ELECTRON-MICROSCOPIC STUDY OF VARIOUS SALTS OF PROTEOGLYCAN AGGREGATES

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UDC 612.398.154.3:577.112.853/.014.2.086.3

KEY WORDS: proteoglycan aggregates; salts of proteoglycans; electron microscopy.

The problem of the connection of proteoglycans with structural elements of tissues, cells, and intracellular organelles has received little study. Carboxyl groups (hyaluronic acid — HUA), sulfuric acid residues (keratan sulfate), present in proteoglycan macromolecules in large quantities, and various groups found in the majority of these biopolymers (chondroitin, heparan, and dermatan sulfates, heparin), together can bind electrovalently with cations. Under these circumstances the carboxyl and sulfate groups of the same macromolecule may bind with different bases present in the system, on the nature and quantity of which in the compound formed will depend to a certain extent the conformational features of the glycosaminoglycan components of the proteoglycans [2-4]. In addition, macromolecules of the same proteoglycan can react with each other and with macromolecules of other proteoglycans through nonelectrovalent and specific interactions, with the formation of complex supramolecular structures, HUA, in the presence of protein-chondroitin keratan sulfate (PCKS), and with or without binding protein, forms proteoglycan aggregates (PGA) with correspondingly different degrees of stability [3]. In vivo, as a result of contact between proteoglycans and the morphological elements of tissues and cells, interaction takes place between these biopolymers and the ultrastructure of these elements, and between the latter and the structure of associates formed from proteoglycan macromolecules [3, 4]. In relation to isolated normal potassium salts of HUA and PCKS (soluble fraction) it has been shown by electron-microscopic methods that the ultrastructure of associates of their macromolecules depends on the presence of cytochrome C, with which these proteoglycans are electrovalently bound, and also on the mutual effect of the proteoglycans when present together [1].

The aim of the present investigation was to study individual preparations of various salts PGA by electron-microscopic methods, which is essential for the discovery of factors determining the supramolecular organization of structures created by PGA in tissues. Only the acid salt of guanidine-PGA was used in the electron-microscopic investigations [5, 7].

EXPERIMENTAL METHOD

PGA were isolated from tracheal rings, purified from surrounding tissues, obtained from men aged 40-50 years dying from mechanical trauma or from cardiovascular diseases, and also from bovine tracheal rings, by extraction with 4 M guanidine hydrochloride solution. Acid (free carboxyl group) and normal K^+-, Mg^{++}-, and Ca^{++}-salts of PGA were obtained by the method developed previously [2]. The acid salt guanidine$^+$-PGA was used in the ready-made form, for PGA were isolated from cartilage in the form of this salt [4]. The PGA preparations studied contained a certain quantity of soluble proteoglycans, not included in PGA, and this brought the experimental conditions more in line with those pertaining in the tissues. Preparations of proteoglycans for electron-microscopic study were dissolved at the rate of 2 mg/ml in 1 M ammonium acetate solution, pH 5.0. The solutions were mixed for 4 h in the cold, cytochrome C (Fluka-Aly), was added to a final concentration of 0.01%, and the sample was well mixed. The test solution in a volume of 0.1 ml was introduced into a Teflon tray filled with 0.3 M ammonium acetate solution. The resulting film was transferred to copper grids, coated with formvar and sprayed with carbon. The grid was stained for 1 min in a 10^{-4} M solution of uranyl acetate in acetone. The specimens were examined under the EM-420 electron microscope (Philips) with accelerating voltage of 80 and 100 kV.

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 744-746, December, 1988. Original article submitted February 20, 1988.

0007-4888/88/0012-1789$12.50

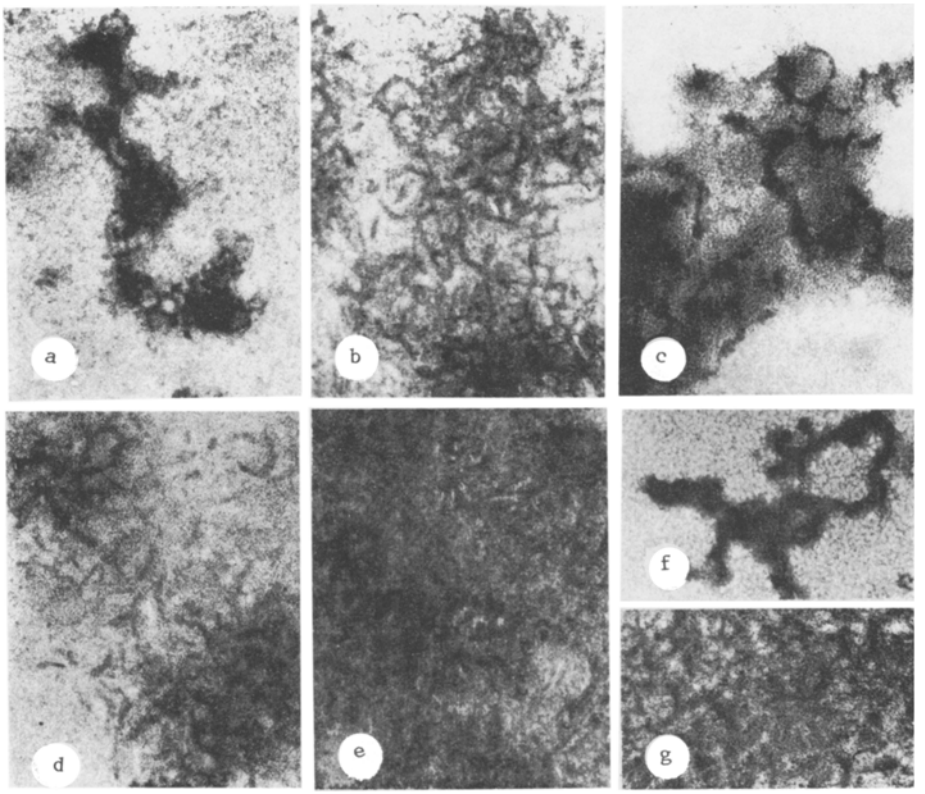

Fig. 1. Acid form and PGA salts. a) Accumulation of closely interwoven PGA macromolecules, around the periphery of which can be seen separate macromolecules, in which monomers are bound in the transverse direction to the hyaluronic acid chain curved along the long axis, and the monomers also are curved along their own protein core; b, c, f) acid Mg^{++}-, Ca^{++}-, and guanidine^{+}-salts of PGA. Concentrations with well-marked interwoven macromolecules (b, c) are uniformly distributed and only very slightly different in electron density. Accumulations of guanidine^{+}-PGA macromolecules are very clearly demarcated against the weak background of their separate macromolecules, uniformly distributed (f); d, e, g) normal Mg^{++}-, Ca^{++}-, and K^{+}-salts of PGA. Uniform distribution of interwoven macromolecules without any marked dense accumulations; separate macromolecules are distinguishable (d). A somewhat smaller distribution, but without any marked boundaries, with boundaries of macromolecules superposed one above another (e, g). Magnification: a) 135,000, b) 105,000, c) 175,000 , d) 135,000, e) 86,000, f) 63,500, g) 175,000 ×.

EXPERIMENTAL RESULTS

Preparations of the acid form of PGA under the electron microscope could be distinguished as separate formations consisting of monomers, arranged transversely relative to the chain of the hyaluronate macromolecule, constructed of chondroitin sulfates and keratan sulfate, covalently bound with the protein core. These structures were curved to different degrees along the hyaluronate, and the monomers in turn were curved along the protein core. Separate macromolecules were interwoven and formed a honeycombed structure. Large, clearly formed electron-dense accumulations of closely interwoven macromolecules also were present in large numbers (Fig. 1a). Acid Mg^{++}-, Ca^{++}-, and guanidine salts of PGA were characterized electron microscopically, like the acid form of PGA, not only by a uniformly distributed network of mutually interweaving separate molecules, but also by the presence of variously unevenly distributed and relatively more electron-dense accumulations. At the periphery of these accumulations there were various separate macromolecules (Fig. 1b, c, f). Normal Mg^{++}-, Ca^{++}-, and K^{+}-salts of PGA differed electron-microscopically from all the preceding PGA preparations by the uniform distribution of these interwoven macromolecules. No clearly distinguished electron-dense accumulations were present. There were only diffusely distributed double-layered accumulations in which seperate molecules were clearly distinguishable (Fig. 1d, e, g). In all the prepara-

tions mentioned above some degree of contamination was present by free monomers, especially in preparations of the acid form of PGA.

It will be evident that structures revealed electron-microscopically in the PGA preparations studied depend on the quantity and nature of the cations connected with their sulfate and carboxyl groups. In the presence of free sulfate and carboxyl groups in the acid form of PGA, and of carboxyl groups only in the acid salts of these biopolymers, their structure is revealed electron-microscopically in the form of distinct, locally arranged associates of macromolecules. In normal Mg^{++}-, Ca^{++}-, and K^{+}-salts of PGA, these clearly distinguishable separate associates are not observed, and only certain diffusely arranged condensations in which separate macromolecules of these salts of PGA are clearly visible, can be observed. In this case the normal K^{+}-salt of PGA differs from the first two in the more uniform distribution of its accumulations of macromolecules. It must be recalled that electron-microscopic images are two-dimensional, whereas the structures studied are three dimensional systems, and this introduces certain elements of indeterminacy into the description of the factual data and their interpretation.

By the electron-microscopic method used in this investigation the PGA preparations studied were initially electrovalently bound with cytochrome C, leading to the formation of a complex of this preparation with cytochrome C. In the case of the acid form of PGA, a large proportion of the sulfate and carboxyl groups took part in complex formation of this kind. A certain number of carboxyl groups of this biopolymer, which formed intramolecular and, in particular, intermolecular hydrogen bonds, may be inaccessible for complex formation with cytochrome C. As a result, not only individual macromolecules of the acid form of PGA took part in complex formation, but also their supramolecular associates, formed by means of hydrogen bonds, as was revealed electron-microscopically by the electron-dense accumulations mentioned above. In acid Mg^{++}- and Ca^{++}-salts of PGA these cations can bind sulfate groups of two separate PGA macromolecules, thus creating a gigantic complex of many PGA macromolecules, containing free carboxyl groups. Such a complex, interacting electronically due to the above-mentioned groups with cytochrome C, forms large accumulations, clearly distinguishable electron-microscopically. Around the accumulations of the acid form of PGA and the acid salts of these biopolymers observed, diffusely distributed interwoven macromolecules of them could be seen, and could be explained by the presence of a certain number of macromolecules, which did not take part in intermolecular complex formation, which can occur in such interactions [4]. The possibility of formation of supramolecular electrovalent complexes in normal Mg^{++}- and Ca^{++}-salts of PGA is even greater than during the formation of acid salts. Such salts can react with cytochrome C mainly nonelectrovalently, as a result of which there is a more uniform distribution of supramolecular complexes of these PGA salts and their separate macromolecules on the surface of this protein, as is shown by electron microscopy. Supramolecular complexes are not formed with the normal K^{+}-salt of PGA on account of the common cation, and electronic interaction with cytochrome C is excluded, as is shown electron-microscopically by the uniform distribution of the above-mentioned protein of that salt, like other normal salts of PGA, over the film.

According to the results of the electron-microscopic investigations of the isolated individual PGA preparations mentioned above, differences were clearly revealed between a group including PGA in the form of the free acid and acid salts of PGA, and the group of normal salts of these biopolymers. Within each such group, besides the individual differences between the preparations there is also a certain similarity between the structures, as is revealed by the electron-microscopic investigation and is connected with the number, nature, and distribution of cations connected with PGA between the sulfate and carboxyl groups. On the basis of these results and also of those of infrared spectroscopic investigations of various PGA salts, which showed that the conformational features of their macromolecules depend on the number and nature of cations bound with them, it can be postulated that the unique, electron-microscopically revealed structures of PGA salts are to a large extent associated with their conformational features. The possibility of variations in the spatial structures of PGA and other proteoglycans present in tissues is much greater than in the case of isolated proteoglycans, for here, besides inorganic cations, various organic bases also are present. Cartilage, carboxyl and sulfate groups of PGA are probably bound with different bases, so that 3-4 M solutions of salts extract them from this tissue in the form of the acid salt, with unsubstituted protons of carboxyl groups [4]. This must be taken into account when the results of electron-microscopic investigations of proteoglycans present in tissues are analyzed.

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ULTRASTRUCTURAL CHANGES IN STRIATED MUSCLES UNDER THE INFLUENCE OF SPACE FLIGHT FACTORS

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UDC 612.74.086.3.06:629.78

KEY WORDS: soleus, gastrocnemius, and diaphragm muscles; muscle fibers; atrophy; ultrastructure.

Human sojourn in space on a space ship is accompanied by functional disturbances in the muscular system, which are based on changes in its structural organization and metabolism [1, 2]. The character and severity of the changes in the muscles are variable and depend on their functional role [3, 5, 6], and for that reason the influence of factors arising during a 7-day space flight on muscle can best be studied in relation to the structural response of different types of muscles.

EXPERIMENTAL METHOD

Pieces of the soleus, gastrocnemius, and diaphragm muscles of six rats, kept on board the "Kosmos-1667" biosatellite for 7 days, and seven rats of the animal house control, were taken for ultrastructural analysis (all the rats were of the Wistar strain, SPF colony). The animals were killed by decapitation 4-8 h after returning to earth.

Material was prefixed in 4% formaldehyde, buffered to pH 7.4 with acetate-Veronal buffer, with the addition of iso-osmotic sucrose; postfixation was carried out in 1% OsO_4 solution and embedding in Araldite. Sections were stained by Reynolds' method and examined in the JEM-7A electron microscope.

EXPERIMENTAL RESULTS

Changes affecting virtually all its structural elements — myofibrils, nuclei, mitochondria, sarcoplasmic reticulum — were discovered in the muscle fibers of the soleus muscle. The most widespread type of change was atrophy of the myofibrils and muscle fibers. Thinning of the myofibrils and widening of the spaces between them were observed, with the formation of slits, vacuoles, and cavities (Fig. 1a). Fibers with focal destruction of the sarcomeres, a zigzag arrangement of the material of the Z-lines (Fig. 1b), and sometimes complete destruction of myofilaments with the formation of homogeneous pale finely granular fields, in which the nuclei, small dense mitochondria, fragments of membranes, and myelin-like formations were chaotically arranged (Fig. 1c). In some muscle fibers there was in increased number of fat droplets and subsarcolemmal and intermyofibrillar rows of glycogen granules.

Research Institute of General Pathology and Pathological Physiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR G. N. Kryzhanovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 12, pp. 746-749, December, 1988. Original article submitted December 24, 1987

 0007-4888/88/0012-1792$12.50