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Inhibition of 5′-Nucleotidase from Ehrlich Ascites-Tumour Cells by Nucleoside Triphosphates

By A. W. MURRAY and BEVERLY FRIEDRICHS
School of Biological Sciences, The Flinders University of South Australia, Bedford Park, S. Austral., Australia

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1. 5′-Nucleotidase activity was obtained in a soluble form after treatment of a particulate fraction from Ehrlich ascites-tumour cells with deoxycholate. The relative rates of hydrolysis of 6-thioinosine 5′-phosphate, UMP, AMP, CMP, GMP, IMP, xanthosine monophosphate, thymidine monophosphate and 2′,3′-AMP were 180, 129, 100, 93, 83, 79, 46, 41 and 3 respectively. 2. Values found for the Michaelis constant were: AMP, $67 \pm 12\,\mu$M; IMP, $111 \pm 8\,\mu$M; GMP, $93\,\mu$M. 3. ATP and thymidine triphosphate were competitive inhibitors of AMP hydrolysis (inhibitor constants 0·4 and $4{\cdot}8\,\mu$M respectively); UTP, GTP and CTP were mixed competitive and non-competitive inhibitors. Thymidine triphosphate was a competitive inhibitor of IMP hydrolysis (inhibitor constant $14{\cdot}4\,\mu$M) and ATP, UTP and GTP showed mixed competitive and non-competitive inhibition. 4. ATP, thymidine triphosphate, UTP, GTP and CTP did not completely inhibit hydrolysis of AMP, IMP and UMP; the concentrations of ATP required to inhibit AMP and IMP hydrolysis by 50% were 12 and $230\,\mu$M respectively. 5. Non-hyperbolic curves relating activity to UMP concentration were obtained in the presence and absence of triphosphates. 6. After fractionation on Sephadex G-200 columns a single peak of 5′-nucleotidase activity (particle weight 120000–125000) was obtained with AMP, IMP and GMP as substrates. UMP hydrolysis was catalysed by enzyme in this peak and in two slower peaks corresponding to apparent particle weights of 32000 and 16000; a single component (particle weight 120000), reacting with UMP and insensitive to UTP inhibition, was obtained when the column was eluted with buffer containing 1mM-UMP. 7. The possible significance of the results in the regulation of tumour-cell 5′-nucleotidase is discussed.

5′-Nucleotidases (5′-ribonucleotide phosphohydrolase, EC 3.1.3.5) have been isolated from a number of animal, plant and bacterial sources (for references see Ipata, 1968; Neu, 1967). Although there is little direct evidence it has been generally assumed that the rather non-specific hydrolysis of 5′-mononucleotides by nucleotidase from a particular source is catalysed by a single enzyme. Strict control of the activity of 5′-nucleotidases would be expected, either by compartmentation or regulation of enzymic activity, to prevent unregulated catabolism of nucleotides required for nucleic acid and coenzyme synthesis. There is little information available on the regulation of 5′-nucleotidase activity; Ipata (1967, 1968) has shown that the hydrolysis of AMP by a soluble 5′-nucleotidase from sheep brain is strongly inhibited by ATP, UTP and CTP and that the inhibitions are allosteric in nature.

This paper describes the preparation of 5′-nucleotidase in a soluble form from a particulate fraction of Ehrlich ascites-tumour cells and the inhibition of nucleotidase activity by nucleoside 5′-triphosphates.

EXPERIMENTAL

Substrates and inhibitors. [8-^{14}C]AMP, [8-^{14}C]GMP and [4-^{14}C]UMP were obtained from The Radiochemical Centre, Amersham, Bucks. [8-^{14}C]IMP was prepared by deamination of [8-^{14}C]AMP. [8-^{14}C]AMP ($16{\cdot}4\,\mu$moles; $10\,\mu$c) was dissolved in 0·5ml. of 2N-acetic acid. Sodium nitrite (48mg.) was dissolved in 0·3ml. of water and added dropwise to the solution of [8-^{14}C]AMP. After 6hr. at room temperature the mixture was applied as a band to Whatman 3MM paper and chromatographed in isobutyric acid–aq. 0·19N-NH_3 (661:339, v/v). After the paper had been dried the area containing [8-^{14}C]IMP (R_F 0·20) was washed with propan-2-ol, dried and eluted with water; the yield of [8-^{14}C]IMP was 65%. All of the above 8-^{14}C-labelled nucleotides were radiochemically pure after chromatography

in the above solvent and scanning in a gas-flow counter (Actigraph III; Nuclear–Chicago Inc., Des Plaines, Ill., U.S.A.).

The barium salt of 6 - thioinosine 5′ - phosphate (Montgomery & Thomas, 1961) was purified as described by Atkinson, Morton & Murray (1963); all other unlabelled nucleotides were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A.

Preparation of extract containing 5′-nucleotidase activity. The preparation of nuclear membranes from Ehrlich ascites-tumour cells containing 5′-nucleotidase activity was carried out essentially as described by Paterson & Hori (1963). Ehrlich ascites-tumour cells were collected by centrifuging for 5 min. at 2000*g* and washed once with cold 0·9% NaCl. The cells were suspended in 9 vol. of chilled water and after 10 min. at 4° lysis was completed with ten complete strokes with a homogenizer rotating at 500 rev./min. Portions (15 ml.) were layered over 12 ml. of 0·25 M-sucrose–2 mM-$CaCl_2$ and centrifuged at 600*g* for 10 min., and the residues washed once with the sucrose–$CaCl_2$ solution. Each residue was suspended in 5 ml. of 0·25 M-sucrose–2 mM-$CaCl_2$, homogenized for 5 sec. with an Ultra-Turrax homogenizer and centrifuged at 5000*g* for 10 min. The supernatant was centrifuged at 30000*g* for 20 min., and the residue was suspended in 8 ml. of 50 mM-tris–1% sodium deoxycholate (adjusted to pH 7·5 with HCl) and stirred for 60 min. at 4°. The suspension was centrifuged at 105000*g* for 30 min. and the supernatant, containing 5′-nucleotidase activity, was stored at −15°.

Assays of 5′-nucleotidase activity. (*a*) Separation of reaction products by chromatography. Solutions containing 20 μmoles of tris chloride, pH 7·5, were mixed with 0·05 ml. of enzyme preparation containing about 0·4 mg. of protein. After 2 min. at 37° a solution of [8-^{14}C]nucleotide (specific radioactivity 1 μc/μmole) in 0·05 ml. was added to give a nucleotide concentration of 0·32 mM (unless otherwise stated) in a final volume of 0·3 ml. After the required incubation time the reaction was stopped by the addition of 0·01 ml. of 10 N-HCl and the radioactivity associated with nucleoside was measured by liquid-scintillation counting after separation of the reactants by paper chromatography as described by Murray (1968). This assay was used as a routine unless otherwise stated in the text.

(*b*) Measurement of inorganic phosphate released by 5′-nucleotidase activity. Concentrations of reactants were the same as described above, but in a final volume of 1·2 ml. and with unlabelled 5′-nucleotides. The reactions were stopped by the addition of 0·04 ml. of 10 N-HCl and orthophosphate was measured after extraction into 2-methylpropan-1-ol as phosphomolybdate (Weil-Malherbe & Green, 1951). Blank assays were carried out in which nucleotide was omitted from the reaction mixture.

Assay of non-specific phosphatase activity. Phosphatase activity was measured with 2 mM-*p*-nitrophenyl phosphate–50 mM-tris chloride, pH 7·5. *p*-Nitrophenol liberated was measured from the extinction at 400 mμ in alkali (Andersch & Szcypinski, 1947).

Nature of the products of the 5′-nucleotidase reaction. Routine assays of 5′-nucleotidase activity with [8-^{14}C]-AMP, -GMP, -IMP and [4-^{14}C]UMP were carried out for 1 hr. as described above. The reaction mixtures were chromatographed in butan-1-ol–acetic acid–water (20:3:7, by vol.) and the nucleoside areas were cut from the dried chromatograms and eluted with water. After concentration the eluates were subjected to electrophoresis in 0·05 M-citrate–tris, pH 4·8, and the distribution of radioactivity was determined by scanning with a gas-flow counter.

Fractionation of 5′-nucleotidase on Sephadex columns. Samples of solubilized 5′-nucleotidase preparation (2 ml.) were passed through a column of Sephadex G-200 (17 cm. × 7 $cm.^2$; equilibrated with 0·05 M-tris chloride, pH 7·5) at 4° and elution was carried out at about 0·5 ml./min. with 0·05 M-tris chloride, pH 7·5. Fractions (about 5 ml.) were collected and assayed for 5′-nucleotidase activity with [8-^{14}C]-AMP, -IMP, -GMP and [4-^{14}C]UMP as substrates; nucleotide concentrations were 0·32 mM in each case. The column was calibrated with Blue Dextran, yeast alcohol dehydrogenase, bovine serum albumin and cytochrome *c* as described by Andrews (1965).

RESULTS

Assay of 5′-nucleotidase. Under standard assay conditions the rate of hydrolysis of AMP, GMP, IMP and UMP was constant for up to 30 min.; a 10 or 20 min. incubation time was used as a routine in these studies. Enzyme activity was also proportional to concentration of enzyme (with concentrations up to 0·95 mg. of protein/assay).

Analysis of the reaction products (see the Experimental section) showed that the only detectable radioactive products of the hydrolysis of [8-^{14}C]-AMP, -GMP, -IMP and [4-^{14}C]UMP were [8-^{14}C]-adenosine, -guanosine, -inosine and [4-^{14}C]-uridine respectively. Extracts were also assayed for adenosine kinase activity as described by Murray (1968); similar assays were carried out for uridine kinase. In the presence of 0·3 mM-adenosine and 2·5 mM-ATP the 5′-nucleotidase preparation catalysed the formation of 0·10–0·13 mμmole of AMP/min./mg. of protein at 37°. The 5′-nucleotidase activity with AMP varied in the range 1·9–4·4 mμmoles of adenosine formed/min./mg. of protein at 37° and a standard assay (see the Experimental section) resulted in the formation of about 7·6–15·6 mμmoles of adenosine. The resulting maximum concentration of adenosine in assay mixtures (25–51 μM) would be sufficient to give maximum rates of adenosine kinase activity (see Murray, 1968). However, the presence of adenosine kinase did not interfere with the 5′-nucleotidase assay as the addition of 5 mM-magnesium chloride to assay media inhibited the kinase activity by 72% (see Murray, 1968) but did not significantly increase the 5′-nucleotidase activity. No detectable uridine kinase activity was present in the 5′-nucleotidase preparations.

Adenylate kinase assays were carried out as described by Murray (1968). In the presence of 0·6 mM-[8-^{14}C]AMP and 0·28 mM-ATP less than 2% of the radioactivity was associated with ADP and ATP after 10 min. incubation. Similarly no kinase activity could be detected when thymidine tri-

phosphate or UTP replaced ATP or when [8-^{14}C]-GMP, -IMP or [4-^{14}C]UMP replaced [8-^{14}C]AMP.

The 5′-nucleotidase preparations catalysed the release of about 0·002mμmole of *p*-nitrophenol/min./mg. of protein from *p*-nitrophenyl phosphate at pH 7·5 (see the Experimental section). The small amount of non-specific phosphatase activity could be removed by passing 8ml. of the solubilized nucleotidase preparation through a column of DEAE-cellulose (9cm. × 1cm.^{2}; equilibrated with 0·05M-tris chloride, pH 7·5). The 5′-nucleotidase activity was eluted with 0·05M-tris chloride, pH 7·5, whereas the non-specific phosphatase activity remained on the column. The 5′-nucleotidase used in these experiments had not been further purified by fractionation on DEAE-cellulose columns.

Assays to detect the presence of phosphatases catalysing the hydrolysis of ATP were carried out by measuring the rate of release of radioactivity not adsorbed by charcoal from ATP labelled with ^{32}P in the γ-position as described by Murray & Wong (1967). In the presence of 0·5mM-ATP, the 5′-nucleotidase preparations catalysed the hydrolysis of about 0·02mμmole of ATP/min./mg. of protein.

In common with other 5′-nucleotidases (Heppel, 1961; Paterson & Hori, 1963; Song & Bodansky, 1966, 1967; Ipata, 1968), the tumour-cell enzyme was strongly inhibited by Zn^{2+}; in assays with 0·64mM-AMP addition of 1mM-zinc chloride resulted in 85% inhibition of AMP hydrolysis. The reaction of 0·32mM-AMP with 5′-nucleotidase was stimulated 3% and 15% by 0·1mM- and 1mM-*p*-chloromercuribenzoate respectively; 1mM-*p*-chloromercuribenzoate had no effect on the inhibition of AMP hydrolysis by triphosphates (see below). When stored at −15° the 5′-nucleotidase activity with all substrates (see below) was stable for at least 3 months with repeated thawing and freezing.

Catalytic properties of the 5′-nucleotidase preparation. Assays to determine the specificity of 5′-nucleotidase activity were carried out by measuring the release of inorganic phosphate (see the Experimental section); the final concentration of all substrates tested was 0·67mM. The relative activities found were: 6-thioinosine 5′-phosphate, 180; UMP, 129; AMP, 100; CMP, 93; GMP, 83; IMP, 79; xanthosine monophosphate, 46; thymidine monophosphate, 41; 2′,3′-AMP (mixed isomers), 3.

Values of the Michaelis constant, K_m, found by using the radioactive nucleotide assay were: AMP, 67 ± 12μM (six determinations); IMP, 111 ± 8μM (four determinations); GMP, 93μM (one determination); K_m values for UMP could not be determined, as non-hyperbolic responses to UMP concentration were obtained with this nucleotide (see below). The

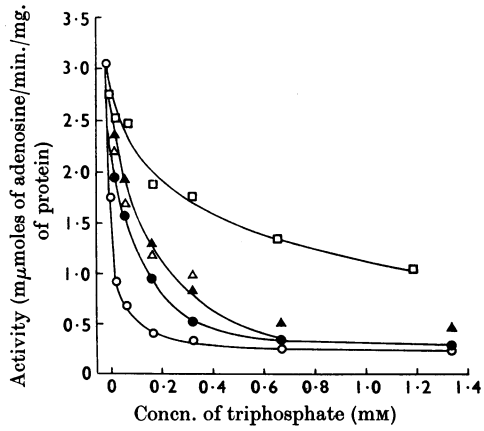

Fig. 1. Inhibition of AMP hydrolysis by triphosphates. Assays were carried out in the presence of 0·32mM-[8-^{14}C]-AMP and various concentrations of ATP (○), thymidine triphosphate (●), UTP (△), GTP (▲) and CTP (□).

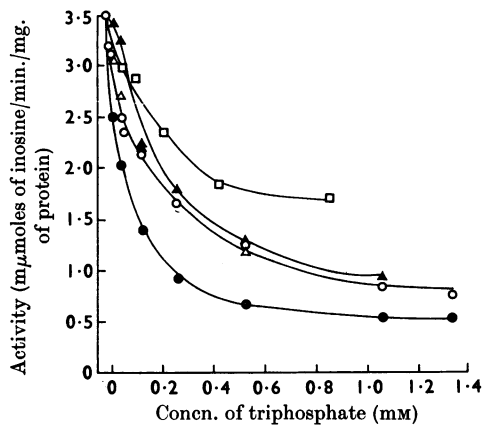

Fig. 2. Inhibition of IMP hydrolysis by triphosphates. Assays were carried out in the presence of 0·32mM-[8-^{14}C]-IMP and various concentrations of ATP (○), thymidine triphosphate (●), UTP (△), GTP (▲) and CTP (□).

hydrolysis of AMP was competitively inhibited by UMP and IMP (K_i 194 and 115μM) and reaction with IMP was competitively inhibited by AMP and UMP (K_i 69 and 160μM).

Inhibition of AMP and IMP hydrolysis by nucleoside triphosphates. The effects of increasing concentrations of triphosphates on the hydrolysis of AMP and IMP are shown in Figs. 1 and 2 respectively. It is clear that in each case the inhibitions increase to a finite value with increasing concentrations of triphosphates. In addition hydrolyses of AMP and of IMP differ in their

Table 1. *Concentrations of triphosphates required for 50% inhibition of 5′-nucleotidase with AMP or IMP as substrate*

The results were obtained from the experiments shown in Figs 1 and 2.

Triphosphate	Concn. of triphosphate for 50% inhibition (μM)	
	AMP hydrolysis	IMP hydrolysis
ATP	12	230
Thymidine triphosphate	65	80
UTP	100	270
GTP	120	270
CTP	470	680

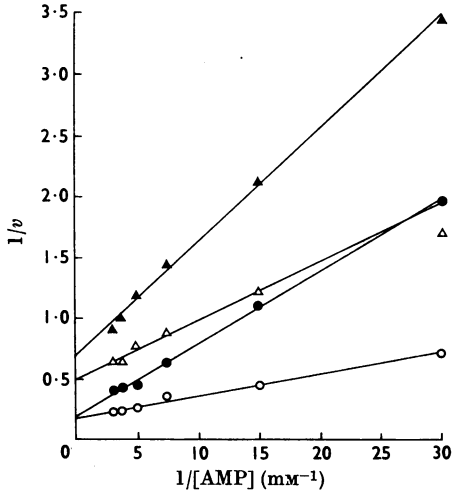

Fig. 3. Plots of $1/v$ against 1/[AMP] in the absence of inhibitor (○) and in the presence of 11 μM-ATP (●), 134 μM-GTP (△) or 134 μM-UTP (▲). v, mμmoles of adenosine formed/min./mg. of protein.

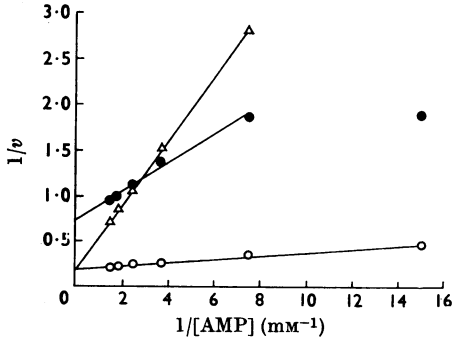

Fig. 4. Plots of $1/v$ against 1/[AMP] in the absence of inhibitor (○) and in the presence of 346 μM-CTP (●) or 67 μM-thymidine triphosphate (△). v, mμmoles of adenosine formed/min./mg. of protein.

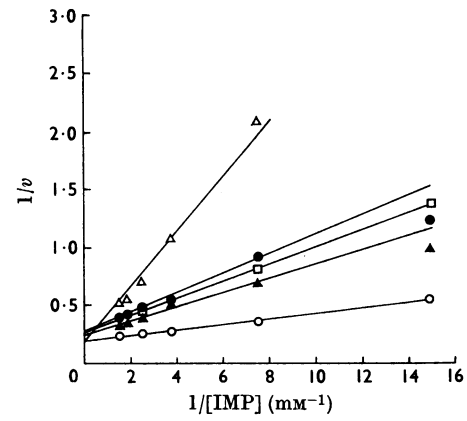

Fig. 5. Plots of $1/v$ against 1/[IMP] in the absence of inhibitor (○) and in the presence of 134 μM-ATP (●), 134 μM-thymidine triphosphate (△), 134 μM-GTP (▲) or 134 μM-UTP (□). v, mμmoles of inosine formed/min./mg. of protein.

sensitivity to triphosphate inhibition (see Table 1). This is most pronounced with ATP; in the presence of 0·32 mM-AMP or 0·32 mM-IMP 50% inhibition of hydrolysis was obtained with 12 μM- and 230 μM-ATP respectively. The inhibitory effects of the triphosphates were not additive with either AMP or IMP as substrate, and at saturating concentrations of inhibitors (1 mM) combinations of ATP, thymidine triphosphate, UTP and GTP gave the same inhibitions as the nucleotides tested separately. Phosphate at 5 mM did not inhibit the reaction with IMP and inhibited the reaction with AMP by 12% (AMP and IMP concentration 0·32 mM); this concentration of phosphate had no effect on the triphosphate inhibitions. It had previously been reported that the inhibition of brain 5′-nucleotidase (with AMP as a substrate) by ATP was overcome by low concentrations of phosphate (Ipata, 1968).

The effects of triphosphates on the hydrolysis of AMP and IMP are shown in Figs. 3, 4 and 5. ATP and thymidine triphosphate were competitive inhibitors of AMP hydrolysis (K_i 0·4 and 4·8 μM respectively); UTP, GTP and CTP were mixed competitive and non-competitive inhibitors. Hydrolysis of IMP was competitively inhibited by thymidine triphosphate (K_i 14·4 μM), and inhibition by ATP, UTP and GTP was of the mixed competitive and non-competitive type (Fig. 5). Ipata (1968) reported that inhibition by ATP, UTP and CTP of hydrolysis of AMP by brain 5′-nucleotidase was of the mixed competitive and non-competitive type.

Inhibition of UMP hydrolysis by nucleoside

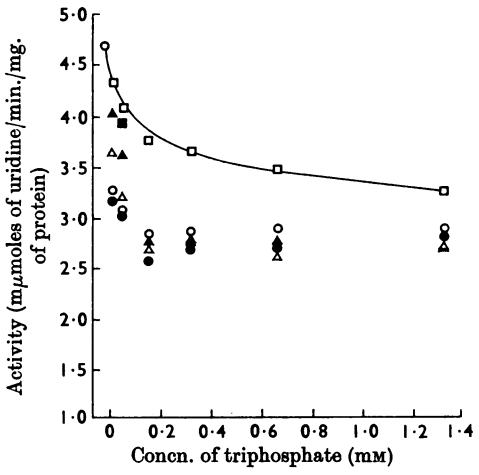

Fig. 6. Inhibition of UMP hydrolysis by triphosphates. Assays were carried out in the presence of 0·32 mM-[4-^{14}C]-UMP and various concentrations of ATP (○), thymidine triphosphate (●), UTP (△), GTP (▲) or CTP (□).

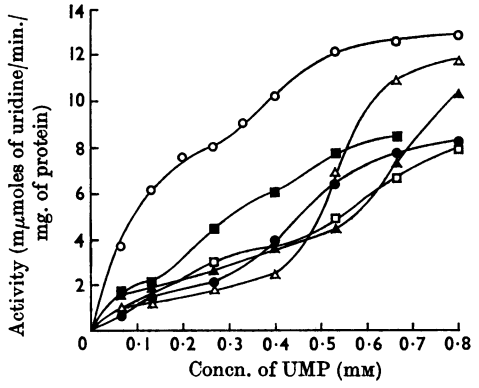

Fig. 7. Plots of the rate of UMP hydrolysis against UMP concentration in the absence of inhibitor (○) and in the presence of 67 μM-ATP (●), 67 μM-UTP (△), 67 μM-GTP (▲), 67 μM-thymidine triphosphate (□) or 87 μM-CTP (■).

triphosphates. The effect of increasing triphosphate concentration on the hydrolysis of UMP is shown in Fig. 6. As with AMP and IMP hydrolysis, the inhibitions were asymptotic to a finite value, but the degree of inhibition obtained (about 40%) was less. Combinations of inhibitors at 0·67 mM caused no further decrease in 5'-nucleotidase activity with this substrate. Phosphate at 5 mM did not inhibit the reaction with UMP and did not affect the triphosphate inhibitions. In separate experiments non-hyperbolic responses were obtained when the concentration of UMP was varied in the presence

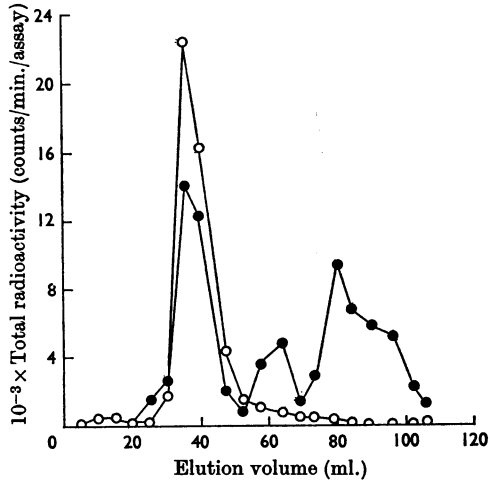

Fig. 8. Fractionation of 5'-nucleotidase on Sephadex G-200. The column was eluted with 0·05 M-tris chloride, pH 7·5, fractions (approx. 5 ml.) were collected as described in the Experimental section and 0·1 ml. samples were assayed with 0·32 mM-[8-^{14}C]AMP (○) or 0·32 mM-[4-^{14}C]-UMP (●) for 20 min. at 37°.

and absence of triphosphates (see Fig. 7). The shape of these curves suggested that increasing concentrations of UMP converted the 5'-nucleotidase reacting with UMP into a form less susceptible to triphosphate inhibition. To test this possibility a 2 ml. portion of solubilized 5'-nucleotidase preparation was fractionated on a Sephadex G-200 column as described in the Experimental section. The elution profiles for 5'-nucleotidase activity with AMP and UMP are shown in Fig. 8; the profiles for IMP and GMP hydrolysis were identical with that shown for AMP. The major peak of activity with both AMP and UMP corresponded to a particle weight of about 120 000; the two smaller peaks of UMP activity had particle weights of about 32 000 and 16 000. With some extracts a fourth peak of UMP activity was obtained with a particle weight of 66 000. In a separate experiment a 2 ml. portion of enzyme preparation was made 1 mM with respect to UMP and elution was carried out with 0·05 M-tris–1 mM-UMP (adjusted to pH 7·5 with hydrochloric acid). In this case only a single peak of UMP and AMP activity was obtained (Fig. 9) corresponding to a particle weight of about 125 000. In four experiments activity in this peak was not inhibited by UTP concentrations up to 0·95 mM (in the presence of 0·3 mM-UMP). However, one experiment showed a response to UTP concentration similar to that obtained with unfractionated enzyme (see Fig. 6), possibly resulting from re-equilibration of the different molecular forms hydrolysing UMP. This could not

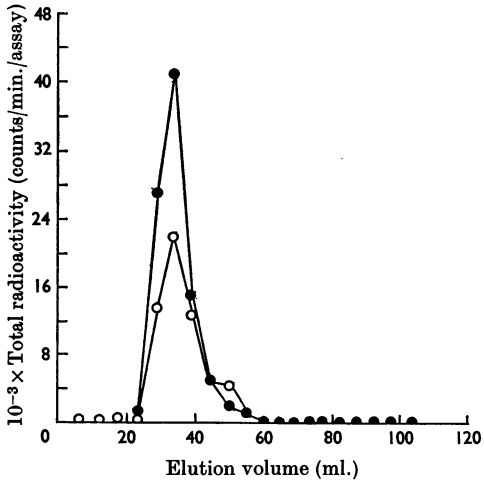

Fig. 9. Fractionation of 5′-nucleotidase on Sephadex G-200 in the presence of UMP. The column was eluted with 0·05 M-tris–1 mM-UMP (adjusted to pH 7·5 with HCl), fractions (approx. 5 ml.) were collected as described in the Experimental section and 0·1 ml. samples were assayed with 0·32 mM-[8-^{14}C]AMP (○) or 0·32 mM-[4-^{14}C]UMP (●) for 20 min. at 37°.

be tested by refractionating the individual peaks on the Sephadex columns as attempts to concentrate 5′-nucleotidase activity in peak tubes, by treatment with dry Sephadex G-25 or by dialysis filtration, resulted in loss of the enzymic activity. The molecular weight of the major 5′-nucleotidase component found here (120000–125000) is close to that determined by Ipata (1968) by Sephadex-gel filtration (134000) or sucrose-density-gradient centrifuging (142000) for sheep brain 5′-nucleotidase reacting with AMP.

DISCUSSION

The results reported in this paper suggest that hydrolysis of UMP by 5′-nucleotidase of Ehrlich ascites-tumour cells is regulated differently from hydrolysis of AMP and IMP. Initial experiments with increasing triphosphate concentrations on the rate of UMP breakdown showed that about 60% of the 5′-nucleotidase activity was not inhibited by triphosphates, either alone or in combination. This result would be expected if there were a single allosteric site for binding triphosphates resulting in only partial inhibition at saturating inhibitor concentrations. An alternative explanation would be the existence of different molecular species of 5′-nucleotidase that react with UMP and that have differing susceptibility to triphosphate inhibition. The latter possibility is supported by experiments demonstrating different molecular species after Sephadex fractionation and the presence of only one high-molecular-weight species on treatment with UMP. It is likely that the high-molecular-weight 5′-nucleotidase is less sensitive to triphosphate inhibition as indicated by direct measurement and by the response curves to UMP concentration in the presence of triphosphates (Fig. 7). However, the possibility remains that UMP acts to prevent the breakdown of enzyme into lower-molecular-weight components during fractionation on the Sephadex column.

Studies on brain 5′-nucleotidase (Ipata, 1968) indicated that inhibition by triphosphates was allosteric in nature. Thus with AMP as substrate sigmoidal response curves to increasing concentrations of ATP, CTP and UTP were found and combinations of triphosphates resulted in co-operative inhibition. In addition, inhibition by ATP and not that by CTP and UTP was overcome by inorganic phosphate; desensitization to ATP and UTP inhibition was obtained with *p*-chloromercuribenzoate. None of these effects was observed with 5′-nucleotidase from tumour cells, and the only evidence for the non-identity of substrate- and inhibitor-binding sites is the finite inhibitions obtained with increasing triphosphate concentration. As indicated above this could also be explained if a form of 5′-nucleotidase insensitive to triphosphate inhibition were present in the enzyme preparation.

In these studies ATP was a very strong inhibitor of AMP hydrolysis catalysed by 5′-nucleotidase (K_m/K_i ratio 168) and, if operating *in vivo*, this would mean that only small amounts of AMP could be degraded by this enzyme, unless there were a drastic fall in ATP concentration or compartmentation of ATP and enzyme occurred. Strong inhibitions of AMP hydrolysis in brain by ATP have been reported by Ipata (1968) and implied by the results of Burger & Lowenstein (1967) in heart and lung tissue. It may be significant that IMP hydrolysis is less sensitive to ATP inhibition than is AMP hydrolysis if the properties of the tumour-cell 5′-nucleotidase are similar to those of enzyme from other tissues. By the known pathways hydrolysis of IMP to inosine followed by phosphorolysis to hypoxanthine provides an essential link between purine nucleotides and bases required for oxidation to uric acid. Overgaard-Hansen (1965) showed that depletion of the adenine nucleotide pool after treatment of Ehrlich ascites-tumour cells with glucose or 2-deoxyglucose was accompanied by accumulation of inosine. No adenosine production was observed, although the intracellular AMP concentration remained at least as high as the IMP concentration for several minutes after addition of glucose or 2-deoxyglucose; thus even with lowered ATP concentrations (to about 30%

of the initial concentration) AMP hydrolysis was negligible. Similar results have been reported with Ehrlich ascites-tumour cells (Wu & Racker, 1959) and with Krebs ascites-tumour cells (McComb & Yushok, 1964). Burger & Lowenstein (1967) have shown that addition of ATP to extracts of rat heart decreased AMP hydrolysis to a greater extent than IMP hydrolysis. In addition, it has been reported (Mager, Hershko, Zeitlin-Beck, Shoshani & Razin, 1967) that rabbit erythrocytes have a rapid turnover of purine nucleotides, and further (Hershko, Razin, Shoshani & Mager, 1967) that several purine bases are incorporated into nucleotide material, but that the major base released by the erythrocyte is hypoxanthine. Mager *et al.* (1967) suggested that the erythrocyte may be involved as a carrier of purines to other tissues. These observations suggest that in some tissues hydrolysis of IMP occurs to a significant extent and this may be reflected in its decreased sensitivity to triphosphate inhibition.

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