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IRREVERSIBLE INHIBITION OF HUMAN *S*-ADENOSYLMETHIONINE DECARBOXYLASE BY THE PURE DIASTEREOMERIC FORMS OF *S*-(5′-DEOXY-5′-ADENOSYL)-1-AMMONIO-4-METHYLSULFONIO-2-CYCLOPENTENE (AdoMac)

YONG QIAN WU and PATRICK M. WOSTER*

Department of Pharmaceutical Sciences, College of Pharmacy and Allied Health Professions, Wayne State University, Detroit, MI 48202, U.S.A.

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Abstract—The *S*-adenosylmethionine (AdoMet) analogue AdoMac [*S*-(5′-deoxy-5′-adenosyl)-1-ammonio-4-methylsulfonio-2-cyclopentene], an enzyme-activated, irreversible inhibitor of the *Escherichia coli* form of *S*-adenosylmethionine decarboxylase (AdoMet-DC), also acts as a potent inhibitor of the human form of the enzyme. This analogue has been resolved recently into its four possible diastereomeric forms, and each pure diastereomer has now been evaluated as an inhibitor of human AdoMet-DC. As was the case for the bacterial enzyme, kinetic analysis revealed that the four pure diastereomeric forms of AdoMac differentially inhibit human *S*-adenosylmethionine decarboxylase (K_i values ranging between 11 and 63 μM). Although the human and bacterial forms of the enzyme each discriminated between the four diastereomers of AdoMac, each form appeared to bind optimally to a distinctly different diastereomer of the inhibitor. These data suggest that the active sites of human and bacterial AdoMet-DC are distinctly different, and that it may be possible to design inhibitors that are specific for a given form of the enzyme.

Key words: *S*-adenosylmethionine; decarboxylase; enzyme inactivation; conformational analysis; antiparasitic; AdoMac

The enzyme AdoMet-DC† (EC 4.1.1.50) is a controlling step in the biosynthesis of polyamines in a variety of organisms, and as such has become an important target for the development of novel antitumor and antiparasitic agents [1]. The catalytic subunit of both *Escherichia coli* and human AdoMet-DC contains a covalently bound pyruvate cofactor that must form an imine linkage with the substrate, AdoMet (Fig. 1), prior to decarboxylation to form dc-AdoMet [2–4]. We and others have taken advantage of this mechanistic feature to design specific, enzyme-activated, irreversible inhibitors of AdoMet-DC [1, 5–10]. Our group recently described the synthesis and biological evaluation of AdoMac (Fig. 1), a conformationally restricted analogue of AdoMet, which acts as a potent, irreversible inhibitor of AdoMet-DC from *E. coli* [10]. AdoMac has the potential to exist in four distinct diastereomeric forms arising from chirality at the 1 and 4 positions of the cyclopentene ring. It was postulated that, since each configurational isomer would represent a distinct conformational mimic, the pure diastereomeric forms of AdoMac could be used as conformational probes for the catalytic site of AdoMet-DC. To this end, the diastereomeric forms of AdoMac have been synthesized recently via a versatile chemoenzymatic pathway, and individually evaluated as enzyme-activated, irreversible inhibitors of the *E. coli* form of AdoMet-DC [11]. AdoMet-DC was able to discriminate between the four diastereomeric forms of AdoMac, which exhibited K_i values ranging between 4 μM (*cis*-1*S*,4*R* diastereomer) and 40 μM (*trans*-1*R*,4*R* diastereomer). However, the rate of AdoMet-DC inactivation did not vary significantly between the diastereomeric forms of AdoMac, with k_{inact} values ranging between 0.064 and 0.099 min^{-1}. The dihydro form of AdoMac, H_2-AdoMac (Fig. 1), and the unmethylated precursor, nor-AdoMac (Fig. 1), do not possess the mechanistic driving force to act as irreversible inactivators of AdoMet-DC, and were found to be weak competitive inhibitors of the enzyme.

Previous studies have shown that there is little sequence homology between the *E. coli* and human forms of AdoMet-DC [4, 8, 12, 13], raising the possibility that, apart from the requisite terminal pyruvate moiety and a few other critical residues, there may be distinct differences in the amino acid composition of the catalytic sites within the various forms of the enzyme. In addition, the allosteric

* Corresponding author. Tel. (313) 577-1523; FAX (313) 577-2033.

† Abbreviations: AdoMet, *S*-adenosylmethionine; dc-AdoMet, decarboxylated *S*-adenosylmethionine; AdoMet-DC, *S*-adenosylmethionine decarboxylase; AdoMac, *S*-(5′-deoxy-5′-adenosyl)-1-ammonio-4-methylsulfonio-2-cyclopentene; H_2-AdoMac, *S*-(5′-deoxy-5′-adenosyl)-1-ammonio-4-methylsulfoniocyclopentane; nor-AdoMac; *S*-(5′-deoxy-5′-adenosyl)-1-ammonio-4-mercapto-2-cyclopentene; AbeAdo, 5′-{[(*Z*)-4-amino-2-butenyl] methylamino}-5′-deoxyadenosine; DTT, dithiothreitol; and MTA, methylthioadenosine.

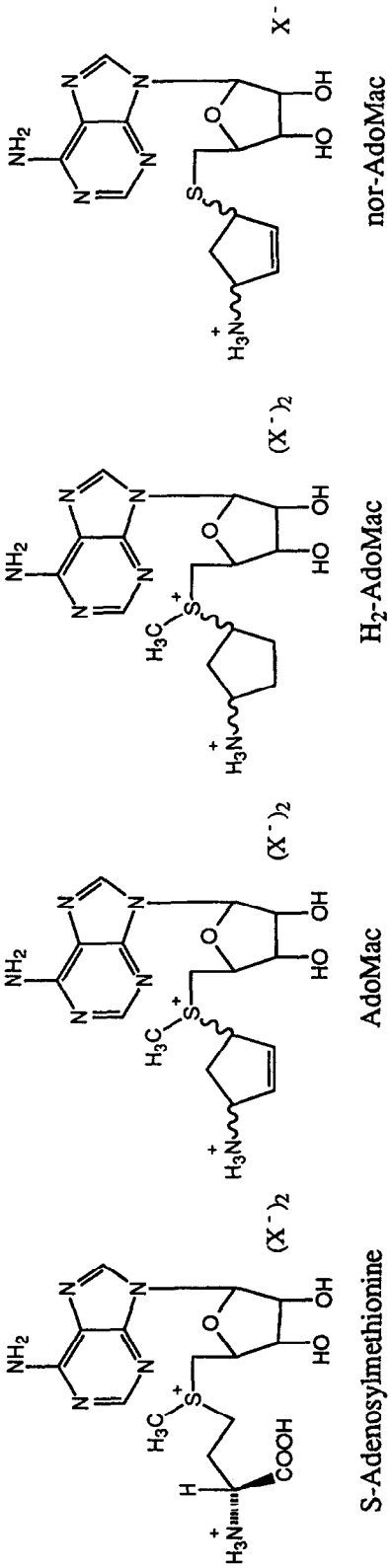


Fig. 1. Structures of *S*-adenosylmethionine (AdoMet), AdoMac, H_2-AdoMac and nor-AdoMac.

control mechanisms are different for these two forms of the enzyme, since the bacterial enzyme is strongly activated by Mg^{2+}, while the human form is activated by putrescine. To determine whether topological differences between the bacterial and human catalytic sites exist, the pure diastereomeric forms of AdoMac were also evaluated as inhibitors of human AdoMet-DC. We now report the results of the kinetic analysis for the inhibition of human AdoMet-DC by the pure diastereomers of AdoMac, and compare these results with those obtained from evaluation of these conformational mimics against the *E. coli* form of the enzyme.

MATERIALS AND METHODS

Chemicals. The four pure diastereomeric forms of AdoMac, as well as *cis*-1*S*,4*R*-H_2-AdoMac and nor-AdoMac, were synthesized as previously reported [10, 11]. All (*R*)- and (*S*)-stereochemical assignments made below are with respect to the cyclopentenyl or cyclopentanyl ring systems present in the molecules. *S*-Adenosyl-L-[^{14}C-COOH]methionine was purchased from Amersham Life Science Division, Arlington Heights, IL, and *E. coli* (3/4 log phase) was purchased from the Grain Processing Corp., Muscatine, IA. Reagents for the determination of protein using the method of Bradford [14] were purchased from the Bio-Rad Corp., Rockville Centre, NY. All other reagents were purchased from the Aldrich Chemical Co., Milwaukee, WI or the Sigma Chemical Co., St. Louis, MO, and were used without further purification. Centrifugation was carried out on a Beckman L7 ultracentrifuge. Radioactivity was measured using an LKB 1209 Rackbeta liquid scintillation counter. Sonic disruption of *E. coli* was carried out using a Beckman 4710 series ultrasonic homogenizer.

Enzyme isolation and assay procedure. AdoMet-DC was isolated from *E. coli* using a modification of the methylglyoxal-*bis*-guanylhydrazone (MGBG)-Sepharose affinity column procedure of Anton and Kutny [4] as previously described [10]. The resulting AdoMet-DC was greater than 90% pure as determined by gel electrophoresis, and the specific activity was typically determined to be 0.80 μmol/min/mg protein at 37°. The bacterial enzyme was stored in 20 mM potassium phosphate, 0.1 M KCl, 0.5 mM EDTA and 0.5 mM DTT, pH 7.4, at 4°. Protein concentrations were measured by the method of Bradford [14] using bovine serum albumin as a standard. For the *E. coli* form of the enzyme, AdoMet-DC activity was monitored by following the evolution of $^{14}CO_2$ from *S*-adenosyl-L-[^{14}C-COOH]methionine using a modification of the procedure of Markham *et al.* [3], as previously described [10].

The human form of AdoMet-DC was a gift from Dr. Anthony E. Pegg of the Penn State University Hershey Medical Center, Hershey, PA. For studies involving the human form of AdoMet-DC (sp. act. 0.3 μmol/min/mg protein), enzyme activity was determined using a modification of the procedure described for the bacterial enzyme assay [10]. Each reaction mixture contained 50 μg of AdoMet-DC,

20 μL of 1 mM *S*-adenosyl-L-[^{14}C-COOH]methionine (0.9 mCi/mmol, 20 μM final concentration) in buffer (0.1 M sodium phosphate, 1 mM putrescine, 2.5 mM DTT, pH 7.5), with a final volume of 1 mL. All reactions were carried out in a tightly closed scintillation vial, and radiolabeled CO_2 was trapped on a filter disk in the vial cap soaked with 20 μL of 1 M hyamine hydroxide. Assay incubations were performed at 37° and were initiated by addition of enzyme and terminated after 20 min by addition of 1 N $HClO_4$. After an additional 20 min at 37°, the filter disk was placed in a scintillation vial with 10 mL of scintillation fluid and counted (counting efficiency 95% or greater). In all cases, enzyme activity (V) is expressed as micromoles of $^{14}CO_2$ produced per minute per milligram of protein $\times 10^{-7}$. Each data point reported below represents the average of two determinations, which in each case differed by less than 5%.

Kinetic analysis of human AdoMet-DC inhibition. Time-dependent decreases in enzyme activity were monitored in buffer (0.1 M sodium phosphate, 1 mM putrescine, 2.5 mM DTT, pH 7.5) for at least four concentrations of each inhibitor (typically, 5–50 μM) over a period of 25 min. The rate of irreversible inhibition was monitored by withdrawing aliquots for assay at 4–5 time intervals following partial inactivation in the presence of the appropriate concentration of inhibitor and the absence of AdoMet. These aliquots were then used to initiate the enzymatic reaction in the assay system described above. In each case, the time-dependent increase in activity (expressed as ln % activity) was linear, and a pseudo-first-order rate constant was derived from each line. Replotting of the rate constants (k_{obs}), using the method of Kitz and Wilson [15], then allowed the k_i and k_{inact} values to be determined graphically.

HPLC analysis of MTA from enzyme assay mixtures. AdoMet-DC was inactivated at an inhibitor concentration of 100 μM in the assay medium described above at 4°, with a total volume of 0.5 mL. Samples were incubated in duplicate for 2-, 6- and 12-hr time intervals for each determination. The resulting solutions were filtered through a Centricon-30 device at 5000 × *g*, and 30 mL of the supernatant was analyzed by HPLC (Waters C-18 Novapak, 0.80 × 10 cm, 4 μm) according to the reversed-phase ion pairing assay procedure of Wagner *et al.* [16].

Molecular modeling of the diastereomeric forms of AdoMac. The pure diastereomeric forms of AdoMac were modeled on a Silicon graphics 4D 220 GTX workstation using the SYBYL molecular modeling software package (Tripos & Associates, St. Louis, MO). Each diastereomer was assigned charges using the Gasteiger–Hückel option within the SYBYL program, and energy minimized (including electrostatics) using the Tripos force field equation. Following assignment of rotatable bonds, each diastereomer was subjected to a systematic conformational search of all allowed conformations, such that conformers with a total energy of 10 kcal/mol greater than the base structure were rejected. Each rotatable bond was searched in 5 degree increments over a range of 360 degrees, and no distance geometry limitations, constraints or coordinate maps were imposed other than standard Van der Waals' factors (general = 0.95, 1,4 = 0.87, H-bond = 0.65). The resulting conformers were then plotted, and in each case a single least-energy conformer was identified. The appropriate least-energy conformation was then re-minimized as described above prior to RMS fitting (FIT subroutine) and distance measurement.

RESULTS

The human form of AdoMet-DC exhibits prominent differences from that of the *E. coli* form in terms of primary sequence (only about 10% sequence homology), subunit structure, and in the requirement of putrescine rather than Mg^{2+} for activation. As is the case for the bacterial enzyme, the precise amino acid composition of the human AdoMet-DC active site is unknown. Thus, a study of the conformational requirements of this enzyme was undertaken using the pure diastereomeric forms of AdoMac, in the hope that preliminary information pertaining to the catalytic site, and to the differences between the human and bacterial forms of AdoMet-DC, could be gained. As was observed for the inactivation of *E. coli* AdoMet-DC, each pure diastereomer of AdoMac inactivated the human enzyme in a time- and concentration-dependent manner. In each case, a pseudo-first-order rate constant of inactivation, k_{obs}, was obtained graphically, as shown in Fig. 2, and the resulting data were replotted using the Kitz–Wilson method [15], as shown in Fig. 3. The kinetic parameters thus obtained are summarized in Table 1, along with those previously determined for the isolated *E. coli* enzyme. The K_i values for the four diastereomeric forms of AdoMac ranged between 11 and 63 μM, while the corresponding k_{inact} values did not vary significantly, and were similar to those observed for the inactivation of the bacterial enzyme.

The value of the partition ratio (i.e. the ratio of k_{cat}/k_{inact}) for *cis*-1*R*,4*S*-AdoMac, the most potent diastereomeric inactivator of human AdoMet-DC, was evaluated indirectly by the titration method. Increasing amounts of *cis*-1*R*,4*S*-AdoMac were added to a known amount of enzyme, and the reaction was allowed to go to completion (24-hr incubation time). The mixture was then assayed to determine the percent activity remaining. Figure 4 shows a plot of the ratio of moles of inactivator per mole of enzyme ([I]/[E]) versus the percent enzyme activity remaining, in which the [I]/[E] ratios were varied from 1 to 30. Product inhibition arising from the presence of MTA could be observed, as indicated by the deviation from linearity at higher concentrations of the inhibitor. However, at ratios of [I]/[E] of 8.0 or less, a linear relationship was observed. Extrapolation of this region by linear regression revealed a turnover number of 9.84 ± 1.0, and thus the partition ratio was determined to be 8.84. This observation then allowed for the calculation of the k_{cat} value for *cis*-1*R*,4*S*-AdoMac, which was found to be 0.645 min^{-1}.

As was observed for the *E. coli* form of the enzyme [11], human AdoMet-DC was protected from inactivation by the pure diastereomers of AdoMac when the enzyme had been preincubated

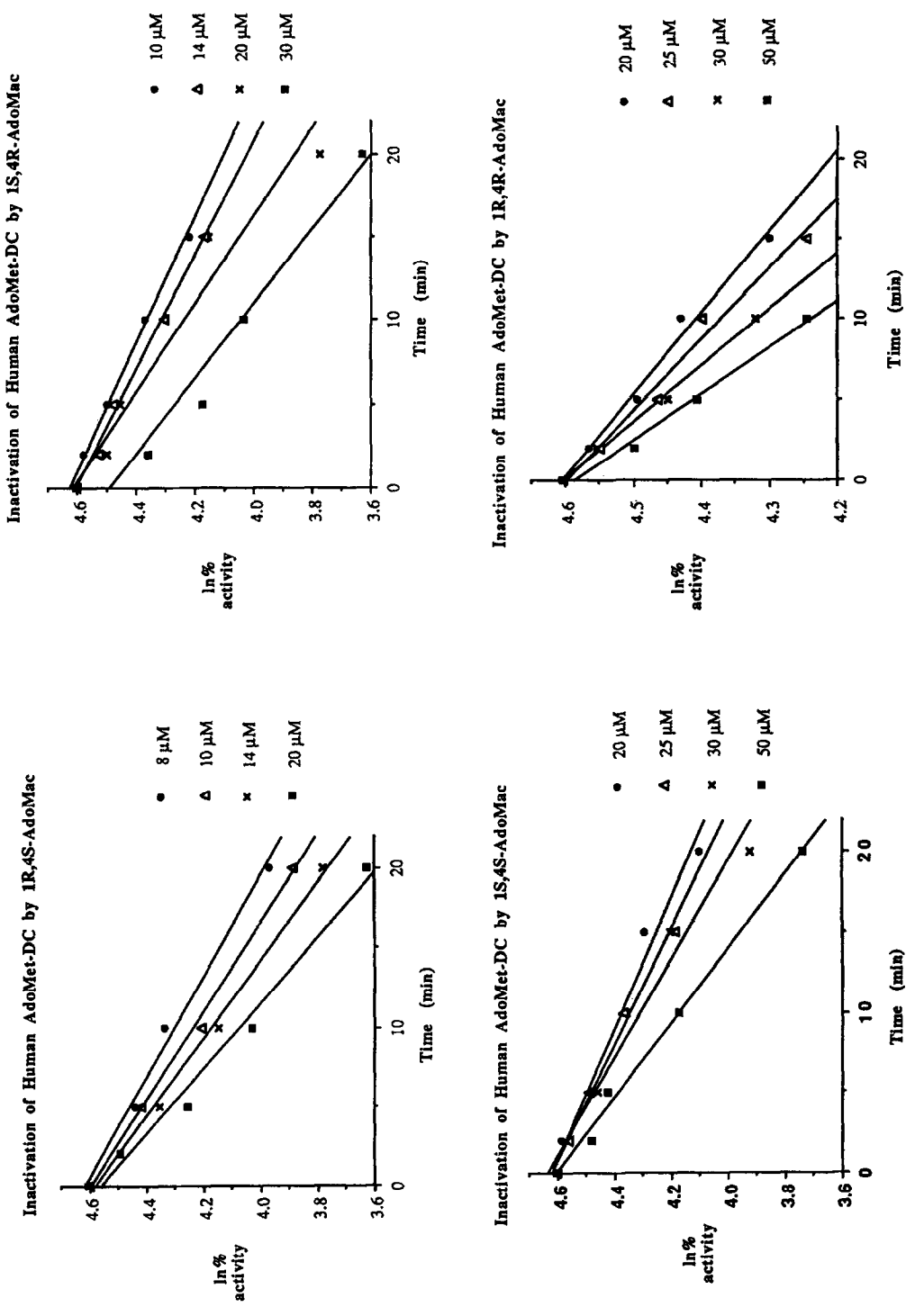


Fig. 2. Time-dependent inactivation of human AdoMet-DC by the pure diastereomers of AdoMac. AdoMet-DC was incubated with the indicated concentration of inhibitor over a 20-min time period. Samples were withdrawn at various time points and assayed for enzyme activity as described in Materials and Methods. Each data point is the average of two determinations, which in each case differed by 5% or less.

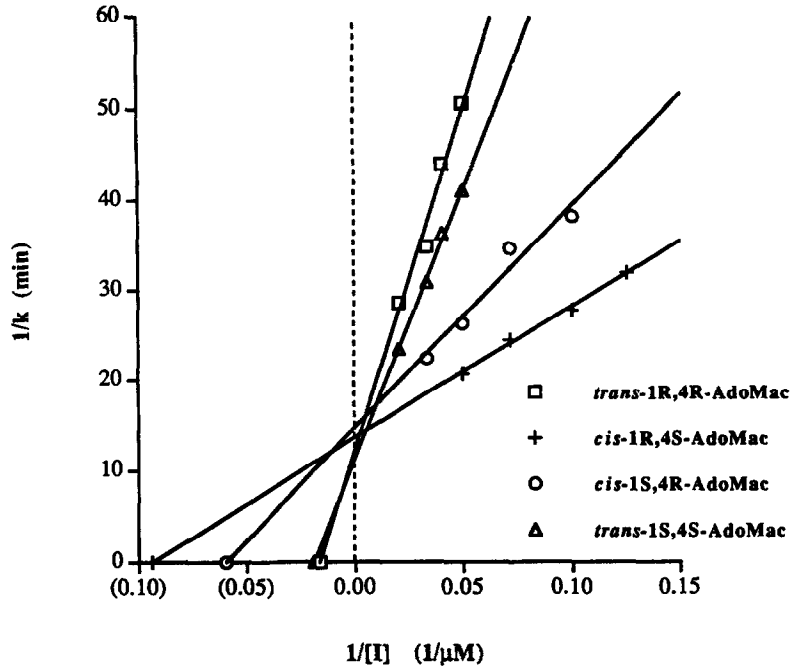


Fig. 3. Kitz–Wilson plot for the inactivation of human AdoMet-DC by the pure diastereomers of AdoMac. The appropriate k_{obs} values derived from the time activation studies shown in Fig. 2 were replotted using the method of Kitz and Wilson [15]. Values for K_i and k_{inact} were then determined by linear regression.

Table 1. Comparison of the kinetic parameters for the inactivation of human and *Escherichia coli* AdoMet-DC by the pure diastereomeric forms of AdoMac, *cis*-1*S*,4*R*-H_2-AdoMac and *cis*-1*R*,4*S*-nor-AdoMac

Inhibitor	K_i (human)	k_{intact} (human)	K_i (*E. coli*)	K_{intact} (*E. coli*)	Time dependence	MTA generation
cis-1*R*,4*S*-AdoMac	11 μM	0.073 min^{-1}	8 μM	0.099 min^{-1}	Yes	Yes
cis-1*S*,4*R*-AdoMac	17 μM	0.068 min^{-1}	4 μM	0.064 min^{-1}	Yes	Yes
trans-1*S*,4*S*-AdoMac	53 μM	0.088 min^{-1}	24 μM	0.068 min^{-1}	Yes	Yes
trans-1*R*,4*R*-AdoMac	63 μM	0.082 min^{-1}	40 μM	0.079 min^{-1}	Yes	Yes
cis-1*S*,4*R*-H_2-AdoMac	72 μM		93 μM		No	No
cis-1*R*,4*S*-nor-AdoMac	307 μM		293 μM		No	No

with the known competitive inhibitor MGBG. In addition, the inhibition produced by each diastereomer was irreversible, as demonstrated by the inability to dialyze away the inhibitor following binding to AdoMet-DC. HPLC product analysis of the enzymatic reaction mixture for each diastereomer of AdoMac was undertaken to determine whether the expected by-product, MTA, was generated during inactivation of the enzyme. These experiments revealed the time-dependent appearance of a peak that co-eluted with MTA, and the corresponding disappearance of the peak co-eluting with AdoMac [11], suggesting that MTA was generated from AdoMac in the enzymatic reaction as predicted. No other metabolites related to AdoMet could be detected under the assay conditions. To rule out the possibility of non-enzymatic generation of MTA from AdoMac under the assay conditions described, control experiments were carried out in the absence of AdoMet-DC, and in the presence of AdoMet-DC that had been inactivated by boiling at 100° for 5 min. Under these conditions, no generation of MTA or any other metabolite of AdoMac was observed during the 20-min assay period.

In an effort to verify the mechanism of inactivation of human AdoMet-DC by AdoMac, the related analogues H_2-AdoMac and nor-AdoMac were designed as putative inhibitors of human AdoMet-DC. In theory, these analogues should also form an imine linkage with the terminal pyruvate of AdoMet-DC. However, neither of these analogues possesses the driving force for elimination of MTA and formation of a latent electrophile within the AdoMet-DC catalytic site. Thus, it was reasoned that these

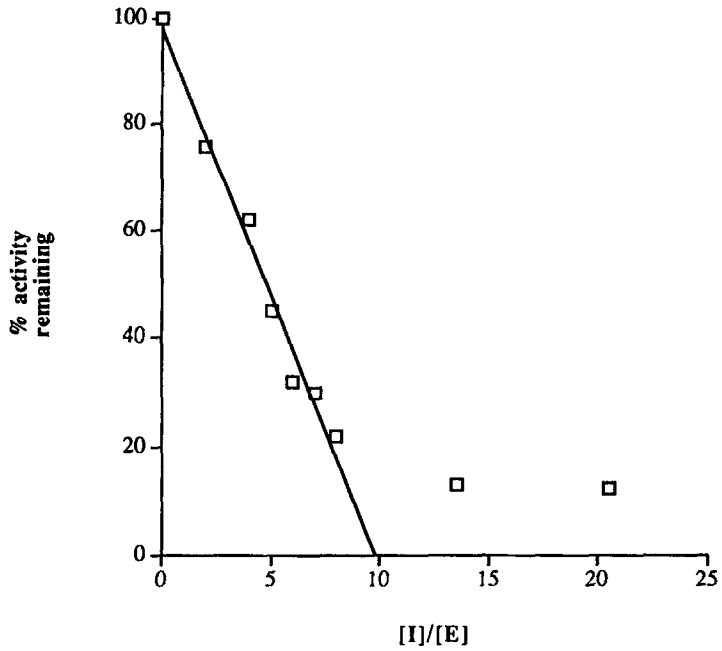


Fig. 4. Partition ratio determination for the inactivation of human AdoMet-DC by *cis*-1*R*,4*S*-AdoMac. The partition ratio for the interaction of *cis*-1*R*,4*S*-AdoMac with human AdoMet-DC was determined by the titration method. Enzyme activity was measured following 24-hr incubations at various inactivator to enzyme ratios. Each data point is the average of two determinations, which in each case differed by 5% or less.

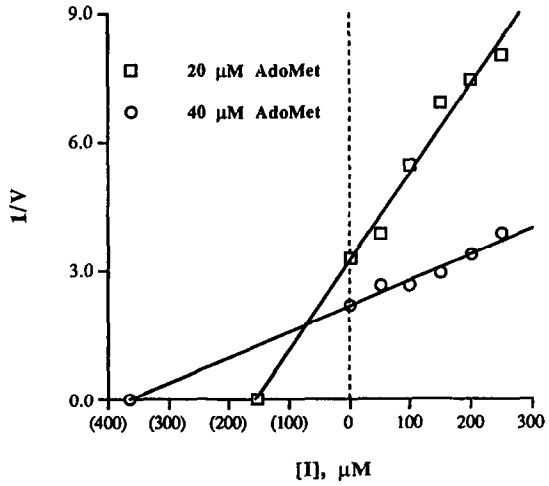


Fig. 5. Dixon plot demonstrating the reversible inhibition of human AdoMet-DC by *cis*-1*S*,4*R*-H_2-AdoMac. Enzyme activity was determined at various concentrations of inhibitor, and at two concentrations of the substrate, AdoMet. The inhibitor constant K_i was then determined by linear regression. Each data point is the average of two determinations, which in each case differed by 5% or less.

analogues would act as competitive inhibitors of the enzyme, and would thereby serve as suitable control compounds with respect to AdoMac. To test this hypothesis, the analogues *cis*-1*S*,4*R*-H_2-AdoMac and *cis*-1*R*,4*S*-nor-AdoMac, each of which possesses the same absolute stereochemistry as the most potent diastereomer *cis*-1*R*,4*S*-AdoMac, were evaluated for inhibitory activity against AdoMet-DC, as described above. The rate of reversible AdoMet-DC inhibition was determined at various concentrations of inhibitor and at two different concentrations of AdoMet (typically 20.0 and 40.0 μM). The resulting data were analyzed using Dixon plots to determine the apparent K_i value of each inhibitor. As expected, *cis*-1*S*,4*R*-H_2-AdoMac reversibly inhibited human AdoMet-DC with a K_i value of 72 μM (Fig. 5, Table 1). Likewise, *cis*-1*R*,4*S*-nor-AdoMac acted as a weak competitive inhibitor of human AdoMet-DC, with a K_i value of 307 μM (Fig. 6, Table 1). The inhibition observed in the presence of H_2-AdoMac and nor-AdoMac was not time dependent, and no generation of MTA or any other related metabolite was detected in the enzymatic reaction mixture following exposure to these analogues.

DISCUSSION

The primary amino acid sequences for the *E. coli* and human forms of AdoMet-DC are known, and the two forms of the enzyme have been shown to exhibit little sequence homology [8, 12]. Thus, it is reasonable to assume that significant differences

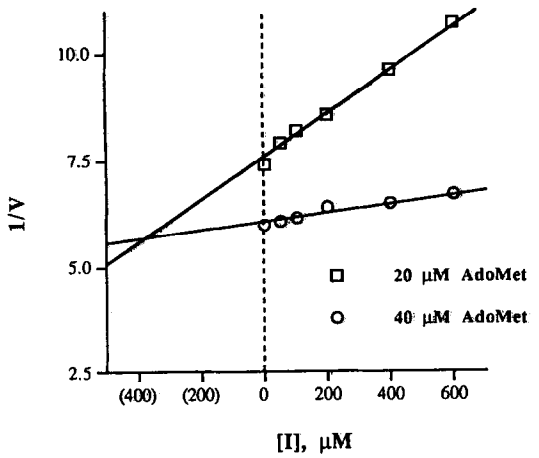


Fig. 6. Dixon plot demonstrating the reversible inhibition of human AdoMet-DC by *cis*-1*R*,4*S*-nor-AdoMac. Enzyme activity was determined at various concentrations of inhibitor, and at two concentrations of the substrate, AdoMet. The inhibitor constant K_i was then determined by linear regression. Each data point is the average of two determinations, which in each case differed by 5% or less.

may exist between the catalytic sites of these two forms of the enzyme. A number of recent studies have identified specific amino acid residues that are thought to participate in the function of human and/or bacterial AdoMet-DC [1, 4, 8, 12, 13, 17, 18]. In one of these studies, a terminal pyruvate-containing peptide fragment was isolated and identified following formation of an adduct between human AdoMet-DC and the known inhibitor 5′-deoxy - 5′ - [3 - hydrazinopropyl)methylamino]-adenosine (MHZPA) [8]. These studies shed light on the catalytic mechanism of AdoMet-DC, but in the absence of a crystal structure for any of the known forms of the enzyme, they can predict little concerning the structural and stereochemical requirements of the active site. Casara *et al.* [6, 19] have described AbeAdo, which acts as an irreversible, enzyme-activated inhibitor of the *E. coli* (K_i = 0.3 μM, k_{inact} = 3.6 min^{-1}) and rat liver (K_i = 0.56 μM) forms of AdoMet-DC. Although AbeAdo does not exhibit a significant difference in K_i between the bacterial and mammalian form of AdoMet-DC, there is a significant difference in potency between the Z- and E-forms of the analogue. Specifically, the Z-form of AbeAdo is 1000-fold more potent against the *E. coli* form of AdoMet-DC [6], and 100-fold more potent against the rat liver form of the enzyme [19], when directly compared with the E-form. These observations suggest that conformationally restricted analogues such as AdoMac, when isolated in pure diastereomeric form, could be useful as topological probes for the active site of AdoMet-DC. As shown in Table 1, both the human and *E. coli* forms of AdoMet-DC are able to discriminate between the four diastereomers of AdoMac, and in each case the *cis* diastereomers (with respect to the cyclopentene ring) are significantly more potent than the *trans*. However, human AdoMet-DC prefers the *cis*-1*R*,4*S*-diastereomer, while the bacterial enzyme preferentially binds to *cis*-1*S*,4*R*-AdoMac. When subjected to computer-assisted molecular mechanics analysis, it is evident that these two molecules possess significantly different least-energy conformations, as shown in Fig. 7. In each case, the appropriate *cis* diastereomer was subjected to a systematic conformational search, as described in Materials and Methods, resulting in the identification of a single least-energy conformer for each diastereomer. The two *cis* conformers were then superimposed using the RMS fitting routine by specifying the atoms in the conformationally restricted aminopropyl sidechain. As seen in Fig. 7, RMS fitting of these diastereomers results in relatively poor overlap, with a mean root square distance of 0.557 Å. Since the charged ammonium and methylsulfonium moieties are reversed in the two diastereomers, the doubly

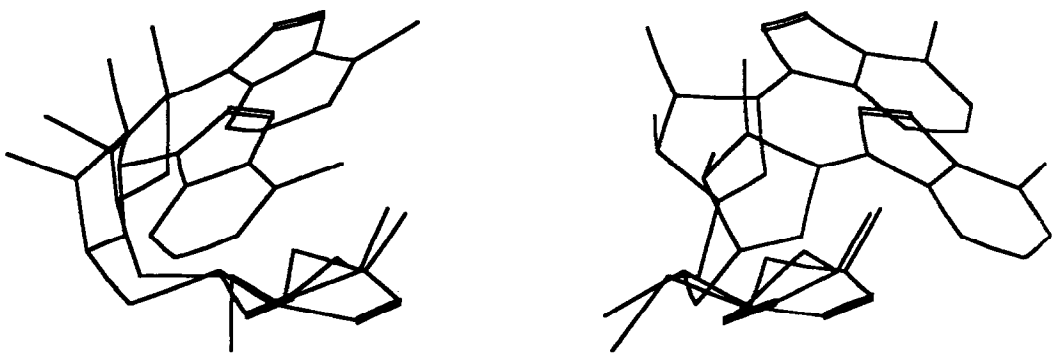
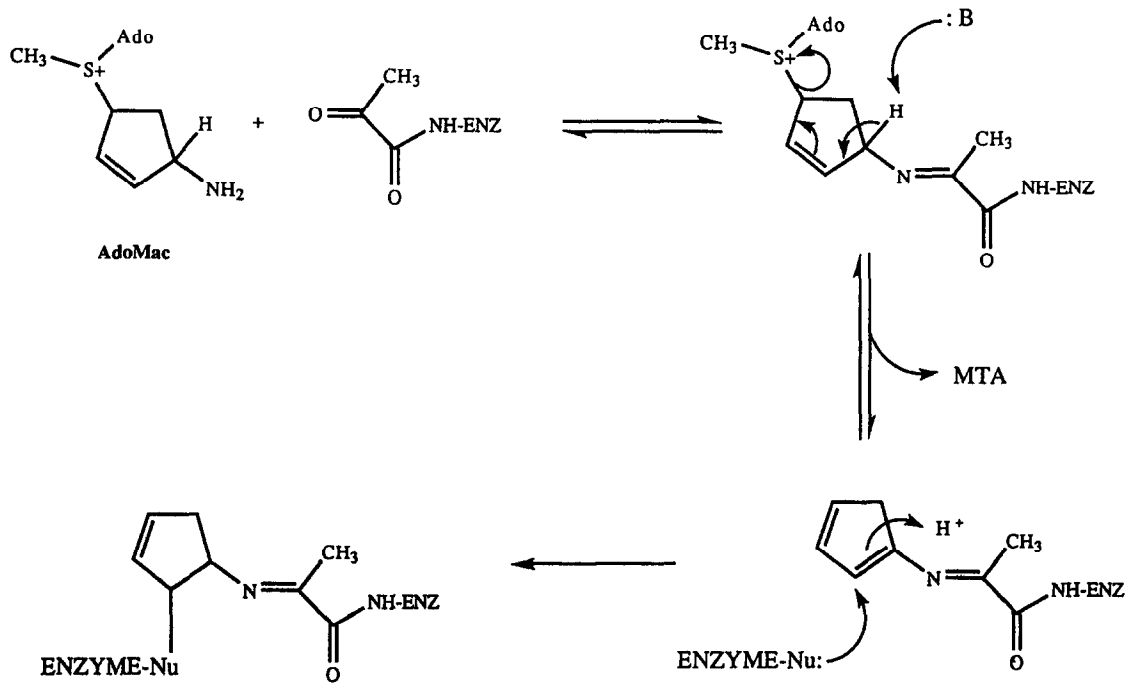


Fig. 7. Results of RMS fitting experiments for the *cis*-1*R*,4*S*- and *cis*-1*S*,4*R*-diastereomers of AdoMac. The appropriate molecules were subjected to systematic conformational analysis, and then energy minimized as described in the text. The resulting conformers were then compared using a standard RMS fitting routine. Hydrogens have been removed from the structures displayed above for clarify. Left: normal view. Right: orthogonal view.



Scheme I.

bonded carbons in the cyclopentene ring lie on opposite sides of a plane containing these charged moieties. As a result, the 1*R*,4*S*- and 1*S*,4*R*-methylene carbons at C5 are separated by a distance of 1.1 Å following RMS fitting. Overlap in the adenosyl portion of the molecule is even poorer, with a separation of 1.6 Å between the ribose ether oxygens, and 3.8 Å between the adenosine N^6-amino groups. Thus, computer simulation suggests that there are significant conformational differences between the *cis*-1*R*,4*S*- and *cis*-1*S*,4*R*-diastereomers of AdoMac, which may account for the observed variation in the K_i values between the human and bacterial forms of the enzyme.

It has been postulated that AdoMac inactivates AdoMet-DC by rearrangement to an electrophilic species within the catalytic site, driven by the elimination of MTA, as shown in Scheme I. The results of the present study strongly support this mechanism, since MTA was detected as the sole by-product of the reaction for each diastereomer of AdoMac, and for both the human and bacterial forms of AdoMet-DC. This by-product was not detected following reversible inhibition by *cis*-1*S*,4*R*-H_2-AdoMac, or by *cis*-1*R*,4*S*-nor-AdoMac, neither of which possesses the driving force for the elimination of MTA. In addition, the present findings suggest the possibility that AdoMac inhibits the bacterial and human forms of the enzyme by the same mechanism, and possibly through a common intermediate, since the k_{inact} values are strikingly similar for each determination. However, additional studies are required to determine the validity of these hypotheses. Interestingly, the inhibitor AbeAdo probably inhibits the human and *E. coli* forms of AdoMet-DC by different mechanisms, since the corresponding by-product methylamino-adenosine (MAA) is detected following inactivation of the bacterial enzyme [6], but is not directly formed after inactivation of human AdoMet-DC [8]. Inactivation of the human enzyme by AbeAdo is now thought to occur by transamination of the terminal pyruvate to alanine, proceeding via a mechanism that produces a by-product that ultimately may or may not be metabolized to MAA [8].

The data presented in Table 1 support the contention that there are distinct differences in the conformational requirements of the catalytic sites of human and *E. coli* AdoMet-DC. These differences can potentially be exploited to design analogues that are specific for a given form of the enzyme. Development of an active site model for the isozymic forms of AdoMet-DC would be facilitated by the design, synthesis, evaluation and computer modeling of a series of conformationally restricted analogues of AdoMet, and efforts along these lines are

underway in our laboratories. The exploitation of conformational differences between isozymes of AdoMet-DC would be of particular value in the case of parasitic organisms such as *Trypanosoma brucei*, which possess yet another distinct form of putrescine-activated AdoMet-DC [20]. To act as trypanocidal agents, AdoMet analogues must enter trypanosomes using a recently discovered adenosine transport system [21]. We have shown recently that AdoMac and related analogues act as parasite-specific toxins, since they appear to enter trypanosomes via this adenosine transport system, but do not affect human cultured cell lines [22]. Inhibition of trypanosomal growth by conformationally restricted analogues of AdoMet also appears to be strongly conformation dependent in all of the strains tested to date (unpublished observations), with IC_{50} values ranging between 0.9 and 100 μM. These data suggest that conformationally restricted analogues of AdoMet may also be useful to probe the conformational requirements of the adenosine transport protein and/or the trypanosomal form of AdoMet-DC. These studies, which may aid in the development of a novel series of antiparasitic agents, are currently underway in our laboratories.

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