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MEMBRANE MARKER ENZYMES: ISOLATION, PURIFICATION, AND PROPERTIES OF 5'-NUCLEOTIDASE FROM RAT CEREBELLUM

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SUMMARY

A 5'-nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) from rat cerebellum was purified 855-fold by $(NH_4)_2SO_4$ fractionation and column chromatography on Sephadex G-100 and G-200. The enzyme was activated by divalent cations with Mg^{2+}, Co^{2+}, and Mn^{2+} being the most efficient activators. The 5'-nucleotidase had an optimum pH of 6.8 $\pm$ 0.2. The relative rates of hydrolysis of 5'-AMP, 5'-UMP, 5'-CMP, 5'-GMP and 5'-IMP were 100, 46, 35, 17 and 9, respectively; there was no hydrolysis of 2'- or 3'-nucleoside monophosphates. Values for the Michaelis constant were 5'-AMP, 0.08 mM; 5'-UMP, 0.82 mM; 5'-CMP, 0.92 mM; 5'-GMP, 1.41 mM; and 5'-IMP, 2.1 mM. Using 5'-AMP as substrate the 5'-nucleotidase was inhibited by NaF, $HgCl_2$, $CuCl_2$, *p*-chloromercuribenzoate, and *p*-hydroxymercuribenzoate. The 5'-nucleotidase was inhibited by $5 \cdot 10^{-3}$ M thymidine, guanosine and inosine but not by uridine or adenosine. The concentrations of ATP, UTP, ITP, CTP and GTP required to inhibit the hydrolysis of 5'-AMP by 50% were 10, 68, 240, 250 and 720 μM, respectively.

INTRODUCTION

5'-Nucleotidase (5'-ribonucleotide phosphohydrolase, EC 3.1.3.5) has been identified and studied in a variety of tissues including bull semen[1], potato tubers[2], soybeans[3], soil bacteria[4], *Clostridium sticklandii*[5], rat liver[6–11], *Clostridium propionicum*[12], snake venom[13], *Escherichia coli*[14–16], calf intestine[17], sheep brain[18,19], human liver[20] chicken liver[21] and Ehrlich ascites tumor cells[22]. The enzymes in the mentioned studies differ in many instances with respect to substrate specificities, ionic cofactors, pH optimum, inhibitors, *etc.* Of particular importance is the subcellular localization of the 5'-nucleotidase. 5'-Nucleotidase has been referred to as a surface enzyme in *Escherichia coli*[14], a lysosomal enzyme in rat liver[9], plasma membrane of rat liver[7] and microsomes of rat liver[6]. Recently 5'-nucleotidase has been determined to reside almost exclusively in the plasma membrane of cells[7,23,24] and the enzyme has been used as a plasma membrane marker enzyme[25,26] for identification of plasma membrane frag-

ments in cell fractionation studies. Furthermore, 5'-nucleotidase has been described as a peak (as opposed to a continuous or step) enzyme in synchronized mouse leukemia cells, L5178Y (ref. 27). Since 5'-nucleotidase is present in cerebral cortices[28] and since it has been used therein as a plasma membrane marker enzyme (although it is present to a limited extent in microsomes)[28] it was deemed important to determine substrate specificity and properties of a purified brain 5'-nucleotidase. The present communication describes results of an investigation on 5'-nucleotidase activity in rat cerebellum.

MATERIALS AND METHODS

Materials

Substrates were purchased from Sigma Chemical Co. Enzyme grade $(NH_4)_2SO_4$ was purchased from Mann Chemicals Co. Distilled water was first deionized and then distilled in a glass still; all solutions were made with this water. Uniformly ^{14}C-labelled 5'-AMP, diammonium salt (specific activity 400 Ci/mole) was purchased from New England Nuclear. All nucleotides were tested for purity and found to be >98% pure.

Enzyme assay

The 5'-nucleotidase was assayed routinely in the following incubation mixture: 100 μmoles of Tris–maleate, 10 mM $MgCl_2$, 3 mM 5'-AMP and enzyme preparation in a total volume of 1.110 ml. The incubation mixture was incubated at 37° for 1 h in a Dubnoff metabolic shaker. The reaction was terminated by the addition of 1 ml of 10% trichloroacetic acid. The precipitated protein was removed by centrifugation at 2000 × *g* for 4 min, and an aliquot of the supernatant fluid was used for P_i determination by the method of FISKE AND SUBBAROW[29]. The 5'-nucleotidase activity was then based on the released P_i. In each series, blanks of substrate without enzyme and enzyme without substrate were incubated simultaneously and any P_i liberated subtracted from the assay in order to eliminate P_i present in the enzyme or P_i liberated by non-enzymatic hydrolysis of the substrate. All experiments were performed in duplicate.

Protein

Protein was determined according to the method of LOWRY *et al.*[30]. Crystalline bovine serum albumin was used as a standard.

Purification of 5'-nucleotidase from rat cerebellum

All steps of purification were carried out at 4° unless otherwise specified. Six independent preparations of enzyme were made. Fractions from the column were assayed for protein by the method of LOWRY *et al.*[30]. All 0.1% Triton X-100 was made in 0.1 M Tris–HCl buffer (pH 7.6). At several steps (Steps 2, 4 and 5 below), solutions were concentrated by lyophilization.

Step 1. Extraction. 18 cerebella were removed from rats (200–250 g) which had been starved for 16 h with water *ad libitum*. They were minced in 5 vol. of the 0.1% Triton X-100 solution and homogenized for 30 strokes in a Ten Broeck homogenizer.

Step 2. Centrifugation. This homogenate was then centrifuged at 40 000 × *g* for 10 min.

Step 3. $(NH_4)_2SO_4$ precipitation. To the supernatant was added sufficient $(NH_4)_2SO_4$, with stirring to make the solution 20% in $(NH_4)_2SO_4$. This was stirred at 4° for 30 min and then allowed to sit for 30 min at 4°. The suspension was centrifuged at 20 000 × *g* for 20 min. Both the supernatant and pellet were exhaustively dialyzed against the 0.1% Triton X-100 solution at 4°.

Step 4. Gel filtration on Sephadex G-100. A 10-ml sample (487 mg protein) from the 20% $(NH_4)_2SO_4$ dialyzed precipitate in the 0.1% Triton X-100 solution was applied to a Sephadex G-100 column (2.5 cm × 45 cm) packed in the same solution. The column was eluted with the same buffer and fractions of 5 ml were collected.

Step 5. Gel filtration on Sephadex G-200. A 5-ml sample (7 mg protein) of the peak from the previous step was applied to the Sephadex G-200 column (45 cm × 2.5 cm) packed in the 0.1% Triton X-100 solution. The column was developed with the same buffer and 3-ml fractions were collected.

Nature of product of rat cerebellum 5'-nucleotidase reaction

Assays were performed in the usual manner except 2 μCi of 5'-[^{14}C]AMP were added to the incubation. The supernatant after precipitation of the protein was chromatographed in 1-butanol–acetic acid–water (20:3:7, by vol.) and the nucleoside areas were cut from the dried chromatograms and eluted with glass-distilled water. The eluates were lyophilized and subjected to electrophoresis in 0.05 M citrate–Tris (pH 4.8) and the distribution of radioactivity was determined by cutting the strips and counting in a liquid scintillation counter by the procedure of MURRAY AND FRIEDRICHS[22].

Enzyme characterization

All experiments were performed with the purified enzyme; purified enzyme from several purification runs were pooled for the experiments.

TABLE I

PURIFICATION AND ISOLATION OF 5'-NUCLEOTIDASE ACTIVITY BY $(NH_4)_2SO_4$ PRECIPITATION AND COLUMN CHROMATOGRAPHY

All procedures were carried out at 0–4°. The experiments were repeated 5 times and were quantitatively and qualitatively reproducible. Procedures were performed as given in MATERIALS AND METHODS.

Step	*Total protein (mg)*	*Total activity (μmoles/h)*	*Recovery (%)*	*Specific activity (μmoles/h per mg protein)*	*Purification factor*
1. 0.1% Triton X-100 extract	4544	8000	100	1.8	1.0
2. 40 000 × *g* supernatant	2326	6210	78	2.7	1.5
3. 20% $(NH_4)_2SO_4$ precipitate dialyzed	487	5210	65	11.7	6.5
4. Sephadex G-100	7	5011	63	720	400
5. Sephadex G-200	3	4612	58	1540	855

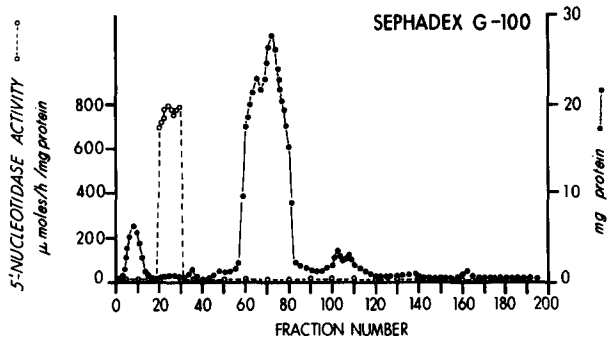


Fig. 1. Chromatography of the rat cerebellum 5'-nucleotidase activity on Sephadex G-100. Enzyme (5210 μmoles/h, 487 mg protein) in 10 ml of 0.1% Triton X-100 in 0.1 M Tris–HCl buffer (pH 7.6) was applied to the column (45 cm × 2.5 cm). Elution was with 0.1% Triton X-100 in 0.1 M Tris–HCl buffer (pH 7.6); 5-ml fractions were collected. All procedures were carried out in a jacketed column at 4°.

RESULTS AND DISCUSSION

Purification of 5'-nucleotidase from rat cerebellum

The purification scheme described above yielded a preparation purified 855-fold over the original homogenate. The scheme was based on centrifugation, $(NH_4)_2SO_4$ precipitation and column chromatography on Sephadex G-100 and Sephadex G-200. 58% of the total activity was recovered by these procedures (Table I). The highest purification step was achieved with the Sephadex G-100 column chromatography (Fig. 1). A further 2-*plus*-fold purification was achieved by the Sephadex G-200 column chromatography (Fig. 2). Repeated attempts to further purify the enzyme by column chromatography on DEAE-cellulose were unsuccessful.

pH optimum of the enzyme

The effect of pH on the catalytic activity of the 5'-nucleotidase of rat cerebellum

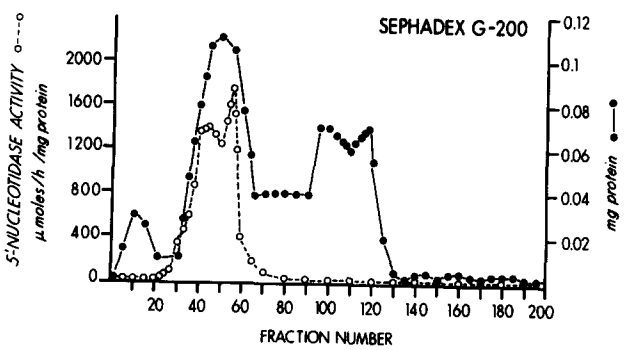
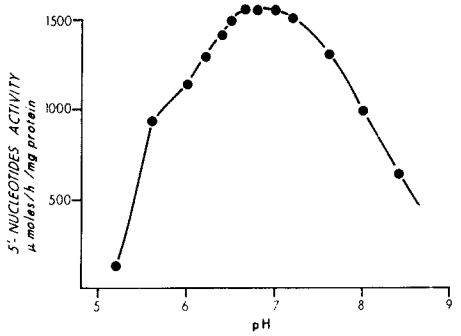


Fig. 2. Chromatography of the rat cerebellum 5'-nucleotidase activity on Sephadex G-200. Enzyme (5011 μmoles/h, 7 mg protein) in 5 ml of 0.1% Triton X-100 in 0.1 M Tris–HCl buffer (pH 7.6) was applied to the column (45 cm × 2.5 cm). Elution was with 0.1% Triton X-100 in 0.1 M Tris–HCl buffer (pH 7.6); 3-ml fractions were collected. All procedures were performed in a jacketed column at 4°.



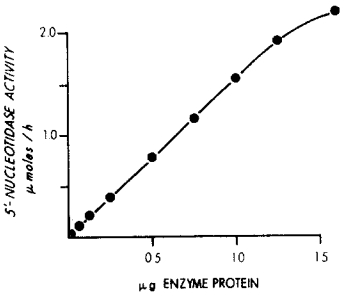


Fig. 3. Influence of pH on the activity of the rat cerebellum 5′-nucleotidase. Enzyme (1 μg), substrate, cofactor and buffer were incubated under standard conditions and the P_i released was determined as given in the text. The buffer was in all instances Tris–maleate buffer.

Fig. 4. Effect of enzyme concentration on the activity of the rat cerebellum 5′-nucleotidase. Incubation conditions with 5′-AMP as the substrate were as described in the text.

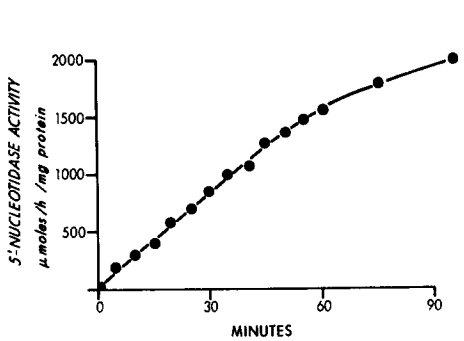
was studied using 5′-AMP as the substrate in Tris–maleate buffers ranging from pH 5.2 to 8.4. The pH activity profile shown in Fig. 3 indicates a pH optimum of 6.8 ± 0.2 for the enzyme. This pH optimum can be compared to that found for 5′-nucleotidase of rat liver microsomes[6] of pH 6.5–8.0, of *Clostridium propionicum*[12] of pH 6.0–9.2, of

TABLE II

EFFECT OF CATIONS ON ACTIVITY OF RAT CEREBELLUM 5′-NUCLEOTIDASE

Experiments were performed with 5′-AMP as substrate as described in the text. "Without $MgCl_2$" refers to experiments in which the indicated ion was substituted for the $MgCl_2$ in the assay. "With $MgCl_2$" refers to experiments in which the indicated ion was added in the presence of the 10 mM $MgCl_2$ normally in the assay. The activity of the 10 mM $MgCl_2$ addition to the "without $MgCl_2$" system was arbitrarily set to 100 and other values are activities relative to this activity. All solutions were made in 0.1 M Tris–HCl buffer (pH 7.6). Any balancing of assay volumes necessary was made with 0.1 M Tris–HCl buffer (pH 7.6).

Ion or addition	*Concn. (mM)*	*Without $MgCl_2$*	*With $MgCl_2$*
$MgCl_2$	1	33	100
	10	100	100
$CoCl_2$	1	32	100
	10	121	112
$CuCl_2$	1	7	72
	10	7	14
$FeCl_3$	1	0	90
	10	0	78
$HgCl_2$	1	0	11
	10	0	0
$PbCl_2$	1	8	92
	10	38	65
$MnCl_2$	1	41	110
	10	116	125
$CaCl_2$	1	30	100
	10	86	92
$CdCl_2$	1	8	89
	10	34	70
EDTA	10	2	7



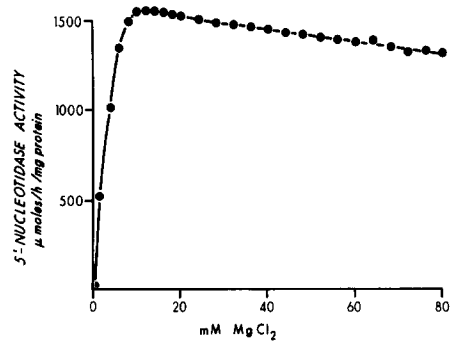


Fig. 5. Time-course of the P_i liberation from 5'-AMP. The enzyme (1 μg) and the substrate were incubated for various time intervals and the P_i released was determined as given in the text.

Fig. 6. Influence of $MgCl_2$ concentration on the activity of 5'-nucleotidase from rat cerebellum. Enzyme (1 μg), substrate, and buffer were incubated under standard conditions and the P_i released was determined as described in the text.

calf intestine[17] of pH 6.0–6.5, of sheep brain[19] of pH 6.5–7.5, of rat liver[11] of pH 6.3 and of chicken liver[21] of pH 6.5.

Effect of added enzyme protein

The activity of the rat cerebellum 5'-nucleotidase was linear with respect to enzyme protein up to 1.25 μg of enzyme protein as shown in Fig. 4.

Time-course

Fig. 5 show that the rate of P_i liberation from 5'-AMP is linear up to 60 min incubation and that the rate of hydrolysis decreases thereafter.

Effect of cations on rat cerebellum 5'-nucleotidase

At 10 mM, $MgCl_2$, $CoCl_2$, and $MnCl_2$ each activated the 5'-nucleotidase of rat cerebellum to about the same degree. $CaCl_2$ also activated the 5'-nucleotidase to 86% the rate with the $MgCl_2$ (Table II). $PbCl_2$ and $CdCl_2$ at 10 mM also slightly activated the rat cerebellum 5'-nucleotidase to 38 and 34% the activity with 10 mM $MgCl_2$. $FeCl_3$, $CuCl_2$ and $HgCl_2$ did not at all activate the 5'-nucleotidase at 1 or 10 mM. In the presence of 10 mM $MgCl_2$, 1 mM $HgCl_2$ inhibited the 5'-nucleotidase activity to 11% the control activity while 10 mM $HgCl_2$ completely inhibited the rat cerebellum 5'-nucleotidase activity. At 10 mM $CuCl_2$ the rat cerebellum 5'-nucleotidase activity was inhibited to 14% the control rate in the presence of 10 mM $MgCl_2$. EDTA at 10 mM almost completely abolished the rat cerebellum 5 -nucleotidase activity in the presence or absence of 10 mM $MgCl_2$. The rat cerebellum 5 -nucleotidase was highly dependent on $MgCl_2$ and was activated over a broad concentration range of $MgCl_2$ (Fig. 6). Other 5'-nucleotidases and the ion reported to activate them to the greatest extent include the following: rat liver[7,10,21], $Mg2^+$; chicken liver[21], Mg^{2+}; calf intestine[17], Mn^{2+}, Mg^{2+}, Co^{2+}; *E. coli*[15], Co^{2+}; *C. sticklandii*[5], Fe^{3+}; and rat liver lysosomal[9], none.

Analysis of reaction product

Analysis of the reaction product (see MATERIALS AND METHODS) showed that the

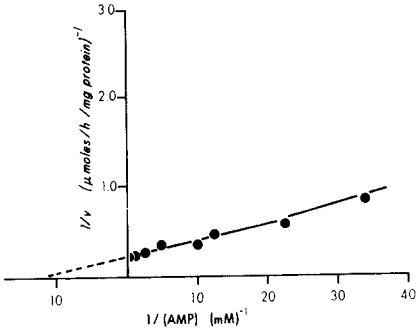


Fig. 7. Lineweaver–Burk plot of 5′-nucleotidase from rat cerebellum. Assay was carried out under the conditions described in the text.

TABLE III

SUBSTRATE SPECIFICITY OF RAT CEREBELLUM 5′-NUCLEOTIDASE

All experiments were performed as given in the text. For relative activity the substrates were all tested at 3 mM. Michaelis constants were determined from plots of $1/v$ *vs.* $1/[S]$ as given in Fig.7.

Substrate	*Relative activity*	K_m *(μM)*
5′-AMP	100	80
5′-UMP	46	820
5′-CMP	35	920
5′-IMP	9	2100
5′-GMP	17	1410
5′-ADP	0	—
5′-UDP	0	—
5′-CDP	0	—
5′-IDP	0	—
5′-GDP	0	—
5′-ATP	0	—
5′-UTP	0	—
5′-CTP	22	1210
5′-ITP	12	2040
5′-GTP	0	—
5′-dAMP	40	880
5′-dUMP	14	1900
5′-dIMP	4	3800
5′-dCMP	60	560
5′-dGMP	56	610
5′-dADP	0	—
5′-dUDP	10	2060
5′-dCDP	11	2010
5′-dGDP	0	—
5′-dATP	8	3080
5′-dUTP	8	3160
5′-dCTP	22	1190
5′-dGTP	6	3420
2′-AMP	0	—
3′-AMP	0	—
2′-CMP	0	—
3′-CMP	0	—
2′-GMP	0	—
3′-GMP	0	—
2′-UMP	0	—
3′-UMP	0	—

only detectable radioactive product of the hydrolysis of the 5′-[^{14}C]AMP was [^{14}C]-adenosine.

Effect of substrate concentration on enzyme catalysis

By varying substrate concentrations of 5′-AMP, a Lineweaver–Burk plot was obtained. The plot of $1/v$ *versus* $1/[S]$ showed a straight line relationship. The value for the apparent K_m as computed from this plot was 80 μM and that of v_{max}, 5.0 μmoles/mg protein per h (Fig. 7).

Substrate specificity of rat cerebellum 5′-nucleotidase

The enzyme had the highest relative activity with 5′-AMP as a substrate and also hydrolyzed other 5′-nucleotides; there was no activity with 2′- or 3′-nucleotides as substrates (Table III). The rat cerebellum 5′-nucleotidase did not have any activity with 5′-nucleoside diphosphates as substrates but had slight activity with 5′-deoxynucleoside diphosphates (5′-dUDP and 5′-dCDP). Two of the 5′-nucleoside triphosphates tested (5′-CTP and 5′-ITP) and all of the 5′-deoxynucleoside triphosphates tested (5′-dATP, 5′-dUTP, 5′-dCTP and 5′-dGTP) had activity with the purified rat cerebellum 5′-nucleotidase. The order of activity for 5′-nucleoside monophosphates as substrates for the purified rat cerebellum 5′-nucleotidase was 5′-AMP > 5′-UMP > 5′-CMP > 5′-GMP > 5′-IMP. The order of activity for the 5′-deoxynucleoside monophosphates was 5′-dCMP > 5′-dGMP > 5′-dAMP > 5′-dUMP > 5′-dIMP. Activity of 5′-dCMP and 5′-dGMP as substrates was higher than 5′-CMP and 5′GMP, respectively. Calculation of apparent K_m values indicated the Michaelis constant for 5′-AMP of 80 μM was the lowest; relatively low K_m values were obtained with 5′-UMP, 5′-CMP, 5′-dAMP, 5′-dCMP and 5′-dGMP (Table III). The specificity of the enzyme is similar to that found in *Bothrops atrox* in which 5′-AMP was the most active substrate[13]. The 5′-nucleotidase from *E. coli* had highest activity with 5′-GMP and also showed slight activity with 3′-AMP[15]. The order of relative activity of 5′-nucleoside monophosphates as substrates for calf intestinal 5′-nucleotidase was 5′-UMP > 5′-GMP > 5′-CMP > 5′-AMP > 5′-TMP. 5′-dAMP, 5′-dCMP and 5′-dGMP showed lower activity but no activity of 3′-AMP was found[17]. The order of relative activity for the chicken liver 5′-nucleotidase was 5′-IMP > 5′-GMP > 5′-dGMP > 5′-XMP > 5′-UMP > 5′-AMP > 5′-CMP; 2′(3′)-IMP and 2′(3′)-AMP (mixed isomers) showed very slight activity[10]. The soluble 5′-nucleotidase of rat liver has the following relative activity of substrates: 5′-dGMP > 5′-GMP > 5′-IMP > 5′-UMP > 5′-AMP > 5′-CMP > 5′-dUMP > 5′-dTMP > 5′-dAMP > 5′-dCMP; mixed 2′(3′)-nucleoside monophosphates also showed some activity[11]. The 5′-nucleotidase of rat cerebellum showed at least as great a specificity as previously reported 5′-nucleotidases. In general, it seems that 5′-nucleotidases show highest activity with 5′-AMP or 5′-IMP, hydrolyze most nucleoside monophosphates and show relatively lower activity with deoxynucleotides.

Inhibition of 5′-AMP hydrolysis

Of the several potential inhibitors tested the following had little or no inhibitory effect on the rat cerebellum 5′-nucleotidase hydrolysis of 5′-AMP: eserine, bovine serum albumins, glutathione, iodoacetate, benzoyl chloride, *N*-ethylmaleimide and L-cysteine (Table IV). NaF at $1 \cdot 10^{-2}$ M was a potent inhibitor (21% of control

TABLE IV

EFFECT OF POTENTIAL INHIBITORS ON ACTIVITY OF RAT CEREBELLUM 5'-NUCLEOTIDASE

All experiments were performed as given in the text. The activity was measured with 5'-AMP as substrate. The activity with no addition was arbitrarily set equal to 100%.

Inhibitor	*Concn. (mM)*	*% Control activity*
None	–	100
Uridine	5	96
Thymidine	5	56
Guanosine	5	52
Adenosine	5	96
Inosine	5	56
Eserine	1	100
Bovine serum albumin	7.5 mg/ml	100
Glutathione	1	100
Iodoacetate	1	90
Benzoyl chloride	1	90
N-Ethylmaleimide	1	100
L-Cysteine	1	103
p-Chloromercuribenzoate	1	62
Sodium fluoride	10	21
p-Hydroxymercuribenzoate	1	63
CTP	6	26
GTP	6	30
UTP	6	16
ITP	6	20
ATP	6	10
CTP	0.6	39
GTP	0.6	46
UTP	0.6	26
ITP	0.6	30
ATP	0.6	16

activity); the two *para*-substituted mercuribenzoates were also effective inhibitors. *p*-Chloromercuribenzoate and NaF have been demonstrated to inhibit rat liver 5'-nucleotidase[10] and chicken liver 5'-nucleotidase[21]. $5 \cdot 10^{-4}$ M *p*-hydroxymercuribenzoate inhibited rat liver lysosomal 5'-nucleotidase 99%, and calf intestinal 5'-nucleotidase was inhibited to 22% of the control activity by $6 \cdot 10^{-3}$ M NaF, but was not inhibited at all by $1 \cdot 10^{-3}$ M *p*-chloromercuribenzoate[17].

Nucleosides at $5 \cdot 10^{-3}$ M inhibited the rat cerebellum 5'-nucleotidase. Uridine and adenosine inhibited the activity only slightly, thymidine, guanosine and inosine at $5 \cdot 10^{-3}$ M inhibited the activity almost 50% (Table IV). Similar inhibition of 5'-nucleotidase activity by nucleosides has been reported for chicken liver 5'-nucleotidase[21] and rat liver 5'-nucleotidase[6,10]. At $3 \cdot 10^{-3}$ M, inosine inhibited rat liver 5'-nucleotidase 42%, guanosine 28%, adenosine 6% and hypoxanthine 0% (ref. 10). At $3 \cdot 10^{-3}$ M inosine inhibited chicken liver 5'-nucleotidase 50%, guanosine 28%, adenosine 8% and hypoxanthine 1% (ref. 21).

The nucleoside triphosphates were extremely inhibitory to rat cerebellum 5'-nucleotidase activity (Table IV). The data presented in Table V indicate that the order of inhibition of the nucleoside triphosphates was ATP > UTP > ITP > CTP > GTP. 10 μM ATP caused a 50% inhibition of rat cerebellum 5'-nucleotidase (Table V). MURRAY AND FRIEDRICHS[22] reported that 5'-nucleotidase from Ehrlich ascites tumor

TABLE V

CONCENTRATIONS OF TRIPHOSPHATE REQUIRED FOR 50% INHIBITION OF 5′-NUCLEOTIDASE FROM RAT CEREBELLUM

All experiments were performed as given in the text with 5′-AMP as substrate. Triphosphate concentrations ranged from 6 mM to 1 μM.

Triphosphate	*Triphosphate concn. yielding 50% inhibition (μM)*
ATP	10
UTP	68
ITP	240
CTP	250
GTP	720

cells was inhibited by nucleoside triphosphates; the order of greatest inhibition of AMP hydrolysis was ATP > TTP > UTP > GTP > CTP. IPATA[18] has reported that 5′-nucleotidase from sheep brain was inhibited by ATP > UTP > CTP; GTP was without effect. Thus the nucleoside triphosphate inhibition of 5′-nucleotidase seems to be a general phenomenon. ATP seems to be the most inhibitory nucleoside triphosphate. The inhibition by ATP and other nucleoside triphosphates may be a control mechanism for the function of the 5′-nucleotidase.

The present communication describes the characterization of a highly purified 5′-nucleotidase from rat cerebellum. The enzyme is similar to some other 5′-nucleotidases with respect to ion activation, optimum pH, substrate specificity and inhibition but is dissimilar with respect to other 5′-nucleotidases. It should be noted that since 5′-AMP was used for the routine assay, other 5′-nucleotidases with different specificities may have not been selected for in the purification scheme.

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