Intracellular Distribution of 5′ Nucleotidase in Rat Liver*

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ABSTRACT

The intracellular distribution of 5′ nucleotidase was investigated in rat liver by biochemical analysis of cell fractions obtained by differential centrifugation. The enzymatic activity was measured by determination of the inorganic phosphorus liberated from 5′ nucleotides. The 5′ nucleotidase activity was mainly found in the nuclear and microsomal fractions. An attempt to extract the enzyme from these fractions with Mg^{++} ion solutions was unsuccessful. It is concluded that 5′ nucleotidase is actually present in the nuclear and microsomal fractions of rat liver cells.

INTRODUCTION

The enzyme 5′ nucleotidase has been shown by histochemical methods to be present in the nucleus of various cell types in the mouse, rat, fowl, and man (1–4). Its presence in the cytoplasm of various cell types in the man has also been reported (5).

In the present work, the intracellular distribution of this enzyme was investigated in rat liver by biochemical analysis of cell fractions obtained by differential centrifugation. The liver nuclear fraction was found very active in 5′ nucleotidase. The cytoplasmic activity was found localized in the microsomal fraction and this fraction showed a 5′ nucleotidase activity comparable with that of the nuclear fraction.

Methods

Preparation of Tissue and Cell Fractions.—Adult rats of the Wistar strain (average body weight 225 gm.) were used. The animals were killed by decapitation and bled. The livers were excised, chilled on ice, blotted on filter paper, and weighed. The organs were minced with a cold plexiglass squeezer. The tissue pulp was weighed and homogenized in 0.25 M sucrose with a glass homogenizer and the final volume was adjusted to give a 10 per cent homogenate. The cellular fractions, namely the nuclear (N), the mitochondrial (M), the microsomal (Mc) fractions, and the supernatant fluid (S), were obtained by differential centrifugation according to the method of Hogeboom and Schneider (6). When the whole sedimentable part of the liver was required, the homogenate (H) was centrifuged at 140,000 *g* for 30 minutes. A modified procedure of differential centrifugation was employed (7) for studying the intracellular distribution of nucleotidase in a large number of cellular fractions. Refrigerated centrifuges, International PR 2, and Spinco Model L, was employed.

Enzyme Assay.—The 5′ nucleotidase was assayed in the following incubation mixture: 0.2 ml. of 1 M glycine-NaOH buffer pH 8.5, 0.2 ml. of 0.1 M $MgCl_2$, 0.2 ml. of a 0.015 M solution of substrate, a suitable amount of homogenate or tissue fraction, and distilled water to a final volume of 3.0 ml. Two different nucleotides were used as substrates, namely 5′ adenylic and 5′ inosinic acids. The incubation was carried out during 15 minutes at 37°C., and the reaction was stopped by adding an equal volume of a 10 per cent trichloroacetic acid solution (8). An aliquot was used for inorganic phosphorus determination by the method of Fiske and SubbaRow (9). The enzyme activity was expressed as micromoles of inorganic phosphorus liberated per minute per hundred milligrams of fresh tissue or fresh tissue equivalents. Nitrogen was determined by micro-Kjeldahl procedure.

Extraction Assay.—The whole sedimentable part of the liver cell or the nuclear and microsomal fractions were isolated from homogenates prepared in 0.25 M sucrose or 0.25 M sucrose containing 10 milliequivalents

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of $MgCl_2$. These fractions were resuspended and washed twice with either solution. Nucleotidase activity was measured on both the extracted fractions and the combined washings.

RESULTS AND DISCUSSION

Activity and Intracellular Distribution.—Table I shows the activity and intracellular distribution of 5′ nucleotidase in rat liver homogenate, using either 5′ adenylic and 5′ inosinic acid as substrates. The results indicate that 5′ adenylic acid was hydrolyzed twice as fast as 5′ inosinic acid (1.03 and 0.51 micromoles respectively), but the intracellular distribution was the same using either substrate. The 5′ nucleotidase activity was found mainly in the nuclear (N) and microsomal (Mc) fractions: these fractions showed 40.2 and 37.9 per cent, respectively, of the total activity using 5′ adenylic acid as substrate, and 47.1 and 33.3 per cent, respectively, using 5′ inosinic acid. The mitochondrial fraction (M) and supernatant fluid (S) each showed about 10 per cent of the homogenate (H) activity. It has been found previously, that on the average, 11 per cent of the total number of mitochondria was present in the isolated nuclear fraction (10). The very low level of enzymatic activity actually observed in the mitochondrial fraction indicated that the contaminating mitochondria in the nuclear fraction did not significantly influence the 5′ nucleotidase activity of this fraction.

TABLE I

Distribution of 5′ Nucleotidase Activity in Cell Fractions Isolated from Rat Liver Homogenates

Liver fractions	Substrates					
	5′ Adenylic acid			5′ Inosinic Acid		
	Activity*	Percentage	Specific activity	Activity*	Percentage	Specific activity
H	1.03 ±0.20	100	0.35 ±0.06	0.51 ±0.15	100	0.15 ±0.03
N	0.41 ±0.05	40.2 ±7.4	0.65 ±0.15	0.24 ±0.05	47.1 ±5.5	0.27 ±0.03
M	0.14 ±0.04	13.7 ±3.3	0.21 ±0.02	0.05 ±0.02	9.8 ±5.4	0.06 ±0.03
Mc	0.39 ±0.12	37.9 ±3.8	0.67 ±0.17	0.17 ±0.06	33.3 ±9.3	0.31 ±0.12
S	0.09 ±0.02	9.2 ±3.3	0.08 ±0.01	0.05 ±0.03	9.8 ±5.3	0.03 ±0.01
Recovery		101.0 ±7.9			100.0 ±9.8	

* The activity is expressed as micromoles of inorganic phosphorus liberated per minute per 100 mg. of tissue or tissue equivalents. The values are the mean of 6 assays with the standard deviations.

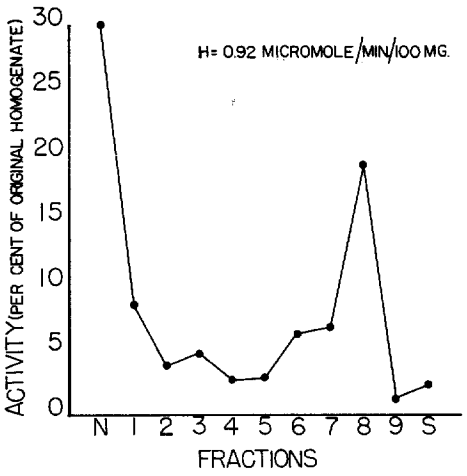

FIG. 1. Intracellular distribution of 5′ nucleotidase in cell fractions isolated from rat liver homogenates prepared in 0.88 M sucrose.

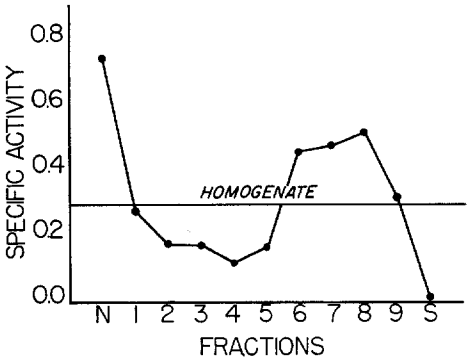

FIG. 2. Specific activity of 5′ nucleotidase in cell fractions isolated from rat liver homogenates prepared in 0.88 M sucrose.

Expressing the results as specific activities of 5′ nucleotidase in liver fractions, *i.e.* activities per mg. of nitrogen, further emphasizes the above conclusions. The specific activities of the nuclear and microsomal fractions (Table I) were approximately twice that of the homogenate. The 5′ nucleotidase activity thus appears to be especially concentrated in these fractions. The specific activities in the mitochondrial fraction and supernatant fluid, on the other hand, was much lower

than that of the whole homogenate, suggesting negligible enzyme activities in these fractions.

Fig. 1 shows the 5′ nucleotidase activity of a large number of cellular fractions isolated from homogenate prepared in 0.88 M sucrose (7). The whole homogenate showed the same activity whether it was prepared in 0.25 M or 0.88 M sucrose, the activities being respectively 1.03 and 0.92 μmole per minute per 100 mg. of fresh tissue. The results, presented in Fig. 1 (mean of two different assays), show that 5′ nucleotidase is mainly found in the nuclear fraction and in fraction 7 and 8, which correspond to the microsomal fraction obtained by the classical method of differential centrifugation (6). This confirms the results presented in Table I.

Fig. 2 shows the specific activity of 5′ nucleotidase in the eleven cellular fractions isolated from homogenates prepared in 0.88 M sucrose. The nuclear fraction had a specific activity of 2.5 times that of the homogenate, and the fraction 8 (microsomes) one of 1.7. These values compare well with those calculated for the nuclear and microsomal fractions isolated by the classical methods of differential centrifugation (Table I). Fractions 1 to 4, which correspond to the mitochondrial fraction (7), and the supernatant fluid had specific activities lower than that of the homogenate, confirming the data obtained for mitochondrial fraction and supernatant fluid isolated from homogenates prepared in 0.25 M sucrose (Table I).

The intracellular distribution of 5′ nucleotidase as determined by differential centrifugation is thus in accordance with the results obtained by histochemical means. In both cases the enzymatic activity was found in the nucleus and in the cytoplasm of liver cells (5). The present biochemical analysis further revealed that the cytoplasmic activity is due mainly to the presence of 5′ nucleotidase in the microsomes.

Extraction Assays of 5′ Nucleotidase from Sedimentable Cell Fractions.—Rosenthal *et al.* have reported that arginase can be extracted from nuclear and microsomal fractions with solutions containing Mg^{++} ions at concentrations equal to or lower than those found in the tissue. These authors concluded from this and other evidence that arginase actually belongs to the soluble fraction of the cell as the other enzymes of the arginine condensing system (11).

Since most enzymes of the purine catabolyzing system have been found previously in the soluble fraction of liver cells (12, 13), it was thought that the peculiar distribution of 5′ nucleotidase might represent a case similar to that of arginase.

TABLE II

Extraction of 5′ Nucleotidase from the Sedimentable Fraction of Rat Liver Homogenates

No. of assays	H	Sediment	Sediment washings	S*	Percentage recovery	Activity retained
						per cent
4‡	0.75 ±0.14	0.69 ±0.12	0.08 ±0.03	0.02 ±0.01	105.3 ±11.5	89.3 ±5.7
3§	0.75 ±0.03	0.69 ±0.13	0.03 ±0.02	0.04 ±0.01	101.3 ±5.9	96.0 ±3.2

* Supernatant obtained by centrifugation of the original homogenate at 140,000 *g* during 30 minutes.

‡ The homogenate was prepared in 0.25 M sucrose, and the sediment was washed twice with 0.25 M sucrose or 0.25 M sucrose containing 10 milliequivalents of $MgCl_2$.

§ The homogenate was prepared in 0.25 M sucrose containing 10 milliequivalents of $MgCl_2$, and the sediment was washed twice with 0.25 M sucrose or 0.25 M sucrose containing 10 milliequivalents of $MgCl_2$.

TABLE III

Extraction of 5′ Nucleotidase from the Nuclear and Microsomal Fractions of Rat Liver Homogenates

No. of assays	N	Sediment washings	Activity retained	Mc	Sediment	Activity retained
			per cent			
3*	0.44 ±0.01	0.08 ±0.08	84.6 ±15.1	0.22 ±0.03	0	100

* The nuclear and microsomal fractions isolated from an homogenate prepared in 0.25 M sucrose were washed twice with a solution of 0.25 M sucrose containing 10 milliequivalents of $MgCl_2$.

Tables II and III summarize the results obtained on the extraction assays carried out on the sedimentable fractions of the cell. Table II shows that the sediments isolated from homogenates prepared in 0.25 M sucrose alone or 0.25 M sucrose containing 10 milliequivalents of $MgCl_2$ retained practically all their enzymatic activity, even after two successive washings with sucrose alone or sucrose containing the electrolyte. In two different series of assays (Table II), the sedimentable fraction of the cell retained 89.3 and 96

per cent of the original homogenate activity. Similar results were obtained when the extraction was carried out on isolated nuclear and microsomal fractions (Table III). The nuclear fraction retained 84.6 per cent of the activity, and the microsomal fraction 100 per cent. These results strongly suggest that, unlike the other enzymes of the purine catabolizing system, 5′ nucleotidase is actually present in the nuclear and microsomal fractions.

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