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ELECTROPHORETIC INVESTIGATION OF NUCLEAR MEMBRANE RIBONUCLEASES OF RAT LIVER AND HEPATOMA-27 CELLS

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Preparations of the nuclear membranes were obtained from purified nuclei of rat liver and hepatoma-27 cells, and from them enzyme-containing extracts of acid-soluble proteins were then prepared. The protein extracts were subjected to disk electrophoresis in 15% polyacrylamide gel. Ribonucleases (RNases) which are constituents of the acid-soluble proteins of the nuclear membranes of normal liver were found to be present as several different components which differed in their electrophoretic mobility and in several physicochemical properties from crystalline bovine RNase and the RNase of nuclear chromatin.

KEY WORDS: electrophoresis; polyacrylamide gel; nuclear membranes; ribonucleases; rat liver; hepatoma-27

Besides the many ribonucleases (RNases) contained in the cytoplasm and its organoids, a high proportion of RNase activity is also contained in the cell nuclei [8, 16]. Despite the comparatively low relative proportion of the nuclear depolymerases, their role in the metabolism of the cellular RNA is evidently very important [17]. By now several RNases hydrolyzing different types of RNA have been found in the protein composition of the cell nuclei, and in their properties these enzymes differ significantly from the cytoplasmic enzymes [13]. Since polyribonucleotide transport from nucleus into cytoplasm probably takes place through the nuclear pores [1, 2], the nuclear membranes must play an important role in the enzymic degradation of RNA.

It has been shown [6, 7] that the layers of the nuclear membranes possess high RNase activity. There is some evidence that the nuclear membranes contain not one, but several enzymes degrading RNA [7]. The study of this problem could be important for a detailed explanation of the mechanism of transport and the realization of the genetic potentials of the cell nucleus. After fractionation in polyacrylamide gel (PAG) the activity of the RNases is still preserved and can be detected by electrophoresis [9, 18].

The object of the present investigation was an electrophoretic study of RNases found in the cell membranes of rat liver and hepatoma-27.

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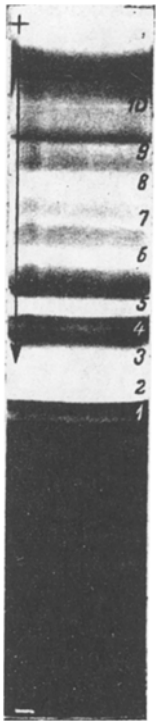

Fig. 1. RNase from purified liver cell nuclei, direct identification of enzyme activity in blocks of gel.

EXPERIMENTAL METHOD

Noninbred male rats weighing 150-200 g were used. Cell nuclei were isolated from rat liver in concentrated sucrose [11]. In individual experiments chromatin preparations were obtained from purified nuclei [10]. The nuclear membranes were isolated by the flotation method [3] or by layering after osmotic shock in 0.02 M phosphate buffer, pH 7.2 [5]. Membranes washed to remove sucrose were extracted with 0.25 N H_2SO_4 at 4°C for 1 h. Sucrose was added to the acid-soluble proteins to a final concentration of 5% and urea to a final concentration of 4 M. After determination of the protein concentration by Lowry's method [12], samples (20-40 μl) were applied to PAG.

Nuclei and nuclear membranes from the tissue of a subcutaneously transplanted rat hepatoma-27 were obtained by the method described above. In this case, however, longer homogenization was needed [4].

A modified system of Reisfeld et al. [14] was used for electrophoretic fractionation of the proteins. The 15% fractionating gel contained 0.5% methylenebisacrylamide and 2.5 M urea. The 5% concentrating gel contained 5% N,N'-bisacrylamide. The pH of fractionation was 4.5 and the duration of electrophoresis 1.5-2 h. Fractionation was carried out in rectangular blocks of gel with dividing partitions, enabling several samples to be applied and analyzed simultaneously. After the end of electrophoresis some of the blocks were treated with 0.1% Amido black or Coomassie brilliant blue solution. To detect RNase activity the blocks were incubated in buffer solutions containing 0.5% RNA (a commercial preparation of tRNA from Sigma, USA) for 1 h at 37°C [18]. The blocks of gel were then washed with cold 0.01 N acetic acid and stained with 0.1% toluidine blue solution in 1 N acetic acid. The stained blocks were decolorized in the same solution not containing the dye.

EXPERIMENTAL RESULTS

During electrophoretic fractionation of the acid extracts from the liver cell nuclei followed by detection of the protein components with Amido black examination of the blocks showed the characteristic picture of nuclear histones; removal of the membranes by osmotic shock caused virtually no change in the content of histone proteins. If RNase activity was revealed by electrophoresis, a large number (up to 10) of zones of enzyme activity was observed (Fig. 1).

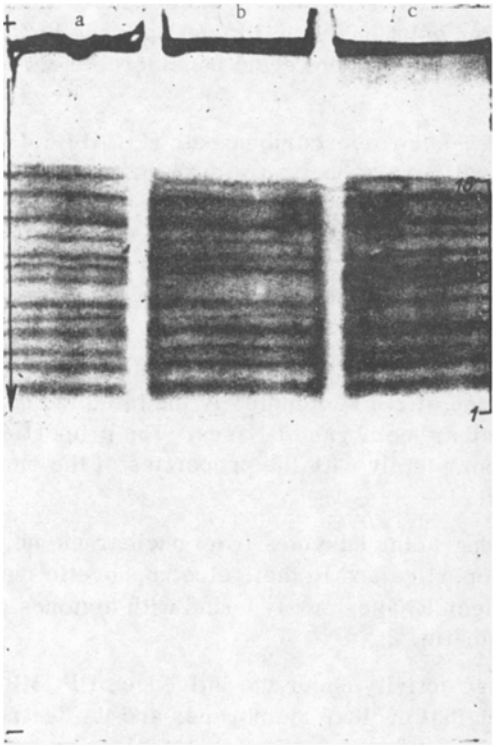

Fig. 2

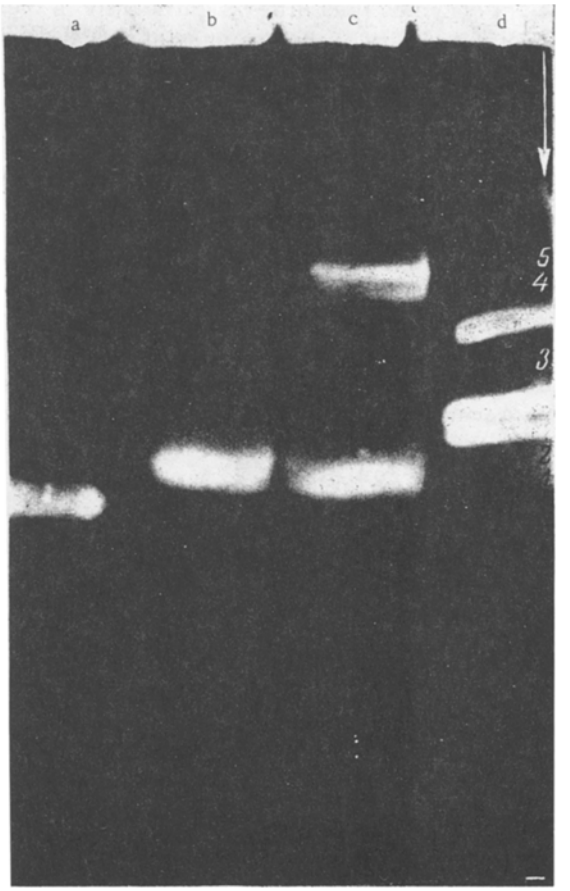

Fig. 3

Fig. 2. Electrophoretic fractionation of acid-soluble nuclear membrane proteins obtained by various methods. a, b) Method of layering above a gradient; c) floatation method.

Fig. 3. Electrophoresis of RNases in a common gel block. a) Crystalline RNase from bovine pancreas (Merck, West Germany); b) RNases from nuclear membranes of hepatoma cells; c) the same from liver cells of healthy animals; d) total preparation of acid-soluble chromatin proteins from rat liver cell nuclei; 1-5) zones of enzyme activity along run "c".

During electrophoretic fractionation of acid extracts from the nuclear membranes the pattern of distribution of the proteins differed considerably from preparations of acid-soluble chromatin proteins (Fig. 2). In this case 12 or 13 protein components, containing about equal amounts of protein in each component and migrating in PAG at about equal distances apart, were found. These proteins had much lower mobility than histones and no histone proteins were found in them. The assortment of proteins in membrane preparations obtained by different methods (floatation [3] or layering [5]) were virtually indistinguishable. The method of direct detection of RNases in the PAG blocks revealed that the RNases of the nuclear membranes were represented by several enzymes differing in their mobility in PAG. Under normal conditions (Fig. 3a, c) two groups of bands with enzyme activity were found. The group of bands 1 and 2, consisting of 2 or 3 components, had relatively high electrophoretic mobility; it was followed by component 3 with low activity and, finally, by two components 4 and 5, with relatively low electrophoretic mobility. The two last components were absent or were present in only very small amounts in preparations from the nuclear membranes of the tumor cells (Fig. 3b).

Comparison showed that the electrophoretic mobility of the RNases from nuclear membranes and of the enzyme extracts obtained from nuclei or from washed chromatin (Fig. 3c, d) differed substantially. The RNases from the nuclear membranes migrated faster (zones 1 and 2) or much slower (zones 3 and 4) than the enzymes associated with the acid-soluble chromatin proteins. The electrophoretic mobility of crystalline bovine pancreatic RNase was appreciably higher than the mobility of enzymes from the nuclei and nuclear membranes.

All three types of enzymes thus differ significantly in their behavior in PAG, as is manifested by differences in the electrophoretic mobility of the enzymes of this type and their heterogeneity. Pancreatic RNase moved in the chosen system as a single homogeneous band; this suggests that the heterogeneity of the other enzyme preparations was not an artefact.

The next step was accordingly to study some other properties of the acid-soluble RNases from nuclear membranes. On incubation of fragments of the blocks in buffer solutions with different pH, the highest enzyme activity in all zones of the blocks was found in the region of pH 7.2-7.5; however, considerable enzyme activity still remained at pH 5.5 also.

Treatment of the blocks before incubation with 0.3 mM p-chloromercuribenzoate (PCMB) did not change activity in any zone. Enzyme activity was preserved after preliminary heating of the blocks to 95°C for 10 min. No enzyme activity was found if the RNA was replaced by DNA as substrate. Preliminary treatment of the blocks in 0.15 M monoiodoacetate, whether at 56°C or at 20°C, for 1 h led to total loss of enzyme activity. By contrast with the pancreatic enzyme, nuclear membrane RNases did not show any significant change in their activity after preliminary reduction with mercaptoethanol (1% mercaptoethanol, 8 M urea, 8-12 h at 37°C before application to the gel).

Another difference was that enzymes of the nuclear membranes showed considerable resistance to the action of ions of the heavy metals. Cu^{2+} ions in a concentration of 2 mM completely inhibited the pancreatic enzyme but had no significant effect on the activity of the nuclear membrane RNases. The properties of the nuclear membrane RNases from hepatoma-27 cells agreed completely with the properties of the enzymes from normal liver cells.

It can thus be concluded from these results that RNA-degrading enzymes from nuclear membranes constitute a special group of enzymes which differ in certain properties and in their electrophoretic mobility in PAG both from bovine pancreatic RNase and from other nuclear RNases partly bound with histones and, in particular, from other acid-soluble proteins of nuclear chromatin.

In these investigations no change was found in the RNase activity under the influence of PCMB, although this does not rule out the possibility of an inhibitor in the original nuclear membranes and its destruction during extraction or electrophoretic fractionation. The fact must also be borne in mind that nuclear membranes may also include other types of RNases which are not extracted by acid or which lose their activity during acid extraction.

These differences in the activity of individual enzymes found in the composition of the nuclear membranes of normal and malignantly changed hepatocytes may reflect differences in the metabolism of certain types of RNA arising as a result of malignant transformation.

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