ISOLATION OF Ca,Mg-DEPENDENT NUCLEAR ENDONUCLEASE AS A PRINCIPAL COMPONENT OF THE NUCLEASE COMPLEX OF HUMAN LYMPHOCYTE CHROMATIN

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KEY WORDS: Ca,Mg-dependent endonclease; cell nuclei; lymphocytes; chromatin

The presence of a whole series of endonuclease activities has been demonstrated in animal tissue cell nuclei [10]. The causes of their diversity and their presence in cell nuclei have not yet been explained. Attention has been drawn to the fact that one particular endonuclease, whose properties are similar in different tissues of the body and in different groups of animals, is present in virtually all samples which have so far been studied, namely Ca,Mg-dependent endonuclease.

The universality of distribution of this endonuclease (or of the group of Ca,Mg-dependent endonucleases) points to its possible role in cell nuclear function and suggests that this enzyme is widely represented among the endonucleases of cell nuclei. To study the ratio between the content of this enzyme and other endonucleases, and also to examine other problems, it is essential to isolate the endonucleases in a highly purified form.

This paper gives data on isolation of a highly purified enzyme from cell nuclei of human splenic lymphocytes and some evidence of its predominant representation (compared with other endonucleases) in the nuclease complex of lymphocyte chromatin.

EXPERIMENTAL METHOD

Human spleens (obtained at splenectomy for cirrhosis of the liver) were used. Cell nuclei were isolated from the splenic lymphocytes by the method described by the authors previously [1], using the detergent NP-40 to produce lysis of the lymphocyte plasma membranes.

The following adsorbents were used to isolate the enzyme: CM-Sephadex S-25 and Blue sepharose (from Pharmacia, Sweden), DEAE-cellulose DE-52 (from Whatman, England); DNA-cellulose (denatured) was prepared by the method in [2]. Electrophoresis of proteins in 12.5% polyacrylamide gel was carried out in the presence of dodecylsulfate by Laemmli's method [6], followed by staining with silver [8]. Activity of the enzyme was determined from the degree of degradation of superhelical DNA of plasmid pBR 322; the unit of activity was taken to be the quantity of enzyme which converts 1 μg of superhelical plasmic DNA into the linear form during incubation for 1 h at 37°C in the presence of 5 mM $MgCl_2$ and 1 mM $CaCl_2$.

EXPERIMENTAL RESULTS

Lymphocytes are interesting as an object with which to study the specific properties and function of endonucleases associated with chromatin. This interest is linked with the well-confirmed data on structural changes in the genome taking place in these cells and the conjectural role of endonucleases, or of proteins with endonuclease activity, in the corresponding processes [5]. Data on endonucleases of human lymphocyte nuclei and, in particular, on Ca,Mg-dependent endonuclease, are virtually not to be found in the literature. A procedure for isolating the enzyme from human splenic lymphocyte nuclei was therefore developed, and its principal stages are indicated in the scheme. With the aid of this scheme it was possible to obtain enzyme preparations with a degree of purity 1860 times higher than the original extracts of cell nuclei. Incidentally, schemes for isolation of Ca,Mg-dependent endonuclease from pig and rat liver cells nuclei and also from calf thymus yield preparations purified by 200-660 times [4, 9, 11].

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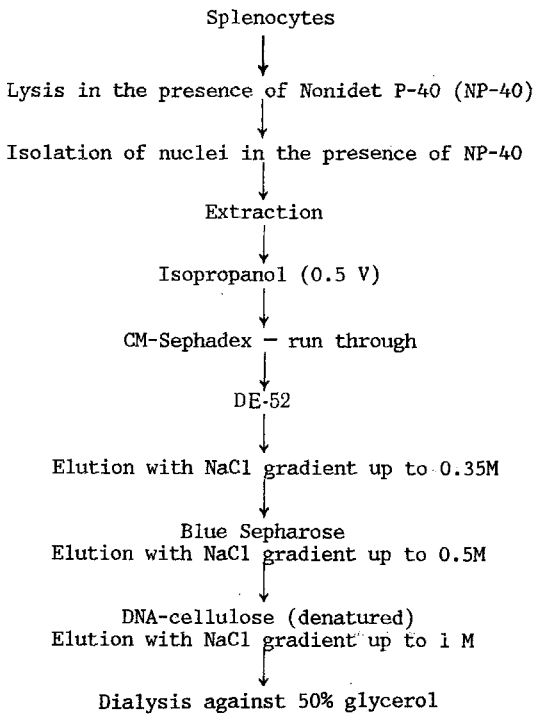
Scheme of Isolation of Ca,Mg-Dependent Endonuclease from Human Spleen Cell Nuclei



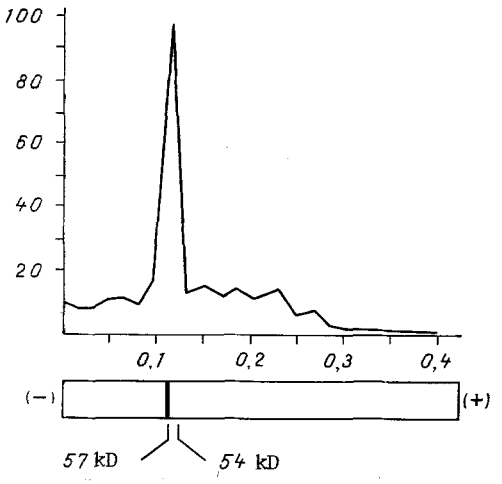


Fig. 1. Scheme of electrophoresis of purified Ca,Mg-dependent endonuclease. Top graph shows distribution of enzyme activity in gel. Abscissa, relative mobility; ordinate, enzyme activity (in percent).

To characterize the protein spectrum of the resulting preparation it was subjected to electrophoresis with parallel scanning of activity in the gels. The results are given in Fig. 1. The enzyme preparation clearly contains a dominant polypeptide with mol. wt. of 57 kilodaltons (kD) and a polypeptide with mol. wt. of 54 kD. Enzyme activity coincided with the position of these proteins. No other proteins could be discovered in the preparation. The 54 kD polypeptide, incidentally, is more probably a product of restricted proteolysis of the 57 kD polypeptide.

Preparations of Ca,Mg-dependent endonuclease described in the literature have about half the molecular weight (27-32 kD) [9, 11], from which it may be concluded that they arise from this high-molecular-weight form as a result of nonspecific proteolysis during isolation or specific processing *in vivo*.

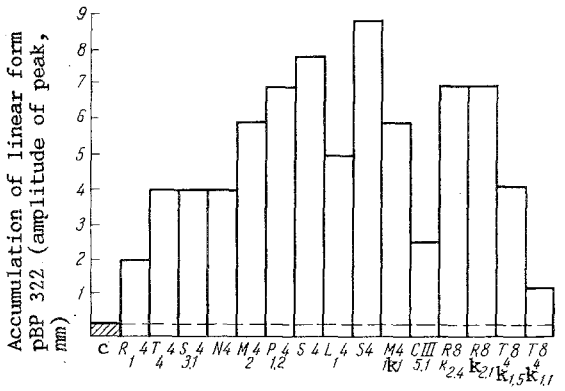


Fig. 2. Binding of antibodies produced by different hybridoma lines (indicated along abscissa) with preparation of Ca,Mg-dependent endonuclease. Ordinate, quantity of linear pBP 322 DNA formed during incubation (amplitude of peak in mm). C) Control.

The study of activity of Ca,Mg-dependent endonuclease compared with that of other possible endonucleases from cell nuclei can be conducted in preparations of isolated cell nuclei or in extracts incubated in the presence of various bivalent cations and at different pH values. The degree of hydrolysis of endogenous or exogenous DNA can be used as the measure of activity in such experiments. However, the absence of specific inhibitors of individual endonucleases makes the results of the corresponding experiments rather indirect.

Another approach is by chromatographic separation of endonucleases [7]. In this case also, however, it is difficult to conclude whether there is marked heterogeneity of the enzymes or multiple or modified forms of one or more enzyme.

We used an approach based on binding of Ca,Mg-dependent nuclease with antibodies produced by hybridomas. The idea of this approach was that if an animal is immunized with a total extract of cell nuclei, conjecturally containing different endonucleases, hybrid cell lines obtained independently, and arising through fusion of immune splenocytes with lymphoma cells, will produce antibodies that interact with different enzymes and will not give "crossed" reactions. Conversely, if one dominant endonuclease is present, the majority of independent hybridoma lines will produce antibodies binding with the same proteins.

Data on binding of antibodies produced by 9 independently obtained hybridoma lines and their derivatives from animals immunized with total extract of human lymphocyte cell nuclei with Ca,Mg-dependent endonuclease, isolated as described above from the same source, are given in Fig. 2. Clearly antibodies produced by all lines interacted with this enzyme. We regard these data as direct proof of predominant representation of Ca,Mg-dependent endonuclease in the nuclease complex of the lymphocyte chromatin. Of course this does not mean that it is the only nuclease, but it suggests: 1) that the content of other types of endonucleases in lymphocyte nuclei is significantly (according to the experimental conditions, by at least an order of magnitude) less than that of the given enzyme; 2) that some metal-dependent endonucleases found in cell nuclei may be products of alternative processing of the high-polymer form of Ca,Mg-dependent endonuclease. A similar situation has been described for *Neurospora crassa* [3].

The results provide an opportunity for the detailed study of Ca,Mg-dependent endonuclease from lymphocyte nuclei and emphasize its functional importance as the principal component of the nuclease complex of lymphocyte chromatin.

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INTERACTION BETWEEN ISOLATED RAT BRAIN SYNAPTIC VESICLES AND PLANAR BILAYER MEMBRANES

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The hypothesis is now generally accepted that mediator secretion takes place by exocytosis, which consists of several stages: 1) coupling of depolarization and secretion of mediators and intracellular accumulation of Ca^{++} ions, 2) Ca-dependent transport of synaptic vesicles (SV) to the active zone of synapses, with the participation of cytoskeletal contractile proteins, 3) Ca-dependent joining of SV with complementary specialized sites on the inner surface of presynaptic membranes (pre-SM), Ca-dependent approximation and fusion of SV with pre-SM, i.e., intermembranous interaction [2]. Interaction of SV with pre-SM may take place through protein structures [3]. However, models of this interaction based only on the properties of phospholipids exist [8]. In the study of exocytosis of mediators it is interesting to investigate systems which model it *in vitro* and, in particular, systems of isolated SV-artificial bilayer lipid membranes (BLM). This system resembles most closely the liposomes-BLM system in which many aspects of intermembranous interactions were studied previously [5, 6, 11, 12]. Methods of studying interaction of BML with liposomes [4] and with lipoprotein complexes [7] have now been developed for systems including BLM. These methods are based on analysis of changes in some electrical characteristics of BLM, induced by the interactions being studied, and determined by changes in parameters of BLM such as capacity, conductivity, the intermembrane potential jump, and the modulus of elasticity.

In the investigation described below changes in conductivity of laminar BLM were measured during their interaction with isolated SV from whole rat brain. By this method it is possible to work with native SV, by which it compares favorably with that described in [10], in which SV filled with the fluorescent dye calcein were obtained, as a result of which their volume was increased by 50-100 times. Development of the model of mediator exocytosis, not yet described in the literature, may be useful for the study of secretory processes both under normal conditions and when disturbed as a result of various forms of pathology of the nervous system.

EXPERIMENTAL METHOD

The SV fraction was isolated from whole brain of rats weighing 150-200 g [1]. For this purpose, after removal of the nuclei from a 20% brain homogenate (0.32 M sucrose, 20 mM Tris-HCl, pH 7.4, 0.1 mM EDTA), unpurified synaptosomes were obtained (10,000g, 10 min), and were subjected to osmotic shock by suspending the residue in distilled water (at the rate of 4 ml of water to residue obtained from 1 g brain tissue). After freezing at −20°C and thawing the suspension was incubated in 1 mM EDTA solution for 10 min, then centrifuged for 30 min

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