Vol. 108, No. 2, 1982 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
September 30, 1982 Pages 687-695

5'-METHYLTHIOADENOSINE METABOLISM AND METHIONINE SYNTHESIS IN MAMMALIAN CELLS GROWN IN CULTURE

Peter S. Backlund, Jr.[1,2] and Roberts A. Smith

Department of Chemistry, Division of Biochemistry, and
the Molecular Biology Institute, University of California,
Los Angeles, California 90024

Received August 11, 1982

The biosynthesis of methionine from 5'-methylthioadenosine was examined in a number of human and mouse cell lines. 5'-Methylthioadenosine added to the culture medium was rapidly converted to methionine, accumulating in cell protein. J111 cells and mouse spleen fibroblasts grew significantly in a medium in which 5'-methylthioadenosine replaced methionine. L1210 cells, which lack 5'-methylthioadenosine phosphorylase, did not grow in this medium, and human breast fibroblasts did not grow either, even though these cells have normal levels of 5'-methylthioadenosine phosphorylase.

Methionine can be synthesized from 5'-methylthioadenosine (MeSAdo)[3] in a variety of both prokaryotic and eukaryotic organisms (1-5). We have previously demonstrated methionine synthesis from MeSAdo in rat liver homogenates (1,6). The methionine produced contains carbons from the ribose portion of the molecule, in addition to the sulfur and the methyl group (1-3).

In mammals, the first step in this pathway is the formation of 5-methylthioribose-1-phosphate (1,6), by the enzyme 5'-methylthioadenosine phosphorylase (7). 5-Methylthioribose-1-phosphate is converted to the α-keto acid of methionine, which is then converted to methionine by a transamination reaction (6). In the process oxygen is consumed, and formate released (8).

In mammalian cells, MeSAdo is produced by direct cleavage of AdoMet (9), or as a stoichiometric by-product of the synthesis of the polyamines,

1. Current address; Laboratory of General and Comparative Biochemistry, NIMH, Bldg. 36, Rm 3A-19, Bethesda, MD 20205.
2. To whom correspondence should be addressed.
3. Abbreviations: MeSAdo, 5'-methylthioadenosine; AdoMet, S-adenosyl-L-methionine; TCA, trichloroacetic acid.

0006-291X/82/180687-09$01.00/0

spermidine and spermine (10). However, if a pathway for methionine synthesis from MeSAdo is functioning in the cell, no net consumption of methionine would result. This may be an important source of methionine for rapidly growing or malignant cells.

Previous studies in mammals have only been done using rat liver homogenates (1,6,8). In order to study methionine synthesis with intact cells, we examined several human and mouse cell lines. Significant methionine synthesis from MeSAdo was found in some cell lines, supporting the proposal that this is a significant salvage pathway for methionine in mammalian cells.

MATERIALS AND METHODS

MeSAdo was purchased from Sigma, and was used without further purification. [^{14}C-methyl]-MeSAdo was prepared from [^{14}C-methyl]-AdoMet by the method of Parks and Schlenk (11).

Growth of Cells in Culture: J111, human monocytic leukemia; HeLa, human uterine cervical carcinoma; HBrF, human breast fibroblasts; L1210, mouse lymphatic leukemia; and MSpF, mouse spleen fibroblasts; were maintained on McCoy's 5a (modified) medium (Grand Island Biological) with 10% or 15% fetal calf serum and gentamicin sulfate (50 mg/L), in 25 cm^2 flasks (Falcon).

For experiments using methionine free media, a reconstructed medium was used, which was the same as McCoy's 5a (modified), except that methionine and bactopeptone were not added. In addition, the fetal calf serum was dialized at 4°C against three changes of buffer containing: NaCl (8 g/L), KCl (0.4 g/L), and $NaHCO_3$ (0.35 g/L), then sterilized by filtration. For growth experiments, cells were plated at 75,000 to 150,000 cells/flask, and the protein was quantitated by the method of Lowry et al. (12). For L1210 cells, cell growth was determined by counting the cells in a hemocytometer, using trypan blue exclusion to indicate viable cells.

Incorporation of [^{14}C-methyl]-MeSAdo into Cells: Cells were grown in 75 cm^2 flasks (Falcon). The medium was then changed to one containing either 0.05 mM [^{35}S]-L-methionine (1 μCi/ μmol) or 0.05 mM [^{14}C-methyl]-MeSAdo (1.6 μCi/ μmol), and the cells allowed to grow another 24 hours. The cells were washed 3 times with ice cold Delbecco's phosphate buffered saline, extracted with 2.5 ml of cold 5% TCA, and the radioactivity in the supernatant and the precipitate was determined. To measure radioactive methionine in cell protein, the TCA precipitate was hydrolyzed in 6 N HCl for 24 hr at 110°C and the amino acids were separated by paper chromatography, using Whatman #1 paper and a solvent system of n-butanol:acetic acid:water (15:3:5).

5'-Methylthioadenosine Phosphorylase Assay: Cells were removed from 75 cm^2 flasks, rinsed with 5 ml Delbecco's phosphate buffered saline, and resuspended in 1.5 ml of 0.05 M potassium phosphate, pH 7.2, 0.5 mM reduced glutathione. The cells were then lyophilized, resuspended in 1 ml of water, and centrifuged at 50,000 x g for 1 hr at 4°C. The supernatant was used to assay for enzyme activity, in an assay mixture containing: 50 mM sodium phosphate, pH 7.0, 30 mM 2-mercaptoethanol, 0.1 mM [^{14}C-methyl]MeSAdo (5,000 cpm/nmol), in a final volume of 0.1 ml. The reaction was started by addition of the cell extract (0.05 to 0.25 mg protein) and was incubated at 37°C for 30 min before stopping the reaction with cold methanol.

MeSAdo was separated from the products by high pressure liquid chromatography, using a μ-Bondapak C_{18} reverse phase column (3.9 mm x 30 cm, Waters Assoc.) with an elution buffer of 4 mM sodium heptane sulfonate, 7% methanol, 2% acetonitrile, adjusted to pH 3.2 with acetic acid, with a flow rate of 1.5 ml/min at 40°C.

RESULTS

To demonstrate the conversion of MeSAdo to methionine with intact cells, several mammalian cell lines were grown for up to 5 days in a culture medium where 0.1 mM L-methionine was replaced with 0.1 mM MeSAdo (Fig 1 & 2). When 0.1 mM MeSAdo was added instead of methionine, varied growth rates were observed with different cell lines. J111 cells (Fig 1A), and mouse spleen

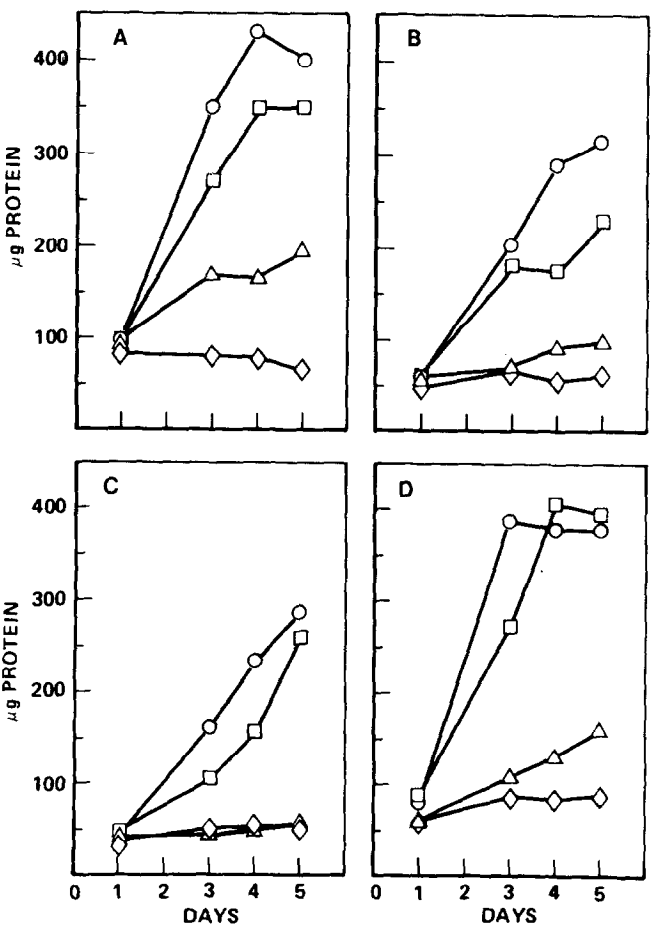

Figure 1. Cell growth with methionine or MeSAdo in medium. Cells were grown in methionine deficient medium, as described. Growth of: A. J111 cells, B. HeLa cells, C. Human breast fibroblasts, D. Mouse spleen fibroblasts. The following additions were made to the medium: 0.1 mM L-methionine, ○ ; 0.1 mM MeSAdo, △ ; 0.1 mM L-methionine and 0.1 mM MeSAdo, □ ; no addition, ◇ .

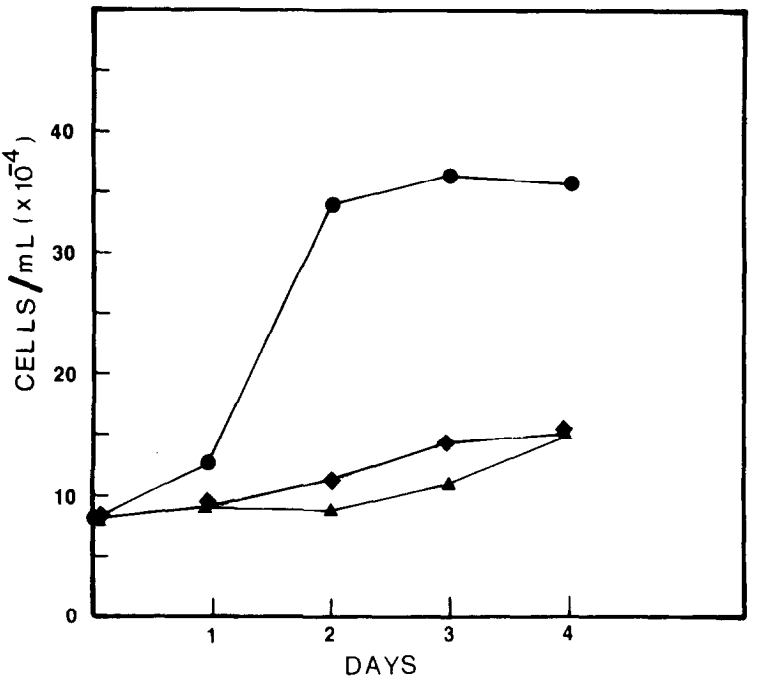

Figure 2. Growth of L1210 cells with methionine or MeSAdo in medium. L1210 cells were grown under the conditions described, with the following additions to the medium: 0.1 mM L-methionine, ● ; 0.1 mM MeSAdo, ▲ ; no addition, ◆ .

fibroblasts (Fig 1D) both showed significant growth in the medium containing MeSAdo, but the rate was not as high as with methionine present. HeLa cells grew only marginally with MeSAdo present (Fig 1B). The human breast fibroblasts (Fig 1C) and L1210 cells (Fig 2) did not grow at all with MeSAdo.

Cells were also grown with both methionine and MeSAdo in the medium to observe the combined effects of both compounds. The addition of 0.1 mM MeSAdo to the medium which already contained 0.1 mM methionine consistantly caused a slight decrease in cell growth (Fig 1).

Since J111 cells grew well with MeSAdo in the absence of methionine, the effect of MeSAdo concentration on J111 cell growth was examined. The initial growth rate for each concentration was calculated from the slope of a plot of the logarithm of cell protein vs. time. The growth rate vs concentration of MeSAdo was then plotted (Fig 3). Cell growth showed a hyperbolic dependence on the concentration of MeSAdo or methionine, with the growth rate appearing to saturate between 0.05 mM and 0.1 mM for either compound.

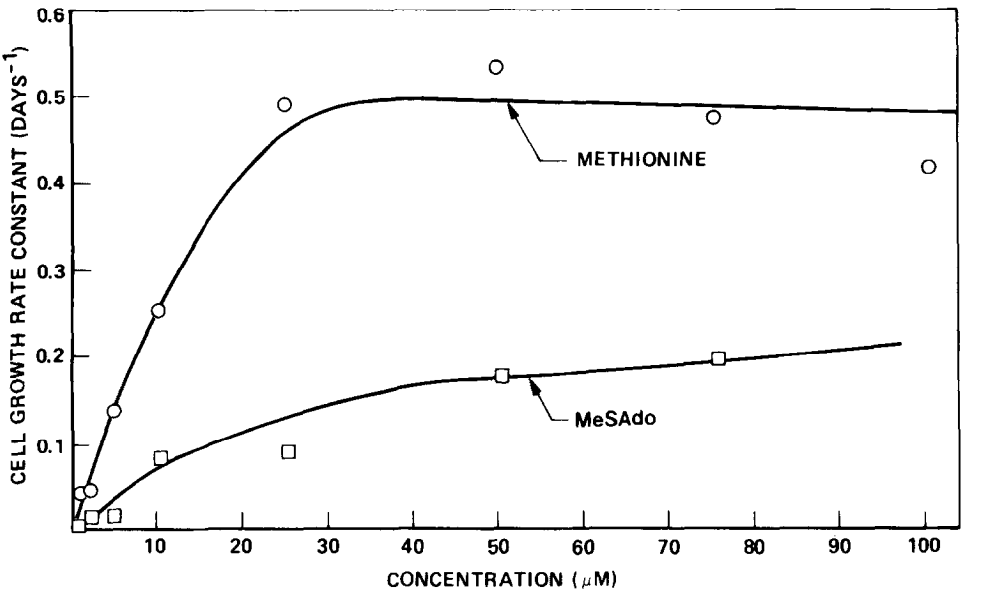

Figure 3. J111 cell growth vs concentration of methionine or MeSAdo. Cells were plated a 100,000 cells/flask and the indicated concentrations of methionine or MeSAdo were added. Growth of the cells was measured for four days and the growth rate determined from the slope of the plot of the logarithm of cell protein vs time. methionine, ○ ; MeSAdo, □ .

The maximal growth rate with MeSAdo was about one third the maximal growth rate with methionine (Fig 3). Therefore, J111 cells could replace a significant portion of their methionine requirement with MeSAdo from the medium.

To directly measure the conversion of MeSAdo into methionine, J111 and HeLa cells were grown in the presence of [^{14}C-methyl]-MeSAdo. When either [^{14}C-methyl]-MeSAdo or [^{35}S]-methionine were added, radioactivity was rapid-

Table I

Incorporation of 5'-Methylthioadenosine and Methionine into Cells

Cell Line	Cell Fraction	Labeled Compound in Medium			
		[^{35}S]-methionine		[^{14}C-methyl]-MeSAdo	
		cpm/mg protein	% Total	cpm/mg protein	% Total
HeLa	5% TCA soluble	9,300	21	6,300	5
	5% TCA ppt.	34,400	79	122,200	95
J111	5% TCA soluble	5,600	16	6,600	6
	5% TCA ppt.	29,300	84	104,000	94

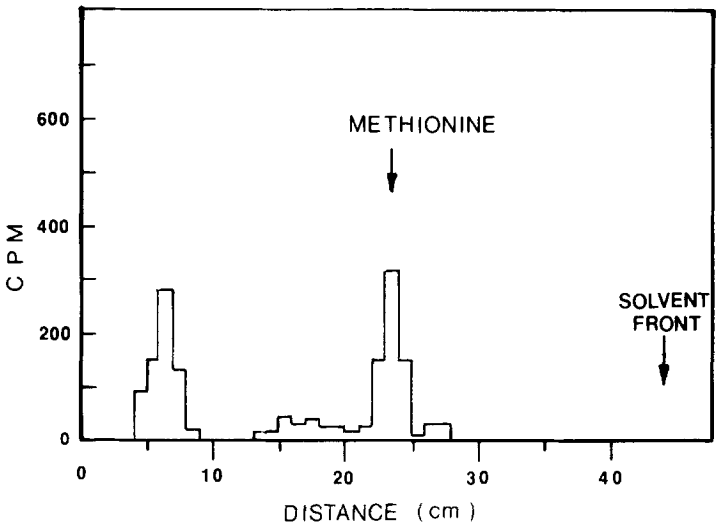

Figure 4. Paper chromatography of acid hydrolysate of protein from J111 cells grown with [^{14}C-methyl]-MeSAdo. Cell protein from J111 cells grown with [^{14}C-methyl]-MeSAdo was hydroylzed, and the radioactive hydrolysate chromatographed on paper. Standard methionine was also chromatographed and detected with ninhydrin, with its migration indicated by the arrow.

ly incorporated into the cells, accumulating in the cell fraction which precipitated with 5% cold TCA (Table I). The distribution of radioactivity between the 5% TCA soluble and insoluble fractions was similar for both MeSAdo and methionine. The incorporation of radioactivity into the 5% TCA insoluble fraction was actually greater with MeSAdo than with methionine (Table I). The 5% TCA precipitate from J111 cells was hydrolyzed in 6 N HCl and the amino acids then chromatographed on paper. A peak of radioactivity co-migrating with methionine was observed (Fig 4), indicating that MeSAdo had been converted to methionine and incorporated into protein by the cells.

It has been reported that L1210 cells and several leukemic cell lines lack the enzyme 5'-methylthioadenosine phosphorylase (14-16). Since this enzyme is the first step for methionine synthesis from MeSAdo, cells lacking this enzyme should not grow when MeSAdo replaces methionine in the medium. When 5'-methylthioadenosine phosphorylase activity was determined for each cell line, L1210 cells lacked enzyme activity, while the other cell lines had high levels of activity (Table II), including human breast fibroblasts which did not grow on MeSAdo. Therefore, the absence of HBrF growth with MeSAdo must be attributed to a lack of some other step in this pathway.

Table II

5'-Methylthioadenosine Phosphorylase Activity in Cell Lines

Cell Line	MeSAdo Phosphorylase Activity (nnol/mg protein/hr)	Growth on MeSAdo
J111	119	Yes
HBrF	130	No
HeLa	119	Slight
MSpF	87	Yes
L1210	< 10	No

DISCUSSION

The ability of J111 cells and mouse spleen fibroblasts to grow with MeSAdo added to a methionine deficient medium, indicates that these cells synthesize methionine from MeSAdo taken up from the medium. Significant amounts of methionine can be synthesized by this pathway, and it is the major product of MeSAdo metabolism for these cells in vivo, as indicated by the radioactivity incorporated into methionine. However, the growth rates with MeSAdo never equaled the rates observed with methionine in the medium. J111 cells grew well with MeSAdo in the medium, while HeLa cells grew very slowly under the same conditions. The difference in growth rates may be due to differences in MeSAdo transport, accumulation of MeSAdo metabolites which inhibit growth, or these cells may require higher levels of free methionine.

The metabolism of MeSAdo to methionine provides the cell with a mechanism to prevent accumulation of MeSAdo, a possibly harmful end-product of polyamine biosynthesis. The addition of MeSAdo inhibits phytohemagglutinin stimulated transformation of lymphocytes (17,18), and the growth of SV40 transformed 3T3 cells (19). However, it has been proposed that accumulation of MeSAdo might offer a selective growth advantage for cells lacking MeSAdo phosphorylase (15,16). We found that MeSAdo consistently inhibited cell growth (Fig 1), but the effect was not large for 0.1 mM MeSAdo. The results

shown here, and those previously reported by others (17-19), suggest that the accumulation of MeSAdo inhibits cell growth.

An alternate pathway for MeSAdo metabolism has been proposed by Toohey (14,20), in which MeSAdo produces a "methylthio" product necessary for cell growth. Since Toohey used growth conditions in which adequate methionine was supplied in the medium, his observations are different from this methionine salvage pathway, and probably represents an alternate pathway for MeSAdo metabolism. However, methionine synthesis appears to be the major route of MeSAdo metabolism, in vivo, for J111 and HeLa cells, based on the accumulation of labeled methionine from MeSAdo.

Methionine is an essential amino acid for mammals, and the production of MeSAdo in the cell could represent a significant loss of methionine unless it was possible to salvage this compound into the methionine pool. Further investigation is required to determine the role of this pathway in the overall methionine and sulfur balance in various types of mammalian cells.

ACKNOWLEDGMENTS

We would like to thank Mrs. Dorothy Hardy for maintaining the cell lines, and for her assistance with the cell culture experiments. This work was supported in part by the Julius and Dorothy Fried Foundation and by Cellular and Molecular Biology USPHS Training Grant GM 07185.

REFERENCES

1. Backlund, P.S., Jr., and Smith, R.A. (1981) J. Biol. Chem. 256, 1533-1535.
2. Shapiro, S.K., and Schlenk, F. (1980) Biochim. Biophys. Acta 633, 176-180.
3. Shapiro, S.K., and Barrett, A. (1981) Biochem. Biophys. Res. Comm. 102, 302-307.
4. Sugimoto, Y., Toraya, T., and Fukui, S. (1976) Arch. Microbiol. 108, 175-182.
5. Giovanelli, J., Mudd, S.H., and Datko, A.H. (1981) Biochem. Biophys. Res. Comm. 100, 831-839.
6. Backlund, P.S., Jr., Chang, P.C., and Smith, R.A. (1982) J. Biol. Chem. 257, 4196-4202.
7. Ferro, A.J., Wrobel, N.C., and Nicolette, J.A. (1979) Biochim. Biophys. Acta 570, 65-73.
8. Trackman, P.C., and Abeles, R.H. (1981) Biochem. Biophys. Res. Comm. 103, 1238-1244.
9. Swiatek, K.R., Simon, L.N., and Chao, K.L. (1973) Biochem. 12, 4670-4674.
10. Tabor, C.W., and Tabor, H. (1976) Ann. Rev. Biochem. 45, 285-301.
11. Parks, L.W., and Schlenk, F. (1958) Arch. Biochem. Biophys. 75, 291-292.
12. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., and Randall, R.J. (1951) J. Biol. Chem. 193, 265-275.

13. Garbers, D.L. (1978) Biochim. Biophys. Acta 523, 82-93.
14. Toohey, J.I. (1978) Biochem. Biophys. Res. Comm. 83, 27-35.
15. Kamatani, N., and Carson, D.A. (1980) Cancer Res. 40, 4178-4182.
16. Kamatani, N., Nelson-Rees, W.A., and Carson, D.A. (1981) Proc. Natl. Acad. Sci. USA 78, 1219-1223.
17. Ferro, A.J., Vandenbark, A.A., and Marchitto, K. (1979) Biochim. Biophys. Acta 588, 294-301.
18. Vandenbark, A.A., Ferro, A.J., and Barney, C.L. (1980) Cell. Immun. 49, 26-33.
19. Pegg, A.E., Borchardt, R.T., and Coward, J.K. (1981) Biochem. J. 194, 79-89.
20. Toohey, J.I. (1977) Biochem. Biophys. Res. Comm. 78, 1273-1280.