Biochimica et Biophysica Acta, 523 (1978) 82—93

BBA 68387

DEMONSTRATION OF 5'-METHYLTHIOADENOSINE PHOSPHORYLASE ACTIVITY IN VARIOUS RAT TISSUES

SOME PROPERTIES OF THE ENZYME FROM RAT LUNG

DAVID L. GARBERS

Departments of Pharmacology and Physiology, Vanderbilt University School of Medicine, Nashville, Tenn. 37232 (U.S.A.)

(Received August 29th, 1977)

Summary

An enzyme (5'-methylthioadenosine phosphorylase) that catalyzes the phosphorolytic cleavage of 5'-methylthioadenosine to 5-methylthioribose-1-phosphate and adenine was found in various rat tissues. Liver and lung had the highest enzyme activities and heart the lowest, most of the activity (>90%) was recovered in soluble tissue fractions.

The enzyme from rat lung was purified about 30-fold by pH treatment $(NH_4)_2SO_4$ fractionation, and gel filtration. The enzyme did not require an added metal-ion for activity, and was not inhibited by EDTA. Many compounds were tested for their inhibitory effects; of these, ribose 1-phosphate, 2-deoxyribose 1-phosphate, fructose 1-phosphate, adenine and guanine were shown to inhibit. Kinetic patterns on reciprocal plots were linear as a function of the concentration of either 5'-methylthioadenosine or phosphate.

More detailed kinetic studies suggested that the rat lung 5'-methylthioadenosine phosphorylase catalyzes an equilibrium-ordered reaction, and that 5'-methylthioadenosine is the first substrate to bind and 5-methylthioribose-1-phosphate is the first product to be released.

Introduction

Although 5'-methylthioadenosine can be synthesized by a number of biochemical pathways [1—6], its concentration in tissues is low [7,8]. In a number of microorganisms, 5'-methylthioadenosine can be degraded by a nucleosidase to 5-methylthioribose and adenine [1,7,9—11]. The activity of this nucleosidase is greater in phosphate buffer then in other buffers [6], but the reaction is not dependent on phosphate and appears to be of hydrolytic rather than of a phosphorolytic type. This contrasts to the situation in prostatic tissue where

phosphate was shown to be absolutely required for the metabolism of 5′-methylthioadenosine [11]. The products of the phosphate-dependent reaction in prostatic tissue were not identified, but it was suggested that 5′-methylthioadenosine was degraded to 5-methylthioribose-1-phosphate and adenine by a phosphorolytic cleavage [11]. Purified purine nucleoside phosphorylase from spleen and erythrocytes, however, were unable to catalyze a phosphorolytic cleavage of 5′-methylthioadenosine [11].

In this study, the enzyme-catalyzed phosphorolytic cleavage of 5′-methylthioadenosine is shown together with an assay method for 5′-methylthioadenosine phosphorylase. The enzyme is observed in various rat tissues, the basic kinetic mechanisms of the partially purified enzyme from rat lung are observed, and some of the enzyme specificity characteristics are described.

Experimental Procedure

Preparation and source of materials. [5′-^{3}H]methylthioadenosine was prepared from *S*-adenosyl-L-[^{3}H]methionine (3 Ci/mmol) (from Amersham/Searle or I.C.I. Pharmaceuticals) by the method of Schlenk et al. [12]. [5-^{3}H]methylthioribose was prepared from [5-^{3}H]methylthioadenosine [4]. The various bases, nucleosides and nucleotides were obtained from Sigma or CalBiochem. Polyethyleneimine-cellulose was purchased from Accurate Chemical and Scientific Corp. and Dowex-50 (AG50W-X8, 100—200 mesh) and BioGel A 0.5 m from BioRad Laboratories. Cel-300 thin layer sheets were obtained from Brinkman Instruments, Inc.

Tissue preparation. For studies on the tissue distribution of the enzyme, the various tissues were removed from male Sprague-Dawley rats (300—350 g). The tissues were diluted to 10% (w/v) with a solution containing 25 mM triethanolamine buffer at pH 7.4 and 1 mM dithiothreitol, diced with scissors, and then homogenized with an Ultraturrax homogenizer (Stauffen, Germany) (10 s each burst, 10 times) at 0—4° C. The suspension was filtered through cheesecloth and centrifuged at 36 000 × *g* for 2 h. The clear supernatant fluid was saved, and the pellet was resuspended in the triethanolamine dithiothreitol buffer to the original volume. These two fractions are the crude soluble and particulate fractions of the cell homogenate.

Enzyme assay. The assay mixture for 5′-methylthioadenosine phosphorylase activity contained 58 mM Tris · HCl (pH 7.4)/111 μM [5′-^{3}H]methylthioadenosine, and 24 μl of the enzyme preparation in a final volume of 225 μl. Reactions were terminated by the addition of 2 ml ice-cold 50 mM acetic acid. The contents of the reaction vessels were then applied to polyethyleneimine-cellulose columns (0.7 cm × 10 cm) and washed, successively, with 50 mM acetic acid, H_2O, 20 mM LiCl and 0.1 M LiCl. The [5-^{3}H]methylthioribose 1-phosphate was then eluted with 0.2 M LiCl. The collected fractions were mixed with Aqueous Counting Scintillant (Amersham/Searle) and counted.

The recoveries of [5-^{3}H]methylthioribose 1-phosphate from the polyethyleneimine-cellulose columns were estimated by the reapplication of previously purified [5-^{3}H]methylthioribose-1-phosphate samples to parallel columns. Recovery of 65—70% were observed, and all data are corrected for this loss.

The degree of degradation of [5-^{3}H]methylthioribose 1-phosphate by various

tissue extracts was estimated by the addition of purified [5-^{3}H]methylthioribose-1-phosphate to normal assay incubations. Some degradation was observed, but it accounted for a loss of less then 8% of the [5-^{3}H]methylthioribose 1-phosphate formed in various tissue homogenates, and less than 2% of the [5-^{3}H]methylthioribose 1-phosphate formed in various tissue supernatant fractions.

Under all of the conditions presented, product accumulation was linear as a function of time and protein concentration at 37° C.

Other methods. Protein was determined by the method of Lowry et al. [13].

Results

Identification of 5-methylthioribose 1-phosphate as a product

After incubation of [5'-^{3}H]methylthioadenosine and phosphate with various rat tissues, a ^{3}H-labeled product was formed, that could be isolated by ionexchange chromatography on polyethyleneimine-cellulose columns. The ^{3}H-containing product could be eluted with 0.2 M LiCl, whereas [5'-^{3}H]methylthioadenosine migrated at the column front (Table I). Nucleoside mono-, but no di- or triphosphates also are eluted by 0.2 M LiCl. No ^{3}H product was formed in the absence of added phosphate, indicating an absolute requirement for phosphate. When the ^{3}H-labeled product isolated from polyethyleneimine-cellulose columns was applied to Dowex-50(H^+) columns and eluted with 0.1 M HCl it migrated at the column front. Based on these findings, it could be concluded that the ^{3}H-labeled compound was acidic.

TABLE I

ELUTION OF [5-^{3}H]METHYLTHIORIBOSE 1-PHOSPHATE FROM POLYETHYLENEIMINECELLULOSE COLUMNS BY 0.2 M LiCl

The incubation mixtures contained 11 μM [5'-^{3}H]methylthioadenosine/6 · 10^5 dpm of [5'-^{3}H]methylthioadenosine/150 mM triethanolamine buffer/22 mM (or none) phosphate and 25 μl (or none) of the rat lung soluble (non-purified) enzyme preparation in a final volume of 225 μl (pH 7.6). Incubations were at 37°C for 30 min. The reactions were stopped and the samples were applied to the columns as described in Experimental Procedure. The polyethyleneimine-cellulose columns were eluted, successively, with 5 ml and 7 ml of 50 mM acetic acid, and 7 ml each of H_2O, H_2O, 20 mM LiCl, 0.1 M LiCl, 0.2 M LiCl, 0.5 M LiCl and 1.0 M LiCl.

Eluting agent	Concentration	ml added	cpm recovered		
			No enzyme 22 mM phosphate	Enzyme (25 μl) no phosphate	Enzyme (25 μl) 22 mM phosphate
Acetic acid	50 mM	5	120 000	123 000	99 000
Acetic acid	50 mM	7	1 700	1 600	1 800
Water	—	7	670	840	760
Water	—	7	580	520	524
LiCl	20 mM	7	480	485	520
LiCl	20 mM	7	670	595	660
LiCl	0.1 M	7	1 120	985	1 250
LiCl	0.2 M	7	95	125	23 000
LiCl	0.5 M	7	80	72	75
LiCl	1.0 M	7	48	60	52

Heating the purified ^{3}H-containing product at 95—98° C at neutral pH for 5 min resulted in no apparent alteration of the ^{3}H-containing molecule, since it did not migrate differently on polyethyleneimine-cellulose and Dowex-50(H^+) columns before or after the heat treatment. However, when the solution was made weakly acidic (0.001 M HCl) and then heated at 95—98° C for 5 min, the purified ^{3}H-containing molecule migrated at the front of both polyethyleneimine-cellulose and Dowex-50(H^+) columns. Thus, heating at acid, but not neutral, pH converted the ^{3}H-containing molecule to an apparently neutral molecule. Ribose 1-phosphate is known to be extremely unstable in weak acid and therefore resembles the labeled material described above [14]. In other experiments, authentic [5-^{3}H]methylthioribose, the product formed by dephosphorylation of [5-^{3}H]methylthioribose-1-phosphate, was shown to migrate at the front of both polyethyleneimine-cellulose and Dowex-50(H^+) columns.

Incubation of the purified [^{3}H]-containing compound with alkaline phosphatase converted the molecule to an apparently neutral compound, since the tritiated material again migrated at the front of both polyethyleneimine-cellulose and Dowex-50(H^+) columns after incubation.

That a single mole of phosphate was being incorporated per mole of tritiated product was confirmed by the incubation of [5′-^{3}H]methylthioadenosine and $^{32}P_i$ with the ling enzume. Based on the specific activity of [5′-^{3}H]methylthioadenosine and of $^{32}P_i$, one mole of phosphate was shown to be incorporated per mole of 5′-methylthioadenosine degraded to the acidic product.

The ^{3}H-labeled methyl group, unlike the phosphate group, was relatively stable. Boiling the ^{3}H-labeled compound in 0.1 M HCl for 1 h did not cause release of the [^{3}H]methyl as [^{3}H]methanol. These data indicate that the [^{3}H]-methyl did not migrate to the anomeric carbon.

The elution of the tritiated product could be retarded by borate ion, whereas the elution of 3′-*O*-methyl GMP was unaffected (Fig. 1). These results indicated the presence of cis-hydroxyl groups.

Finally, after mild acid or alkaline phosphatase treatment, the tritiated unknown was shown to migrate with authentic [5-^{3}H]methylthioribose on cellulose thin layer plates developed in 60% Butanol/25% H_2O/15% acetic acid (v/v).

Based on the above results, the ^{3}H-containing molecule formed by the incubation of [5′-^{3}H]methylthioadenosine with phosphate was identified as [5-^{3}H]-methylthioribose 1-phosphate.

Tissue distribution

5′-Methylthioadenosine phosphorylase activity was highest in liver and lung, but was found in all of the rat tissues examined (Table II). Most, if not all, of the enzyme activity was associated with the soluble fractions of the various tissue homogenates (Table II). The slightly higher enzyme activity in some soluble fractions may be due to removal of inhibitors or to the low rate of hydrolysis of 5-methylthioribose 1-phosphate in the homogenate.

Partial purification

The partial purification of the rat lung enzyme consisted of pH treatments, $(NH_4)_2SO_4$ fractionation, and gel filtration. The rat lung supernatant fluid was slowly titrated with ice-cold 0.1 M acetic acid to pH 5.2. The resulting suspen-

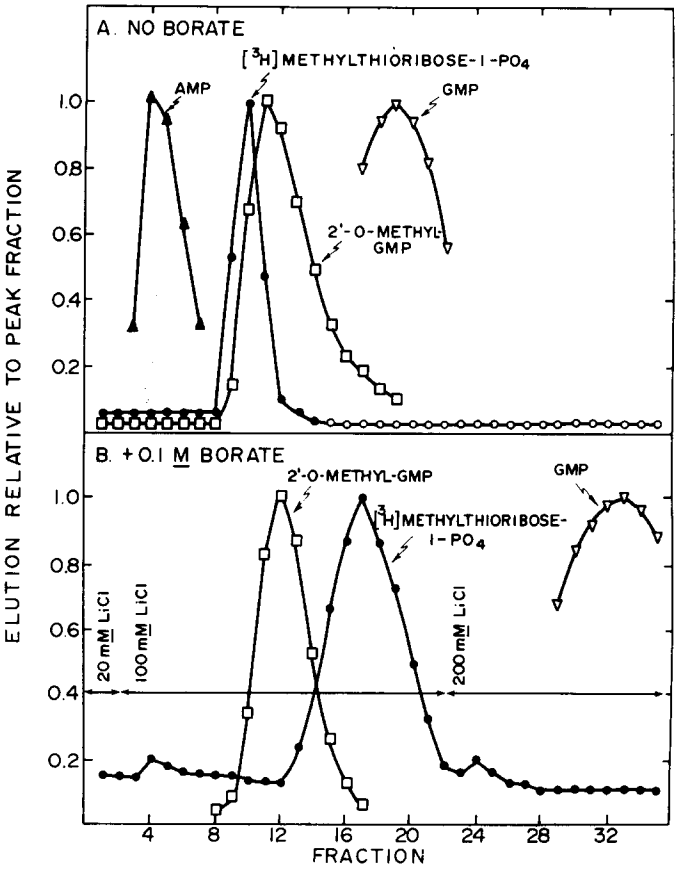


Fig. 1. Retardation of the elution of [5-^{3}H]methylthioribose 1-phosphate by borate ion. The incubations were as described in the legend to Table I. After application of the sample, the polyethyleneiminecellulose columns were either eluted with LiCl as shown in the figure or with LiCl/0.1 M borate (pH 6.0).

TABLE II

THE 5'-METHYLTHIOADENOSINE PHOPHORYLASE ACTIVITY IN VARIOUS MAMMALIAN TISSUES

The incubation mixtures (175 μl) contained 143 μM [^{3}H]methylthioadenosine/28.6 mM KH_2PO_4/75 mM Tris · HCl (pH 7.6), and 25 μl of a 10% equivalent of the tissue homogenate. All incubations were for 5—20 min at 37°C. Data, expressed as pmol 5-methylthioribose 1-phosphate formed/min, represent the mean ± S.E. of 6 determinations on tissue preparations from 3 different experiments.

Tissue	Homogenate	Soluble fractions	Particulate fractions
Liver	91.0 ± 7.0	97.9 ± 2.7	4.7 ± 0.6
Lung	87.4 ± 4.0	101 ± 4.2	6.3 ± 0.4
Spleen	66.7 ± 1.7	74.4 ± 3.4	5.8 ± 0.3
Testes	34.9 ± 0.3	34.7 ± 0.6	3.4 ± 0.3
Kidney	30.4 ± 2.4	40.7 ± 1.8	1.4 ± 0.2
Heart	14.3 ± 0.9	16.2 ± 0.6	0.4 ± 0.1

sion was stirred for 15 min at 0—4° C and centrifuged at 36 000 × *g* for 1 h. The resulting supernatant fluid contained the 5'-methylthioadenosine phosphorylase activity (105% recovery, 3.9-fold purification). The supernatant fluid was adjusted to pH 7.0—7.2 with Tris base, and then $(NH_4)_2SO_4$ was added slowly to 40% of saturation (4° C). The suspension was centrifuged at 36 000 × *g* for 15 min, and the supernatant fluid was saved. $(NH_4)_2SO_4$ was then slowly added to the supernatant fluid to 65% of saturation (4° C). The suspension was centrifuged at 36 000 × *g* for 15 min and the resulting supernatant fluid was discarded. The pellet was dissolved in a solution containing 25 mM triethanolamine buffer at pH 7.4 and 1 mM dithiothreitol (106% recovery, 10-fold purification) and applied to a BioGel A0.5 column. The 5'-methylthioadenosine phosphorylase activity was eluted in the same area as ovalbumin (Fig. 2). The recovery of enzyme activity after this step was 47% with a 30-fold purification. The enzyme was stable at —70° C for at least 4 months after the gel filtration step.

pH optimum

Maximal enzyme activity was observed over pH range of 7.4—8.0.

Effects of nucleosides and other agents.

The partially purified enzyme was inhibited by ribose 1-phosphate, 2-deoxy-

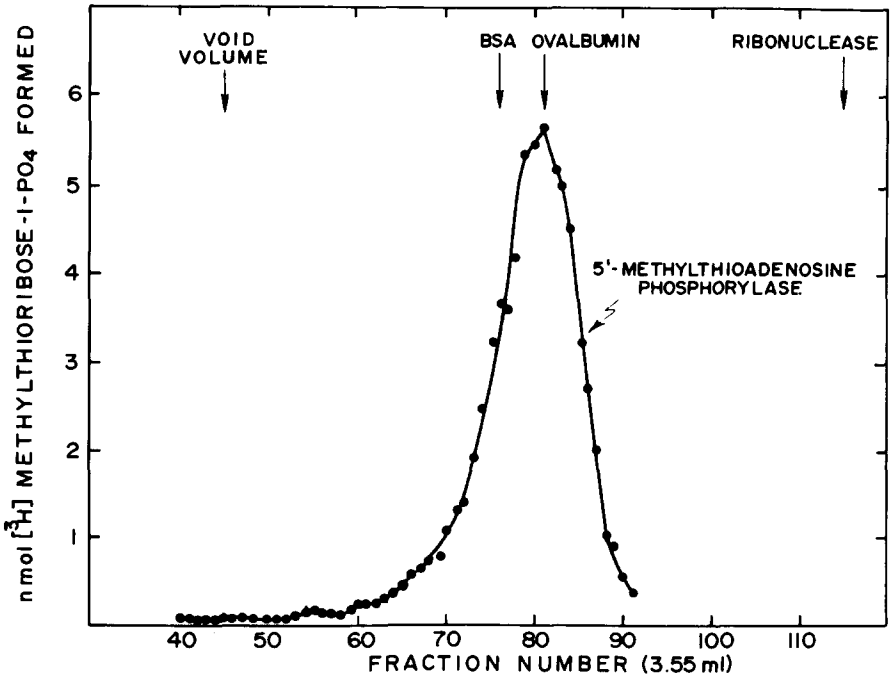


Fig. 2. Gel filtration of 5'-methylthioadenosine phosphorylase on a BioGel A0.5 m column (2.5 cm × 90 cm). The rat lung enzyme obtained after $(NH_4)_2SO_4$ fractionation (5 ml) was applied to a gel column previously equilibrated with 25 mM triethanolanine (pH 7.4)/1 mM dithiothreitol/50 mM NaCl. Enzyme activity was estimated in each fraction as described in Experimental Procedure. Standard proteins and Dextran Blue T 2000 obtained from Pharmacia also were run, and their respective peak elution volumes are indicated by the arrows.

ribose 1-phosphate, fructose 1-phosphate, adenine, guanine and various methylated analogues of guanine, but not by other sugar-phosphates (Table III). Cytosine and theophylline also did not inhibit the enzyme. Various nucleosides

TABLE III

THE EFFECTS OF VARIOUS COMPOUNDS ON 5'-METHYLTHIOADENOSINE PHOSPHORYLASE ACTIVITY

The incubation mixtures contained 111 μM 5'-methylthioadenosine/22.2 mM KH_2PO_4/58 mM Tris · HCl (pH 7.5), the concentration of added substance indicated below, and 206 μg of the Bio-Gel A0.5 m partially purified enzyme protein. Incubations were at 37°C for 10 min, and all data are expressed as pmol methylthioribose-1-*P* formed/min. The values represent the mean ± S.E. of 6 observations.

Addition	Concentration (mM)	Methylthioribose-1-*P* formed
None	—	294 ± 6.5
Ribose	2.2	307 ± 7.6
2-deoxyribose	2.2	297 ± 6.8
Methylthioribose	2.2	317 ± 1.0
Ribose-5-*P*	2.2	344 ± 6.9
2-deoxyribose-5-*P*	2.2	329 ± 3.4
Ribose-1-*P*	2.2	159 ± 2.5
2-deoxyribose 1-*P*	2.2	144 ± 7.1
Rubulose-5-*P*	2.2	331 ± 3.2
Glucose-6-*P*	2.2	326 ± 9.5
Glucose-1-*P*	2.2	310 ± 9.5
Fructose-1-*P*	2.2	157 ± 2.8
Adenine	0.2	126 ± 0.3
Guanine	0.2	323 ± 3.5
Guanine	2.2	164 ± 3.5
Cytosine	2.2	311 ± 5.1
1-Me-guanine	2.2	157 ± 3.6
3-Me-guanine	2.2	189 ± 7.0
7-Me-guanine	2.2	184 ± 6.5
9-Me-guanine	2.2	160 ± 2.5
Theophylline	2.2	317 ± 8.5
Adenosine	0.2	309 ± 6.3
Adenosine	2.2	313 ± 4.3
Guanosine	2.2	283 ± 5.8
Inosine	2.2	331 ± 1.9
2'-OMe-adenosine	2.2	294 ± 4.1
3'-OMe-adenosine	2.2	303 ± 3.5
XMP	2.2	294 ± 12.0
IMP	2.2	310 ± 12.0
Cyclic AMP	4.3	307 ± 3.9
Cyclic GMP	2.2	291 ± 1.4
ATP	2.2	297 ± 6.2
GTP	2.2	297 ± 10.5
EDTA	11.1	309 ± 6.1
Fluoride	8.9	320 ± 5.6
$MgCl_2$	4.4	326 ± 9.5
$CaCl_2$	4.4	321 ± 2.8
$MnCl_2$	4.4	321 ± 11.3
Azide	22.2	303 ± 1.7
Dinitrophenol	0.2	287 ± 4.2
Cadaverine	2.2	304 ± 6.5
Putrescine	2.2	301 ± 3.8

(adenosine, guanosine, inosine) and nucleotides (IMP, XMP, cyclic AMP, cyclic GMP, ATP, GTP) failed to inhibit suggesting that the enzyme was not a general purine nucleoside phosphorylase. Neither added metals (Mg^{2+}, Ca^{2+}, Mn^{2+}) nor EDTA affected enzyme activity. The enzyme appeared not to be regulated by polyamines, since putrescine and cadaverine did not affect enzyme activity.

5'-Methylthioadenosine and phosphate kinetics

Linear intersecting patterns on reciprocal plots were observed when data were plotted as a function of the concentration of either 5'-methylthioadenosine or phosphate (Fig. 3). A rather unusual effect, however, was the intersection of the lines on the ordinate when the concentration of 5'-methylthioadenosine was varied (Fig. 3). These data are consistent with an equilibrium-ordered reaction mechanism [15—20] where 5'-methylthioadenosine binds to the enzyme first and phosphate second. The general rate equation fitting these data is shown as Eqn. 1, where v = observed velocity, V = maximal velocity, A = [5'-methylthioadenosine], B = [phosphate], K_{ia} = dissociation constant for A, and K_b = Michaelis constant for B.

$$v = {}^{VAB}/K_{ia}K_b + K_bA + AB \qquad (1)$$

The kinetic constant calculated from the reciprocal slope and intercept plots (insets, Fig. 3) were K_{ia} = 40 μM and K_b = 3.0 mM. The Michaelis constant for 5'-methylthioadenosine is defined as zero [15].

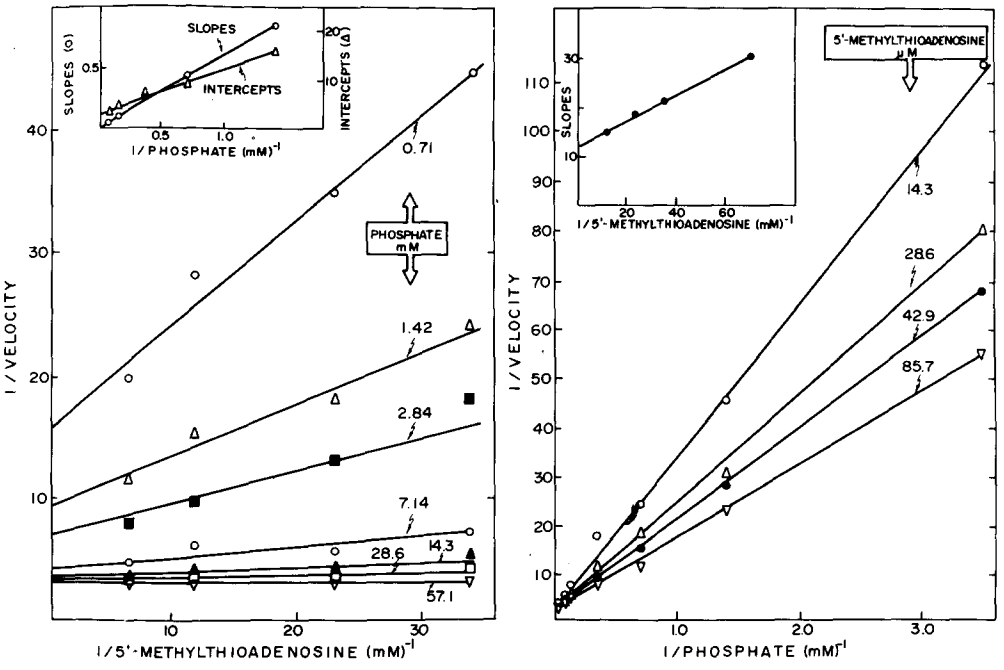


Fig. 3. Reciprocal plots as a function of the concentration of 5'-methylthioadenosine or phosphate. Reactions were run as indicated in Experimental Procedure except that the concentrations of phosphate and 5'-methylthioadenosine were varied as shown. The insets represents the secondary plots of slopes and intercepts from each primary reciprocal plot. The purified enzyme from rat lung was used for these experiments.

Inhibition patterns

Both ribose 1-phosphate and fructose 1-phosphate were studied in more detail as inhibitors of the enzyme. It was assumed that both of these sugar-phosphates inhibited by binding to the 5-methylthioribose 1-phosphate site. Ribose 1-phosphate yielded uncompetitive inhibition patterns with respect to either 5'-methylthioadenosine or phosphate (Fig. 4). These data are compatible with ribose-1-phosphate binding at the 5-methylthioribose 1-phosphate site on the enzyme but only if ribose 1-phosphate is a "dead-end" inhibitor (e.g. ref. 15). The possibility that ribose 1-phosphate is a "dead-end" inhibitor seems likely, since adenosine, the predicted product of ribose 1-phosphate and adenine in the reverse reaction, does not inhibit the enzyme. Fructose 1-phosphate also gave uncompetitive inhibition patterns (Fig. 5), suggesting that it too was bound at the 5-methylthioribose 1-phosphate site as a "dead-end" inhibitor.

If the reaction mechanism is equilibrium-ordered, then the last product to be released should give competitive inhibition patterns with respect to both 5'methylthioadenosine and phosphate [15]. This is an unusual situation, but as shown in Fig. 6, adenine did give competitive inhibition patterns against both 5'-methylthioadenosine and phosphate. Thus, the observed inhibition patterns are consistent with an equilibrium-ordered mechanism.

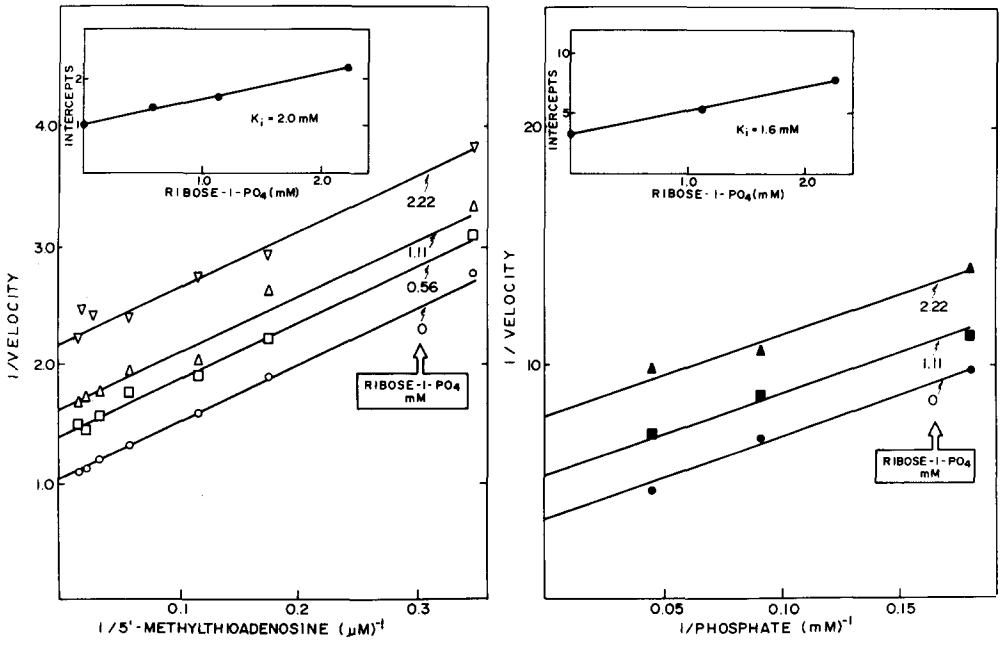


Fig. 4. Uncompetitive inhibition by ribose 1-phosphate. Reactions were run as indicated in Experimental Procedure except that the concentrations of 5'-methylthioadenosine or phosphate were varied at fixed concentrations of ribose 1-phosphate. The incubations, using the partially purified enzyme from rat lung, were for 3 min and the velocity is expressed as the picomoles of 5-methylthioribose-1-phosphate formed per 3 min. The insets represent the secondary plots of the intercepts from each primary reciprocal plot.

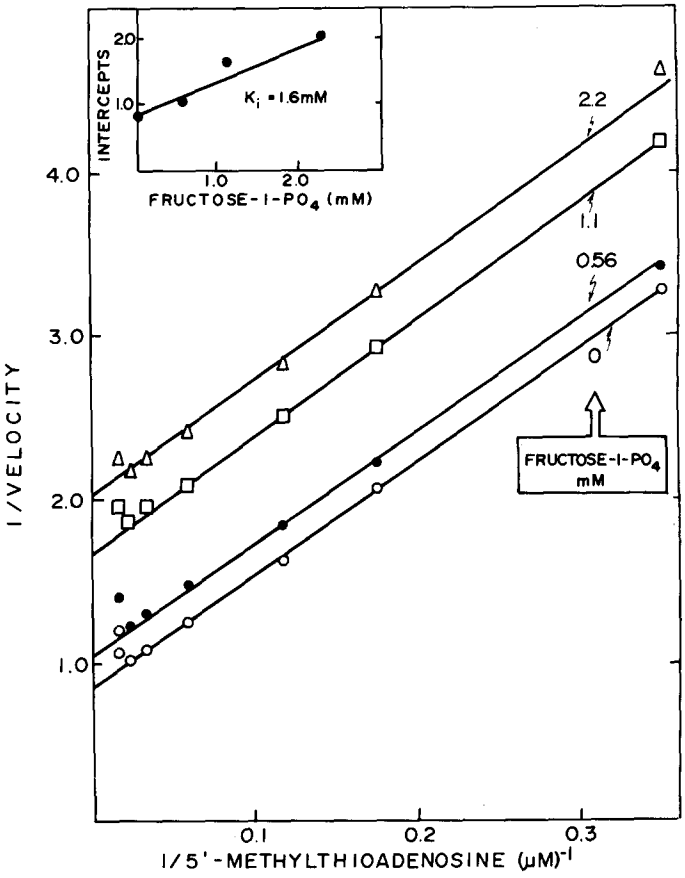
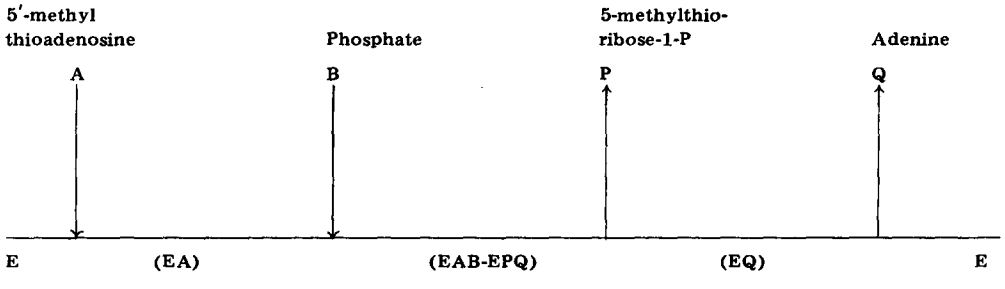


Fig. 5. Uncompetitive inhibition by fructose 1-phosphate. The enzyme reactions were as described in the legend to Fig. 4, except that fructose 1-phosphate was used as the inhibitor. The inset represents the secondary plot of the intercepts from the primary reciprocal plot.

Proposed kinetic mechanism

The kinetics as a function of the two substrates, and the inhibition patterns observed with adenine and ribose 1-phosphate and fructose 1-phosphate suggest an equilibrium-ordered kinetic mechanism, with the ordered binding of substrates and release of products as shown in Scheme I.



Scheme 1.

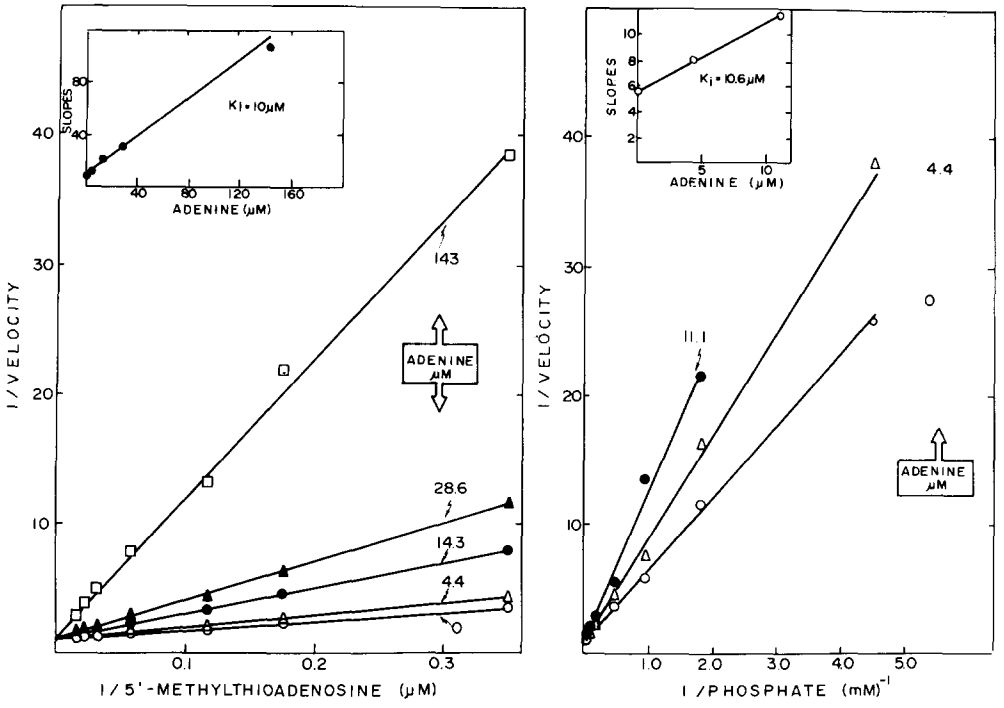


Fig. 6. Competitive inhibition by adenine. Reactions were run as described in the legend to Fig. 4, except that adenine was studied as an inhibitor. The insets represent the secondary plots of the slopes from the primary reciprocal plots.

Discussion

Pegg and Williams-Ashman [11] previously demonstrated an absolute phosphate requirement for the metabolism of 5'-methylthioadenosine in prostatic tissue, and suggested that the nucleoside was metabolized by an initial phosphorolytic cleavage. They were unable, however, to clearly identify whether or not 5-methylthioribose 1-phosphate was formed because of a lack of sufficient material for analyses. Based on several criteria, this study has shown that 5-methylthioribose 1-phosphate is a metabolite of 5'-methylthioadenosine. The phosphorolytic cleavage of the nucleoside contrasts to the metabolism of 5'-methylthioadenosine in microorganisms where a hydrolytic cleavage of the glycosidic bond occurs [1—10]. It was shown earlier [11] that a number of purine nucleoside phosphorylases would not catalyze the phosphorolytic cleavage of 5'-methylthioadenosine, and it is shown that adenosine, guanosine and inosine do not inhibit the 5'-methylthioadenosine phosphorylase activity. Thus, the enzyme appears to show high specificity for the configuration at the 5'-position.

The kinetic mechanism of 5'-methylthioadenosine phosphorylase is somewhat unusual in that it is equilibrium-ordered. Such mechanisms have been observed with other enzymes [15—20], although the binding of a metal ion at equilibrium has generally been the case. It should be noted that kinetic alterna-

tives to the described equilibrium-ordered mechanism, under various conditions, has been presented [21]. Because 5′-methylthioadenosine is at equilibrium with the enzyme, high concentrations of phosphate are capable of shifting essentially all of the enzyme · 5′-methylthioadenosine complex to the ternary complex; thus, the amount of 5′-methylthioadenosine required for maximal reaction becomes essentially equal to the enzyme concentration. If the function of 5′-methylthioadenosine phosphorylase is to maintain low levels of the nucleoside within the cell, the equilibrium-ordered mechanism would offer an advantage, since the Michaelis constant for 5′-methylthioadenosine would be very low at normal cell phosphate concentrations.

The kinetic mechanisms for other types of purine nucleoside phosphorylases have been reported to be both ordered Bi-Bi [22] and Theorell-Chance [23]. Both the Theorell-Chance and the equilibrium-ordered mechanism discussed here are special cases of an ordered Bi-Bi mechanism. Thus, all of the purine nucleoside phosphorylases, including the 5′-methylthioadenosine phosphorylase studied here, have been shown to have ordered kinetic mechanisms [22,23]. Furthermore, in all instances, the order of binding has been the nucleoside binding first followed by phosphate, while the release of products has been the ordered release of the sugar-phosphate followed by the base, respectively [22, 23].

Acknowledgments

The author wishes to express his thanks to Dr. Henry A. Lardy and Joel G. Hardman for their advice during the course of these experiments, and to Ms. Marvist A. Parks and Ms. D. Janette Tubb for their expert technical assistance. This study was supported by NIH grants HD10254, GM16811 and AM07462. The author is an investigator for the Howard Hughes Medical Institute.

References

1 Shapiro, S.K. and Mather, A.N. (1958) J. Biol. Chem. 233, 631—633
2 Tabor, H., Rosenthal, S.M. and Tabor, C.W. (1958) J. Biol. Chem. 233, 907—914
3 Mudd, S.H. (1959) J. Biol. Chem. 234, 1784—1786
4 Schlenk, F., Zydek-Cwick, C.R. and Dainko, J.L. (1973) Biochim. Biophys. Acta 320, 357—362
5 Stoner, G.L. and Eisenberg, M.A. (1975) J. Biol. Chem. 250, 4029—4036
6 Ferro, A.J., Barrett, A. and Shapiro, S.K. (1976) Biochim. Biophys. Acta 438, 487—494
7 Rhodes, J.B. and Williams-Ashman, H.G. (1964) Med. Exp. 10, 281—285
8 Chu, T.M., Mallette, M.F. and Mumma, R.O. (1968) Biochemistry 7, 1399—1406
9 Duerre, J.A. (1962) J. Biol. Chem. 237, 3737—3741
10 Walker, R.D. and Duerre, J.A. (1975) Can. J. Biochem. 53, 312—319
11 Pegg, A.E. and Williams-Ashman, H.G. (1969) Biochim. J. 115, 241—247
12 Schlenk, F., Zydek-Cwick, C.R. and Hutson, N.K. (1971) Arch. Biochem. Biophys. 134, 414—422
13 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
14 Kalckar, H.M. (1947) J. Biol. Chem. 167, 477—486
15 Cleland, W.W. (1970) in Enzymes, 3rd edn. (Boyer, P.D., ed.), Academic Press, N.Y.
16 McClure, W.R., Lardy, H.A. and Kneifel, H.P. (1971) J. Biol. Chem. 246, 3569—3578
17 Morrison, J.F. and Ebner, K.E. (1971) J. Biol. Chem. 246, 3977—3984
18 Morrison, J.F. and Ebner, K.E. (1971) J. Biol. Chem. 246, 3985—3991
19 Warren, G.B. and Tipton, K.F. (1974) Biochem. J. 139, 311—320
20 Garbers, D.L., Hardman, J.G. and Rudolph, F.B. (1974) Biochemistry 13, 4166—4171
21 Dalziel, K. (1969) Biochem. J. 114, 547—556
22 Kim, B.K., Cha, S. and Parks, Jr., R.D. (1968) J. Biol. Chem. 243, 1771—1776
23 Lewis, A.S. and Glantz, M.D. (1976) Biochemistry 15, 4451—4456