VARIATION IN DEOXYRIBONUCLEASE ACTIVITY IN NUCLEI OF SPLENIC LYMPHOCYTES

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UDC 612.411:612.112.94.015.1:577.152.314

The nuclei of splenic lymphocytes were shown to contain nuclease activity whose action is manifested under optimal conditions for different types of DNases: DNase I, micrococcal nuclease, Ca,Mg-dependent endonuclease, and DNase II. No such variety of nuclease activity was found in liver and kidney nuclei. The presence of high nuclease activity in the lymphocytes is responsible for the more intensive degradation of their chromatin by endonuclease than in liver nuclei. The diversity of nuclease activity and the more advanced degree of chromatin degradation by endonuclease in splenic lymphocyte nuclei may perhaps be connected with the rapid renewal of the lymphocyte pool in lymphoid organs and the need for autolysis of the genome of the dying lymphocytes. They may also lie at the basis of the somatic mutagenic mechanism of the diversity of V-genes and of the union of the V- and C-genes of immunoglobulins.

KEY WORDS: splenic lymphocytes; DNase.

Among the particular features of the system of immunoglobulin genes the most puzzling are the mechanisms responsible for the diversity of the V-genes and their union with the C-genes. There is steadily increasing evidence against the view that a large population of V-genes exists beforehand, with the result that an explanation of the diversity of the V-genes is increasingly being sought on account of somatic mutagenesis [5]. Both the somatic mutagenic mechanism of the diversity of V-genes and the union of V-genes with C-genes presuppose the participation of nuclear nucleases in their realization.

Analysis of the distribution of Ca,Mg-dependent intranuclear endonuclease in different organs has shown that this enzyme is not present in all organs. Its activity is high in liver cell nuclei but absent in brain cell and neutrophil nuclei [3, 7]. Since lymphoid organs consist of rapidly renewed cells, and since reorganization of the genome takes place in connection with antibody formation in their lymphocytes, the presence of high nuclease activity in the lymphocyte nuclei can be expected.

Starting from this assumption, endonucleolysis of the nuclear chromatin of splenic lymphocytes of albino rats was investigated under optimal conditions for exhibition of the activity of the various types of nucleases. As an indicator of the degree of endonucleolysis, estimation of the fraction of extractable fragmented chromatin and electrophoretic analysis of the spectra of chromatin fragments obtained by endonucleolysis under different conditions in a polyacrylamide gel (PAG) concentration gradient was used.

EXPERIMENTAL METHOD

Nuclei were isolated from the spleen, liver, and kidney cells of noninbred albino rats by the method described in [6]. Isolated, washed nuclei were suspended for endonucleolysis in the following solutions: I) 0.25 M sucrose, 0.25 M KCl, 0.05 M Tris-HCl, pH 7.5, 0.005 M $MgCl_2$, and 0.002 M $CaCl_2$; II) 0.25 M sucrose, 0.01 M Tris-HCl, pH 7.5, 0.001 M $CaCl_2$; III) 0.25 M sucrose, 0.01 M NaCl, 0.01 M Tris-HCl, pH 7.5, 0.003 M $MgCl_2$; IV) 0.25 M sucrose, 0.01 M Tris-HCl, pH 7.0. These solutions corresponded to optimal conditions of manifestation of activity of Ca,Mg-dependent endonuclease, nuclease of the micrococcal type, DNase I, and DNase II respectively. Incubation of the nuclei in solution I was carried out at 20°C and in solutions II-IV at 57°C. Nucleolysis in solutions I-III was stopped by the addition of EDTA in a final concentration of 0.002-0.005 M and by cooling, and in incubation medium IV

I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR A. A. Smorodintsev.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 87, No. 4, pp. 308-311, April, 1979. Original article submitted June 14, 1978.

0007-4888/79/8704-0307$07.50

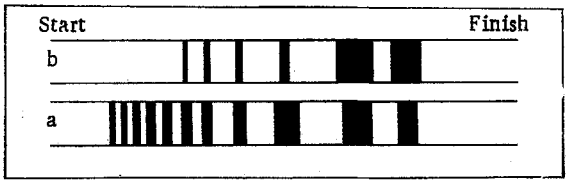

Fig. 1

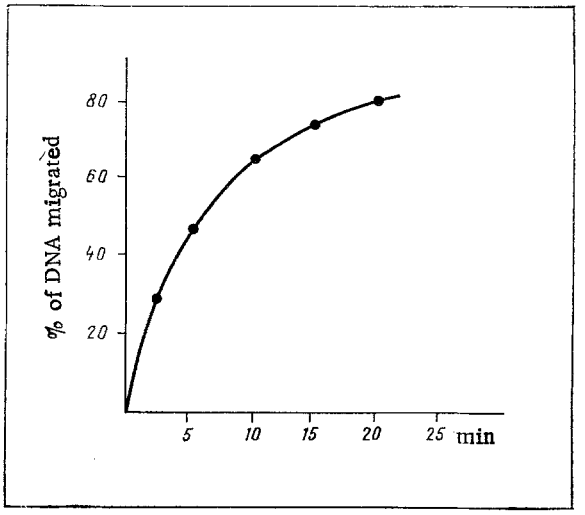

Fig. 2

Fig. 1. Electrophoresis of spectra of chromatin fragments of liver (a) and spleen (b) obtained by endonucleolysis in incubation medium with Ca^{++} and Mg^{++} simultaneously.

Fig. 2. Kinetics of migration of fragmented chromatin from splenic nuclei into incubation medium containing Ca^{++} during nucleolysis.

by cooling. The incubated nuclei were centrifuged and fragmented chromatin was extracted from the residue with the aid of gentle homogenization in a homogenizer with glass pestle, and the fraction of chromosomal DNA in the incubation solution, in the extracted chromatin, and in the residual nuclear material by a spectrophotometric method [4]. The extracted chromatin was subjected to electrophoresis in a concentration gradient of 2.5-7.5% PAG by the method described in [3].

EXPERIMENTAL RESULTS

Data on digestion of splenic chromatin by intranuclear nucleases under different conditions are given in Table 1. The differences in the composition of the incubation media for manifestation of nuclease activity were due mainly to the presence or absence of bivalent cations Ca^{++} and Mg^{++}, which was reflected in the depth of nucleolysis of the splenic chromatin and the yield of DNA in the individual fractions. For instance, whereas if Ca^{++} and Mg^{++} were present simultaneously, most of the chromatin underwent endonucleolysis, and the fraction of chromatin passing into the incubation medium was very small, in the presence of Ca^{++} most of the chromatin which was fragmented left the nuclei. The fraction of digested chromatin in the presence of Mg^{++} alone was substantially smaller, and the fragments of chromatin after endonucleolysis virtually did not leave the nucleus. Without the addition of extra bivalent cations to the incubation medium (which by no means implies their complete absence in the nuclei), endonuclease activity was exhibited to a lesser degree than in the presence of Ca^{++} alone or of Ca^{++} and Mg^{++}, but most of the fragmented chromatin left the nuclei, evidently because of their lysis.

Electrophoretic analysis of the spectra of fragments of splenic chromatin cut under different conditions of nucleolysis revealed differences in their patterns compared with liver chromatin. For instance, in the case of fragmentation of liver chromatin by Ca,Mg-dependent endonuclease, electrophoresis revealed up to 11-13 chromatin fractions, and several subfractions were found in the fast-migrating fraction. The relative values of electrophoretic mobility of the fractions under these circumstances agreed with the natural series of integral values of the logarithmic scale (except the first fast-migrating fraction itself), so that chromatin fragments succeeding one another during electrophoresis could be regarded as multiples of the nucleosome in size, the elementary structural subunit of chromatin [7]. The first fast-migrating fraction is evidently composed of subnucleosomes; the remaining fractions with lower electrophoretic mobility are mono-, di-, trinucleosomes, and so on, respectively.

TABLE 1. Proportions of Chromosomal DNA of Splenic Nuclei Detected in Individual Fractions After Endonucleolysis with Different Combinations of Ca^{++} and Mg^{++} for 1 h

Presence of Ca^{++} and Mg^{++} in incubation mixture		DNA, %		
Ca^{2+}	Mg^{2+}	in incubation medium	in extracted chromatin	in nuclear residue
+	+	2—5	70—80	10—15
+	—	63—67	18—22	11—15
—	+	1—3	48—51	42—45
—	—	12—15	3—5	78—82

On endonucleolysis of splenic chromatin in the presence of Ca^{++} and Mg^{++}, deeper fragmentation of the chromatin was found, as shown by the small quantity of the fractions with slow electrophoretic mobility and predominance of the fast-migrating fractions (Fig. 1). The electrophoretic spectra of fragments of splenic chromatin obtained by nucleolysis under different conditions were similar in the composition of their fractions to the picture obtained by nucleolysis in the presence of Ca^{++} and Mg^{++}, but they differed in relative content of the individual fractions in the spectrum.

The escape of most of the digestive chromatin from the splenic nuclei in the presence of Ca^{++} closely resembles the effect observed from treatment of nuclei with micrococcal nuclease [1], which is known to be a Ca-dependent enzyme. Endonucleolysis of chromatin becomes manifested very quickly, as is shown by the kinetics of migration of the digested chromatin fragments into the incubation medium (Fig. 2).

Since the degree of fragmentation of splenic chromatin and of its extraction are the same whether incubated in medium containing Ca^{++} only or medium containing Ca^{++} and Mg^{++}, the migration of chromatin fragmented by nucleases in the splenic nuclei is perhaps determined by the state of the nuclear membranes, which are permeable to large macromolecules in the presence of Ca^{++} and in the absence of Mg^{++}. Evidently Mg^{++} "blocks" the outlet in the membranes for large molecules and, in particular, for chromatin fragments, for in the presence of Ca^{++} and Mg^{++} simultaneously or only of Mg^{++} the ability of splenic nuclear chromatin to be attacked by intranuclear nucleases is relatively high, and the migration of its fragments into the incubation medium virtually does not occur.

Only activity of Ca,Mg-dependent endonuclease is manifested in nuclei of the liver and kidney (in the kidney, moreover, the activity observed is weaker than in the liver), and activity of other types of nucleases is virtually absent.

Endonuclease activity in splenic lymphocyte nuclei is thus possible both in the presence of Ca^{++} or Mg^{++} only, and in the presence of both simultaneously, as well as in the absence of bivalent cations, in conformity with the conditions for manifestation of activity of nucleases of the micrococcal nuclease, DNase I, Ca, Mg-dependent endonuclease, and DNase II respectively. The manifestation of endonuclease activity in the splenic nuclei in the presence of different combinations of bivalent cations (or their absence) and the known specificity of nucleases toward them are evidence that the nuclease activity of splenic lymphocyte nuclei is evidently due not to one versatile enzyme, but to the presence of different types of endonucleases.

The diversity of DNase activity discovered in splenic lymphocyte nuclei must evidently be taken into consideration when chromatin is isolated from lymphocytes. Solutions with only Mg^{++} in their composition can probably be recommended for the isolation of splenic chromatin, for Mg^{++} prevents the leakage of chromatin material from the nuclei, and endonucleolysis is weaker in intensity in its presence. The isolation of chromosomal material from lymphocytes without endonucleolysis is impossible without the use of nuclease inhibitors.

The diversity of the DNase activity in the spleen can perhaps be attributed to the high mitotic activity of the lymphocytes and the correspondingly high degree of renewal of the lymphocyte pool in the spleen, which may require a high degree of autolysis of dying lymphocytes. High endonuclease activity in the lymphocytes could be connected with somatic mutagenesis giving rise to the diversity of the V-genes and with structural changes in the

chromosomes responsible for union of the V- and C-genes of the immunoglobulins during antibody formation. Regardless of the mechanism which actually causes fusion of the V- and C-genes into one single cistron with template activity (translocation of the V-gene into the region of the C-gene or diminution of the chromatin separating them), there is no question that these structural changes in the chromosomes connected with antibody formation must take place with the participation of endonucleases.

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