MECHANISM OF PROTEOGLYCAN INDUCED STERIC EXCLUSION OF CELLS

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A unique property of proteoglycans (PG) namely their ability to effect steric exclusion of cells and protein and other substances from the space they occupy in solution, and to concentrate all this material in the form of a separate phase, is of great biological importance. This phenomenon is of great interest from the energy point of view, for a more concentrated phase is formed from a dispersed suspension in a solution of macromolecules of proteins, other substances, and cells, with the result that their chemical activity is increased [9-11].

This ability of PG to effect steric exclusion, which is a leading factor in cell aggregation and adhesion, has received little study from quantitative and energy points of view. The investigation described below is devoted to a study of dependence of separation of a solution and cells from a uniform suspension of them in that solution, into separate phases on the PG concentration and the number of cells in it, an essential prelude to the elucidation of the physical nature and energy relationships of PG-induced steric exclusion.

EXPERIMENTAL METHOD

A suspension of rabbit erythrocytes (previously washed with the same solution) in salt solution (0.15 M NaCl, pH 7.2-7.4, phosphate buffer), was used as the experimental model of isolated cells. With this model it is possible to study the steric excluding action of PG, uncomplicated by the simultaneous effect of other factors involved in cell–cell interaction. The results obtained by this model agree with those obtained by tissue culture studies [12]. The Na^+ salt of hyaluronic acid (HUA) and the natural complex protein–chondroitin–keratan–sulfate (PCKS) were chosen from the PG group as being the biopolymers most thoroughly studied from the above point of view [3-7, 12]. Methods of obtaining these preparations, the results of their analysis, and the method of quantitative estimation of fractionation of an erythrocyte suspension in salt solution into phases of solution and cells were described in detail previously [1-3]. To determine how separation of the suspension into phases of solution and cells depends on the number of erythrocytes, suspensions containing different volumes of these cells, estimated from their hematocrit value, were prepared. The initial rate of separation of phases was measured graphically as the tangent of the angle of slope of the curve reflecting the course of separation in time relative to the abscissa.

EXPERIMENTAL RESULTS

Suspensions containing 10, 25, and 50% of erythrocytes by volume, without addition of HUA or PCKS, do not separate into phases of solution and cells during a period of time significantly longer than the duration of the experiments described below. It will be clear from Fig. 1 that with erythrocytes present in a volume equivalent to 10% of the total volume of the suspension, erythrocytes quickly separated out into an independent phase under the influence of HUA as a linear function between concentration limits of 0.5 and 2.0 $mg \cdot ml^{-1}$. With an increase of concentration this rate also increased (Fig. 1a). With erythrocytes present in a volume of 25% of the total volume

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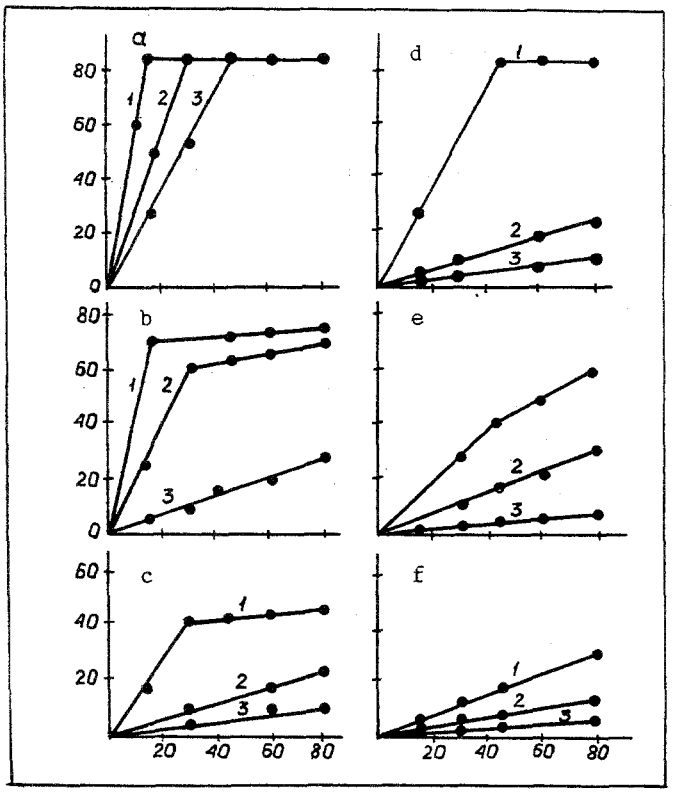

Fig. 1. Time course of separation of suspension of erythrocytes in salt solution into phases of solution and cells, induced by HUA and PCKS, with different relative volumes of solution and erythrocytes. Abscissa, time (in min); ordinate, relative volume (in %) of solution phase above aggregated erythrocytes. a) Volume of erythrocytes in suspension 10%. HUA concentration: 1) 2.0 mg·ml^{-1}; 2) 1.0 mg·ml^{-1}; 3) 0.5 mg·ml^{-1}; b) volume of erythrocytes in suspension 25%. HUA concentration: 1) 2.0 mg·ml^{-1}; 2) 1.0 mg·ml^{-1}; 3) 0.5 mg·ml^{-1}; c) volume of erythrocytes in suspension 50%. HUA concentration: 1) 2.0 mg·ml^{-1}; 2) 1.0 mg. ml^{-1}; 3) 0.5 mg·ml^{-1}; d) volume of erythrocytes in suspension 10%. PCKS concentration: 1) 4.0 mg·ml^{-1}; 2) 2.0 mg·ml^{-1}; 3) 1.0 mg·ml^{-1}; e) volume of erythrocytes in suspension 25%. PCKS concentration: 1) 4.0 mg·ml^{-1}; 2) 2.0 mg·ml^{-1}; 3) 1.0 mg·ml^{-1}; f) volume of erythrocytes in suspension 50%. PCKS concentration: 1) 4.0 mg·ml^{-1}; 2) 2.0 mg·ml^{-1}; 3) 1.0 mg·ml^{-1}.

of the suspension, and with HUA in a concentration of 1.0 and 2.0 mg·ml^{-1}, separation initially took place quickly, after which there was a slow condensation of the phase of erythrocytes to their original volume. With the same ratio between volumes of the phases, when the HUA concentration was 0.5 mg·ml^{-1}, separation of the suspension into phases always took place slowly, as a linear function of time, and did not reach the initial value in the course of the experiment (Fig. 1b). If volumes of the liquid phase and cell phase in the suspension were equal (50%), their relatively rapid separation followed by slow packing of the erythrocytes to their initial volume was observed only with HUA in a concentration of 2.0 mg·ml^{-1}. Under the same conditions, when the HUA concentration was 1.0 and 0.5 mg·ml^{-1} separation into phases took place much more slowly than when the cells were present in a volume of 10% and 25%, with the same concentrations of HUA, and did not reach the initial volume of erythrocytes during the experiment (Fig. 1c).

During steric exclusion of erythrocytes from suspension by PCKS, their rapid and complete isolation into a separate phase took place only if their volume in the suspension was 10% and the concentration of PCKS was 4.0 mg·mg^{-1} (Fig. 1d). With PCKS in concentrations of 2.0 and 1.0 mg·ml^{-1}, in this case separation of the phases

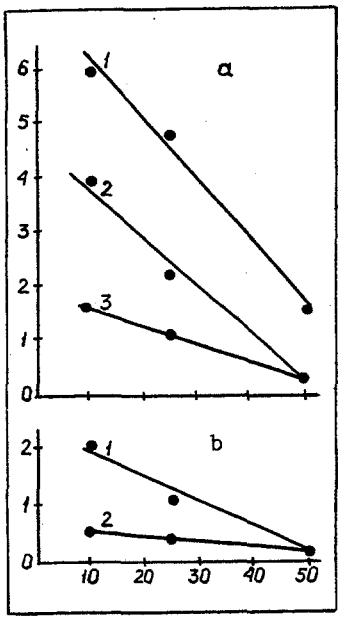

Fig. 2. Initial rate of separation of erythrocyte suspension in salt solution into phases of solution and cells as a function of erythrocyte volume, given constant concentrations of HUA and PCKS. Abscissa, volume of erythrocytes in suspension (in %). Ordinate, relative volume of solution above erythrocytes (in %/min). a) Concentration of HUA: 1) 2.0, 2) 1.0, 3) 0.5 mg·ml^{-1}; b) concentration of PCKS: 1) 4.0, 2) 2.0, 3) 1.0 mg·ml^{-1}.

was a linear function of time and did not reach the initial volume of the erythrocytes during the experiment. The suspension was separated into phases more slowly still when PCKS was present in the same concentrations but the volume of erythrocytes was 25% or 50% (Fig. 1d, e).

It will be clear from the results of calculation of the initial rate of separation of the erythrocyte suspension in salt solution into the above-mentioned phases that if the concentration of HUA and PCKS remains constant, this rate will be inversely proportional to the quantity (volume) of erythrocytes in the test system. Correspondingly, if the quantity of erythrocytes in the suspension is constant, the initial rate of separation into phases of solution and cells will be directly proportional to the concentration of HUA and PCKS in solution (Fig. 2).

This dependence of the initial rate of separation of the test suspension into phases on the number of erythrocytes in the suspension and on the concentration of HUA and PCKS in solution, is in full agreement with the fact that normally erythrocytes do not exist as separate formations, not aggregating with each other and possessing a definite degree of kinetic stability, resisting steric exclusion from the space in the suspension occupied by HUA and PCKS. It will be evident that the larger the space in suspension occupied by the volume of the erythrocytes themselves, the greater will be the total value of this stability, expressed as a decrease in the initial rate of separation into phases with an increase in the number of erythrocytes in suspension. Correspondingly, accordingly, high concentrations of HUA and PCKS are needed to bring about complete displacement of these cells from the solution with an increase in their number in the system.

The results show that with constant concentrations of HUA and PCKS and with a constant number (volume) of erythrocytes in the suspension the course of separation into phases is a linear function of time. Dependence of separation of the suspension into phases on concentration of the specified PG and on the number of erythrocytes also is linear. Hence it follows that steric exclusion of erythrocytes from their suspension in salt solution with the aid of HUA and PCKS is a physical process. Covalent and electrovalent interactions of PG with erythrocytes do not take place. Accumulation of erythrocytes in a reduced space limits their mobility, increases the possibility of contacts between individual cells and others, and endows a whole mass of these cells with a certain structural orderliness, which is expressed as the formation of erythrocyte aggregates. HUA and PCKS contained in the liquid phase prevent the disintegration of these structured formations [9-11, 13].

In the experiments described above HUA and PCKS acted as factors of steric exclusion separately and independently of each other, and also in the absence of a concentration gradient of these PG, independently of the number of erythrocytes also. It was shown previously that if both HUA and PCKS are present, they may form an easily dissociating complex, which acts as a steric exclusion factor more strongly than HUA and PCKS separately [4]. Heparin fractions, one of which contains three (HP-3), the other four (HP-4) sulfate groups per disaccharide unit, which are heparin macromolecules not causing steric exclusion of erythrocytes, strongly affect this action of HUA and PCKS. Fractions HP-3 and HP-4 depress this action of HUA to different degrees. The same action of PCKS is activated by HP-3, but HP-4 activates it in low concentrations and inhibits it in high concentrations. In the combined presence of HP-3 and HP-4 they mutually neutralize each other's effect on the action of PCKS [7]. Various proteins [8] have a quantitatively complex effect on steric exclusion of erythrocytes caused by HUA and PCKS. Under conditions when steric exclusion is complicated by different interactions between individual PG and between them and other biopolymers, separation of the erythrocyte suspension in salt solution into phases can no longer be a linear function of the concentrations of HUA and PCKS and on the number of erythrocytes [7, 8].

Several different PG, which can form macromolecular structures on account of covalent, electrostatic, specific and nonspecific interactions are always present in the intercellular space of the body tissues. Moreover, PG (HUA, PCKS, and others) can combine directly with the surface of certain cells [6, 9]. All these interactions, some of which perform regulatory functions, greatly complicate the action of PG as factors of steric exclusion of cells and biopolymers. Under these conditions, dependence of this phenomenon on PG concentrations and number of cells cannot be linear, as in simplified model experiments, more especially because the concentrations and number of cells become variable values. Of course the physical nature of the steric exclusion exhibited by PG remains unchanged under these circumstances. The same applies also to the mechanism by which PG prevent disintegration of cells, associated into definite structures.

Specific protein factors of cell adhesion can also be excluded sterically from the space in solution occupied by PG, and can be concentrated together with cells in a limited volume, which enhances their chemical and specific activity [10, 11, 13]. As a result complex macromolecular structures are created, the individual components of which may possess high chemical potential. It follows from the facts described above that steric exclusion, induced by PG, is one of the main factors necessary for interaction between cells, whether during tissue formation in embryogenesis or during functioning of tissues already formed.

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