Conversion of 5′-Deoxy-5′-methylthioadenosine and 5′-Deoxy-5′-methylthioinosine to Methionine in Cultured Human Leukemic Cells[1]

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ABSTRACT

5′-Deoxy-5′-methylthioadenosine and 5′-deoxy-5′-methylthioinosine, which are metabolized to the methionine precursor, 5-methylthioribose-1-phosphate, by 5′-deoxy-5′-methylthioadenosine phosphorylase and purine nucleoside phosphorylase, respectively, can serve as sources of methionine for cultured HL-60 promyelocytic leukemia cells. CCRF-CEM T-cell leukemia cells, which lack 5′-deoxy-5′-methylthioadenosine phosphorylase, convert 5′-deoxy-5′-methylthioinosine (but not 5′-deoxy-5′-methylthioadenosine) to methionine; this conversion is blocked by purine nucleoside phosphorylase inhibitors. Therefore, the pathway for the conversion of 5-methylthioribose-1-phosphate to methionine is present in both human leukemic lines.

INTRODUCTION

MTA[3] is formed from AdoMet as a by-product in the synthesis of the polyamines spermidine and spermine (16, 17). MTA is metabolized in mammalian tissues by the enzyme MTAPase, which catalyzes the following reaction:

$$\text{MTA} + P_i \rightleftharpoons \text{Adenine} + \text{5-methylthioribose-1-phosphate.}$$

Although adenine can be salvaged back to adenine nucleotide pools, the fate of the other product, MTR-1-P, was unclear until recently. Early studies on nonmammalian cells hinted that the methylthio-containing portion of MTA was converted in some manner to methionine (1, 22, 23, 25). Recently, Backlund and Smith (3) showed that all carbon atoms from the ribose moiety of MTA except C(1′) are incorporated together with the methylthio group into methionine by rat liver homogenates. Subsequently, it was shown that MTR-1-P can be converted to formate and α-keto-γ-methylthiobutyrate, the α-keto acid of methionine, in a series of reactions requiring 2 oxidative steps and accompanied by the consumption of O_2 (27). α-Keto-γ-methylthiobutyrate then undergoes a glutamine-dependent transamination, yielding methionine and α-ketoglutaramate (2, 6).

The conversion of MTA to methionine has been studied in a limited number of mammalian tissues (3, 4, 13, 20, 26, 27). Previously, it has been shown that some human and murine cell lines are capable of utilizing MTA as a methionine source while others are not (4). Here we have observed the conversion of MTR-1-P to methionine in an MTAPase-deficient line, the CCRF-CEM human T-cell leukemia (10), and a line containing MTAPase, the HL-60 human promyelocytic leukemia. In addition to contrasting MTA metabolism in these 2 lines, the ability of these cells to utilize MTI to methionine was also evaluated. MTI is metabolized to the methionine precursor MTR-1-P and hypoxanthine by the enzyme PNP (EC 2.4.2.1) (24) and is therefore a potential source of methionine to PNP-containing cells. Portions of this work have been communicated in preliminary form (13, 20).

MATERIALS AND METHODS

Materials. The following chemicals were purchased from Sigma Chemical Co., St. Louis, Mo.: D,L-dithiothreitol; glutamine; reduced glutathione; MTA; methionine; iodoplatinate spray reagent; ninhydrin; and xanthine oxidase, Grade III. 8-Aminoguanosine and 5′-chloroformycin B were gifts of Dr. Shih-Hsi Chu of Brown University. Baker-flex cellulose microcrystalline thin-layer chromatographic plates were purchased from J. T. Baker Chemical Co., Phillipsburg, N. J. [Methyl-^{14}C]AdoMet was purchased from New England Nuclear, Boston, Mass. [Methyl-^{14}C]MTA was synthesized from [methyl-^{14}C]AdoMet by the method of Schlenk and Ehninger (21) and isolated by reversed-phase HPLC using a Varian Model 4200 HPLC system equipped with a C_{18}-μBondapak column (3.9 mm inside diameter x 30 cm; Waters Associates, Milford, Mass.) MTA was eluted off the column with a linear gradient of 0 to 50% methanol over an 11-min period and then held at 50% methanol for 6 min. The retention time of MTA is approximately 15.3 min in this system. MTI was synthesized from MTA by nitrous acid deamination. Ten mg of MTA were dissolved in 1.25 ml of 2 N acetic acid and chilled on ice; to this solution, 0.75 ml of 100 mg $NaNO_2$ in H_2O was added dropwise with stirring; the mixture was allowed to stand for 2 to 3 hr at room temperature. MTI generated in this manner was isolated by reverse-phase HPLC (retention time of MTI under the above conditions, 12.3 min). [Methyl-^{14}C]MTI was prepared from [methyl-^{14}C]MTA in a similar manner. [Methyl-^{14}C]MTR-1-P used for the determination of R_f values was enzymatically synthesized from [methyl-^{14}C]MTA by reaction with partially purified MTAPase from Sarcoma 180 cells (19).

Enzyme Assays. Spectrophotometric assays were used to determine the MTAPase (19) and PNP (12) activities in 105,000 $\times$ g supernatant fluids of cells sonicated in 15 mM sodium phosphate (pH 7.3) and 1 mM D,L-dithiothreitol. PNP-associated MTI cleavage activity was measured by following the conversion of hypoxanthine (generated by the phosphorolysis of MTI) to uric acid (ΔE_{293} nm = 12.5 $\times$ 10^3 molar absorbance units) in reaction cuvets containing 400 μM MTI, 100 mM potassium phosphate (pH 7.4), 0.05 μM xanthine oxidase, and appropriate amounts of high-speed supernatant fluids of cell extracts. Protein concentrations were determined using kits obtained from Bio-Rad Laboratories (Richmond, Calif.) with bovine γ-globulin as standards.

Cell Culture Studies. HL-60 (8) and CCRF-CEM (7) cells are routinely grown in a humidified 37° incubator maintained in an atmosphere of 95% air:5% CO_2 in RPMI 1640 Medium (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal calf serum, penicillin (100 units/ml), and streptomycin (100 μg/ml). For the methionine-deficient

experiments, cells were washed twice in 0.9% NaCl solution and intro-
duced into 16- x 125-mm culture tubes (Falcon Plastics) containing 3 ml
of methionine-deficient RPMI 1640 Medium (made up from RPMI 1640
Medium Selectamine kits available from Grand Island Biological Co.)
supplemented with 7.5% horse serum. The initial cell concentration was
10^5 cells/ml. Horse serum was used in all growth studies because, unlike
fetal calf serum, it lacks MTAPase activity (9). After 120 hr of incubation,
cell concentrations were determined using a hemacytometer.

Tracer Studies. Assay conditions for the conversion of either [*methyl*-
^{14}C]MTA or [methyl-^{14}C]MTI to methionine were as detailed by Backlund
and Smith (3) with the following modifications: each 500-μl reaction
mixture contained whole-cell extracts (2 mg protein); and either 15 μM
[*methyl*-^{14}C]MTA (specific activity, 15,000 to 20,000 cpm/nmol) or 75 μM
[*methyl*-^{14}C]MTI (specific activity, 6,000 to 8,000 cpm/nmol). At appro-
priate times, aliquots of 50 μl were inactivated with 3 volumes of ice-
cold methanol. The conversion of [*methyl*-^{14}C]MTA to methionine was
monitored by spotting 50 μl of the methanolic extracts on cellulose thin-
layer sheets which were developed in 1-butanol:acetic acid:H_2O
(60:15:25). Chromatograms were visualized under 254 nm UV (for the
detection of nucleosides) and sprayed with potassium iodoplatinate (for
the detection of sulfur-containing compounds) and ninhydrin (for the
detection of amino acids). Individual spots were cut out, and radioactivity
was determined by liquid scintillation counting. The presence of methio-
nine was also verified by its reaction with cyanogen bromide according
to the method of Clark *et al.* (5). [*Methyl*-^{14}C]MTI conversion to methio-
nine was determined in a similar fashion except that only 20 μl were
spotted, and the plates were developed using distilled water as the
solvent (R_f values: MTA, 0.70; MTI, 0.46; methionine, 0.80; MTR-1-P,
0.89).

RESULTS AND DISCUSSION

The levels of MTAPase, PNP, and PNP-associated MTI cleav-
age activities of the 2 human leukemia cell lines are compared in
Table 1. Based on these enzyme patterns, it can be predicted
that HL-60 cells would produce MTR-1-P from both MTA and
MTI, whereas CCRF-CEM cells would produce this intermediate
only from MTI. Therefore, if these cell lines have the pathway for
the conversion of MTR-1-P to methionine, HL-60 cells should be
able to utilize both MTA and MTI as sources of methionine;
CCRF-CEM cells, in contrast, should be able to use MTI but not
MTA.

These predictions were borne out when these cell lines were
cultured in methionine-deficient medium supplemented with
either MTA or MTI (Chart 1). In the HL-60 line, MTA and MTI
were as effective as was methionine itself in supporting the
growth of these cells, at least up to a concentration of 50 μM.
The decreasing ability of MTA to support growth at concentra-
tions above 50 μM was probably related to the growth-inhibitory
activity of MTA toward these cells,[4] an effect noted in other cell
systems (14, 18, 28, 29). Because growth rates were similar at
suboptimal concentrations of methionine, MTA, or MTI, the
conversion of the nucleosides to methionine must be approxi-
mately stoichiometric.

As expected, MTAPase-deficient CCRF-CEM cells were una-
ble to utilize MTA as their sole methionine source (Chart 1*B*).
However, that these PNP-containing CCRF-CEM cells retain the
pathway for the conversion of MTR-1-P to methionine is dem-
onstrated by the fact that they grew in a methionine-deficient
medium supplemented with the PNP substrate, MTI.

MTI, used here as a biochemical tool, may be a metabolite of

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[4] T. M. Savarese, unpublished data.

MTA (11). Although MTA cannot be converted directly to MTI by
mammalian adenosine deaminase (15), MTR-1-P released from
the MTAPase reaction can react with free hypoxanthine as
catalyzed by PNP to form MTI (11). The physiological significance
of MTI is unknown. In any case, these studies provide the first
evidence that mammalian cells can convert this nucleoside to
methionine (20).

The metabolism of [*methyl*-^{14}C]MTA and [*methyl*-^{14}C]MTI
were studied in cell-free extracts of each cell line to demonstrate
directly their ability to convert these nucleosides to methionine
(Chart 2). The MTAPase-containing HL-60 cell extracts con-
verted [*methyl*-^{14}C]MTA to methionine with a kinetic pattern
similar to that originally observed in rat liver homogenates (3),
i.e., a rapid conversion of MTA to MTR-1-P, followed by a slow
increase in label associated with methionine as MTR-1-P levels
decline (Chart 2*A*). This cell line converted [*methyl*-^{14}C]MTI to
methionine in a similar manner (Chart 2*B*). In both cases, there
was virtually a complete conversion of labeled nucleosides to
methionine within the 4-hr period of study. The maximal rate of
formation of methionine in this cell line under these conditions
(determined from the data obtained with [*methyl*-^{14}C]MTI) was
15.5 nmol/hr/mg protein.

As predicted, extracts of the MTAPase-deficient CCRF-CEM
cells were unable to metabolize [*methyl*-^{14}C]MTA to MTR-1-P or
methionine (Chart 2*C*). The finding that MTA levels remained
unchanged after 4 hr of incubation in the presence of these
extracts suggests that there is no alternative route of metabolism
for this compound in this cell line. Nevertheless, these PNP-
containing cells were able to form MTR-1-P and methionine from
[*methyl*-^{14}C]MTI (Chart 2*D*), although MTI degradation occurred
at a slower rate than in HL-60 extracts, reflecting the 3-fold
higher PNP activity of the latter cell line (Table 1). The role of
PNP in the pathway for the formation of methionine from MTI in
these cells was further demonstrated by the following experi-
ment. Inhibitors of PNP were added to cultures of CCRF-CEM
cells utilizing MTI as their sole source of methionine. These
inhibitors of PNP, namely, 8-aminoguanosine ($K_i = 17 \mu$M) and
5'-chloroformycin B ($K_i = 10 \mu$M) (24), were able to produce a
significant inhibition of MTI-dependent cell growth under these
conditions, although these PNP inhibitors by themselves were
not cytotoxic (Table 2). Thus, inhibiting the formation of MTR-1-
P from MTI by establishing a blockade of PNP limits the synthesis
of methionine.

These studies demonstrate the critical roles of MTAPase and
PNP in the conversion of MTA and MTI, respectively, to methi-
onine in intact cells. The common product generated by both
these enzymes from their respective substrates is the methionine
precursor, MTR-1-P. Methionine synthesis fails to occur when

Chart 1. Growth of human leukemia cells in a methionine-deficient medium supplemented with either MTA or MTI. *A*, HL-60 promyelocytic leukemia cells; *B*, CCRF-CEM T-cell leukemia cells. *Points*, mean of 4 samples run in 2 independent experiments; *bars*, S.D. Experimental details are described under "Materials and Methods."
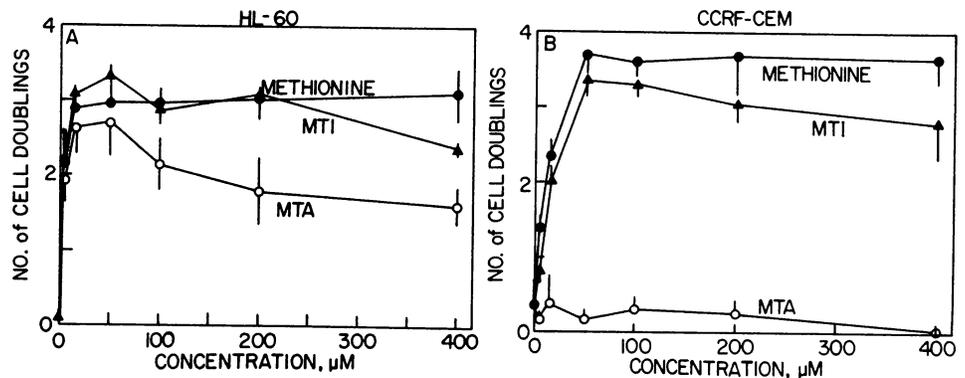

Chart 2. Conversion of [*methyl*-^{14}C]MTA or [*methyl*-^{14}C]MTI to MTR-1-P and methionine in sonicates of HL-60 (*A* and *B*) or CCRF-CEM (*C* and *D*). *Points*, mean of 2 samples run in 2 independent experiments; *bars*, range. Experimental details are described under "Materials and Methods."
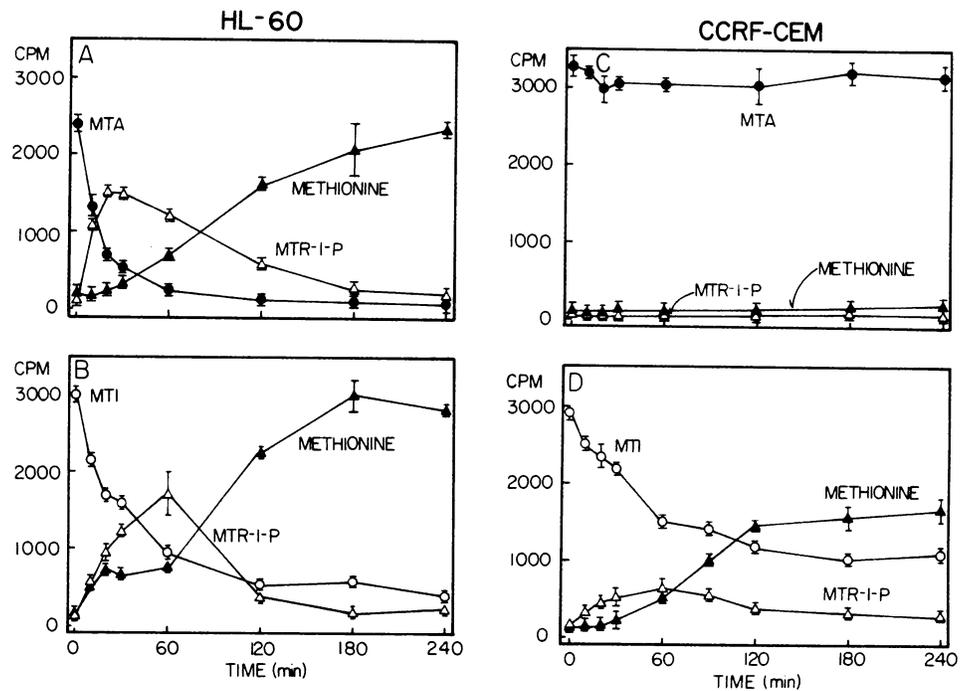
Table 2
Effect of inhibitors of PNP on the rescue of methionine-deprived CCRF-CEM leukemia cells by MTI

	No. of cell doublings
No addition	0.24 ± 0.08[a]
Methionine (50 µM)	3.50 ± 0.14
MTI (50 µM)	3.26 ± 0.06
MTI (50 µM) + 8-aminoguanosine (100 µM)	0.66 ± 0.15
MTI (50 µM) + 5′-chloroformycin B (100 µM)	1.00 ± 0.09
Methionine (50 µM) + 8-aminoguanosine (100 µM)	3.45 ± 0.08
Methionine (50 µM) + 5′-chloroformycin B (100 µM)	3.47 ± 0.03

[a] Mean ± S.D. of 6 samples run in 2 independent experiments.

the formation of MTR-1-P from MTA is prevented by MTAPase deficiency, *e.g.*, in CCRF-CEM cells or L1210 murine leukemia cells (4). A pharmacological blockade of PNP likewise decreases methionine formation from MTI. Nevertheless, the presence of MTAPase or PNP does not guarantee that a particular cell will convert MTA or MTI to methionine. A subline of the DLD-1 human colon carcinoma contains both enzymes but is unable to convert MTR-1-P to methionine (13, 20); this line is deficient in a single enzyme in the MTR-1-P-to-methionine pathway.[5] Also, a

[5] L. Y. Ghoda, et al., manuscript in preparation.

MTAPase-containing human breast fibroblast line has been shown to be unable to grow using MTA as a methionine source (4). Of course, cells grown in culture, where methionine is provided in excess, are not subjected to any selection pressure to retain or operate this pathway. Thus, metabolic deficiencies along the MTA-to-methionine route among cultured cell lines may not be uncommon. However, this pathway may be important in maintaining overall methionine and sulfur homeostasis *in vivo*, where methionine is frequently a limiting amino acid.

In the HL-60 cell line, the MTR-1-P-to-methionine pathway is active enough to convert either MTA or MTI to methionine in an apparently stoichiometric manner. This suggests that, at least in these cells, there is no alternative route for MTR-1-P metabolism other than its conversion to methionine. If this finding for HL-60 cells is generally true, one wonders why CCRF-CEM cells retain the enzymatic machinery for converting MTR-1-P to methionine despite the fact that these cells are unable to generate MTR-1-P from the natural metabolite, MTA. The existence of the pathway in CCRF-CEM cells is revealed through the use of MTI, which was demonstrated in these studies to be a useful biochemical tool for providing MTR-1-P to PNP-containing cells. Further studies are being undertaken to determine the preva-

lence and importance of the MTR-1-P-to-methionine pathway in normal tissues.

ACKNOWLEDGMENTS

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