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Purification and Properties of Methanol:5-Hydroxybenzimidazolylcobamide Methyltransferase from *Methanosarcina barkeri*

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Methanol:5-hydroxybenzimidazolylcobamide methyltransferase from *Methanosarcina barkeri* has been purified to approximately 90% homogeneity by ion-exchange chromatography on DEAE-cellulose and QAE-A50 Sephadex columns. The molecular weight, estimated by gel electrophoresis, was found to be 122,000, and the enzyme contained two different subunits with molecular weights of 34,000 and 53,000, which indicates an $\alpha_2\beta$ structure. The enzyme contains three or four molecules of 5-hydroxybenzimidazolylcobamide, which could be removed by treatment of the enzyme with 2-mercaptoethanol or sodium dodecyl sulfate. In both cases the enzyme dissociated into its subunits. For stability, the enzyme required the presence of divalent cations such as Mg^{2+}, Mn^{2+}, Sr^{2+}, Ca^{2+}, or Ba^{2+}. ATP, GTP, or CTP was needed in a reductive activation process of the enzyme. This activation was brought about by a mixture of H_2, ferredoxin, and hydrogenase, but also by CO, which is thought to reduce the corrinoid chemically. The CO dehydrogenase-like activity of the methyltransferase is discussed.

Methanosarcina barkeri is a methanogenic bacterium that can grow on various one-carbon compounds such as CO_2, methylamines, methanol, and CO and on acetate (3, 10, 11, 23). Growth on methanol has been reported in both the presence and absence of H_2; in the latter case the reduction equivalents needed in methanogenesis were derived from the oxidation of part of the methanol to CO_2 (11).

The reduction of methanol to CH_4 in cell-free extracts of *M. barkeri* was found to depend on the presence of coenzyme M (2-mercaptoethanesulfonic acid; HS-CoM) and ATP under an atmosphere of H_2 (12). First, HS-CoM is methylated to 2-(methylthio)ethanesulfonic acid (methylcoenzyme M; CH_3S-CoM) (18, 21). CH_3S-CoM is subsequently reduced to methane by a methylreductase system that contains an enzyme-bound coenzyme MF_{430} (7, 8, 13).

The involvement of two distinct methyltransferases in the formation of CH_3S-CoM from methanol was recently reported (21). Methanol:5-hydroxybenzimidazolylcobamide methyltransferase (MT_1) binds the methyl group of methanol to a corrinoid bound to this enzyme (22). The enzyme is subject to activation and inactivation. Inactivation is brought about by O_2 and other oxidizing agents, and activation is achieved in the presence of ATP and H_2 (21). Activation of the partially purified MT_1 requires also the presence of hydrogenase and ferredoxin and leads to the formation of a Co(I) corrinoid (B_{12_s}) (22b). The role of the catalytic amount of ATP in this activation has not been elucidated. The second methyltransferase, methylcobalamin:HS-CoM methyltransferase (MT_2), is oxygen stable, and ATP is not required in its activity (20). It transfers the methyl group of the bound corrinoid of MT_1 to HS-CoM. The activity of MT_2 is not limited to the bound methylated corrinoid of MT_1; free methylcorrinoids with either 5-hydroxybenzimidazole (HBI) (16) or 5,6-dimethylbenzimidazole (DMBI) as the α-ligand could be demethylated (19, 20).

Here we report on the purification and properties of MT_1 from *M. barkeri* and on the effects of ATP, various other nucleoside triphosphates, and some inhibitors and inactivators. The functioning of MT_1 in the conversion of methanol and the activation and inactivation of the enzyme are discussed and presented in a model.

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MATERIALS AND METHODS

Culture methods and preparation of cell-free extracts. Cells of *M. barkeri* strain MS (DSM800) were mass cultured in a 350-liter fermentor with a mineral medium and methanol as the substrate under an atmosphere of N_2-CO_2 (4:1, vol/vol) as described before (21). Cells were harvested in the late exponential phase and stored under N_2 at −80°C.

Cell extracts were prepared in 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.2) containing 15 mM $MgCl_2$ by passage through a French pressure cell and centrifugation for 30 min at 20,000 × *g* at 4°C as described before (21). Extracts were stored under N_2 at −20°C in 15-ml serum vials closed with black butyl rubber stoppers and sealed with aluminum caps.

MT_1 assay. Incubation mixtures were prepared in an anaerobic glove box with an oxygen concentration below 1 ppm. A typical reaction mixture contained the following (final volume, 100 μl): 12.5 mM methanol, 12.5 mM HS-CoM, 9.35 mM ATP, 6.25 mM $MgCl_2$, 50 μM 2-bromoethanesulfonic acid to prevent possible enzymic reduction of CH_3S-CoM to methane (21), 7.0 mM 2-propanol as an internal standard, 10 μl of MT_2 (60 μmol of CH_3B_{12} converted per min per ml), and a sufficient amount of MT_1 and 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (pH 7.2). The reaction was performed in 10-ml serum vials, closed with red rubber stoppers and aluminum seal caps under a given gas phase (21). The reaction was started at 37°C; at various time intervals a vial was placed on ice, and air was injected. Activity was measured gas chromatographically by measuring the methanol concentration.

In experiments with partially purified MT_1, hydrogenase (5 μl; 22 μmol of benzylviologen reduced per min per ml) and ferredoxin (10 μl; 0.6 μmol of disulfide reduced per min per ml in the test system described earlier (22b) were added.

TABLE 1. Influence of metal ions on the activity of MT_1 from *M. barkeri* after treatment with EDTA

Addition (10 mM)	Activity (%)[a]		
	Direct	After 1 week[b]	
		No addition	$MgCl_2$ added[c]
None	0	0	0
Na^+	6	10	28
K^+	2	ND	ND
Mg^{2+}	66	44	59
Sr^{2+}	20	4	60
Ca^{2+}	35	3	33
Ba^{2+}	27	0	55
Mn^{2+}	27	9	20
Ni^{2+}	21	0	6
Co^{2+}	46	0	7
Cu^{2+}	0	ND	ND
Zn^{2+}	5	ND	ND
Fe^{2+}	5	ND	ND
Cr^{3+}	0	ND	ND

[a] The activity of untreated MT_1 was set at 100% and was equivalent to 0.35 μmol of methanol converted per min per mg of protein. ND, Not determined.
[b] The indicated metal ions were added to metal-free MT_1, and the stability of the enzyme was tested upon storage for 1 week at −20°C.
[c] Activity was measured in the presence of $MgCl_2$ in the assay mixture.

Purified MT_1 was tested in the presence of these components and an unknown enzymic component (component S; 10 μl; 9.6 mg of protein per ml) that stimulated the activity of MT_1.

Purification of MT_1. All purification procedures were carried out in an anaerobic glove box with buffers and column materials that were freed of oxygen by several cycles of evacuation and gassing.

Crude cell-free extract (160 ml) was applied to a DEAE-cellulose (DE-52) column (14 by 4.8 cm) equilibrated with 10 mM *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid buffer (pH 7.2) containing 15 mM $MgCl_2$ (buffer A). Elution with buffer A yielded a hydrogenase-containing fraction that was used for activation of MT_1 without further purification. Elution with buffer A containing 0.15 M NH_4Cl yielded MT_2, which was purified further as described earlier (20). Elution of MT_1 was performed with a linear gradient of NH_4Cl in buffer A (0.15 to 0.6 M, 1.6 liters). Fractions eluted between 0.25 and 0.30 M NH_4Cl contained MT_1 activity. Ferredoxin was obtained at 0.35 M NH_4Cl and was purified as described before (22b). MT_1-containing fractions were pooled and concentrated by ultrafiltration (Amicon, PM 30 filter) under N_2. The concentrated MT_1 solution was applied to an identical DEAE-cellulose column and eluted with a linear gradient of NH_4Cl (0.1 to 0.4 M, 1 liter). Active MT_1 fractions were pooled and applied to a QAE-A50 column (13 by 4.8 cm) equilibrated with buffer A. Elution was performed with a linear gradient of NH_4Cl (0.25 to 0.8 M, 1 liter). Fractions eluted between 0.4 and 0.5 M contained corrinoids, but were inactive in the MT_1 assay. After concentration of these fractions and washing on an Amicon filter (PM 30) with buffer A, activity could be measured. The concentrated pool (about 8 ml) was applied to a Sephacryl S-200 column (100 by 2.6 cm) equilibrated with buffer A, and elution was performed with the same buffer at a flow rate of 6 to 7 ml h^{-1}. Fractions of 4 ml were collected and assayed for MT_1 activity in the presence of component S. Active fractions were pooled and concentrated.

Analytical methods. The molecular weight of the purified native MT_1 was determined by polyacrylamide gel electrophoresis with various gel concentrations (6, 8, 10, and 12%) by the method of Hedrick and Smith (9). The following reference proteins (molecular weights within parentheses) were used: trypsin inhibitor (20,000); α-amylase (45,000); and bovine serum albumin monomer (68,000), dimer (136,000), and trimer (204,000). Molecular weight determination of MT_1 subunits was performed with sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis by the method of Laemmli (14) with cytochrome *c* (12,400), trypsin inhibitor (20,000), lactate dehydrogenase (36,000), bovine serum albumin (68,000), and phosphorylase B (94,000) as references. Protein was determined with Coomassie brilliant blue G-250 by the method of Sedmak and Grossberg (17) with bovine serum albumin as the standard. Methanol was measured with a Pye Unicam GCD gas chromatograph as described before (21). Corrinoids were analyzed by high-pressure liquid chromatography with a reversed-phase system on a 10-μm C_{18} LiChrosorb-RP18 column. Samples were prepared by extraction in 80% methanol at 80°C in the dark as described before (22).

Materials. Black butyl rubber stoppers were obtained from Rubber B. V., Hilversum, The Netherlands. Red rubber stoppers were from Helvoet B. V., Alken, Belgium. DEAE-cellulose, grade DE-52, was purchased from Whatman, Maidstone, England. QAE-A50 Sephadex and Sephacryl S-200 were obtained from Pharmacia Fine Chemicals A. B., Uppsala, Sweden. Serva Blue G, used for protein determination, was from Serva Feinbiochemica, Heidelberg, Federal Republic of Germany. *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid, vitamin B_{12} (CN-B_{12}-DMBI), ATP, and other nucleotide triphosphates were from Sigma Chemical Co., St. Louis, Mo. HS-CoM was obtained from Merck-Schuchard A. G., Darmstadt, Federal Republic of Germany. 2-Bromoethanesulfonic acid was purchased from Aldrich Europe, Beerse, Belgium. Methanol (high-pressure

TABLE 2. Purification of MT_1 from *M. barkeri*

Purification step	Total protein (mg)	Sp act (μmol min^{-1} per mg of protein)[a]			Total activity[b] (μmol min^{-1})	B_{12} content[a] (nmol per mg of protein)	Purification factor (fold)[c]	Recovery (%)
		No addition	Hydrogenase and ferredoxin added	Hydrogenase, ferredoxin, and component S added				
Crude extract	1,560	0.24	0.24	0.24	375	5.1		100
DEAE-cellulose I	354	0.52	0.67	0.70	248	16.0	3.1	66
DEAE-cellulose II	260	0.01	0.67	ND[d]	ND	19.8	3.9	ND
QAE-A50 Sephadex	218	0.00	0.52	0.76	166	28.5	5.6	44

[a] Activity and B_{12} content were determined as given in the text.
[b] Calculated on the basis of the specific activity of MT_1 tested in the presence of hydrogenase, ferredoxin, and component S.
[c] The purification was calculated on the basis of B_{12} content.
[d] ND, Not determined.

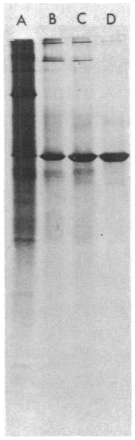

FIG. 1. Polyacrylamide gel electrophoresis of the pooled MT_1-containing fractions obtained after the various purification steps. Lanes: A, crude cell-free extract of *M. barkeri*; B, DEAE-cellulose (I) chromatography; C, DEAE-cellulose (II) chromatography; D, QAE-A50 Sephadex chromatography. Electrophoresis was on a 10% polyacrylamide gel.

liquid chromatography grade) was obtained from Baker, Deventer, The Netherlands. Reference proteins for molecular weight determinations were obtained from Boehringer, Mannheim, Federal Republic of Germany. Gases were obtained from Hoek Loos, Schiedam, The Netherlands. To remove traces of oxygen, H_2 was passed over a catalyst (BASF R0-20) at room temperature, and N_2 was passed over a prereduced catalyst (R3-11) at 150°C. Both catalysts were a gift from BASF, Ludwigshafen, Federal Republic of Germany.

RESULTS

Metal ion requirement. MT_1 obtained after the first DEAE-cellulose purification step (see above) was freed of metal ions by the addition of 50 mM EDTA and subsequent removal of the EDTA-metal complex by Sephadex G-25 gel filtration. The activity of the metal-free MT_1 preparation was tested immediately in the presence of various metal ions (Table 1). The addition of Mg^{2+} restored 66% of the original activity, whereas the addition of Co^{2+}, Ca^{2+}, Mn^{2+}, Ba^{2+}, Ni^{2+}, and Sr^{2+} yielded activities between 20 and 46% of that of the untreated enzyme. In the absence of any metal ion no activity was found. The effect of the various metal ions on the stability of MT_1 was also studied (Table 1). Various metal ions were added to a metal-free MT_1 preparation, which was then stored at −20°C. The activity was measured after 1 week in both the presence and absence of extra added $MgCl_2$. Only the MT_1 preparation stored in the presence of $MgCl_2$ showed activity (44%) without extra $MgCl_2$ addition. The presence of extra $MgCl_2$ during incubation revealed the stabilization of MT_1 in the presence of Ca^{2+}, Sr^{2+}, Mn^{2+}, Ba^{2+}, and Na^+ ions. Because of the activating and stabilizing effect, $MgCl_2$ was added to all buffers used in handling MT_1.

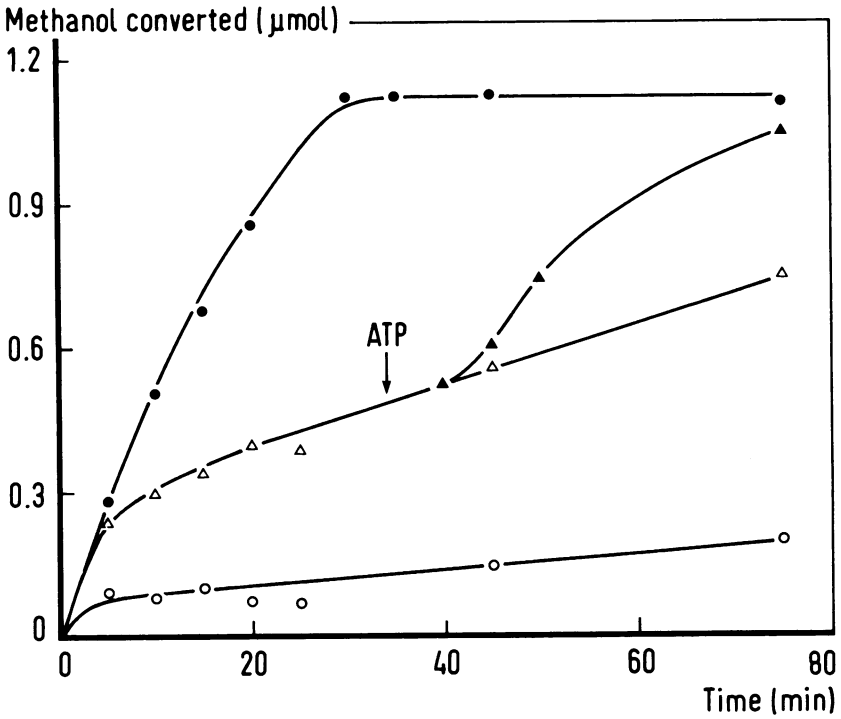

FIG. 2. Effect of the removal of ATP on the activity of MT_1 from *M. barkeri*. The activation mixture (total volume, 100 μl) contained the compounds given in the text for the MT_1 assay including 22.5 μl of MT_1 (taken from the first DEAE-cellulose step; 3.5 μmol min^{-1} per ml), but HS-CoM and methanol were absent. Activation of MT_1 was achieved by preincubation for 30 min at 37°C under H_2-N_2 (50:50, vol/vol). Where indicated, ATP was then removed by incubation with a mixture of adenylate kinase (4 mU), hexokinase (4 mU), and glucose (24 mM) for 30 min at 30°C. After the addition of the substrates methanol and HS-CoM, the reaction was started at 37°C under H_2-N_2 (50:50, vol/vol). Symbols: (●) ATP was not removed; (△) ATP was removed; (▲) ATP was removed, and at the time indicated by the arrow extra ATP (9.4 mM) was added; (○) before the activation, ATP was converted by the adenylate kinase mixture, as a control of the effectiveness of ATP removal.

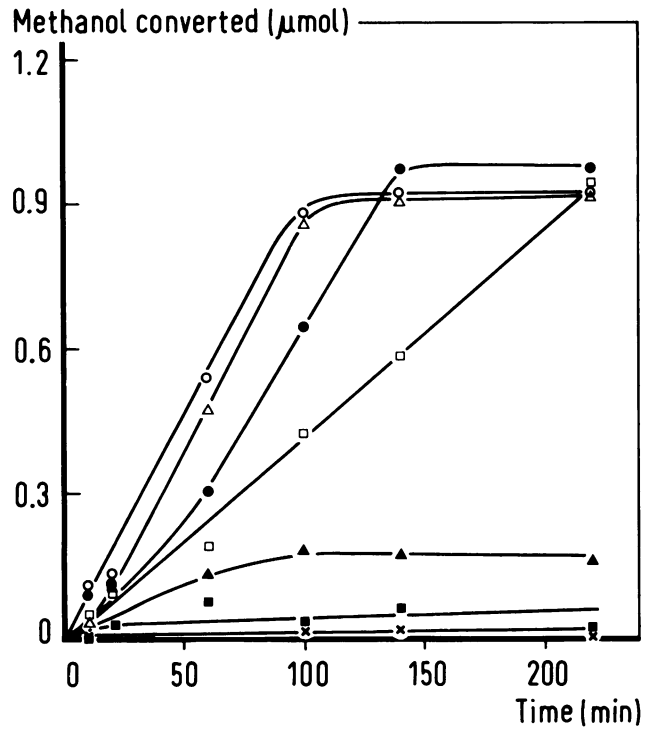

FIG. 3. Influence of various nucleotide triphosphates on the activity of MT_1 from *M. barkeri*. The reaction mixtures (total volume, 100 μl) were as described in the text, and 15 μl of MT_1 (taken from the QAE-A50 Sephadex step; 0.6 μmol min^{-1} per ml) was used. The following nucleotide triphosphates (9.4 mM) were used: (○) ATP, (△) GTP, (●) CTP, (□) UTP, (▲) ITP, (■) TTP, and (×) none. Incubation was performed at 37°C under H_2-N_2 (50:50, vol/vol) as the gas phase.

Purification of MT_1. MT_1 was purified as described above (Table 2). During the purification procedure the specific activity decreased drastically. The addition of hydrogenase and ferredoxin resulted in a reactivation in all purification steps, but only a 2.8-fold increase of specific activity was obtained. The addition of an unknown component (component S), which was eluted just in front of MT_1 at the first DEAE-cellulose purification step, stimulated the transmethylation when added together with hydrogenase and ferredoxin. During Sephacryl S-200 gel filtration a very drastic decrease of MT_1 activity took place; the specific activity dropped from 0.52 to 0.01 μmol min^{-1} per mg of protein tested in the presence of hydrogenase and ferredoxin. The specific activity was only partly restored to 0.13 μmol min^{-1} per mg of protein when component S was added.

On the basis of the B_{12}-HBI content per milligram of protein, a five- to sixfold purification factor was achieved. Measurement of the corrinoid showed that the observed loss of enzyme activity upon Sephacryl S-200 gel filtration was probably due to loss of the bound corrinoid from the enzyme; the B_{12} content was reduced from 28.5 to 6.2 nmol per mg of protein.

Polyacrylamide gel electrophoresis of the MT_1 fractions after the successive purification steps showed an almost pure MT_1 preparation after QAE-A50 Sephadex chromatography (Fig. 1).

Component S was found to be sensitive to boiling, but insensitive to oxygen. The molecular weight was estimated to be larger than 30,000, but less than 100,000, on the basis of its behavior during gel filtration and Amicon filtration.

Subunit structure of MT_1. The molecular weight of the native MT_1 molecule was estimated by gel electrophoresis with different gel concentrations and was found to be 122,000. MT_1 could be observed before staining of the gels as a red band due to the presence of bound B_{12}-HBI. MT_1 contained two different subunits of 34,000 and 53,000 molecular weight as estimated by SDS-polyacrylamide gel electrophoresis. No red bands could be seen even at high MT_1 concentrations on these gels, but a red band migrated just in front of the bromophenol blue marker.

The B_{12}-HBI content of MT_1 was calculated from the amount of B_{12}-HBI present in the QAE-A50 Sephadex fraction and was 3.4 mol of B_{12}-HBI per mol of enzyme with a molecular weight of 122,000.

As was clear from SDS-gel electrophoresis, B_{12}-HBI was removed by treatment of MT_1 with SDS and 2-mercaptoethanol. Gel electrophoresis in the presence of only one of these compounds showed that 2-mercaptoethanol could dissociate the enzyme partially into a corrinoid and some protein products (data not shown). SDS had a similar, but somewhat stronger, effect.

Nucleotide requirement and specificity. The methylation of HS-CoM from methanol depends on the presence of catalytic amounts of ATP (18, 21). In cell-free extracts the number of CH_3S-CoM molecules formed per ATP molecule reach values up to 80. Upon increase of the ATP concentration in the 0 to 0.3 mM range, both the amount of methanol converted and the rate of this conversion were enhanced. This rate plotted as a function of the catalyst ATP showed a hyperbolic curve and a half-maximal activity at 0.16 mM ATP for purified MT_1 preparation obtained after QAE-A50 Sephadex chromatography. When ATP and ADP were removed by a trap of glucose, hexokinase, and adenylate kinase after a preincubation period of 30 min, the initial activity was similar to the activity in the absence of the trap, but after a short period of time the activity declined (Fig. 2). The addition of extra ATP during the incubation resulted in an increased activity.

The specificity of MT_1 toward ATP was studied by the addition of various other nucleotides instead of ATP (Fig. 3). Replacement of ATP by GTP resulted in the same activity after a short time. CTP was also active, but after a much longer lag time (28 min), and the addition of UTP resulted in only about 43% of the maximal activity. The addition of ITP, TTP, and dATP (data not shown) did not result in significant activity at all.

Inhibition and inactivation of MT_1. Various compounds were tested for their ability to inhibit CH_3S-CoM formation from methanol (Table 3). Possible inhibitors can be divided into the following three groups: (i) those acting on the MT_1 corrinoid, (ii) those acting on the MT_1 enzyme, and (iii) those inhibiting MT_2 or one of the enzymes involved in MT_1 activation (i.e., hydrogenase, ferredoxin, component S).

None of the compounds tested was able to inhibit MT_2 in the conversion of CH_3-B_{12}-DMBI to CH_3S-CoM. Compounds that acted on the B_{12}-HBI of MT_1 were NH_4^+, NO_2^-, and SO_3^{2-}. High concentrations of NH_4^+ ions inhibited MT_1, probably due to the formation of NH_3-B_{12}-HBI, since high-pressure liquid chromatographic analysis showed that this compound was the predominant B_{12} derivative present in the enzyme under these conditions (15). In the presence of 0.1 mM NO_2^- only NO_2-B_{12}-HBI was found in samples of MT_1. Upon the addition of SO_3^{2-} to the incuba-

tion mixture, equimolar amounts of CH_3-B_{12}-HBI and SO_3-B_{12}-HBI were found in the enzyme.

The inhibitory effects of NH_2OH and benzylviologen were transitory and disappeared upon reduction of these compounds by the H_2-hydrogenase-ferredoxin system. The formation of NH_4^+ could be demonstrated in the former case.

The effect of sulfhydryl-reactive reagents was observed only at rather high concentrations. 5,5'-Dithiobis-(2-nitrobenzoic acid) could not be tested because it was converted by the reducing system.

Pyridoxal phosphate inhibits transmethylation in crude extracts (21) and also inhibits the conversion of methanol to CH_3S-CoM by the purified enzymes. It does not inhibit the activity of MT_2, and high-pressure liquid chromatographic analysis did not show the formation of a B_{12}-HBI derivative other than CH_3-B_{12}-HBI. The addition of extra ATP (6 mM) or CH_3-B_{12}-DMBI (0.1 mM) could not prevent the inhibition. The inhibiting effect could be increased by preincubation of MT_1 with pyridoxal phosphate; the addition of a large (50-fold) molar excess of lysine abolished the inhibition.

Activation of MT_1 by carbon monoxide. The transmethylation of methanol to HS-CoM in crude extracts requires the presence of H_2 or an H_2-donating system such as pyruvate plus coenzyme A for maximal activity (21). The activation of partially purified MT_1 was dependent on the presence of H_2 together with hydrogenase and ferredoxin (22b). A chemical reduction of B_{12}-DMBI to the Co(I) level by CO was reported before (2), and one might expect an activation of MT_1 by CO. This was found by the use of CO instead of H_2 as the gas phase (Table 4). The presence of ferredoxin was not needed for activation, whereas the presence of hydrogenase resulted in an extra stimulation. The effect of 1 mM cyanide on the activation differed for the two gases: with CO only 20% activity was observed, and with H_2 full activity was observed. The presence of CO dehydrogenase-like activity was measured as described by Diekert and Thauer (5) by the reduction of methylviologen. This activity was found in the MT_1 preparation, obtained after the first DEAE-cellulose purification step (0.21 μmol of methylviologen reduced per min per mg of protein) as well as in the hydrogenase fraction (0.11 μmol of methylviologen reduced per min per mg of protein).

TABLE 3. Effect of various compounds on the activity of MT_1 from *M. barkeri*

Compound	Concn (mM)	Activity[a] (%)
None		100
Pyridoxal phosphate	1	26
$HgCl_2$	0.1	90
$HgCl_2$	1.0	10
N-Ethylmaleimide	1.0	90
p-Chloromercuribenzoate	0.1	100
$NaNO_2$	0.1	10[b]
Na_2SO_3	1.0	50
NH_2OH	0.1	22 to 100[c]
NH_4Cl	400	7
Benzylviologen (oxidized)	0.05	0 to 100[d]
Benzylviologen (reduced)	0.05	100

[a] The activity measured in the absence of the compounds listed was used as reference activity set at 100% and amounted to 0.52 μmol of methanol converted per min per mg of protein.
[b] An apparent K_i of 0.04 mM was determined.
[c] An increase of activity was observed after the addition of extra hydrogenase.
[d] The activity increased during the incubation because of reduction of the benzylviologen.

TABLE 4. Effect of the gas phase on the activity of partially purified MT_1[a] from *M. barkeri*

Changes of the reaction mixture[b]	Gas phase[c]	Activity[d] (%)
None	N_2	0
None	H_2	100
None	CO	65
− Ferredoxin	CO	60
− Hydrogenase	CO	30
− Ferredoxin and hydrogenase	CO	30
− Ferredoxin and hydrogenase	H_2	10
+ KCN[e]	CO	20
+ KCN	H_2	100

[a] An MT_1 preparation obtained from the first DEAE-cellulose step was used.
[b] The complete reaction mixture is described in the text.
[c] Pressure of 1 atm (ca. 101.3 kPa).
[d] The activity of the complete reaction mixture under H_2 was set at 100% and was equivalent to 0.49 μmol min^{-1} per mg of protein.
[e] A concentration of 1 mM was used.

DISCUSSION

MT_1 from *M. barkeri* was purified to about 90% homogeneity as judged by gel electrophoresis in the presence or absence of SDS. On the basis of the specific activity, a 2.8-fold purification was obtained by four purification steps, and a 5.6-fold purification was obtained on the basis of the B_{12}-HBI content per milligram of protein. These results suggest that a rather large (about 15%) part of the protein in the crude extract might consist of this enzyme. MT_1 with a molecular weight of 122,000 is composed of two different subunits of 34,000 and 53,000. Approximately three or four molecules of B_{12}-HBI were found per molecule of protein. Dissociation of the enzyme into its subunits by SDS plus 2-mercaptoethanol resulted in a complete loss of the bound corrinoids, and a partial dissociation and loss of the corrinoids was observed upon treatment of MT_1 with 2-mercaptoethanol. The specific activity and the B_{12}-HBI content of MT_1 decreased drastically upon gel filtration with Sephacryl S-200.

The properties of MT_1 are schematically given in Fig. 4. As was previously reported (21, 22) MT_1 activity is dependent upon the presence of catalytic amounts of ATP. The required ATP concentration for maximal MT_1 activity of purified MT_1 was 0.3 mM. In this experiment the B_{12}-HBI concentration, present in a form bound to MT_1, was 0.016 mM. Thus 19 molecules of ATP were present per molecule of bound B_{12}-HBI. Removal of ATP from the reaction mixture before incubation of the enzyme with the substrates was started showed that ATP is not needed for the enzymic reaction itself, but that MT_1 is quickly inactivated in the absence of ATP. It may be concluded that the reductive activation of MT_1 is catalyzed by a process requiring ATP. Since oxygen increases the need for extra ATP (21), it is possible that activation of MT_1, pure or in crude extracts, is based upon the withdrawal of remaining traces of oxygen or other oxidizing agents. By this process the redox potential of the medium is lowered to allow the formation and stability of B_{12s} derivatives needed in the receipt of the methyl group from methanol. The complete process of the suggested reduction of B_{12} to B_{12s} with H_2 at the expense of ATP might be an example of possible routes for ATP-driven reversed electron transport.

MT_1 can be activated by the use of CO as the gas phase. The activation involves most probably a reduction of the B_{12}-HBI to the Co(I) level and might be brought about by CO

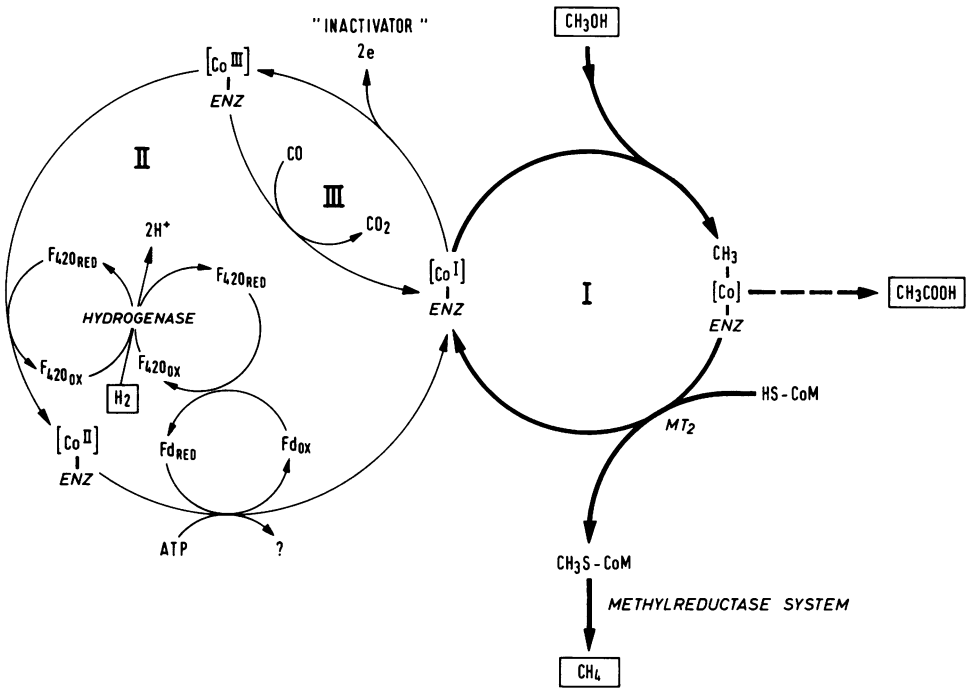

FIG. 4. Carbon and electron flow scheme proposed for the conversion of methanol into CH_3S-CoM in *M. barkeri*. (I) The carbon cycle involves the action of MT_1 (ENZ), which is methylated (22), when it is present in its most reduced state, Co(I) (22b). The methyl group is then transferred to HS-CoM by MT_2 (20) or enters the acetogenic pathway. (II) The flow of electrons to the inactivated corrinoid bound to MT_1. Coenzyme F_{420} is reduced by H_2 and hydrogenase. First the corrinoid is reduced to the Co(II) level (van der Meijden and van Aerts, unpublished results), and a subsequent reduction to the active Co(I) level is mediated by H_2, hydrogenase, coenzyme F_{420} (van der Meijden and van Aerts, unpublished results), and ferredoxin (22b). The presence of an oxidator causes oxidation to the Co(III) level and inactivation of MT_1 (22b). (III) CO dehydrogenase-like activity displayed by MT_1. Inactive MT_1 is reduced and activated by the presence of CO. If both CO and a suitable electron acceptor are present, MT_1 (or solely the MT_1-bound corrinoid) catalyzes the oxidation of CO to CO_2.

in a chemical way. The CO dehydrogenase-like activity measured with the purified sample of MT_1 is probably the net result of a reduction of the enzyme-bound corrinoid by CO and the oxidation of the reduced corrinoid by methylviologen. The inhibition of this corrinoid reduction by cyanide might be explained by the fact that CO is not able to react with a Co-C bond, whereas CN-B_{12}-DMBI can be reduced by the action of hydrogenase, ferredoxin, and H_2 (22b). These results indicate that the measurement of CO dehydrogenase activity in enzyme-bound corrinoid-containing extracts and cells should be interpreted with caution. However, a corrinoid-free Ni^{2+}-containing CO dehydrogenase is found in methanogenic and acetogenic bacteria (3, 6).

Inhibition of the transmethylation from methanol to HS-CoM by NO_2^-, SO_3^{2-}, NH_2OH, and NH_4^+ may be explained by the fact that these compounds can be bound to the Co atom of the corrinoid (15) and thus prevent reduction and subsequent methylation. Possibly the transient effects of NH_2OH and SO_3^{2-} result from a reduction of these compounds by a combined action of the corrinoid and hydrogenase, as is the case in N_2O inhibition of methionine synthetase in rat liver, which results in the oxidation of Cob(I) alamin to Cob(III) alamin and the concomitant production of N_2 (1, 4).

The observation that only rather high concentrations of the sulfhydryl-reactive reagents $HgCl_2$, *p*-chloromercuribenzoate, and *N*-ethylmaleimide inhibit MT_1 indicates, in contrast to the suggestion of Wood et al. (25), that no sulfhydryl groups are involved in the enzymatic process.

From the observation that pyridoxal phosphate inhibits the MT_1 reaction, but neither reacts with the corrinoid nor influences the corrinoid-protein binding, one may conclude that pyridoxal phosphate binds to the enzyme and alters its configuration in such a way that the enzyme becomes less active.

The corrinoid-containing methyltransferase of *Clostridium thermoaceticum* (24), an anaerobic acetogen, is involved in acetate formation from CO_2 and has some properties similar to MT_1 from *M. barkeri*. It is quite labile under aerobic conditions and requires ATP for a reductive activation. Gel electrophoresis in the absence of SDS resulted in a dissociation of the enzyme into its subunits (24). MT_1 is also a labile enzyme with respect to its subunit structure since treatment with 2-mercaptoethanol resulted in a partly dissociated enzyme preparation as was shown by means of gel electrophoresis.

Eubacterium limosum, an anaerobic acetogen that uses CO_2 as well as methanol for acetate production, contains a methyltransferase that is active with methanol and is very much like MT_1 from *M. barkeri* with respect to its functioning and activation and the inactivation phenomena described for it (22a).

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