ISOLATION AND CHARACTERISTICS OF PLASMA DNA FROM BLOOD DONORS AND PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by a unique assortment of different types of antibodies and autoantibodies in the blood serum and tissues. Antibodies against DNA are found most frequently and in the largest amounts. The causes of formation of these antibodies are unknown. At the same time, it has been shown that injection of DNA subjected to chemical and physical treatment into animals causes antibody formation [7-9]. Tissue destruction, due to certain agents and accompanied by the liberation of DNA into the blood stream, also stimulates antibody formation [2]. In this connection, the study of the physicochemical properties of the plasma DNA of patients with SLE is an extremely important stage in the study of the development of autoimmune processes.

In the investigation described below DNA was isolated from plasma of blood donors and patients with SLE and its physicochemical properties were studied.

EXPERIMENTAL METHOD

DNA was isolated from plasma by two different methods. In the first method DNA was isolated from blood plasma diluted 1:2 with physiological saline [5]. The second method consisted of two stages: removal of the main mass of plasma proteins from the DNA (by adsorption on hydroxyapatite and elution) and deproteinization (with a mixture of chloroform and isoamyl alcohol).

As a first step the adsorption-elution properties of different types of hydroxyapatite (Biorad, Sigma, Calbiochem) were tested. Hydroxyapatite from Sigma was used in the experiments. Adsorption and elution were carried out in bulk by Kupriyanova's method [1, 4]. To the blood plasma, half its volume of a 50% suspension of hydroxyapatite, equilibrated with 0.05M Na-phosphate buffer (SPB) at pH 6.9 was added drop by drop. The unadsorbed material was removed after centrifugation for 2-3 min at 1000g. The residue of hydroxyapatite was washed with 0.05M SPB, pH 6.9, with spectrophotometric monitoring of the protein concentration. Elution was carried out in batches with 0.25M SPB, pH 6.9, each of which amounted to one-quarter of the original volume. After dialysis, the eluates were deproteinized with a mixture of chloroform and isoamyl alcohol (24:1) in the presence of 0.25M NaCl.

Dialyzed plasma from blood donors and patients was treated with nucleases (DNase, RNase) and pronase. Before the experiments the RNase was heated for 10 min at 80°C. The final concentration of DNase and RNase was 500 μg/ml. Incubation with pronase was carried out in two stages: first, incubation for 4 h, followed by readdition of the enzyme (final concentration 0.9 mg/ml), after which the incubation was continued for a further 16 h at 37°C. Incubation was followed by dialysis. Pronase subjected to autodigestion (to remove nuclease activity) and undigested pronase were used in the experiments.

Cryoprecipitins [10] were isolated from the blood serum of patients with SLE and incubated with pronase in the manner described above. After incubation with the enzyme the immune complexes were dialyzed. The DNA concentration in the initial samples, after incubation with enzymes, and later after dialysis, was determined by a spectrofluorometric method [3] and the protein concentration by Lowry's method.

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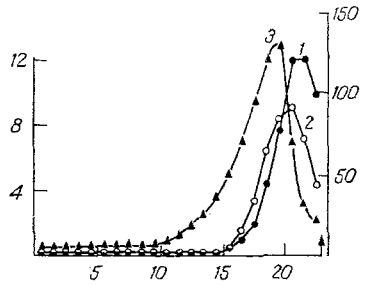

Fig. 1. Distribution of blood plasma DNA and sturgeon sperm DNA by fractions after centrifugation in sucrose concentration gradient. Abscissa, sucrose concentration (in %); ordinate, DNA content (in μg); on left, for donor and patient; on right, for marked DNA. 1) DNA from blood donors' plasma; 2) DNA from plasma of patients with SLE; 3) marker DNA with sedimentation constant of 6S.

The sedimentation constant of the plasma DNA was determined by centrifugation of the preparations in a 5-20% sucrose concentration gradient at 29,000 rpm for 20-24 h at 20°C. The sucrose solutions were made up in buffer containing 0.1M NaCl, 0.001M EDTA, and 0.01M Tris-HCl, pH 7.4. The DNA concentration in the fractions of the gradient was determined spectrofluorometrically.

EXPERIMENTAL RESULTS

In a preliminary series of experiments the attempt was made to detect DNA in the blood plasma of patients with SLE and of healthy donors. For this purpose plasma was treated with ethidium bromide and ultracentrifuged. As marker of the sedimentation constant, sturgeon sperm DNA with a sedimentation constant of 6S was used. The results (Fig. 1) show that DNA of both donors and patients with SLE is heterogeneous and has mean sedimentation constants, calculated by the standard method [6] of 2.6 and 3.6S, respectively; most of the material remains in the light part of the gradient.

When DNA was isolated from blood plasma of donors and patients with SLE by the method of Ledoux and Charles [5], very small amounts of it were obtained, insufficient for characterization of the physicochemical properties of the DNA.

In subsequent experiments a different method was used to isolate DNA from blood plasma. The main difficulty in isolation of DNA from blood plasma is due to its low content compared with the very high protein content. It was therefore decided to use hydroxyapatite, which enables selective sorption of nucleic acids.

As a first step the adsorptive power of the hydroxyapatite was tested with double-helical DNA isolated from sturgeon sperm and double-helical DNA mixed with blood plasma. These investigations showed no difference between adsorption and elution of double-helical DNA and of a mixture of it with blood plasma. The yield of DNA from hydroxyapatite was 50%, and this was increased by 10% if 1% sodium dodecyl sulfate was used.

Adsorption of blood plasma on hydroxyapatite followed by elution with phosphate buffer led to 38-fold purification of the DNA from plasma proteins, but despite this fact, the eluates still had a sufficiently high protein content. Later, after dialysis, they were subjected to triple deproteinization. The DNA yield in this experiment was 8.5%. Determination of the sedimentation constant of this DNA preparation revealed a peak of low-molecular-weight DNA similar to that illustrated in Fig. 1.

Treatment of dialyzed blood plasma from donors and patients (with SLE and rheumatism) with DNase and RNase, with different periods of incubation (1-24 h), showed that the plasma DNA is completely resistant to the action of these enzymes.

Preliminary experiments showed that pronase, subjected to autodigestion, hydrolyzes double-helical DNA in the course of 24 h at 37°C by 3%, whereas pronase not treated by autodigestion does so by 25%.

The results of the experiments with pronase showed that if pronase almost free from nuclease activity was used, the DNA concentration in the samples was unchanged after incubation with the enzyme, but after dialysis it was reduced on average by 50%. These results showed that the DNA, in the form of complexes with serum proteins, was dialyzed and that, consequently, it was of low molecular weight. This was found in the case of both patients and healthy donors. When pronase containing nuclease activity was used in the experiments the DNA concentration fell immediately after incubation with pronase and remained practically unchanged after dialysis (15 and 14.3 μg/ml – the initial values for donor and patient with SLE respectively were: 9.1 and 8.3 μg/ml after hydrolysis and 8.9 and 8 mg/ml after dialysis). Cryoprecipitins isolated from the blood serum of patients with SLE were incubated with pronase subjected to prior autodigestion.

The DNA content in the samples in this case was unchanged both after incubation with the enzyme and after dialysis. Consequently, the DNA contained in immune complexes was nondialyzable.

It can be concluded from these experiments that the DNA which circulates in the blood plasma of healthy persons and patients with SLE is mainly of low molecular weight and exists in the form of complexes with serum proteins. Meanwhile, a DNA of higher molecular weight may also be present in the serum of patients with SLE. This DNA forms complexes with other serum proteins (immunoglobulins).

LITERATURE CITED

1. N. S. Kupriyanova and M. Ya. Timofeeva, Mol. Biol., 7, 140 (1973).
2. G. J. Fournie, P. H. Lambert, and P. A. Miescher, J. Exp. Med., 140, 1189 (1974).
3. R. C. Kamm and A. G. Smith, Clin. Chem., 18, 519 (1972).
4. N. S. Kupriyanova and M. Ya. Timofeeva (M. Ja. Timofeeva), Eur. J. Biochem., 44, 59 (1974).
5. L. Ladoux and P. Charles, Exp. Cell. Res., 45, 498 (1967).
6. R. A. Martin and B. N. Ames, J. Biol. Chem., 236, 1372 (1961).
7. C. J. Plescia, W. Braun, and N. C. Palczuk, Proc. Natl. Acad. Sci. USA, 52, 279 (1964).
8. A. M. Poverenny, V. E. Podgorodnichenko, and D. B. Saprygin, Immunochemistry, 10, 577 (1973).
9. H. V. Vunakis, E. Seaman, et al., Biochemistry (Washington), 5, 3986 (1966).
10. J. B. Winfield, D. Koffler, and H. G. Kunkel, J. Clin. Invest., 56, 563 (1975).

SPIN PROBE INVESTIGATION OF THE STATE OF BLOOD PLASMA

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Spin probes and labels have recently found wide application in molecular biophysics for the study both of relatively simple systems (proteins, nucleic acids, bilayer lipid membranes) and of certain complex supramolecular formations, especially biological membranes, by the use of electron paramagnetic resonance (EPR) [2].

The object of this investigation was to develop approaches to the use of the EPR method for the study of a heterogeneous system such as blood plasma, one of the most frequently tested objects in medicine and experimental biology. Rabbits with alimentary atherosclerosis were used as the experimental animals, and a hydrophobic probe of the benzocarboline series, binding of which with albumin, the principal plasma protein, has been investigated in detail previously [3], was used as the spin probe.

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