ULTRASTRUCTURAL LOCALIZATION OF NAD-PYROPHOSPHORYLASE IN MOUSE LIVER NUCLEI

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The ultrastructural localization of NAD-pyrophosphorylase in mouse liver nuclei was studied by means of a new method suggested by the writers for the electron-histochemical detection of the activity of this enzyme. NAD-pyrophosphorylase activity is localized in the chromatin structures of the nucleus and in the nucleolonema of the nucleolus.

NAD-pyrophosphorylase (E.C. 2.7.7.1), the enzyme responsible for the final stage of NAD synthesis, is located chiefly in the chromatin fraction. For this reason NAD-phosphorylase is used as a marker to indicate the presence of nuclear or chromatin fragments in cytoplasmic fractions [4]. Besides in the chromatin, NAD-pyrophosphorylase has also been found in the nucleoli of starfish oocytes [3] and rat liver nucleoli [7]. However, data on the extraction of nuclei with salt solutions may be evidence that this enzyme is also to be found in nuclear sap [6].

Mouse liver nuclei were isolated by Chauveau's method with certain modifications [1] and prefixed at room temperature for 10 min in 2% formaldehyde in 0.05 M Tris-maleate buffer (pH 7.6) and 0.25 M sucrose, or in 70° ethanol, or successively in these two solutions, with two final washings in 0.25 M sucrose for 5-10 min each time.

After this treatment the nuclei were incubated at 38°C for 60 min in medium of the following composition: 0.05 M tris-maleate buffer (pH 7.5), 0.25 M sucrose, 10 mM $Mg(CH_3COO)_2$, 0.2 M nicotinamide, 2 mM $Pb(CH_3COO)_2$, 2 mM ATP, and 2 mM nicotinamide-mononucleotide (NMN). At the end of incubation the nuclei were washed with 0.25 M sucrose with 3 mM $Mg(CH_3COO)_2$, fixed for 10 min in 1.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.4) and 0.5 M sucrose, washed again in 0.25 M sucrose with magnesium, and fixed for 30 min in 1% OsO_4. The nuclei were then imbedded in 1% agar at about 40°C. Small pieces of material, 1-2 mm^3 in volume, were cut out of the solidified agar and kept in 70° ethanol overnight at 4°C. Next day the pieces were dehydrated and embedded in Epon 812. Ultrathin sections were cut on the LKB-111 ultratome and examined in the JEM-7 electron microscope.

EXPERIMENTAL RESULTS

NAD-pyrophosphorylase is responsible for the final stage of NAD synthesis from NMN and ATP in accordance with the following equation:

$$NMN + ATP \rightleftharpoons NAD + pyrophosphate.$$

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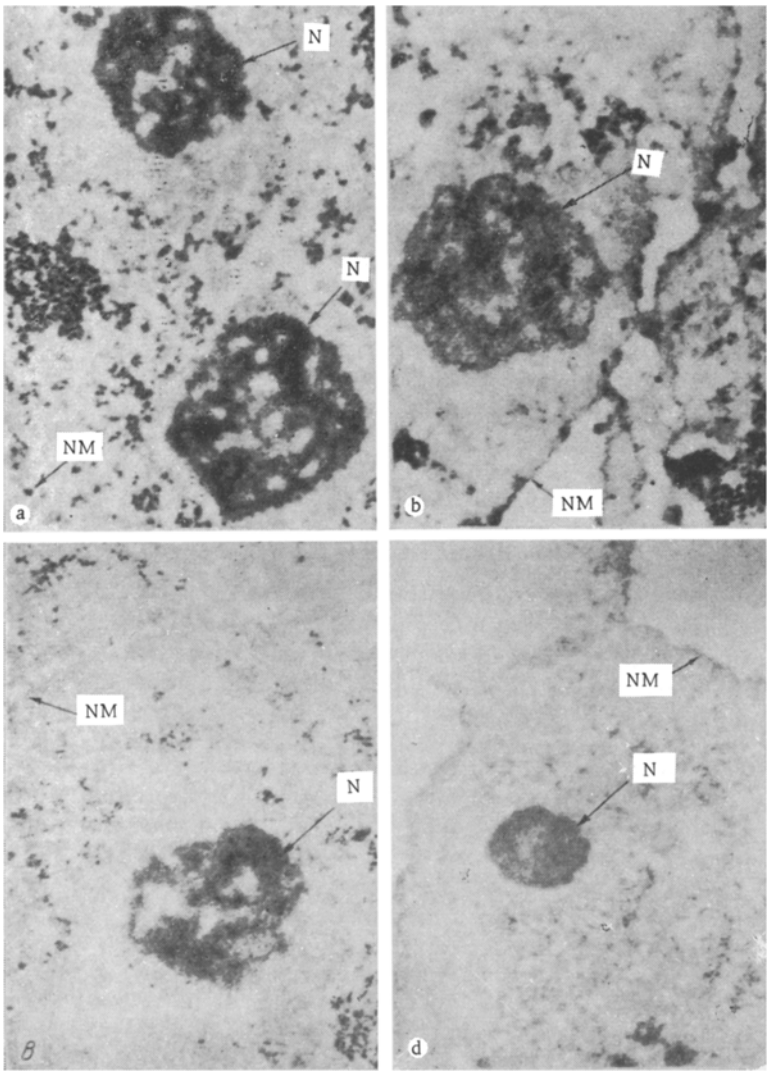

Fig. 1. Isolated mouse liver nuclei: a) prefixation in 2% formaldehyde and incubation in complete incubation medium for detecting NAD-pyrophosphorylase (20,000 ×); b) prefixation in 70° ethanol and incubation in complete incubation medium (23,000 ×); c) prefixation in 2% formaldehyde and in 70° ethanol consecutively and incubation in complete incubation medium (33,000 ×); d) prefixation consecutively in 2% formaldehyde and 70° ethanol and incubation in medium without NMN and nicotinamide (20,000 ×). N) Nucleolus, NM) nuclear membrane.

The method of electron-histochemical detection of NAD suggested by the writers is based on precipitation of the pyrophosphate as its lead salt by means of lead acetate added to the incubation medium suggested previously by Kornberg [5] for biochemical purposes.

The main difficulty in the way of detecting NAD-pyrophosphorylase in accordance with this principle was competition between NAD-pyrophosphorylase and ATPase for one of the substrates (ATP), for which the conditions (temperature and pH, presence of activating Mg^{++} ions) are optimal in the incubation medium used. Removal of the terminal phosphate of ATP by ATPase in the presence of lead ions leads to the formation of a precipitate which cannot be differentiated from the histochemical end product of the NAD-pyrophosphorylase reaction – lead pyrophosphate.

To inhibit the ATPase reaction the nuclei were prefixed in formaldehyde or ethanol. As the writers showed previously, during the biochemical determination of nuclear ATPase and NAD-pyrophosphorylase

activity, prefixation in formaldehyde inhibits ATPase by 55% and prefixation in ethanol by 88%, whereas double prefixation in both formaldehyde and ethanol inhibits ATPase activity by 94%. The NAD-pyrophosphorylase activity was not reduced under these circumstances.

The electron-microscopic examination of preparations of isolated nuclei incubated in medium for detecting NAD-pyrophosphorylase shows that prefixation in formaldehyde (Fig. 1a) permits better preservation of the ultrastructure of the nucleus than prefixation in ethanol (Fig. 1b).

However, during incubation of control preparations of nuclei in medium without NMN and nicotinamide, i.e., under the conditions for detection of ATPase, significantly worse inhibition of ATPase activity was obtained by formaldehyde. Accordingly, double prefixation in formaldehyde and ethanol consecutively was used. This treatment resulted in good morphological preservation and adequate inhibition of ATPase activity (Fig. 1c, d).

It will be clear from the illustrations that NAD-pyrophosphorylase activity was localized in the chromatin structures of the nucleus and in the nucleolonema of the nucleolus. No activity of this enzyme was found in the nuclear sap.

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