*Biochemical Pharmacology*, Vol. 31, No. 4, pp. 503–507, 1982.
Printed in Great Britain.

0006-2952/82/040503–05 $03.00/0


# STRUCTURAL ANALOGS OF 5′-METHYLTHIOADENOSINE AS SUBSTRATES AND INHIBITORS OF 5′-METHYLTHIOADENOSINE PHOSPHORYLASE AND AS INHIBITORS OF HUMAN LYMPHOCYTE TRANSFORMATION

MICHAEL W. WHITE*, ARTHUR A. VANDENBARK†‡, CAROLYN L. BARNEY† and ADOLPH J. FERRO*§

*Department of Microbiology, Oregon State University, Corvallis, OR 97331, U.S.A., †Surgical Research Laboratory, Veterans Administration Medical Center, Portland, OR 97201, and ‡Department of Microbiology and Immunology and Department of Neurology, University of Oregon, Health Sciences Center, Portland, OR 97201, U.S.A.

(*Received* 21 *March* 1981; *accepted* 24 *June* 1981)

**Abstract**—5′-Deoxy-5′-methylthioadenosine (MTA) phosphorylase was purified 13.4-fold from human peripheral lymphocytes. The enzyme demonstrated normal Michaelis–Menten kinetics with $K_m$ values of 26 $\mu$M and 7.5 mM for the two substrates, MTA and phosphate, respectively. The rate of MTA degradation was temperature dependent, 47° being the optimum temperature. Five structural analogs served as alternative substrates with $K_m$ values ranging from 31 to 53 $\mu$M while two compounds, 5′-deoxy-5′methylthiotubercidin (MTT) ($K_i$ = 31 $\mu$M) and adenine ($K_i$ = 172 $\mu$M), were inhibitory. These same analogs were examined as inhibitors of mitogen-induced human lymphocyte blastogenesis. MTT was found to be the most effective inhibitor of lymphocyte transformation with an $I_{50}$ of 80 $\mu$M.

The progression of cells from a quiescent to a proliferating state is accompanied by marked increases in the cellular levels of polyamines [1] as well as in the activities of the enzymes involved in their biosynthesis [2]. 5′-Deoxy-5′-methylthioadenosine (MTA)‖ is synthesized in mammalian tissue in stoichiometric quantities with the production of spermidine and spermine [3]. MTA, however, does not appear to normally accumulate intracellularly. Seidenfeld *et al.* [4] demonstrated recently that MTA levels in rat tissue are one order of magnitude smaller than those of *S*-adenosylmethionine and nearly two orders of magnitude lower than the levels of spermidine plus spermine in the same tissue. The rapid degradation of MTA in mammalian tissue is accomplished via MTA phosphorylase, an enzyme shown to exist in a variety of normal and transformed cells [5–10] and to be distinct from purine nucleoside phosphorylase [10].

Vandenbark *et al.* [11] described the cytostatic action of MTA to human peripheral lymphocyte cultures stimulated with mitogens, antigens, or allogeneic cells. The MTA-mediated inhibitory effect also was shown to be dose-dependent and readily reversible. Ferro *et al.* [8] subsequently found that MTA phosphorylase increased markedly during blastogenesis and that the 7-deaza analog of MTA, 5′-deoxy-5′-methylthiotubercidin (MTT), was a more potent inhibitor of the lymphocyte enzyme. The non-reversible inhibition of lymphocyte transformation by MTT, as compared to the reversibility of MTA, was suggested to be due, in part, to the resistance of the analog to degradation by MTA phosphorylase.

The present study was designed to further investigate the effects of MTA, MTT and additional structural analogs of MTA on lymphocyte transformation and to compare this to the substrate specificity of the MTA phosphorylase from human lymphocytes. In addition, a further characterization of the lymphocyte enzyme is described.

## MATERIALS AND METHODS

*Compounds.* 5′-[$^{14}$C-*methyl*]Methylthioadenosine was prepared by the procedure of Schlenk *et al.* [12] from *S*-adenosyl-L-[$^{14}$C-*methyl*]methionine (>40 Ci/mmole) which was obtained from the Amersham Corp., Arlington Heights, IL. MTA, tubercidin, adenosine, adenine and inosine were purchased from

§ To whom correspondence should be sent.

‖ Abbreviations: MTA, 5′-deoxy-5′-methylthioadenosine; ETA, 5′-deoxy-5′-ethylthioadenosine; PTA, 5′-deoxy-5′-propylthioadenosine; iPTA 5′-deoxy-5′-isopropylthioadenosine; BTA, 5′-deoxy-5′-butylthioadenosine; iBTA (SIBA), 5′-deoxy-5′-isobutylthioadenosine; MTT, 5′-deoxy-5′-methylthiotubercidin; MTI, 5′-deoxy-5′-methylthioinosine; DMTA, 5′-deoxy-5′-dimethylthioadenosine; MTR, 5-methylthioribose; MTR-1-P, 5-methylthioribose-1-phosphate; APRT, adenine phosphoribosyltransferase (EC 2.4.2.7); HEPES, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid; and PHA, phytohemagglutinin.

the Sigma Chemical Co., St. Louis, MO. ETA, PTA, iPTA, BTA, iBTA, and MTI were synthesized by established methods [13–15], as were MTT [16] and DMTA [17, 18].

*Lymphocyte transformation.* Lymphoctye transformation was carried out using purified cells from normal human donors as described previously [19].

*Preparation of cell extracts.* Purified lymphocytes were centrifuged at 15,000 *g* for 15 min, washed, and resuspended in 0.05 M sodium HEPES buffer (pH 7.2) containing 0.05 M $K_2HPO_4$, 3 mM mercaptoethanol, and 10% glycerol. The cells were freeze-thawed five times in liquid nitrogen, centrifuged at 7000 *g* for 20 min, and then acidified and heated at 60° according to the procedure of Toohey [20]. This procedure resulted in a 2.3-fold purification with a 95% yield. The pH heat-treated extract was precipitated with ammonium sulfate at 40, 50, and 60% saturations. The great majority of the enzyme activity was found in the precipitate of the 60% saturation. This precipitate was dissolved in the HEPES buffer (pH 7.2) and dialyzed against 20 volumes of the same buffer. This final preparation represented a 13.4-fold purification and 30% yield, as compared to the crude extract, and was utilized as the source of enzyme in the MTA phosphorylase assays.

*MTA phosphorylase assays.* MTA phosphorylase activity was determined by: (1) measuring the conversion of [$^{14}$C-*methyl*]MTA to [$^{14}$C-*methyl*]MTR-1-P [21] and/or (2) measuring the conversion of MTA to adenine. The standard reaction mixture for both methods, unless otherwise stated, contained in a total volume of 250 μl, 23 mM Na HEPES, 23 mM $K_2HPO_4$, 1.4 mM mercaptoethanol protein and either 70 μM [$^{14}$C-*methyl*]MTA ($6.6 \times 10^6$ cpm/μmole) or 70 μM MTA. The assay mixture was incubated at 47° for 30 min, the reaction was terminated by the addition of 50 μl of 1.8 M trichloroacetic acid, and the precipitate was removed by centrifugation at 11,000 *g* for 5 min. When [$^{14}$C-*methyl*]MTA was utilized as the substrate, a 0.2-ml aliquot of the centrifuged reaction mixture was applied to a Dowex 50 $H^+ \times 4$ (100–200 mesh) column (1 × 4 cm) equilibrated with 3 N $H_2SO_4$. The product, [$^{14}$C-*methyl*]MTR-1-P, is eluted directly into scintillation vials with 3 ml water and the radioactivity determined [21]. When unlabeled MTA was used as substrate, either MTA depletion or adenine formation was measured by high pressure liquid chromatography. An aliquot of the centrifuged assay mixture was injected into a Waters model M-6000A HPLC system equipped with a Waters μBondapak $C_{18}$ column and a model 440 detector (254 nm). The solvent used was 10 mM potassium acetate, 10% $CH_3CN$ with Pic B7 (Waters paired complex), pH 4.0, at 1.0 ml/min and a pressure of 1500 psi. Peak heights of MTA, its analogs and/or adenine were measured, and concentration values were determined from a standard curve run the same day. Standard curves were linear over a range of 0.01 to 1.0 nmoles of MTA or adenine with *r* values typically greater than 0.99. Protein concentrations were determined by the commercially available BioRad protein assay using bovine serum albumin as a protein standard [22].

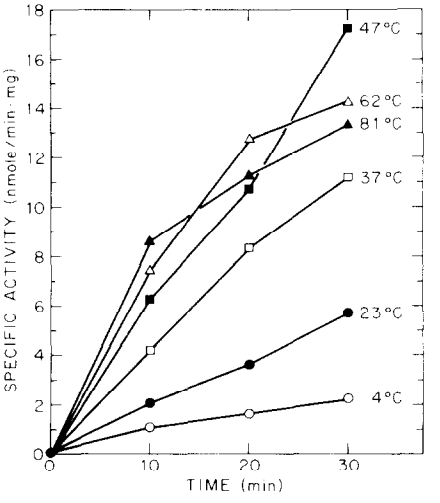


Fig. 1. Effect of temperature of incubation on MTA phosphorylase activity. Enzyme activity was determined by Method 1 as described in Materials and Methods at 4° (○), 23° (●), 37° (□), 47° (■) 62° (△), and 81° (▲) at the designated times. In each case controls were run to correct for any non-enzymatic breakdown of MTA.

## RESULTS

Initial experiments were designed to further characterize human lymphocyte MTA phosphorylase. Utilizing the 13-fold purified enzyme preparation, the effect of incubation temperature on enzyme activitiy was studied (Fig. 1). The data reveal that the rate of MTA breakdown was increased markedly by temperature elevation. At 4°, 23°, 37° and 47° the degradation rate was linear throughout the 30-min incubation period, whereas at 62° the rate was linear for only 20 min at 81° linearity did not exceed 10 min. Of the incubation temperatures which exhibited linearity throughout the 30-min period, the highest enzyme activity after 30 min was observed at 47°. Under these conditions (47° for 30 min) the rate of MTA degradation also was linear for the concentrations of protein and substrate used and, therefore, they were utilized for all further experiments. The enzyme was quite stable under the standard assay conditions; when the 13-fold purified enzyme was preincubated for 20 min at 47° and then assayed for activity after 30 min more of incubation at 47°, no loss in activity was observed. At 62°, however, loss in activity was observed beginning at 20 min of preincubation, while at 81° enzyme stability was decreased by preincubation for 10 min.

To further characterize the lymphocyte enzyme, the effects of MTA and phosphate concentration on the reaction rate were determined. The reaction velocity of MTA phosphorylase showed normal Michaelis–Menten kinetics with either MTA or phosphate as the variable substrate. Apparent $K_m$ values for MTA of 26 μM (Fig. 2) and for phosphate of 7.5 mM (Fig. 3) were calculated from double-reciprocal plots.

To analyze the substrate specificity of MTA phosphorylase, several MTA analogs and derivatives were tested as substrates and inhibitors. Since the

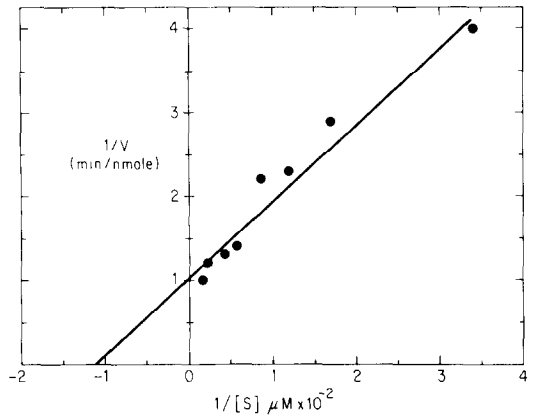


Fig. 2. Double-reciprocal plot of initial reaction velocity versus MTA concentration.

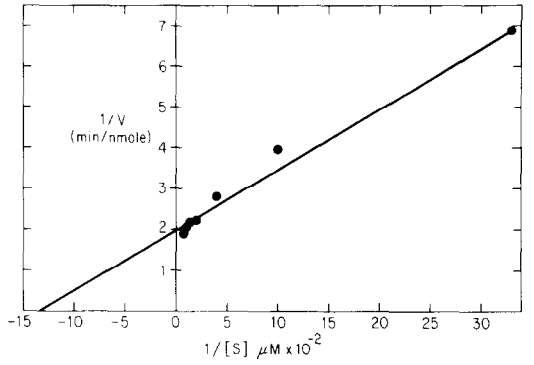


Fig. 3. Double-reciprocal plot of initial reaction velocity versus phosphate concentration.

synthesized analogs were not radioactively labeled, a new method to measure MTA phosphorylase activity was devised. The reaction mixture and conditions were the same as for the radioactively labeled assay except that unlabeled substrates were substituted for the [$^{14}CH_3$]MTA. High pressure liquid chromatography, as described in Materials and Methods, was employed to measure the quantity of free adenine and/or the quantity of remaining substrate present in the reaction mixtures. Neither the enzyme preparation nor the analogs contained any detectable adenine. Under the conditions described, the retention times of MTA and some of its analogs by h.p.l.c. are shown in Table 1.

Of the compounds tested, six served as substrates for MTA phosphorylase (Table 2). Three of these compounds (MTA, iPTA and ETA) served as better substrates than did iBTA, BTA or PTA. The naturally occurring substrate, MTA, had the lowest $K_m$ (26 μM) while the propyl analog had the highest $K_m$ (53 μM). The 7-deaza (MTT), the dimethyl (DMTA), and the deaminated (MTI) analogs, or adenine and adenosine did not serve as substrates for MTA phosphorylase. These latter compounds, therefore, were tested also for inhibitory activity. DMTA, MTI, and adenosine were without any significant effect, while MTT ($K_i$ = 31 μM) and adenine ($K_i$ = 172 μM) were found to be inhibitors of the lymphocyte enzyme activity.

Table 1. Retention times of MTA and some if its derivatives in potassium acetate–$CH_3CN$–Pic B7*

| Compound | Retention time (min) |
|---|---|
| Adenine | 3.2 |
| Adenosine | 3.3 |
| 5'-Deoxy-5'-dimethylthioadenosine | 4.9 |
| 5'-Deoxy-5'-methylthioinosine | 5.1 |
| 5'-Deoxy-5'-methylthioadenosine | 6.9 |
| 5'-Deoxy-5'-ethylthioadenosine | 11.4 |
| 5'-Deoxy-5'-methylthiotubercidin | 12.3 |
| 5'-Deoxy-5'-isopropylthioadenosine | 21.0 |
| 5'-Deoxy-5'-propylthioadenosine | 25.8 |
| 5'-Deoxy-5'-isobutylthioadenosine | 51.6 |
| 5'-Deoxy-5'-butylthioadenosine | 59.4 |

* Flow-rate, 1.0 ml/min.

Table 2. Kinetic constants of MTA and MTA analogs for MTA phosphorylase from human lymphocytes*

| Compound | $K_m$ (μM) | $K_i$ (μM) |
|---|---|---|
| 5'-Deoxy-5'-methylthioadenosine | 26 | |
| 5'-Deoxy-5'-isopropylthioadenosine | 28 | |
| 5'-Deoxy-5'-ethylthioadenosine | 31 | |
| 5'-Deoxy-5'-isobutylthioadenosine | 40 | |
| 5'-Deoxy-5'-butylthioadenosine | 46 | |
| 5'-Deoxy-5'-propylthioadenosine | 53 | |
| 5'-Deoxy-5'-methylthiotubercidin | | 31 |
| Adenine | | 172 |
| 5'-Deoxy-5'-dimethylthioadenosine | | |
| 5'-Deoxy-5'-methylthioinosine | | |
| Adenosine | | |

* All $K_m$ and $K_i$ values were determined by h.p.l.c. as described in Materials and Methods.

Table 3. Effects of MTA and MTA analogs on lymphocyte transformation*

| Compound | $I_{50}$ (μM) |
|---|---|
| 5'-Deoxy-5'-methylthiotubercidin (MTT) | 80 |
| 5'-Deoxy-5'-butylthioadenosine (BTA) | 210 |
| 5'-Deoxy-5'-isobutylthioadenosine (iBTA) | 330 |
| 5'-Deoxy-5'-propylthioadenosine (PTA) | 370 |
| 5'-Doxye-5'-isopropylthioadenosine (iPTA) | 450 |
| 5'-Deoxy-5'-methylthioadenosine (MTA) | 500 |
| 5'-Deoxy-5'-ethylthioadenosine (ETA) | 630 |
| Adenosine | 850 |
| 5'-Deoxy-5'-dimethylthioadenosine (DMTA) | >1000 |
| 5'-Deoxy-5'-methylthioinosine (MTI) | >1000 |
| Adenine | >1000 |

* PHA (15 μg) was added at $T_0$, [$^3$H]thymidine at $T_{48}$, and uptake of [$^3$H]thymidine determined at $T_{72}$. Nonstimulated controls: 800 cpm ± 0.2; stimulated controls: 94,000 cpm ± 9.3. All compounds were added at $T_0$ at the following concentrations: 62.5, 125, 250, 500, and 1000 μM.

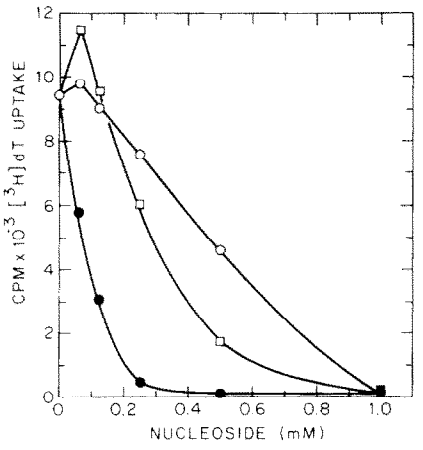


Fig. 4. Dose-dependent inhibition of PHA-stimulated human lymphocytes by MTA (○), SIBA (□), and MTT (●). Each point represents the mean of four replicate cultures. Individual values did not vary more than 10% from the mean.

MTA and its structural analogs were also tested as inhibitors of PHA-induced lymphocyte transformation as measured by [$^3$H]thymidine uptake. All compounds were added to the medium at the same time as the mitogen and were tested at various concentrations between 62.5 $\mu$M and 1 mM. Typical dose–response curves for MTA, iBTA (SIBA), and MTT are shown in Fig. 4 and the 50% inhibitory concentration ($I_{50}$) for each of these compounds was calculated (Table 3). The 7-deaza analog (MTT) was the most potent compound tested ($I_{50}$ = 80 $\mu$M) and was followed, in decreasing order of potency by, BTA, iBTA, PTA, iPTA, MTA, ETA, and adenosine. 5′-Deoxy-5′-methylthioinosine(MTI), 5′-deoxy-5′-dimethylthioadenosine (DMTA), and adenosine were not inhibitory ($I_{50}$ values = > 1 mM). 5′-Methylthioribose-1-phosphate (1.0 mM) and 5-methylthioribose (1.0 mM) were also tested and found not to exert any inhibitory effects under the same conditions.

## DISCUSSION

The apparent $K_m$ for MTA was found to be 26 $\mu$M, while the $K_m$ for phosphate was 7.5 mM. The $K_m$ for MTA, therefore, is similar to the values obtained from rat liver [23], human prostate [7] and Sarcoma 180 cells [9], and suggests that the low levels of this nucleoside in mammalian tissues are attributable to the high affinity that the phosphorylase has for its substrate. Cacciapuoti *et al.* [6] demonstrated that the rate of MTA breakdown by the human placenta enzyme was increased markedly by temperature elevation; a maximum rate of degradation occurred at 67° when the reaction mixture was incubated for 60 min. We also have observed increased enzyme activity associated with increased temperature of incubation for the lymphocyte enzyme but, in addition, have found that over a 30-min incubation period the reaction is linear only at temperatures of 47° and below. At higher temperatures (62° and 81°), the reaction is linear for only shorter periods of time (20 min and 10 min respectively). Although the rate of degradation of MTA by the phosphorylase at 4° is only 13% of the rate at 47° this suggests that storage of cell extracts in phosphate-containing buffer at this low temperature will result in the degradation of any MTA present in the extracts. This may be particularly detrimental to those investigators wishing to determine the endogenous levels of this nucleoside.

The specificity of the human lymphocyte MTA phosphorylase for its substrate is rather strict compared to the *Escherichia coli* enzyme [21]. The replacement of the 6-amino group with a hydroxy group (MTI), the replacement of the bivalent sulfur in the thioether by a charged sulfonium group (DMTA), or the replacement of N-7 of adenine with a methinic radical (MTT) all result in complete loss of activity. Zappia *et al.* [7] have postulated that the human prostate MTA phosphorylase interacts with MTA at three sites: (1) the amino group of the adenine moiety, (2) N-7 of the purine ring, and (3) the sulfur atom in thioether conformation. Recently, however, Savarese *et al.* [24] found that 5′-deoxyadenosine was also a substrate for MTA phosphorylase from Sarcoma 180 cells, demonstrating that the substrate for the phosphorylase may be a non-sulfur containing compound. The human prostate enzyme also was found to be non-competitively inhibited by DMTA and it was suggested that this sulfonium compound may bind to a non-catalytic site on the enzyme [7]. Our data with the human lymphocyte enzyme, however, are not consistent with this since DMTA was not found to be inhibitory. Whether or not this apparent difference is due to differences in tissue specificities has not yet been determined.

Coward *et al.* [16] found that MTT is an inhibitor but not a substrate of rat ventral prostate MTA phosphorylase, while Zappia *et al.* [7] reported similar data with the human prostate enzyme. The data presented here are consistent with these reports. Three lines of evidence suggest that MTA need not be degraded to exert its inhibitory effect on lymphocyte blastogenesis: (1) neither adenine, MTR, nor MTR-1-phosphate, degradation products of MTA, is inhibitory to lymphocyte transformation; (2) the 7-deaza analog, MTT, is not degraded by the human lymphocyte phosphorylase, yet it is still a potent inhibitor of the blastogenesis process (it is possible, however, that MTT and MTA have different modes of action and/or that MTT is degraded *in vivo* by a heretofore undefined pathway); and (3) of the compounds which served as substrates for the lymphocyte MTA phosphorylase, MTA, iPTA and BTA had lower $K_m$ values than did iBTA, BTA, or PTA. If these $K_m$ values are compared to the $I_{50}$ values obtained for these same compounds, the group of compounds which served as the better substrates (MTA, iPTA, ETA) was also found to be the least inhibitory to the transformation process, while the group (iBTA, BTA, PTA) which had the higher $K_m$ values was found to be more inhibitory to lymphocyte blastogenesis. Although this is not an exact correlation, it does suggest that MTA and its analogs need not be degraded to inhibit lymphocyte

transformation. The inability to obtain a direct correlation between $K_m$ and $I_{50}$ values may be due to other requirements for inhibition, such as the rate of transport of each of the compounds and the specificity of the analogs at the site of action.

Adenine has been shown previously to be an inhibitor of MTA phosphorylase activity in rat lung [5] and rat liver [23] extracts. That adenine is an inhibitor of the *in vitro* human lymphocyte enzyme activity, yet has not effect on lymphocyte transformation is not surprising since adenine is found only in very low levels in human tissues due to its rapid conversion to AMP via adenine phosphoribosyltransferase (APRT). Individuals deficient in APRT activity have been shown to accumulate elevated levels of adenine and 2,8-dihydroxyadenine [25], but any effect of this deficiency on MTA phosphorylase activity remains to be elucidated.

5'-Deoxy-5'-isobutylthioadenosine (SIBA) is a powerful antiproliferative drug shown to inhibit the growth of transformed mouse mammary cells [26], cell transformation induced by oncogenic RNA or DNA viruses [27, 28], mitogen-induced lymphocyte blastogenesis [29], and the capping of herpes virus mRNA [30]. SIBA was originally synthesized as an analog of *S*-adenosylhomocysteine [31]. Zappia and co-workers [32], however, have suggested that this nucleoside more closely resembles MTA and found that human placenta MTA phosphorylase utilized SIBA as a substrate. Our data are consistent with the contention that SIBA is an MTA analog in that the human lymphocyte MTA phosphorylase can also utilize SIBA as a substrate. The pharmacological actions of this drug may therefore be ascribed to its structural similarity to MTA. Our data indicating that MTA need not be degraded to inhibit cellular proliferation also suggest, by analogy, that SIBA also need not be further metabolized to exert its cytostatic effects. It is noteworthy, in this respect, that MTA, ETA, and MTT were all found to be powerful *in vitro* inhibitors of both spermidine synthase and spermine synthase from the rat ventral prostate [33]. Recently, MTA, MTT, and SIBA were each shown to decrease the content of spermidine in virally transformed mouse fibroblast cells; cell growth, however, could not be restored by the exogenous addition of spermidine [34]. This suggests that these nucleosides have other inhibitory actions in addition to that observed on polyamine biosynthesis. Interestingly, we have found that MTA is an irreversible inactivator of *S*-adenosylhomocysteine hydrolase [35], which is the key enzyme involved in the degradation of *S*-adenosylhomocysteine, a potent inhibitor of transmethylation reactions.

*Acknowledgements*—This research was supported by Grants CA25756 and CA24151 from the National Cancer Institute, DHEW, and by a Research Career Development Award (CA00617) to A. J. F. from the National Cancer Institute.

## REFERENCES

1. H. T. Rupniak and D. Paul, in *Advances in Polyamine Research* (Ed. R. A. Campbell), Vol. 1, p. 117. Raven Press, New York (1978).
2. D. R. Morris, in *Advances in Polyamine Research* (Ed. R. A. Campbell), Vol. 1, p. 105. Raven Press, New York (1978).
3. A. E. Pegg and H. G. Williams-Ashman, *J. biol. Chem.* **244**, 682 (1969).
4. J. Seidenfeld, J. Wilson and H. G. Williams-Ashman, *Biochem. biophys. Res. Commun.* **95**, 1861 (1980).
5. D. L. Garbers, *Biochim. biophys. Acta* **523**, 82 (1978).
6. G. Cacciapuoti, A. Oliva and V. Zappia, *Int. J. Biochem.* **9**, 35 (1978).
7. V. Zappia, A. Oliva, G. Cacciapuoti, P. Galletti, G. Mignocci and M. Carteni-Farina, *Biochem. J.* **177**, 77 (1978).
8. A. J. Ferro, A. A. Vandenbark and K. Marchitto, *Biochim. biophys. Acta* **588**, 294 (1979).
9. T. Savarese, G. W. Crabtree and R. E. Parks, *Biochem. Pharmac.* **28**, 2227 (1979).
10. A. E. Pegg and H. G. Williams-Ashman, *Biochem. J.* **115**, 241 (1969).
11. A. A. Vandenbark, A. J. Ferro and C. L. Barney, *Cell. Immun.* **49**, 26 (1980).
12. F. Schlenk, C. R. Zydiek-Cwick and N. K. Hutson, *Archs. Biochem. Biophys.* **142**, 144 (1971).
13. K. Kikugawa and M. Ichino, *Tetrahedron Lett.* 87, (1971).
14. R. T. Borchardt, J. A. Huber and Y. S. Wu, *J. org. Chem.* **41**, 565 (1976).
15. S. K. Shapiro and F. Schlenk, *Biochim. biophys. Acta* **633**, 176 (1980).
16. J. K. Coward, N. C. Moola and I. D. Moyer, *J. med. Chem.* **20**, 500 (1977).
17. G. Toennies and J. J. Kolb, *Analyt. Chem.* **23**, 823 (1951).
18. L. W. Parks and F. Schlenk, *J. biol. Chem.* **230**, 295 (1958).
19. M. Jondal, G. Holm and H. Wigzell, *J. exp. Med.* **136**, 207 (1972).
20. J. I. Toohey, *Biochem. biophys. Res. Commun.* **83**, 27 (1978).
21. A. J. Ferro, A. Barrett and S. K. Shapiro, *Biochim. biophys. Acta* **438**, 487 (1976).
22. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
23. A. J. Ferro, N. C. Wrobel and J. A. Nicolette, *Biochim. biophys. Acta* **570**, 65 (1979).
24. T. M. Savarese, G. W. Crabtree and R. E. Parks, Jr., *Biochem. Pharmac.* **30**, 189 (1981).
25. K. J. Van Acker, H. A. Simmonds, C. Potter and J. S. Cameron, *New Engl. J. Med.* **297**, 127 (1977).
26. C. Terrioux, M. Crepin, F. Gros, M. Robert-Gero and E. Lederer, *Biochem. biophys. Res. Commun.* **83**, 673 (1978).
27. M. Robert-Gero, F. Lawrence, G. Farrugia, A. Berneman, P. Blanchard, P. Vigier and E. Lederer, *Biochem. biophys. Res. Commun.* **65**, 1242 (1975).
28. A. Raies, F. Lawrence and M. Robert-Gero, *Fedn. Eur. Biochem. Soc. Lett.* **72**, 48 (1976).
29. C. Bona, M. Robert-Gero and E. Lederer, *Biochem. biophys. Res. Commun.* **70**, 622 (1976).
30. B. Jacquemont and J. Huppert, *J. Virol.* **22**, 160 (1977).
31. J. Hildesheim, R. Hildesheim and E. Lederer, *Biochimie* **53**, 1067 (1971).
32. M. Carteni-Farine, Della Ragione, G. Ragosta, A. Oliva and V. Zappia, *Fedn. Eur. Biochem. Soc. Lett.* **104**, 266 (1979).
33. H. Hibasami, R. T. Borchardt, S. Chen, J. K. Coward and A. E. Pegg, *Biochem. J.* **187**, 419 (1980).
34. A. E. Pegg, R. T. Borchardt and J. K. Coward, *Biochem. J.* **194**, 79 (1981).
35. A. J. Ferro, A. A. Vandenbark and M. R. MacDonald, *Biochem. biophys. Res. Commun.* **100**, 523 (1981).