1969), in press.
[13] I.Olsson, Biochim. Biophys. Acta 177 (1969) 241.
[14] J.C.Fratantoni, C.W.Hall and E.F.Neufeld, Proc. Natl. Acad. Sci. 60 (1968) 699.
[15] U.Lindahl and L.Roden, J. Biol. Chem. 241 (1966) 2113.
[16] F.C.Van Hoof and H.G.Hers, European J. Biochem. 7 (1968) 34.