Functions of the Carboxyl and Sulfate Groups of Proteoglycans

S. M. Bychkhov and S. A. Kuz'mina

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The extraction of hyaluronic acid, the natural protein-chondroitin-keratan sulfate complex, and proteoglycan aggregates from tissues is studied as a finction of the ionic strength of the extracting solution. It is shown that the strength of binding with the tissue diminishes in the following series: proteoglycan aggregates, protein-chondroitin-keratan sulfate, hyaluronic acid. Binding of proteoglycans with tissue structures is realized mainly by electrovalent interactions between sulfate groups of proteoglycan aggregates and protein-chondroitin-keratan sulfate, and positively charged tissue structures. It follows from data of infrared spectra that carboxyl groups of the macromolecules participate in the formation of both inner and outer hydrogen bonds with acetamide groups of the macromolecules. Carboxyl groups also play a role in the generation of soluble complexes with various bases. Rupture of linkage of proteoglycans with the tissue structures can result in their entry in the plasma and erythrocyte aggregation, and serve as evidence of a pathological process.

Key Words: *proteoglycans; proteoglycan aggregates; carboxyl and sulfate groups; complexes*

It is known that acidic properties of hyaluronic acid (HUA) are determined only by the carboxyl groups of HUA residues present in its macromolecules, while properties of keratan sulfate are connected to the content only of sufate groups in its macromolecules. However, the acidic properties of other known proteoglycans are mediated by hexuronic and sulfuric acid residues [4,5]. Apparently, these differences account for the multiple biochemical properties of individual biopolymers of this type [3,5,13]. The goal of the present investigation was to elucidate the biochemical significance of the functional peculiarities of HUA carboxyl groups, and of carboxyl and sulfate groups in the natural protein-chondroitin-keratan sulfate complex (PCKS) and proteoglycan aggregates (PA).

Scientific and Experimental Enterprise of the "Eye Microsurgery" Associated Scientific and Technical Complex, Moscow. (Presented by D. S. Sarkisov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

HUA was isolated from human umbilical cord [1]; PCKS and PA were isolated from bovine tracheal cartilage [2]. The efficacy of PCKS and PA isolation from tissues depends on the ionic strength of the salt solution used for their extraction. This gives an indication of the strength of binding of these components with the tissue structures. Proteoglycans were extracted with water, and then the tissue was treated consecutively with 0.15 M NaCl and 4 M guanidine×HCl solutions. After extraction with water and salt solutions the proteoglycans were precipitated and dried [1,2]. The water extracts from umbilical cord practically all HUA contained in this tissue and a certain portion of proteoglycan sulfates. The remaining portion of these is extracted with 0.15 M NaCl. The same strength of NaCl solution extracts from the cartilage the soluble fraction of PCKS. Subsequent treatment with 4 M guanidine×HCl solution leads to practically total extraction of tissue PA.

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The preparations obtained were assayed for the content of nitrogen, hexosamines, hexurinic acids, and sulfate groups [1,2]. The covalently bound protein was quantitated on the basis of the amount of nitrogen in the preparation and in N-acetylhexosamines. Moreover, the spectra of infrared absorption in the 4000-400 cm^{-1} region were recorded for all tested proteoglycan and PA preparations using a method described earlier [4,6].

RESULTS

As is shown in Table 1, the content of components included in the macromolecular structures of isolated HUA, PCKS, and PA preparations is in agreement with the literature [1,2,6]. Preparations were free of electrovalently bound protein products, this attesting to the individuality and purity of HUA, PCKS, and PA obtained. Infrared spectroscopy of all mentioned proteoglycans revealed a clearly manifested absorption at 750-700 cm^{-1}, corresponding to a hydrogen bond between the carboxyl and acetamide groups in their macromolecules. With the use of the same method, cartilage-derived PA revealed free carboxyl groups (absorption at 1760 cm^{-1}). On the whole, the infrared spectra of all obtained HUA, PCKS, and PA preparations are identical to the spectra of the same biopolymers obtained earlier [4,6]. Regarding the strength of binding with the tissues, the proteoglycans studied rank in descending order as follows: PA, PCKS (soluble fraction), HUA. Evidently, the ability of individual proteoglycans to be released from tissue as a result of treatment with water and salt solutions of different ionic strength is governed by the nature and amount of anionic groups residing in macromolecules, and by the composition, structure, and physical parameters of these macromolecules.

Thus, PA assembled from HUA macromolecules, with PCKS residing along them with the help of a linking protein, are most firmly bound to the tissues. The anionic groups of these proteoglycans remain free, which points to their biochemical significance. The peculiar role of HUA in the PA structure is explained by its linear form and the absence of branched structures and sulfate groups [3]. Since PA can be extracted from tissues only through the use of concentrated salt solutions, and isolated PA carry free carboxyl groups, the connection between PA and tissues is realized mainly via interaction between their sulfate groups with positively charged tissue structures [4,6]. The contribution of carboxyl groups in this linkage is weak if it occurs at all, and plays a negligible role in the tissue fixation of PA. The soluble PCKS complex is bound with tissues in a similar manner, but much less firmly than PA, as follows from the readier extraction of PCKS from the tissues.

The mechanism of PA and PCKS complex formation with tissue structures is not restricted to electrovalent interactions. Since both PA and PCKS contain a large number of sulfate groups, complex formation may be accompanied by anticooperation processes, i.e., the occupation of certain regions of tissue structures by the biopolymers leads to a lowered affinity of adjacent regions for proteoglycans. Evidently, there are other, as yet unknown factors at work in the mechanism of PA and PCKS binding to tissues.

It is known that sulfate groups of PA and PCKS, besides participating in tissue binding, are involved in cation, in particular, Ca^{2+} transport. Due to structural and physical differences, PA and PCKS obviously differ also in the ratio of the number of sulfate groups responsible for tissue binding to the number of groups involved in Ca^{2+} transport. As a rule, PA and soluble PCKS are represented in tissues by several fractions differing in molecular weight and other parameters, thereby broadening the spectrum of activity without changing the main biochemical properties [3,5].

PA, PCKS, and HUA differ in composition, structure, and physical parameters. However, they belong to the same type of biopolymers and are able to perform the same physical actions, such as

Table 1. Analysis of Proteoglycan Preparations (%; $M \pm m$; $n = 8$)

Preparation	Solvent used for extraction	Content			
		hexosamine	HUA	$-O-SO_3^-$	Protein (by nitrogen content)
HUA	Water	42.00±0.12	43.00±0.10	0.00	1.20
PCKS	0.15 M NaCl	28.40±0.80	28.00±0.10	14.60±0.50	14.60
PA	Guanidine × HCl 4 M solution	29.40±0.70	21.20±0.60	11.20±0.50	12.00

Note. n – number of preparations.

water and ion transport, spatial exclusion of cells, and prevention of cell dissociation [3,5,14]. On the basis of this one may assume that PA is a strictly regulated, polyfunctional system with a high chemical potential, that coordinates the above-mentioned functions intrinsic in individual proteoglycans in the organism. Such a system has to be stably fixed in the tissues, and it is the sulfate groups that ensure this, as mentioned earlier.

As follows from the data of infrared spectroscopy, one of the functions of proteoglycan carboxyl groups is the formation of intra- and intermolecular hydrogen bonds with acetamide groups of hexosamines, resulting in the appearance of tertiary complexes consisting of HUA and HUA plus PCKS macromolecules. This process induces conformational changes of proteoglycan macromolecules and possibly leads to the formation of hydrogen bonds between their carboxyl groups [3,13]. HUA, which is predominant in a number of tissue structures, can accumulate in individual sites of such structures via the formation of hydrogen bonds with the resident sulfated proteoglycans. PA-unbound HUA is present in tissues in the form of salts of various bases and of electrovalent complexes with protein substances, including collagen.

Carboxyl and sulfate groups of HUA, PCKS, and PA are not directly involved in the spatial exclusion of cells as components of electrovalent interactions [5,14]. The mentioned proteoglycan compounds can influence this process via the formation of Ca^{2+}- and Mg^{2+}-salts and electrovalent complexes with protein substances. These new compounds differ from the initial biopolymers in a higher molecular weight and a number of conformational peculiarities, as was shown in our earlier study [9].

Umbilical cord and vitreous body contain HUA and very few sulfated proteoglycans. However, this amount is sufficient for the fulfillment of specialized functions in these organs. In cartilage characterized by a relatively high level of metabolism, HUA and PCKS are present mainly as constituents of PA. In parallel with the quantitative and qualitative increase of proteoglycan functions in the systems of the organism, the role of PA as a necessary component of biochemical reactions requiring the presence of sulfate groups of these biopolymers also increases. The significance of sulfate groups in these reactions is confirmed by the fact that in the animal organism no structures containing only HUA in the total absence of sulfated proteoglycans are to be found. Evidently, certain minimal amounts of sulfated proteoglycans, as in the case of umbilical cord and vitreous body, are necessary for the regulation of HUA function.

The ability of tissues to bind in an electrovalent form individual proteoglycans and PA can wax and wane depending on the physiological status of the organism. This is most clearly expressed during embryogenesis and tissue regeneration. In pathological processes (especially of infectious origin) associated with damage to tissue structures that bind PA, HUA and PCKS in the mentioned manner, the biopolymers are released in soluble form and can enter the blood stream and accumulate in various tissues, thereby impairing their functioning [8,13]. Following entry into the plasma, which, under normal conditions, contains proteoglycans in a very low concentration, they may act as factors of spatial exclusion and lead to erythrocyte aggregation, thus limiting the normal dispersion of these cellular elements of the blood [7,8,10-12]. In such cases the degree of erythrocyte aggregation can serve as a sensitive indicator of the derangement of the PA, HUA, and PCKS electrovalent interactions with the positively charged tissue structures.

REFERENCES

1. S. M. Bychkov and V. N. Kharlamova, *Biokhimiya*, **33**, 840-846 (1968).
2. S. M. Bychkov and M. F. Kolesnikova, *Ibid.*, **34**, 204-208 (1969).
3. S. M. Bychkov and S. A. Kuz'mina, *Vopr. Med. Khimii*, № 9, 227-237 (1986).
4. S. M. Bychkov and S. A. Kuz'mina, *Byull. Eksp. Biol. Med.*, **112**, № 12, 480-482 (1991).
5. S. M. Bychkov and S. A. Kuz'mina, *Usp. Sovr. Biol.*, **112**, 273-280 (1992).
6. S. M. Bychkov and S. A. Kuz'mina, *Byull. Eksp. Biol. Med.*, **114**, № 9, 246-249 (1992).
7. S. M. Bychkov and S. A. Kuz'mina, *Ibid.*, **115**, № 3, 240-242 (1993).
8. S. M. Bychkov and S. A. Kuz'mina, *Ibid.*, № 6, 604-606 (1993).
9. S. M. Bychkov and S. A. Kuz'mina, *Ibid.*, **117**, № 6, 596-599 (1994).
10. A. Engstron-Laurent, *Circulating Sodium Hyaluronate*, Uppsala (1985).
11. J. K. E. Fraser, T. C. Laurent, H. Petrof, and E. Baxter, *Biochem. J.*, **200**, 415-424 (1981).
12. J. K. E. Fraser, L. J. Appelgren, and T. C. Laurent, *Cell Tissue Res.*, **233**, 285-293 (1989).
13. L. Kjellen and U. Lindahl, *Ann. Rev. Biochem.*, **60**, 448-475 (1991).
14. J. E. Morris, *Exp. Cell Res.*, **120**, 141-153 (1979).