Specific Roles of Methylcobamide:Coenzyme M Methyltransferase Isozymes in Metabolism of Methanol and Methylamines in Methanosarcina barkeri*

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An immunochemical approach was employed as a direct test for functional activities of isozymes of methylcobamide:coenzyme M methyltransferase (MT2-M and MT2-A) in the metabolic pathways of methane formation from: methanol, acetate, monomethylamine, dimethylamine, and trimethylamine. Specific removal of the MT2 isozymes from buffer soluble cell extracts of Methanosarcina barkeri was accomplished by use of immobilized, affinity-purified, ovine polyclonal antibodies. Extracts of methanol-grown cells depleted of MT2-M lost entirely the ability to carry out conversion of methanol to 2-(methylthio)ethanesulfonate (methyl-CoM). Methanol:CoM methyl transfer activity was completely restored by addition of purified MT2-M, but no activity was recovered by addition of MT2-A. In contrast, the activity of trimethylamine-grown cell extracts to convert monomethylamine and dimethylamine to methyl-CoM was lost almost entirely by immunosorptive removal of MT2-A. Addition of purified MT2-A, but not MT2-M, to the MT2-A-depleted extract fully reconstituted methyl-CoM formation from both mono- and dimethylamine. Interestingly, in extracts resolved of MT2-A, trimethylamine-dependent methylation of coenzyme M was observed at approximately 20% of the rate of controls not treated with antibody. Furthermore, both isozymes were effective in full restoration of trimethylamine conversion. Tests indicated that neither of the two MT2 isozymes are involved in methane formation from acetate. The results establish that MT2-A plays a specific role in metabolism of methylated amine substrates, whereas, MT2-M functions in methane formation from trimethylamine and methanol.

Methanosarcina barkeri is a methanogenic archaeal species that is capable of growth on a relatively wide range of substrates (1). Although Methanosarcina spp. generally share in common with most methanogens the ability to use hydrogen for reduction of CO₂ to methane, these species have been most useful in the study of methylotrophic methanogenesis. Many of the catabolic substrates of M. barkeri are compounds from which methyl groups are converted essentially intact to methane (2). These compounds include acetate, methanol, trimethylamine (TMA), dimethylamine (DMA), and monomethylamine (MMA). In many microbial ecosystems, these compounds serve as the primary precursors of methane (1, 3).

Detailed studies have been carried out on the metabolic pathways by which methane is produced from methanol, acetate, and CO₂. However, pathways of methanogenesis from methylated amines are still not well characterized. All methanogenic substrates ultimately serve to methylate 2-mercaptoethanesulfonate (CoM), thereby forming the final metabolic intermediate in methanogenesis 2-(methylthio)ethanesulfonate (methyl-CoM). The problem of understanding methane formation from the various growth substrates requires knowledge of the mechanisms by which each of these methylated substrates is converted to methyl-CoM.

Methanol utilization was one of the earlier studied pathways of methanogenesis (4, 5). The overall pathway for methane formation from methanol is shown in Scheme I. Conversion of chiral [(H,3,²H) methanol to methyl-CoM in extracts of M. barkeri was shown to proceed with overall retention of stereochemical configuration, consistent with a pathway that contains an even number of S_N2 displacement reactions (6). Two different enzymes that catalyze separate methyl group transfer reactions in the pathway have been identified. The first enzyme, designated methyltransferase I (MT1), consists of two subunits with molecular weights of 53,000 and 34,000 (7). The enzyme contains a noncovalently bound 5-hydroxybenzimidazolylcobamide prosthetic group, which becomes methylated by interaction with methanol (Scheme I). Methyl-CoM is then produced by the second enzyme in the pathway, termed methyltransferase II (MT2), which catalyzes transfer of the methyl group from the MT1-bound methylcob(II)amide to CoM. The MT2 enzyme is a monomeric protein of approximately 35 kDa that does not contain a prosthetic group distinguishable by UV-visible spectroscopy. A convenient assay for MT2 activity has been developed based on the finding that MT2 also catalyzes methyl group transfer from methylcobalamin to coenzyme M, as shown in Equation 1.

\[
\text{HS-CoM} + \text{CH}_3\text{cob(II)alamin} \rightleftharpoons \text{CH}_3\text{S-CoM} + \text{cob(I)alamin} + \text{H}^+ \\
\text{(Eq. 1)}
\]

Since the MT2 reaction is analogous to a part of the overall reaction catalyzed by microbial and mammalian cobalamin-dependent methionine synthase enzymes, studies on the methanogenic system may have potential to provide insight into the function of the human enzyme.

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During the course of investigations on the pathway of acetate conversion to methane it was discovered that extracts of acetate-grown *M. barkeri* contained two different isozyme forms of MT2 (8). The isozymes had nearly identical molecular masses as judged by SDS-polyacrylamide gel electrophoresis, but exhibited different electrophoretic mobilities under nondenaturing conditions, indicating that they differ in overall charge (8). The two isozymes are also distinguishable based upon their relative affinities for hydroxylapatite and based on their different reactivities with polyclonal antibodies directed against each of the two proteins (8). One of the isozymes was predominant in acetate-grown cells and was termed the “A” isozyme (MT2-A) (8). The other form accounted for nearly all of the MT2 activity in cells grown on methanol and was designated the “M” isozyme (MT2-M) (8). It is likely that MT2-M was the major form of the enzyme originally described as being involved in the pathway of methanol conversion to methyl-CoM. Discovery of a different form of MT2 in cells grown on acetate, MT2-A, suggested at first that this form might be specifically involved in metabolism of acetate; however, a specific metabolic function of MT2-A was not demonstrated. Later it was found that MT2-A was also the major form of the enzyme present in cells grown on TMA or H$_2$/CO$_2$ (9). Since methanogens grow autotrophically low levels of MT2 activity, it was hypothesized that MT2-A functioned in methanogenesis from TMA (9).

Recently two proteins, a 29-kDa protein that contains a corrinoid cofactor, and MT2-A were demonstrated to be involved in methane formation from MMA (10). Both MMA and D2A are produced during methanogenesis from TMA by either cell cultures (11) or extracts (12), and subsequently serve as methanogenic substrates. However, it was unknown whether MT2-A might function in methanogenesis from the other methylamines as well. Furthermore, it was unknown to what extent different pathways might exhibit specific requirements for either one of the MT2 isozymes.

In this paper, an immunochemical approach is employed in order to test for specificity of involvement of individual MT2 isozymes in methyl-CoM formation from various substrates: acetate, methanol, monomethylamine, dimethylamine, and trimethylamine. Resolution and reconstitution of the individual MT2 isozymes in extracts active in conversion of the different substrates is performed. Direct evidence is obtained demonstrating that MT2-A is capable of functioning in the transformation of all three methylated amine substrates, whereas MT2-M acts only in the conversion of methanol and trimethylamine.

**Functional Roles of Methyltransferase Isozymes**

**Scheme 1.** Methyl transfer reactions in the pathway of methanol conversion to methane.

![Scheme 1](http://www.jbc.org/)

**MATERIALS AND METHODS**

**General Reagents—**CNBr-activated Sepharose 4B, and GammaBind™ G-Sepharose (a preparation of recombinant streptococcal protein G covalently immobilized on Sepharose 4B) were obtained from Pharmacia Biotech Inc. Ovine antibodies specific for MT2-A and MT2-M were raised in two separate animals as described previously (8). The y-globulin fraction from the immune serum of each animal was prepared by ammonium sulfate precipitation, as described earlier (8), and was maintained frozen at −70 °C.

**Cell Culture and Preparation of Cell-free Extracts—**Methanosarcina barkeri was grown at 37 °C on sodium acetate (13), trimethylamine hydrochloride (10) or methanol. Cell extracts used in the experiments for methanol- and acetate-dependent CoM methylation were prepared by centrifugation at 38,000 × g of French pressure cell homogenates as described previously (14). Experiments involving methylamine-dependent CoM methylation employed extracts prepared in MOPS buffer, pH 7.0, subjected to a second step of centrifugation at 150,000 × g (15). Protein concentration was estimated by the method of Bradford (16).

**Enzyme Assays—**Two methods of analysis of MT2 activity were used. In one method, the demethylation of 1 mM methylcobalamin in the presence of 4 mM CoM was measured by use of the cyanide derivatization method described previously (8). One unit of enzyme is defined as the amount required for methyl group transfer at 1 μmol/min under the conditions described (8). In the other method, relative amounts of activity were measured spectrophotometrically based on the increase in absorbance at 620 nm due to the demethylation of methylcobalamin and the appearance of cob(I)alamin and cob(II)alamin. Reactions were carried out in 1-ml cuvettes with a 1-cm path length containing 50 mM Tris-HCl, pH 8.0, 4 mM CoM, and 0.5 mM methylcobalamin. The initial rate of increase in absorbance was proportional to the amount of enzyme added to the solution.

Assays of methyl-CoM formation from CoM and methylamine substrates in extracts were performed by measurement of the substrate-dependent loss of the free thiol group of CoM over time by use of Ellman’s reagent, 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB). All assays were carried out in anaerobic sealed 2-ml vials under an atmosphere of H$_2$; the reaction mixtures contained 12.5 mM CoM, 10 mM ATP, 20 mM MgCl$_2$, 3.2 mM BES, 0.4 mM Ti$^{3+}$-citrate, and 16 mM methyl donor substrate (TMA, DMA, or MMA). The total volume of the assay was 25 μl, made up of 45 μl of cell-free extract of TMA-grown cells, 55 μl of 50 mM MOPS, pH 7.0, containing 1 mM Ti$^{3+}$-citrate, 20 μl of a solution containing ATP, MgCl$_2$, CoM, and BES, and 5 μl of mono-, di-, or trimethylamine substrate in water. The vials were loaded with extract samples to be analyzed and sealed inside an anaerobic chamber (Coy Laboratory Products, Inc., Grass Lake, MI). The vials were removed from the chamber, chilled on ice, and all other reagents were added under an atmosphere of 100% H$_2$, maintained by use of a gassing manifold. The reactions were initiated by transfer of the vials from ice to a shaking water bath at 37 °C. Samples (3 μl) were removed at specific time points and mixed in wells of a microtiter plate containing 250 μl of 0.5 mM DTNB in 150 mM Tris-HCl, pH 8.0. Absorbance at 410 nm was measured by use of an MR700 enzyme immunoassay plate reader (Dynatech Laboratories, Inc.). Calculations of thiol concentration were based on the value of 13.6 nm$^{-1}$ cm$^{-1}$ as the molar absorptivity of the 2-nitro-5-thiobenzoate diion at 412 nm (17).

The assays for CoM production from acetyl phosphate in extracts from acetate-grown *M. barkeri,* and from methanol in extracts of cells grown on methanol, were similarly based on measurement of the time-dependent consumption of CoM by use of DTNB reagent.

**Affinity-purified Anti-MT2 Isozyme Antibodies Immobilized on Protein G-Sepharose—**Affinity purification of antibodies against the MT2
isozyme A was carried out by a modification of the methods described by Kincaid (18). A column containing MT2-A linked to Sepharose 4B was constructed by reaction of 2.5 mg of the pure MT2-A with 1 g of CNBr-activated Sepharose 4B prepared for coupling in pH 8.3 sodium bicarbonate solution according to the procedure recommended by the manufacturer. Affinity purification from approximately 1700 mg of total IgG was carried out in three separate runs and yielded a total of approximately 10 mg of affinity-purified antibody. The IgG fraction was applied at a concentration of approximately 71 mg/ml, and the column was subsequently washed with 50 ml of 0.1 M Tris-HCl, 0.5 M NaCl, pH 8.0. Thorough washing of the column was confirmed by monitoring of the effluent absorbance at 280 nm. Elution of the bound antibodies was then carried out with 0.1 M sodium acetate, pH 3.5, in 10% glycerol. Fractions were promptly neutralized with an equal volume of 0.8 M Tris-HCl, pH 8.0. The unbound IgG was reapplied in subsequent runs in order to remove residual antibody reactivity, as judged by the eventual loss of ability of the unbound IgG to function in Western blot analyses. Affinity-purified antibodies against MT2-M were prepared in a similar manner, by use of an immobilized antigen column constructed from 1 g of CNBr-activated Sepharose and 5.7 mg of MT2-M.

Prior to immobilization on GammaBind G-Sepharose, affinity-purified antibody preparations were concentrated by ultrafiltration and adjusted to contain 50 mM MOPS, 100 mM NaCl, pH 7.2. Affinity-purified anti-MT2-M and anti-MT2-A antibody-GammaBind G-Sepharose gels were separately prepared containing 11 and 14 mg of affinity-purified antibody/ml of gel, respectively. The control IgG-GammaBind G-Sepharose matrix (1.0 ml) contained 16 mg of nonspecific IgG, obtained during affinity purification as the IgG fraction that did not bind to the MT2-A matrix.

Immunosorptive Depletion of MT2 Isozymes from Cell-free Extracts—The strategy used to bring about efficient removal from the extract of either one of both of the isozymes of MT2 was designed to minimize dilution of the extract preparations. The buffer-soluble cell extracts were incubated with IgG-protein G-Sepharose gel preparations for 25 min at approximately 25 °C with gentle mixing under strictly anaerobic conditions inside a Coy anaerobic chamber containing approximately 3% H2 in N2. The suspension was then centrifuged for 2–3 min, and the supernatant was analyzed for total MT2 activity, and for specific activity of methyl-CoM formation in assays of the overall conversion of a particular methanogenic substrate (either methanol, acetyl phosphate, MMA, DMA, or TMA). Measurement of the remaining MT2 activity was performed routinely in order to assess both the time required, and the amount of immunosorbent gel necessary to obtain the maximal extent of MT2 removal from each of the different cell extracts. The time course of depletion showed that removal of MT2 activity reached apparent completion after approximately 15–20 min. The minimum amount of antibody-linked gel required to accomplish the maximal extent of MT2 removal was found to vary depending upon the amount of MT2 activity present in the extract. The largest amount of gel was required when concentrated extracts (25 mg of protein/ml) from cells grown on trimethylamine were employed. Maximal depletion of MT2 activity from the trimethylamine-grown cell extract was achieved by use of a volume of gel approximately equal to that of the extract.

Control incubation experiments were carried out in which the specific activity of methanogenic substrate conversion to methyl-CoM was measured following incubation of the extract with samples of the control gel (nonspecific IgG bound to protein G-Sepharose). The effect of incubation on the overall methyl-CoM formation activities were also measured in the absence of gel, with or without dilution with 50 mM MOPS buffer, pH 7. Dilution of the extracts by up to 50% with 50 mM MOPS buffer alone was found to have no effect on specific activity of the extracts in conversion of any of the methanogenic substrates tested. Similarly, no decrease in specific activity was observed in mixtures that contained samples of the control gel incubated under conditions that were otherwise identical to those which contained the specific antibody-linked gel preparations.

RESULTS

Functional Analysis of MT2 Isozymes in Methylamine Metabolism—In order to perform direct measurements of MT2 isozyme function in pathways of methyl-CoM formation, extracts were depleted of either one or both of the MT2 isozymes by incubation with immobilized, affinity-purified anti-MT2 antibody preparations. Immunosorptive depletion of MT2 from extracts of TMA-grown cells was performed by use of an anti-MT2-A isozyme matrix, as described under Materials and Methods. The procedure resulted in removal of 95% (±2%, n = 6) of the MT2 specific activity initially present in the extract. In contrast, relatively little MT2 activity was removed by incubation with a control matrix containing nonspecific IgG.

Samples of the depleted extract were used, both directly and after addition of specified amounts of the purified MT2-A and MT2-M isozymes, for analysis of methyl-CoM formation from TMA, DMA, and MMA. The reaction mixtures remaining after the assays were completed were frozen and thereafter subjected to SDS-polyacrylamide gel electrophoresis and Western blot analyses. Results of one such analysis are shown in Fig. 1. A band on a gel stained for protein was observed at the same position in the crude extract (Fig. 1A, lane 1) as in the purified MT2-A (Fig. 1A, lane 5). The intensity of this band was significantly diminished after treatment with the anti-MT2-A matrix, as shown in Fig. 1A, lanes 2 and 4. The MT2-A isozyme was specifically revealed on Western blots developed with antibodies raised against MT2-A, as shown in Fig. 1B, lanes 1–5. Anti-MT2-A antibodies did not react with MT2-M, as shown in Fig. 1B, lane 6, verifying the specificity of the anti-MT2-A antibody preparation. Anti-MT2-M antibodies showed little cross-reaction with MT2-A (cf. lanes 5 and 7 in Fig. 1C). Moreover, MT2-A was almost completely eliminated from the ex-
TABLE I

| Extract treatment | % MT2 activity | Activity in conversion of substrates:<br> | TMA | DMA | MMA |
|-------------------|---------------|------------------------------------------|-----|-----|-----|
| None              | 100           | CoM methylated nmol/min/mg              | 405 | 445 | 213 |
| Nonspecific IgG matrix | 92          | CoM methylated nmol/min/mg              | 449 | 516 | 231 |
| Anti-MT2-A IgG matrix | 4.9        | CoM methylated nmol/min/mg              | 88  | 15  | 7   |
| Anti-MT2-A matrix + MT2-A | 114    | CoM methylated nmol/min/mg              | 387 | 495 | 200 |
| Anti-MT2-A matrix + MT2-M | 96.2    | CoM methylated nmol/min/mg              | 282 | 46  | 9.7 |

* Specific activity of 100% corresponded to 2.2 U/mg protein.
* CoM methylated nmol/min/mg protein.

extract after treatment with immobilized antibody, whereas the level of MT2-M was unchanged (Fig. 1, B and C).

Methyl-CoM formation from TMA, DMA, and MMA was assayed in antibody matrix-treated and untreated extracts as described under "Materials and Methods." Treatment of the TMA-grown cell extract with immobilized anti-MT2-A resulted in a large decrease in the specific activity of CoM methylation from all three methylamine substrates, as shown in Table I. Loss of activity correlated with the observed decrease in specific activity of MT2. Methyl-CoM formation from MMA was found to be highly dependent on the presence of MT2-A (Fig. 2A). As shown in Table I, removal of MT2-A led to a 97% decrease in the rate of CoM methylation (from 231 to 7 nmol/min/mg). Addition of purified MT2-A to the depleted extract resulted in regain of MMA:CoM methyl transfer activity. However, activity was not recovered when purified MT2-M was added, indicating that the requirement for MT2-A was specific. Similar results were obtained when MT2-A depleted extracts were tested for DMA:CoM methyl transfer activity (Table I). Elimination of MT2-A from the extract also resulted in approximately 97% loss of DMA-dependent CoM methylation activity (from 516 to 15 nmol/min/mg). Addition of exogenous, purified MT2-M to the extract did not reconstitute the activity. However, addition of pure MT2-A resulted in complete restoration of DMA:CoM methyl transfer activity (Table I).

Whereas MT2-A was required specifically for MMA- and DMA-dependent CoM methylation pathways, both MT2 isozymes were able to participate in CoM methylation by TMA (Table I and Fig. 2B). In contrast to the virtually complete elimination of methyl-CoM formation from MMA and DMA, extracts depleted of MT2-A continued to carry out TMA-dependent methylation of CoM at 88 nmol/min/mg, about 20% of the rate (449 nmol/min/mg) in undepleted extracts. As noted for the other amine substrates, TMA-dependent CoM methylation activity was reconstituted by addition of exogenous MT2-A (Table I and Fig. 2B). However, unlike the DMA- and MMA-dependent reactions, a substantial amount (approximately 43%) of TMA:CoM methyl transfer activity was restored by addition of MT2-M.

In control experiments, the extracts were treated with a matrix-containing nonspecific IgG, prepared as described under "Materials and Methods." Treatment with similar volumes and amounts of control IgG resulted in no decrease in the specific activities of the MMA-, DMA-, or TMA-dependent methylation of CoM (Table I and Fig. 2).

**Fig. 2.** MMA-dependent (A) and TMA-dependent (B) coenzyme M methylation by TMA-grown cell-free extracts treated with immobilized IgG preparations. The assays were performed as described under "Materials and Methods" with untreated extract (C), extracts treated with nonspecific antibodies (D), anti-MT2-A antibody matrix treated extracts (E), and anti-MT2-A matrix-treated extracts supplemented with either 2.3 units of MT2-A/mg of extract protein (F) or 2.1 units of MT2-M/mg of extract protein (G).

Titration of DMA:CoM Methyl Transfer Activity by Adjustment of the Level of MT2-A in Extracts—The effect of varying the amounts of the MT2-A isozyme on the rate of DMA conversion to CH₃-CoM was tested in two ways. In one experiment the extract was incubated with decreasing amounts of the immobilized anti-MT2-A antibody to produce samples containing different levels of residual MT2 activity. In the other experiment, 95% of initial MT2 activity was removed and then increasing amounts of either MT2-M or MT2-A were added back to the extract. The results of both experiments are shown in Fig. 3. A strong correlation was found between the amount of MT2 activity remaining in the extract, and the rate of DMA-dependent methylation of CoM. The response curve (Fig. 3) indicated that saturation occurs at levels of MT2-A somewhat higher than originally present in the extract. The curve extrapolated to zero activity as MT2-A approached zero, indicating that dependence on MT2-A was absolute. No increase in DMA:CoM methyl transfer activity was produced in extracts depleted of MT2-A by addition of MT2-M to 250% of the initial MT2 specific activity, again indicating specificity for the MT2-A isozyme.

Function of MT2-M in Methylation of CoM by Methanol—The involvement of MT2 in methanogenesis was first demonstrated for the methanol-dependent methylation of CoM (7). However, both MT2 isozymes are present in cells grown on this substrate. In order to determine whether specificity existed for either MT2 isozyme in methanogenesis from methanol, an extract of
methanol-grown cells was depleted of MT2-M by use of immobi-
лизованных, аффинити-очищенной MT2-M, как указано в "Материалах и методах." Специфичный иммунный матрикс был инкубирован с экстрактом метанол-как произведено для 92% MT2-активности (MT2-M в метаноле была оценена для 89% MT2-активности в клетках, выращенных на метаноле (8)). Этот подход полностью лишил меченый-депонированный MT2-M метилирования, как показано в табл. 1. Добавление 0.36 unit of purified MT2-M to the MT2-M depleted extract resulted in an increase of the methanol:CoM methyl transfer activity to 500 nmol/min/mg of extract protein. In contrast, addition of 0.24 unit of purified MT2-A did not reconstitute the activity, and the rate of meth-
анол-депонированный MT2-M метилирования remained undetectable. The results indicated a specific requirement for the MT2-M isozyme in conversion of methanol to methyl-CoM.

Effect of MT2 Depletion on Coenzyme M Methylation by Ac-
etate—In order to determine whether MT2 isozymes are in-
volved in methane formation from acetate, both isozymes were removed from extracts of cells grown on acetate by incubation with a mixture of immobilized anti-MT2-A and anti-MT2-M antibodies. Incubation was carried out with suffi-
cient immune matrix to remove a minimum of 97% of MT2 activity. The depleted extract was then tested for acetyl phos-
phat-depentent methylation of CoM. As shown in Table I, the specific activity of acetyl phosphate conversion remained un-
changed after treatment with the mixed immune matrix. Fur-
thermore, no stimulation of the rate of methyl-CoM formation was noted by addition of either MT2-A or MT2-M back to the depleted extract. The results indicated that neither one of the MT2 isozymes plays a role in methane formation from acetate.

DISCUSSION

Herein the first direct evidence is given of MT2-A involve-
ment in the formation of methyl-CoM from either DMA or TMA. These results also confirm recent data, indicating that MT2-A is involved in the MMA:CoM methyl transfer pathway (10). MT2-A is a versatile enzyme, since it is capable of partic-
ipating in the methylation of CoM by all three of the methyl-
amines tested. MT2-A may be considered as the amine-specific isozyme of MT2, since it does not function in the methylation of CoM by either methanol or acetate. In contrast, MT2-M is able to participate in both the TMA- and methanol-dependent path-
ways of methanogenesis.

The methylation of CoM by DMA or MMA exhibited speci-
ficity for MT2 isozyme A. MT2-M was unable to replace MT2-A in either one of these pathways. The overall rate of CoM meth-
ylation by DMA was also adjustable simply by variation of the amount of MT2-A present in the extract, suggesting a possible means for regulation of the pathway. In contrast, the TMA-de-
pendent pathway did not display strict dependence on the MT2-A isozyme. Removal of MT2-A markedly restricted the TMA:CoM methyl transfer pathway; however, a significant percentage of the initial activity remained following immu-
nodepletion. Whereas previous studies have provided the hy-
thesis that MT2-A is involved in metabolism of TMA (9), our data provide definite proof that this is the case and further establish the functional capabilities of both of the MT2 isozymes in metabolism of trimethylamine.

The involvement of methylcobamide:CoM methyltransferase isozymes in methyl-CoM formation from MMA, DMA, or TMA implies that one or more corrinoid proteins are also involved in these pathways. As mentioned earlier, MT2 activity may be measured by use of methylcobalamin as a methyl donor sub-
strate. However, there is little cobamide in Methanosarcina that is not bound to protein. Both isozymes of MT2 have K_m values of approximately 14 μM for free methylcobalamin, suggesting that in vivo both MT2 isozymes would recognize pri-
marily determinants found on the protein rather than on the corrinoid coenzyme. It is very likely that specificity of protein-
protein interactions is responsible for the specificity shown here for functional activity of the two MT2 isozymes in path-
ways of methanol, MMA and DMA conversion.

Proteins with corrinoid prosthetic groups are almost cer-
tainly involved in the methylation of CoM by each of the methyl-
amines, most probably in a manner analogous to the pathway of methanol conversion (Scheme 1). Perhaps the simplest hy-
thesis is that, like the methanol-dependent MT1 corrinoid enzyme, these methylamine MT1-type enzymes automethylate the enzyme bound corrinoid by reaction with a specific methyl-
amine substrate. The corrinoid cofactor would then be dem-
ethylated by the requisite MT2 isozyme, catalyzing transfer of the methyl group to CoM.

Available data indicate that the corrinoid proteins of the TMA (3) and MMA (10) pathways are separate enzymes. Each pathway of methanogenesis from the different methylamines is

* Specific activity of CH_3-CoM formation, nmol/min/mg protein.
* The immunosorbent gel contained only anti-MT2-M antibodies.
* The immunosorbent gel contained a mixture of anti-MT2-A and anti-MT2-M antibodies.

| Extract treatment | Activity in conversion of substrates: |
|-------------------|-------------------------------------|
|                   | Methanol | Acetyl phosphate |
| None              | 245       | 36               |
| Anti-MT2 IgG matrix | 0       | 35               |
| Anti-MT2 IgG matrix + MT2-A | 0     | 32               |
| Anti-MT2 IgG matrix + MT2-M | 499     | 29               |

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separately inducible by its substrate, and cells grown on MMA
do not convert TMA to methane at a high rate (12). The differ-
ence in the MT2 isozyme requirement for methyl-CoM forma-
tion from TMA versus DMA and MMA also suggests that the
TMA pathway must diverge from that of DMA and MMA uti-
лизization. One of our laboratories isolated a 29-kDa corrinoid
protein involved in the MMA:CoM methyl transfer pathway
(10). This protein did not appear to function in the utilization
of TMA. Another corrinoid protein has recently been isolated
which appears to be involved in the TMA:CoM methyl transfer
pathway. Both corrinoid proteins may serve as MT1-type en-
zymes in the utilization of their respective substrates. Al-
though the DMA pathway shares the same MT2 isozyme with
the MMA pathway, it is unknown whether or not a common
MT1 cobamide component is involved.

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