ROLE OF GLYCOSAMINOGLYCANS AND PROTEOGLYCANS IN RED CELL AGGREGATION AND ADHESION

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The action of potassium hyaluronate (HUA) and protein-chondroitin-4-sulfate (PCS) on aggregation and adhesion of rabbit red cells suspended in physiological saline was studied. The ability of HUA and PCS to produce nonspecific and reversible aggregation of red cells was shown to be attributable to the property of these biopolymers of creating complex structures of the osmotic mesh and molecular sieve type in solutions, which displace the cells from the space they occupy and concentrate them in the smallest possible volume. Various fractions of heparin, which do not create such structures in solutions, do not cause the formation of separate, clearly demarcated aggregates of red cells but prevent the aggregating action of HUA and PCS when the concentrations of these biopolymers are insufficient for complete red cell aggregation. It is suggested that the aggregating action of HUA and PCS, which is essential for adhesion to take place, is one of the universal biological functions and is manifested not only toward red cells, but also toward other cells and various tissue elements.

KEY WORDS: *hyaluronate; protein-chondroitin-4-sulfate; heparin; red blood cells; aggregation; adhesion.*

The role of glycosaminoglycans and proteoglycans in cell aggregation and adhesion has received little study. It is postulated that these biopolymers act as cementing agents and connect single cells into definite aggregates [10, 12-15]. During the separation of a suspension of red cells in physiological saline the decisive factor is the ability of glycosaminoglycans and proteoglycans to create structures of the osmotic mesh and molecular sieve type in solutions [1, 2].

In the investigation described below a quantitative study was made of the action of hyaluronic acid (HUA) and protein-chondroitin-4-sulfate (PCS), which form aggregates in solutions [2], and two heparin fractions (one containing 3 (HP-3S) and the other 4 (HP-4S) sulfuric acid residues per glucosamine residue) without ability to create structurized solutions

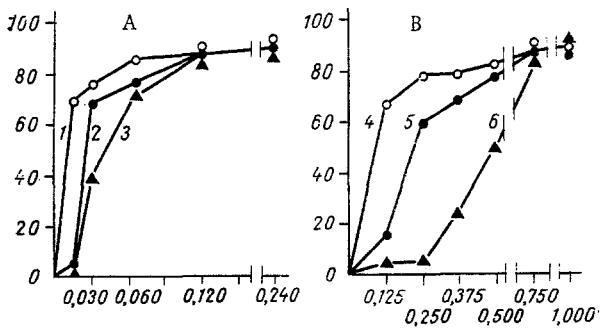

Fig. 1. Effect of concentration of HUA and PCS on aggregation of red cells after 60 min. A: 1) HUA; 2) HUA + HP-3S; 3) HUA + HP-4S; B: 4) PCS; 5) PCS + HP-3S; 6) PCS + HP-4S. Abscissa, concentration of biopolymer (in %); ordinate, aggregation (in %). Concentration of HP-3S and HP-4S in these and in all experiments illustrated in Figs. 2 and 3 was 1.0%.

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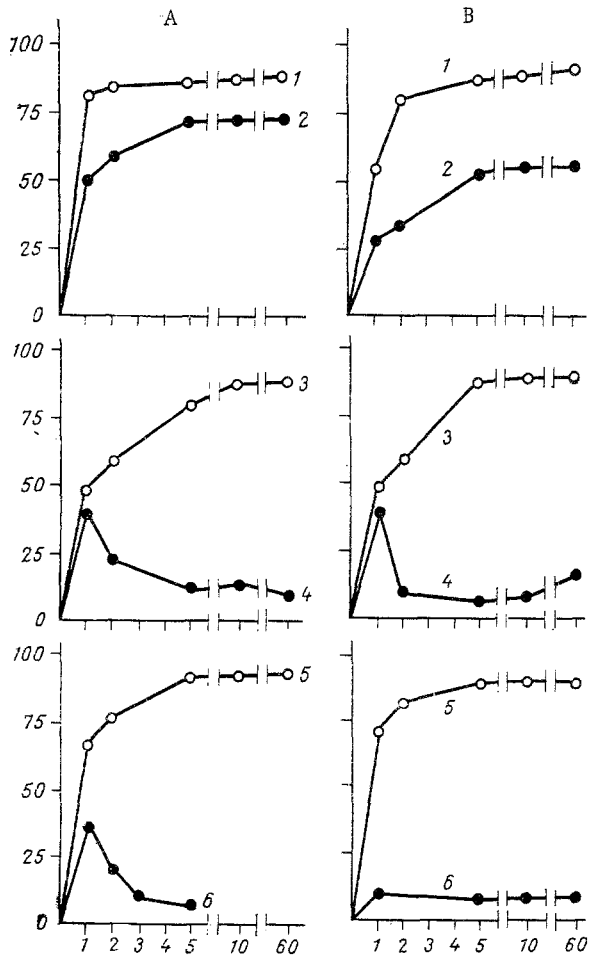

Fig. 2. Kinetics of aggregation of red cells in presence of HUA and PCS and effect of HP-3S and HP-4S on it. A: 1) 0.125% HUA; 2) 0.015% HUA; 3) 0.125% HUA + HP-3S; 4) 0.15% HUA + HP-3S; 5) 0.125% HUA + HP-4S; 6) 0.015% HUA + HP-4S; B: 1) 0.750% PCS; 2) 0.125% PCS; 3) 0.750% PCS + HP-3S; 4) 0.125% PCS + HP-3S; 5) 0.750% PCS + HP-4S; 6) 0.125% PCS + HP-4S. Abscissa, time (in min); ordinate, aggregation (in %).

on aggregation of red blood cells, chosen as a model of isolated cells. These investigations are essential for elucidation of the role of the principal components of connective tissue in cell aggregation and adhesion.

EXPERIMENTAL METHOD

Highly purified high-polymer preparations of HUA were isolated from human umbilical cords [3], and of PCS from bovine cartilaginous rings [4]. Individual fractions of heparin were isolated from commercial preparations (Spofa, Czechoslovakia) [5, 6]. All the biopolymers were used as their potassium salts.

Rabbit red cells were washed three times with physiological saline and suspended in the same solution in a concentration of 1% (by volume). A known volume of the red cell suspension was treated with the biopolymer chosen for study, dissolved in 0.14 M NaCl. The reaction of the mixture was neutral (pH 7.20-7.30) in all the experiments. The mixture was introduced into a Goryaev chamber and photographed under the microscope (magnification 120) after various time intervals. With the aid of a projector, the total number of red cells, the number of aggregates, and the number of single cells in each sample were counted visually in each frame. The degree of aggregation of the red cells was expressed by the ratio (in %) of the number of aggregated cells to the total number. The dependence of the initial rates of red cell aggregation on the concentration of HUA and PCS was calculated graphically as the tangent of the angle of slope of the curve expressing the change in aggregation with time for a given concentration of the biopolymers relative to the abscissa. In all the experiments, a suspension of red cells in 0.14 M NaCl was used as the control.

EXPERIMENTAL RESULTS AND DISCUSSION

No aggregation of red cells took place in the control samples during 1 h. HP-3S and HP-4S in a concentration of 1% did not cause the formation of discrete aggregates.

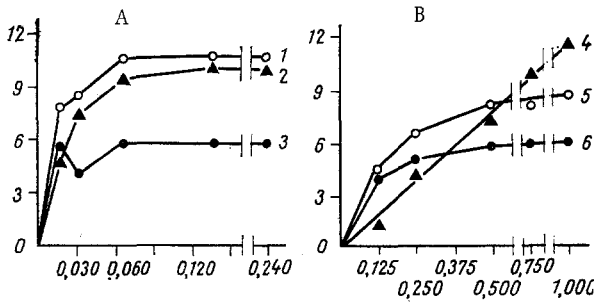

Fig. 3. Effects of concentration of HUA and PCS on initial rate of red cell aggregation. A: 1) HUA + HP-4S; 2) HUA; 3) HUA + HP-3S; B: 4) PCS + HP-4S; 5) PCS; 6) PCS + HP-3S. Abscissa, concentration of biopolymer (in %); ordinate, initial rate of aggregation (% of aggregation × sec^{-1}).

In the presence of 0.015% HUA, 70% of the red cells were joined into discrete, clearly isolated aggregates after 60 min; and in the presence of 0.120% HUA the corresponding number was about 90%. An increase in the HUA concentration did not cause any increase in aggregation (Fig. 1A). HP-3S and HP-4S reduced the aggregating action of HUA if its concentration was below 0.12%. With higher concentrations of HUA, its action was not affected by HP-3S or HP-4S.

Over the same period, PCS in a concentration of 0.125% aggregated 75% of the red cells, and in higher concentrations it aggregated 80-90% of the red cells. In a concentration of over 0.75% PCS caused no increase in red cell aggregation. The weakening action of HP-3S and HP-4S on the effects of PCS was much stronger than on the effects of HUA. If the concentration of PCS was 0.75% or higher, no weakening effect of HP-3S and HP-4S on the action of this proteoglycan was observed (Fig. 1B). In every case, HP-4S weakened aggregation of the red cells by a greater degree than HP-3S.

Aggregation of the erythrocytes ended in the course of 1 min in the presence of HUA in a concentration of 0.125%, but in a concentration of 0.015% it took place in two phases. The rapid phase lasted 1 min and the subsequent, slower, phase was complete after 4 min. In this case, complete aggregation was effected in 5 min (Fig. 2A). In the presence of HP-3S and HP-4S and of HUA in a concentration of 0.12%, aggregation took place in two phases. The rapid phase, whether HP-3S or HP-4S was used, was complete in 1 min and the slow phase after the end of 10 and 15 min, respectively. In the presence of HUA in a concentration of 0.015% and of HP-3S and HP-4S, to begin with only the rate of aggregation was reduced, but later rapid destruction of the formed aggregates of red cells took place (Fig. 2A).

Aggregation of red cells caused by PCS took place in two phases in the presence of all concentrations and was complete after 5 min. In the presence of PCS in a concentration of 0.75% HP-3S revealed the slow phase of aggregation more sharply, but in the presence of PCS in a concentration of 0.125% it destroyed the red cell aggregates formed during the first minute, just as in the presence of HUA. HP-4S speeded up the action of PCS in a concentration of 0.75% and sharply reduced the rate of red cell aggregation by PCS in a concentration of 0.125% (Fig. 2B).

It follows from these results that in the presence of HUA in concentrations of over 0.06% the rate of red cell aggregation is independent of the concentration of the biopolymer (Fig. 3A). The rate of red cell aggregation was independent of the PCS concentration if it was high (0.5%; Fig. 3B). HP-3S retarded aggregation in the presence of HUA and PCS in all concentrations studied, whereas HP-4S reduced the rate of aggregation only in the presence of these biopolymers in certain concentrations. Outside those ranges of concentration of HUA and PSA the rate of red cell aggregation was increased in the presence of HP-4S. HP-4S also reduced the rate of aggregation in the presence of HUA and PCS in concentrations too low to produce complete aggregation of the red cells (Fig. 1A, B).

In a physiological solution, the surface of the red cell membrane has a negative electric

charge due to the presence of acid groups of sialic acid [11, 14]. Sulfated macropolyanions can completely or partly inhibit dissociation of the acid groups and react electrovalently with the basic groups of the red cell membranes. With respect to this property, the macropolyanions studied are arranged in the following series: HP-4S > HP-3S > PCS. HUA is unable to significantly depress dissociation of the acid groups of the red cell membrane, for the glucuronic acid found in the macromolecule of this biopolymer is weaker than sialic acid [7]. Ability to cause aggregation of red cells, however, is a property best developed in HUA and, to a somewhat lesser degree, in PCS, which do not interact (or do so only weakly) electrostatically with red cells but possess well-marked ability to create structurized solutions [2]. The principal factors in red cell aggregation in the presence of HUA and PCS are evidently flexible structures formed in solutions by these biopolymers; acting as osmotic measures and molecular sieves, these structures displace the red cells from the space filled by them. In addition, separation into phases is facilitated by the fact that the electric charges on the red cells and macropolyanions are of the same character.

Electrovalent compounds of red cells with HP-3S and HP-4S can lead to the formation of complexes whose negative charge is greater than that of the cells because of the large number of anionic groups of HP-3S and HP-4S remaining unused after neutralization of the positive charges on the surface of the red cell membrane. Association of these complexes into discrete, clearly demarcated aggregates can be ruled out because of their mutual electrostatic repulsion, and also because HP-3S and HP-4S do not form structures capable of overcoming the energy barrier of this repulsion in solutions.

The decrease in the degree and rate of aggregation in low concentrations of HUA and PCS in the presence of HP-3S and HP-4S can be explained by the formation of red cell–heparin complexes, with strong resistance to the aggregating action of the first two macropolyanions, under these conditions. Meanwhile, in low concentrations of HUA and PCS, structures formed by them in solution in the presence of HP-3S and HP-4S are evidently insufficiently stable to overcome the resistance to aggregation of these red cell complexes carrying a high negative charge. Destruction of red cell aggregates formed initially in low concentrations of HUA and PCS in the presence of HP-3S and HP-4S is probably the result of the relaxing effect of heparin on the structures of HUA and PCS which they form in solutions, a process that takes place more slowly than aggregation. The weakened and unstable structures cannot withstand the powerful repulsion of the red cell–heparin complexes thus produced. This also explains the increase in the duration of the slow phase of aggregation in the presence of HP-3S and HP-4S in higher concentrations of HUA and PCS, as well as S shapes of the curves expressing the degree of aggregation as a function of the concentration of HUA and PCS (Fig. 1A, B). Disintegration of the red cell aggregates formed initially points to the reversibility of their aggregation induced by HUA and PCS [8]. The fact that both heparin fractions inhibit the action of PCS on red cell aggregation by a greater degree than the corresponding action of HUA may be attributable to the weaker ability of PCS than of HUA to create structurized solutions. The stronger inhibition of red cell aggregation by HP-4S than by HP-3S when produced by the action of both HUA and PCS is due to the stronger charge on the red cell–HP-4S complex, which creates a high energy barrier that must be overcome before aggregation can take place. In the presence of high concentrations of HUA and PCS, when their structures in solutions are sufficiently strong and stable, they completely overcome the resistance of the red cell–heparin complexes to aggregation. It is not yet clear what lies at the basis of the synergism found in the action of HP-4S on the rate of red cell aggregation induced by HUA and PCS, when this rate is independent of their concentration.

The role of HUA and PCS in the nonspecific and reversible aggregation of red cells is thus that, although they do not react chemically with the membranes of these cells, they displace the cells from a uniformly distributed suspension into a separate phase, concentrating them in the smallest possible volume, so that the cell membranes can interact with each other on account of van der Waals–London forces, hydrophobic forces, and other factors, i.e., so that adhesion of the cells can take place. Since the property of HUA and PCS of creating structurized solutions is one of their important physicochemical features, it can tentatively be suggested that the consequent ability of these biopolymers to induce aggregation of red cells is a universal property, exhibited also toward other cells, as a tissue element, biopolymer, and low-molecular-weight substances [9]. It must be assumed that aggregation of red cells in the presence of HUA and PCS is of great importance in the formation of postoperative thrombi, when, as a result of mechanical action on the tissues, microfoci of high concentration of these biopolymers may arise.

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SOME KINETIC FEATURES OF MEMBRANE-BOUND MONOAMINE OXIDASE

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Initial reaction-velocity versus substrate-concentration curves for serotonin oxidation catalyzed by monoamine oxidase (MAO) from fragments of rat liver or bovine brain mitochondrial membranes have a complex, nonhyperbolic shape; this is regarded as a kinetic manifestation of substrate cooperativeness for membrane-bound MAO. The possibility of interaction between different types of MAO based on conformational changes in the membrane itself is discussed.

KEY WORDS: *monoamine oxidase; biological membranes; substrate cooperativeness; serotonin.*

In the modern view [11] membrane-bound monoamine oxidases (MAO) of type A (selectively blocked by chlorgyline) specifically oxidize serotonin; however, serotonin binds with the active sites of type B MAO which are selectively blocked by Deprenil [8] and which specifically oxidize β-phenylethylamine [5].

In this investigation the effect of substrate concentration [S] on the initial reaction velocity (v) of serotonin oxidation catalyzed by MAO of fragments of mitochondrial membranes was studied because information on the kinetics of this reaction is contradictory: The curves of v as a function of [S] were either strictly hyperbolic in shape [7, 10] or of a complex, biphasic character [14].

EXPERIMENTAL METHOD

Mitochondrial fractions were obtained from rat liver or bovine brain by differential centrifugation [13], followed by freezing and thawing and sedimentation of the fragments of the mitochondrial membranes at 40,000g (1 h). The suspension of the residue in 0.01 M phosphate

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