STRUCTURAL-MECHANICAL PROPERTIES AND SWELLING OF PROTEIN COMPLEXES OF HYALURONATE AND PROTEIN-CHRONDROITIN-4-SULFATE

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Structural-mechanical strength and swelling of gels consisting of gelatin and potassium hyaluronate (PHY) or potassium protine–chondroitin-4-sulfate (PPCS) were shown to depend on the ratio between the components. With low concentrations of PHY and PPCS the minimum of structural-mechanical strength coincided with the maximum of swelling of the gels. In zones of neutralization of the positive electric charges of gelatin by macropolyanions, high structural-mechanical strength of the gels coincided with the minimum of swelling. In high concentrations of PHY, structural-mechanical strength and swelling of the gel became equal to the values characteristic of a gel consisting of gelatin only, but in high concentration of PPCS there was an additional parallel increase in this strength and in swelling of the gel.

KEY WORDS: hyaluronate; protein–chondroitin-4-sulfate; gelatin; structural-mechanical strength and swelling of gels.

The participation of glycosaminoglycans and proteoglycans in the binding and transport of water in connective tissue is determined by the ability of these biopolymers to dissolve without limit in water, thereby creating complex structures which function as osmotic cells and molecular sieves, and to interact with proteins and low-molecular-weight bases [2, 4].

To study the molecular mechanism of the binding and transport of water in connective tissue the structural-mechanical properties and swelling of gels consisting of potassium hyaluronate (PHY) or potassium protein–chondroitin-4-sulfate (PPCS) and gelatin, chosen as a collagen-like protein, were investigated.

EXPERIMENTAL METHOD

The PHY was isolated from human umbilical cords [3] and PPCS from cartilaginous rings of bovine trachea [4]. Gelatin was purified by Loeb's method. Gels were prepared by mixing gelatin solution with solutions of PHY and PPCS in different quantitative proportions. The structural-mechanical strength of the gels was measured by the tangentially moving plate method, in which the plate is immersed in the gel until it solidifies [5, 7]. The limiting shearing stress P, characterizing the structural-mechanical strength of the gel, corresponds to the force F at which the plate shifts through an area S cm^2, i.e., P = F/dynes $\cdot$ cm^{-2}. The investigations were carried out at 20-21°C after the gel had stood for 24 h at 5°C. The volume of the gels in all experiments was 25 ml. Swelling was determined by immersing pieces of the film of gel (10 × 10 mm), dried at 4-5°C, and then weighing them on a microbalance after known time intervals to establish equilibrium. The volume of water absorbed by the film was expressed as a percentage of the increase in weight of the film relative to its dry weight. Gels and films prepared from gelatin alone were used as the control. In all experiments the reactions of the gels were neutral.

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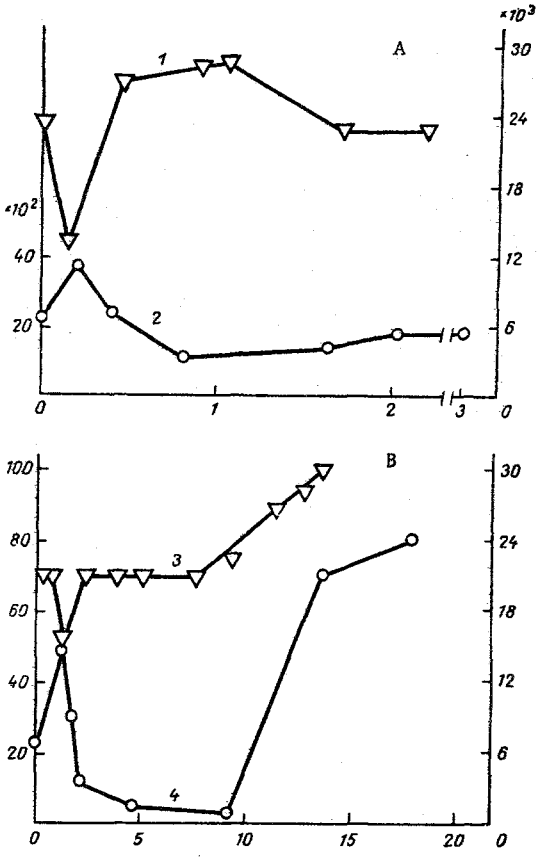

Fig. 1. Graph showing relationship of P (1, 3) and swelling (2, 4) to concentration of PHY (A) and PPCS (B). A) Gelatin concentration 5%; B) gelatin concentration 4%. First points on ordinate show control (pure gelatin of same concentration as in experiment). Results of analysis of PHY and PPCS respectively: nitrogen 2.8 and 5.0%, aminosugar 38.3 and 28.0%, hexuronic acids 40.0 and 28.0%, sulfate 0.0 and 9.0%, sialic acids 0.0 and 1.0%. Ordinate: left, swelling (in %), right, P (in dynes · cm^{-2}); abscissa, concentration of PHY (A) and PPCS (B) (w/w, %).

EXPERIMENTAL RESULTS AND DISCUSSION

If the PHY content in the gel was low, the value of P was much lower than that of pure gelatin and reached a minimum when the PHY concentration was 0.2%, after which P began to rise just as rapidly to a maximum in PHY in a concentration of 0.5-1%. An increase in the PHY concentration in the gel above 1% was accompanied by a decrease in the value of P down to the value characteristic of a gel of gelatin only (Fig. 1A, curve 1). The volume of water absorbed by the gelatin film rose sharply if PHY was present in it, to reach a maximum (3800%) if its concentration was 0.2%, when a minimum of structural-mechanical strength was observed. An increase in the concentration of this glycosaminoglycans led to an initially sharp, but later gradual decrease in swelling, down to a minimum (1200%) in the presence of PHY in a concentration of 0.9%, less than the swelling of a gel consisting of gelatin only. A subsequent increase in the PHY concentration led to an increase in swelling of the gel up to the level of swelling of a gelatin gel (Fig. 1A, curve 2).

The value of P of gel of gelatin and PPCS, with low concentrations of PPCS at first did not differ from P for gelatin, but when the concentration of the proteoglycan reached 2.2%, P reached a minimum. With an increase in the PPCS concentration, P rose sharply up to a limit, at which it remained unchanged until a concentration of 7.5%; higher concentrations of this proteoglycan in the gel led to a second increase in its structural-mechanical strength (Fig. 1B, curve 3). The swelling of the gelatin rose sharply in the presence of PPCS to reach a maximum (5000%) when the concentration of the biopolymer was at the level at which structural- mechanical strength of the gel reached a minimum. An increase in the PPCS concentration caused a decrease in

swelling of the gel to a minimum, and when the concentration of PPCS was between 5 and 10% the swelling remained at the 200-500% level, i.e., much less than the swelling of gelatin alone; this was followed by a second increase in swelling, which was virtually parallel to the increase in P, which was not observed when a gel consisting of gelatin and PHY was used (Fig. 1B, curve 4).

The structural-mechanical strength and swelling of gels composed of gelatin and containing PHY or PPCS were thus interconnected and depended on the concentration of the components. In low concentrations of PHY and PPCS (0.2 and 2.2% respectively) coincidence of the minimum of structural-mechanical strength with the maximum of swelling of the gels was evidently due to changes in the secondary and quaternary structures of the protein caused by the action of these macropolyanions on it and also by changes in the structures of the osmotic cells and molecular sieves which PHY and PPSC form in aqueous solutions, under these conditions, leading to an increase in swelling and a decrease in strength of the gel. The increase in structural-mechanical strength of the gels in higher concentrations of PHY and PPCS was the result of the formation of complexes of gelatin with these biopolymers. The slight swelling of the gels in such cases was due to some extent to the strength of their structure. In the zone of great structural-mechanical strength and weak swelling of the gels the positive electric charges of the gelatin were neutralized and the macromolecules of the complexes became electronegative to a varied degree on account of the carboxyl groups of gelatin and also, perhaps, the anionic group of PHY and PPCS not used up in combination with the positively charged groups of this protein. The minimum of swelling and the coincident maximum of structural-mechanical strength of the gels cannot therefore be regarded as points of equivalence of the positive and negative charges of the components. Furthermore, the minimum and maximum mentioned above occurred at higher concentrations of PPCS than of PHY, regardless of the fact that the number of anionic groups of the former is much greater than of the latter.

The difference between the action of PHY and PPCS on the structural-mechanical properties and swelling of gels arose only in relatively high concentrations of these macropolyanions (which differed for PHY and PPCS).

In a gel composed of gelatin and PHY with high concentrations of PHY, because of the decrease in free space between the macromolecules of this glycosaminoglycans the gelatin molecules were displaced, i.e., microstratification of the gel occurred [1, 6], as expressed by the equality of P and swelling to the values characteristic of a gel consisting of gelatin only, for PHY itself does not form gels [2]. In a gel of gelatin and PPCS with very high concentrations of PPCS, however, additional formation of two more intricate complexes took place, as shown by the increase in structural-mechanical strength of those gels. The increase in swelling observed under these circumstances was possibly due to the large quantity of PPCS not incorporated into the complexes and mechanically trapped in the structure of the gel. In low concentrations PHY affected the structure and swelling of the gels more strongly than PPCS, since the molecular weight of the former is greater than the molecular weight of the latter. The presence of sulfate groups in PPCS, together with a relatively large quantity of covalently bound protein, with a negative electric charge, the density of which was much greater than in PHY, and other special features distinguishing this proteoglycan make a wider variety of interactions possible between PPCS and proteins [2, 8]. The second increase in structural-mechanical strength of the gel in high concentrations of PPCS was evidently connected with this fact.

The principles thus discovered must play an important role in the processes of hydration of connective tissue and of water transport in it.

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