Subcellular Distribution and Activity in Different Rat Tissues of a Deoxyinosine-Activated Nucleotidase*

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A nucleotidase (EC 3.1.3.31) isolated previously from rat liver cytosol was specifically measured in 14 different rat tissues and in subcellular fractions of liver and spleen, taking advantage of the stimulation exerted on it by deoxyinosine. The intracellular distribution studies showed that the enzyme is located almost entirely in the soluble cytoplasm except for the possible presence of 1-2% of the enzyme in the nucleus. The enzyme was present in various amounts in all the tissues studied. Spleen, thymus and intestinal mucosa showed higher specific activities than any other tissue. On a per cell basis spleen, liver and intestinal mucosa had the highest enzyme activity, whereas bone marrow, brain, thymus, heart and skeletal muscle had activities in the lower range. The results may suggest that the enzyme plays a role in the recovery of endogenous nuclear material for nucleic acid synthesis.

Previous studies (Fritzson & Smith, 1971) have shown the presence in rat liver of a nucleotidase (EC 3.1.3.31) that hydrolyses certain 5'-deoxyribo-nucleotides as well as various 3'-nucleotides of both the ribo and deoxyribo series. The activity towards the 3'-nucleotides was stimulated about threefold by deoxyinosine and deoxyguanosine, and to a smaller extent by other deoxyribonucleic acid constituents (Fritzson, 1972, 1973). The activation exerted by deoxyinosine provides a convenient means of specific measurement of the enzyme in crude tissue preparations. So far the enzyme has only been studied in the cytosol of rat liver. In the present work the intracellular location of the enzyme has been further investigated and its distribution in a wide variety of rat tissues explored.

Materials and Methods

Materials

Nucleosides, nucleotides, coenzymes, calf thymus DNA, bovine serum albumin, xanthine oxidase and yeast alcohol dehydrogenase were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Dithiothreitol (Cleland's reagent) was from Calbiochem (San Diego, Calif., U.S.A.).

Tissue preparations

Male Wistar rats, about 60 days old and weighing about 200g, were used. Liver homogenization was

* The enzyme is activated by several deoxyribonucleic acid constituents, but deoxyinosine is the most effective activator, and for simplicity and to distinguish the enzyme from other nucleotidases it has been termed deoxyinosine-activated nucleotidase.

carried out in 0.25M-sucrose for about 30s with a Teflon-glass homogenizer of the Potter-Elvehjem type having a clearance of 0.41mm, as described previously (Fritzson, 1967). For homogenization of the other tissues an all-glass homogenizer having a clearance of 0.23mm was used. The particle-free supernatant (cytosol) was prepared by centrifugation for 1h at 105000g.

The intestinal mucosa was obtained by scraping the washed intestine with a scalpel. Bone-marrow cells were flushed from tibiae of the rats. Blood was collected after decapitation, and 0.2vol. of 0.25M-sucrose containing 75mM-EDTA was added to prevent clotting. Platelets and cells were separated from plasma by centrifugation for 10min at 10000g and 0°C.

Preparation of subcellular fractions

For subcellular fractionation of liver 1mM-EDTA was included in the homogenization medium (Beaufay *et al.*, 1954). The nuclear fraction was prepared by centrifugation at 600g for 10min and was further purified by sedimentation through 2.3M-sucrose (Blobel & Potter, 1966). Fractionation of the cytoplasm was accomplished by centrifugation at 3300g for 10min to sediment the mitochondria, at 12500g for 20min to sediment the lysosomes, and at 105000g for 60min to sediment the microsomal fraction.

Subcellular fractions from spleen were prepared essentially as for liver, except that 0.25M-sucrose was replaced by 0.2M-KCl (Bowers & de Duve, 1967). The mitochondria and lysosomes were sedimented together by centrifugation at 20000g for 5min (Bowers, 1974).

Enzyme assays

The nucleotidase activity was measured with Urd-3'-P as substrate as described previously (Fritzson & Smith, 1971), except that the total volume of the incubation mixture was 250 μ l. In crude enzyme preparations the rate of dephosphorylation of 3'-UMP was determined in the presence and absence of 3 mM-deoxyinosine, and the nucleotidase activity was calculated as the difference between the two values divided by 2.05. This method is based on the finding that 3 mM-deoxyinosine increases the activity of the pure enzyme by 205 % (Fritzson, 1973). No significant splitting of deoxyinosine occurred during the nucleotidase measurements, as revealed by analysing 50 μ l portions of the reaction mixtures for hypoxanthine by means of xanthine oxidase (Kalckar, 1947). The nucleotidase activity determined by this method was constant with time and proportional with the amount of tissue preparation within the ranges tested (up to 30 min and up to 10 units of enzyme).

Lactate dehydrogenase (EC 1.1.1.27) was determined as described by Wroblewski & La Due (1955).

One unit of enzyme is defined as that amount which produces 1 nmol of product/min under the given assay conditions. Specific activity denotes units per mg of protein.

Assay of protein and DNA

Protein was determined by the method of Lowry *et al.* (1951) with crystalline bovine serum albumin as the standard. Protein samples that contained substances which interfere with the assay, were dialysed

as described previously (Fritzson & Smith, 1971). Nucleic acids were extracted and DNA was measured by the method of Burton (1956), with calf thymus DNA as standard.

Results

Intracellular location of the enzyme

Table 1 shows a typical result of determinations of the activity of the nucleotidase and the cytoplasmic marker enzyme lactate dehydrogenase in different subcellular fractions from liver and spleen. It can be seen that the activity of the nucleotidase is small in all the particulate fractions compared with the activity in the cytosol. The percentage activity of the nucleotidase in the different particulate fractions is not significantly higher than that of lactate dehydrogenase except for the liver nuclear fraction (N_1) sedimentable in 2.3 M-sucrose (purified nuclei) which shows a nucleotidase activity that is more than 100 times higher than that of lactate dehydrogenase. This difference has been consistently observed in repeated experiments and may be taken to indicate that a certain fraction of the nucleotidase is localized in the nucleus. The finding that in spleen the percentage nucleotidase activity of purified nuclei is only twice the value for lactate dehydrogenase may be ascribed to the difficulties involved in preparing the particulate fractions from this organ due to extensive agglutination. This is reflected in the high percentage protein content of the nuclear fractions N_1 and N_2 compared with the liver values. The total nucleotidase activity of the purified nuclei from both liver and spleen is approx. 1.5 % of the total activity of the homogenate.

Table 1. Intracellular distribution of nucleotidase and lactate dehydrogenase in liver and spleen

Subcellular fractions were prepared from approx. 2 g of tissue and assayed for enzyme activities as described in the Materials and Methods section. The presence of 1 mM-EDTA in the tissue homogenates and the use of Triton X-100 (0.1 %) in the incubation mixture for solubilization of mitochondrial and lysosomal enzymes did not significantly influence enzyme assays. The absolute values for enzyme activities in total homogenate are expressed in units, and protein is given in mg. The enzyme activities and the protein content in the subfractions are expressed as percentage of the total in the whole homogenate. N_1 and N_2 are obtained from the nuclear fraction by centrifugation through 2.3 M-sucrose; N_1 denotes the sedimentable and N_2 the non-sedimentable fraction. M = mitochondrial fraction, L = lysosomal fraction, P = microsomal fraction, and S = cytosol. Spleen M and L were isolated as a single fraction.

Enzyme	Absolute values of total homogenate (units)	Enzyme activities						
		Percentage values						Recovery
		N_1	N_2	M	L	P	S	
Liver								
Nucleotidase	724	1.4	2.8	0.6	0.7	0.4	89.6	95.5
Lactate dehydrogenase	1299×10^6	<0.01	2.0	0.1	0.1	1.8	77.9	81.9
Protein	375	6.5	16.1	16.0	9.4	15.0	31.5	94.5
Spleen								
Nucleotidase	6908	1.7	3.6	0.7	0.3	67.2	73.5	
Lactate dehydrogenase	34.1×10^6	0.9	4.1	0.9	1.2	68.0	75.1	
Protein	162	14.2	30.0	10.0	6.4	36.0	96.6	

Table 2. *Distribution of the nucleotidase in rat tissues*

The cytosol was prepared from the tissue homogenates and assayed for nucleotidase activity as described in the Materials and Methods section. Enzyme values are means $\pm$ S.E.M. of the numbers of experiments indicated in parentheses.

Tissue	Enzyme activity		
	(units/g of tissue)	(units/mg of soluble protein)	(units/mg of DNA)
Spleen	3360 $\pm$ 240 (7)	66 $\pm$ 3 (6)	168 $\pm$ 4 (4)
Thymus	1400 $\pm$ 60 (4)	51 $\pm$ 4 (6)	42 $\pm$ 6 (4)
Small-intestinal mucosa	1200 $\pm$ 60 (5)	32 $\pm$ 2 (6)	151 $\pm$ 12 (4)
Bone marrow	—	16 $\pm$ 3 (10)	9 $\pm$ 1 (4)
Pancreas	600 $\pm$ 52 (5)	13.1 $\pm$ 0.6 (9)	87 $\pm$ 12 (6)
Kidney	518 $\pm$ 55 (4)	9.7 $\pm$ 0.4 (7)	93 $\pm$ 14 (5)
Lung	462 $\pm$ 36 (6)	8.3 $\pm$ 0.7 (6)	140 $\pm$ 31 (5)
Testis	356 $\pm$ 39 (3)	14.2 $\pm$ 1.1 (4)	105 $\pm$ 11 (4)
Liver	333 $\pm$ 11 (25)	5.2 $\pm$ 0.2 (36)	117 $\pm$ 8 (16)
Heart	85 $\pm$ 17 (4)	3.0 $\pm$ 0.6 (7)	59 $\pm$ 13 (6)
Brain	74 $\pm$ 14 (4)	3.4 $\pm$ 0.4 (6)	26 $\pm$ 4 (5)
Skeletal muscle	44 $\pm$ 10 (5)	1.4 $\pm$ 0.3 (5)	84 $\pm$ 15 (4)
Adipose tissue	13 $\pm$ 3 (4)	5.3 $\pm$ 0.5 (4)	38 $\pm$ 6 (3)
Blood	—	0.13 $\pm$ 0.02 (4)	—
Blood cells	—	1.9 $\pm$ 0.3 (4)	—
Blood plasma	—	$\leq$ 0.03 (6)	—

Tissue distribution of the enzyme

Table 2 shows the activity of the nucleotidase in a number of different rat tissues, calculated per g of tissue, per mg of soluble protein, and per mg of DNA. It can be seen that the enzyme is present in all the tissues studied except for blood plasma. The activity per g of tissue shows that the enzyme is particularly abundant in spleen, thymus and intestinal mucosa. Pancreas, kidney, lung, testis and liver occupy an intermediate position. Heart, brain, skeletal muscle, adipose tissue and blood cells constitute a group with low enzyme activity. The distribution pattern of the enzyme is almost the same when the activity is calculated per mg of soluble protein. Bone marrow belongs to the group of tissues with intermediate activity. Liver, which has so far been used for purification and studies of the properties of the enzyme (Fritzson & Smith, 1971; Fritzson, 1972, 1973), has a specific activity in the low activity range.

The enzyme activity per mg of DNA may give a relative measure of the per cell activity of the tissues. The cellular complement of DNA is about 6.7 pg in rat somatic tissues except for in the liver which has a value of 9.4 pg (Vendrey, 1955). Taking this higher DNA value into account the relative per cell enzyme value of the liver will be 164. It is thus indicated that on a per cell basis liver is a high-activity tissue together with spleen and intestinal mucosa. Thymus, on the other hand, has an activity about four times lower than that of the spleen. Bone marrow and brain have the lowest activities of all the tissues studied, and adipose tissue has a value similar to that of brain.

Also heart and skeletal muscle are tissues with enzyme activities in the lower range.

The fact that the assay of the nucleotidase activity of the different tissues was based on the activation exerted by deoxyinosine, a property which is not exhibited by any other known nucleotidases, clearly indicates the identity of the enzymes in the different tissues. The identity of the spleen enzyme and the previously investigated liver enzyme (Fritzson & Smith, 1971) was further established by their essentially identical purification patterns, similar molecular weights determined by gel filtration on Sephadex G-100, relative activity toward 11 different nucleotides, stimulating effects of 8 nucleosides and nucleotides, Mg^{2+} requirements, pH optima, as well as K_m values for 3'-UMP and dTMP.

Discussion

The finding that the nucleotidase is widely distributed in the different rat tissues suggests that it has a general and possibly essential function in cell metabolism. Its intracellular location is mainly in the cytosol. Indications were also obtained by comparing the intracellular distribution of the nucleotidase and the cytoplasmic marker enzyme lactate dehydrogenase that small amounts (1–2%) of the nucleotidase may be situated in the nucleus. The possibility cannot be excluded, however, that the small activity is due to artificial adsorption of the enzyme on nuclear material during homogenization as a result of disruption of intracellular membranes.

The observation that the enzymes from proliferating tissues such as spleen, thymus and intestinal mucosa had the highest specific activity may suggest that a high enzyme activity is related to active DNA synthesis. However, the fact that bone marrow, which is a rapidly proliferating tissue, showed low activity speaks against a relationship between the enzyme activity and the rate of DNA synthesis. This conclusion is in accordance with results obtained previously with regenerating rat liver (Fritzson, 1967) and parasynchronously growing mouse fibroblasts (Magnusson, 1971), although in these studies a less specific enzyme assay based on high concentrations of dTMP as substrate, was used. The results with regenerating liver have been confirmed (Tjernshaugen & Gautvik, 1975) by the specific enzyme test described in the present paper. The possibility exists, however, that the nucleotidase activity per cell is related to the turnover rate of nucleic acids. Thus spleen and intestinal mucosa contain cells with a short lifespan (Jandl *et al.*, 1965; Leblond & Stevens, 1948), and both have a high activity per cell, whereas tissues with fixed cell populations such as brain, heart and skeletal muscle have enzyme activities in the lower range. Should such a relationship exist, it is tempting to speculate that the nucleotidase plays a role in re-utilization of endogenous nuclear material. However, at the present stage of research the validity of this interpretation is uncertain because the actual rates of DNA and RNA turnover in the different tissues are largely unknown.

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