RIBONUCLEASE ACTIVITY OF THE OUTER NUCLEAR MEMBRANE OF RAT HEPATOCYTES

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The outer nuclear membrane was studied by treating isolated nuclei from the rat liver with 0.5% Triton X-100 solution followed by centrifugation in a sucrose density gradient. Electron microscopy showed that the outer layer of the nuclear membrane has the characteristic structure of membranes of the endoplasmic reticulum, with clusters of ribosomes attached to them. The fraction of the nuclear membrane contains 55% protein, 34% phospholipids, 6.8% RNA, and about 1% DNA (calculated per dry weight) and has no glucose-6-phosphatase tivity. Preparations of the nuclear membrane possess ribonuclease activity with pH optimum of 7.8. Activity of the enzyme in the membrane fraction was 2.6 times higher than in the isolated nuclei. Treatment with p-chloromercuribenzoate and urea increased the ribonuclease activity by 2.0 and 2.2 times respectively, indicating the latent state of most of the enzyme in the membrane. Cytoplasmic inhibitor completely suppresses the ribonuclease activity of the outer layer of the rat liver nuclear membrane.

Information is available on RNA-splitting enzymes in cell nuclei and nuclear structures. However, there is no information in the literature on the ribonuclease activity of the nuclear membrane of animal cells. However, investigation of nuclear membranes and, in particular, of the outer membrane, has now become possible with the development of methods for their isolation [1, 3, 4].

In the investigation described below, ribonuclease was demonstrated in the isolated outer nuclear membrane of the rat liver and some of its properties were studied.

EXPERIMENTAL METHOD

Nuclei were isolated from the liver of albino rats weighing 170-200 g by the sucrose method [5]. The purity of the nuclear preparations was verified by phase-contrast and electron microscopy and by biochemical tests. The isolated nuclei had a double membrane with attached ribosomes and were not contaminated with mitochondria or cell fragments. The glucose-6-phosphatase and 5'-nucleotidase activity was 0.13 and 0.27% respectively of the total activity of the enzymes in the homogenase; the RNA/DNA ratio was 0.19.

TABLE 1. Action of p-CMB and Urea on Ribonuclease Activity of Preparations of Outer Nuclear Membrane and Nuclei of Rat Liver (in ΔE_{260}/mg protein in 30 min at 37°C; n = 5)

Fraction	Control	p-CMB ($3 \cdot 10^{-4}$ M)	Urea (4M)
Nuclei . . .	0,72±0,07	1,02±0,10	0,96±0,15
Nuclear membrane	1,80±0,22	3,60±0,32	4,0±0,52

The outer nuclear membrane was obtained by treating the isolated nuclei with 0.5% Triton X-100 solution [4] and subsequent centrifugation in a sucrose density gradient [1].

Electron-microscopic analysis showed that the preparations had the characteristic structure of membranes of the endoplasmic reticulum, with clusters of ribosomes attached to them (Figs. 1 and 2).

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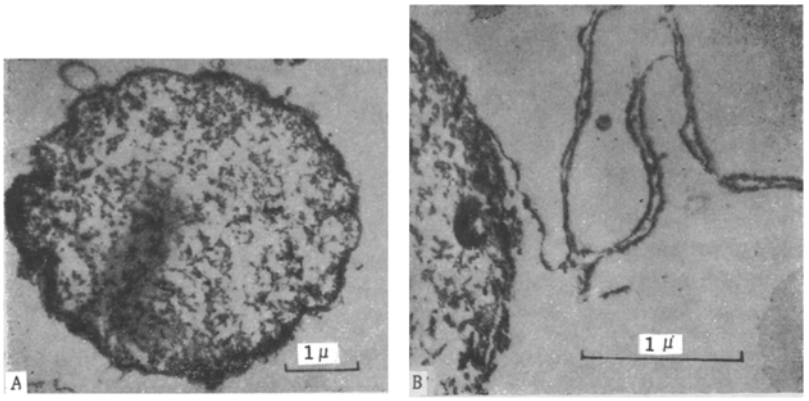

Fig. 1. Electron micrograph of isolated cell nuclei from rat liver. A) Nucleus before treatment with 5% Triton X-100 solution (1200×); B) nucleus after such treatment (40,000×); detached layer of outer nuclear membrane with ribosomes can be seen. Fixation with 2% glutaraldehyde and 1% osmic acid solution, staining with 1% uranyl acetate and lead hydroxide by Watson's method.

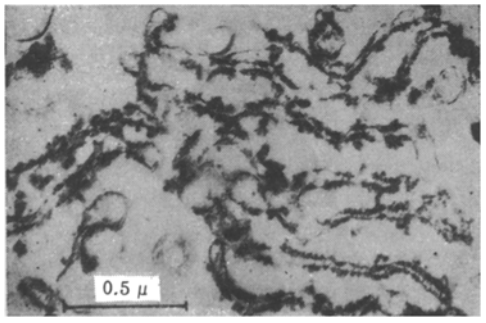

Fig. 2. Electron micrograph of isolated outer nuclear membrane from rat liver. Fixation and staining as in Fig. 1 (54,000×); nuclear membrane with attached ribosomes can be seen.

The membrane material contained 55% protein, 34% phospholipids, 6.8% RNA, and about 1% DNA calculated per dry weight. The RNA of the membrane fraction constituted 7% of the total RNA of the original nuclei. The test material did not possess glucose-6-phosphatase activity.

Ribonuclease activity in the preparations was determined by the standard method [2]. The protein content in the sample was 0.4–0.6 mg. Ribonuclease activity was expressed in ΔE_{260}/mg protein in 30 min at 37°C. Cytoplasmic ribonuclease inhibitor was isolated from rat liver [2]; one unit activity of the inhibitor corresponded to 50% inhibition of 0.05 μg crystalline ribonuclease from bovine pancreas (Reanal, Hungary).

EXPERIMENTAL RESULTS

The experiments showed that the preparations of outer nuclear membrane possessed ribonuclease activity with pH optimum of 7.8. At pH 5.6 and 8.4 50% of the maximal enzyme activity remained. Until 60 min the quantity of acid-soluble hydrolysis products of RNA was directly dependent on the incubation time of the samples. Monovalent cations (K^+, Na^+) in concentrations of 1×10^{-1} and 1×10^{-2} activated, while bivalent cations (Ca^{++}, Mg^{++}), in the same concentration, slightly inhibited ribonuclease activity.

The action of p-chloromercuribenzoate (p-CMB) and urea on the ribonuclease activity of the nuclear membrane and nuclei was studied (Table 1).

The activity of the enzyme in the membrane fraction was 2.6 times higher than in the isolated nuclei. The membrane ribonuclease was activated by 2.0 and 2.2 times respectively by p-CMB (3×10^{-4} M) and urea (4 M). Ribonuclease activity in the isolated nuclei, however, was increased by only 40% and 30% respectively in the presence of p-CMB and urea. This shows that most of the ribonuclease in the nuclear membrane is in the latent state and is evidently blocked by the natural inhibitor.

Accordingly experiments were carried out to titrate the ribonuclease of the nuclear membrane (0.5 mg protein) with purified inhibitor. As Fig. 3 shows, the percentage inhibition of ribonuclease was directly dependent on the quantity of inhibitor. Activity of the enzyme was completely suppressed by the addition of three units of inhibitor.

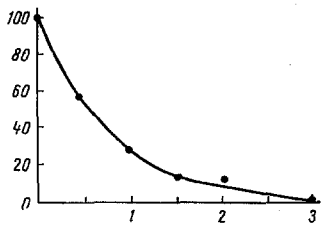

Fig. 3. Effect of inhibitor on ribonuclease activity of isolated outer nuclear membrane of rat liver (incubation mixture contained 0.5 mg membrane protein and 0-3 units of inhibitor). Abscissa, content of inhibitor (in units); ordinate, inhibition of ribonuclease activity (in %).

The results of these experiments thus showed that the isolated outer nuclear membrane of the rat liver contains ribonuclease, much of it bound with inhibitor and present in the latent form.

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