Presence of Heparan Sulfate Proteoglycan in Thyroid Tissue

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Abstract

Glycosaminoglycan (GAG) was extracted from the porcine thyroid gland with a buffer comprising 5.3 M guanidine-HCl and proteolytic enzyme inhibitors and was fractionated by subsequent isodensity CsCl centrifugation. 60% of uronic acid positive materials was accumulated in the bottom one-fourth fraction with high buoyant density. More than 90% of this uronic acid positive material in the thyroid tissue was heparin or heparan sulfate (sensitive to nitrous acid treatment) and the rest was chondroitin sulfate or dermatan sulfate (sensitive to chondroitinase ABC treatment). When the accumulated high buoyant density GAG was analyzed on a Sepharose CL-6-B column, approximately 14% of the heparin sulfate were in the macromolecular portion as a form of proteoglycan because it was destroyed by the papain digestion or alkaline borohydride treatment which extensively digests protein or releases GAG from protein by the elimination reaction, respectively. This study demonstrates the existence of heparan sulfate proteoglycan in thyroid tissue for the first time.

It has long been known that the state of the thyroid influences the mucopolysaccharide (glycosaminoglycan, abbreviated to GAG) metabolism. For example, Schiller et al. reported an accumulation of GAG in connective tissue of myxedema (1962). Winand et al. reported an increase in the urinary excretion of GAG in Graves' exophthalmos patients (1968). The presence of GAG in the thyroid tissue itself was recently reported by Van Dessel in a form of abstract (1978) but its functional role still remains unknown. Those GAGs are known to be synthesized by their parent cells as components of proteoglycans (Roden, 1980). Accordingly, in the present study, we sought to examine the presence of proteoglycan in porcine thyroid tissue.

Materials and Methods

Approximately 50 g of porcine thyroid glands were collected at the Tokyo Metropolitan Slaughterhouse and were transported to the laboratory on ice. After trimming off extraneous tissue and fat, the thyroid glands were weighed and minced with scissors to fine pieces. The minced tissue was extracted with three times the volume of buffer comprising 5.3 M guanidine-HCl, 0.13 M 6-aminohexuronic acid, 67 mM benzamidine HCl, 33 mM EDTA, pH 5.8 for 48 h at 4°C with continuous stirring. After the extraction, tissue fragments were removed by centrifugation and the supernatant was fractionated by CsCl density-gradient centrifugation. Dissociative CsCl density-gradients in 4 M guanidine HCl (initial density 1.46 g/ml) were formed in a Hitachi 65P RPS-50 rotor by centrifugation at 37,000 rpm at 10°C for 48 h as described by Hascall et al. for cartilage proteoglycans (1974 a and b). Four equal fractions designated as D1 through D4 from bottom to top.

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were prepared using a Beckman tube slicer as described by Heinegard (1972). The bottom one forth fraction designated as D1 was dialyzed against 0.5 M sodium acetate, pH 7.0, for 48 h and then against distilled water for another 48 h at 4°C. The dialysate was lyophilized and kept refrigerated until further analysis.

Lyophilized specimens were reconstituted with an appropriate buffer and subjected to either enzymatic treatments or alkaline borohydride treatment. Papain digestion was carried out at 65°C for 4 h in 0.1 M sodium acetate, pH 7.0 containing 5 mM sodium DETA and 5 mM cysteine hydrochloride. Digestion with chondroitinase ABC (0.25 μg/ml of sample) was performed in a buffer consisting of 0.1 M Tris and 0.1 M sodium acetate, pH 7.3, incubating for 3 h at 37°C. Treatment with alkaline borohydride was performed in 0.05 M NaOH at 45°C for 24 h with 1 M sodium borohydride, which was known to prevent the degradation of sugar chains by the "peering" reaction (Carlson, 1968). Excess borohydride was destroyed by neutralization of the solution with glacial acetic acid. Nitrous acid treatment was done at room temperature with 0.24 M NaNO2 in 0.18 M acetic acid for 80 min (Lindahl et al., 1973). The reaction was stopped by the addition of the same volume of 2 M ammonium sulfamate. Enzyme digests, alkaline borohydride-treated or nitrous acid treated samples were chromatographed immediately or after storage at −20°C. The specimens were applied to an analytical Sepharose CL-6B column (110 ×0.7 cm) and eluted with 0.5 M sodium acetate, pH 7.0.

Protein was measured by the method of Lowry et al. (1951) with bovine serum albumin (BSA) as a standard. The hexuronic acid concentration was determined by the carbazol reaction (Dische, 1947). Sialic acid was measured by the periodate-resorcinol method of Jourdian et al. (1971). Triiodothyronine (T₃) was measured with a radioimmunoassay kit supplied by Eiken Immunochemical Laboratories (Tokyo).

Results

The D1 fraction obtained by CsCl density gradient centrifugation contained approximately 60% of the uronic acid positive materials in the thyroid gland. The amount of material in the D1 fraction was about 0.2 mg per g wet weight thyroid. Table 1 summarizes the result of analysis of the

Table 1. Components of D1 fraction

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Porcine thyroid</th>
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<tr>
<td>Protein</td>
<td>260 mg/g D1 fraction</td>
</tr>
<tr>
<td>Hexuronic acid</td>
<td>433 mg/g D1</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>116 mg/g D1</td>
</tr>
</tbody>
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* The D1 fraction content was approximately 0.2 mg/g wet weight thyroid tissue.

Fig. 1. Elution profile of porcine thyroid D1 fraction by Sepharose CL-6B chromatography. Each fraction is 0.6 ml. Panel A shows the elution profile of protein, hexuronic acid and sialic acid. Panel B shows the elution profile of triiodothyronine immunoreactivity in addition to that of hexuronic acid.
D1 fraction for the content of protein, glycosaminoglycan (calculated based on the uronic acid measurement) and sialic acid. When the D1 fraction was chromatographed on Sepharose CL-6B, three major uronic acid peaks were observed. They are designated as peak I (PI) eluting at $K_\text{av}=0.21$, peak II (PII) at $K_\text{av}=0.56$ and peak III (PIII) at $K_\text{av}=0.82$ as illustrated in Fig. 1 and 2. The PI, PII and PIII contained approximately 14, 71 and 15% of the uronic acid, respectively. Only a little protein or sialic acid was eluted in the PIII. The PIII also contained triiodothyronine measured by radioimmunoassay (Fig. 1B). After papain or alkaline borohydride treatment, the PI disappeared (Fig. 2B and C). Papain digests proteins extensively and the alkaline borohydride treatment releases GAGs from protein by the elimination reaction. The disappearance of the PI following these treatments suggests that this peak contains GAG as a form of proteoglycan. On the other hand, the elution position of the PII was not altered by either of these treatments, indicating that GAGs in the PII are present mainly as single GAG chains with very small amounts of covalently linked peptide, if any. The molecular weight of GAGs in the PII was estimated as approximately 16,000 based on the published data on the calibration of Sepharose 6B by chondroitin sulfate chains of known molecular weight (Wasteson, 1971).

Chondroitinase ABC treatment of the D1 sample released about 9% of the total uronic acid as low molecular weight material eluted close to the column total volume (Fig. 2D) indicating the presence of chondroitin sulfate or dermatan sulfate. The PI was not degraded by chondroitinase ABC treatment. Nitrous acid treatment degraded the majority of the uronic acid containing material in both PI and PII (Fig. 2E). Degraded fragments eluted at
Kay = 0.80. A big peak at the column total volume (Fig. 2E) was due to the non-specific color reaction caused by nitrous acid and ammonium sulfamate used for chemical reaction. The result indicates that majority of the uronic acid containing material in the PI and PII is heparin or heparan sulfate.

**Discussion**

The present study confirmed the presence of GAG in the thyroid tissue as reported by Van Dessel et al. in an abstract form (1978). The amount of GAG in the thyroid tissue was similar to that reported for other parenchymatous tissues such as liver, kidney and spleen (Slavkin et al., 1975) and far less than that in various cartilagenous tissues (Hascall et al., 1977). More than 90% of the GAG in the thyroid tissue was heparin or heparan sulfate (sensitive to nitrous acid) and the rest was chondroitin sulfate or dermatan sulfate (sensitive to chondroitinase ABC). This report demonstrated, for the first time, that a part of the heparin or heparan sulfate (approximately 14%) is present as proteoglycans (PI in Sepharose CL-6B analysis) in the thyroid gland. The elution position of the PI on Sepharose CL-6B was very similar to that reported for the heparan sulfate proteoglycans isolated from rat liver cell membrane (Oldberg et al., 1979), suggesting a similar molecular weight of 70,000-80,000. The presence of heparan sulfate, extracellular matrix and basement membrane etc. (Brandford-White and Hardy, 1977, Kanwar, et al. 1981, Kraemer, 1971, Mutoh, et al., 1978, Norling, et al., 1981 and Oldberg, et al., 1979). The location of heparin or heparan sulfate proteoglycans in the thyroid tissue remains to be studied.

Despite the use of strong denaturing conditions of 5.3 M guanidine HCl and protease inhibitors throughout the extraction and purification procedures, most of the GAG was present as a single chain species (PII), suggesting proteolytic degradation of proteoglycans. The nature of such proteolysis, whether this represents physiological processes or experimental artifacts, remains to be studied further.

The PIII contained most of the protein and sialic acid in the D1 fraction. It is therefore possible that uronic acid reaction in this peak can be due to non-specific color reaction to these molecules and may not indicate the presence of GAG. The presence of triiodothyronine immunoreactivity in this peak suggests that these molecules are derived from thyroglobulin. The presence of such polypeptides which contain thyroxine has been reported by other investigators (Haeberli 1979, Chernoff et al., 1981, Dunn et al., 1981). The high density of these molecules probably is the result of high iodine and carbohydrate content of the peptides. The physiological significance of this fraction remains to be elucidated.

**References**


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