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COMBINED ACTION OF PROTEIN-CHONDROITIN-4-KERATAN-SULFATE AND HYALURONIC ACID ON AGGREGATION AND ADHESION OF RED CELLS

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The rate and degree of aggregation of red cells produced by a mixture of protein-chondroitin-4-keratan-sulfate (PCKS) and hyaluronic acid (HUA) were found to be greater than the sum of the values of the corresponding indices for the separate action of these proteoglycans on red cell aggregation in the same concentrations as in the mixtures. It is suggested that this effect is due to the formation of a hybrid PCKS–HUA complex in the mixture which is more accurate as regards red cell aggregation than the separate components.
KEY WORDS: protein-chondroitin-4-keratan-sulfate; hyaluronic acid; complexes; red blood cells; aggregation.

Studies of the role of proteoglycans in the aggregation and adhesion of cells, using red blood cells as a model of isolated cells have shown that the ability of protein-chondroitin-4-keratan-sulfate (PCKS) and hyaluronic acid (HUA) to induce nonspecific reversible aggregation of red cells is due mainly to the property of these biopolymers of creating supramolecular complexes and three-dimensional structures in solutions which displace the red cells from the space they occupy into a separate phase. Electrostatic interaction between the red cell surface and these macropolyanions evidently plays a less important role in red cell aggregation [2, 3]. It has been suggested that red cell aggregation induced by PCKS and HUA is an expression of common properties of these proteoglycans of concentrating various tissue elements in a definite and limited space, and thus enabling all forms of interaction between them to be manifested [2, 3, 5].

In order to probe deeper into the role of proteoglycans in cell adhesion, in the investigation described below the combined action of PCKS and HUA on red cell aggregation was studied, for in many types of connective tissue these two substances coexist in various amounts and, by combining with each other, they may form hybrid complexes [6-10].

EXPERIMENTAL METHOD

PCKS was isolated from the cartilaginous rings of the bovine trachea [4] and HUA from human umbilical cords [1]. Both biopolymers were used in the experiments as their potassium salts.

Rabbit red blood cells were washed with physiological saline and a 1% (by volume) suspension of these cells was prepared in the same solution. Proteoglycans dissolved in 0.16 M NaCl were added to a known volume of the suspension in sufficient quantity to obtain the necessary final concentration of the proteoglycan. The mixture was quickly stirred and part of it transferred to a counting chamber, after which it was photographed at various time intervals under the microscope (magnification 120). The total number of cells was counted visually, by means of a projector, from the photographic frames taken during the first 1-2 min. The number of single red cells, i.e., unaggregated, was counted in the same frames obtained subsequently. The difference between the initial total number of red cells and the number of cells still remaining single at the subsequent times gave the number of red cells forming aggregates. Aggregation was expressed by the number of aggregated red cells as a percentage of their total number. A suspension of red cells in physiological saline, photographed after the same time intervals as the experimental samples, served as the control. Aggregation of the red cells was not observed in the control samples.

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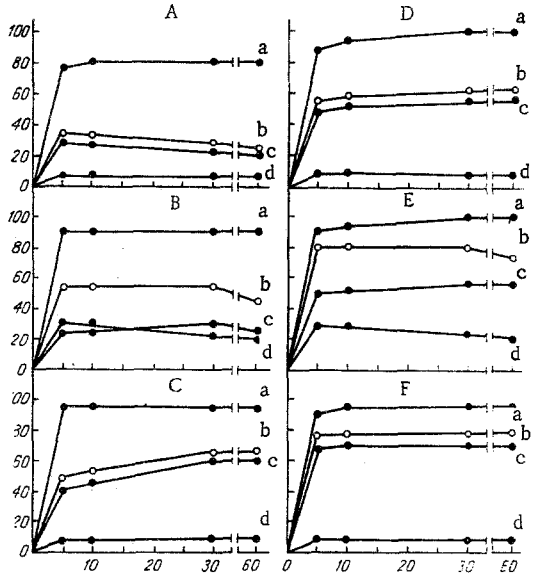

Fig. 1

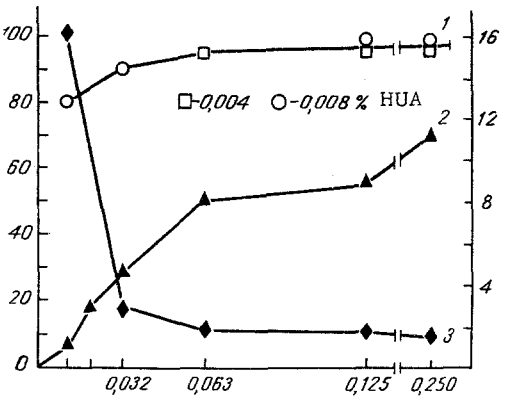

Fig. 2

Fig. 1. Kinetics of red cell aggregation on combined action of PCKS and HUA. A: a) 0.008% PCKS + 0.008% HUA, b) sum of c and d, c) 0.008% HUA, d) 0.008% PCKS; B: a) 0.032% PCKS + 0.008% HUA, b) sum of c and d, c) 0.032% PCKS, d) 0.008% HUA; C: a) 0.063% PCKS + 0.004% HUA, b) sum of c and d, c) 0.063% PCKS, d) 0.004% HUA; D: a) 0.125% PCKS + 0.004% HUA, b) sum of c and d, c) 0.125% PCKS, d) 0.004% HUA; E: a) 0.125% PCKS + 0.008% HUA, b) sum of c and d, c) 0.125% PCKS, d) 0.008% HUA; F: a) 0.250% PCKS + 0.004% HUA, b) sum of c and d, c) 0.250% PCKS, d) 0.004% HUA. Abscissa, time (in min): ordinate, aggregation (in %).

Fig. 2. Dependence of red cell aggregation on concentration of PCKS in the presence of HUA. 1) PCKS + HUA; 2) PCKS; 3) ratio between degree of aggregation in mixtures of PCKS + HUA and that observed in the absence of HUA. Abscissa, PCKS concentration (in %); ordinate: left – aggregation after 60 min (in %), on right – ratio between values of aggregation.

The reaction of the solution in all the experiments was close to neutral (pH 7.2-7.4)

EXPERIMENTAL RESULTS

The study of the kinetics of red cell aggregation gave the following results: If, in the case of the separate addition of PCKS and HUA, the concentration of the former was insufficient to cause aggregation, and the concentration of the latter was such that only slow and a very slight degree of aggregation was observed, if both these macropolyanions were present in the same solution and in the same amounts, rapid aggregation of these cells took place, to the extent of 80% during the first 5 min (Fig. 1A). A mixture consisting of quantities of PCKS and HUA which separately gave a weak action of this sort caused total aggregation (95-100%) in 5 min (Fig. 1B, E). With PCKS in concentrations of 0.063, 0.125, and 0.250% the degree of aggregation of the red cells after this period was 40, 60, and 70%, respectively. Addition of 0.004% HUA, which in this concentration by itself had no effect of this sort on the red cells, to the above concentrations of PCKS caused rapid and complete (100%) aggregation of red cells in all these mixtures (Fig. 1C, D, F).

The kinetic data show that the rate and degree of red cell aggregation following the combined action of PCKS and HUA were always greater than the sum of the indices obtained when the two biopolymers acted separately and independently on aggregation, in the same concentrations in each case (Fig. 1).

A study of the dependence of the degree of red cell aggregation on the concentration of PCKS in the presence of HUA in amounts which themselves do not induce aggregation or do so only to a very slight degree showed that the degree of aggregation in such mixtures is relatively independent of the PCKS concentration in them (Fig. 2). Even with PCKS in concentrations of 0.008 to 0.032%, giving rise to 5-30% aggregation, if the mixtures contained 0.004-0.008% HUA the degree of aggregation reached 80-90%, i.e., practically the same as occurred under the same conditions but with PCKS in a concentration of 0.063-0.250%. The relative increase in

the degree of red cell aggregation on account of HUA was thus greatest in the presence of low concentrations of PCKS, which themselves induced only a very slight degree of aggregation. In mixtures of PCKS and HUA containing 0.008% of each macropolyanion the degree of aggregation was 16 and 4 times greater than the degree observed when PCKS and HUA respectively acted independently. With an increase in the PCKS concentration the relative increase in aggregation in the presence of HUA initially fell sharply, but later, starting with PCKS in a concentration of 0.032%, aggregation was only 3-1.5 times greater than that produced by PCKS alone (Fig. 2). It should be noted that such small concentrations of HUA had a very powerful effect on red cell aggregation in the presence of PCKS.

The greater rapidity and degree of aggregation of red cells in a mixture of PCKS and HUA than when they act indepedently are thus not the result of summation of the separate actions of the two proteoglycans. The phenomenon observed is evidently due to the formation of a hybrid PCKS–HUA complex, which is perfectly feasible under the experimental conditions used, for such complexes readily form when solutions of these two proteoglycans are mixed [7]. Giant macromolecules of the PCKS–HUA complex, while preserving certain individual properties of each component, creates more complex structures in solution than PCKS and HUA separately. By virtue of this fact, structures of the PCKS–HUA complex displace the red cells from the space they occupy in the solution into an isolated phase much more effectively than structures composed of PCKS and HUA alone. The rate and degree of aggregation of red cells in a solution of mixtures of PCKS and HUA are thus greater than the sum of the values of these indices for the independent action of PCKS and HUA in this respect.

In mixtures with low and approximately equivalent concentrations of PCKS and HUA, after the formation of the PCKS–HUA complex only negligible amounts of these proteoglycans remain in the free form. Under these conditions the only biopolymer producing aggregation of the red cells is this complex, and a rough idea of its aggregating activity can be obtained from the values of the rate and degree of aggregation in this case. With high concentrations of PCKS and low concentrations of HUA in the mixtures, when all the available amount of the latter is combined into a complex, but some of the PCKS in the mixture still remains free, aggregation of red cells may perhaps take place through the additive action of the PCKS–HUA complex and free PCKS. Under these conditions, however, more complex relations between the PCKS–HUA complex, PCKS, and the red cells cannot be ruled out. For this reason, and also because of the considerable variability in the content of the two components in the PCKS–HUA complex [7, 8], it is impossible at this stage to give a strictly quantitative description of the relationship between the rate and degree of aggregation of red cells and the ratio between the concentrations of PCKS and HUA in the mixtures.

The results of this investigation suggest that one of the biological functions of the ground substance of connective tissue is to limit the dispersion of certain tissue elements and concentrate them in particular morphological structural formations by means of the mechanism the essence of which has been indicated above, and which was described in greater detail in the writers' previous communications [3, 5].

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