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CHEMOTHERAPEUTIC POTENTIAL OF METHIONINE ANALOGUE INHIBITORS OF TUMOR-DERIVED METHIONINE ADENOSYLTRANSFERASES

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Abstract—Two isozymes of ATP:L-methionine *S*-adenosyltransferase (MAT) were fractionated from rat Novikoff solid hepatoma. Their K_m values for L-methionine and/or their inhibition constants for various L-methionine analogues were significantly different from the kinetic constants obtained for three isozymes fractionated from normal rat liver. K_i values for cycloleucine and (±)-2-aminobicyclo[2.1.1]hexane-2-carboxylic acid, presented for each tumor and liver isozyme, indicate that (±)-2-aminobicyclo[2.1.1]hexane-2-carboxylic acid was the more potent inhibitor. Dixon plots were also used to test a series of amino acid analogues [cycloleucine, 1-aminocyclobutanecarboxylic acid, 1-aminocyclohexanecarboxylic acid, (±)-2-aminobicyclo[2.1.1]hexane-2-carboxylic acid, L-2-amino-4-hexynoic acid, (*Z*)-L-2-amino-5-chloro-*trans*-4-hexenoic acid, L-ethionine, *S*-*n*-propyl-DL-homocysteine, *S*-*n*-butyl-DL-homocysteine, and seleno-DL-ethionine] of methionine for inhibitory potency. Fixed L-methionine concentrations were used to determine the concentration of inhibitor necessary to inhibit the MAT reaction by 50%. Differential inhibitory activities of the amino acid analogues were noted between the tumor and rat liver isozymes thus supporting the suggestion that tumor-derived MAT isozymes may provide an exploitable target for cancer chemotherapy.

S-Adenosyl-L-methionine (Ado-Met) is the principal methyl donor for transmethylation reactions and is the source of the propylamine moieties in the synthesis of spermidine and spermine [1–6]. Due to the importance of Ado-Met in intermediary metabolism, Talalay and coworkers [7–11] suggested the chemotherapeutic potential of selective inhibition of isofunctional ATP:L-methionine *S*-adenosyltransferases (MATs) (EC 2.5.1.6). These investigators determined in structure–activity relationship experiments the steric, electronic, and conformational requirements of analogues of methionine which were essential for their function as substrates or inhibitors of the enzymatic synthesis of Ado-Met [7–11]. In these early reports, isofunctional MATs were partially purified from yeast, *Escherichia coli*, and rat liver. However, it was not then evident to these investigators that multiple forms of the enzyme are present in microorganisms [12–14] and mammalian tissues [15–18]. From kinetic and physical studies [18], and from experiments utilizing dimethyl sulfoxide (DMSO) [15], it has become increasingly evident that multiple forms of MAT exist in mammalian tissues.

Early studies also suggested that a variety of mouse and rat neoplasms, with the exception of certain Morris hepatomas, do not contain detectable amounts of MATs [19, 20]. However, Lombardini and Talalay [21] clearly demonstrated that MAT activity was present in a variety of rodent tumors such as the Walker 256 tumor, the B-16 melanoma, and the Lewis lung tumor. Recent studies have further demonstrated that tumor MAT displays kinetic and physical properties [22–24] and responses to methionine analogue inhibitors or substrates [25–27] that are different than those found for MATs in normal tissues, thus implying altered active site regions [26, 27]. Liau *et al.* [16] also observed significant amounts of a tumor-derived MAT in Novikoff ascites hepatoma.

Recently our laboratories have renewed interest in extending some of the earlier observations of Talalay and colleagues [7, 8] by defining and comparing the topographic features of the L-methionine binding site of MAT isozymes from rat liver and that of solid Novikoff hepatoma to determine whether this enzyme might be selectively exploited for antineoplastic purposes [25]. Thus, examination of apparent differences between the active site regions of the two recently discovered MAT isozymes present in the Novikoff solid hepatoma [25] and those of the three isozymes present in normal rat liver [28] form a preliminary basis for our ultimate aim, which is the design of selective inhibitors of tumor MAT isozymes for chemotherapeutic purposes. In the present work the separation of two isozymes from Novikoff solid hepatoma is reported along with the

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re-evaluation of a series of methionine analogue inhibitors, the majority of which were first tested for inhibitory potency more than 10 years ago in a rat liver MAT preparation of unknown isozymic composition. Inhibitory potencies for the methionine analogue inhibitors of MAT have been tested in three rat liver isozyme preparations and two solid Novikoff hepatoma isozyme preparations.

MATERIALS AND METHODS

Materials. The following chemicals were obtained from commercial sources: 1-aminocyclopentanecarboxylic acid (cycloleucine) (II), L-methionine (I) and seleno-DL-ethionine (XI) (Sigma Chemical Co., St. Louis, MO); L-ethionine (VIII) (Fluka Chemical Co., Columbia, SC); [^{14}C-methyl]-L-methionine (60.2 mCi/mmole) and [^{35}S]-L-methionine (1220 Ci/mmole) (Amersham Corp., Arlington Heights, IL); Aquasol (New England Nuclear Corp., Boston, MA); phenyl Sepharose (CL-4B) (Pharmacia, Piscataway, NJ); and DEAE Bio-Gel A (100–200 mesh) (Bio-Rad Laboratories, Richmond, CA). The following compounds were gifts from Dr. Paul Talalay, The Johns Hopkins University School of Medicine: *S-n*-propyl-DL-homocysteine (IX), *S-n*-butyl-DL-homocysteine (X), L-2-amino-4-hexynoic acid (VI), and (*Z*)-L-2-amino-5-chloro-*trans*-4-hexenoic acid (VII). 1-Aminocyclobutanecarboxylic acid (III), 1-aminocyclohexanecarboxylic acid (IV), and (±)-2-aminobicyclo[2.1.1]hexane-2-carboxylic acid (V) (aminobicyclohexanecarboxylic acid) were synthesized according to published procedures [10, 29, 30].

Separation of MAT isozymes from Novikoff solid hepatoma cells. Two MAT isozymes were separated from each other by phenyl Sepharose chromatography according to the procedures of Kunz *et al.* [31]. All operations were conducted at 0–4°. Novikoff solid hepatoma cells (20 g), harvested from seven rats (Wistar strain, female) injected 7 days prior with Novikoff hepatoma cells (obtained from Dr. Douglas Stocco, Texas Tech University Health Sciences Center), were disrupted in 2 vol. of 10 mM Tris–HCl buffer, pH 7.5, containing 10 mM mercaptoethanol, with a Potter–Elvehjem tissue grinder. The homogenate was centrifuged for 20 min at 10,000 *g;* the supernatant fraction was recentrifuged for 60 min at 144,000 *g*. Solid ammonium sulfate (final concentration 1.14 M) was slowly added to 50 ml of the 144,000 *g* supernatant fraction, stirred for 15 min, and centrifuged for 20 min at 10,000 *g*. The supernatant fraction was chromatographed on a phenyl Sepharose column (2.6 × 18 cm) previously equilibrated with 1.14 M ammonium sulfate and 10 mM Tris–HCl buffer, pH 7.5, containing 10 mM mercaptoethanol. Tumor isozymes I and II were eluted from the phenyl Sepharose column by low ionic strength buffer (10 mM Tris–HCl buffer, pH 7.5, containing 10 mM mercaptoethanol). No further isozymes were eluted when DMSO (40%) was added to the low ionic strength buffer, and chromatography continued for another 7 hr (280 ml of DMSO buffer). The peak fractions of MAT activity were pooled and concentrated with solid ammonium sulfate (5.7 M). The pellet, formed upon centrifugation at 10,000 *g* for 15 min, was dissolved in a small volume of buffer containing 20% glycerol and dialyzed overnight against buffer containing 20% glycerol. Each isozyme was then partially purified by chromatography on DEAE Bio-Gel A (1.5 × 30 cm column equilibrated with buffer containing 20% glycerol) utilizing a linear KCl gradient (0 to 0.5 M) for elution of the MAT. These procedures resulted in approximately a 50-fold purification over the high speed supernatant fraction.

Separation of MAT isozymes from rat liver. Isozymes of MAT obtained from 30 g of rat liver (Wistar strain, female) were separated by phenyl Sepharose chromatography according to the procedures of Hoffman and Kunz [28]. Three isozymes (I, II, and III) were isolated utilizing a gradient generated by a 9 chamber mixer. Chambers 1–3 each contained 100 ml of 10% DMSO, and 10 mM Tris–HCl buffer, pH 7.5, containing 10 mM mercaptoethanol and 0.6 M ammonium sulfate; chambers 4–9 contained similar components, but with 40% DMSO. Each isozyme was further purified according to the procedures used for the tumor isozymes.

Enzymatic assays. MAT activity was measured as described by Chou and Lombardini [32]. Assays were carried out in a final volume of 0.1 ml containing, in addition to enzyme protein, the following components in μmoles; Tris–HCl, pH 8.0, 10; KCl, 30; $MgCl_2$, 2.4; dithiothreitol, 0.4; ATP, 0.5; and L-methionine (^{35}S or ^{14}C-methyl), amount depending upon isozyme preparation. Methionine analogue inhibitors were tested for inhibitory potency as previously described [7]. Optimal assay conditions were chosen which gave linear product formation with both time and enzyme protein.

Calculations. Concentrations of methionine analogues observed to inhibit the MAT isozymes by 50% (I_{50}) were calculated from Dixon plots [33]. Kinetic parameters and I_{50} values were determined by least square regression analyses. Data are presented as single determinations or means ± S.E.M.

RESULTS

The separation of two isozymes of MAT from rat Novikoff solid hepatoma by phenyl Sepharose chromatography is shown in Fig. 1. The designation isozyme I and II denotes the order of elution. Stepwise elution with DMSO (40%) or a DMSO gradient as in the separation of three isozymes from rat liver did not unmask any additional isozymes in the Novikoff hepatoma preparation.

The K_m values of L-methionine for the three rat liver and two Novikoff solid hepatoma isozymes are shown in Table 1. Structures of all compounds are shown in Fig. 2. The K_m value for rat liver III isozyme is only approximate as the kinetics demonstrated positive cooperativity, and thus reciprocal plots of the data did not yield easily calculable kinetic parameters. Hoffman and Kunz [28] reported similar values. The isozymes of MAT obtained from Novikoff hepatoma demonstrated classical Michaelis–Menten kinetics, that is, they did not display cooperativity; K_m values of 11.0 ± 1.6 μM for isozyme I and 5.78 ± 0.24 μM for isozyme II have thus been calculated.

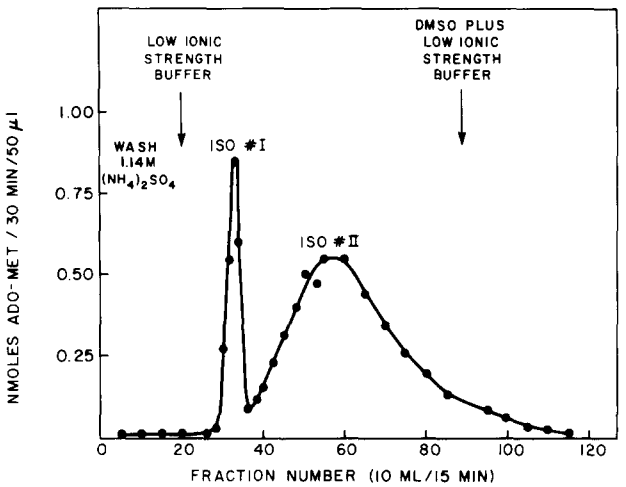

Fig. 1. Phenyl Sepharose chromatography of methionine adenosyltransferase isozymes from Novikoff solid hepatoma. Low ionic strength (10 mM) Tris–HCl buffer, pH 7.5, containing 1.14 M ammonium sulfate and 10 mM mercaptoethanol eluted two isozymes (I and II). Enzyme activity is expressed as the amount of Ado-Met formed, in nmoles per 30 min of incubation. Samples of fifty µl of each fraction were used in the assay. L-Methionine concentration was 37.5 µM; ATP concentration was 5 mM.

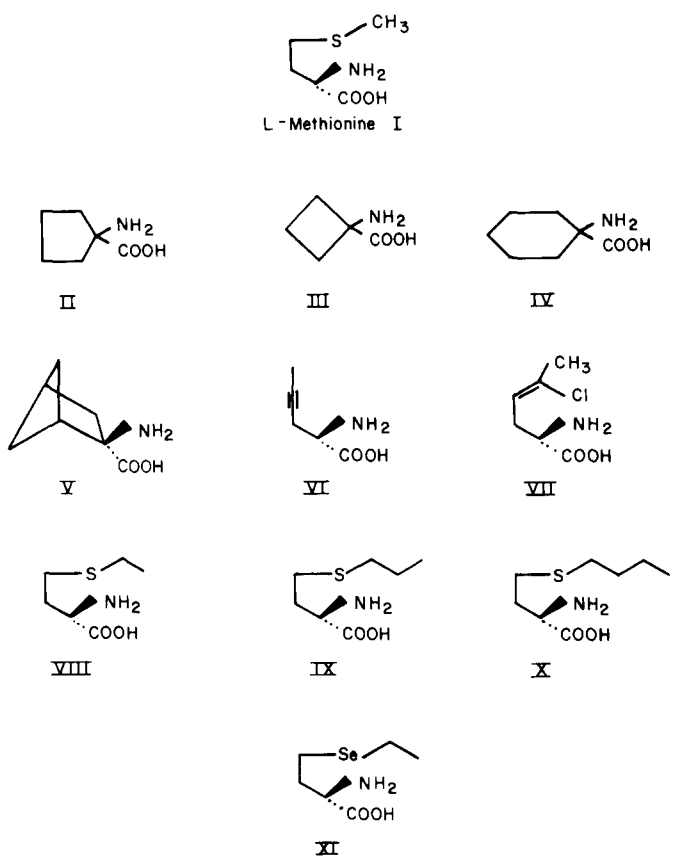

Fig. 2. Structures of methionine and methionine analogues [II, 1-aminocyclopentanecarboxylic acid (cycloleucine); III, 1-aminocyclobutanecarboxylic acid; IV, 1-aminocyclohexanecarboxylic acid; V, (±)-2-aminobicyclo[2.1.1]hexane-2-carboxylic acid; VI, L-2-amino-4-hexynoic acid; VII, (*Z*)-L-2-amino-5-chloro-*trans*-4-hexenoic acid; VIII, L-ethionine; IX, *S*-*n*-propyl-DL-homocysteine; X, *S*-*n*-butyl-DL-homocysteine; and XI, seleno-DL-ethionine]. Compounds V, VII, IX, X, and XI were tested as racemic mixtures; the structures which are presented represent what is assumed to be the active isomer of the mixture. The structures of these active isomers have been indicated in previous studies [7–11].

Table 1. Kinetic constants for L-methionine and analogue inhibitors of isozymes of ATP:L-methionine *S*-adenosyltransferase prepared from rat liver and Novikoff solid hepatoma*

Compound No.	Compound	Kinetic constants (μM)				
		Rat liver isozymes			Novikoff hepatoma isozymes	
		I	II	III	I	II
I	L-Methionine (K_m)	34.5 ± 4.4	11.1 ± 3.3	93 ± 5	11.0 ± 1.6	5.78 ± 0.24
II	1-Aminocyclopentanecarboxylic acid (cycloleucine) (K_i)	516 ± 36	263 ± 22	1633 ± 73	382 ± 131	209 ± 10
V	(±)-2-Aminobicyclo[2.1.1]hexane-2-carboxylic acid (K_i)	132 ± 26	104 ± 13	435 ± 10	51 ± 5	78 ± 11

* ATP concentration was 5 mM. Values are the means ± S.E.M. obtained from double-reciprocal plots in a minimum of three experiments.

Table 2. Inhibitory potencies of L-methionine analogues towards isozymes of ATP:L-methionine *S*-adenosyltransferase prepared from rat liver and Novikoff solid hepatoma*

		Rat liver isozymes			Novikoff hepatoma isozymes	
		I	II	III	I	II
L-Methionine concentration (μM)		5.0	1.0	37.5	1.0	1.0
Compound No.	Compound	Concentration (mM) required for 50% inhibition				
II	1-Aminocyclopentanecarboxylic acid (cycloleucine)†	0.29 ± 0.02	0.18 ± 0.01	2.50 ± 0.14	0.19 ± 0.03	0.14 ± 0.01
III	1-Aminocyclobutanecarboxylic acid	2.2	1.5	12.4	3.6	1.2
IV	1-Aminocyclohexanecarboxylic acid	9.7	8.2	57.4	4.0	7.6
V	(±)-2-Aminobicyclo[2.1.1]hexane-2-carboxylic acid	0.15	0.08	0.68	0.18	0.078
VI	L-2-Amino-4-hexynoic acid	0.16	0.09	0.80	0.23	0.10
VII	(*Z*)-L-2-Amino-5-chloro-*trans*-4-hexenoic acid	0.18	0.12	1.10	0.26	0.15
VIII	L-Ethionine‡	1.64 ± 0.27	0.79 ± 0.17	15.4 ± 3.15	0.21 ± 0.02	0.25 ± 0.01
IX	*S*-*n*-Propyl-DL-homocysteine	39§	50§	113§	44.4§	41§
X	*S*-*n*-Butyl-DL-homocysteine	234§	149§	NA	NA	NA
XI	Seleno-DL-ethionine‡	4.28 ± 0.58	1.98 ± 0.34	11.1 ± 1.95	0.21 ± 0.02	0.22 ± 0.01

* ATP concentration was 5 mM. I_{50} values were obtained from Dixon plots [33]. (NA = not active).

† Cycloleucine was utilized as a standard test inhibitor each time a methionine analogue was measured for inhibitory potency. For the three rat liver isozymes, the number of experiments utilizing cycloleucine was nineteen; for the two tumor isozymes, cycloleucine was tested in ten experiments.

‡ L-Ethionine and seleno-DL-ethionine were tested in three experiments.

§ Values were determined by extrapolation and were not bracketed by experimental observations.

Talalay and coworkers [5, 7] were the first to report that 1-aminocyclopentanecarboxylic acid (II; cycloleucine), a nonmetabolizable amino acid transport monitor [34–36] and tumor inhibitor [37–39], was a potent inhibitor of MAT. In the present studies, cycloleucine was used as the standard by which comparisons of inhibitory potency for all other methionine analogue inhibitors were made. Cycloleucine had K_i values ranging from 209 to 1633 μM in the isozymes of rat liver and tumor (Table 1).

The K_i values of (±)-2-aminobicyclo[2.1.1]-hexane-2-carboxylic acid (aminobicyclohexanecarboxylic acid; V) were approximately 2- to 7-fold lower than the K_i values of cycloleucine, depending upon the isozyme and its tissue source. Since compound V is a racemic preparation, it is assumed resolution would afford an enantiomer of even higher potency. This is based on previous studies [11] which have indicated that the enantiomer of V with the 2R configuration is the active isomer.

The inhibitory potencies of nine methionine analogues are shown in Table 2. The values are presented as the concentrations required to inhibit the MAT activity by 50%. To allow measurement of the inhibitory potency of the methionine analogues at competitive levels of L-methionine, concentrations of L-methionine for assay of each MAT isozyme were chosen which were no more than 40% of their respective K_m values.

As first reported more than 10 years ago for MATs of microbial and mammalian origin [7], ring size was again found to be critically important for all rat liver and Novikoff hepatoma MAT isozymes, since 1-aminocyclobutanecarboxylic acid (III) and 1-aminocyclohexanecarboxylic acid (IV) were considerably weaker inhibitors than cycloleucine. The I_{50} values of (±)-aminobicyclohexanecarboxylic acid indicate that it is approximately two to three times more potent than cycloleucine as an inhibitor for rat liver isozymes I and II and tumor isozymes I and II.*

Mudd [6] first examined the geometric isomers of DL-2-amino-4-hexenoic acid as inhibitors of MAT of yeast. Talalay and coworkers [7] confirmed the observations of Mudd that the *trans* isomer was the more potent isomer in homologous enzymes of yeast, *E. coli* and rat liver. These investigators incorporated additional unsaturation into the molecule to produce L-2-amino-4-hexynoic acid (VI). During this period the chloro-derivative, *Z*-L-2-amino-5-chloro-*trans*-4-hexenoic acid (VII), was also synthesized and tested. In the present study, the acetylenic (VI) and chloro (VII) analogues were approximately equal in inhibitory potency toward the rat liver and Novikoff hepatoma isozymes. However, both compounds were approximately 2- to 3-fold more potent than cycloleucine toward the rat liver isozymes while being as potent as cycloleucine toward the Novikoff hepatoma isozymes.

The higher analogues of methionine, that is, L-ethionine (VIII), *S*-*n*-propyl-DL-homocysteine (IX), *S*-*n*-butyl-DL-homocysteine (X) and seleno-DL-ethionine (XI), in general did not bind as well as cycloleucine to the L-methionine active site. Of the four compounds, L-ethionine and seleno-DL-ethionine were the more potent inhibitors (Table 2) and are also substrates for all five isozymes [26].

DISCUSSION

Two tumor isozymes (I and II) have been separated and partially purified from Novikoff solid hepatoma, in contrast to one tumor isozyme detected previously in the ascites form of the Novikoff hepatoma by Liau *et al.* [16]. Differences in these two isozymes have been observed not only in their chromatographic patterns on phenyl Sepharose but also in their K_m values for L-methionine (11.0 ± 1.6 vs 5.78 ± 0.24 μM) ($P < 0.02$). Furthermore, five of the ten methionine analogue inhibitors that were tested were more potent in tumor II isozyme than in tumor I isozyme as demonstrated by lower I_{50} values. Four of the analogues, L-ethionine, seleno-DL-ethionine, *S*-*n*-propyl-DL-homocysteine, and *S*-*n*-butyl-DL-homocysteine had approximately equal inhibitory potency for tumor isozymes I and II while 1-aminocyclohexane-1-carboxylic acid was more potent in isozyme I.

Comparisons of the inhibitory potencies of 1-aminocyclobutane-1-carboxylic acid, 1-aminocyclopentane-1-carboxylic acid, and 1-aminocyclohexane-1-carboxylic acid towards rat liver isozymes I, II, and III and tumor isozymes I and II indicate optimum binding affinity of the cyclic 5-membered ring amino acid for all five isozymes. This phenomenon, observed previously for other isofunctional methionine adenosyltransferases prepared from rat liver, yeast and *E. coli* [7], delineates a region in the active site which is highly complementary to a specific conformation of the cyclopentane ring, and indicates a region of high topographic homology for these five isozymes. It is of interest that this observation can be extended to include the tumor MAT isozymes.

(±)-Aminobicyclohexanecarboxylic acid and the acetylenic L-methionine analogue (L-2-amino-4-hexynoic acid), compounds which are structurally very dissimilar, had approximately equal inhibitory potencies, although based on prior studies [11] it is assumed that the former, tested as a racemate, has one enatiomer whose activity is significantly higher than that of its optical isomer. The low K_i and I_{50} values of racemic compound V towards both tumor isozymes indicate that chemical resolution of compound V to obtain the active isomer [11] would be advantageous.

The next two higher homologues of L-methionine, that is, L-ethionine and *S*-*n*-propyl-DL-homocysteine, inhibited the MAT isozymes and thus are capable of binding at the L-methionine active site. However, *S*-*n*-butyl-DL-homocysteine lacked activity in rat liver

* The K_i value for (±)-aminobicyclo[2.1.1]-hexanecarboxylic acid was smaller for tumor iso I than for tumor iso II, whereas the I_{50} value was larger for tumor iso I than for tumor iso II. This discrepency is most likely related to the fact that the bicyclohexane amino acid, V, is a mixture of two enantiomers, each with differing inhibitory activities towards the two tumor isozymes. Separation of the enantiomers appears warranted, not only to resolve this specific paradox, but also because the K_i of the racemic mixture is sufficiently low as to suggest significantly high inhibitory activity for the active isomer in this mixture.

isozyme III and in Novikoff hepatoma isozymes I and II while in the other liver isozymes it was a very weak inhibitor. Thus, for the Novikoff tumor as well as the normal rat liver isozymes, steric accommodation of the *S*-ethyl substituent at the active site is possibly but, as this group becomes larger in the *n*-propyl and *n*-butyl moieties, steric hindrance is encountered, binding becomes more difficult, and less inhibition is observed.

An important question to be answered is whether either or both of the Novikoff solid hepatoma isozymes (I and II) are the same as any of the three rat liver isozymes. Initial observations by Liau *et al.* [16] of elution position on Sephadex G-150 and DEAE-cellulose and K_m values of two rat liver isozymes and one Novikoff (ascites) isozyme suggested the possible correspondence of one of the rat liver isozymes with the tumor isozyme. However, in this communication we report that the rat liver isozymes are different from the Novikoff tumor isozymes. For instance, K_m values for tumor isozyme I ($11.0 \pm 1.6\ \mu M$) and II ($5.78 \pm 0.24\ \mu M$) were significantly lower than for rat liver isozyme I ($34.5 \pm 4.4\ \mu M$) and III ($93 \pm 5\ \mu M$). In addition, inhibitory potencies of various methionine analogues demonstrated differences between rat liver isozyme II and Novikoff isozyme I and II. This is of particular significance from a chemotherapeutic standpoint since Hoffman and Kunz [28] have found that rat liver iso II is the major form of methionine adenosyltransferase in non-liver tissues. First, the K_i value of (±)-aminobicyclohexanecarboxylic acid for tumor I ($51 \pm 5\ \mu M$) was 2-fold lower than that obtained for rat liver isozyme II ($104 \pm 13\ \mu M$). Second, both L-ethionine and seleno-DL-ethionine were more potent inhibitors of tumor isozyme II (0.21 ± 0.02; $0.21 \pm 0.02\ \mu M$) than for rat liver isozyme II (0.79 ± 0.17; $1.98 \pm 0.34\ \mu M$) as demonstrated by I_{50} values measured at $1\ \mu M$ L-methionine.

Another issue which has been clarified by this study is related to the pharmacologic potential of developing selective inhibitors of the enzymatic synthesis of Ado-Met. The previous studies of Talalay and coworkers [5, 7–11] were carried out at a time when it was assumed that, in general, isofunctional methionine adenosyltransferases were enzymes with high K_m values for L-methionine of approximately 0.4 to 2.5 mM [40]. Accordingly, inhibitory assays were carried out at an L-methionine concentration of $37.5\ \mu M$ which, for example, was assumed to be a subsaturating level for the rat liver enzyme but, in fact, is such only for rat liver iso III. The identification of low K_m isozymes in both rat liver and Novikoff hepatoma, and a re-examination of inhibitory potencies under modified assay conditions, indicates that these L-methionine analogue inhibitors are more potent than was initially apparent. This favorable re-evaluation of inhibitory activities, as well as our preliminary indications that tumor-derived MAT isozymes not only differ from those found in the normal host tissue but also exhibit low K_m (L-methionine) kinetic constants, has significant implications for the mechanism of tumor growth and the pharmacologic development of antitumor agents. The synthesis and evaluation of new analogues of L-methionine as potent inhibitors of MAT isozymes in normal and neoplastic tissues are currently in progress [27, 41].

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REFERENCES

1. G. L. Cantoni, *J. biol. Chem.* **204**, 403 (1953).
2. S. H. Mudd and G. L. Cantoni, in *Comprehensive Biochemistry* (Eds. M. Florkin and E. H. Stotz), Vol. 15, p. 1. Elsevier, Amsterdam (1964).
3. H. Tabor and C. W. Tabor, *Pharmac. Rev.* **16**, 245 (1964).
4. H. G. Williams-Ashman, A. E. Pegg and D. H. Lockwood, *Adv, Enzyme Regulat.* **7**, 291 (1969).
5. J. B. Lombardini and P. Talalay, *Adv. Enzyme Regulat.* **9**, 349 (1971).
6. S. H. Mudd, in *Transmethylation and Methionine Biosynthesis* (Eds. S. K. Shapiro and F. Schlenk), p. 33. University of Chicago Press, Chicago (1965).
7. J. B. Lombardini, A. W. Coulter and P. Talalay, *Molec. Pharmac.* **6**, 481 (1980).
8. A. W. Coulter, J. B. Lombardini and P. Talalay, *Molec. Pharmac.* **10**, 293 (1974).
9. A. W. Coulter, J. B. Lombardini and P. Talalay, *Molec. Pharmac.* **10**, 305 (1974).
10. A. W. Coulter, J. B. Lombardini, J. R. Sufrin and P. Talalay, *Molec. Pharmac.* **10**, 319 (1974).
11. J. R. Sufrin, A. W. Coulter and P. Talalay, *Molec. Pharmac.* **15**, 661 (1979).
12. H. Cherest and Y. Surdin-Kerjan, *Molec. gen. Genet.* **163**, 153 (1978).
13. P. K. Chiang and G. L. Cantoni, *J. biol. Chem.* **252**, 4506 (1977).
14. S. K. Shapiro and A. J. Ferro, in *The Biochemistry of Adenosylmethionine* (Eds. F. Salvatore, E. Borek, V. Zappia, H. G. Williams-Ashman and F. Schlenk), p. 58. Columbia University Press, New York (1977).
15. J. L. Hoffman and G. L. Kunz, *Biochem. biophys. Res. Commun.* **77**, 1231 (1977).
16. M. C. Liau, G. W. Lin and R. B. Hurlbert, *Cancer. Res.* **37**, 427 (1977).
17. J. J. Tallan, *Biochem. Med.* **21**, 129 (1979).
18. G. Okada, H. Teraoka and K. Tsukada, *Biochemistry* **20**, 934 (1981).
19. B. Shield and E. Bilik, *Cancer Res.* **28**, 2512 (1968).
20. R. L. Hancock, *Cancer Res.* **26**, 2425 (1966).
21. J. B. Lombardini and P. Talalay, *Molec. Pharmac.* **9**, 542 (1973).
22. M. C. Liau, C. F. Chang, L. Belanger and A. Grenier, *Cancer Res.* **39**, 162 (1979).
23. M. C. Liau, C. F. Chang and F. F. Becker, *Cancer Res.* **39**, 2113 (1979).
24. M. C. Liau, C. F. Chang and B. C. Giovanella, *J. natn. Cancer Inst.* **64**, 1071 (1980).
25. J. R. Sufrin and J. B. Lombardini, *Fedn Proc.* **39**, 1908 (1980).
26. J. R. Sufrin and J. B. Lombardini, in *Biochemistry of S-Adenosylmethionine and Related Compounds* (Eds. E. Usdin, R. T. Borchardt and C. R. Creveling), p. 687. Macmillan, London (1982).

27. J. R. Sufrin and J. B. Lombardini, *Molec. Pharmac.* in press.
28. J. L. Hoffman and G. L. Kunz, *Fedn Proc.* **39**, 1690 (1980).
29. R. J. Cremyln, *J. chem. Soc.* 3977 (1962).
30. L. Munday, *J. chem. Soc.* 4372 (1961).
31. G. L. Kunz, J. L. Hoffman, C-A. Chia and B. Stremel, *Archs Biochem. Biophys.* **202**, 565 (1980).
32. T-C. Chou and J. B. Lombardini, *Biochim. biophys. Acta* **267**, 399 (1972).
33. M. Dixon, *Biochem. J.* **55**, 170 (1953).
34. H. Akedo and H. N. Christensen, *J. biol. Chem.* **237**, 113 (1962).
35. H. N. Christensen and J. C. Jones, *J. biol. Chem.* **237**, 1203 (1962).
36. W. R. Sterling and J. F. Henderson, *Biochem. Pharmac.* **12**, 303 (1963).
37. F. Martel and L. Berlinguet, *Can. J. Biochem. Physiol.* **37**, 433 (1959).
38. T. A. Connors, L. A. Elson, A. Haddow and W. C. J. Ross, *Biochem. Pharmac.* **5**, 108 (1960).
39. R. B. Ross, C. I. Noll, W. C. J. Ross, N. V. Nadkarni, E. H. Morrison, Jr. and H. W. Bond, *J. mednl Pharm. Chem.* **3**, 1 (1961).
40. J. R. Sufrin, D. A. Dunn and G. R. Marshall, *Molec. Pharmac.* **19**, 307 (1981).
41. J. R. Sufrin, J. B. Lombardini and D. D. Keith, *Biochem. biophys. Res. Commun.* **106**, 251 (1982).