ULTRASTRUCTURAL LOCALIZATION OF β-GLYCEROPHOSPHATASE IN MOUSE LIVER NUCLEI

I. B. Bukhvalov and É. Unger

UDC 612.351.11

The localization of phosphatase activity was studied in isolated nuclei of the mouse liver within the pH range from 5.6 to 8.8. Hydrolysis of sodium β-glycerophosphate was most marked at pH 6.4. Enzyme activity was localized in the chromatin, nuclear membranes, and perinuclear space. The nuclear sap and nucleolus were not stained.

Many investigators consider that phosphatase activity in the cell is localized entirely in cytoplasmic structures. A positive reaction for phosphatase in the nuclei in histochemical investigations has been explained by diffusion of the enzyme or the histochemical reaction product [2, 7, 9]. On the other hand, it is stated that acid phosphatase is localized in the chromatin of hepatoma cell nuclei [3], in the chromatin and discs of chromosomes, and the nuclear membrane of corn and pea root cap cells [10], in the perinuclear space of certain ciliates [6], and so on [5].

The object of this investigation was to examine the ultrastructural localization of phosphatase activity in mouse liver nuclei.

EXPERIMENTAL METHOD

Mouse liver nuclei were isolated by Chauveau's method with certain modifications [1] after preliminary fixation in glutaraldehyde. The liver of decapitated mice was cut up finely with scissors in a cold fixing mixture of the following composition: 0.25 M sucrose, 3 mM $Mg(CH_3COO)_2$, 1.5% glutaraldehyde, and 0.05 M cacodylate buffer, pH 7.2. This material was homogenized in a glass homogenizer with Teflon pestle and motor for 1 min in the same fixing mixture, filtered through four layers of gauze, and centrifuged for 5 min at 3000 g. The material was in the fixing mixture for not more than 15 min. The residue was then washed twice in 0.25 M sucrose with 3 mM $Mg(CH_3COO)_2$, homogenized in 2.3 M sucrose with 3 mM $Mg(CH_3COO)_2$ (one or two runs of the pestle), centrifuged for 50 min at 22,000 g, and washed in 0.25 M sucrose with 3 mM $Mg(CH_3COO)_2$. In some experiments nuclei were obtained in the usual way without prefixation in glutaraldehyde as described earlier [1].

Nuclei thus obtained were incubated in a medium of the following composition: 5 ml 0.25 M Tris-malate buffer, 2 ml 2.3 M sucrose, 2.5 ml 1.25% sodium β-glycerophosphate solution, and 0.75 ml 2% $Pb(CH_3COO)_2$ solution. Incubation continued for 15 min at 30°C at the following pH values: 5.6, 6.2, 6.4, 6.7, 7.2, 7.5, 8.8. The nuclei were then washed with cold isotonic sucrose with Mg^{++}, fixed for 15 min in 1.5% glutaraldehyde solution in 0.25 M sucrose and 0.05 M cacodylate buffer, pH 7.4, again washed in isotonic sucrose solution, postfixed for 30 min in 1% OsO_4 solution in 0.3 M sucrose and 0.05 M cacodylate buffer, pH 7.4, and embedded in 1% agar at about 40°C. After the agar had solidified small pieces of material measuring about 1-2 mm^3 were cut out and kept overnight in 70° ethanol. Next day the material was dehydrated and embedded in Epon 812. Ultrathin sections were cut on the LKB-111 ultratome and examined in the IEM-7 electron microscope.

Laboratory of Histochemistry and Electron Microscopy, Department of Pathological Anatomy of Human Tumors, Institute of Exptl. and Clinical Oncology, Academy of Medical Sciences of the USSR, Moscow. Laboratory of Biochemistry, Institute of Biology of Development, Academy of Sciences of the USSR, Moscow. Laboratory of Investigation of the Cell, Central Institute of Microbiology and Experimental Therapy, East German Academy of Sciences, Jena. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. A. Kraevskii.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 77, No. 5, pp. 117-118, May, 1974. Original article submitted July 12, 1973.

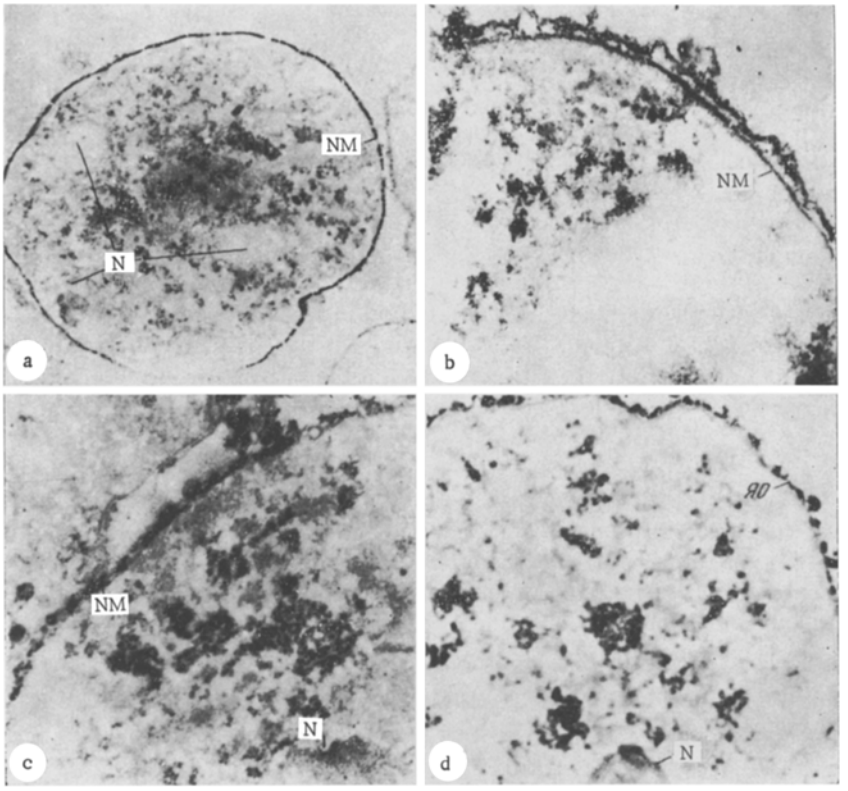

Fig. 1. Isolated mouse liver nuclei: a,b) isolation of nuclei with prefixation in 1.5% glutaraldehyde and incubation in complete incubation medium for detecting β-glycerophosphatase; c) isolation of nuclei without prefixation and incubation in complete incubation medium for detecting β-glycerophosphatase; d) isolation of nuclei without prefixation and incubation in complete incubation medium with the addition of 0.01 M NaF. N) Nucleolus, NM) nuclear membrane. Magnification in: a) 10,000×, in b and c) 30,000×, in d) 20,000×.

EXPERIMENTAL RESULTS

Maximal hydrolysis of β-glycerophosphate in nuclei obtained after preliminary fixation in glutaraldehyde within the pH range tested was observed at pH 6.4. Activity of the enzyme was lower at pH 6.2 and 6.7, and at other pH values no staining whatever developed. The histochemical reaction product could be observed in many of the nuclei. It was located chiefly in the perinuclear space (Fig. 1a) and to a lesser extent in the chromatin and nuclear membranes (Fig. 1b). The addition of 0.01 M NaF to the incubation medium completely suppressed the enzyme activity in the perinuclear space but had virtually no effect on development of the stain in the chromatin.

In nuclei obtained without prefixation in glutaraldehyde the histochemical reaction product at pH 6.4 also was located in the chromatin, the perinuclear space, and the nuclear membranes; the intensity of the reaction in the chromatin and on the outer nuclear membrane was much higher than in nuclei prefixed in glutaraldehyde (Fig. 1c). This reaction was not abolished in the presence of 0.01 M NaF (Fig. 1d). The intensity of the reaction in the perinuclear space in these nuclei, on the other hand, was lower than in the prefixed nuclei. This reaction was completely abolished by NaF (Fig. 1d).

Besides the control with NaF, a specific acid phosphatase inhibitor, the specificity of the histochemical reaction was verified by incubating the nuclei without the substrate or by replacing the substrate by an equimolar quantity of Na_2HPO_4. No color developed in either of these controls. This shows that under the conditions used for the reaction nonspecific staining with lead or nonspecific deposition of diffused lead phosphate on the nuclear structures took place.

Differences in sensitivity to NaF may indicate that the phosphatases of the chromatin and perinuclear space are isozymes. These enzymes also differ in their sensitivity to glutaraldehyde: the intensity of the reaction in the nuclear chromatin, not prefixed in glutaraldehyde, was much higher than in the prefixed nuclei. On the other hand, the intensity of the reaction in the perinuclear space was incomparably higher in the prefixed nuclei, and this can be explained by stabilization of the enzyme or of the membranous structures surrounding it by the glutaraldehyde.

The discovery of phosphatase activity in the chromatin confirms the view that not only specific, but also nonspecific phosphatases can participate in nucleic acid metabolism [3, 8]. The localization of acid phosphatase in the perinuclear space suggests that this cell structure plays an important role in initiating the synthesis of certain enzymes found in the cisterns of the endoplasmic reticulum and in the vesicular organelles.

LITERATURE CITED

1. I. B. Bukhvalov, I. B. Zbarskii, N. T. Raikhlin, et al., Tsitologiya, 13, 654 (1971).
2. L. V. Danilova, Izvest. Akad. Nauk SSSR (Seriya Biol.), No. 1, 104 (1970).
3. V. G. Pinchuk, L. A. Zotikov, and V. D. Monastyrskaya, Tsitologiya, 8, 520 (1966).
4. A. M. Catesson and Y. Czaninski, J. Microscopie, 6, 509 (1967).
5. E. Essner and A. Novikoff, J. Biochem. Biophys. Cytol., 9, 773 (1961).
6. S. Goldfisher, N. Carasso, and P. Favard, J. Microscopie, 2, 621 (1963).
7. A. G. E. Pearse, Histochemistry, Williams and Wilkins.
8. C. C. Richardson, Ann. Biochem., 38, 795 (1969).
9. B. Sandström, Acta Histochem. (Jena), 38, 193 (1970).
10. R. Sexton, J. Gronshaw, and J. L. Hall, Protoplasma (Vienna), 73, 417 (1971).