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METABOLISM TO METHIONINE AND GROWTH STIMULATION BY 5'-METHYLTHIOADENOSINE AND 5'-METHYLTHIOINOSINE IN MAMMALIAN CELLS

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Viable human and murine lymphoblasts, and normal human tissue extracts, converted the thioether nucleosides 5'-methylthioadenosine (MeSAdo) and 5'-methylthioinosine (MeSIno) to methionine. Both MeSAdo and MeSIno, but not homocysteine, supported the short-term growth of human or murine lymphoblasts in methionine deficient medium. However, MeSAdo at concentrations greater than 25 μM inhibited cell growth. MeSIno was non-toxic at concentrations up to 200 μM, and supported the long-term growth of lymphoblasts in methionine-free medium.

The utilization of S-adenosylmethionine by mammalian cells for transmethylation reactions and polyamine synthesis, respectively, yields the thioether nucleosides adenosylhomocysteine (AdoHcy) and MeSAdo. Neither compound normally accumulates intracellularly. AdoHcy is cleaved by AdoHcy hydrolase to adenosine and homocysteine (1). MeSAdo is metabolized by MeSAdo phosphorylase to adenine and 5-methylthioribose-1-phosphate (2).

The conversion of homocysteine to methionine by mammalian cells has been studied extensively, and has been presumed to be the major methionine recycling pathway (3). However, Backlund and Smith recently showed that rat liver homogenates, and certain mammalian cells in tissue culture, synthesized methionine from 5-methylthioribose-1-phosphate (4-6). The latter compound may also condense with hypoxanthine to yield MeSIno, in a reaction catalyzed by purine nucleoside phosphorylase (7,8).

Abbreviations:
MeSAdo, 5'-deoxy-5'-methylthioadenosine; MeSIno, 5'-deoxy-5'-methylthioinosine.

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The extent to which various normal mammalian cells and tissue homogenates can generate methionine from MeSAdo and MeSIno is not known. Moreover, the relative importance of the homocysteine to methionine, and MeSAdo to methionine, biosynthetic pathways in proliferating mammalian cells has not been established.

In the present investigations, we have measured the conversion of MeSAdo and MeSIno to methionine by viable human and murine cells, and by normal human tissue extracts. We have also determined the relative capacities of MeSAdo, MeSIno and homocysteine to support cell proliferation under conditions of methionine limitation. The results indicate that the route for the biosynthesis of methionine from MeSAdo (and MeSIno) is widespread among human cell types, and may exceed in activity the homocysteine to methionine biosynthetic pathway.

MATERIALS AND METHODS

Cell Lines: The human B lymphocyte cell lines WI-L2 and 679 were obtained and grown as described previously (7). The murine L-1210 lymphoid leukemic cell line came from Dr. D. Jacobsen of this institution, and was maintained under the same conditions.

Methionine Synthesis from MeSAdo and MeSIno by Cells and Tissues: Logarithmically growing cells were washed twice in methionine free RPMI-1640 medium (prepared from a Selectamine kit, Grand Island Biological Company, Grand Island, NY) and then were resuspended in methionine-free and serum-free RPMI-1640 medium, supplemented as described by Darfler et al (9). To 4.0×10^6 cells in 0.25 ml of the above medium was added either 0.5 µCi [methyl-^{3}H]-MeSAdo (10 mCi/mmol), or 0.5 µCi [methyl-^{3}H]-MeSIno (10 mCi/mmol). After three hours at 37°C the cultures were terminated by the addition of ice cold perchloric acid to a final concentration of 0.4 N. After removal of precipitates by centrifugation (1,000 X g, 4°C, 15 mins.), the supernatants were neutralized with Alamine-Freon solution (7,10), and then were applied to a Partisil SCX column (Waters Associates, Milford, MA) that was eluted at a flow rate of 1 ml/min with water adjusted to pH 2.4 with trifluoroacetic acid. Absorption at 215 nm was monitored. One ml fractions were collected directly into scintillation vials, and radioactivity was determined at an efficiency of 25%, using a toluene based scintillation fluid. Methionine emerged at 13.5 minutes. When analyzed by thin layer chromatography in five solvent systems, the radioactive methionine fraction migrated with the same R_F as authentic methionine.

Fresh human tissues obtained at surgery were quickly frozen at -70°C. Subsequently, they were rapidly thawed in a 37°C

water bath, and then were homogenized at 4°C in an equal volume of water, using a Servall Omni-mixer (Sorvall Inc., Norwalk, CA). Particulate material was removed by centrifugation (14,000 X g, 4°C, 60 minutes). MeSAdo phosphorylase activities in the extracts were determined by the radiochemical method of Pegg and Williams-Ashman (2). To measure the conversion of MeSAdo to methionine, approximately 20 µg of protein in 100 µl of 7 mM sodium phosphate pH 7.0, 1 mM dithiothreitol, 1 mM $MgCl_2$, and 1 mM glutamine, was incubated with 1.5 µCi [methyl-^{3}H]-MeSAdo for 1 hour at 37°C. The incubations were terminated, and methionine formation was measured following high performance liquid chromatography, exactly as described above for cell lines. Protein was determined by the Coomasie blue method (11).

Cell Growth in Methionine Deficient Medium: Cells in logarithmic growth were washed twice in methionine deficient RPMI-1640 medium, and were suspended at a density of 10^5 cells/ml in the same medium supplemented with 10% dialyzed horse serum, 2 mM glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. Replicate cultures were incubated for three days with 3.6 µM to 200 µM MeSAdo or MeSIno, or with 15 µM to 1.5 mM homocysteine. At that time, the viable cell number was enumerated in a hemocytometer, using 0.1% trypan blue. Control cultures contained RPMI-1640 medium with 100 µM methionine.

Materials: Radioactive MeSAdo was prepared from [methyl-^{3}H]-S-adenosylmethionine (sp. act. 10 mCi/mmol, New England Nuclear, Boston, MA) by the method of Parks and Schlenk (12), and was purified before use by high performance liquid chromatography (7). The [methyl-^{3}H]-MeSIno was made from MeSAdo by the action of non-specific adenosine deaminase, and was also purified by high performance liquid chromatography.

RESULTS AND DISCUSSION

Conversion of MeSAdo to MeSIno and to Methionine by Intact Cells and Tissue Extracts: The WI-L2 lymphoblastoid cell line, that contains both MeSAdo phosphorylase and purine nucleoside phosphorylase (7), converted [methyl-^{3}H]-MeSAdo and [methyl-^{3}H-] MeSIno to methionine (Table 1). The MeSAdo phosphorylase deficient L-1210 cell line (13) synthesized methionine from [methyl-^{3}H]-MeSIno, but not from MeSAdo. In a reciprocal fashion, the purine nucleoside phosphorylase deficient 679 cell line (7) generated methionine from MeSAdo, but not from MeSIno. All of seven normal human tissue extracts contained substantial MeSAdo phosphorylase, and detectably converted [methyl-^{3}H]-MeSAdo to methionine (Table 2). The same cell extracts also converted MeSIno to methionine (results not shown), and contained abundant purine nucleoside phosphorylase (14).

TABLE 1

CONVERSION OF [^{3}H-METHYL] MeSAdo AND [^{3}H-METHYL] MeSIno

TO METHIONINE BY INTACT CELLS

Cell Line	Phosphorylase (nmol/min/mg)		dpm product per 10^7 cells per hour	
	MeSAdo	Ino	MeSAdo-Met	MeSIno-Met
WI-L2	1.58	108	216,800	203,000
L-1210	0.001	111	2,500	61,200
679	2.08	<1	154,500	0

4.0×10^6 cells were incubated with 0.25 ml serum-free RPMI-1640 medium lacking methionine, and supplemented with either 0.50 μCi [methyl-^{3}H]-MeSAdo , or [methyl-^{3}H]-MeSIno. The dpm incorporated into MeSIno, and into Met, were determined after three hours as described in the Methods. Ino, Inosine; Met, methionine.

Growth of Cell Lines in Methionine Deficient Medium: To estimate the quantitative importance of the various methionine biosynthetic pathways in viable lymphoblasts, we measured cellular proliferation in methionine deficient medium supplemented with various concentrations of MeSAdo, MeSIno, or homocysteine (Figure 1). The proliferation of the WI-L2, L1210 and 679 lymphoblastoid cell lines was markedly retarded in medium lacking supplemental methionine. When increasing concentrations of either MeSAdo or MeSIno were added to the same medium, the three cell lines responded differently. Both

TABLE 2

ENZYMATIC ACTIVITIES IN HUMAN TISSUES

	MeSAdo Phosphorylase (nmols/min/mg protein)	MeSAdo-Methionine (dpm/hour/mg protein)
Spleen	0.19	27,000
Colon	0.13	29,700
Lung	0.20	8,060
Breast	0.12	26,560
Thymus	0.17	40,500
Esophagus	0.60	38,080
Prostate	0.88	31,500

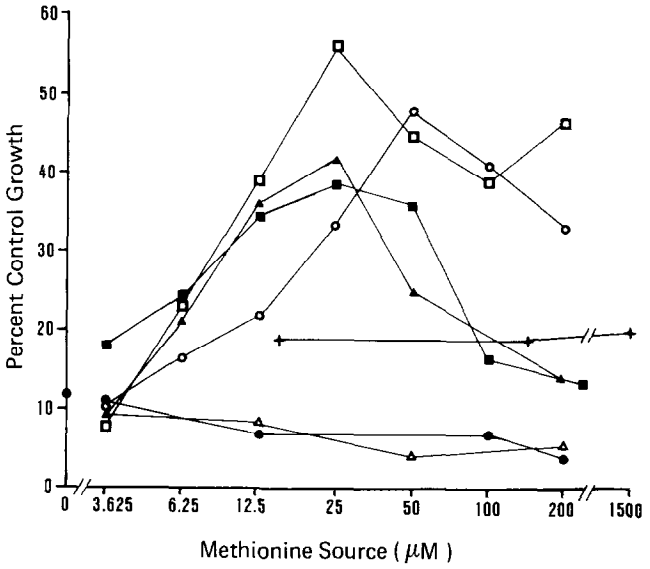

Figure 1: Cell growth in methionine deficient medium. Cells were grown for three days in methionine deficient RPMI-1640 medium supplemented with 10% dialyzed horse serum, and the indicated concentrations of either MeSAdo, MeSIno or homocysteine. Control cultures contained RPMI-1640 plus 10% dialyzed horse serum and 100 μM methionine. Percent control growth =

$$100 \frac{\text{Cells per ml in methionine deficient medium}}{\text{Cells per ml in methionine supplemented medium}}$$

WI-L2 in MeSAdo (■), WI-L2 in MeSIno (□), WI-L2 in homocysteine (+), 679 in MeSAdo (▲), 679 in MeSIno (△), L1210 in MeSAdo (●), L1210 in MeSIno (o).

MeSAdo and MeSIno augmented the growth of the WI-L2 cell line, that contains both MeSAdo phosphorylase and purine nucleoside phosphorylase. The proliferation of the MeSAdo phosphorylase deficient L-1210 cell line was enhanced by MeSIno but not MeSAdo. Finally, the growth of the purine nucleoside phosphorylase deficient 679 cell line was promoted by MeSAdo, but not MeSIno. Under the same conditions, 15 μM - 1.5 mM exogenous homocysteine did not support the growth of the cell lines. Recently, Kano et al also reported that many normal mammalian hematopoietic cell lines did not grow in a methionine-depleted medium that was supplemented with homocysteine (15). Thus, the failure to use homocysteine efficiently as a methionine source is not confined to malignant cells, as earlier reports had suggested (3).

In agreement with previous reports (16-18), MeSAdo at concentrations greater than 25 μM dose dependently inhibited the growth of all three cell lines in regular medium. Approximately 50-100 μM MeSAdo impeded growth by 50%. The toxicity of MeSAdo limited its ability to stimulate short-term cell growth in methionine deficient medium (Figure 1), and rendered impossible the prolonged maintenance of cells in MeSAdo supplemented medium. In contrast, MeSIno was non-toxic to cell lines at concentrations up to 200 μM, and therefore supported cell growth in methionine deficient medium more efficiently than did MeSAdo (Figure 1). Indeed, we have maintained L-1210 cells for more than 50 days in methionine free medium supplemented with 50 μM MeSIno. During this entire period, the lymphoblasts proliferated at approximately 40% of the rate of the same cells grown in methionine supplemented medium.

Taken together, these results indicate (i) that the ability to convert 5-methylthioribose-1-phosphate to methionine is widespread among mammalian cells and tissues, and (ii) that this pathway may exceed in quantitative importance the homocysteine to methionine biosynthetic pathway, at least in lymphoblastoid cell lines.

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