MeaB Is a Component of the Methylmalonyl-CoA Mutase Complex Required for Protection of the Enzyme from Inactivation*

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Adenosylcobalamin-dependent methylmalonyl-CoA mutase catalyzes the interconversion of methylmalonyl-CoA and succinyl-CoA. In humans, deficiencies in the mutase lead to methylmalonic aciduria, a rare disease that is fatal in the first year of life. Such inherited deficiencies can result from mutations in the mutase structural gene or from mutations that impair the acquisition of cobalamin. Recently, a human gene of unknown function, MMAA, has been implicated in methylmalonic aciduria (Dobson, C. M., Wai, T., Leclerc, D., Wilson, A., Wu, X., Dore, C., Hudson, T., Rosenblatt, D. S., and Gravel, R. A. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 15554–15558). MMAA orthologs are widespread in bacteria, archaea, and eukaryotes. In Methylobacterium extorquens AM1, a mutant defective in the MMAA homolog, was unable to grow on C3 and C2 compounds because of the inability to convert methylmalonyl-CoA to succinyl-CoA (Korotkova N., Chistoserdova, L., Kuksa, V., and Lidstrom, M. E. (2002) J. Bacteriol. 184, 1750–1758). Here we demonstrate that this defect is not due to the absence of adenosylcobalamin but due to an inactive form of methylmalonyl-CoA mutase. The presence of active mutase in double mutants defective in MeaB and in the synthesis of either R-methylmalonyl-CoA or adenosylcobalamin indicates that MeaB is necessary for protection of mutase from inactivation during catalysis. MeaB and methylmalonyl-CoA mutase from M. extorquens were cloned and purified in their active forms. We demonstrated that MeaB forms a complex with methylmalonyl-CoA mutase and stimulates in vitro mutase activity. These results support the hypothesis that MeaB functions to protect methylmalonyl-CoA mutase from irreversible inactivation.

Coenzyme B12 (5’-deoxyadenosylcobalamin) (AdoCbl) serves as a cofactor for radical-based isomerization reactions (1). Methylmalonyl-CoA mutase (MCM), isobutyryl-CoA mutase, B12-dependent amino mutases, α-methylene-γ-lactate mutase, and diol dehydratase are members of this family of enzymes (2–6).

MCM catalyzes the reversible isomerization of R-methylmalonyl-CoA and succinyl-CoA. Genes for MCM from Propionibacterium shermanii (7), Streptomyces cinnamonensis (8), Escherichia coli (9), humans (10), and mice (11) have been cloned and sequenced. The P. shermanii and S. cinnamonensis MCM contain two homologous but non-identical subunits: a-subunit (~79 kDa) and β-subunit (~65 kDa) (7, 8). The human (10), mouse (11), and E. coli (9) enzymes are homodimers. Crystal structures of P. shermanii MCM were determined (12, 13) that revealed that both α and β subunits consist of two principal domains: an eight-stranded α/β triose phosphate isomerase barrel (αβ)2 and a flavodoxin-like AdoCbl-binding fold. The cofactor is sandwiched between the (αβ)2 barrel and the flavodoxin-like AdoCbl-binding fold (12).

The enzyme has a broad distribution among living organisms and has been found in both bacterial and animal cells (14). MCM plays an essential role in the conversion of propionyl-CoA to succinyl-CoA, an intermediate of the tricarboxylic acid cycle. In higher animals, MCM is involved in the breakdown of the amino acids valine, isoleucine, methionine, and threonine as well as thymine, cholesterol, and odd-chain fatty acids (15, 16). In Streptomyces, the enzyme is involved in polyketide biosynthesis (17). In the methylo trophic bacterium Methylobacterium extorquens, MCM is part of the glyoxylate regeneration pathway, an essential element of methylostrophic metabolism (18).

In humans, inherited defects that impair the activity of MCM lead to methylmalonic aciduria. Such a disorder can result from mutations in the gene encoding MCM or from mutations that impair the assimilation of cobalamin (CbI) into the MCM cofactor, AdoCbl (19, 20). A newly described gene, MMAA, involved in conversion of methylmalonyl-CoA to succinyl-CoA has recently been identified in humans (21). A mutation in MMAA causes a disorder classically associated with vitamin B12-responsive methylmalonic aciduria (22). Fibroblast cultures from patients with this kind of disorder show decreased propionate incorporation into cellular protein and reduced synthesis of AdoCbl, but they show a normal concentration of methylcobalamin (23). A role of MMAA in the transport of vitamin B12 into mitochondrion has been proposed (21).

M. extorquens AM1 contains a homolog of MMAA, called meaB (18). This bacterium synthesizes AdoCbl de novo and utilizes AdoCbl as the cofactor for two enzymes, MCM and MeaA, a mutase involved in the conversion of butyryl-CoA to propionyl-CoA (18, 24). Mutation in meaB has no effect on the function of MeaA, suggesting that meaB is not involved in AdoCbl biosynthesis in this organism (18). MeaB contains a consensus-binding sequence for GTP/ATP, based on analysis with the COG program (18). Orthologs of meaB are present in many archaeal, bacterial, and eukaryotic genomes for which sequences are available. In most cases, meaB orthologs are located in close proximity to genes encoding orthologs of propi
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ononyl-CoA carboxylase, methylmalonyl-CoA epimerase, and MCM (9, 25).

Other B12-dependent enzymes (glycerol and diol dehydratase) undergo suicide inactivation by the substrate during catalysis (6, 26, 27). The inactivation involves irreversible cleavage of the C-C bond of AdoCbl, forming 5'-deoxyadenosine and an alkylcobalamin-like species (27). The modified coenzyme remains tightly bound to the enzyme and is not exchangeable with free intact cofactor. The inactivated enzyme is released, perhaps by exchange of the modified coenzyme for intact AdoCbl in the presence of a thionine-like protein (reactivase) and ATP (6). The reactivation takes place in two steps: ADP-dependent cohalamine release and ATP-dependent dissociation of the apoenzyme-reactivating factor complex (6). Although reactivases have not been reported for any MCMs, the MCM of P. shermanii has been shown to be subject to inactivation during turnover under aerobic conditions. The inactivation occurred at a much slower rate than glycerol and diol dehydratases, with a half-life for inactivation of ~170 min (28). However, in the absence of substrate the enzyme was stable (28). These precedents suggested that MeaB might play a role in protection or reactivation of MCM. In this report, we provide evidence that MeaB does not function as a reactivase but is involved in protection of the MCM from suicide inactivation, possibly by a stabilization function.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Plasmids, and Growth Conditions—E. coli JM109 (Promega), Top 10 (Invitrogen), S17-1 (29), and BL21 DE3 (Novagen) used in this study were grown in Luria Bertani medium in the presence of appropriate antibiotics as described by Maniatis et al. (30). M. extorquens AM1 and Burkholderia fungorum LB400 were grown in the minimal medium described previously (31, 32). Succinate (20 mM), methanol (100 mM), ethylamine (20 mM), propionate (10 mM), or citrate (20 mM) was used as the substrate. The following antibiotic concentrations of appropriate antibiotics as described by Maniatis for B. fungorum and M. extorquens were added: 50 mM HEPES, pH 7, 50 mM NaCl, and 15% (v/v) glycerol, and stored at −70 °C.

DNA Manipulations—Plasmid isolation, E. coli transformation, and restriction enzyme digestion or ligation was carried out as described by Maniatis et al. (30). The chromosomal DNA of M. extorquens AM1 was isolated with the procedure of Saito and Miura (34).

DNA Sequencing—DNA sequencing from both strands was carried out with an Applied Biosystems automated sequencer by the Department of Biochemistry Sequencing Facility at the University of Washington.

Computer Analysis—Translations and analyses of DNA and DNA-derived polypeptide sequences were carried out using Genetic Computer Group (Wisconsin) and ORF Finder (NCBI) programs (www.ncbi.nlm.nih.gov).

Mutant Generation—Insertion mutations in meaD (AY388648), meaB (AAAL8272.1), and rpmC (YPO0031472.1) were generated in vitro using pCM184 with a Km' gene cartridge as described earlier (32). Tc' colonies were chosen as potential double-crossover recombinants, whereas Tc' colonies were assumed to be single-crossover recombinants. The identity of the double-crossover mutants was confirmed by diagnostic PCR.

The plasmid pCM157 was introduced by conjugation into ΔMeaB ΔrpmC ΔmcmA ΔmcmB ΔmcmC mutants using the helper plasmid pRK2073. Tc' strains were streaked for purity until the resulting strain produced only Km' colonies. pCM157 was removed from the strains by several transfers on medium lacking Tc. The deletion of the Km cassette from ΔmeaD ΔrpmC mutants was confirmed by diagnostic PCR. Unmarked ΔmeaD ΔrpmC strains were used to generate ΔmeaD ΔrpmC ΔmcmA ΔmcmB ΔmcmC mutants in vitro using pCM184 with a Km' gene cartridge as described (32).

Matings—Triparental or biparental matings between E. coli and M. extorquens AM1 or B. fungorum LB400 were performed overnight on nutrient agar at 30 °C. Cells were then washed with sterile minimal medium and plated on selective minimal medium at appropriate dilutions. In triparental matings, pRR2013 (33) was used as a helper plasmid.

Cloning, Expression, and Purification of Proteins—The genes encoding meaB, mcmA, and mcmB were PCR-amplified from chromosomal DNA isolated from M. extorquens AM1. Oligonucleotides containing the first and last 24 bases of each gene and restriction endonuclease sites at their 5'-ends were used as primers. After restriction with NcoI and MluI, the meaB product was cloned into the pET21d vector (Novagen) in the 5'-NcoI and 3'-XhoI restriction sites. The PCR product containing mcmB was restricted with NdeI and SalI and cloned into pET22b (5'-NdeI and 3'-XhoI restriction sites), generating pNK11. To express the native form of MCM, the following strategy was used. The mcmB gene with the T7 promoter was PCR-amplified from pNK11 with the following primer pair: 5'–CCTGGGATCCATGAGGCCTTAG-3' and 5'-CTGTGAGGATCCATGCTGCTGAACAG-3'. The PCR product was restricted with SphI and BamHI and then ligated to the pET21d vector that had been restricted with SphI and BglII to produce pNK12. The mcmA gene was PCR-amplified from chromosomal M. extorquens AM1 DNA and cloned into pNK12 that had been restricted with NheI and NotI to produce pNK13 containing mcmA and mcmB, each transcribed by a separate T7 promoter.

All plasmids were transformed into the expression strain E. coli BL21 DE3. One isolate obtained from each transformation was used for expression. The expression strains were grown on Luria Bertani supplemented with appropriate antibiotic at 37 °C with shaking. Cells were grown to 0.4–0.7 A at 600 nm. Then expression of the target protein resulted in the formation of 1.5 mM (1M KH2PO4, pH 4.5, as a solvent) overnight under aerobic conditions. The inactivation resulted in the same activity as the full amount, suggesting that the MCM activity was a measure of relative AdoCbl levels in the cell extracts tested, able to assess decreased AdoCbl. ApoMCM (0.2 unit activity in this assay was a gift from M. Rasche, University of Florida (36). The purified proteins were concentrated, exchanged into 20 mM HEPES, pH 7, 50 mM NaCl, and 15% (v/v) glycerol, and stored at −70 °C.

Enzyme Assays—Enzyme activities were determined in M. extorquens AM1 and B. fungorum LB400 crude extracts obtained by passing cells through a French press or by centrifugation at 10,000 × g. Methylmalonyl-CoA mutase activity was determined by two methods: a radiolabel-based assay (35) was used to monitor the MCM activity in the mutants, and an HPLC method (36) was used to characterize the MCM activity of purified proteins. To test for the presence of AdoCbl in cell extracts of the mutants and the wild type, 0.1 μM apoMCM was added to the reaction mixture, and MCM activity was monitored by the radiolabeling assay. Addition of half this amount of apoMCM to the wild type extract resulted in the same activity as the full amount, suggesting that the apoMCM was saturating under these conditions. Therefore, the MCM activity in this assay was a measure of relative AdoCbl levels in the cell extracts tested, able to assess decreased AdoCbl. ApoMCM (0.2 unit specific activity) was a gift from M. Rasche, University of Florida (36).

Spectrophotometric methods (37) were used for protein determination. GTPassive Activity—The reaction was performed at 25 °C for the indicated time periods in a reaction mixture containing (in a final volume of 25 μl): MeaB, 40 μg; GTP, 5 mM; [32P]GTP, 1 μc; MgCl2, 5 mM; KCl, 39.5 mM; and potassium phosphate buffer (pH 8.0), 50 mM. The reaction was terminated by addition of ethanol (25 μl), and the precipitated protein was removed by centrifugation. The products formed via the hydrolysis of GTP by MeaB were analyzed by TLC on a polyethyleneimine/ cellulose plate (Merek) with 1 x K2HPO4, pH 4.5, as a solvent system. The plates were dried and exposed to x-ray film, and then radioactive spots were cut out. Radioactive counting in a liquid scintillation counter (model LS 8010, Beckman, CA). The chromatographic positions of GTP and GDP were verified with standards by localization of UV absorbing areas.

PAGE—To analyze complex formation between MeaB and MCM, the MCM-AdoCbl and the MCM-CNcbl complexes were prepared by incu-
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The ability to form succinyl-CoA from methylmalonyl-CoA in crude extracts of the wild type and the mcmC mutant was determined using a radiolabeling method. Cell extracts of the mcmC mutant failed to form succinate. Under the same conditions the MCM activity of the B. fungorum wild type was 10–12 milliunits, suggesting that McmC encodes active MCM in B. fungorum.

Genes that become fused into a single gene in an organism are likely to encode polypeptides that interact in other organisms (41). The finding that MeaB is fused with MCM into one polypeptide in several bacteria implies that MeaB may bind MCM in the organisms in which these proteins are separated.

MCM Activity in M. extorquens Mutants Defective in MeaB and Mea—In previous studies we demonstrated that M. extorquens AM1 mutants in meaB and meaD grew normally on succinate but lost the ability to grow on C1 and C2 compounds (18, 42). The maeB mutant failed to form succinyl-CoA from methylmalonyl-CoA, intermediates of the glyoxylate regeneration pathway of M. extorquens AM1 (18). A maeB mutant had a similar phenotype, in that it could only grow on C1 and C2 compounds in the presence of glyoxylate. Thus, the mutant showed a phenotype characteristic of mutants defective in glyoxylate regeneration, and the possibility was explored that it might function together with MeaB. However, MaeD was found to have homology (38% identity) to ATP:cohalamin adenosyltransferase from humans (20, 43), suggesting that this protein might be involved in AdoCbl synthesis. MCM activity was studied in M. extorquens AM1 wild type and maeB and meaD mutants with different assay conditions. No MCM activity was detected in extracts of meaD and meaB mutants when AdoCbl was omitted from the reaction mixture (Table I). Under the same conditions, the activity of MCM in the wild type was 1–2 milliunits. When 50 μM AdoCbl was added to the reaction mixture, wild type MCM activity increased almost an order of magnitude, indicating that most of the MCM in M. extorquens AM1 extract was present in the apoenzyme form. This result is consistent with an observation that only 5–13% of total MCM occurred in the holoenzyme form after MCM purification from M. extorquens NR-1 (44). Activity similar to the wild type was detected in the meaD mutant (7–12 milliunits) in the presence of AdoCbl, but activity was not detectable in the meaB mutant, suggesting it was below 0.1 milliunits, the level of detection (Table I). These results further supported the suggestion that MeaD is involved in cofactor biosynthesis for MCM in M. extorquens.

To test for the presence of AdoCbl in the meaB and meaD mutants, 0.1 μM apoMCM was added to the cell extracts of the mutants and the wild type. Under these conditions the meaB mutant and the wild type demonstrated the same level of MCM activities, whereas MCM activity in the meaD mutant was only 29–31% of the wild type activity (Table I). These results support the hypothesis that the meaD mutant contains decreased AdoCbl.

These data suggest that in the meaB mutant MCM is present in an inactive form, and this form is not due to a lack of AdoCbl. Therefore, MeaB must have a function that either protects
MCM from some type of inactivation or reactivates MCM after it has become inactivated. The inactivation might be a result of damaging products generated during catalysis, as reported for other B12-dependent enzymes (6), or might reflect inactivation due to proteolysis or poor protein folding. Therefore, it is possible that MeaB functions as a reactivase (6) for MCM, although MeaB does not show identity to known reactivase enzymes (data not shown). It does however, contain a GTPase/ATPase motif.

**Mutation in \( \Delta mcmA \Delta mcmB::kan \) and \( \Delta mcmD\Delta mcmB::kan \)—If MeaB functions to protect MCM from inactivation that occurs as part of the catalytic cycle, then meaB mutants should contain active MCM under conditions in which no in vivo activity occurs. Alternatively, if MeaB protects MCM from proteolysis or enhances protein folding as a chaperone, no active MCM should be present under those conditions. To distinguish between these alternatives, a M. extorquens AM1 double mutant was constructed defective in both MeaB and in methylmalonyl-CoA epimerase. This mutant is defective in the ability to synthesize R-methylmalonyl-CoA, the substrate of MCM. A second double mutant was also constructed, defective in both meaB and meaD. This mutant should have low in vivo activity due to low AdoCbl content.

Both double mutants grew on succinate but lost the ability to grow on methanol and ethylamine unless glyoxylate was supplied in the medium, the same phenotype as the meaB mutant. No MCM activity was detected in any of these mutant extracts to homogeneity by nickel affinity chromatography. The reaction was performed at 25°C, and MgCl\(_2\) was present. These data indicate that MeaB catalyzes the hydrolysis of GTP to GDP and that it binds GTP.

**Expression and Purification of MCM—**To investigate the possibility of an interaction between MCM and MeaB, MCM from M. extorquens AM1 was expressed in E. coli. Attempts to express the individual subunits and reconstitute activity in vitro were not successful. Therefore, the two genes were expressed from one vector, each under a separate T7 promoter, and the MCM complex was purified from cell-free extracts to homogeneity by nickel affinity chromatography. The results of the expression and purification were monitored by SDS-PAGE (Fig. 3). The purified MCM showed two bands (Fig. 3). The observed molecular masses are in excellent agreement with those derived from the sequences of mcmA and mcmB (78 and 64 kDa, respectively). Purified MCM had a specific activity of 1.4 ± 0.2 units/mg.

**Complex Formation between MCM and MeaB—**The gene fusion results from B. fungorum as well as the results in M. extorquens suggested the possibility that MCM and MeaB might form a complex. Complex formation between MCM and MeaB was analyzed by non-denaturing PAGE (Fig. 4). When apoenzyme was incubated with MeaB, a new band appeared (Fig. 4, E-MeaB). This band was developed in the second dimension by SDS-PAGE: McmA, McmB, and MeaB were detected (Fig. 5), indicating that this band is a complex between MCM and MeaB.

After incubating MCM with either AdoCbl or CNCbl to generate either MCM-AdoCbl or MCM-CNCbl, a new major band appeared (Fig. 4). To confirm that this band contained MCM bound to Cbl species, the band was developed in the second dimension by SDS-PAGE, and McmA and McmB were detected.

Formation of the MCM-MeaB complex was also observed when MeaB was incubated with MCM-AdoCbl or MCM-CNCbl

**GTP-hydrolyzing Activity of MeaB—**MeaB homologs have a consensus binding motif of GTPase/ATPase with a conserved aspartate residue for GTPases, suggesting that their actual substrate is GTP rather ATP (45). To test this hypothesis we assayed the GTPase activity of purified MeaB. When GTP was incubated with MeaB, a time-dependent and MgCl\(_2\)-dependent decrease of GTP and formation of GDP were observed by TLC (Fig. 2). Purified MeaB was incubated with [\( \alpha^{32}\)P]GTP and then resolved in a non-denaturing gel. A radioactive band was observed at the same place in the gel as MeaB (data not shown). No band was observed unless MgCl\(_2\) was present. These data indicate that MeaB catalyzes the hydrolysis of GTP to GDP and that it binds GTP.
**FIG. 3.** Expression of MCM in *E. coli* BL21 (DE3). Proteins were separated by SDS-PAGE. Lane 1, markers; lane 2, total proteins of *E. coli* carrying the plasmid with *mcmA* and *mcmB* before induction of IPTG; lane 3, total proteins of *E. coli* carrying the plasmid with *mcmA* and *mcmB* after 4 h of induction by IPTG; lane 4, soluble fraction; lane 5, insoluble fraction; lane 6, eluate containing purified His$_6$-tagged McmA:McmB complex. The arrows denote the McmA and McmB bands.

**FIG. 4.** Analyses of complex formation between MeaB and MCM by PAGE. The MCM-AdoCbl (holoE) and the MCM-CNCbl (E-CNCbl) complexes were prepared by incubation of 12 µg of apoenzyme (apoE) with 50 µM AdoCbl or CNCbl, respectively, at 4 °C for 30 min in 100 mM Tris-HCl buffer (pH 7.0), 5 mM MgCl$_2$, and 0.05 mM methylmalonyl-CoA in the dark. 12 µg of MeaB were added to each complex, and apoenzyme and the mixtures were incubated at 25 °C for 5 min. They were further incubated with either GTP or GDP (all at 5 mM) for 5 min. The mixtures were subjected to PAGE under non-denaturing condition in a 4–20% gradient gel (Bio-Rad) over 12 h. A, lane 1, markers; lane 2, MeaB; lane 3, MeaB+GDP; lane 4, MeaB+GTP; lane 5, apoMCM; lane 6, MCM-AdoCbl; lane 7, apoMCM+GTP; lane 8, apoMCM+MeaB; lane 9, apoMCM+MeaB+GDP; lane 10, apoMCM+MeaB+GTP; lane 11, MCM-AdoCbl+MeaB; lane 12, MCM-AdoCbl+MeaB+GDP; lane 13, MCM-AdoCbl+MeaB+GTP. B, lane 1, markers; lane 2, MeaB; lane 3, apoMCM; lane 4, MCM-CNCbl; lane 5, MCM-CNCbl+GTP; lane 6, apoMCM+MeaB; lanes 7 and 8, MCM-CNCbl+MeaB; lane 9, MCM-CNCbl+MeaB+GTP. Positions of MCM-AdoCbl (holoE), apoMCM (apoE), MCM-CNCbl complex (E-CNCbl), MeaB and their complexes (E-MeaB) are indicated with arrows.
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MCM plays an important role in the central metabolism of many living organisms (15). In humans, MCM deficiency results in an often fatal methylmalonic aciduria and causes neuropsychiatric symptoms (19, 23, 46, 47). Methylmalonic aciduria can result from mutations in the MCM structural genes or from defects in the acquisition of the required cofactor AdoCbl (23, 46, 47).

Recently, it was shown that mutations in the gene MMAA (a meaB homolog), whose function is unknown, are responsible for methylmalonic aciduria (21). A role of MMAA in the transport of vitamin B_{12} into the mitochondrion was suggested based on the annotation of the MMAA homologue (ArgK) in E. coli (21). ArgK was assigned as the ATPase participating in the regulation of lysine-arginine-ornithine transport in E. coli (48). This functional assignment was challenged later, because the orthologs of argK (meaB) are found clustered with the other genes involved in methylmalonyl-CoA rearrangement in the genomes of many organisms (9, 25). In addition, meaB orthologs are widespread in all genomes where genes encoding MCM are present, including archaea, bacteria, and eukaryotes. Because MeaB might play an important role in the MCM pathway, carrying out a function conserved in all life domains, the identification of the MeaB function constitutes an important challenge.

In previous work, we showed that the M. extorquens meaB mutant was not able to grow on C_{1} and C_{2} compounds because of the inability to convert methylmalonyl-CoA to succinyl-CoA, intermediates of the glyoxylate regeneration pathway (18). Here we demonstrated that this defect is due to the lack of an MCM active form, but not the lack of the AdoCbl cofactor. Moreover, the absence of the substrate (R-methylmalonyl-CoA) or the cofactor (AdoCbl) partially restores the MCM function in the meaB mutant. Based on these data we suggest that MeaB functions in the protection of MCM from suicide inactivation. We also demonstrated that MeaB binds to MCM in a complex with or without cofactor. The finding that a gene we showed was functional as an MCM contained a MeaB homologue fused with McmA domains in B. fungorum further supported the hypothesis that MeaB is a binding partner of MCM in the organisms in which these proteins are separated.

As noted previously, diol dehydratase undergoes suicide inactivation involving production of inactivated cofactor. This inactive enzyme requires reactivation by removal of the damaged cofactor by a reactivase (6, 26, 27). Although MeaB shows no sequence homology to this reactivase, it was possible that it might carry out an analogous function. However, the mechanism of the complex formation between MCM and MeaB is different from that of diol dehydratase-reativase. It has been reported that ADP is required for the complex formation between diol dehydratase and reactivase and that ATP stimulates the dissociation of the complex (6). Moreover, formation of the apoenzyme-reativase complex was much reduced in the absence of nucleotides, and the reactivase was not associated with the apoenzyme after release of the inactive AdoCbl (6). MeaB was found to bind MCM in the absence and the presence of nucleotides, though GTP/GDP slightly stimulated formation of the complex. We demonstrated that MeaB alone catalyzes the hydrolysis of GTP to GDP and is able to bind GTP. At this time, we do not know the role of GTP hydrolysis in MeaB function. However, it might induce conformational changes in MeaB to modulate its affinity for MCM. Moreover, MeaB was unable to catalyze detectable cofactor exchange in the absence and the presence of either GTP or GDP and unable to reactivate MCM in the meaB mutant. Hence, it appears that MeaB

![Fig. 5. SDS-PAGE electrophoresis of the MeaB-MCM complex.](image)
does not function as a reactivating factor of MCM under the conditions tested.

It has been shown that the MCM of P. shermanii dissociated progressively into its two subunits, leading to a decrease in enzymatic activity (7, 49). Native enzyme and the separated subunits establish a rapid monomer-dimer equilibrium (7). The dissociation of the enzyme into α-β-subunits probably exposes the bound cofactor to attack by O2/water/highly reactive radical intermediates and generates the inactive form of MCM (7). Therefore, a possible role for MeaB might be to stabilize the dimer form of the enzyme. Alternatively, MeaB might also play a role in the protection of the cofactor from attack by oxygen or adventitious nucleophiles during catalysis.

In summary, these data demonstrate a crucial role played by MeaB in the methylmalonyl-CoA rearrangement reaction. MeaB binds MCM, and its role may be to stabilize the dimer form of the enzyme. Alternatively, MeaB might also play a role in the protection of the cofactor from attack by oxygen or adventitious nucleophiles during catalysis.

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