INTERACTION BETWEEN ACID GLUCOSAMINOGLUCANS AND THE RED CELL SURFACE

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The ability of hyaluronic acid and the protein-chondroitin-keratin sulfate complex to separate a suspension of red cells in physiological saline rapidly into the solution and cell phases has been shown to be largely determined by the property of these biopolymers to form three-dimensional structures in solution. Heparin and polypeptide-chondroitin-4-sulfate, which do not possess this property, inhibit the separation of a red cell suspension into these two phases induced by the first two biopolymers.

The problem of the role of acid glucosaminoglucans in adhesion of cells has received little investigation despite its current importance [9-11]. The writers have found that several of these biopolymers have the ability to induce nonspecific reversible adhesion of red cells and their rapid precipitation from blood plasma and from suspension in physiological saline as a separate phase [1-3].

The object of this investigation was to study the importance of the negative electrical charge, the macromolecular nature, and the hydrophilic properties of hyaluronic acid (HA), the hybrid biocomplex protein-chondroitin-keratin sulfate (PCKS), polypeptide-chondroitin-4 sulfate (PCS), and heparin (HP) during interaction with the outer surface of the red cell. These glucosaminoglucans can be arranged in the following order of size and charge density of their molecules: HP > PCS > PCKS > HA. In their molecular weight and the ability to form three-dimensional structures in solution and to bind water in various ways they can be arranged in the opposite order to the first series: HA > PCKS > PCS > HP [4, 5].

EXPERIMENTAL METHOD

A suspension of rabbit red cells (6% by volume) in physiological saline was used in all the experiments. The red cells were first washed three times with the same solution. The substances for testing were added to the solution used for preparing the suspension. A suspension of red cells without the test substance acted as the control. The rate of separation of the suspension into red cells and solution was measured by observing the movement of the partition boundary between the phases at recorded times in graduated pipettes fixed in the vertical position and placed in a water thermostat (30°C). The formation of red cell aggregates was observed in a drop of the test mixture under the microscope under low power.

Highly purified preparations of HA were isolated from human umbilical cords [7]. PCKS was isolated from the cartilage of bovine tracheal rings [6]. PCS was obtained by removal of the protein component from PCKS by the action of papain [6]. HP was used as the sodium salt prepared by purification of the commercial preparation (Spofa, Czechoslovakia) of this glucosaminoglucan. Details of the chief structural components of the preparations used are given in Table 1.

EXPERIMENTAL RESULTS

A suspension of red cells in physiological saline separates very slowly into the cell and solution phases (Fig. 1). The presence of HP and TCS in the suspension delayed this separation into layers still more (Fig. 1A and B). Rapid separation of the suspension into red cells and solution was induced by HA

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TABLE 1. Results of Analysis of Glucosaminoglucan Preparations

Preparation	Composition (in %)					
	nitrogen	glucosamine	galactosamine	hexuronic acid	sialic acid	SO_3
HA	2,95	43,00	0	43,00	0	0
HP	1,58	21,45	—	—	0	18,75
PCKS	4,06	3,70	22,20	24,00	2,70	10,85
PCS	2,14	Her	29,00	34,60	0	4,75

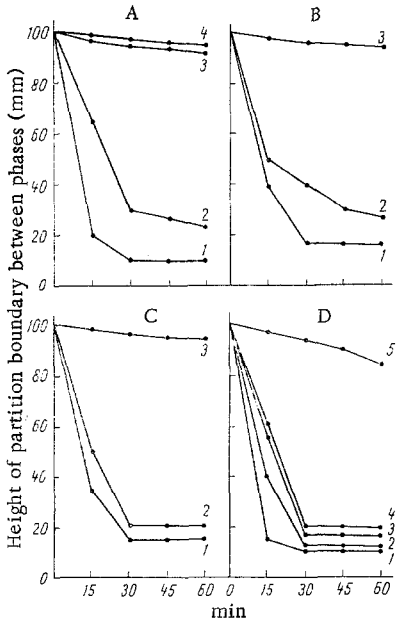

Fig. 1. Rate of separation of a suspension of red cells into solution and cells. A: 1) 0.25% HA; 2) 0.25% HA + 3.00% HP; 3) control; 4) 3.00% HP. B: 1) 0.50% PCKS; 2) 0.50 PCKS + 1.00% HP; 3) 1.00% HP. C: 1) 0.50% PCKS; 2) 0.50% PCKS + 1.00% PCS; 3) 1.00% PCS. B: 1) 0.12% HA; 2) 0.07% HA; 3) 0.12% HA + 1.00% PCS; 4) 0.07% HA + 0.12% PCKS; 5) 0.12% PCKS.

and PCKS. The action of the last two glucosaminoglucans is strongly inhibited by HP (Fig. 1A and B) and PCS (Fig. 1D and C). If HA and PCKS were present together in the solution, the separation of the red cell suspension took place more closely because of its inhibition of the effect of HA. With all investigated combinations of the mixture of glucosaminoglucans, the final volume of red cells determined after the lapse of 1 h was greater than after the action of HA and PCKS independently. In the last cases the volume of the red cell phase was more tightly compressed (Fig. 1).

In the presence of HA, PCKS, PCS, and HP, and also of the mixtures of these biopolymers indicated above, in all concentrations investigated aggregation of the red cells took place to a varied degree; i.e., nonspecific adhesion occurred between the cells [3].

Chemical interaction between glucosaminoglucans and the red cell surface can take place mainly by union of their anionic groups with the cationic groups of the outer pair of the membrane of this cell. PCKS, PCS, and HP, which contain sulfuric acid residues, can in addition suppress the dissociation of acid groups present on the red cell surface, thereby increasing the possibility of interaction with the cationic groups of this surface. Being polyanions, the macromolecules of these glucosaminoglucans are centers of aggregation of large numbers of red cells, with the result that aggregates are formed. These associations of red cells have a negative charge, produced by the acid groups of the glucosaminoglucans which are not used up in neutralizing the positive charges of the red cell surface. As a result, HP and PCS increase the stability of the suspension of aggregated red cells in the solution. HP has a stronger action in this respect than PCS, for it contains many more anionic groups. PCKS gives the aggregates of red cells a smaller charge than HP and PCS (see above). HA is the weakest agent from this point of view, yet it was HA and PCKS which induced the most rapid and complete separation of the red cells as an independent phase. Since HA and PCKS possess to a very great degree the ability to form complex three-dimensional structures, binding large quantities of water in various ways, in solutions, it is evident that one of the chief factors inducing the separation of a suspension of red cells into solution and cells is this particular property of the biopolymers. HA and PCKS, which bind large quantities of water, expel the red cells from the solution. The electric charge on the red cell aggregates, which possibly arises in the presence of HA and, in particular, of PCKS, has only a weak action in preventing the separation of these aggregates from the suspension into an isolated phase. The reason for this is dominance of the factors determining the formation of three-dimensional structures to correspond to the binding of water by these biopolymers. Destruction of the protein component of PCKS, leading to the formation of PCS which does not form such complex structures in solution, and which cannot bind large quantities of water, like the original biopolymer, increases the stability of the red cell suspension and does not induce its separation into layers.

The reason for the inhibitory effect of HP and PCS on the action of HA and PCKS and also for the weakening of the action of HA by accompanying PCKS is the resistance of the red cell aggregates to their expulsion from the solution into a separate phase as a result of the relatively high electric charge on these aggregates. The large final volume of the red cell phase determined in mixtures of HA with HP, PCS, and

PCKS, and also in mixtures of PCKS with HP and PCS compared with the volume in the experiments in which HA and PCKS acted independently of the other glucosaminoglucans, can be explained in the same way.

The participation of acid glucosaminoglucans of the HA and PCKS type in the formation of certain tissue structures of the body can be considered to be determined not only by the ability of these biopolymers to form electrovalent bonds with many components, but also by their ability to bind (retain) large quantities of water. As a result of this phenomenon, macromolecules and complexes expelled from the space occupied by the glucosaminoglucans may be concentrated into a smaller volume, thereby increasing the probability of various types of interaction between them [8].

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