COLLAGEN – POLYSACCHARIDE GEL AS A MODEL OF THE GROUND SUBSTANCE OF CONNECTIVE TISSUE

V. A. Lazarev

UDC 612.751.3.001.57

Samples of gel formed at 37°C in solutions containing tropocollagen and various polysaccharides were studied with the electron microscope. Contracting gel clots were formed in solutions containing chondroitin sulfate, proteoglycan from tracheal cartilage, and gum arabic as the polysaccharide component. Examination under the electron microscope showed that these clots were permeated by transversely striated collagen fibrils with a period of 64 nm. The connection between the density of the gel thus formed and the nature of the polysaccharide component is discussed. Gel forming in solutions containing tropocollagen and various polysaccharides can be regarded as a suitable model of the ground substance of connective tissue.
KEY WORDS: connective tissue; simulation of ground substance; collagen; polysaccharides; electron microscopy.

The ground substance of connective tissue is a gelatinous substance formed on a basis of fibrous structures and interstitial material containing acid mucopolysaccharides of the hyaluronic acid and chondroitin sulfate type, which are usually in the form of complexes with noncollagen proteins. In different types of connective tissue the relationship between these components varies, and as they vary so also do the physical properties of the ground substance: its density, strength, elasticity, and hydrophilic properties.

Some workers have regarded the gel obtained in vitro and containing acid mucopolysaccharides and collagen fibrils [4, 9, 13] as a model of the ground substance of connective tissue. Interaction of tropocollagen with mucopolysaccharides and proteoglycans and also the effect of polysaccharides and of protein–polysaccharide complexes on the formation of collagen fibrils in vitro have frequently been investigated [1, 2, 5, 7, 8, 10-12, 14, 15].

In this investigation an attempt was made to simulate the ground substance of connective tissue by using a gel formed during combined precipitation of tropocollagen and various polysaccharides from solution.

EXPERIMENTAL METHOD

Solutions of tropocollagen were obtained by extracting the protein from the skin of albino rats with citrate buffer, pH 3.4 [3]. The protein concentration was about 0.1%. The solutions were purified by filtration. The polysaccharides used included: hyaluronic acid (Reanal, Hungary), chondroitin sulfate A (Schuchardt, West Germany), gum arabic (Merck, West Germany), amylose, and also proteoglycan isolated by extraction with water from bovine tracheal cartilage [6]. To avoid uncontrollable changes of pH in the solution of tropocollagen on the addition of the polysaccharides, solutions of tropocollagen and polysaccharides with weakly acid or neutral pH values were made up separately, after which the solutions were mixed and the pH of the mixture determined. The polysaccharides were dissolved in citrate buffer. Besides tropocollagen the solutions contained only one polysaccharide component. The concentration of hyaluronic acid was 0.5-2%, of chondroitin sulfate 5-10%, gum arabic 5-15%, amylose 5-15%, and proteoglycan 1-2%. The mixture of tropocollagen and polysaccharides was incubated at 37°C for a few days. For electron-microscopic investigation the material precipitated

Laboratory of Histophysiology, Institute of Clinical and Experimental Medicine, Academy of Medical Sciences of the USSR, Siberian Branch, Novosibirsk. (Presented by Academician of the Academy of Medical Sciences of the USSR V. P. Kaznacheev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 10, pp. 1216-1218, October, 1976. Original article submitted March 23, 1976.

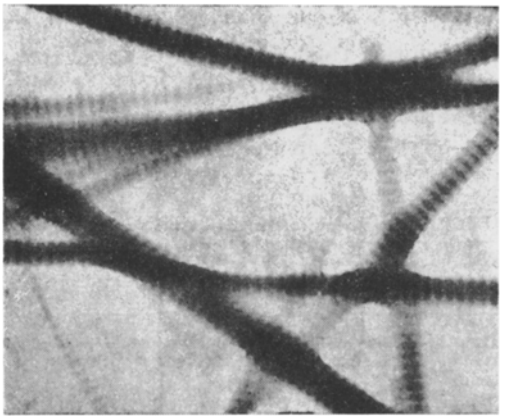

Fig. 1. Collagen fibrils in gel formed in solution containing tropocollagen and chondroitin sulfate. Stained with phosphotungstic acid, 30,000 ×.

from the solution was transferred by means of a bacteriological loop to grids covered with collodion film stabilized with carbon. The structure of some specimens of gel was investigated in ultrathin sections cut from material embedded in Araldite. The sections were stained with a 3% aqueous solution of phosphotungstic acid (pH 6.5). They were examined in the JEM- 100B electron microscope.

EXPERIMENTAL RESULTS

In some of the tubes containing the mixed solutions, gel clots were formed after a few days of incubation at 37°C, and in the course of time they contracted. Clots were formed in solutions containing chondroitin sulfate and gum arabic at pH 6.0-6.5. The densest clots were formed in solutions with chondroitin sulfate, less dense in a solution containing proteoglycan. No gel was formed in solutions containing amylose, even in high concentrations (up to 15%). A gel formed in solutions containing hyaluronic acid but did not condense into clots. Examination in the electron microscope showed that the collagen–polysaccharide gel formed in solutions containing chondroitin sulfate, hyaluronic acid, gum arabic, and proteoglycan was permeated by cross-striated collagen fibrils with a period of 64 nm, forming a fibrillary felt (Fig. 1). The diameter and length of the fibrils depended on the concentration of polysaccharides added; with an increase in concentration the diameter decreased but the length increased. In particular, in the absence of added polysaccharides tropocollagen in citrate solution (at pH about 6.0) formed fibrils or tactoids over 1 μ in diameter, but in a 10% concentration of chondroitin sulfate it formed fibrils only 100-200 nm in diameter, i.e., the collagen fibrils were of about the same diameter as in connective tissue. The addition of amylose also led to a decrease in the thickness of the fibrils formed, although no gel was formed in this case. The influence of mucopolysaccharides on the diameter of collagen fibrils formed in vitro has also been noted by other workers [10, 15]. The results suggest that the effect of mucopolysaccharides on the diameter of collagen fibrils formed in their presence can be the explanation why fibrils 50-150 nm in diameter are found in connective tissue, whereas the diameter of fibrils or tactoids formed in tropocollagen solutions may reach tens of microns. Fibrous structures and the ground substance of connective tissue are evidently indissolubly connected.

As has already been stated, no gel was seen to form in tropocollagen solutions containing amylose, even if the polysaccharide was present in high concentrations. All polysaccharides in the presence of which gel clots were formed contained ionogenic groups – in the case of gum arabic these were carboxyl groups, in chondroitin sulfate carboxyl and also sulfate groups. However, in solutions containing hyaluronic acid, a noncontracting gel was formed, despite the presence of carboxyl groups in this polymer. The explanation of this fact must probably be sought in the high hydrophilic property of the hyaluronic acid, which is capable of binding large quantities of water. In the presence of proteoglycan from tracheal cartilage, weakly contracting gel clots were formed, and this may also be attributed to the farily high hydrophilic property of the protein–polysaccharide complex.

The degree of contraction of the gel clots formed in solutions containing tropocollagen and polysaccharides was thus determined, first, by the character and quantity of ionogenic groups in the polysaccharides and, second, by the hydrophilic property of the polysaccharides, connected with their molecular weight.

Because of these findings it is natural to try to associate the formation of connective tissue with a varied degree of contraction with the nature and quantity of mucopolysaccharides present during this process. During the formation of dense forms of connective tissue (cartilege, bone, dermis, tendon) large quantities of sulfated mucopolysaccharides are present. Loose connective tissue, synovial fluid, certain connective-tissue components of the eye, and Wharton's jelly are formed in the presence of large quantities of hyaluronic acid or of incompletely sulfated hexosaminoglycans. The hyaluronic acid prevents contraction of these tissues, so that their degree of hydration remains adequate.

The study of the collagen–polysaccharide gel formed under certain conditions in solutions containing tropocollagen and polysaccharides can thus explain some of the properties of connective tissue; such a gel can be regarded as a model of the ground substance of connective tissue.

LITERATURE CITED

1. S. M. Bychkov, Dokl. Akad. Nauk SSSR, 75, 83 (1950).
2. S. M. Bychkov and V. A. Fomina, Vopr. Med. Khim., No. 1, 59 (1958).
3. V. N. Orekhovich and V. O. Shpikiter, Biokhimiya, 20, 438 (1955).
4. J. H. Sessler, Biochem. J., 76, 124 (1960).
5. D. A. Lowther, B. P. Toole, and A. C. Herrington, in: Chemistry and Molecular Biology of the Intercellular Matrix (ed. by E. A. Balazs), Academic Press, New York (1970), p. 1135.
6. J. Malawista and M. Schubert, J. Biol. Chem., 230, 535 (1958).
7. M. B. Mathews, Biochem. J., 96, 710 (1965).
8. M. B. Mathews and L. Decker, Biochem. J., 109, 517 (1968).
9. F. A. Meyer, W. D. Comper, and B. N. Preston, Biopolymers, 10, 1351 (1971).
10. B. Öbrink, Europ. J. Biochem., 34, 129 (1973).
11. B. Öbrink and A. Wasterson, Biochem. J., 121, 227 (1971).
12. V. Podrazky et al., Biochim. Biophys. Acta, 229, 690 (1971).
13. M. Schubert, in: Structure and Function of Connective and Skeletal Tissue, London (1965), p. 124.
14. B. P. Toole and D. A. Lowther, Biochem. J., 109, 857 (1968).
15. G. C. Wood, Biochem. J., 75, 605 (1960).