INTERACTION BETWEEN THE STRUCTURAL GLYCOPROTEIN OF CONNECTIVE TISSUE AND PROTEOGLYCANS AND GLYCOSAMINOGLYCANS

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UDC 612.751.3:612.398.7

Structural glycoprotein (SGP) of connective tissue isolated from bovine heart valves and nasal septal cartilage forms water-soluble complexes with protein-chondroitin-4-sulfate and with various fractions of heparin. SGP does not form these complexes with hyaluronic acid. It is postulated that this phenomenon plays an important role in the formation of collagen and elastic fibers.

KEY WORDS: structural glycoprotein of connective tissue; complexes with protein-chondroitin-4-sulfate and heparin; hyaluronic acid.

The structural glycoprotein (SGP) of connective tissue plays an important role in the formation of collagen and elastic fibers; it enters into their composition [8, 12, 15] and is one of the most powerful antigens of that tissue [11]. However, the physical and mechanical nature of the SGP and its relationship to proteoglycans, glycosaminoglycans, and other components of the ground substance of connective tissue have received little study, despite the direct relevance of this question to the elucidation of the biochemical role of this glycoprotein.

In this investigation the chemical composition of SGP preparations isolated from bovine heart valves and nasal septal cartilage and also its interaction with protein-chondroitin-4-sulfate (PCS) and with individual fractions of heparin containing three (HP-3S) and four (HP-4S) sulfate residues to one residue of glucosamine and hyaluronic acid (HUA), was investigated.

EXPERIMENTAL METHOD

SGP was isolated by the method of Robert and Dische [14] with some modifications. The original tissue was washed free of blood, first with cold running tap water and then with distilled water, after which it was minced twice in a mincer (4°C). Five volumes of buffer solution (1 M $CaCl_2$, Tris-citrate, pH 7.6) were then added to 0.5-1 kg of the resulting mass. The mixture was homogenized at 14,000g (3 min, 4°C); allowed to stand for 24 h (4°C); the residue was separated by centrifugation (6000g, 20 min, 4°C); it was again treated with 5 volumes of the above buffer, homogenized, and centrifuged, and all these operations were repeated 8 to 10 times. The final residue was washed with water and treated with 5 volumes (relative to the weight of the original tissue) of 3% TCA at 90°C for 5, 15, 25, and 30 min consecutively, each time separating the cold residue by centrifugation (6000g, 15-20 min, 18°C); the residue was then washed with water to remove the acid. The washed residue was treated with 10 volumes of 8 M urea and homogenized (8000g, 3 min). After the mixture had been allowed to stand for 24 h at 18°C, the supernatant containing SGP was separated by centrifugation (57,000g, 40 min), and the extraction was repeated 5 times. The pooled extracts were dialyzed against water to remove the urea. The residue formed during dialysis was separated by centrifugation (6000-10,000g, 15 min). The SGP was separated from the transparent solution by lyophilization. The yield of SGP was 1.5-3g.

PCS was obtained by isolation from bovine cartilaginous tracheal rings or nasal septal cartillage [1], and hyaluronic acid from a human umbilical cord [2]. Heparin (Spofa, Czechoslovakia) was separated into individual fractions [3].

The content of protein [13], hydroxyproline [7], aminosugars [4], hexoses (anthrone method), and hexuronic [9] and sialic (resorcin method) acids were determined in the SGP preparations.

Institute of Rheumatism, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 12, pp. 1430-1432, December, 1976. Original article submitted May 21, 1976.

TABLE 1. Results of Analysis (%) of SGP Preparations

Component	SGP preparation	
	from heart valves	from nasal septal cartilage
Protein	87,0	97,0
Hydroxyproline	0,2	0,3
Hexoses	4,0	6,6
Amino sugar	1,6	2,1
Hexuronic acids	0,1	0,1
Sialic acids	0,0	0,0

Interaction between SGP and proteoglycans and glycosaminoglycans was studied by adding different quantities of these biopolymers to a suspension of SGP in 0.01 M buffer (glycine–HCl, pH 3.5), containing 4 mg of the given glycoprotein in 1 ml of solution. The total volume of the mixture was 2 ml. The contents of the mixture were stirred for 5-10 min at 4°C, after which it was centrifuged (4500g, 15 min, 4°C). The residue was washed several times with the above-mentioned buffer solution and dried over phosphoric anhydride in vacuo to a constant weight. The quantity of precipitate was determined gravimetrically and expressed as a percentage of the quantity of SGP taken. The content of hexuronic acids was determined in the residues and in the pooled supernatant and washings.

EXPERIMENTAL RESULTS AND DISCUSSION

Analysis of the SGP preparations showed that the predominant structural elements of the carbohydrate component of this glycoprotein were an amino sugar and hexoses. Hexuronic, sialic, and sulfuric acids were not present in the SGP preparations. Hydroxyproline was virtually absent from the protein component of SGP, which accounted for about 90% of the total. Differences in the contents of these components between SGP isolated from heart valves and nasal septal cartilage varied within the limits of error of the preparative and analytical methods (Table 1). The absence of hydroxyproline and of sialic and hexuronic acids in the SGP preparations proves that these preparations were uncontaminated by collagen proteins, other glycoproteins, or glycosaminoglycans. A comparatively low carbohydrate content was a feature of the SGP obtained from connective tissue.

SGP isolated as described above had low solubility in water and salt solutions. On standing in the cold (4°C) this biopolymer separated rapidly from solution, evidently as a result of the formation of complex aggregates. These aggregates did not dissolve at 20°C in solutions of sodium and calcium chlorides (0.01-0.10 M), sodium pyrophosphate (0.1-1.0 M), hydrochloric and sulfuric acids (0.01-0.10 M), perchloric (0.1-1.0 M) and trichloroacetic (0.3-1.0 M) acids. The features of SGP mentioned above distinguish it from the remaining glycoproteins of the animal body.

A study of interaction between SGP, on the one hand, and PCS, HP-3S, and HP-4S on the other hand, showed that these biopolymers solubilize this glycoprotein (Fig. 1). Sodium and calcium chlorides reduced the solubility of SGP in the presence of PCS, HP-3S, and HP-4S. These chlorides reduced the solubility of SGP much more strongly if PCS was present in the solution than if HP-3S and HP-4S were present. No significant difference was found in the action of salts on the solubility of SGP in the presence of HP-3S or HP-4S (Fig. 2). In SGP residues remaining in the samples because the quantities of PCS and heparin fractions were insufficient to cause complete solution of this glycoprotein, and also in residues formed in the presence of salts from solutions of mixtures of SGP and the above-mentioned biopolymers, no hexuronic acids were found.

HUA had no action whatsoever on the solubility of SGP even in relatively high concentrations (Fig. 1). The residues left behind under these conditions contained no HUA. Solubilization of SGP in the presence of PCS, HP-3S, and HP-4S was due to the formation of soluble complexes of this glycoprotein with these macropolyanions as a result of electrovalent interaction between the sulfate groups of these macropolyanions and the basic groups of the SGP. This is proven by the decrease in formation of soluble complexes in the presence of salts screening the charges on the components. Salts cause liberation of free, undissolved SGP from the dissolved complex into the residue, as is confirmed by the absence of PCS or heparin fractions in such residues. In the absence of salts in the solution, no insoluble complexes of SGP with PCS, HP-3S, and HP-4S likewise appeared, for the residues left behind if the amounts of PCS, HP-3S, and HP-4S were insufficient to dissolve all the SGP did not contain these macropolyanions. Complexes of SGP with PCS, HP-3S, and HP-4S cannot associate into insoluble aggregates because of their high negative charge, created by the anionic groups of the

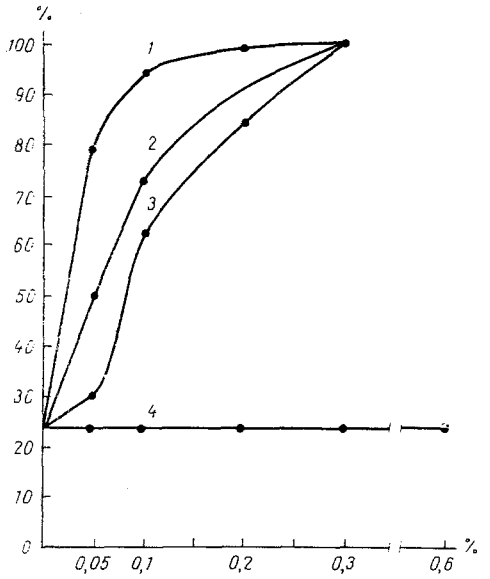

Fig. 1

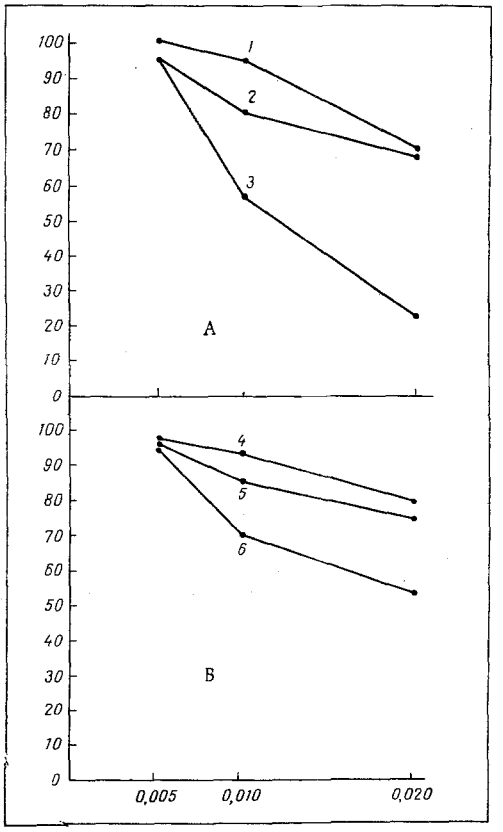

Fig. 2

Fig. 1. Formation of soluble complexes of SGP with PCS, HP-3S, and HP-4S: 1) SGP + HP-3S; 2) SGP + HP-4S; 3) SGP + PCS; 4) SGP + HUA. Ordinate, solubility of SGP (in %); abscissa, concentration of macropolyanion (in %).

Fig. 2. Formation of complexes of SGP with PCS, HP-3S, and HP-4S as a function of ionic strength of solution: A) complex formation in presence of sodium chloride: 1) SGP + HP-3S; 2) SGP + HP-4S; 3) SGP + PCS; B) complex formation in presence of calcium chloride: 4) SGP + HP-3S; 5) SGP + HP-4S; 6) SGP + PCS.

macropolyanions left undecomposed during the formation of the compound with SGP. Such differences as were found in the amounts of soluble complexes formed by SGP with PCS, HP-3S, and HP-4S when the concentrations of these macropolyanions were insufficient to cause complete conversion of the SGP into a soluble complex (Fig. 1), were due to the physical and chemical differences between these various macropolyanions. The molecular weight of PCS is much higher than that of HP-3S and HP-4S; furthermore, the PCS macromolecule contains much more of the covalently bound protein component than the heparin fractions. This probably explains why complete conversion of SGP into a soluble complex takes place in much lower molecular concentrations of PCS than of HP-3S or HP-4S, as well as the stronger influence of salts on complex formation in that case.

The sulfated proteoglycans, which are evidently present in much larger quantities than HUA in the ground substance of mature connective tissue, evidently form a soluble complex with SGP; by interacting with tropocollagen, this complex participates in the formation of the collagen fiber. In the ground substance of embryonic connective tissue, on the other hand, because it contains predominantly HUA, the greater part of the SGP does not form these soluble complexes but associates into microfibrils [17] which, by interacting with tropoelastin, convert it into an elastin fiber [16]. The presence of large quantities of HUA in embryonic connective tissue facilitates the association of SGP into microfibrils for, by creating three-dimensional structures acting as osmotic cells and molecular sieves in solutions, this acid concentrates the various tissue elements within the smallest possible volume [5, 6].

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ACTION OF VASOPRESSIN ON ATPase ACTIVITY OF MICROSOMAL FRACTIONS OF RABBIT HEART AND LIVER

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UDC 612.434'14.018.2:[612.17+612.35

Intravenous injection of vasopressin in a dose of 5 pressor units/kg body weight led after 1 h to changes in the ATPase activity of rabbit heart and liver microsomes. These changes differed in direction: Mg- or Ca-activated ATPase activity of the cardiac microsomes was very slightly increased, whereas ATPase activity of the hepatic microsomes was reduced.

KEY WORDS: ATPase activity; microsomes of heart and liver; vasopressin.

In recent years, vasopressin in combination with macromolecular dextran has been used in order to produce acute experimental disturbances of the coronary circulation. This use was based on the ability of vasopressin to constrict the small coronary vessels, to change the permeability of biological membranes, and thereby to increase the aggregating effect of macromolecular dextran [4-6]. Under these conditions changes were observed in cardiac function, accompanied by marked shifts in the protein and energy metabolism of the myocardium [2].

Since muscle contraction is influenced by the state of the sarcoplasmic reticulum (SPR) and, in particular, of its ATPase systems, changes in Ca- and Mg-ATPase activity of SPR fragments from heart muscle were studied during the action of vasopressin. To emphasize the role of SPR in the contractile activity of the cell,

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(Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 12, pp. 1433-1434, December, 1976. Original article submitted May 21, 1976.