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Steric Mapping of the L-Methionine Binding Site of ATP:L-Methionine S-Adenosyltransferase

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SUMMARY

SUFRIN, J. R., D. A. DUNN, AND G. R. MARSHALL. Steric mapping of the L-methionine binding site of ATP:L-methionine *S*-adenosyltransferase. *Mol. Pharmacol.* **19**:307–313 (1981).

Three-dimensional steric mapping of the L-methionine binding site of mammalian (rat liver) ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6) has been accomplished by computing the combined occupied volume of six inhibitory, conformationally rigid, amino acid analogues that have been shown in previous studies, which used an enzyme preparation of undetermined isozymic composition, to bind to the enzyme surface in competition with L-methionine. [Coulter, A. W., J. B. Lombardini, J. R. Sufrin, and P. Talalay. *Mol. Pharmacol.* **10**:319–334 (1974); Sufrin, J. R., A. W. Coulter, and P. Talalay. *Mol. Pharmacol.* **15**:661–677 (1979).] These six amino acids, all of which are derivatives of 1-aminocyclopentane-1-carboxylic acid, include (1R,2R,4S)-2-aminonorbornane-2-carboxylic acid, (1S,2S,4R)-2-aminonorbornane-2-carboxylic acid, (1R,2S,4S)-2-aminonorbornane-2-carboxylic acid, (1R,2R,4S)-2-amino-5,6-*exo*-trimethylenenorbornane-2-carboxylic acid, 7-aminonorbornane-7-carboxylic acid, and 9-aminofluorene-9-carboxylic acid. This has generated an "enzyme-excluded" volume map, which defines that region of the active site known to be available for binding by substrates or inhibitors structurally related to L-methionine. Volume mapping of three conformationally rigid analogues which possess the minimal structural requirements for recognition, but nevertheless are inactive, has been carried out to determine their unique volume requirements that are not associated with the enzyme-excluded volume. These inactive amino acids are (1S,2R,4R)-2-aminonorbornane-2-carboxylic acid, (1R,2R,4S)-2-aminobicyclo[3.2.1]octane-2-carboxylic acid, and 2-aminoadamantane-2-carboxylic acid. Intersection of the unique volume segments of the three inactive analogues shows one region of unique volume overlap for all three molecules and defines an "enzyme-essential" region, i.e., a region required by the enzyme itself and not available for ligand binding.

INTRODUCTION

The biosynthetic conversion of L-methionine and ATP to *S*-adenosyl-L-methionine, the principal biologic methyl donor, is catalyzed by methionine adenosyltransferase[2] (EC 2.5.1.6). The strategic role of adenosylmethionine-mediated methylation in effecting a diversity of biochemical and regulatory functions has become more widely appreciated in recent years, and has been accompanied by a greater understanding of the factors and mechanisms which control its biosynthesis (1).

A detailed investigation of amino acid analogue inhibitors of isofunctional methionine adenosyltransferases catalyzing adenosylmethionine biosynthesis has been carried out by Talalay and co-workers (2–7). Analysis of the salient structural and electronic features of these enzyme inhibitors has enabled deductions to be made about the conformation of L-methionine at the active site and has also provided information about the topographic features of the complementary region on the enzyme surface at the L-methionine binding site (2–7). As an extension of these studies, steric mapping of the L-methionine binding site of mammalian methionine adeno-

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[2] The abbreviations used are: methionine adenosyltransferase, ATP:L-methionine *S*-adenosyltransferase (EC 2.5.1.6); cycloleucine, 1-aminocyclopentane-1-carboxylic acid.

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syltransferase found in rat liver and whose isozymic composition is undetermined has been accomplished by computing the combined occupied volume of six inhibitory, conformationally rigid amino acid analogues, all of which are structurally related to 1-aminocyclopentane-1-carboxylic acid, thus generating an "enzyme-excluded" volume map[3] of this region. Volume mapping of three conformationally rigid amino acid analogues which possess the minimal requirements for recognition by the enzyme (i.e., the 3-dimensional arrangement of the H_3^+N-C-CO_2^- functionality), but are nevertheless inactive, has been carried out to determine the unique volume associated with them that is not found in the "enzyme-excluded" volume. These "unique" volume segments of inactive analogues have been analyzed to provide information about possible "enzyme-essential"[3] regions in the active site. The purpose of this study is to define more precisely the topographic features of the L-methionine binding site within the active site region. This should enable predictions about inhibitory activity prior to synthesis of new analogues and could provide important refinements for the systematic design of inhibitors of isofunctional methionine adenosyltransferases with possible chemotherapeutic potential. It is recognized and assumed that the observed activity of each amino acid analogue, inhibitory or noninhibitory, is dependent upon contributions from a variety of physical parameters. However, the set of amino acid analogues under consideration here has been specifically chosen because their steric properties appear to be the physical parameter which presents significant and well-defined differences among an otherwise similar group of molecules.

EXPERIMENTAL PROCEDURE

Computer-generated volume maps. The inhibitory potencies of nine rigid amino acid analogues toward rat liver methionine adenosyltransferase have been described previously (6, 7). Six of these analogues display inhibitory activity and their combined occupied volume generates the enzyme-excluded volume map. The structures of these six active analogues appear in Fig. 1 and include the following compounds: (1R,2R,4S)-2-aminonorbornane-2-carboxylic acid (I), (1S,2S,4R)-2-aminonorbornane-2-carboxylic acid (II), (1R,2S,4S)-2-aminonorbornane-2-carboxylic acid (III), (1R,2R,4S)-2-amino-5,6-*exo*-trimethylenenorbornane-2-carboxylic acid (IV), 7-aminonorbornane-7-carboxylic acid (V), and 9-aminofluorene-9-carboxylic acid (VI). Three inactive amino acid analogues are represented in Fig. 1 and include (1S,2R,4R)-2-aminonorbornane-2-carboxylic acid (VII), (1R,2R,4S)-2-aminobicyclo[3.2.1]octane-2-carboxylic acid (VIII), and 2-aminoadamantane-2-carboxylic acid (IX).

[3] The "enzyme-excluded" volume map defines that region of the enzyme-active site available for binding by substrate, substrate analogues, or nonsubstrate analogues, and therefore not occupied by the enzyme itself. Conversely, volume occupied by the enzyme, and therefore not available for occupancy by other molecules, is defined as "enzyme-essential" volume. A molecule requiring volume in an enzyme-essential region is unable to bind to the enzyme surface and is therefore inactive.

It is important to note that the measurements of inhibitory potencies for Compounds I through IX were obtained using a partially purified preparation of methionine adenosyltransferase from rat liver according to described procedures (2, 3). Recently, rat liver methionine adenosyltransferase has been fractionated, yielding three isozymes: two "low K_m" forms and a "high K_m" form (8). The isozymic composition of the rat liver preparation used to determine the inhibitory potencies of the amino acid analogues utilized for these mapping studies remains unknown. Thus, we are aware that steric mapping of the L-methionine binding site using the published data provides some ambiguities, and the following possibilities must be kept in mind. (a) The computed enzyme-excluded volume is that of either low K_m Isozyme I, low K_m Isozyme II, or the high K_m Isozyme. (b) The obtained enzyme-excluded volume is valid for both low K_m isozymes and the high K_m isozyme. (c) The generated enzyme-excluded volume represents one of the possible unions of different enzyme-excluded volume maps of the three isozymes.

Steric analysis. The conformations of the molecules were generated by using Cartesian coordinates from X-ray crystallographic structural determinations and, whenever necessary, were spplemented by the appropriate addition of fragments of standard geometry. This is indicated for each molecule as follows.

The structure of I was determined from the X-ray crystal data of Apgar and Ludwig (9); the structures of II, III, V, and VII were generated from I by transposition of the amino-carboxyl fragment to the appropriate carbon atom. The structure of IV was generated from I by attachment of a trimethylene fragment of standard geometry to C-5, followed by the use of a ring closure program. The structure of VI was built from X-ray crystallographic data (10) of the fluorenyl framework, and attachment at position C-9 of a H_3^+N—C—CO_2^- fragment of standard geometry. The structure of IX was constructed using the X-ray crystallographic data reported by Chacko and Zand (11).

Comparisons of molecules were made on an interactive real-time molecular graphics system (MMS-X) developed at Washington University, St. Louis, Mo. This system permits rotation, translation, and display of each structure (12). Comparison of the molecules required proper alignment using a least-squares fitting program which superimposed the H_3^+N—C—CO_2^- functionalities of all nine amino acid analogues. The carboxylate oxygen atoms and the ammonium hydrogen atoms in each molecule were rotated to the same position, but it should be kept in mind that their enzyme-bound orientation is not known.

For each aligned molecule, an electron density volume map was generated using Gaussian functions which were surface-contoured at the level representative of van der Waals radii[4] (13). The union of the density maps of the

[4] For comparative purposes the volume maps were generated by three different methods: (1) volume maps were generated after removal of hydrogen atoms from the molecules; (2) volume maps were generated from molecules with hydrogen atoms, and with overlap of the electron density; (3) volume maps for molecules were constructed with hydrogen atoms but without overlap of electron density. Maps generated by these

six active analogues allowed construction of the enzyme-excluded volume map, i.e., that volume which is available at the binding site as indicated by the volume requirements of the active molecules.

Inactive molecules were examined for unique volume requirements not associated with the enzyme-excluded volume. This was done by first performing an intersection of the volume map of an inactive molecule with the enzyme-excluded volume map, to give the common volume of active molecules plus inactive molecules. Subtraction of this common volume from the volume map of the inactive analogue leaves excess volume, unique to the inactive analogue, not occupied by the set of active molecules. This procedure was carried out for each inactive molecule.

RESULTS AND DISCUSSION

Molecular recognition is an essential requirement for the binding of these nonsubstrate amino acid analogues to the enzymatic surface of methionine adenosyltransferase. Coulter and co-workers (6) have suggested that the polar NH_3^+ and CO_2^- groups of these analogues are the functional groups which are recognized and which initiate the interactions with the enzyme surface; their stereospecific 3-dimensional arrangement, which includes the α-carbon atom to which they are attached, defines the pharmacophore for recognition by this enzyme. As a logical extension of the volume-mapping techniques, the three inactive rigid amino acid analogues VII, VIII, and IX, which meet this essential pharmacophoric requirement for recognition, are assumed to be inactive because they have unique volume requirements not fitting the enzyme-excluded volume. For each inactive analogue, this unique volume (or a segment of it) is assumed to contain "enzyme-essential" volume—i.e., a region occupied by the enzyme and therefore not available for binding by other molecules. Careful analysis of the unique volume segments of these inactive analogues presenting the pharmacophore can help to identify which segments are indeed enzyme-essential and can assist in defining the location of the enzyme with respect to the binding molecule. Once an "enzyme-essential" region is identified, any analogue possessing steric bulk in this region may be predicted to be inactive prior to synthesis.

Steric mapping of the L-methionine binding site of rat liver methionine adenosyltransferase has been accomplished by computing the combined volume occupied by six rigid amino acid analogues which were shown in Talalay's laboratory to be inhibitors of a rat liver enzyme preparation of unknown isozymic composition (6, 7). These amino acids include (1R,2R,4S)-2-aminonorbornane-2-carboxylic acid (I), (1S,2S,4R)-2-aminonorbornane-2-carboxylic acid (II), (1R,2S,4S)-2-aminonorbornane-2-carboxylic acid (III), (1R,2R,4S)-2-amino-5,6-*exo*-trimethylenenorbornane-2-carboxylic acid (IV), 7-aminonorbornane-7-carboxylic acid (V), and 9-aminofluorene-9-carboxylic acid (VI). The structures of those molecules are represented in Fig. 1. These active molecules were chosen specifically for these mapping studies because their rigid structures eliminate conformational ambiguities and allow for precise definition of their occupied volume.

The enzyme-excluded volume map obtained from the union of the six active analogues is represented in Fig. 2. The point of attachment of the amino and carboxyl groups to the enzyme's surface most certainly identifies an "enzyme-essential" region. As is seen in Fig. 3, the inactive norbornane analogue VII presents unique volume in only one region not included in the enzyme-excluded volume, and this unique volume segment is therefore considered enzyme-essential and responsible for the inability of VII to bind to the enzyme surface. Both VIII and IX, also seen in Fig. 3, require new volume in more than one region. However, intersection of the unique volumes associated with all three inactive analogues VII, VIII and IX, as seen in Fig. 3, shows one region of unique volume overlap for all three molecules. This is a segment of the region delineated as enzyme-essential by the inactive norbornane analogue VII. It is apparent, then, that the inactivity of VIII and IX could have been predicted prior to synthesis. In other words, the inactivity of all three analogues can be rationalized by their assumption of one small segment of volume required by the enzyme. The remaining unique volume segments of VIII and IX do not necessarily represent enzyme-essential regions since occupied volume in one

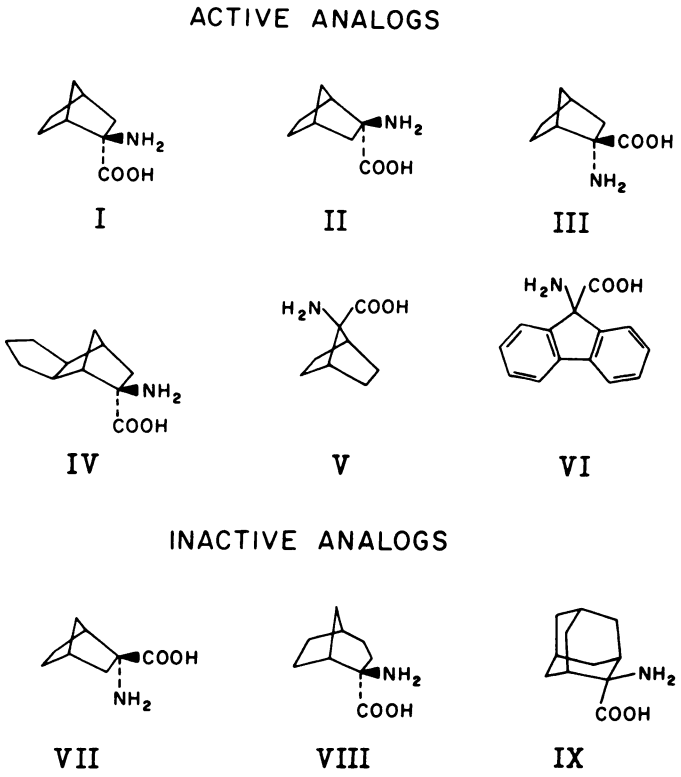

FIG. 1. *Structures of active and inactive amino acid analogues used in volume mapping of the* L *-methionine binding site of methionine adenosyltransferase*

three methods gave similar results—the differences, as expected, were indicated only by a relative displacement of surface contours and volume segments. The figures presented in this study show volume maps generated from molecules with hydrogen atoms but without addition of their overlapping electron densities.

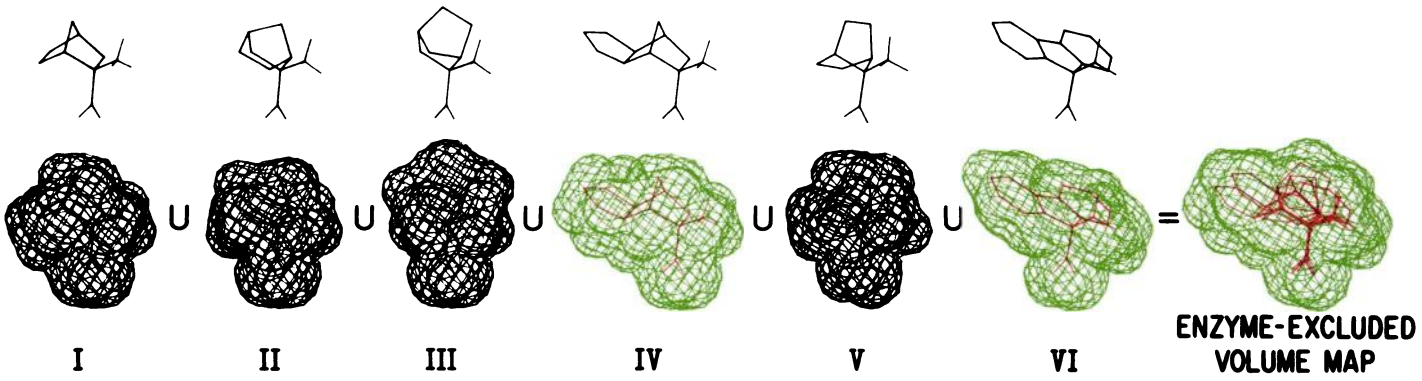

FIG. 2. *Enzyme-excluded volume map*

The structures of the six active analogues are shown on *top*; *below* each structure is the electron density map for that molecule. The union of these six individual electron density maps gives the enzyme-excluded volume map and defines that region of the methionine binding site available for binding by substrate, substrate analogues, or nonsubstrate analogues.

enzyme-essential region is sufficient to cause inactivity of a given molecule.

Two boundaries of substrate-enzyme interface have been computed and are designated as the A region and the B region. Enzyme-essential region A represents the region in which the primary event of molecular recognition of the amino and carboxyl groups of L-methionine or a nonsubstrate amino acid analogue occurs. Therefore, it would be expected that, in this region, critical steric requirements exist for identification of this pharmacophoric grouping. We have included the α-carbon as a member of the three-point pharmacophore because small deviations in its accessibility have had drastic effects on the binding ability of a given analogue. Evidence for this is provided by the observation by Lombardini *et al.*[2] that α-methyl-L-methionine did not bind to the enzyme surface despite the fact that its α-methyl substituent *could* assume a position corresponding to a β-methylene group in the inhibitory analogue 1-aminocyclopentane-1-carboxylic acid. It is entirely possible that assumption of this "allowed" position for the α-methyl substituent of α-methyl-L-methionine would prevent the remaining thioalkyl chain from folding into its receptor-bound orientation.

The enzyme-essential region B (see Fig. 3) had been identified previously as a region in which small increments in steric bulk had large effects on inhibitory potency (7). For this reason it had been concluded that this was a region in which the steric requirements were critical for complementary fit to the enzyme surface. The computer mapping studies now enable a more precise definition of the enzyme surface boundary which must not be exceeded in this region if inhibitory activity is to be retained in a given amino acid analogue, and assists in predicting inactivity prior to synthesis.

It must be pointed out that region B, which has been defined as enzyme-essential, may not really be so, but might alternatively prove to be second substrate-essential, i.e., it might infringe upon the volume requirements for ATP at the active site region and be inactive for this reason. It would be of interest to determine whether the enzyme or ATP is the occupant in this area, but for the predictive purposes of drug design it is not essential to know.

Regions A and B indicate "real" boundaries of the L-methionine binding site. Refinement and expansion of the remaining surface of the enzyme-excluded volume map await synthesis and assay of inhibitory potencies of additional rigid amino acid analogues.

It is our hypothesis that any rigid amino acid analogue presenting the pharmacophore, and whose volume requirements are accommodated within the enzyme-ex-

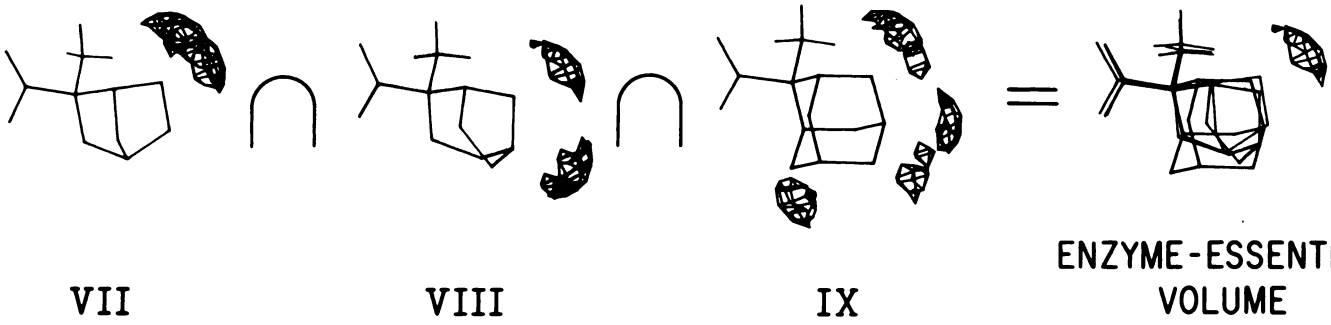

FIG. 3. *Enzyme-essential volume map*

Each inactive molecule is shown with its unique volume segments that are not part of the enzyme-excluded volume map. Intersection of the unique volume segments of each inactive analogue gives one region of unique volume overlap for all three molecules which defines the enzyme-essential volume, i.e., a region occupied by the enzyme and therefore not available for occupancy by other molecules.

cluded volume map, will be inhibitory. This prediction of activity is strictly qualitative and functional. The degree of inhibitory potency, which is a function of numerous other parameters, cannot be predicted from this conformational approach alone.

Computer graphics examination of the active rigid amino acid analogue 9-aminofluorene-9-carboxylic acid (VI) has provided significant details about the receptor-bound orientation of this molecule. The observed highly inhibitory properties of VI toward rat liver adenosyltransferase were in marked contrast to the low inhibitory levels seen for its analogue (R,S)-1-aminoindane-1-carboxylic acid (6), in which one of the phenyl groups has been removed from VI. It has been suggested that the planar conformation of VI produced large regions for possible hydrophobic interactions with the enzyme which contributed to its inhibitory properties (6). A more precise location of these hydrophobic areas on the enzyme surface was delineated when it was observed that IV was more inhibitory than I, suggesting that the *exo*-trimethylene region of IV participates in hydrophobic interactions that increase the molecule's binding capacity (7). Graphic comparision of VI and IV shows some overlap of the *exo*-cyclopentyl ring of IV with one of the phenyl rings of VI as seen in Fig. 4. The two overlapping rings are nearly coplanar and the distance between them is approximately 1.52 A, as determined from measurements of computer-generated diagrams. The observed overlap of the two rings is significant since it suggests that both the *exo*-cyclopentyl ring of IV and the phenyl ring of VI lie in a hydrophobic pocket at the active site, that this hydrophobic region is quite extensive in size, and that it includes, at minimum, the volume occupied by the union of the *exo*-cyclopentyl ring of IV and the overlapping phenyl ring of VI. The second phenyl ring of VI may serve to "anchor" the other phenyl ring within the hydrophobic region or may itself also be accommodated within this same region. The low inhibitory activity of (R,S)-1-aminoindane-1-carboxylic acid (6), whose cyclopentyl ring is nonplanar, indicates that the resultant shift in the position of its phenyl ring does not allow the same positive hydrophobic interactions as seen for the phenyl ring(s) of VI.

A significant observation emerges from computer-aided, steric mapping of the L-methionine binding site of rat liver methionine adenosyltransferase. Despite the small steric requirements of the natural substrate L-methionine or of the inhibitor, cycloleucine, there is an extremely large volume available for binding of either molecule at the active site. This might provide a partial explanation for the relatively low binding capacity of L-methionine to several isofunctional methionine adenosyltransferases isolated from a variety of microbial and mammalian sources: although the kinetic and mechanistic complexities of the enzymatic synthesis of adenosylmethionine have produced deviations from Michaelis-Menten kinetics (14, 15) and, therefore, have often precluded accurate determinations of K_m (L-methionine) for isofunctional methionine adenosyltransferases, a range of apparent K_m (L-methionine) values has been reported. Table 1 shows some of these values and it appears that these isofunctional enzymes can be classified as low K_m or high K_m forms. For the latter group, with K_m values in the millimolar range, L-methionine is not tightly bound at the active site. Deductions about the active-site conformation of L-methionine have been made by Lombardini *et al.* (1), through consideration of a series of straight-chain analogues of the amino acid substrate, as well as cyclic analogues, and the proposed planar, extended conformation of L-methionine would leave large regions still unoccupied at the L-methionine binding site. It remains to be seen whether low K_m isoenzymes have smaller enzyme-excluded volume maps than those of high K_m adenosyltransferases, or whether electronic interactions that increase binding affinity are primarily responsible for the higher substrate specificity of the low K_m forms.

The ultimate goal of these computer mapping studies

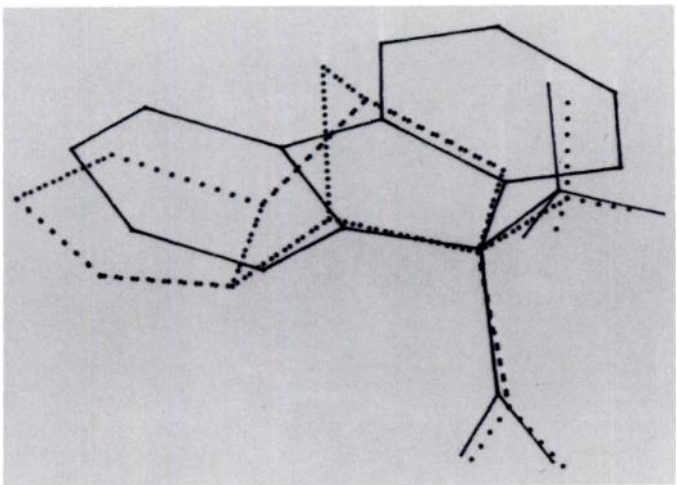

FIG. 4. *Graphic comparision of active analogues IV and VI*

Superimposition of IV and VI shows, at left, overlap of the *exo*-cyclopentyl ring of IV with a phenyl ring of VI. Compound IV is represented as a *dotted* structure; compound VI, as a *line* structure. The overlap of the *exo*-cyclopentyl and phenyl rings of the two molecules suggests that both of these rings lie in a hydrophobic pocket of the enzyme-active site.

TABLE 1

Apparent K_m (L-methionine) values for "high K_m" and "low K_m" isofunctional methionine adenosyltransferases

Enzyme source	High K_m (L-methionine)	Low K_m (L-methionine)	Reference
Yeast[a]	0.42–0.55 mM		14
Yeast[a]	0.31–1.4 mM		16
Yeast[a]			
Iso-I	0.11 mM		
Iso-II	0.14 mM		17
Rat liver			
Iso-I		30 μM	8
Iso-II		6 μM	8
Iso-III	0.2 mM (approx)		8
Rabbit liver	2.2 mM		18
Human liver		23.6–507 μM	19
Human liver: patient with hyperme-thioninemia		6.1–51.3 μM	19

[a] The differences in reported K_m (L-methionine) values for methionine adenosyltransferases in yeast reflect significant differences in enzyme assay conditions.

is to define the precise volume requirements for binding of amino acid analogues at the methionine binding site of the active site region of methionine adenosyltransferases from varying sources, so that this may be directly exploited in drug design. The rationale that selective methionine adenosyltransferase inhibitors could have pharmacologic utility, as proposed by Lombardini *et al.* (2), has been increasingly supported by the rapid expansion of knowledge about the cellular functions of *S*-adenosyl-L-methionine (1) and by recent biochemical evidence of multiple forms of methionine adenosyltransferase within cells of microbial and mammalian origin (8, 17, 20–24).

Clinical studies of children with persistent hypermethioninemia, who are deficient in hepatic methionine adenosyltransferase, have been reported by several investigators (19, 25–27). Finkelstein *et al.* (19) have found that, in a patient with this deficiency, the residual hepatic enzyme displays unusual kinetic properties, suggesting that it is a mutant enzyme. Tallan (25) has shown that, despite their deficiency in this hepatic enzyme, these patients have normal complements of methionine adenosyltransferases in their erythrocytes, skin fibroblasts, and lymphoid cells. Tallan's observation (25) provides evidence for the existence in humans of different forms of methionine adenosyltransferases under separate genetic control and suggests that differences in methionine adenosyltransferase isoenzymes might be greater than previously suspected. If these differences include differing volume requirements at the L-methionine binding site, then computer-aided mapping studies could provide strategic information for the future synthesis of amino acid analogue inhibitors of methionine adenosyltransferases with chemotherapeutic potential.

CONCLUSIONS

Computer-aided mapping of the combined occupied volume of six active amino acid analogues has generated an enzyme-excluded volume map of the L-methionine binding site of rat liver methionine adenosyltransferase. Three inactive rigid analogues have been examined to determine whether they require new volume not fitting into the enzyme-excluded volume map. All three inactive molecules present unique volume not found for the set of six active molecules which generate the enzyme-excluded volume map. Analysis of the unique volume segments required by these three inactive molecules has demonstrated an enzyme-essential region responsible for the inability of these molecules to bind. A self-consistent model has emerged from these mapping studies, i.e., each inactive molecule does not fit entirely into the enzyme-excluded volume map, as predicted, but instead requires additional volume responsible for negative steric interactions with the enzyme surface that contribute to its inactivity. Inhibitory potencies of the set of six active rigid analogues I–VI and three inactive rigid analogues VII–IX can be used to carry out mapping studies of other isofunctional or isozymal methionine adenosyltransferases in order to define differences in active site topography that can be exploited chemotherapeutically. Inhibitory data on additional rigid amino acid analogues can be used to refine the surface contours of the L-methionine binding site of rat liver methionine adenosyltransferase that have been computed in these studies.

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