

Short Communications

Deoxyinosine-activated Nucleotidase Purified from Rat Spleen. Evidence for Its Identity with Liver Enzyme

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Previous studies¹ have shown the presence in rat liver of a nucleotidase (nucleotide phosphohydrolase, EC 3.1.3.31), which catalyzes the dephosphorylation of certain deoxyribonucleoside 5'-phosphates as well as various ribo- and deoxyribonucleoside 3'-phosphates. This enzyme does not fit in all aspects with the description given at this entry in the catalogue of enzyme nomenclature (see Ref. 3). The activity toward the 3'-nucleotides was increased 225 % by deoxyinosine, and activation was also achieved by certain other deoxyribonucleosides and their 5'-monophosphates.^{2,3} The enzyme was shown to be located in the soluble space of liver cells⁴ and was different from the acid 5'(3')-nucleotidase found in rat liver lysosomes⁵ and in postmicrosomal supernatant of rat spleen and liver.⁶ Using a specific enzyme assay based on deoxyinosine activation, the nucleotidase was shown to be present in a large number of tissues, and was particularly abundant in the spleen, which had a specific activity 10–20 times higher than that of liver.⁴ The regulation by nucleic acid constituents and the variations in activity observed during cell growth have led to speculations about the role of the enzyme in metabolism.^{7,8} The high activity of deoxyinosine-activated nucleotidase in lymphoid tissues has prompted further studies on the possible function of the enzyme in such tissues. The present work describes purification of the spleen enzyme and the comparison with the enzyme from liver.

Twelve male Wistar rats weighing approximately 200 g were used in the enzyme purification experiments. The animals were stunned by a blow on the head and killed by decapitation. Spleens (total weight 8–10 g) were washed in cold 0.25 M sucrose and coarsely minced with scissors. Cold 0.25 M sucrose (about 3 ml/g of spleen) was added, and the mixture was homogenized with 12 pestle strokes in an all-glass homogenizer of the Potter-Elvehjem type (Kontes Glass Co., Vine-

land, N.J., U.S.A.). The pestle, which had a clearance of 0.23 mm, was rotated at a speed of 1600 rev min⁻¹ and was moved up and down (one stroke) 25 times per min. The resulting homogenate was diluted to approximately 8 ml/g with 0.25 M sucrose. The particle-free supernatant fluid (cytosol) was prepared by centrifugation in a Spinco Model L centrifuge at an average *g* value of 123 000 for 1 h and diluted with an equal volume of 0.04 M Tris-maleate buffer pH 6.3, giving fraction E 1.

Purification was carried out essentially as described by Fritzon and Smith¹ for the liver enzyme. The results of a typical purification experiment are shown in Table 1. It may be seen that the relative increase in specific activity during purification is about the same for spleen and liver¹ enzyme, but due to the far higher specific activity in spleen cytosol a correspondingly higher specific activity was obtained for the purified spleen enzyme. The 2800-fold purification indicates a high degree of purity of the spleen enzyme preparation though gel electrophoresis showed several protein bands. The purity is also indicated by the constant rate of activation by deoxyinosine (Table 1) and the constant ratio between the activities toward different substrates (3'-UMP, 5'-dTMP,* and *p*-nitrophenyl phosphate) during purification.

The molecular weight of the spleen enzyme was determined by gel filtration on Sephadex G-100, both with the crude supernatant and with the purified preparation (Fig. 1), the former as a control for possible changes in molecular size during purification. The column (2.5 × 38 cm) was equilibrated with a buffer mixture containing 50 mM Tris-HCl (pH 7.5), 10 mM (NH₄)₂SO₄, 1 mM MgCl₂, and 1 mM dithiothreitol. A 2 ml aliquot of enzyme fraction E 5 containing 0.1 mg/ml of bovine serum albumin was applied, and the column eluted with the buffer mixture. The fraction size was 4 ml and flow rate 16 ml/h. Enzyme activity was measured with 5'-dTMP as substrate as earlier described.¹ The column was calibrated with the following marker proteins: Cytochrome *c*, MW 12 400; horse myoglobin, MW 17 800; chymotrypsinogen A, MW 25 000; egg albumin, MW 45 000; bovine serum albumin, MW 67 000. The two sources of enzyme gave

* Nucleic acid constituents are abbreviated according to the CBN rules, see *Eur. J. Biochem.* 15 (1970) 203.

Table 1. Purification of deoxyinosine-activated nucleotidase from rat spleen. Determination of deoxyinosine-activated nucleotidase and definition of the enzyme unit are explained in an earlier publication.⁴ Specific enzyme activity is expressed as units/mg protein. Protein was quantified by the method of Lowry *et al.*⁹

Fraction	Total volume/ml	Total protein/mg	Total enzyme activity	Specific activity	Activation by 5 mM deoxyinosine
E 1 (123 000 g supernatant)	116	458	33.6	0.0734	195
E 3 (Supernatant after dialysis at pH 6.0) ^a	8.5	5.3	14.2	2.68	225
E 4 (Supernatant after calcium phosphate gel adsorption)	9.3	1.0	9.6	9.58	226
E 5 (DEAE-Sephadex A-50 eluate) ^b	19.2	0.09	6.0	67.6	223
E 6 (Sephadex G-100 eluate) ^b	36	0.026	5.3	204	225

^a Fraction E 2, ammonium sulfate precipitate, was not assayed for enzyme activity. ^b Peak fractions only.

virtually identical results: $41\,000 \pm 1\,800$ dalton from a total of 5 determinations, which is not significantly different from the 45 000 value reported for the rat liver enzyme.¹ Accordingly only one peak of activity was found when liver and spleen enzymes were run through the column together.

The pH-activity curves obtained with 5'-dTMP or 3'-UMP, the latter both in the absence of activator and in the presence of deoxyinosine, are shown in Fig. 2. They all agree well with those of the liver enzyme.^{1,8} Using the same substrates, both in the presence and absence of deoxyinosine, the curve for dephosphorylating activity against Mg(II) concentration (data not shown) was identical with

that for the liver enzyme.¹ Relative activity towards the substrates tested, including nucleoside 2'-,3'-, and 5'-monophosphates of all the common ribo- and deoxyribonucleosides, *p*-nitrophenyl phosphate, deoxythymidylyl 3'→5'-deoxythymidine, and deoxythymidine 3',5'-bisphosphate, was within $\pm 5\%$ of the values

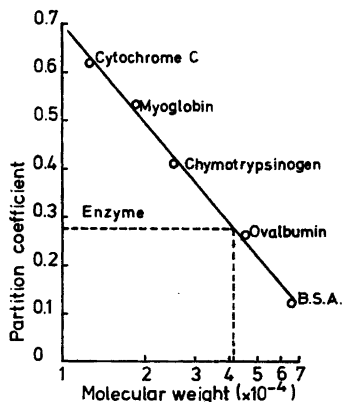


Fig. 1. Estimation of molecular weight by gel filtration on Sephadex G 100. Elution volume (V_e) for each protein species, total bed volume (V_t), and void volume (V_o) were determined and the partition coefficient $(V_e - V_o)/(V_t - V_o)$ calculated.

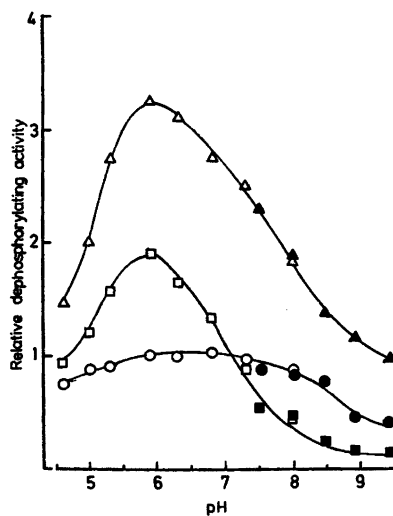


Fig. 2. The effect of pH on enzyme activity. Fraction E 5 was incubated with the stated substrates as described, except for the varying pH; open symbols mean Tris-maleate buffer, closed symbols, glycine buffer. pH was adjusted to the desired value with NaOH and measured at 20 °C in the complete incubation mixture. O, ●, 3'-UMP (2.5 mM); Δ , \blacktriangle , 3'-UMP (2.5 mM) + dIno (5.0 mM); \square , \blacksquare , 5'-dTMP (5.0 mM).

for the liver enzyme.¹ Also the same increase in the rate of 3'-nucleotide dephosphorylation was found with deoxyribonucleosides,² the activators being, in decreasing order of effectiveness, dIno > dGuo > dThd > dUrd > Ino. Slight inhibition by dAdo and dCyd was observed.

Apparent K_m -values were determined by double reciprocal plots of reaction velocity against substrate concentration.¹ The values obtained were 0.17 mM for 3'-UMP and 2.0 mM for 5'-dTMP. The corresponding values reported for the liver nucleotidase are 0.16 mM¹ and 2.2 mM,³ respectively.

The data presented seem to provide good evidence for the identity of the spleen and liver form of deoxyinosine-activated nucleotidase. Furthermore, the identical data obtained for molecular weight and catalytic properties with crude supernatant enzyme and final preparation show that the enzyme does not undergo significant changes during the purification procedure. The total activity of one liver is not very different from that of one spleen, but due to the much higher specific enzyme activity of spleen it has been shown to be superior to the liver as a source of deoxyinosine-activated nucleotidase by giving preparations of higher specific activity.

1. Fritzson, P. and Smith, I. *Biochim. Biophys. Acta* 235 (1971) 128.
2. Fritzson, P. *Eur. J. Biochem.* 27 (1972) 38.
3. Fritzson, P. *Eur. J. Biochem.* 38 (1973) 408.
4. Tjernshaugen, H. and Fritzson, P. *Biochem. J.* 154 (1976) 77.
5. Arsenis, C. and Touster, O. *J. Biol. Chem.* 243 (1968) 5702.
6. Tjernshaugen, H. *Biochem. J.* 169 (1978) 597.
7. Tjernshaugen, H. and Gautvik, K. M. *J. Cell. Physiol.* 88 (1976) 13.
8. Fritzson, P. *Adv. Enzyme Regul.* 16 (1978) 43.
9. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. *J. Biol. Chem.* 193 (1951) 265.

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