ISOLATION AND PROPERTIES OF A SOLUBLE CHONDROITIN SULFATE PROTEOGLYCAN FROM BRAIN

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SUMMARY: A proteoglycan in which the glycosaminoglycans are predominantly chondroitin sulfate has been isolated from the soluble fraction of rat brain by ion exchange chromatography and gel filtration. Glycoprotein oligosaccharides are also present, and result in adsorption of the proteoglycan by Concanavalin A-Sepharose. The proteoglycan-glycoprotein complex eluted from the affinity column by α-methylglucoside floats near the top of a cesium chloride density gradient run under dissociative conditions (4 M guanidine), but after β-elimination of the chondroitin sulfate polysaccharide chains from their low buoyant density glycoprotein complex they sediment to the bottom of the gradient. These results suggest that relatively few polysaccharide chains are covalently linked to a large protein core in the dissociated chondroitin sulfate proteoglycan "subunit" from brain, and that the proteoglycans are closely associated with soluble glycoproteins.

Approximately 90% of the brain glycoproteins occur as components of cell membranes, and only 10% are found in the soluble fraction after high-speed centrifugation of a brain homogenate. In contrast, a much larger proportion (40%) of the brain glycosaminoglycans occur as soluble proteoglycans, in which the polysaccharide chains of the sulfated glycosaminoglycans are covalently linked to a protein core (1,2). Moreover, much of the glycosaminoglycan associated with very low density microsomal membrane subfractions (2) can be solubilized by various types of mild washing procedures, and may be closely related to the strictly soluble glycosaminoglycans. Because chondroitin sulfate accounts for over 80% of the soluble glycosaminoglycans of adult brain, we have investigated the properties of the chondroitin sulfate proteoglycan and the form in which it occurs in brain.

MATERIALS AND METHODS: Brains from 30 to 60 day-old Sprague-Dawley rats were homogenized in 9 volumes of cold 5 mM phosphate buffer, pH 7.2, containing 0.15 M NaCl, using 10 up-down strokes of a motor-driven glass Duall tissue grinder (Kontes) operated at 400 rpm. The soluble fraction was then obtained by centrifugation for 2 hours at 146,000g. For the preparation of sulfate-labeled proteoglycan, rats were injected with [^{35}S]sodium sulfate and killed after 24 hours. The supernatant was dialyzed against deionized water, lyophilized, and redissolved in 50 mM Tris buffer, pH 8.0. After centrifugation to remove undissolved protein (containing less than 10% of the chondroitin sulfate) the sample, at a concentration of 5-10 mg protein/ml, was applied to a column of DEAE cellulose, which was then eluted with a linear gradient of NaCl in the same buffer. Pooled fractions were dialyzed, lyophilized, extracted with chloroform-methanol, and digested with Pronase. The fractionation, quantitation, and carbohydrate analysis of the glycosaminoglycans and glycopeptides was then performed as described previously (2).

For cesium chloride density gradient fractionation, the proteoglycan, at a concentration of 1-3 mg/ml, was dissolved in a solution having a final concentration of 4 M guanidine HCl, 3 M CsCl, 0.15 M potassium acetate, pH 6.3, and was centrifuged in a fixed angle rotor at 128,000g for 40 hours at 5°C. Gradient fractions were dialyzed to remove cesium chloride and guanidine, and aliquots were hydrolyzed for 22 hrs in 6 N HCl at 110°C for hexosamine analysis.

RESULTS AND DISCUSSION: A single sharp peak of chondroitin sulfate was eluted from the DEAE cellulose column between 0.24 M and 0.5 M NaCl, after the prior elution of most of the proteins and glycoproteins. The proteoglycan peak represented 2-3% of the protein in the original sample. Its composition (exclusive of nucleic acid) was 79% protein, 11% glycosaminoglycans and 10% glycoprotein carbohydrate. Pronase digestion and cetylpyridinium chloride fractionation revealed that 61% of the total hexosamine was present in the form of glycosaminoglycans, comprising 85% chondroitin sulfate, 8% heparan sulfate and 7% hyaluronic acid. The remaining hexosamine (39%) was a constituent of glycoprotein oligosaccharides containing glucosamine, galactosamine, galactose, mannose, sialic acid and fucose, in molar ratios of 1.00:0.23:1.34:0.76:0.80:0.16.

Sulfate-labeled proteoglycan incubated for 1 hr at 37° with 10 mM dithiothreitol in 1% SDS[1] did not enter 7% polyacrylamide gels (run in 0.1 M phosphate buffer, pH 7.1, containing 0.1% SDS),

[1]Abbreviation used: SDS, sodium dodecyl sulfate.

although a number of unlabeled proteins staining with Coomassie Blue did migrate in the gels. Some of these bands, comprising 25-30% of the total protein in the proteoglycan fraction from the DEAE column, could be separated from the proteoglycan by treatment with 10 mM dithiothreitol and gel filtration on Sephadex G-200 (in 0.2 M phosphate buffer, pH 7.1, containing 1 mM dithiothreitol). All of the sulfate-labeled proteoglycan appeared in the void volume, well separated from retarded peaks of protein and nucleic acid. Similar results were obtained if the sample was prepared as described above for gel electrophoresis and eluted with buffer containing 0.1% SDS. However, the proteoglycan was eluted as a broad and partially retarded peak on Sepharose 4B (using 0.1 M acetate buffer, pH 5.5, without SDS or dithiothreitol).

Either before or after purification by gel filtration on Sephadex G-200, the proteoglycan was quantitatively adsorbed on a column of Concanavalin A-Sepharose (equilibrated with 25 mM Tris buffer, pH 7.8, containing 1 mM each of $CaCl_2$, $MgCl_2$ and $MnCl_2$), and could be eluted with 0.2 M α-methylglucoside. (Inclusion of 1 M NaCl in the elution buffer together with α-methylglucoside increased the recovery of proteoglycan to approx. 80%.) Sulfated glycosaminoglycans obtained from the DEAE cellulose column proteoglycan peak were not retained by Con A-Sepharose affinity columns after release of the polysaccharide chains from the protein core by base treatment or Pronase digestion, indicating that mannose-containing oligosaccharides closely associated with the proteoglycans were responsible for their adsorption to Concanavalin A.

The proteoglycan was also fractionated by equilibrium density gradient centrifugation in 3 M CsCl-4 M guanidine HCl. Although these conditions are known to dissociate cartilage proteoglycans from accompanying proteins and glycoproteins (3), only a very small pro-

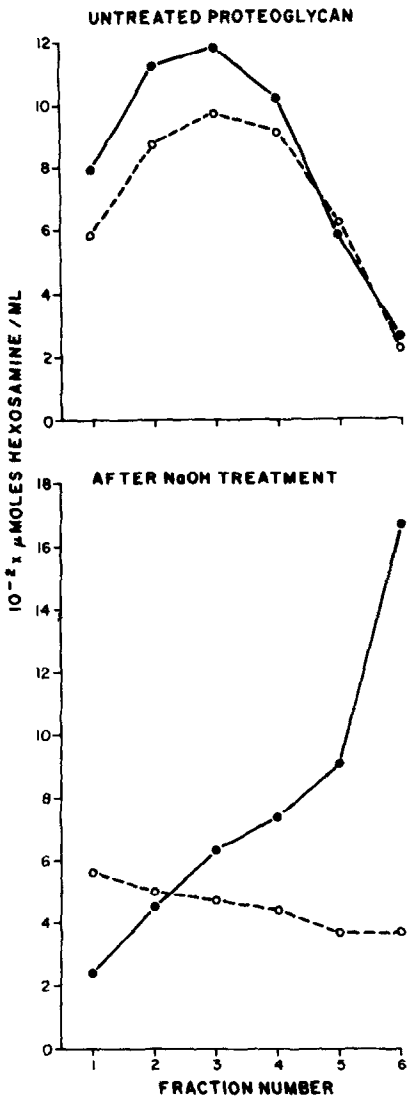

Fig. 1. Cesium chloride density gradient centrifugation of soluble chondroitin sulfate proteoglycan eluted from Concanavalin A-Sepharose. The distribution of glucosamine (o-----o) and galactosamine (●———●) was determined before and after alkali treatment (see text). Fraction 1 is from the top of the gradient. Approximately 90% of the galactosamine in the sample is present in chondroitin sulfate, and 80% of the glucosamine is in glycoprotein.

portion of the chondroitin sulfate proteoglycan from brain is found at the bottom of the gradient (Fig. 1, top). We have previously demonstrated that the chondroitin sulfate polysaccharide chains in brain are O-glycosidically linked to serine residues in the protein

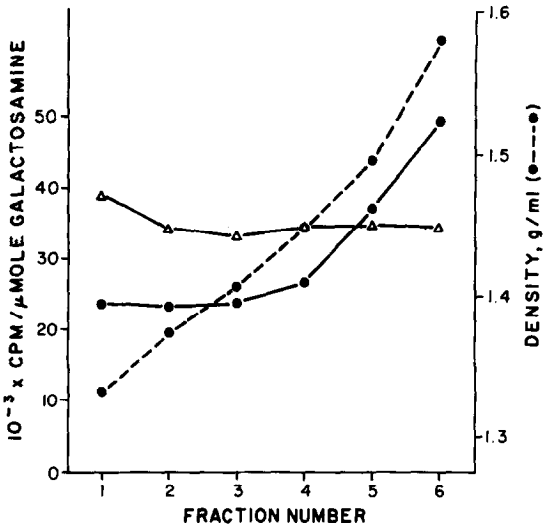

Fig. 2. Fraction densities and relative labeling (sulfate cpm/μmole galactosamine) of proteoglycan before (●———●) and after (Δ———Δ) base treatment.

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core, from which they can be released by a β-elimination reaction in the presence of NaOH or alkaline borohydride (1). In agreement with these results, we now find that after base treatment (0.2 N NaOH, 48 hr, 25°C) to release the chondroitin sulfate chains from their low buoyant density complex with glycoprotein, most of the galactosamine sediments to the bottom fractions of the gradient (Fig. 1).

The glucosamine found in the bottom two gradient fractions before base treatment is approximately equimolar with galactosamine, and could represent either heparan sulfate, or glycoproteins associated with a (possibly oversulfated) chondroitin sulfate proteoglycan containing a relatively low proportion of protein. The greater ratio of sulfate radioactivity to chondroitin sulfate galactosamine in the high density proteoglycans before base treatment (Fig. 2) is consistent with either explanation, and a definitive answer will therefore have to await the isolation of this small proportion of high density proteoglycan in quantities sufficient for a detailed study of its composition.

Our data indicate that the soluble chondroitin sulfate proteoglycan from brain is considerably different from that present in cartilage, insofar as the glycosaminoglycan chains are covalently linked to a relatively large protein moiety, with which glycoproteins are also closely associated. Such a composition would account both for its adsorption by Concanavalin A affinity columns as well as its low buoyant density when centrifuged in a cesium chloride density gradient under dissociative conditions. We are currently investigating the possibility that glycosaminoglycans and glycoprotein oligosaccharides may both be covalently linked to a common protein core in brain.

The exact localization and function of these soluble proteoglycans is not yet clear, although histochemical studies indicate that chondroitin sulfate and other glycosaminoglycans are present in both the cytoplasm (4,5) and extracellular space (6,7) of brain. In view of the relative sizes of the intracellular and extracellular compartments, and our previous finding that the concentration of glycosaminoglycans in neurons and astrocytes isolated in bulk from brain is 40% greater than their concentration in whole cerebrum (8), we believe that most of the soluble proteoglycans are probably cytoplasmic constituents. However, preliminary studies indicate that these proteoglycans have lectin activity, as demonstrated by their ability to agglutinate formalinized sheep and chicken erythrocytes, raising the possibility that they are partly present as an extracellular ground substance in nervous tissue where they may play a role in various types of cell-cell interactions. The presence of a similar type of agglutinin in embryonic chick brain has also been reported (9).

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REFERENCES

1. Margolis, R.U., Margolis, R.K., and Atherton, D.M. (1972) J. Neurochem., 19, 2317-2324.

2. Margolis, R.K., Margolis, R.U., Preti, C., and Lai, D. (1975) Biochemistry, 14, 4797-4804.

3. Hascall, V.C., and Sajdera, S.W. (1969) J. Biol. Chem., 244, 2384-2396.

4. Castejón, H.V. (1970) Acta Histochem., 35, 161-172.

5. Castejón, H.V., and Castejón, O.J. (1976) Acta Histochem., 55, 300-316.

6. Bondareff, W. (1967) Z. f. Zellforsch., 81, 366-373.

7. Tani, E., and Ametani, T. (1971) J. Ultrastruc. Res., 34, 1-14.

8. Margolis, R.U., and Margolis, R.K. (1974) Biochemistry, 13, 2849-2852.

9. Barondes, S.H., and Rosen, S.D. (1976) in Neuronal Recognition (S.H. Barondes, Ed.) pp. 331-356, Plenum Press, New York.