STRUCTURAL CHANGES IN NUCLEOPROTEIN SYSTEMS UNDER THE INFLUENCE OF BLOOD SERA OF HEALTHY AND SICK PERSONS

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Interaction between model DNP systems of chromatin and blood sera of healthy and sick persons was investigated by a thermomechanical method. Healthy human blood serum was shown to cause decondensation of DNP systems. The intensity of this action varied when pathological sera with modified composition were used: The sera of patients with systemic lupus erythematosus increased the degree of condensation, whereas sera from patients with schizophrenia increased the decondensation of the DNP systems compared with healthy sera. The action of the sera was unconnected with activity of the serum enzymes. The role of serum components in the regulation of the structural and functional properties of chromatin is discussed.

KEY WORDS: *Thermomechanical method; condensation; nucleoprotein systems.*

The functional activity of cells and, in particular, of their nuclear chromatin is known to depend on several exogenous and endogenous physiologically active substances. Such substances may be components of the blood serum, as is confirmed by the stimulation of synthetic processes in cell nuclei observed after addition of blood serum to the culture medium [11, 15]. Indirect proof of the participation of serum components in processes of regulation of nuclear functions is given by changes in chromatin activity found when autologous serum in the culture medium is replaced by homologous serum, modified either in various diseases or as a result of regenerative processes *in vivo* [10, 12-14]. It is not yet clear how the active serum factors exert their action: whether they do so directly and modify the functional properties of the chromatin by interaction with it.

The object of this investigation was to study whether blood sera can in principle influence the structure of nucleoprotein complexes as the result of direct contact with them in model systems. It was hoped to discover whether the character of this interaction is changed if the blood sera used have their composition modified as a result of certain diseases.

EXPERIMENTAL METHOD

Experiments were carried out with the blood serum of 60 healthy donors and, in the pathological group, blood serum from 28 patients with systemic lupus erythematosus (SLE) and 27 schizophrenics, for these diseases are known to be accompanied by qualitative and quantitative disturbances of the composition of the blood serum [4, 5]. Tests were carried out on preparations of DNP isolated from calf thymus in 0.7 M NaCl.

The action of the blood sera on DNP was investigated by a thermomechanical method, consisting essentially of the obtaining of DNP structures in fiber form in a physiological medium [6]. On heating, the DNP systems undergo structural changes: irreversible deformation at 55-80°C and "melting" at temperatures above 80°C. The two processes were evaluated by

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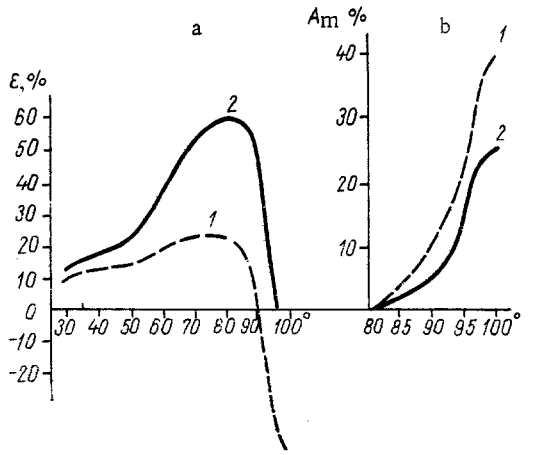

Fig. 1. Thermomechanical curves of values of E and A_m as functions of temperature in medium containing blood serum (2) and in control solution of 0.14 M NaCl (1). Here and in Fig. 2, ordinate: a) E (in %), b) A_m (in %); abscissa, temperature.

TABLE 1. Effect of Blood Serum on Thermomechanical Parameters of DNP Systems (M ± m)

Incubation medium	$\frac{A_g}{A^*_g}$	$\frac{A_m}{A^*_m}$
Blood serum (dilution 1/150)	1,85±0,3†	0,71±0,04†
Serum + protease inhibitors (DFP $1 \cdot 10^{-3}$ %)	1,90±0,1†	0,74±0,02†
Soy trypsin inhibitor (1 mg/ml serum)	2,05±0,15†	0,72±0,02†
Serum + 0.015 M citrate	2,00±0,35†	0,71±0,01†

*Test parameter measured in 0.14 M NaCl.
†$P < 0.05$ compared with corresponding control.

the following parameters: 1) the relative length of the DNP fibers $E = \frac{l_t - l_0}{l_0} \cdot 100\%$, 2) the relative degree of irreversible deformation $A_d = \frac{l_m - l_0}{l_0} \cdot 100\%$, 3) the relative amplitude of melting $A_m = \frac{l_m - l_T}{l_m} \cdot 100\%$, where l_0, l_t, l_m, and l_T are the lengths of the DNP fibers after incubation in the medium before heating, at the given temperature (t°C), before the beginning of melting (80°C), and at the melting temperatures (over 80°C), respectively.

The incubation medium contained the test serum diluted 150 times with physiological saline. This degree of dilution lowers the concentration of bivalent ions to values that have no effect on DNP systems, so that the action of the other serum components can be tested.

The significance of differences between parameters of the DNP systems in media with sera and in physiological saline and also in the sera of the sick individuals compared with the healthy was determined in each experiment by means of Wilcoxon's criterion [2].

EXPERIMENTAL RESULTS AND DISCUSSION

The presence of serum in the physiological medium increased the degree of irreversible deformation and reduced the amplitude of melting of the DNP fibers (Fig. 1, Table 1). An

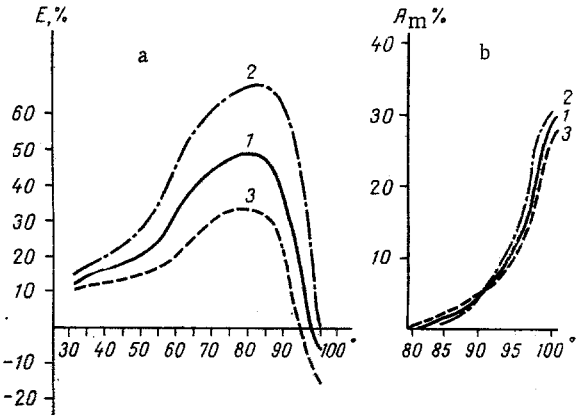

Fig. 2. Further mechanical curves of values of E and A_m as functions of temperature in media with sera of healthy donors (1), schizophrenics (2), and patients with systemic lupus erythematosus (3). DNP concentration 0.25 mg/ml; protein/DNA ratio 1.64; characteristic viscosity 37 dl/g.

increase in the parameter A_d in medium with serum indicates that the serum contains factors weakening protein–protein and protein–DNA interaction in the DNP system, i.e., decondensation of the system [6, 7]. An increase in A_m is also characteristic of the decondensation process. On the contrary, however, in the presence of serum A_m was reduced on the average by 30%. This phenomenon during a rise of temperature can, in the writers' opinion, be attributed to an increase in the number of reactive groups of the serum proteins, mainly albumin, interacting with the DNP system.

To analyze the factors reducing interaction between the components of the DNP structures it had to be discovered whether this was the result of the action of serum proteases and DNases. For this purpose, enzyme inhibitors were added to the medium with serum: To inhibit proteases di-isopropylfluorophosphate (DFP) and soy trypsin inhibitor were added, and DNases were inhibited by Na citrate. The results showed that the character of action of the serum on the DNP system was unchanged.

The action of the sera on the DNP system was thus due not to enzymes, but to the activity of other components, the content of which varied in different types of pathology. This was confirmed by investigation of the action of sera of patients with SLE and schizophrenia (Fig. 2). The character of their effects on the DNP systems differed: Sera from patients with SLE reduced (condensed) whereas sera from schizophrenics increased (decondensed) A_d compared with healthy human serum. The amplitudes of melting in both cases were unchanged. Absence of correlation must be noted between the intensity of action of the sera of the SLE patients and some of their serological parameters: the complement level, titers of anti-DNA and anti-DNP antibodies, and total protein concentration.

It is very important to note that the character of the structural changes in chromatin in cells and in model DNP systems under the influence of sera was the same: It has been shown that in schizophrenia the chromatin structure has features typical of activated cells [1, 3], i.e., an increased degree of decondensation; this agreed with the present observations. Changes in chromatin structure toward increased condensation under the influence of sera in cells and models were observed previously by the writers in another pathological condition, namely Down's syndrome [8]. A similar correlation exists also for other agents which change the degree of chromatin condensation [6, 7].

From that has been said, the existence of components capable of interacting with chromatin and thereby controlling the degree of its condensation, and so modifying its functional activity [7, 9], in blood serum can be postulated.

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ISOLATION OF CELLS MOST ACTIVELY SYNTHESIZING DNA FROM HEMATOPOIETIC ORGANS BY FRACTIONATION IN AN ALBUMIN DENSITY GRADIENT

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Fractionation of suspensions of rat bone marrow or spleen cells yielded fractions in which activity of DNA-dependent DNA-polymerase and aspartate-carbamoyl transferase and incorporation of ^{14}C-thymidine were two or three times greater than in the initial suspension. Depending on the osmotic concentration of the original 35% solution of bovine serum albumin, the cells synthesizing DNA most actively were concentrated in fractions Nos. 5-6 (370 milliosmoles) or Nos. 2-3 (380 milliosmoles).

KEY WORDS: *DNA biosynthesis; DNA-dependent DNA-polymerase; aspartate-carbamoyl transferase; bone marrow; spleen; fractionation; bovine serum albumin gradient.*

The problem of isolation, identification, and comprehensive investigation of the biochemical properties of individual types of cells for subpopulations from hematopoietic organs has become increasingly urgent in hematology, radiobiology, and radiation medicine. Recently several methods of fractionation of heterogeneous cell suspensions have been suggested. By their use fractions partially enriched by cells of a given type can be obtained. The most promising method of its kind seems to be that of cell fractionation in a bovine serum albumin (BSA) density gradient [1].

The object of this investigation was to isolate cells from hematopoietic organs of rats synthesizing DNA most actively. The intensity of DNA biosynthesis was judged from the ac-

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