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Escherichia coli *S*-adenosylhomocysteine/5′-methylthioadenosine nucleosidase

Purification, substrate specificity and mechanism of action

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S-Adenosylhomocysteine/5′-methylthioadenosine nucleosidase (EC 3.2.2.9) was purified to homogeneity from *Escherichia coli* to a final specific activity of 373 μmol of 5′-methylthioadenosine cleaved/min per mg of protein. Affinity chromatography on *S*-formycinylhomocysteine–Sepharose is the key step of the purification procedure. The enzyme, responsible for the cleavage of the glycosidic bond of both *S*-adenosylhomocysteine and 5′-methylthioadenosine, was partially characterized. The apparent K_m for 5′-methylthioadenosine is 0.4 μM, and that for *S*-adenosylhomocysteine is 4.3 μM. The maximal rate of cleavage of *S*-adenosylhomocysteine is approx. 40% of that of 5′-methylthioadenosine. Some 25 analogues of the two naturally occurring thioethers were studied as potential substrates or inhibitors of the enzyme. Except for the analogues modified in the 5′-position of the ribose moiety or the 2-position of the purine ring, none of the compounds tested was effective as a substrate. Moreover, 5′-methylthioformycin, 5′-chloroformycin, *S*-formycinylhomocysteine, 5′-methylthiotubercidin and *S*-tubercidinylhomocysteine were powerful inhibitors of the enzyme activity. The results obtained allow the hypothesis of a mechanism of enzymic catalysis requiring as a key step the protonation of N-7 of the purine ring.

INTRODUCTION

It is well known that AdoMet-dependent methyltransfer reactions are involved in a wide variety of important biological processes in both prokaryotes and eukaryotes (Cantoni & Chiang, 1980) and that AdoHcy is a 'general' inhibitor of this class of reactions (Zappia *et al.*, 1969; Cox *et al.*, 1977; Ueland, 1982). Thus a regulatory role of the AdoHcy cellular concentration (or AdoHcy/AdoMet ratio) on the methylation-dependent processes has been proposed (Cantoni & Chiang, 1980).

The catabolism of AdoHcy is different in various phyla: in eukaryotes and plants the thioether is hydrolysed to adenosine and L-homocysteine by AdoHcy hydrolase (EC 3.3.1.1) (de la Haba & Cantoni, 1959), whereas in several prokaryotes AdoHcy is cleaved to adenine and *S*-ribosylhomocysteine by a specific nucleosidase (EC 3.2.2.9) (Duerre, 1962).

Many studies have been carried out on the occurrence, distribution, mechanism of action and substrate specificity of mammalian AdoHcy hydrolase (Richards *et al.*, 1978; Palmer & Abeles, 1979; Ueland & Saebo, 1979; Fujioka & Takata, 1981). Conversely, few reports describing a partial purification and some properties of the bacterial enzyme have so far been published (Duerre, 1962; Ferro *et al.*, 1976). It is noteworthy that the bacterial enzyme is also responsible for the removal of MTA, a thioether formed from AdoMet by several independent pathways (Duerre, 1962).

This molecule has been considered as a 'regulator' of cellular growth, rather than as a simple side-product of polyamine biosynthesis (Cartenì-Farina *et al.*, 1983). Indeed, MTA exerts several metabolic effects, including a powerful inhibition of mammalian and bacterial spermidine synthase and spermine synthase (Pajula & Raina, 1979; Hibasami *et al.*, 1980), a suicide-like inhibition of AdoHcy hydrolase (Ferro *et al.*, 1981) and an inhibition of DNA methylases (Linn *et al.*, 1977). Moreover, the thioether is a growth inhibitor of many cell systems (Wolford *et al.*, 1981; Pegg *et al.*, 1981; Cartenì-Farina *et al.*, 1983).

On the basis of these observations AdoHcy/MTA nucleosidase, which regulates both methylation-dependent processes (via the AdoHcy concentration) and polyamine biosynthesis (via that of MTA), may play a significant role in the metabolism of prokaryotes.

The present study reports for the first time the purification to homogeneity of *Escherichia coli* AdoHcy/MTA nucleosidase and some properties of this enzyme. Particular attention has been given to the substrate specificity and inhibition of the enzyme. The results are discussed in terms of substrate requirements for enzyme recognition and of the mechanism of enzymic catalysis.

Preliminary results of this work have been reported (Della Ragione *et al.*, 1984*a*; Zappia *et al.*, 1985).

Abbreviations used: AdoMet, *S*-adenosylmethionine; AdoHcy, *S*-adenosylhomocysteine; MTA, 5′-methylthioadenosine.

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EXPERIMENTAL

Materials

AdoMet was prepared from cultures of *Saccharomyces cerevisiae* and isolated by ion-exchange chromatography (Zappia *et al.*, 1968). *S*-Adenosyl-L-[*Me*-^{14}C]methionine and [8-^{14}C]adenosine were supplied by The Radiochemical Centre, Amersham, Bucks., U.K. MTA and [*Me*-^{14}C]MTA were prepared as described by Zappia *et al.* (1978) from labelled and unlabelled AdoMet. *S*-[8-^{14}C]Adenosylhomocysteine was synthesized from [8-^{14}C]adenosine and L-homocysteine (Cacciapuoti *et al.*, 1983). *S*-Adenosyl-L-homocysteine, *S*-adenosyl-D-homocysteine, tubercidin (7-deaza-adenosine), formycin A (8-aza,9-deaza-adenosine), adenine, guanine, cytidine and hypoxanthine were supplied by Sigma Chemical Co, St. Louis, MO, U.S.A. 5'-Isobutylthioadenosine, 5'-n-butylthioadenosine and 5'-methylthiotubercidin were synthesized by established methods (Coward *et al.*, 1983), as were 5'-chloroformycin (Savarese *et al.*, 1981), *S*-formycinylhomocysteine and 5'-methylthioformycin (Cacciapuoti *et al.*, 1983). *S*-Tubercidinylhomocysteine, *S*-adenosyl-3-thiopropylamine (decarboxy-AdoHcy), *S*-adenosyl-4-thiobutyrate (deaminated AdoHcy), *S*-2-aza-adenosylhomocysteine, *S*-8-aza-adenosylhomocysteine, *S*-adenosylhomocysteine sulphoxide, 2'-*O*-[(*R*)-formyl(adenin-9-yl)methyl]-3'-*S*-(*R*)-homocysteinyl-3'-deoxy-(*S*)-glyceraldehyde (AdoHcy dialdehyde), 2'-*O*-[(*R*)-hydroxymethyl(adenin-9-yl)methyl]-3'-[*S*-(*R*)-homocysteinyl]-3'-deoxy-(*S*)-glycerol (2',3'-acyclic AdoHcy), 5-[5'-deoxy-5'-(*C*)-adenosyl]-L-ornithine (Sinefungin), 5-[5'-deoxy-5'-(*C*)-4',5'-didehydroadenosyl]-L-ornithine (compound A9145C), N^6-methyl-3-deaza-adenosylhomocysteine, N^6-dimethyl-3-deaza-adenosylhomocysteine, *S*-inosylhomocysteine, *S*-cytidylhomocysteine and *S*-guanosylhomocysteine were kindly given by Dr. R. T. Borchardt, Department of Biochemistry, University of Kansas, Lawrence, KS, U.S.A. 5'-Isobutylthioinosine, 5'-n-butylthioinosine and 5'-methylthioinosine were prepared by enzymic deamination of the corresponding adenosyl derivatives with a non-specific adenosine deaminase from *Aspergillus oryzae* (Schlenk & Zydeck-Cwick, 1968; Zappia *et al.*, 1978). 5'-Methylthio-3-deaza-adenosine and 5'-isobutylthio-3-deaza-adenosine were kindly given by Dr. G. Stramentinoli, BioResearch Co., Linate, Italy.

All other chemicals were of the purest commerical grade.

S-Adenosylhomocysteine/5'-methylthioadenosine nucleosidase assay

AdoHcy/MTA nucleosidase activity was determined by measuring: (1) the formation of [*Me*-^{14}C]methylthioribose from [*Me*-^{14}C]MTA; (2) [8-^{14}C]-adenine formed from *S*-[8-^{14}C]adenosylhomocysteine; and (3) the formation of the purine base from MTA, AdoHcy or their analogues.

The standard reaction mixture used for Method (1), unless otherwise stated, contained (in a total volume of 200 μl) 50 mM-potassium phosphate buffer, pH 7.0, 200 μM-[*Me*-^{14}C]MTA (5×10^5 c.p.m./μmol) and the enzymic preparation. The assay mixture was incubated at 37 °C for 20 min; the reaction was stopped by the addition of 50 μl of 3 M-trichloroacetic acid, and a 0.2 ml sample was loaded on a column (0.6 cm × 2 cm) of Dowex-50 (H^+ form) equilibrated in water. The [*Me*-^{14}C]methylthioribose produced was eluted directly into scintillation vials with 2 ml of 0.1 M-HCl. Throughout the kinetic and purification studies the amount of protein was adjusted so that no more than 10% of the substrate was converted into methylthioribose, and the reaction rate was strictly linear as function of time and protein.

When labelled AdoHcy or unlabelled MTA, AdoHcy and their analogues were used as substrates, the formation of adenine (or its analogues) or depletion of the compounds was measured by h.p.l.c. In this case the assay mixture contained, unless otherwise stated (in a total volume of 100 μl), 50 mM-potassium phosphate buffer, pH 7.0, 500 μM substrate and the enzyme protein. The reaction was incubated at 37 °C and was stopped by adding 200 μl of methanol. A sample (50–100 μl) of the centrifuged (15000 *g*, 15 min) assay mixture was injected into a Beckman h.p.l.c. system (equipped with a Ultrasil ODS RP-18 column and a model 100-1 detector) and eluted as reported by Della Ragione *et al.* (1981) and Cacciapuoti *et al.* (1983). The rate of enzyme-catalysed reaction is expressed as μmol of substrate cleaved/min at 37 °C.

Preparation of *S*-formycinylhomocysteine–Sepharose

A portion (2 g) of 6-aminohexanoic acid–Sepharose 4B (Pharmacia Fine Chemicals, Upsala, Sweden) was swollen overnight in 200 ml of 0.5 M-NaCl and washed with 4 × 200 ml of 0.5 M-NaCl and then with 1 litre of distilled water in order to remove the salt. The washed gel (6 ml) was mixed with 1 mmol of *S*-formycinylhomocysteine (Cacciapuoti *et al.*, 1983), and the total volume adjusted to 20 ml with distilled water. The mixture was stirred slowly while 400 mg of 1-ethyl-3[(3-dimethylamino)propyl]carbodi-imide dissolved in water was added dropwise. The pH was maintained at 5.5 and the mixture was shaken gently at room temperature for 24 h. The gel was then washed with 2 litres of 0.5 M-NaCl and stored in 50 ml of 50 mM-Tris/HCl, pH 7.0, containing 1 M-NaCl and 0.02% NaN_3 at 4 °C until use.

Purification of *E. coli* AdoHcy/MTA nucleosidase

A portion (300 g) of frozen cell paste of *E. coli* B (Miles Laboratories) was resuspended in 600 ml of 50 mM-sodium phosphate buffer, pH 7.4 (buffer A), and homogenized by a single pass through a French press at 15000 lb/in^2 (100 MPa). The resulting extract was centrifuged at 15000 *g* for 1 h, and the supernatant was adjusted to 40% saturation by the addition of crystalline $(NH_4)_2SO_4$. The precipitate was discarded by centrifuging (15000 *g*, 20 min) and additional $(NH_4)_2SO_4$ was added to the supernatant to give 60% saturation. The resulting precipitate was collected by centrifugation (15000*g*, 20 min), dissolved in the smallest possible volume of 10 mM-sodium phosphate (pH 7.4)/100 mM-KCl (buffer B), and dialysed overnight against 100 volumes of the same buffer. The dialysed sample was then applied to a DEAE-Sephadex A-50 column (2.5 cm × 50 cm) previously equilibrated with buffer B at a flow rate of 30 ml/h. The column was washed with 500 ml of the equilibration buffer and a linear gradient (2 litres total volume) of 0.1–0.4 M-KCl was applied. AdoHcy/MTA nucleosidase was eluted between 0.3 M-and 0.35 M-KCl, and the active fractions were concentrated by ultrafiltration and dialysed against 100 vol. of 10 mM-sodium phosphate buffer, pH 7.4. The sample was applied on to a hydroxyapatite column (2.5 cm × 10 cm) equilibrated

Table 1. Purification of *S*-adenosylhomocysteine/5'-methylthioadenosine nucleosidase

A unit is the amount of the enzyme that cleaves 1 μmol of substrate/min at 37 °C.

Step	Total protein (mg)	Specific activity (units/mg) Towards MTA	Towards AdoHcy	Ratio MTA/AdoHcy	Purification (fold)	Yield (%)
Supernatant at 15000 *g*	12000	0.038	0.0152	2.5	1	100
$(NH_4)_2SO_4$ (40–60% satn.)	8300	0.045	0.018	2.5	1.2	81
DEAE-Sephadex A-50	500	0.7	0.287	2.43	15.5	76
Hydroxyapatite	70	4.2	1.8	2.33	110	64
Sephacryl S-200	15	15.3	6.27	2.44	400	50
S-Formycinylhomocysteine–Sepharose	0.5	373	156	2.39	9810	40

with 10 mM-sodium phosphate, pH 7.4, at a flow rate of 20 ml/h, and, after a washing with 200 ml of the equilibration buffer, a linear gradient (500 ml total volume) of 10–150 mM-sodium phosphate was applied. The active fractions were concentrated to 2 ml by ultrafiltration and dialysed against 10 mM-sodium phosphate (pH 7.4)/200 mM-KCl (buffer C). The sample was then applied at a flow rate of 6 ml/h on to a Sephacryl S-200 column (1.5 cm × 95 cm) equilibrated and eluted with buffer C. The fractions containing AdoHcy/MTA nucleosidase activity were pooled and applied at a flow rate of 10 ml/h on to a *S*-formycinylhomocysteine–Sepharose column (1 cm × 5 cm) pre-equilibrated with buffer C. The column was washed with 50 ml of buffer C and then with 100 ml of 10 mM-sodium phosphate (pH 7.4)/1 M-KCl. The enzyme activity was eluted with 10 mM-sodium phosphate (pH 7.4)/1 M-KCl/4 mM-MTA. The active fractions were pooled and concentrated to 1 ml, dialysed extensively against 50 mM-sodium phosphate, pH 7.4, to remove MTA, and stored in small fractions at −20 °C.

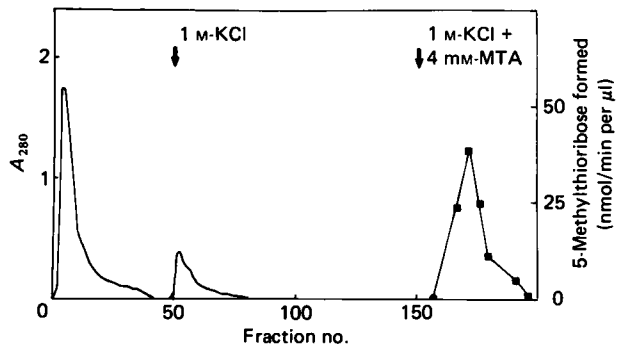

Fig. 1. Elution pattern of *S*-adenosylhomocysteine/5'-methylthioadenosine nucleosidase from *S*-formycinylhomocysteine–Sepharose

The enzyme was applied to the column in 10 mM-sodium phosphate (pH 7.4)/200 mM-KCl, and the column was washed successively with 10 mM-sodium phosphate, (pH 7.4)/1 M-KCl and 10 mM-sodium phosphate (pH 7.4)/1 M-KCl/4 mM-MTA. Fractions (1 ml) were collected and the A_{280} (——) and the nucleosidase activity (■) measured.

Polyacrylamide-gel electrophoresis

Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis was carried out at room temperature by using 10%- or 12.5%-acrylamide resolving gel (11 cm) and 3%-acrylamide stacking gel (3 cm) (Laemmli, 1970). Samples containing about 15 μg of protein were incubated for 5 min at 100 °C in 0.125 M-Tris/HCl, pH 6.8, containing 1% sodium dodecyl sulphate, 1% 2-mercaptoethanol, 8 M-urea and 0.001% Bromophenol Blue. After being cooled, the samples were layered directly on the stacking gel, and electrophoresis was carried out at 5 mA/gel for about 5 h. The gels were then fixed in 40% (v/v) methanol/10% (v/v) acetic acid overnight and stained with 0.02% Coomassie Brilliant Blue in the same solution. The gels were destained at room temperature with 10% acetic acid/10% methanol. Non-denaturing gel electrophoresis was carried out as described by Della Ragione & Pegg (1982), except that no spermidine was added in the buffers. After electrophoresis, the gels were stained as described above. In some cases the gels were not stained, but were sliced into 2 mm sections, and each of these was incubated overnight with 150 μl of a solution containing 50 mM-sodium phosphate buffer, pH 7.0, and 150 μg of bovine albumin at 4 °C. AdoHcy/MTA nucleosidase activity was determined in each sample as described above by method 1 (MTA as substrate) or Method 2 (AdoHcy as substrate).

Protein determination

This was done as described by Bradford (1976), with human γ-globulin as standard. The absorbance of column eluates was monitored at 280 nm.

RESULTS

Enzyme purification and stability

AdoHcy/MTA nucleosidase was purified 9800-fold with a 40% yield (Table 1), by using affinity chromatography on S-formycinylhomocysteine–Sepharose as the key step (Fig. 1). This was selected as affinity absorbant on the basis of the remarkable affinity of the enzyme toward the formycinyl derivatives (see under 'Inhibition of AdoHcy/MTA nucleosidase' below). The specific activity of the final preparation was 373 μmol of MTA or 156 μmol of AdoHcy cleaved/min per mg of protein.

The enzyme was homogeneous as judged by poly-

Table 2. Substrate specificity of *E. coli* *S*-adenosylhomocysteine/5′-methylthioadenosine nucleosidase

The assays were carried out as described in the Experimental section by Method 3. The reaction rates are expressed as percentages of that found with MTA as substrate (373 μmol/min per mg).

Compound	Maximal rate of cleavage (%)
(1) Analogues of methylthioadenosine	
5′-Methylthioadenosine	100
5′-Isobutylthioadenosine	52
5′-n-Butylthioadenosine	40
5′-Methylthioinosine	< 0.1
5′-Isobutylthioinosine	< 0.1
5′-Isobutylthio-3-deaza-adenosine	< 0.1
5′-Methylthio-3-deaza-adenosine	< 0.1
5′-Chloroformycin	< 0.1
5′-Methylthioformycin	< 0.1
5′-Methylthiotubercidin	< 0.1
(2) Analogues of adenosylhomocysteine	
S-Adenosyl-L-homocysteine	42
S-Adenosyl-D-homocysteine	26
Decarboxylated adenosylhomocysteine	18
Deaminated adenosylhomocysteine	33
S-Formycinylhomocysteine	< 0.1
S-Tubercidinylhomocysteine	< 0.1
S-Inosylhomocysteine	< 0.1
S-Guanosylhomocysteine	< 0.1
S-Cytidylhomocysteine	< 0.1
S-2-Aza-adenosylhomocysteine	27
S-8-Aza adenosylhomocysteine	< 0.1
S-N^6-Methyl-3-deaza-adenosylhomocysteine	< 0.1
S-N^6-Dimethyl-3-deaza-adenosylhomocysteine	< 0.1
Sinefungin	< 0.1
Compound A9145C	< 0.1
S-Adenosylhomocysteine dialdehyde	< 0.1
Acyclic adenosylhomocysteine	< 0.1

acrylamide-gel electrophoresis under denaturing conditions with sodium dodecyl sulphate. Moreover, a single protein band which co-migrated with the enzyme activity was obtained when the enzyme was subjected to polyacrylamide-gel electrophoresis under 'native' conditions. The material eluted from the gel slices was active towards AdoHcy and MTA, thus further confirming that the same enzyme is responsible for the catabolism of both the thioethers.

The enzyme stored at −20 °C did not show significant loss of activity for at least 2 months. Conversely AdoHcy/MTA nucleosidase was rapidly inactivated after exposure for 15 min at 60 °C. These data agree very well with those previously reported by Duerre (1962).

Michaelis constants and substrate specificity

When the effect of MTA and AdoHcy concentrations on the reaction rate was investigated, a K_m value of 0.4 μM for MTA was obtained by extrapolation, and that for AdoHcy was 4.3 μM. It is noteworthy that considerably higher K_m values for MTA (1.8 mM) and for AdoHcy (3 mM) were previously reported by Duerre (1962). Such a discrepancy is probably due to the different sensitivity of the assay methods used, as well as to the extent of purification. Conversely the Michaelis constant for MTA obtained by Ferro *et al.* (1976) (0.31 μM) is in good agreement with the kinetic constant of the homogeneous enzyme. The maximal rate of cleavage of AdoHcy was approx. 40% of that of MTA, thus confirming previous data of Ferro *et al.* (1976).

To test several analogues of MTA and AdoHcy as substrates of the homogeneous enzyme, h.p.l.c. methodology which allows the separation of thioethers and purine bases has been employed (see the Experimental section). The sensitivity of the method (0.1% of substrate cleavage) makes the procedure suitable for enzymic assay.

Only six compounds of the 25 tested served as substrates (Table 2). All the reported modifications in the purine moiety were not compatible with the catalytic process, except for the 2-aza analogue of AdoHcy, which was a good substrate of the enzyme. Conversely, the analogues modified in the side chain at the 5′-position, i.e. the butyl (isobutyl and n-butyl) derivatives of MTA and the decarboxy and the deaminated analogues of AdoHcy, were active as substrates, although to different extents. It is noteworthy that Sinefungin and compound A9145C, powerful inhibitors of AdoMet-dependent reactions and envisaged as AdoHcy analogues (Pugh *et al.*, 1978), are not substrates of AdoHcy/MTA nucleosidase.

Inhibition of AdoHcy/MTA nucleosidase

All the compounds resistant to enzymic cleavage were tested as inhibitors (Table 3). As shown in Table 3, the analogues assayed can be grouped into two categories:

Table 3. Inhibition of *E. coli* *S*-adenosylhomocysteine/5′-methylthioadenosine nucleosidase

The assays were carried out as described in the Experimental section, by Method 1, with MTA as substrate. The I_{50} values (concentration giving 50% inhibition) were calculated with a concentration of MTA of 0.7 μM.

Compound	I_{50} (μM)	K_i (nM)
Strong inhibitors		
5′-Methylthioformycin	0.06	27
5′-Chloroformycin	0.4	322
S-Formycinylhomocysteine	0.02	9.7
5′-Methylthiotubercidin	7.7	4100
S-Tubercidinylhomocysteine	3.2	1900
S-8-Aza-adenosylhomocysteine	1.8	928
Poor inhibitors		
5′-Isobutylthioinosine	132	
5′-n-Butylthioinosine	94	
5′-Methylthioinosine	> 500	
5′-Methylthio-3-deaza-adenosine	79	
5′-Isobutylthio-3-deaza-adenosine	13	
S-Inosylhomocysteine	> 500	
S-Guanosylhomocysteine	> 500	
S-*N*^{6}-Methyl-3-deaza-adenosylhomocysteine	20	
S-*N*^{6}-Dimethyl-3-deaza-adenosylhomocysteine	> 500	
S-Adenosylhomocysteine sulphoxide	69	
S-Adenosylhomocysteine dialdehyde	> 500	
Acyclic adenosylhomocysteine	> 500	
Sinefungin	24	
Compound A9145C	> 500	
S-Cytidylhomocysteine	> 500	
Adenine	216	
Hypoxanthine	> 500	
Guanine	> 500	
AMP	> 500	

poor inhibitors ($I_{50} > 10$ μM) and good inhibitors ($I_{50} < 10$ μM). A detailed kinetic analysis has been performed only on those compounds with $I_{50} < 10$ μM.

The poor inhibitors include MTA and AdoHcy analogues modified in the pyrimidine ring of the aglycone moiety or modified in the ribose ring (AdoHcy dialdehyde and acyclic AdoHcy).

Conversely, the compounds modified in the imidazole ring of the purine base, including 5′-methylthiotubercidin and *S*-tubercidinylhomocysteine (in which N-7 is replaced by a carbon atom) and *S*-8-aza-adenosylhomocysteine (where C-8 is replaced by a nitrogen atom) cause powerful inhibition. The seemingly conservative change of the imidazole moiety of MTA and AdoHcy (N-7, C-8, N-9) into the pyrazole ring (N-7, N-8, C-9) of formycinyl derivatives results in powerful inhibitors, with K_i values markedly lower than the apparent K_m for MTA.

A kinetic analysis of the inhibition exerted by 5′-methylthioformycin and *S*-formycinylhomocysteine is reported in Figs. 2 and 3. The double-reciprocal plots are indicative of competitive inhibition with respect to MTA, and apparent K_i values of 27 nM and 9.7 nM for 5′-methylthioformycin and *S*-formycinylhomocysteine respectively were calculated.

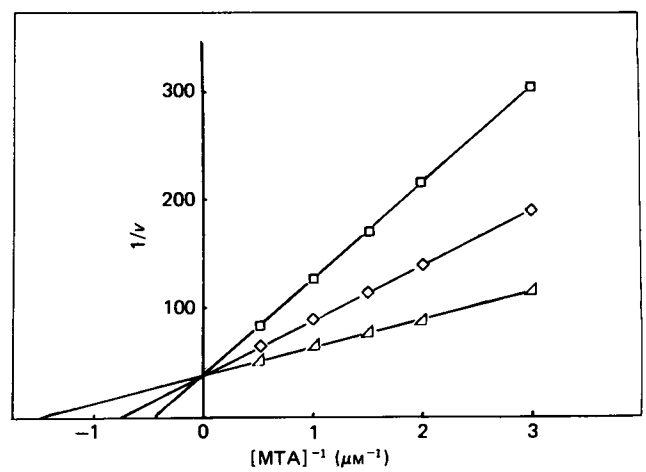

Fig. 2. Inhibition of *S*-adenosylhomocysteine/5′-methylthioadenosine nucleosidase by 5′-methylthioformycin

Assays were carried out in the presence of (△) 0, (◇) 65 nM- and (□) 130 nM-5′-methylthioformycin, and the reciprocal of the initial velocity [*v* (μmol/min)] was plotted against the reciprocal of MTA concentration as shown.

DISCUSSION

The present paper describes the purification to homogeneity, the substrate specificity and the catalytic mechanism of *E. coli* AdoHcy/MTA nucleosidase, a key enzyme in the metabolism of adenosyl-sulphur compounds.

The purified enzyme has a specific activity of 373 μmol of MTA and 156 μmol of AdoHcy cleaved/min per mg of protein. This value is significantly higher than that of

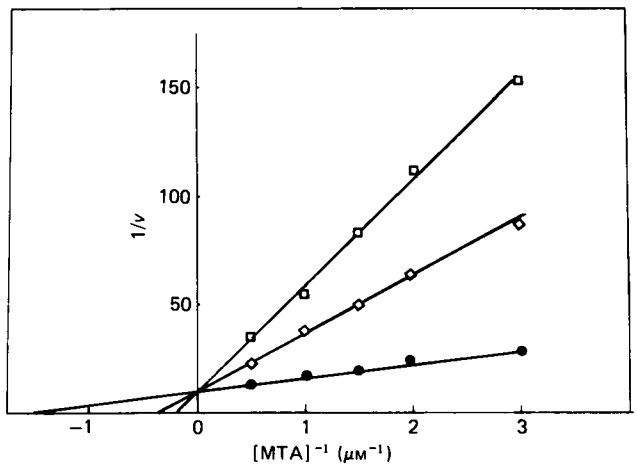

Fig. 3. Inhibition of *S*-adenosylhomocysteine/5′-methylthioadenosine nucleosidase by *S*-formycinylhomocysteine

Assays were carried out in the presence of (●) 0, (◇) 70 nM- and (□) 140 nM-*S*-formycinylhomocysteine and the reciprocal of the initial velocity [v (μmol/min)] was plotted against the reciprocal of MTA concentration as shown.

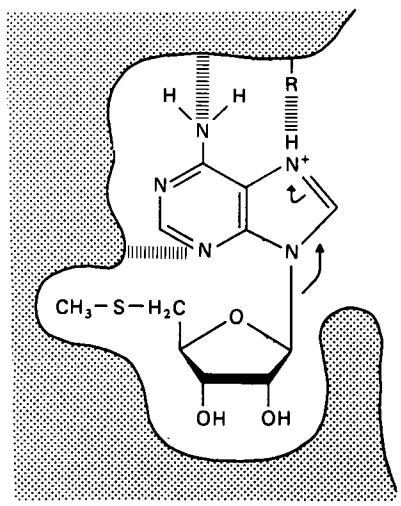

Fig. 4. Hypothetical transition state for 5′-methylthioadenosine catalysis

MTA phosphorylase from human placenta (Della Ragione *et al.*, 1984*b*) and from *Caldariella acidophila* (Cartenì-Farina *et al.*, 1979) and of MTA nucleosidase from *Lupinus luteus* seeds (Guranowski *et al.*, 1981), the only MTA-cleaving enzymes so far purified to homogeneity. However, those enzymes are inactive towards AdoHcy. Moreover, the specific activity of AdoHcy hydrolase ranges between 0.5 and 5 μmol of AdoHcy cleaved/min per mg of protein, which is at least 30-fold less than that of our final preparation (Richards *et al.*, 1978; Palmer & Abeles, 1979; Ueland & Saebo, 1979; Fujioka & Takata, 1981; Kajander & Raina, 1981). These observations support the relevant role played by the enzyme in the regulation of AdoMet-dependent processes in prokaryotes.

The purification to homogeneity of the enzyme provides for the first time a definitive demonstration that a single enzyme is responsible for the catabolism of both AdoHcy and MTA in *E. coli*, confirming the previous hypothesis of Duerre (1962) and Ferro *et al.* (1976).

The enzyme is highly specific with respect to the substrate, as indicated by substrate-specificity and inhibition studies. The lack of inhibitory effect by AdoHcy analogues modified in the sugar moiety (AdoHcy dialdehyde and acyclic AdoHcy) suggests that an intact ribose ring is required for the binding process. Moreover, the poor inhibition by 6-amino or N-3-modified analogues (Table 3) argues for a direct involvement of the 6-amino group and the N-3 atom in the enzyme–substrate binding. Conversely, the results with 5′-side-chain-modified analogues (Table 2) indicate that such a region of the molecule is not relevant in the recognition mechanism, confirming the data reported by Ferro *et al.* (1976) with other analogues.

In conclusion, four recognition sites can be postulated, i.e. the amino group of adenine, N-3 of the purine ring, as well as, possibly, the two hydroxyl groups of the ribose moiety (Fig. 4).

The inhibition experiments reported in Table 3 and Figs. 2 and 3 indicate a mechanism of catalysis requiring as the key step the protonation at N-7 of the substrate, by analogy with the mechanism postulated for the acid hydrolysis of adenosine, which involves the protonation of N-7, followed by an attack by hydrogen on the protonated nucleoside (Garrett & Mehta, 1972). In fact, the 7-deaza analogues of MTA and AdoHcy are not substrates, and exert a powerful competitive inhibition on the enzyme, thus indicating that N-7 is not relevant in the recognition, although it is critical in the catalytic mechanism. Moreover, the high affinity of formycin derivatives (K_i 9.7 nM and 27 nM) can be explained by the occurrence of an N-7 atom protonated at physiological pH (Ward *et al.*, 1969), thus resembling the transition state hypothesized in Fig. 4.

The powerful inhibition exerted by formycinyl analogues on AdoHcy/MTA nucleosidase makes this class of compounds candidates for antibacterial therapy. It is noteworthy in this respect that 5′-methylthioformycin is metabolically stable and permeates biological membranes (Cartenì-Farina *et al.*, 1984). Studies are required to evaluate the effect of these analogues on the bacterial growth.

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