Purification and Properties of S-Adenosyl-\(\text{L}\)-methionine:Methionine S-Methyltransferase from Wollastonia biflora Leaves*

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Franck James, Kurt D. Nolte, and Andrew D. Hanson†

From the Plant Molecular and Cellular Biology Program, University of Florida, Gainesville, Florida 32611

The plant enzyme S-adenosylmethionine:methionine S-methyltransferase (EC 2.1.1.12, MMT) catalyzes the synthesis of S-methylmethionine. MMT was purified 620-fold to apparent homogeneity from leaves of Wollastonia biflora. The four-step purification included fractionation with polyethylene glycol, affinity chromatography on adenosine-agarose, anion exchange chromatography, and gel filtration. Protein yield was about 180 \(\mu\)g/kg of leaves. Estimates of molecular mass from sodium dodecyl sulfate-polyacrylamide gel electrophoresis and native gel filtration chromatography were, respectively, 115 and 450 kDa, suggesting a tetramer of 115-kDa subunits. The 115-kDa subunit was photoaffinity labeled by \(\text{S-}\text{[\(3\text{H}\)]}\text{[2\text{H}]}\text{methionine}. Antibodies raised against \(W.\) biflora MMT recognized a 115-kDa polypeptide in partially purified MMT preparations from leaves of lettuce, cabbage, clover, and maize.

The pH optimum of \(W.\) biflora MMT was 7.2. Kinetic analysis of substrate interaction and product inhibition patterns indicated an Ordered Bi Bi mechanism, with S-adenosylmethionine the first reactant to bind and S-adenosylhomocysteine the last product to be released. The enzyme catalyzed methylation of selenomethionine and ethionine, but not of S-methylcysteine, homocysteine, cysteine, or peptidylmethionine. Tests with other substrate analogs indicated that a free carboxyl group was required for enzyme activity, and that a free amino group was not.

The tertiary sulfonium compound S-methyl-L-methionine (SMM), also termed vitamin \(U\), occurs in a wide variety of plants at levels ranging from about 0.01 to 6 \(\mu\)mol/g dry weight (1-4). SMM is synthesized by the action of AdoMet:Methionine S-methyltransferase (MMT), which catalyzes the following reaction (2).

\[
\text{Met} + \text{AdoMet} \rightarrow \text{SMM} + \text{AdoHcy}
\]

\text{REACTION 1}

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† To whom correspondence should be addressed: Plant Molecular and Cellular Biology Program, 2143 Fifield Hall, University of Florida, Gainesville, FL 32611-0690. Tel.: 904-392-1928; Fax: 904-392-6479.

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This activity has been detected in many phylogenetically diverse plants (2, 3).

We recently demonstrated that SMM is the first intermediate in the biosynthesis of DMSP from Met in the salt-tolerant plant Wollastonia biflora (5). DMSP is a compound with strong osmoprotective properties that may play a role in adaptation to salt and drought stress (6). DMSP is known to accumulate in many marine algae (7) and in a small number of flowering plants; in addition to \(W.\) biflora (family Asteraceae), these include species of Spartina (8, 9) and Saccharum (10) (family Poaceae). As for other osmoprotective compounds (11), genetic engineering of DMSP biosynthesis has been proposed as a strategy to enhance the stress resistance of crop plants (12).

In plants that do not accumulate DMSP, SMM may have two other metabolic fates: reaction with Hcy to give two molecules of Met, and hydrolysis to Hse and dimethyl sulfide (1, 2). The former reaction, catalyzed by SMM:Hcy S-methyltransferase (EC 2.1.1.10), could allow SMM to act as an efficient storage form for Met, and there is evidence for this in senescing flower tissue (13). Mudd and Datko (3) have further proposed that tandem operation of MMT and SMM:Hcy S-methyltransferase, which they referred to as the SMM cycle, could sustain the pool of free Met in the event of an overshoot in the conversion of Met to AdoMet.

Despite its wide distribution among plants, MMT has not previously been purified to homogeneity (2, 14). As might be expected from the high rate of SMM production required for DMSP accumulation (5), \(W.\) biflora leaves proved to be rich in MMT. We report here the purification and characterization of the enzyme from this source. We also show that an MMT polypeptide similar in size and antigenically related to that of \(W.\) biflora is present in various plants that produce SMM but do not accumulate DMSP.

EXPERIMENTAL PROCEDURES

Plant Material

\(W.\) biflora (L.) DC, was grown as described previously (5), or in a greenhouse under natural illumination. Genotype H (5) was used for MMT purification. Maize (Zea mays L.) was greenhouse grown; white clover (Trifolium repens L.) was field grown. Lettuce (Lactuca sativa L.) and red cabbage (Brassica oleracea L.) were from a local market. Mature leaves were devided, washed, frozen in liquid \(N_2\), ground into fragments (about 5 \(\times\) 5 mm), and stored at \(-80^\circ\)C for up to 8 weeks before extraction.

Chemicals

\([\text{methyl-}^{3\text{H}}]\text{Met}\) (1.61 GBq/mmol), [\(\text{methyl-}^{3\text{H}}\text{AdoMet}\) (2.1 GBq/ mmol), and [\(\text{methyl-}^{3\text{H}}\text{AdoMet}\) (3.15 TBq/mmol) were from DuPont NEN. AdoMet was purchased from Sigma and used directly for routine assays; for kinetic studies it was purified as described (15). 3-(Methylthiol)propanoic acid and its methyl ester were from TCI (Tokyo, Japan). 3-(Methylthiol)propylamine was from Chem Service (West Chester, PA); 3-(methylthio)propanol was from Aldrich. Dimethylsulfoxonium compounds were synthesized using iodomethane (16). Other Met analogs,
protein modification reagents, standards to calibrate the gel filtration column, and polyvinylpolypyrrolidone were from Sigma. PEG was from BDH (Ville St-Laurent, Canada). The protein dye reagents and SDS-PAGE protein standards were from Bio-Rad. All other chemicals were of the highest purity available.

Measurement of MMT Activity

Standard Assay Conditions—MMT was assayed essentially as described by Mudd and Datko (3). Enzyme (up to 50 μg) was incubated in a final volume of 100 μl with 1 mM Met (containing 16.3 kBq of [methyl-3H]AdoMet/assay) and 1 mM AdoMet, in a buffer mixture containing 100 mM Tris base, 50 mM Mes free acid, and 50 mM acetic acid (buffer A). The unadjusted pH of this buffer is 7.2, and it was used at this pH. After 15 min at 25 °C, 0.9 ml of ice-cold water was added and the solutions were applied to 0.8-ml columns (9 mm diameter, x 14 mm) of Dowex-50 (NH4⁺), which were then washed with 20 ml of water. [14C]SMM was eluted with 3 ml of 3M NH4OH, which was mixed with 3 ml of Ready Gel (Beckman) for quantification of [3H]. Controls with no AdoMet or no enzyme were included. Enzyme activity is expressed in katal (1 katal = 1 mol of SMM formed/d). The identity and purity of the [14C]SMM product were verified by autoradiography following TLC on 0.25-mm Silica Gel G plates (Polygram, Machery-Nagel, Düren, Germany) developed in methanol:acetone:concentrated HCl (90:10:4, v/v/v) (TLC system 1) and on 0.1-mm cellulose plates (Merck, Darmstadt, Germany) developed in n-butanol:acetic acid:water (60:20:20, v/v/v) (TLC system 2).

Kinetic Analysis and Studies With Met Analogs—Kinetic analysis was performed at pH 7.2 in buffer A, using 0.7 μg of purified MMT per assay. AdoMet concentration was varied from 10 to 500 μM, Met concentration from 0.1 to 1 mM. Incubation was for 5 min, within which time, reaction velocity remained constant at all the substrate concentrations used. Inhibition by analogs was tested in the standard assay using 1.6 μg of MMT/assay and 5 mM analog (50 mM for DMSP). Analogs were tested as substrates in 25-μl reaction mixtures containing 0.8 μg of MMT, 40 μM [methyl-3H]AdoMet (2.1 GBq/mmol), and 10 mM analog in buffer A. After 30 min at 25 °C, reactions were stopped in liquid N₂. Products were analyzed by TLC systems 1 and 2. Standards to calibrate the gel filtration column (Amicon) were described by Mudd and Datko (3). Enzyme (up to 50 μg) was homogenized in a blender (2 min, full speed) with 2 liters of buffer A containing 0.5 M NaCl (10 ml) was incubated in buffer A containing 1 mM EDTA and 5 mM 2-mercaptoethanol in a final volume of 3 ml, 40 g of protein from the Mono Q fraction were mixed with [methyl-3H]AdoMet (74 kBq, 3.15 TBq/mol) in buffer A containing 1 mM Met (containing 16.3 kBq of [14C]AdoHcy, 3.15 TBq/mmol) and 10 μl of [14C]SMM was eluted with 3 ml of 3M NH4OH, which was mixed with 3 ml of Ready Gel (Beckman) for quantification of [14C]. Control with no AdoMet was included. Samples were placed in a microtiter plate on ice and irradiated for 1 h with an ultraviolet lamp (256 nm, 10 watts) positioned 1 cm away (17). After adding SDS sample buffer (18), the samples were boiled for 3 min and then separated by SDS-PAGE. Gels were treated for fluorography with Amplify™ (Amersham Pharmacia Biotech), vacuum-dried, and exposed to preflashed Kodak X-Omat film for 7 days at –80 °C.

Analysis of Kinetic Data

Kinetic data were first analyzed by inspection of Lineweaver-Burk plots and then fitted to the appropriate rate equations using the nonlinear regression program EZ-FIT (DuPont). Substrate interaction data were fitted to the rate equation for a sequential bireactant mechanism, where A and B are the substrates, KA and KB are the respective Michaelis constants, V is the maximum velocity, and KA·KB is an interaction term. Product inhibition data were fitted to the equations for noncompetitive (Equation 2), competitive (Equation 3), or uncompetitive inhibition (Equation 4), where S is the varied substrate (A or B), I is the inhibitory product, K'S and V' are the apparent Michaelis constant and maximum velocity in the presence of the product at each concentration of the nonvaried substrate, and KII and KIII are the slope and intercept inhibition constants, respectively. The nomenclature is that of Cleland (19).

Immunological and Blotting Methods

Polyclonal antibodies were produced by subcutaneous injection of a New Zealand White rabbit with 100 μg of purified native W. biflora MMT emulsified with Freund's complete adjuvant. Boosts (50 μg) were given after 3 and 5 weeks using Freund's complete and incomplete adjuvant, respectively. Serum was collected 1 week after the last injection and stored at −80 °C in 0.2% (w/v) NaNO₃. MMT antiserum was used to prepare an affinity column that was then used to purify MMT from other plants.

Partial Purification of MMT from Other Plants

The first two steps of the protocol given above were used, with these modifications. The quantities of leaves and extraction buffer were reduced to 100 g and 200 ml; the PEG fractionation range was extended to 8–22%. Similar amounts of activity were separated by SDS-PAGE, and stained with Coomassie Blue or used for immunoblots.

Photoaffinity Labeling With [methyl-3H]AdoMet

Samples containing 1.2 μg of protein from the Mono Q fraction were mixed with [methyl-3H]AdoMet (74 kBq, 3.15 TBq/mmol) in buffer A containing 1 mM Met (containing 16.3 kBq of [14C]AdoHcy, 3.15 TBq/mmol) and 10 μl of [14C]SMM was eluted with 3 ml of 3M NH4OH, which was mixed with 3 ml of Ready Gel (Beckman) for quantification of [14C]. Control with no AdoMet was included. Samples were placed in a microtiter plate on ice and irradiated for 1 h with an ultraviolet lamp (256 nm, 10 watts) positioned 1 cm away (17). After adding SDS sample buffer (18), the samples were boiled for 3 min and then separated by SDS-PAGE. Gels were treated for fluorography with Amplify™ (Amersham Pharmacia Biotech), vacuum-dried, and exposed to preflashed Kodak X-Omat film for 7 days at –80 °C.

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used without further purification for immunodetection. Proteins from SDS-PAGE were transferred to nitrocellulose for 1 h at 110 V in a Mini Trans-Blot transfer cell (Bio-Rad). Blots were blocked with Tris-buffered saline plus 0.2% Tween 20 and 5% (w/v) nonfat milk powder. MMT was detected with antiserum (diluted 1000-fold with Tris-buffered saline containing 0.2% Tween 20 and 1% nonfat milk powder) plus goat anti-rabbit immunoglobulin G-alkaline phosphatase conjugate (Bio-Rad).

Other Methods

Protein was estimated by the Bradford method (20) with bovine serum albumin as standard. SDS-PAGE was performed in mini-gels (12.5% polyacrylamide) according to Laemmli (18). Gels were stained for 30 min in 0.2% (w/v) Coomassie Brilliant Blue G-250 in methanol:acetic acid:water (40:10:50, v/v/v) and destained for 2–3 h in this solution without dye. Silver staining (21) was used to verify the purity of the final MMT fraction. Prestained standard proteins were used for immunoblot applications.

RESULTS

Purification of MMT from W. biflora—The results of purification are summarized in Table I and Fig. 1. A high concentration of 2-mercaptoethanol (50 mM) in the extraction buffer and during early separation steps was required to maintain MMT activity. Precipitation with (NH₄)₂SO₄ caused almost complete inactivation of MMT that could not be reversed by desalting. In contrast, precipitation with PEG gave modest enrichment (2.9-fold, 23% yield) while also removing polysaccharides and lipids that interfered with subsequent steps. The resuspended PEG fraction was applied directly to an adenosine-agarose affinity column, in which the adenine was attached to the matrix via the C-8 position. This was a key step, providing 170-fold enrichment; the column bound 98% of the MMT activity applied, but few other proteins. Adenosine-agarose with N-6 attachment, AMP- or ADP-agarose with C-8 attachment, and AdoHcy-Sepharose or Met-agarose with amino attachment were not efficient affinity adsorbents under the same conditions (data not shown). After washing with 200 mM NaCl to remove nonspecifically bound protein, the adenosine-agarose column was eluted by the addition of 1 mM AdoMet (Fig. 1A).

The eluate was concentrated, diluted to lower the NaCl concentration, and separated on a Mono Q column; this step removed AdoMet and contaminating proteins (Fig. 1B). Final purification was achieved by gel filtration on Superdex-200, which gave one peak of MMT activity coincident with a major protein peak (Fig. 1C). The elution volume of MMT corresponded to a molecular mass of 450 kDa (Fig. 1C, inset). Chromatofocusing on Mono P was also tested, but caused large loss of activity.

Protein fractions from the various purification steps were analyzed by SDS-PAGE (Fig. 2A). The efficiency of the affinity step is evident from comparison of lane 4 (non-retained fraction) with lane 5 (retained fraction). The latter included a major polypeptide of molecular mass 115 ± 5 kDa, which was the only one detected in the final enzyme preparation by Coomassie Blue staining (lane 7) or silver staining (not shown). Taken with the native molecular mass of 450 kDa estimated from native gel filtration chromatography, the value of 115 kDa for the denatured enzyme suggests that MMT is a tetramer of identical 115-kDa subunits.

As an additional criterion of chemical homogeneity, purified MMT was subjected to hydrophobic interaction chromatography (Fig. 3). The purified enzyme gave a single protein peak.

### Table I

| Purification step | Volume | Total activity | Total protein | Specific activity | Purification | Recovery of activity |
|------------------|--------|----------------|---------------|------------------|--------------|---------------------|
| Crude extract    | 1550   | 35,300         | 9050          | 3.9              | 1            | 100                 |
| PEG              | 52     | 8230           | 728           | 11.3             | 2.9          | 23                  |
| Adenosine-agarose| 5      | 1920           | 1             | 1920             | 491          | 5.4                 |
| Mono Q           | 3.2    | 1230           | 0.52          | 2360             | 604          | 3.4                 |
| Superdex 200     | 5.0    | 341            | 0.14          | 2430             | 622          | 0.9                 |

Fig. 1. Purification of MMT from W. biflora leaves. The purification involved three column chromatographic steps: A, adenosine-agarose; B, Mono Q; and C, Superdex 200. The dotted lines are A at 280 nm, and the solid circles represent the MMT activity expressed relative to the most active fraction (100) in each chromatographic step. Activities of the most active fractions (pkat/fraction) were: A, 135; B, 490; C, 110. Estimation of native molecular mass of MMT by native gel filtration chromatography is shown in the inset. The column was calibrated with the following standard proteins: 1, thyroglobulin (670 kDa); 2, apoferritin (443 kDa); 3, alcohol dehydrogenase (150 kDa); 4, bovine serum albumin (66 kDa).
Lane 1, molecular mass markers (kDa); lane 2, crude extract (80 μg); lane 3, 9–15% PEG precipitate (95 μg); lane 4, fraction not retained by adenine-agarose (0.2 μg); lane 5, adenosine-agarose (0.7 μg); lane 6, Mono Q (0.2 μg); lane 7, Superdex 200 (0.7 μg). Panel B, photoaffinity labeling of MMT with [methyl-3H]AdoMet with (lanes 2 and 4) or without (lanes 3 and 5) 200 μM AdoHcy. Samples were analyzed by SDS-PAGE, followed by Coomassie Brilliant Blue staining (lanes 2 and 3) or fluorography (lanes 4 and 5).

The lability of MMT, biochemical and kinetic characterization was carried out immediately after purification.

Treatment with various thiols increased MMT activity by about 20–50% whereas the thiol reagents p-hydroxymercuribenzoate and N-ethylmaleimide strongly inhibited activity; this inhibition was reversed by dithiothreitol (Table II). These data suggest that MMT has at least one essential Cys residue. Consistent with this, the Ser and Cys reagent phenylmethylsulfonyl fluoride (22) also inhibited MMT; however, neither iodoacetamide nor iodoacetic acid did so (Table II). Based on these considerations, 2-mercaptoethanol was routinely added to buffers and to purified MMT preparations.

Effect of pH—The pH optimum was determined using purified enzyme and buffer A adjusted to various pH values with HCl or NaOH. The ionic strength of this buffer is virtually independent of pH; the theoretical basis for this is given by Ellis and Morrison (23). MMT activity showed a sharp optimum at pH 7.2; at pH 5.8 and 9.0, the enzyme activity fell to about 50–55% of the optimal value, and to only 1% at pH 5.0.

Photoaffinity Labeling With [methyl-3H]AdoMet—The 115-kDa MMT polypeptide was labeled after irradiation with ultraviolet light in the presence of [methyl-3H]AdoMet with (lanes 2 and 4) or without (lanes 3 and 5) DTT (5 mM). The labeling was completely prevented by AdoHcy, a potent inhibitor of methyltransferases (24). Table II shows that AdoHcy was a competitive inhibitor with respect to AdoMet and noncompetitive with respect to Met, and that SMM was noncompetitive with respect to both substrates (data not shown). At a saturating concentration of Met (1 mM), SMM
acted as an uncompetitive inhibitor with respect to AdoMet (data not shown). These patterns rule out a Steady State Ordered Theorell-Chance or a Rapid Equilibrium Random mechanism but are consistent with an Ordered Bi Bi mechanism in which AdoMet is the first substrate to bind MMT and AdoHcy the last product released (19, 25). Fitting the product inhibition data to Equation 2, 3, or 4, as appropriate, gave the $K_i$ and $K_M$ values shown in Table III. From these values, $K_i$ for AdoHcy and SM were estimated (25) as $13 \pm 1 \mu M$ and $226 \pm 17 \mu M$, respectively.

Met Analogs as Inhibitors and Substrates—The inhibitory activity of most analogs was tested at $5 \mu M$ in the presence of $1 \mu M$ [methyl-$^{14}$C]Met; DMSP was tested at $50 \mu M$ as its physiological concentration may be of this order (5). To assay analogs as substrates, their capacity to acquire label from [methyl-$^{14}$C]AdoMet was tested. Table IV summarizes the results. Most analogs inhibited MMT slightly (up to about 30%) or not at all; selenomethionine, 3-(methylthio)propylamine and 2-hydroxy-4-(methylthio)butyrate were more effective, inhibiting activity by 97, 85, and 58%, respectively. Selenomethionine, ethionine, and 2-hydroxy-4-(methylthio)butyrate were substrates but S-methylCys and the 3-methylthio derivatives of propylamine, propionate and propandiol were not. This indicates (a) that there is an absolute requirement for a 2-carbon side chain, but less specificity for the alkylthio group, and (b) that the Met carboxyl group is essential for activity and the amino group is not. Consistent with the second point, none of the other analogs lacking a free carboxyl group were substrates, whereas those without a free amino group were all methylated to some extent. As would be expected from a requirement for a free carboxyl group, the Met residues of N-formylMet-Ala and Trp-Met-Asp-Phe amide were not methylated. S-Methylation of Hcy and Cys, O-methylation of Hse, and N-methylation of 2,4-diaminobutyrate were not detected. D-Met was apparently weakly active both as an inhibitor and as a substrate; however, the presence of as little as 2% L-Met in the D-Met preparation could explain these results, and such trace contamination cannot be ruled out.

Partial Purification and Immunodetection of MMT from Other Species—MMT was partially purified from leaves of four diverse flowering plants that are known to contain SM (4) but not to accumulate DMSP (10). W. biflora genotypes H and B (5) were included for comparison. The first two steps (PEG fractionation and adenosine-agarose) of the purification procedure developed for W. biflora were used. MMT activity was recovered in the adenosine-agarose eluates from all species, with total activity yields from 30 to 60% of that for W. biflora. SDS-PAGE analysis of comparable amounts of MMT activity showed that the preparations from all species contained a major polypeptide of molecular mass $115 \pm 5$ kDa, of similar staining intensity (Fig. 5, A and B). Specific antibodies raised against W. biflora MMT recognized the 115-kDa band in all species (Fig. 5C). The strength of the cross-reaction varied among species in general agreement with their phylogenetic distance from W. biflora (27). Thus, the strongest signal was given by lettuce, which is from the same family (Asteraceae) as W. biflora, the weakest by maize, a monocotyledon.

**DISCUSSION**

This report presents the first protocol for purifying MMT to electrophoretic homogeneity. The key step, affinity chromatography on adenosine-agarose, was effective for MMT from all plants tested. With minor modification, the protocol should therefore be widely applicable.

The enzyme purified from W. biflora behaved as a homotetramer of 115-kDa subunits. Such a structure is unusual for methyltransferases, which are typically monomers or dimers with subunits of 20–45 kDa (e.g. Refs. 28–30). However, as an immunologically related 115-kDa polypeptide was found in MMT preparations from four other plants, the W. biflora MMT would appear to be representative. Although they lack the capacity to convert SMM to DMSP, these other plants had extractable MMT levels up to 60% of that in W. biflora. The evolution of DMSP synthesis in W. biflora may thus have involved little change in the amount of MMT, even though DMSP production would be expected to raise the net demand for SMM more than 10-fold (5).

In addition to identifying features essential for activity as an MMT substrate, the studies with Met analogs clarified two aspects of plant metabolism. First, as previously inferred but not demonstrated (31), the activity of MMT toward selenomethionine can account for the occurrence of Se-methylselenomethionine in plants exposed to selenium salts. Second, MMT is dearly distinct from the enzyme that catalyzes post-translational methylation of Met residues (32) because the latter has a native molecular mass of only 28 kDa; additionally, MMT did not attack Met residues in small peptides.

The properties of MMT in general resembled those of other methyltransferases. The specificity of the purified enzyme (about 2.5 nkat/mg protein) is about 10-fold higher than those reported for thiol methyltransferases (33, 34), but falls well within the range (about 1–10 nkat/mg) typical of small molecule methyltransferases in general (e.g. Refs. 29, 30, 35, and 36). Activation by thiols and inactivation by thiol reagents, implying one or more essential Cys residues, is also a common feature among methyltransferases (28, 37), as is affinity labeling by [methyl-$^{3}$H]AdoMet (17, 35). An Ordered Bi Bi kinetic mechanism with AdoMet and AdoHcy as the leading reaction partners would likewise be typical (28, 38, 39).

In W. biflora, MMT is the first enzyme in the biosynthetic pathway from Met to DMSP (5), so its in vivo regulation is of particular significance. Some inferences about this can be drawn from our kinetic data if reasonable assumptions are made about physiological levels of the MMT substrates and products, and if the reaction catalyzed by MMT is taken to be essentially irreversible, as has been shown (2). With respect to substrates, reports for various plants suggest concentrations of Met and AdoMet in metabolic compartments of around 100 $\mu M$ (40–42). Using these values, Fig. 6 shows the extent of inhibition expected with various intracellular concentrations of AdoHcy or SMM. Although this analysis is speculative and...
K_{iA} and K_{iB} are the apparent inhibition constants (± standard error) estimated by using nonlinear regression to fit data to Equation 2, 3, or 4 (see "Experimental Procedures").

| Variable substrate | Constant substrate | Product inhibitor | K_{iA} | K_{iB} |
|-------------------|-------------------|-----------------|-------|-------|
| AdoMet 10–100 μM | Met 100 μM | AdoHcy 0–50 μM | 13 ± 1 | 463 ± 82 |
| SMM 0–400 μM | 445 ± 54 | 226 ± 17 |
| Met 100–600 μM | AdoMet 20 μM | AdoHcy 0–50 μM | 20 ± 6 | 16 ± 3 |
| SMM 0–400 μM | 382 ± 41 | 532 ± 89 |

Activity of analogs as inhibitors or substrates of MMT

Inhibition was assayed using 1 mM [methyl-14C]Met and 5 mM analog, and is expressed relative to a control with no analog. Substrate activity was measured using 40 μM [methyl-14C]AdoMet and 10 mM analog; 14C-product formation was analyzed by TLC and is expressed relative to a control with Met as substrate. ND, not determined.

| Analog | Inhibition of MMT activity | Formation of 14C-product |
|-------|--------------------------|------------------------|
| Physiological S and Se compounds | % | % of control |
| L-Met | 0 | 100 |
| Se-Methionine | 97 | 81 |
| 3-(Methylthio)propionate | 14 | <0.1 |
| L-Ethionine | 8 | 17 |
| 3-(Methylthio)propionate methyl ester | 7 | <0.1 |
| S-Methyll-Cys | 0 | <0.1 |
| DL-Hcy | 0 | <0.1 |
| L-Cys | 0 | <0.1 |
| 3-Dimethylsulfiniopropionaldehyde | 1 | ND |
| DMSP (50 mM) | 0 | ND |
| Carboxyl group replaced or modified | | |
| 3-(Methylthio)propionylamine | 85 | <0.1 |
| L-Met methyl ester | 36 | <0.1 |
| L-Met p-nitroanilide | 30 | <0.1 |
| L-Met ethyl ester | 20 | <0.1 |
| L-Methioninamide | 20 | <0.1 |
| L-Methioninol | 17 | <0.1 |
| Amino group replaced or modified | | |
| DL-2-Hydroxy-4-(methylthio)butyrate | 58 | 21 |
| N-2,4-Dinitrophenyl-0L-Met | 20 | 1 |
| Gly-Met | 8 | 13 |
| N-Formyl-L-Met | 3 | 0.5 |
| N-Acetyl-L-Met | 0 | 0.5 |
| Others | | |
| O-Met | 11 | 9.4 |
| 3-(Methylthio)propanol | 3 | <0.1 |
| L-Hse | 0 | <0.1 |
| L-2,4-Diaminon-butyrate | 0 | <0.1 |
| N-Formyl-Met-Ala | 0 | <0.1 |
| Trp-Met Asp-Phe amide | 0 | <0.1 |

* No standard of the methylated product, S-dimethylCys, was available as it cannot be prepared by reacting S-methylCys with CH3I (26).

** MMT activation observed (see Table II).

* DMSP was in the HCl form and was neutralized with KOH. MMT activity in the presence of DMSP was 83% and 113% of that in controls without or with 50 mM KCl, respectively. This is consistent with an osmoregulatory effect of DMSP.

* Trace 3C-incorporation into SMM observed, attributable to ester hydrolysis.

MMT is also likely to be inhibited by SMM. However, as the inhibition curve has a shallow slope, even large accumulations of SMM would be expected to produce modest inhibition. Consistent with this prediction, when W. bifuila leaf disks were supplied with 5 mM external SMM, it was readily absorbed but did not depress SMM synthesis by more than about 50% (5). As MMT was unaffected by a physiological concentration of DMSP (50 μM), it seems unlikely that the DMSP pathway is regulated by feedback inhibition at the MMT step.

Finally, our data have two implications for the proposal (12) to genetically engineer the biosynthesis of DMSP in crop plants. First, the high levels of MMT found in various plants that lack DMSP suggests that in the simplest case it would not be necessary to increase MMT expression as part of the engineering process. This assumes, of course, that the MMT of plants that lack DMSP is localized in the same subcellular compartment(s) and has kinetic properties similar to the enzyme in DMSP accumulators. The second implication concerns the level of AdoHcy, which reflects the balance between its production in transmethylation reactions and its breakdown by AdoHcy hydrolase (24, 44). Engineering DMSP accumulation...
in leaf mesophyll cells might be expected to roughly triple the rate of methyl group transfer (12), and hence the rate of AdoHcy production. In these circumstances, the endogenous AdoHcy hydrolase activity in a non-DMSP accumulating plant might not suffice to prevent AdoHcy accumulation, making it necessary to engineer higher levels of this enzyme.

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