ROLE OF PROTEOGLYCAN SALTS AS STERIC EXCLUSION FACTORS

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UDC 616.155.1-008.939.951.5-38:616.155.1-008.939.2

KEY WORDS: aggregation; adhesion; proteoglycan salts; steric exclusion; erythrocytes.

The ability of hyaluronic acid (HUA), protein–chondroitin–keratan sulfate (PCKS), and aggregates of proteoglycans to exclude cells of an animal, their organelles, and macromolecules of other biopolymers, into a separate phase from the space occupied by them in solution, is determined by the property of the proteoglycans of creating 3-dimensional lattice formations in solutions [3-5, 8, 10]. The structure of such systems depends on many factors, including the conformation of the proteoglycan macromolecules, which is influenced in turn by the nature of the cations bonded to the anionic groups of these biopolymers [6, 7, 11].

The aim of the present investigation was to study dependence of the steric exclusion effect on the nature of the cations connected with HUA and PCKS (soluble fraction), using for this purpose rabbit erythrocytes as a model of isolated cells enabling this effect to be determined quantitatively.

EXPERIMENTAL METHOD

Normal Ca^{++}- and K^{+}-salts of HUA and PCKS were used, and highly purified preparations of these substances were obtained by methods developed by ourselves [1, 2, 7].

Rabbit erythrocytes were washed with salt solution (0.14 M NaCl) and suspended in the same solution (pH 7.2) to concentrations of 6, 10, and 18% of erythrocytes (by volume). Quantitative determination of partition of these suspensions into phases of solution and erythrocytes was carried out at 20°C by the method described previously [3]. The initial rate of partition of the suspension into phases was calculated graphically as the tangent of the angle of slope of the initial segment of the curve reflecting the course of this partition in time, with different concentrations of proteoglycan salts, to the abscissa.

EXPERIMENTAL RESULTS

In control tests suspensions of erythrocytes in salt solution, with the same quantitative ratios between solution and erythrocytes as were used in the experimental samples, but not containing proteoglycans, did not separate into phases at any time of the experiment.

In the presence of K^{+}-HUA between concentrations of 0.08 and 0.32 mg/ml, partition of the erythrocyte suspension in salt solution into phases of solution and cells, when the relative volume of erythrocytes was 6%, took place as a linear function of concentration of the biopolymer. Under these same conditions a suspension containing 18% of erythrocytes separated into these phases less intensively than the 6% suspension, and dependence of this partition on the K^{+}-HUA concentration was nonlinear (Fig. 1A). In the presence of Ca^{++}-HUA in concentrations of between 0.08 and 0.16 mg/ml, in suspensions containing 6 and 18% of erythrocytes, partition into the phases mentioned above took place on a very small scale, but in the presence of concentrations of this salt above 0.16 mg/ml, in a suspension containing 6% of erythrocytes, this partition increased steeply, whereas in suspension containing 18% of erythrocytes, the increase in partition into phases was significantly weaker (Fig. 1B). The difference between the action of K^{+}-HUA and that of Ca^{++}-HUA in respect of steric exclusion of erythrocytes from a stable equilibrium dispersion of the cells in salt solution is that this action was more strongly dependent on the relative volume of the erythrocyte phase in the presence of Ca^{++}-HUA than in the presence of K^{+}-HUA.

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 545-547, November, 1988. Original article submitted January 20, 1988.

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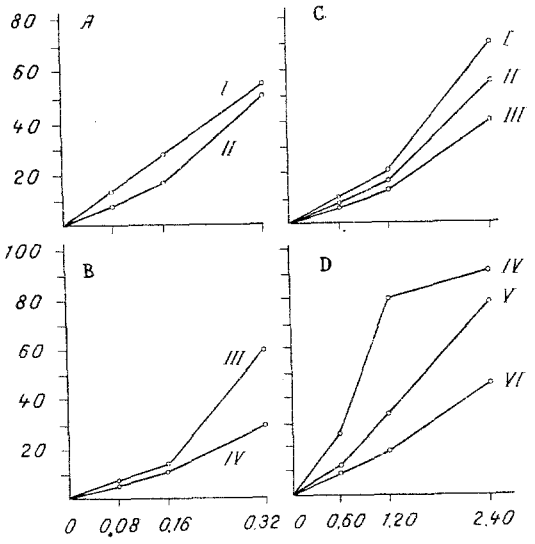

Fig. 1

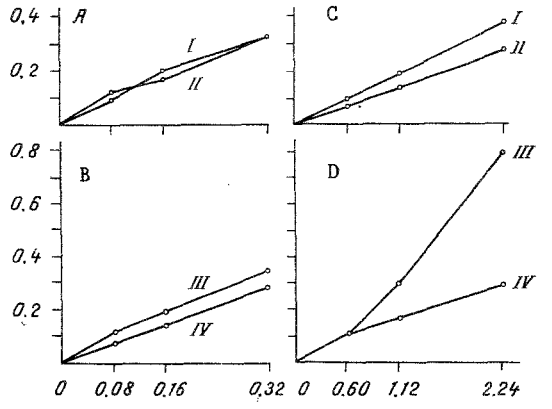

Fig. 2

Fig. 1. Dependence of partition of erythrocyte suspension into phases of solution and cells on concentrations of K^+- and Ca^{++}-salts of HUA and PCKS. Abscissa, concentration of HUA and PCKS salts (in mg/ml); ordinate, relative volume of solution above phase of aggregated erythrocytes (in %), allowed to stand for 120 min. A) K^+-HUA; I) 6% erythrocytes, II) 18% erythrocytes; B) Ca^{++}-HUA; III) 6% erythrocytes, IV) 18% erythrocytes; C) K^+-PCKS; I) 6% erythrocytes, II) 10% erythrocytes, III) 18% erythrocytes; D) Ca^{++}-PCKS; IV) 6% erythrocytes, V) 10% erythrocytes, VI) 18% erythrocytes.

Fig. 2. Dependence of initial rate of partition of erythrocyte suspension in solution into phases on concentration of HUA and PCKS salts. Abscissa, concentration of HUA and PCKS salts (in mg/ml); ordinate, change (in %) in volume of solution above phase of aggregated erythrocytes ($\cdot min^{-1}$). A) K^+-HUA: I) 18% of erythrocytes, II) 6% of erythrocytes; B) Ca^{++}-HUA: III) 6% of erythrocytes, IV) 18% of erythrocytes; C) K^+-PCKS: I) 6% of erythrocytes, II) 18% of erythrocytes; D) Ca^{++}-PCKS: III) 6% of erythrocytes, IV) 18% of erythrocytes.

Partition of the suspension containing 6, 10, and 18% of erythrocytes into phases in the presence of K^+-PCKS was a linear function of concentration of the salt up to a value of 1.20 mg/ml. An increase in the K^+-PCKS concentration to 2.40 mg/ml caused a sharp increase in the intensity of this partition with all concentrations of erythrocytes in the suspensions. With an increase in the erythrocyte concentration, partition of the suspension into phases was less marked, but the character of dependence of this partition on the K^+-PCKS concentration was unchanged (Fig. 1C). The action of Ca^{++}-PCKS as a steric exclusion factor is characterized by stronger dependence on the volume of the erythrocyte phase in the suspension. In a suspension containing 6% of erythrocytes complete partition into phases was observed in 120 min, with a concentration of Ca^{++}-PCKS as low as 1.20 mg/ml, and in this case dependence of partition into phases on the concentration of this biopolymer is nonlinear. With an increase in the relative volume of the erythrocytes (10 and 18%) the intensity of this partition was reduced, and its dependence on Ca^{++}-PCKS concentration approximated to linear within the concentration range of this salt studied (Fig. 1D).

The initial velocity of partition of the erythrocyte suspension into phases, which determine the onset of erythrocyte aggregation, under the influence of K^+-HUA increased as a linear function with an increase in its concentration and was independent of the relative volume of the erythrocyte phase (Fig. 2). The initial velocity of this partition, induced by Ca^{++}-HUA, was rather higher with erythrocytes in a concentration of 6% than of 18%, and in all cases this velocity increased as a linear function with an increase in concentration of this biopolymer between the limits studied. During partition of suspensions containing 6 and 18% of erythrocytes into phases with the aid of K^+-PCKS the initial velocity increased as a linear function with an increase in concentration of the salt, and in the first case (6%) its value was rather higher than in the second (18%). This same velocity of partition into phases un-

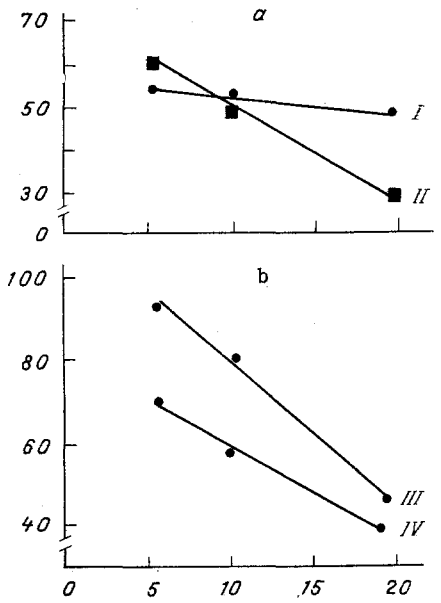

Fig. 3. Dependence of action of HUA and PCKS salts on partition of erythrocyte suspension into phases on erythrocyte concentration. Abscissa, content of erythrocytes in suspension (in % by volume); ordinate, volume of solution above phase of aggregated erythrocytes (in %), after allowing to stand for 120 min. I) K^+-HUA, II) Ca^{++}-HUA, III) K^+-PCKS, IV) Ca^{++}-PCKS. Concentration of HUA salts 0.32 mg/ml, of PCKS salts 2.40 mg/ml.

der the influence of Ca^{++}-PCKS increased much more steeply with an increase in concentration of this biopolymer in the case when the relative volume of erythrocytes was 6% than when it was 18%, when the initial velocity increased as a linear function of its concentration (Fig. 2C, D). It will be clear from these data that the initial velocity of partition of the erythrocyte suspension in a salt solution into phases of solution and cells, when their relative volume was 6%, was significantly higher under the influence of Ca^{++}-HUA and Ca^{++}-PCKS than under the influence of the K^+-salts of these proteoglycans. This difference was particularly clearly manifested in the case of the action of K^+- and Ca^{++}-salts of PCKS (Fig. 2).

The steep rise in the degree of partition of the erythrocyte suspension and of its initial velocity observed in the presence of certain concentrations of Ca^{++}-HUA, K^+-PCKS, and Ca^{++}-PCKS, which was particularly marked in small volumes of these cells, was probably connected with the formation of their larger aggregates under these conditions. It will be clear from Fig. 3 that the intensity of partition of the erythrocyte suspension into phases, in the presence of a constant concentration of the proteoglycan salts studied, was an inverse linear function of the relative volume of erythrocytes in solution. This was particularly marked for the action of Ca^{++}-salts of HUA and PCKS, compared with the action of their K^+-salts.

It will be clear from the results that activity of HUA and PCKS as factors of steric exclusion of erythrocytes from their stable equilibrium suspension in salt solution is affected by the nature of the cation bound with them. This is demonstrated by the stronger dependence of steric exclusion and of its initial velocity on the concentration of Ca^{++}-salts than on the ratio between the volumes of the phases of erythrocytes and salt solution in their suspensions. Differences observed in the action of K^+ and Ca^{++}-salts of HUA and PCKS are evidently not connected with direct interaction of these salts with the outer surface of the erythrocytes [12, 13].

These differences are evidently due to differences in the structure of the macromolecules of these salts. Macromolecules of Ca^{++}-HUA, unlike those of K^+-HUA, are chains consisting of two HUA chains connected with the aid of Ca^{++} through the carboxyl groups of the glucuronic acid residues contained in HUA. Formation of Ca^{++}-PCKS also leads to the appearance of a double chain as a result of interaction of Ca^{++} with carboxyl and sulfate groups of glucuronic and sulfuric acid residues of PCKS [6, 9]. In solutions, Ca^{++}-HUA and Ca^{++}-PCKS create 3-dimensional lattice structures, which differ considerably in their complexity from the structures of the K^+-salts of these proteoglycans, as is reflected in the differences in the ability of these Ca^{++}-salts to exhibit steric exclusion, discovered in the present investigation.

The existence of Ca-dependent and Ca-independent mechanisms of cellular adhesion in vivo has been suggested [9]. Whatever the mechanisms of cellular adhesion in vivo, especially during embryogenesis and regeneration, a leading role in cellular aggregation preceding adhesion is played by HUA, PCKS, and other proteoglycans existing in the form of salts, including their potassium and calcium salts [9]. In this connection the possibility cannot be ruled out that the effect of Ca^{++} on cellular aggregation and adhesion is effected to some degree through HUA and PCKS.

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PREPARATION OF PORCINE MB CREATINE PHOSPHOKINASE HETERODIMER AND STUDY OF ITS BINDING WITH POLYCLONAL ANTIBODIES

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UDC 612.128:577.152.273/.017.1-019/.08

KEY WORDS: creatine phosphokinase heterodimer; polyclonal antibodies; conjugates with peroxidase.

One stage in the investigation of the structural—immunological properties of enzymes is to determine the degree of affinity of antibodies obtained to different molecular forms of an enzyme [3, 5].

The aim of this investigation was to study these properties of porcine creatine phosphokinase, which is a dimer consisting of M and B subunits, which give rise to three corresponding molecular forms of the enzyme: MM, MB, and BB.

EXPERIMENTAL METHOD

The protein content at all stages of isolation and purification of antigens and immunoglobulins and of synthesis of conjugates was determined by Lowry's method [2]. The immunoprecipitation test was carried out by Ouchterlony's method [4]. M creatine phosphokinase was isolated from porcine skeletal muscles. BB creatine phosphokinase was isolated from porcine brain by double ion-exchange chromatography on DEAE-cellulose followed by chromatography on hydroxyapatite. Rabbits were immunized 6 times at intervals of 3 weeks: with 1 mg of enzyme in 1.5 ml of 0.05 M Tris HCl buffer (pH 8.9) with Freund's complete adjuvant. Conjugates of antibodies and antigens were synthesized with horseradish peroxidase [1, 7].

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(Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 11, pp. 548-549, November, 1988. Original article submitted October 26, 1987.