INTERACTION OF A HYBRID PROTEIN – CHONDROITIN – KERATOSULFATE COMPLEX WITH PROCOLLAGEN

S. M. Bychkov, S. S. Nikolaeva, and V. N. Kharlamova

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During interaction between a hybrid protein–chondroitin–keratosulfate (PCKS) biopolymer with procollagen (acid-soluble collagen) the packing density of the chains of this protein in the resulting procollagen–PCKS complex is increased. The procollagen–PCKS complex has a greater capacity than free procollagen, but less than PCKS, for absorbing water.

The participation of acid glycosaminoglycans in the formation of collagen and other protein complexes and in the transportation of water and various ions is due primarily to the extremely high negative electric charge and the well marked hydrodphilicity of their macromolecules [2, 3]. More detailed investigations of the physicochemical properties of these biopolymers and their protein complexes in this direction are essential. This paper describes the x-ray structural analysis, and determination of the density and capacity for absorbing water of one of the most important and widespread hybrid biopolymers in animal tissues, namely protein–chondroitin–keratosulfate (PCKS), and its complex with procollagen (acid-soluble collagen).

EXPERIMENTAL METHOD

PCKS was used as the potassium salt, isolated from the cartilaginous rings of the bovine trachea [5, 6]. The preparation of PCKS used in the work contained (in percent of dry weight): nitrogen 4.1, galactosamine 22.2, glucosamine 3.7, sulfate sulfur 4.3, hexuronic acid 24.0, sialic acid 2.4. The predominant components of its protein moiety were aspartic and glutamic acids and threonine. The procollagen was isolated from the skin of albino rats [9]. The methods of obtaining the complex of procollagen with PCKS are described elsewhere [1, 5, 6]. The content of PCKS in its complex with procollagen was 22-25%.

For the x-ray structural investigations a type URS-55A apparatus with CuK α-ray source and nickel filter was used. The density of the preparations was measured at 25°C with an accuracy of 10^{-5} g/cm^3 by the "electromagnetic float" method, using p-xylene as the medium [11, 15]. The material was first freed from moisture in a vacuum at 10^{-6} mm Hg. The water-absorbing capacity of specimens dried over phosphoric anhydride was determined from the increase in weight after they had been kept at a relative humidity of 95% and a temperature of 20°C [7].

EXPERIMENTAL RESULT

The roentgenogram of PCKS has a diffuse halo ($d_3 - d_4$) and a very low-intensity reflection d_1, namely 10.30 Å, indicating the quasiamorphous state of this biopolymer (Table 1). X-ray structural analysis of procollagen reveals quasicrystalline (d_1, d_2, d_5) and quasiamorphous ($d_3 - d_4$) structures of its molecule. This is in agreement with data in the literature [12]. Interference of d_1 in the procollagen–PCKS complex is 9.64 Å and its intensity is reduced, indicating approximation of the protein chain and disturbance of their quasicrystalline order. In addition, interference of d_2 disappears completely, further evidence of

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TABLE 1. Results of X-Ray Structural Analysis and Measurements of Density

Material	Interplanar distances (in Å along the meridian)					Density (in g/cm³)
PCKS	10,30	—	5,10	4,10	—	1,62940
Procollagen	11,80	7,85	5,90	3,92	2,99	1,38400
Procollagen–PCKS .	9,64	—	5,35	3,79	2,90	1,41287

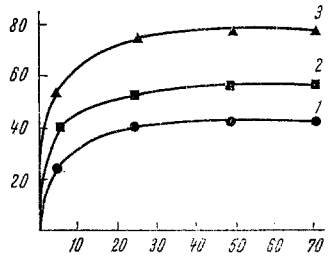

Fig. 1. Absorption of water by PCKS (3), procollagen (1), and the procollagen–PCKS complex (2). Abscissa, time (in h); ordinate, absorption of water (in %).

a disturbance of the quasicrystalline order in the structure of the procollagen part of the complex. However, in the complex there is an increase in the intensity of the diffuse halo ($d_3 - d_4$), due partly to disturbance of this structure of procollagen but mainly to the addition of PCKS to it.

PCKS has a relatively high density (Table 1). The density of procollagen is much lower than that of PCKS. The value of this index in the procollagen–PCKS complex is intermediate between the two values mentioned.

PCKS has the highest water-absorbing capacity and procollagen the lowest, that of the procollagen–PCKS complex being intermediate (Fig. 1).

The results of x-ray structural analysis show that when it interacts electrostatically with procollagen, the PCKS brings the protein molecules closer together and arranges them in a certain manner, thereby facilitating weak interactions between them. As a result, the distance between the protein chains in the procollagen–PCKS complex is reduced.

The higher density of the procollagen–PCKS complex than of the original procollagen is due to addition of the PCKS to it and to the increased packing density of the procollagen chains.

Absorption of water by PCKS, by procollagen, and by the complex, if other conditions are equal, increases with increasing density of the polymers. One of the main factors responsible for this property is the presence of groups capable of forming hydrogen bonds in the polymer. The high density of the PCKS is due not only to its composition and the structural features of its macromolecule, but also, probably, to the existence of hydrogen bonds between the individual chains of the chondroitin-4-sulfate and keratosulfate composing this biopolymer. The greater ability of the procollagen–PCKS complex than of free procollagen to absorb water is due to the presence of the exceptionally hydrophilic glycosaminoglycan in this complex.

Acid glycosaminoglycans thus provide a certain essential content of water in the collagen complex, and under the conditions prevailing in the tissues this is an important factor [13]. Investigations [8, 10, 14] have shown that water is incorporated as a special structural element in the collagen helix. The formation of complexes of the collagen–glycosaminoglycan (acid) type creates a gradual hydration gradient in the tissues between collagen fibers and their surrounding medium containing glycosaminoglycans in the free form [13].

The results of the investigation described above are in full agreement with the fact that removal of all glycosaminoglycans linked by electrovalent bonds from the collagen bundles of the tendon considerably weakens the bond between the collagen fibers and reduces their density [4].

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