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5′-DEOXY-5′-METHYLTHIOADENOSINE PHOSPHORYLASE—III

ROLE OF THE ENZYME IN THE METABOLISM AND ACTION OF 5′-HALOGENATED ADENOSINE ANALOGS*

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Abstract—5′-Deoxy-5′-halogenated adenosines are alternative substrates for 5′-deoxy-5′-methylthioadenosine phosphorylase (MTAPase), an enzyme responsible for the metabolism of 5′-deoxy-5′-methylthioadenosine (MTA), a by-product of polyamine biosynthesis. The relative reactivity of these nucleosides with MTAPase from HL-60 human promyelocytic leukemia cells is MTA > 5′-deoxy-5′-fluoroadenosine (5′-FlAdo) > 5′-chloro-5′-deoxyadenosine (5′-ClAdo) > 5′-bromo-5′-deoxyadenosine (5′-BrAdo) > 5′-deoxy-5′-iodoadenosine (5′-IAdo). In MTAPase-containing cells, the adenine released from the 5′-halogenated adenosine was incorporated into adenine nucleotide pools; cleavage by (MTAPase appeared to be the rate-limiting step in this process. 5′-BrAdo and 5′-IAdo were growth inhibitors (EC_{50} values < 10 μM) of MTAPase-containing cell lines (HL-60 human promyelocytic leukemia and the L5178Y murine lymphoblastic leukemia) but were much less active (EC_{50} values > 65 μM) against MTAPase-deficient cell lines (the CCRF-CEM human T cell leukemia and the L1210 murine leukemia). The full cytotoxicity of these compounds, therefore, appeared to be related to their phosphorolysis by MTAPase. Indirect evidence suggests that 5-halogenated ribose-1-phosphate derivatives of 5′-BrAdo or 5′-IAdo produced by the MTAPase reaction were the active metabolites of these 5′-halogenated adenosines.

5′-Deoxy-5′-methylthioadenosine phosphorylase (MTAPase‖) is responsible in mammalian tissues for the metabolism of 5′-deoxy-5′-methylthioadenosine (MTA), which is produced from *S*-adenosylmethionine primarily during the synthesis of the polyamines spermidine and spermine [1, 2]. The products of the MTAPase reaction, adenine and 5-methylthioribose-1-phosphate, are salvaged to adenine nucleotides and methionine, respectively; the presence of MTAPase, therefore, permits the recycling of the metabolic components of *S*-adenosylmethionine, thus preventing their depletion during polyamine synthesis (for a review, see Ref. 3).

Recently, MTAPase has been suggested as an activating enzyme for antimetabolites [4–6]. The general strategy has been to design analogs of MTA which could be cleaved by MTAPase to cytotoxic metabolites. For example, 2-fluoroadenine and 2,6-diaminopurine-containing derivatives of MTA have been shown to act as alternative substrates of MTAPase, releasing free adenine analog bases which are subsequently converted to cytotoxic adenine analog nucleotides [7]. There is also evidence that MTA-like molecules containing modifications in the 5′-position may also possess antiproliferative activity. Analogs of MTA in which the 5′-methylthio moiety has been replaced by various alkylthio substituents, e.g. 5′-ethylthio-, 5′-*n*-propylthio- or 5′-isobutylthio-, display growth inhibitory effects in a number of systems, including chick fibroblasts [8], a variety of murine leukemias [9], and lectin-stimulated human lymphocytes [10]. We have demonstrated that the inhibitory effects of 5′-deoxyadenosine on a number of metabolic pathways, including glycolysis and purine *de novo* biosynthesis [11], is the result of its cleavage by MTAPase and the metabolism of these cleavage products [4, 12]. This suggested that 5′-substituted MTA analogs other than those containing 5′-alkylthio moieties may act as alternative substrates of MTAPase and have biological activity. A screening of available 5′-substituted adenosine analogs led to the finding that 5′-deoxy-5′-iodoadenosine, a reagent used in the synthesis of 5′-deoxyadenosine, acts as a substrate of MTAPase. Other 5′-halogenated adenosines were subsequently found to be

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‖ Abbreviations: APRT, adenine phosphoribosyltransferase (EC 2.4.2.7); 5′-BrAdo, 5′-bromo-5′-deoxyadenosine; 5′-BrIno, 5′-bromo-5′-deoxyinosine; 5′-ClAdo, 5′-chloro-5′-deoxyadenosine; 5′-FlAdo, 5′-deoxy-5′-fluoroadenosine; 5′-IAdo, 5′-deoxy-5′-iodoadenosine; 5′-IIno, 5′-deoxy-5′-iodoinosine; 5-IR-1-P, 5-iodoribose-1-phosphate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; MTA, 5′-deoxy-5′-methylthioadenosine; MTAPase, 5′-deoxy-5′-methylthioadenosine phosphorylase; and PNP, purine nucleotide phosphorylase (EC 2.4.2.1).

cleaved by MTAPase. In this report, the role of MTAPase in the metabolic activation and antineoplastic activity of 5′-halogenated adenosines is explored. Portions of these findings have been reported in preliminary form [4, 5, 13].

MATERIALS AND METHODS

Materials. 5′-Deoxy-5′-methylthioadenosine, adenine and xanthine oxidase (Grade III) were purchased from the Sigma Chemical Co. (St. Louis, MO); 5′-deoxy-5′-iodoadenosine was purchased from the Aldrich Chemical Co. (Milwaukee, WI). 2-Fluoroadenine and 5′-amino-5′-deoxyadenosine were gifts of Dr. John A. Montgomery of the Southern Research Institute. 5′-Deoxy-5′-fluoroadenosine [14], 5′-chloro-5′-deoxyadenosine [15], 5′-bromo-5′-deoxyadenosine [15], 5′-bromo-5′-deoxyinosine [16] and 5′-deoxy-5′-iodoinosine [17] were synthesized as previously described.

Cell culture. HL-60 human promyelocytic leukemia cells, first established and characterized by Gallagher *et al.* [18], were obtained from Dr. Michael Wiemann of the Roger Williams General Hospital, Providence, RI. HL-60 cells were routinely grown as suspension cultures in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, penicillin (100 units/ml) and streptomycin (100 μg/ml) in a humidified incubator maintained at 37° in an atmosphere of 95% air:5% CO_2. For growth inhibition studies, cells were washed several times in saline and then introduced into 16 × 125 mm culture tubes (Corning Plastics, Corning, NY) in an RPMI 1640 medium containing 15% horse serum and appropriate concentrations of MTA analogs (3 ml final volume). Horse serum, unlike fetal calf serum, contains no MTAPase activity [19] and is, therefore, appropriate for studies involving MTA-like compounds. The initial cell concentration was 10^5 cells/ml. After 120 hr of incubation, cell numbers were determined by light microscopy using a hemacytometer.

Routine culture of the CCRF-CEM human T cell leukemia line [20] was carried out as previously outlined [7]. Growth inhibition studies with the 5′-halogenated nucleosides were performed as described above for the HL-60 cells, with the exception that the RPMI 1640 medium contained the following buffers and antibiotics: $NaHCO_3$ (0.075%), HEPES (10 mM), Tris (10 mM), fungizone (25 μg/ml), and gentamycin (20 μg/ml).

Growth inhibition studies with the L5178Y and L1210 cells were carried out using previously described procedures [21].

Partial purification of MTAPase from HL-60 cells. Approximately 7×10^8 HL-60 cells were grown in culture as described above. Cells were harvested by centrifugation and washed twice in normal saline. The following procedures were carried out at 4°. The cell pellet was resuspended in 6–10 ml of a 25 mM imidazole, 1 mM dithiothreitol (DTT), pH 7.4, buffer and sonicated. The suspension was then centrifuged at 105,000 *g* for 45 min. The supernatant fluid (approximately 9 ml) was transferred to a dialysis bag and rolled in Sephadex G150 powder to reduce volume [22]. The Sephadex powder was replaced

Table 1. Partial purification of MTAPase from HL-60 cells*

Method	Volume (ml)	Protein (mg/ml)	Activity (nmoles/min/ml)	Sp. act. (nmoles/min/mg protein)	Total activity (nmoles/min)	Yield (%)	Fold purification
Crude 105,000 *g* supernatant fluid	7	15	17.7	1.18	128.9	100	1
Chromatofocusing	9	0.48	11	23.1	99	80	19.6
Sephadex G150	20	0.05	2.2	41.5	44	35	35

* Experimental details are described under Materials and Methods.

every 30 min for five to six cycles; this procedure reduced the extract volume by about 60%. The extract was loaded onto a chromatofocusing column (0.9 × 30 cm) containing approximately 18 cm^3 of Polybuffer exchanger 94 resin (Pharmacia) equilibrated in the 25 mM imidazole, 1 mM DTT, pH 7.4, buffer. Before the enzyme preparation was added, the column was pretreated with 5 ml of 7-fold-diluted Polybuffer 74 (Pharmacia), 1 mM DTT, pH 4.0. The enzyme was eluted using the latter buffer at a column flow rate of 0.25 ml/min. Fractions (1.5 ml) were collected. MTAPase-containing fractions were pooled and concentrated using the Sephadex dehydration technique mentioned above. The pH of this pooled fraction was adjusted to 7.4 with 5 N KOH. The enzyme preparation was further purified by Sephadex G150 chromatography. The sample was loaded onto a 1.6 × 40 cm column equilibrated with 100 mM KCl, 1 mM DTT, and 25 mM potassium phosphate, pH 7.4; MTAPase activity was eluted using this buffer. Fractions (3 ml) were collected at a flow rate of 0.3 ml/min. The gel filtration step removes the Polybuffer 74 which was added to the enzyme preparation during the chromatofocusing step. Fractions containing MTAPase activity were pooled, and the volume was reduced by the Sephadex dehydration technique. The resulting samples were stored at −20°. A summary of a typical purification is presented in Table 1.

High performance liquid chromatography. A reversed-phase HPLC method [7] was used to demonstrate the liberation of adenine from 5'-halogenated adenosines. This technique was also used to check the purity of these nucleosides; all compounds used in these studies produced single, homogeneous peaks. An anion-exchange HPLC method [7] was used to quantitate adenine nucleotide pools; authentic adenine nucleotides were used as standards.

RESULTS

5'-Halogenated adenosines as substrates of MTAPase. If 5'-halogenated adenosines are substrates of MTAPase, they should be phosphorolyzed to adenine and a 5-halogenated derivative of ribose-1-phosphate. This should occur in cells with MTAPase activity, but not in cells that lack this enzyme. When extracts of MTAPase-containing (0.81 nmole/min/mg protein) HL-60 human promyelocytic leukemia cells were incubated with 5'-deoxy-5'-fluoroadenosine (5'-FlAdo), 5'-chloro-5'-deoxyadenosine (5'-ClAdo), or 5'-deoxy-5'-iodoadenosine (5'-IAdo), adenine was produced, as detected by reversed-phase HPLC. The results obtained with 5'-FlAdo are shown in Fig. 1, panels A and B. Extracts of the CCRF-CEM human T-cell leukemia line, which lacks MTAPase activity [19, 23], did not generate adenine from 5'-FlAdo (Fig. 1, panels C and D) or from any of the 5'-halogenated adenosines studied. To demonstrate further that MTAPase was responsible for the cleavage of these analogs, this enzyme was partially purified from HL-60 cells. 5'-ClAdo and 5'-IAdo cleaving activities remained associated with MTAPase activity, following an approximately 20-fold purification of the enzyme using chromatofocusing, which separates proteins on the basis of their isoelectric points (Fig. 2). Furthermore, the 5'-halogenated adenosine cleaving activities were not

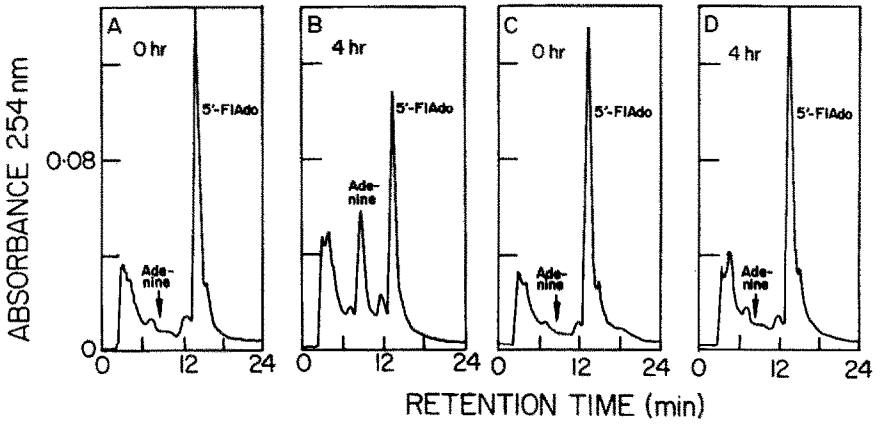

Fig. 1. (A and B) Reversed-phase HPLC profiles demonstrating the liberation of adenine from 5'-FlAdo in the presence of MTAPase-containing HL-60 cell extracts. (C and D) Reversed-phase HPLC profiles demonstrating the inability of MTAPase-deficient CCRF-CEM cells to liberate adenine from 5'-FlAdo. Exponentially-growing HL-60 or CCRF-CEM cells were harvested and washed twice in saline. Cells were suspended in 50 mM potassium phosphate (pH 7.4), 1 mM dithiothreitol, sonicated, and then spun at 105,000 *g* for 45 min. The supernatant fluid was dialyzed against the above buffer for several hours at 4°. Reaction mixtures (1 ml) containing 100 μM 5'-FlAdo, 50 mM potassium phosphate, pH 7.4, and the dialyzed high-speed supernatant fluids of the cell extracts (500 μg protein) were incubated in a shaking water bath at 37°. At the indicated times, samples were prepared for reversed-phase HPLC as described in Ref. 7. Reversed-phase HPLC analysis was carried out using a modification of a previously described method [7]; here the high concentrate eluent was 20% methanol in 5 mM potassium phosphate, pH 5.5. Also, the isocratic portion of the gradient was extended to 11 min in these studies. Adenine was identified based on the retention time of authentic standards.

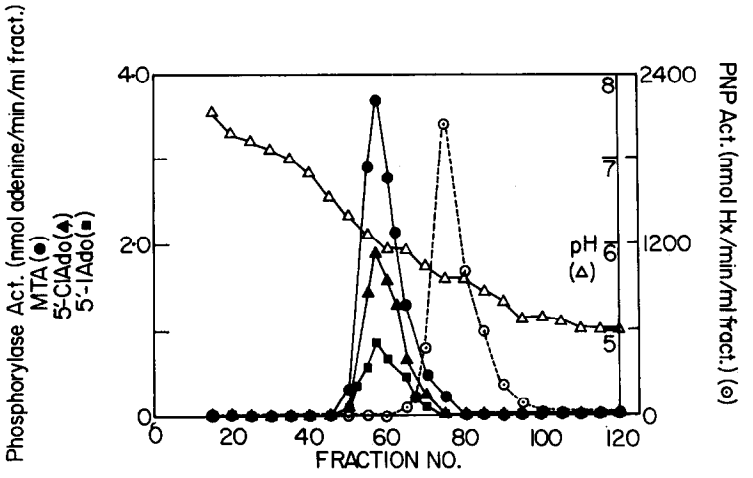

Fig. 2. Association of 5'-ClAdo and 5'-IAdo cleaving activities with MTAPase activity during partial purification of HL-60 cell MTAPase by chromatofocusing. Experimental details are described under Materials and Methods. 5'-ClAdo, 5'-IAdo, and MTA cleaving activities were assayed spectrophotometrically using 100 μM of these nucleosides as described previously [12]. PNP activity was assayed by the method of Kim *et al.* [24].

associated with purine nucleoside phosphorylase (PNP), the only other purine-specific nucleoside phosphorylase known in mammalian tissues.

The kinetic parameters of the 5'-halogenated adenosines with MTAPase partially purified from HL-60 cells are listed in Table 2. As in other tissues [7, 10, 12, 25–27], MTAPase from HL-60 cells had a low Michaelis constant for its natural substrate, MTA. The K_m values for the 5'-halogenated adenosines were also low, ranging within approximately 2-fold of that for MTA. The V_{max} values relative to MTA decreased with increasing size of the halogen atom substituent, i.e., 5'-FlAdo > 5'-ClAdo > 5'-BrAdo > 5'-IAdo.

If these 5'-halogenated adenosines are, in fact, substrates of MTAPase, it would be predicted that in intact cells these compounds would be cleaved to yield free adenine, which would then be converted to adenine nucleotides via APRT and the enzymes of adenine nucleotide interconversion. This should not occur in MTAPase-deficient cells. As shown in Table 3, adenosine triphosphate pools of cultured MTAPase-containing HL-60 cells treated for 2 hr with 100 μM MTA, 5'-FlAdo, 5'-IAdo, and adenine were increased 2.3-, 2.5-, 1.4-, and 2.9-fold, respectively, relative to untreated control cells. Two observations can be made here. First, the rate of incorporation of adenine into adenine nucleotides exceeds that of MTA or its analogs. Second, the analogs are incorporated at rates that reflect the relative V_{max} values of these compounds with MTAPase, e.g. 5'-IAdo, with a V_{max} value relative to MTA of 25%, was incorporated into adenine nucleotide pools at a rate of 28% relative to the rate of incorporation from

Table 2. Kinetic constants of 5'-halogenated adenosine analogs and related compounds with MTAPase from HL-60 cells*

	K_m (μM)	V_{max} (% rel. to MTA)
5'-Deoxy-5'-methylthioadenosine	5.5 ± 1.1	100
5'-Deoxy-5'-fluoroadenosine	11.4 ± 0.7	68 ± 6
5'-Chloro-5'-deoxyadenosine	11.6 ± 4.7	51 ± 9
5'-Bromo-5'-deoxyadenosine	2.4 ± 0.6	36 ± 5
5'-Deoxy-5'-iodoadenosine	8.1 ± 0.4	25 ± 5
5'-Amino-5'-deoxyadenosine	Weak activity†	

* Values were determined using the xanthine oxidase-coupled spectrophotometric assay described in Ref. 12. Reaction mixtures (1 ml) contained 50 mM potassium phosphate, pH 7.4, xanthine oxidase (0.8 μmoles product per min), HL-60 MTAPase (0.5 nmoles adenine formed per min) (partially purified as described in Materials and Methods) and appropriate amounts of nucleoside analogs. Results represent the mean ± S.D. of two to four independent determinations.

† Less than 2% activity relative to MTA at a concentration of 700 μM.

Table 3. Effects of MTA, adenine and 5′-halogenated adenosines on the adenine nucleotide pools of MTAPase-containing (HL-60 promyelocytic leukemia) and MTAPase-deficient (CCRF-CEM T-cell leukemia) cells*

	Nucleotide levels (nmoles/10^6 cells after 2-hr incubation)		
	AMP	ADP	ATP
		HL-60	
Control	0.10 ± 0.03	0.09 ± 0.04	1.32 ± 0.09
100 μM MTA	0.08 ± 0.02	0.17 ± 0.05	3.08 ± 0.06
100 μM 5′-IAdo	0.09 ± 0.01	0.12 ± 0.03	1.82 ± 0.08
100 μM 5′-FlAdo	0.16 ± 0.07	0.14 ± 0.02	3.28 ± 0.18
100 μM Adenine	0.23 ± 0.12	0.21 ± 0.06	3.81 ± 0.31
		CCRF-CEM	
Control	0.09 ± 0.02	0.10 ± 0.01	1.53 ± 0.06
100 μM MTA	0.06 ± 0.02	0.11 ± 0.02	1.44 ± 0.07
100 μM 5′-IAdo	0.10 ± 0.01	0.13 ± 0.01	1.57 ± 0.11
100 μM 5′-FlAdo	0.07 ± 0.03	0.11 ± 0.01	1.52 ± 0.03
100 μM Adenine	0.10 ± 0.02	0.20 ± 0.01	5.46 ± 0.16

* Exponentially-growing HL-60 or CCRF-CEM cells were harvested by centrifugation and washed twice in normal saline. The cells were then suspended in a minimal salt medium containing 50 mM potassium phosphate (pH 7.4), 75 mM NaCl, 2 mM $MgSO_4$, 10 mM D-glucose, 100 units/ml penicillin/streptomycin and the appropriate compound. Final cell concentration was 10^6 cells/ml. After 2 hr of incubation in a 37° shaking water bath, 8-ml aliquots were spun in a refrigerated centrifuge, and all but 0.25 ml of the supernatant fluid was removed. Perchloric acid was added to the cell pellets (final PCA concentration, 4%) on ice. After centrifugation, the supernatant fluid was neutralized to pH 6.5–7.5 with 5 N KOH. Samples were frozen at −20° and later analyzed for adenine nucleotides by a previously described anion-exchange HPLC method [7]. Values represent the mean ± S.D. of three independent determinations.

MTA. These findings indicate that MTAPase is the rate-limiting step in the formation of adenine nucleotides from MTA and these representative 5′-halogenated adenosine analogs.

In contrast, neither MTA nor any of the 5′-halogenated adenosines increased the adenine nucleotide pools of MTAPase-deficient CCRF-CEM leukemia cells under the same conditions (Table 3). Only when the MTAPase step was circumvented, i.e. when the cells were treated with adenine, were increases in the adenine nucleotides observed. All of the above findings are consistent with the concept

Table 4. Growth-inhibitory effects of 5′-halogenated adenosines and 5′-halogenated inosines on MTAPase-containing and MTAPase-deficient human and murine leukemic cell lines

	HL-60	CCRF-CEM	L5178Y	L1210
MTApase act. (nmoles/min/mg protein)	0.8	<0.01	1.1*	<0.01*
PNP act. (nmoles/min/mg protein)	130	43	11*	169*
		EC_{50}† (μM)		
5′-FlAdo	>200	>200	100	>100
5′-ClAdo	200	154	100	>100
5′-BrAdo	9	185	3	>100
5′-IAdo	3	66	3	>100
Adenine	>200	>200	>100	>100
5′-BrIno			>100	8
5′-IIno	56	>200	>100	8
Hypoxanthine	>200	>200	>100	>100

* Data from Ref. 13.

† EC_{50} Values represent the concentration of compound required to inhibit the number of cell doublings by 50%. Experimental details are described under Materials and Methods. Values are the mean of at least two independent determinations.

that 5'-halogenated adenosines are alternative substrates of MTAPase. These results support the findings of Kamatani *et al.* [28], who demonstrated that radioactivity from 5'-ClAdo tritiated in the adenine moiety becomes incorporated into the nucleic acids of MTAPase-containing, but not of MTAPase-deficient cells.

Role of MTAPase in the growth-inhibitory action of 5'-halogenated adenosines. To assess whether the metabolism of the 5'-halogenated adenosines by MTAPase results in the formation of growth-inhibitory metabolites, the antiproliferative activities of these compounds were compared in leukemic cell lines that either have, or are deficient in, MTAPase. In the case of 5'-BrAdo or 5'-IAdo, the growth inhibition exerted by these analogs appeared to be related to their metabolism. 5'-IAdo, for example, was highly active against the MTAPase-containing HL-60 human promyelocytic leukemia and L5178Y murine lymphoblastic leukemia, but was much less active against the MTAPase-deficient human (CCRF-CEM) and murine (L1210) leukemia lines (Table 4). This indicates that MTAPase is required for the full expression of the growth-inhibitory action of 5'-IAdo. This further indicates that one (or both) of the products of the reaction of 5'-IAdo with MTAPase, i.e. adenine and 5-iodoribose-1-phosphate (5-IR-1-P), is responsible for the inhibitory effect. Since adenine was only mildly inhibitory to the growth of all the cell lines (Table 4), 5-IR-1-P is implicated as the active metabolite (or precursor of it). Further evidence for this is obtained in studies with the deaminated derivative of 5'-IAdo, 5'-deoxy-5'-iodoinosine (5'-IIno). 5'-IIno is a substrate for PNP, albeit with a low V_{max} value compared to inosine (0.1%), yielding hypoxanthine and 5-IR-1-P [29]. When the growth inhibitory effect of 5'-IIno was examined, its activity was found to be far greater against those cell lines that have relatively high PNP levels (e.g. the HL-60 and L1210 lines), which would be able to phosphorolyze 5'-IIno more rapidly, than those lines (e.g. CCRF-CEM and L5178Y) that have relatively low levels of this enzyme. Hypoxanthine itself is noninhibitory. Thus, 5-IR-1-P is the common metabolite of both 5'-IAdo and 5'-IIno that may be, in part, responsible for the growth inhibitory effects of these nucleosides. A similar pattern was observed with 5'-BrAdo and 5'-BrIno.

DISCUSSION

These studies demonstrate that 5'-halogenated adenosines are phosphorolyzed by MTAPase, and that this cleavage results in the formation of biologically active metabolites. It is not surprising that 5'-halogenated adenosines behave as analogs of MTA. Halogen atoms resemble the sulfur atom of the methylthio group of MTA in that they have unshared outer electron pairs and act as Lewis bases. In addition, MTAPase apparently has low specificity with regard to the size of the 5' substituent; the methylthio moiety of MTA can be replaced by bulky 5'-*n*-butylthio [9, 10, 30] and 5'-isobutylthio [4, 31] groups as well as by a hydrogen atom, i.e. 5'-deoxyadenosine [4, 12]. The 5' substituents of the 5'-halogenated adenosines, namely the halogen atoms themselves, easily fall into this size range. It should be noted that not all 5'-substitutions are readily accepted by MTAPase; adenosine (i.e. 5'-OH) has a K_m in the millimolar range [30] and 5'-amino-5'-deoxyadenosine has very poor reactivity (see Table 2).

Indirect evidence has been obtained indicating that the 5-halogenated ribose-1-phosphates are primarily responsible for the growth-inhibitory actions of several of the 5'-halogenated adenosines and 5'-halogenated inosines. This postulate is based on the findings that (1) MTAPase-containing cells were at least 20-fold more sensitive to 5'-BrAdo or 5'-IAdo than MTAPase-deficient cells, (2) cell lines with high PNP activity were more sensitive to 5'-BrIno and 5'-IIno than cells with low PNP activity, and (3) the other MTAPase reaction product of 5'-halogenated adenosines, adenine, was not growth inhibitory, nor was hypoxanthine, the second product of the reaction of 5'-halogenated inosines with PNP. In addition, 5'-halogenated derivatives of formycin A, which are inhibitors of MTAPase [5], protect L5178Y cells from the growth inhibitory action of 5'-IAdo (M. Y. Chu, unpublished data). The mechanism of action of 5-halogenated ribose-1-phosphates is unknown, but they might, as homologs, interfere with the metabolism of 5-phosphoribosyl-1-pyrophosphate (PRPP), ribose-1-phosphate, glucose-1-phosphate, or 5-methylthioribose-1-phosphate, including the conversion of the latter to methionine [3]. Preliminary studies in this laboratory on the cyclohexylammonium salt of 5-IR-1-P, synthesized enzymatically from 5'-IIno using PNP, have shown that this compound is inhibitory to several PRPP-metabolizing enzymes at concentrations of 50 μM or less (H. S. Choi, J. D. Stoeckler and R. E. Parks, Jr., unpublished results).

The 5'-halogenated adenosines by themselves may have biological activity. 5'-IAdo, for example, was growth inhibitory to MTAPase-deficient CCRF-CEM cells (Table 4), although there was no evidence for its metabolism by these cells. This nucleoside has been shown by others to be inhibitory to certain enzymes, including 2'-deoxycytidine kinase [32] and *S*-adenosylhomocysteine (SAH) hydrolase [33]. Since MTA itself directly inhibits a number of important reactions, including SAH hydrolase [34, 35], spermine synthase [36], and cyclic AMP phosphodiesterase [37], at least in isolated enzyme systems, it is conceivable that the 5'-halogenated adenosines, as analogs of MTA, may exert some of their effects by similar mechanisms.

The above observations on 5'-halogenated adenosines may aid in the design of "second generation" MTA analogs. For example, doubly-substituted MTA-like compounds may be formulated that would liberate both a cytotoxic adenine analog and a pentose-1-phosphate analog. Since 2-fluoroadenosine-containing MTA analogs are the most active among the adenine-substituted compounds [7] whereas 5'-IAdo is the most active of the 5'-substituted analogs, one can conceive of the hybrid molecule, 5'-deoxy-5'-iodo-2-fluoroadenosine. This compound has been synthesized recently by J. A. Sechrist III and J. A. Montgomery of the Southern Research Institute and is presently under study.

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