Substrate Specificity of 5'-Methylthioadenosine Phosphorylase from Human Prostate

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5'-Methylthioadenosine phosphorylase was purified approx. 340-fold from human prostate by using affinity chromatography by Hg-coupled Sepharose. The enzyme, responsible for the breakdown of 5'-methylthioadenosine into adenine and methylthioribose 1-phosphate, was partially characterized. The apparent K_m for 5'-methylthioadenosine is $25\mu\text{M}$. It is activated by thiols and shows an absolute requirement for phosphate ions. New analogues of 5'-methylthioadenosine were prepared and their activity as substrates or inhibitors of the reaction was investigated. The replacement of the 6-amino group of the adenine moiety by a hydroxy group, as well as the replacement of N-7 by a methinic radical, resulted in an almost complete loss of activity. Otherwise the replacement of sulphur by selenium, as well as that of the methyl group by an ethyl one, is compatible with the activity as substrate. The positively charged sulphonium group also prevents catalytic interaction with the enzyme. The inhibitory effect of 5'-methylthiotubercidin (competitive) and 5'-dimethylthioadenosine sulphonium salt (non-competitive) was also demonstrated. The reported results suggest three binding sites between the substrate and the enzyme.

Among the multiple pathways of biosynthesis of 5'-methylthioadenosine from the common precursor *S*-adenosylmethionine described in microorganisms (Schlenk & Ehninger, 1964; Schlenk, 1965; Tabor & Tabor, 1972; Nishimura *et al.*, 1974; Stoner & Eisenberg, 1975; Nishimura, 1977), only two are also operative in mammalian tissues. The direct cleavage of *S*-adenosylmethionine into 5'-methylthioadenosine and α -amino- γ -butyrolactone, through the action of a specific lyase (Shapiro & Mather, 1958; Pietropaolo *et al.*, 1972; Swiatek *et al.*, 1973), represents the first reaction leading to the formation of the thioether; probably the primary role of this reaction is the control of the cellular concentrations of *S*-adenosylmethionine. The other pathway, which is the most relevant quantitatively, involves the transfer of the propylamine moiety from the decarboxylated *S*-adenosylmethionine to putrescine or spermidine (Pegg & Williams-Ashman, 1970; Bowman *et al.*, 1973; Tabor & Tabor, 1976; Zappia *et al.*, 1977a): 2 mol of 5'-methylthioadenosine is released/mol of spermine and 1 mol/mol of spermidine. Both enzymic routes are virtually irreversible.

Polyamine concentrations in prostate gland are relatively elevated ($5\text{--}7\mu\text{mol/g}$), whereas the con-

centration of 5'-methylthioadenosine is less than $0.2\mu\text{mol/g}$ (Rhodes & Williams-Ashman, 1964): 5'-methylthioadenosine nucleosidase, which represents the main enzyme responsible for the degradation of the thioether in mammalian tissues, is the major factor preventing the cellular accumulation of 5'-methylthioadenosine and plays a primary role in purine salvage. Two classes of 5'-methylthioadenosine nucleosidases have been reported: a hydrolytic nucleosidase that cleaves 5'-methylthioadenosine into adenine and 5-methylthioribose has been described in *Aerobacter aerogenes* (Shapiro & Mather, 1958) and in *Escherichia coli* (Duerre, 1962; Ferro *et al.*, 1976), and a phosphorolytic nucleosidase has been purified from rat ventral prostate (Pegg & Williams-Ashman, 1969). The phosphorolytic cleavage mechanism of the latter enzyme has been implied by the absolute requirement for phosphate ions (Pegg & Williams-Ashman, 1969).

Another enzyme activity involved in the catabolism of the thioether catalyses the release of the methylthio group from 5'-methylthioadenosine and has been described in malignant mammalian cells (Toohey, 1977).

The inhibitory effect exerted by 5'-methylthioadenosine on some methyltransferases (Zappia *et al.*, 1969) suggests that the enzymic cleavage(s) of the thioether can also regulate this class of reactions.

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The present paper reports, for the first time, the occurrence in human prostate of a 5'-methylthioadenosine nucleosidase acting with a phosphorolytic mechanism. The purification and partial characterization of the enzyme are described. To investigate the substrate specificity and the mechanism of the catalytic process, new purine-sulphur compounds have been synthesized and assayed as substrates as well as inhibitors.

Preliminary results of this work have been reported at the 10th International Congress of Biochemistry (Oliva *et al.*, 1976).

Experimental

Chemicals

S-Adenosyl-L-methionine was prepared from cultures of *Saccharomyces cerevisiae* (Schlenk & De Palma, 1957) and isolated by ion-exchange chromatography (Shapiro & Ehninger, 1966; Zappia *et al.*, 1968); S-adenosyl-L-[Me-¹⁴C]methionine was supplied by The Radiochemical Centre, Amersham, Bucks., U.K.; L-[Et-1-¹⁴C]ethionine was supplied by NEN Chemicals G.m.b.H., Frankfurt, Germany. 5'-[Me-¹⁴C]methylthioadenosine was prepared as described by Schlenk & Ehninger (1964) from labelled S-adenosyl-L-methionine. The chemical and radiochemical purity of 5'-methylthioadenosine and S-adenosylmethionine was checked by t.l.c. and high-voltage electrophoresis (Zappia *et al.*, 1969, 1977b). The u.v. quenching for purine compounds, the ninhydrin reaction for amino acids and the iodoplatinate spray (Winegard & Toennies, 1948) for sulphur-containing compounds were used to detect 5'-methylthioadenosine and the analogues and derivatives. 5'-Methylthiotubercidin was a gift from Dr. J. K. Coward (Coward *et al.*, 1977). Seleno-DL-methionine, reduced glutathione, reduced and oxidized dithiothreitol, iodoacetic acid, iodoacetamide and *p*-chloromercuribenzoic acid were supplied by Sigma Chemical Co., St. Louis, MO, U.S.A.

Preparation of derivatives and analogues of 5'-methylthioadenosine

5'-[Me-¹⁴C]Methylthioinosine. 5'-[Me-¹⁴C]Methylthioadenosine was converted into 5'-[Me-¹⁴C]methylthioinosine by enzymic deamination with non-specific adenosine deaminase from *Aspergillus oryzae* (Schlenk & Zydek-Cwick, 1968; Zappia *et al.*, 1974).

5'-Methylselenoadenosine. 5'-Methylselenoadenosine was prepared by acid hydrolysis of S-adenosyl-L-selenomethionine (Schlenk & Zydek-Cwick, 1969). The selenonium compound has been enzymically synthesized from L-selenomethionine and ATP

(Stekol, 1965); the thioether has been isolated from its precursor by ion-exchange chromatography (Zappia *et al.*, 1969).

5'-[Et-1-¹⁴C]Ethylthioadenosine and 5'-[Et-1-¹⁴C]ethylthioinosine. 5'-[Et-1-¹⁴C]Ethylthioadenosine was prepared by acid hydrolysis of S-adenosyl-L-[Et-1-¹⁴C]ethionine at 100°C for 30 min. The sulphonium compound has been obtained from cultures of *Torulopsis utilis* supplemented with L-[Et-1-¹⁴C]ethionine and isolated by ion-exchange chromatography (Parks, 1958). Labelled 5'-ethylthioadenosine was then converted into the inosine analogue as previously described, the kinetics of deamination being similar to that of 5'-methylthioadenosine.

5'-Dimethylthioadenosine sulphonium salt. 5'-Dimethylthioadenosine sulphonium salt was prepared by methylation of 5'-methylthioadenosine with methyl iodide (Toennies & Kolb, 1951). After removal of excess of methyl iodide by evaporation under reduced pressure, the sulphonium compound was purified as described by Parks & Schlenk (1958).

Preparation of Hg-coupled Sepharose

AH (1,6-diaminohexane)-Sepharose 4B was converted into a mercurial resin by a reaction with *p*-chloro-mercuribenzenesulphonyl chloride, a bi-functional reagent prepared as described by Goss & Parkhurst (1974). To 20ml of AH-Sepharose 4B, previously equilibrated in 1M-potassium carbonate buffer, pH9, 20mg of *p*-chloromercuribenzenesulphonyl chloride dissolved in 80ml of tetrahydrofuran was added dropwise. After washing with water, the Hg-coupled Sepharose was equilibrated with 0.2M-potassium phosphate buffer, pH7.4, and mixed with 20ml of Sepharose 4B previously equilibrated in the same buffer. The resin thus obtained had a binding capacity of 10µequiv. of thiol group/ml of settled gel.

Preparation of tissue extracts

Prostate glands from patients with prostatic hypertrophy were frozen at -20°C immediately after surgery. The diagnosis of prostate adenoma was checked histologically and carcinomatous glands were discarded. The frozen glands rinsed with 0.3M-sucrose/0.05M-potassium phosphate buffer, pH7.4, containing 0.5mM-dithiothreitol, were minced in small pieces and homogenized for 4min in a Waring Blendor with 4vol. of the same buffer. A second homogenization of the extract was performed with a Potter-Elvehjem apparatus for 5min at the maximum speed. The homogenate was then centrifuged for 2h at 20000g in a Sorvall refrigerated centrifuge and the resulting supernatant was used as enzyme source.

Enzyme and assays

Non-specific adenosine deaminase from *A. oryzae* was purified from Sanzyme (Calbiochem, La Jolla, CA, U.S.A.) as described by Sharpless & Wolfenden (1967).

5'-Methylthioadenosine nucleosidase activity was determined by measuring 5'-[Me-¹⁴C]methylthioribose 1-phosphate released from 5'-[Me-¹⁴C]methylthioadenosine; ion-exchange chromatography was used for the separation of the two molecules (Pegg & Williams-Ashman, 1969). The assay medium, unless otherwise stated, contained 40 µmol of potassium phosphate buffer, pH 7.4, 0.3 µmol of 5'-[Me-¹⁴C]methylthioadenosine (74 mCi/µmol), 0.8 µmol of dithiothreitol and the enzyme protein in a total volume of 0.8 ml. The reaction was carried out at 37°C for 60 min, then was terminated by the addition of 0.2 ml of 1 M-trichloroacetic acid, and the precipitate was removed by centrifugation (3000 g for 10 min). The supernatant was then applied to a column (0.3 cm × 4 cm) of Dowex 50 (H⁺ form) equilibrated with 0.2 M-trichloroacetic acid: 5-methylthioribose 1-phosphate is eluted with 2 ml of 0.2 M-trichloroacetic acid; in these conditions 5'-methylthioadenosine is quantitatively retained by the resin. 5'-Methylthioinosine, 5'-ethylthioadenosine and 5'-ethylthioinosine were also assayed by the above procedure, since their chromatographic behaviour on Dowex 50 (H⁺ form) is similar to that of 5'-methylthioadenosine. 5-Ethylthioribose is also eluted quantitatively with 2 ml of 0.2 M-trichloroacetic acid.

The enzymic cleavage of 5'-methylselenoadenosine and 5'-dimethylthioadenosine sulphonium salt was followed spectrophotometrically by measuring the increase in A_{305} in the presence of an excess of xanthine oxidase (EC 1.2.3.2) (Klenow, 1952).

The breakdown of 5'-methylthiotubercidin into 5-methylthioribose 1-phosphate and 7-deaza-adenine was followed by t.l.c. on silica gel in two solvent systems: water/aq. NH₃ (sp.gr. 0.88) (999:1, v/v) and butan-1-ol/water (43:7, v/v). After incubation at 37°C for 60 min the reaction was terminated by the addition of 0.2 ml of 1 M-trichloroacetic acid. The protein precipitate was removed by centrifugation

(3000 g for 15 min), and the supernatant was extracted with 2 × 5 vol. of diethyl-ether and evaporated to a small volume. The sample was then applied to the silica-gel sheets and chromatographed.

Determination of radioactivity

Radioactivity was measured in a Tri-Carb liquid-scintillation spectrometer (Packard model 3380) equipped with an absolute radioactivity analyser. For this 3 ml of trichloroacetic acid eluate was mixed in the scintillation vials with 6 ml of Insta-gel (Packard). Quenching was corrected by external standardization. The enzymic activity is expressed as µmol of 5-methylthioribose 1-phosphate released/h per mg of protein.

Protein determination

Protein concentration was determined by the method of Lowry *et al.* (1951), with crystalline serum albumin as standard. The albumin solution was calibrated by using $A_{280}^{0.1\%} = 0.667$ (Schachman & Edelstein, 1966).

Since dithiothreitol interferes with the Lowry *et al.* (1951) method, all the protein determinations have been performed in the absence of the dithiol.

Results*Enzyme purification and stability*

The enzyme was purified 340-fold with 20% yield as shown in Table 1. The purification procedure is that of Cacciapuoti *et al.* (1978) with several modifications. Fig. 1 shows the elution pattern of the enzyme from a column (1 cm × 8 cm) of Sepharose-p-chloromercuribenzenesulphonyl chloride equilibrated with 0.2 M-sodium phosphate buffer, pH 7.4. Contaminating proteins are eluted with 2 M-KCl and 3 mM-2-mercaptoethanol; the enzyme is then recovered with 10 mM-dithiothreitol.

The preparation is nearly homogeneous on the basis of disc polyacrylamide-gel electrophoresis (Davis, 1964). The electrophoresis was performed at 2 mA/tube (5.3 mm × 75 mm), in 7.5% (w/v) gels in

Table 1. *Summary of purification procedure*
Units are expressed as µmol of substrate transformed/h.

Purification steps	Total activity (units)	Specific activity (units/mg)	Purification (-fold)	Yield (%)
Crude extract	172	0.023	1	100
55–75%-satd. (NH ₄) ₂ SO ₄	123.2	0.113	4.9	71.6
Hydroxyapatite	76.5	0.407	17.7	44.5
Sephadex G-200	55	0.86	37.4	32
Hg-coupled Sepharose	33.2	7.85	341.3	19.3

Fig. 1. Elution pattern of 5'-methylthioadenosine phosphorylase from an Hg-coupled Sepharose column. A column (1cm $\times$ 8cm) of Hg-coupled Sepharose was pre-equilibrated with 0.2M-potassium phosphate buffer, pH 7.4. Contaminating proteins are eluted stepwise with 2M-KCl and 3mM-2-mercaptoethanol: 5'-methylthioadenosine phosphorylase was recovered with 10mM-dithiothreitol. The arrows indicate the order of addition of the various eluents. ●, A_{280} ; ○, enzyme activity. Fraction size was 1.8 ml.

0.4 M-Tris/HCl, pH 6.9. The running buffer was 0.05M-Tris/0.4M-glycine, pH 8.3. The phosphorylase activity is associated with one major band at 2.5cm from the start; two faint bands of contaminants with higher mobility are also observable.

The enzyme is stable to repeated freeze-thawing and is rapidly inactivated by exposure for 15 min at 65°C.

Effect of reducing agents

A partial inactivation of the enzyme by exposure to O_2 was observed in preliminary experiments, suggesting the presence of essential thiol groups. Fig. 2 shows the effect of various concentrations of 2-mercaptoethanol and dithiothreitol on the reaction rate. Maximum activation is reached in the presence of 1mM-dithiothreitol, whereas 1mM-2-mercaptoethanol is less effective. Only 50% of the maximum activity is observable in the absence of reducing agents.

The effect of thiol inhibitors, i.e. iodoacetamide, iodoacetic acid and *p*-chloromercuribenzoic acid, has also been tested (Table 2): 1mM-*p*-chloromercuribenzoic acid caused quantitative inhibition, whereas 1mM-iodoacetamide and -iodoacetic acid exerted only 63 and 6% inhibition respectively. The inhibition by iodoacetamide is quantitatively reversed by dithiothreitol, whereas the inhibition by *p*-chloromercuribenzoate is only partially (20%) reversed by the thiol.

Fig. 2. Effect of 2-mercaptoethanol and dithiothreitol on the enzyme activity.

The assay was performed as indicated in Fig. 1, except that dithiothreitol (●) or 2-mercaptoethanol (○) was added at the given concentrations.

Table 2. Effect of thiol-blocking reagents on the enzyme activity both in the absence and in the presence of dithiothreitol

Results are mean values of three separate experiments. The assay was performed as indicated in the legend of Fig. 1, except that the listed compounds were added to the mixture at 1mM. The assay solution contained 100 μ g of enzyme. Abbreviation: N.D., not detectable.

Additions (mM)	Enzyme activity (units/mg)	Relative activity (%)
None	3.1	50
Iodoacetate	2.9	46.7
Iodoacetamide	1.15	18.5
<i>p</i> -Chloromercuribenzoate	N.D.	0
Dithiothreitol	6.2	100
Dithiothreitol + iodoacetamide	3.2	51.6
Dithiothreitol + <i>p</i> -chloromercuribenzoate	0.74	12

Effect of anions on the enzyme activity

Fig. 3 reports the effect of various anions on the 5'-methylthioadenosine nucleosidase activity: an absolute requirement for phosphate is observable. In the presence of 50mM-phosphate the reaction rate is linear with time up to 40 min and then declines; arsenate can partially replace phosphate, whereas sulphate and citrate are inactive.

The product of the reaction, obtained with the purified enzyme, was identified as methylthioribose 1-phosphate by ion-exchange chromatography. The

reaction mixture (400 μ l) was chromatographed through a column (0.5 cm $\times$ 1.5 cm) of AG 1-X8 (Cl⁻ form). Elution of 5'-methylthioadenosine and 5'-methylthioribose is performed with 30 ml of water followed by 20 ml of 30 mM-NH₄Cl; 5'-methylthioribose 1-phosphate is then eluted with 25 ml of 50 mM-NH₄Cl. The methyl-labelled phosphorylated sugar was also identified by t.l.c. on silica gel in butanol/acetic acid/water (12:3:5 by vol.) as solvent system. The *R_F* values are: 0.39 for 5'-methylthioadenosine, 0.58 for 5'-methylthioribose, 0.07 for ribose 1-phosphate and 0.08 for 5'-methylthioribose 1-phosphate. An active phosphatase present in the crude preparations readily converts 5'-methylthioribose 1-phosphate into methylthioribose and phosphate.

Fig. 3. Effect of various anions on 5'-methylthioadenosine phosphorylase activity

The assay medium contained 40 μ mol of sodium diethylbarbiturate buffer, pH 7.4, 0.3 μ mol of 5'-[Me-¹⁴C]methylthioadenosine (74 mCi/ μ mol), 0.8 μ mol of dithiothreitol and 100 μ g of enzyme protein in a final volume of 0.8 ml, also containing: ●, 40 μ mol of NaH₂PO₄; ○, 40 μ mol of Na₂HAsO₄; △, 40 μ mol of Na₂SO₄; □, 40 μ mol of citrate. All of these solutions were adjusted to pH 7.4.

Michaelis constant and substrate specificity

The effect of 5'-methylthioadenosine concentration on the reaction rate is shown in Fig. 4. A maximal rate of cleavage of 5'-methylthioadenosine was observed with 0.3 mM-substrate. From the double-reciprocal plot in the insert an apparent *K_m* of 25 μ M was calculated.

To analyse the substrate specificity, the analogues and derivatives of 5'-methylthioadenosine shown in Fig. 5 were tested. The procedures for the synthesis of these compounds are reported in the Experimental section. Among the analogues assayed only the selenium derivative equals the activity of the natural substrate (Table 3). The modifications of the purine moiety result in a resistance to enzymic hydrolysis: only 9% of activity is retained in 5'-methylthioinosine and the 7-deaza analogue is completely in-

Fig. 4. Effect of substrate concentration on the reaction rate. The assay was performed as indicated in the text, except that the substrate was added at the given concentrations. In the insert, *v* is expressed as μ mol of 5'-methylthioadenosine decomposed/h per mg of protein.

Fig. 5. 5'-Methylthioadenosine (I) and some of its derivatives (II-VII)
For further details of these analogues see Table 3.

Table 3. *Substrate specificity of 5'-methylthioadenosine phosphorylase*

Results are mean values of three separate experiments. Numbers in parentheses refer to Fig. 5. Abbreviation: N.D., not detectable.

Substrate analogues	Concn. (mM)	Enzyme activity (units/mg)	Relative activity (%)
5'-Methylthioadenosine (I)	0.4	5.7	100
5'-Methylselenoadenosine (II)	0.4	5.4	95
5'-Methylthiotubercidin (III)	2.0	N.D.	—
5'-Methylthioinosine (IV)	0.4	0.5	8.9
5'-Dimethylthioadenosine sulphonium salt (V)	1.0	N.D.	—
5'-Ethylthioadenosine (VI)	0.4	3.42	60
5'-Ethylthioinosine (VII)	1.0	N.D.	—

active as substrate. Also the sulphonium compound tested, namely 5'-dimethylthioadenosine sulphonium salt, is inactive.

The replacement of the methyl group by an ethyl one results in a definite decrease in activity: 5'-ethylthioadenosine displays only 60% of the activity obtained with equimolar amounts of the natural substrate; the respective ethylated sugar was chromatographically identified as the reaction product. The doubly modified analogue, i.e. 5'-ethylthioinosine, is also resistant to enzymic cleavage.

The compounds inactive as substrates have been also assayed as inhibitors. Fig. 6 reports the effect of 5'-methylthiotubercidin and 5'-dimethylthioadenosine sulphonium salt on the reaction rate. The 7-deaza compound exerts a competitive inhibition, whereas the sulphonium salt acts as non-competitive inhibitor.

Discussion

It is well known that human prostate contributes the greatest part of seminal spermine (Mann, 1964) and that the amount of this polyamine in such organs (500mg/100g of fresh tissue) exceeds by about 10 times the average content of other tissues (Russell, 1973). Since the formation of spermine is stoichiometric with the synthesis of 5'-methylthioadenosine (2mol of spermine/mol of 5'-methylthioadenosine), the occurrence in human prostate of an enzymic system catalysing the cleavage of the thioether is particularly relevant. The kinetic data here reported suggest that the relatively low concentrations of 5'-methylthioadenosine reported in this organ can be ascribed to the high phosphorylase activity.

The nucleosidase here investigated resembles in many respects the similar enzyme partially purified from rat ventral prostate (Pegg & Williams-Ashman, 1969): the difference in K_m values between the two enzymes (25 μ M for human prostate enzyme and 300 μ M for rat prostate) probably reflects the high degree of purity of our preparation. It is noteworthy in this respect that the cellular concentrations of the

Fig. 6. *Effect of 5'-methylthiotubercidin and 5'-dimethylthioadenosine sulphonium salt on the reaction rate (Hofstee plot)*

5'-Methylthioadenosine phosphorylase was assayed as detailed in the legend of Fig. 1, except that 5'-methylthioadenosine was added at the given concentrations. Reaction velocity (v) is expressed as mol of 5'-methylthioadenosine decomposed/h per mg of protein. ●, Control; ▲, addition of 85mM-5'-methylthiotubercidin; ▽, addition of 90mM-5'-dimethylthioadenosine sulphonium salt.

thioether are saturating with respect to the enzyme, which therefore displays the maximal velocity in physiological conditions.

The enzyme here characterized is markedly activated by reducing agents and inhibited by thiol-blocking reagents: these data suggest an involvement of thiols in the catalytic process. In analogy with the enzyme purified from rat ventral prostate (Pegg & Williams-Ashman, 1969), our preparation shows an

absolute requirement for phosphate ions: the isolation of the phosphorylated sugar demonstrates the phosphorolytic mechanism of the reaction; arsenate can partially replace phosphate ions.

The specificity of human 5'-methylthioadenosine phosphorylase is rather strict compared with that of the enzyme purified from *E. coli* (Ferro *et al.*, 1976). The replacement of the sulphur atom of 5'-methylthioadenosine by selenium and the replacement of the methyl group by an ethyl one are the only substrate modifications compatible with enzymic activity. The rate of breakdown of 5'-methylselenoadenosine equals that of 5'-methylthioadenosine (see Table 3). This finding agrees with the generally accepted view that the enzyme systems that normally utilize sulphur metabolites also convert their selenium analogues, i.e. the interchangeability of methionine and selenomethionine has been demonstrated in protein synthesis (Hoffman *et al.*, 1970) as well as that of *S*-adenosylmethionine and *Se*-adenosylselenomethionine in polyamine biosynthesis (Skupin, 1962).

The ethyl derivative shows 60% of the activity of an equimolar concentration of 5'-methylthioadenosine. Conversely, the replacement of the adenine 6-amino group by a hydroxy group, as well as the replacement of N-7 of adenine by a methinic radical, resulted in an almost complete loss of activity. The resistance of 5'-methylthiotubercidin to enzymic hydrolysis has also been observed by Coward *et al.* (1977) with the enzyme purified from rat prostate.

Replacement of the bivalent sulphur in the thioether conformation by a charged sulphonium group

results in a loss of activity: the positively charged group probably prevents the catalytic interaction with the enzyme. On the other hand, the binding of the sulphonium group of 5'-dimethylthioadenosine sulphonium salt to non-catalytic sites of the enzyme protein could explain the non-competitive inhibition exerted by this molecule.

Three sites of interaction between 5'-methylthioadenosine and the enzyme can be postulated from the reported data. The binding involves the amino group of the adenine moiety, N-7 of the purine ring and a sulphur atom in thioether conformation. This interpretation does not take into account possible conformational changes of the thioether caused by the chemical modifications. A similar binding has been proposed for *S*-adenosylmethionine and *S*-adenosylhomocysteine to methyl-transfer enzymes (Zappia *et al.*, 1969; Borchardt, 1977).

The newly synthesized analogues of 5'-methylthioadenosine, acting as inhibitors of the reaction, are potentially useful tools for the study of the regulatory role exerted by 5'-methylthioadenosine phosphorylase on the metabolic pathways involving 5'-methylthioadenosine and *S*-adenosylmethionine; the metabolic relationships between these two adenosine-sulphur compounds and the investigated enzyme are summarized in Scheme 1.

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Scheme 1. Regulatory role of 5'-methylthioadenosine phosphorylase on the metabolism of adenosine-sulphur compounds. Abbreviations: Ado-Hcy, *S*-adenosylhomocysteine; Ado-Met: *S*-adenosylmethionine. Solid lines indicate the metabolic pathways, broken lines indicate the inhibitory effect.

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