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Sequence and Transcript Analysis of a Novel *Methanosarcina barkeri* Methyltransferase II Homolog and Its Associated Corrinoid Protein Homologous to Methionine Synthase

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The sequence and transcript of the genes encoding a recently discovered coenzyme M methylase in *Methanosarcina barkeri* were analyzed. This 480-kDa protein is composed of two subunits in equimolar concentrations which bind one corrinoid cofactor per αβ dimer. The gene for the α polypeptide, *mtsA*, is upstream of that encoding the β polypeptide, *mtsB*. The two genes are contiguous and overlap by several nucleotides. A 1.9-kb mRNA species which reacted with probes specific for either *mtsA* or *mtsB* was detected. Three possible methanogen consensus BoxA sequences as well as two sets of direct repeats were found upstream of *mtsA*. The 5′ end of the *mts* transcript was 19 nucleotides upstream of the translational start site of *mtsA* and was positioned 25 bp from the center of the proximal BoxA sequence. The transcript was most abundant in cells grown to the late log phase on acetate but barely detectable in cells grown on methanol or trimethylamine. The amino acid sequence of MtsB was homologous to the cobalamin-binding fragment of methionine synthase from *Escherichia coli* and possessed the signature residues involved in binding the corrinoid, including a histidyl residue which ligates cobalt. The sequence of MtsA is homologous to the "A" and "M" isozymes of methylcobamide:coenzyme M methyltransferases (methyltransferase II), indicating that the α polypeptide is a new member of the methyltransferase II family of coenzyme M methylases. All three methyltransferase II homolog sequences could be aligned with the sequences of uroporphyrinogen decarboxylase from various sources. The implications of these homologies for the mechanism of corrinoid binding by proteins involved in methylotrophic methanogenesis are discussed.

Methanosarcina species utilize a number of different methylated compounds for growth and methanogenesis. In this regard, they are unique among methanogens (6). No other genus in this branch of the *Archaea* has the capability to use such a broad range of methane precursors, including acetate, carbon dioxide, trimethylamine, dimethylamine, monomethylamine, methanol (14), dimethylsulfide, methanethiol (16), methylmercaptopropionate (21), tetramethylammonium ion (56), and pyruvate (5). To generate methane from these compounds, organisms such as *Methanosarcina barkeri* must possess the enzymatic assemblage to methylate coenzyme M (CoM) from the growth substrate, since the conversion of methyl-CoM to methane by methylreductase is a major site of energy conservation in methanogens (4, 10, 61).

Four different enzymes capable of methylating CoM have been identified (19, 29, 36, 55, 58, 60). All four CoM methylases either bind corrinoid or interact with proteins binding a corrinoid cofactor. Presumably, in *M. barkeri*, the protein-bound corrinoid is the 5-hydroxybenzimidazole-substituted cobamide which is most abundant in this methanogen (46). In addition, these proteins all carry out CoM methylation by using non-protein-bound methylated corrinoids such as methylcobalamin.

Of the CoM methylases, only the membrane-bound pterin:CoM methyltransferase is found among both the methylotrophic, aceticlastic, and autotrophic methanogens. In *Methanobacterium thermoautotrophicum*, it consists of eight subunits found in one transcriptional unit (23). The α subunit binds a corrinoid cofactor (18, 53). During methyl transfer from methyltetrahydromethanopterin, the corrinoid cofactor is transiently methylated and another subunit with methylcobamide:CoM methyltransferase activity methylates CoM (60). Interestingly, methyl transfer is coupled to the translocation of sodium ion across the membrane (3). The methyl-pterin:CoM methylase is involved in the conversion of acetate and CO_2 to methane, since methyltetrahydromethanopterin or its derivatives are direct intermediates in both of these pathways (10, 15, 20).

Two different CoM methylases are involved in the direct conversion of methylated compounds such as methanol or the methylamines to methane. These enzymes have been identified only in *Methanosarcina* species and are designated as isozymes of methyltransferase II (MT2) (19, 58, 62). The MT2 isozymes are isolated as soluble monomeric methylcobalamin:CoM methyltransferases of approximately 40 kDa without a prosthetic group detectable by UV-visible spectroscopy. The sequences of the two isozymes are 50% similar (22, 35). The "A" isozyme (MT2-A) was originally discovered in cells grown on acetate (19) but has no role in methane formation from acetate (13). MT2-A was hypothesized to be involved in trimethylamine utilization on the basis of its presence in cells grown on this substrate (62). It has since been demonstrated that MT2-A interacts with a 29-kDa corrinoid protein in the monomethylamine:CoM methyl transfer pathway (8, 13) and with a different corrinoid protein during methanogenesis from trimethylamine (12, 13). It also participates in methane formation from dimethylamine (13). The "M" isozyme of methyltransferase II (MT2-M) is most abundant in methanol-grown cells (19, 62). MT2-M interacts with methyltransferase I, a corrinoid-binding protein with subunits of 55 and 34 kDa, during methanogenesis from methanol (59). MT2-M can also interact with the corrinoid protein involved in methanogenesis from trimethylamine (12, 13).

Recently, a fourth enzyme with methylcobalamin:CoM

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methyltransferase activity was identified (55). This soluble 480-kDa protein was originally isolated as one of two corrinoid proteins methylated upon inhibition of methylreductase during methanogenesis in vitro (9). It is expressed at higher levels in cells grown on acetate relative to those grown on methanol or trimethylamine (32) and was recently found to methylate CoM with methylthiols (54). It is composed of equimolar amounts of 41- and 30-kDa subunits, designated as the α and β subunits or polypeptides, respectively. One corrin ring is found per αβ dimer in the isolated protein (33). The α subunit possesses methylcobalamin:CoM methyltransferase activity and is also able to effect methyl transfer from the endogenous methylcorrinoid to CoM (55). The N-terminal 20 amino acids of the α subunit are similar to those of MT2-A and MT2, indicating a possible relationship between these proteins (55).

In this article, we document the transcriptional start site and regulation of the genes encoding the 480-kDa CoM methylase. The sequence of the α subunit (MtsA) identifies this polypeptide as a new member of the MT2 family of CoM methylases with further homology to a heme biosynthetic enzyme. The sequence of the β subunit (MtsB) identifies for the first time a corrinoid binding motif in a methanogenic protein. These homologies provide a general model of how MT2 homologs and their associated corrinoid-binding proteins may interact with corrinoid during methanogenesis from methanol or the methylamines.

MATERIALS AND METHODS

Organisms and culture. *M. barkeri* MS was grown on various substrates in 15- or 40-liter carboys as described previously (8). The cells were stored frozen at −70°C. *Escherichia coli* DH5α was grown on Luria-Bertani broth (49) at 37°C. Competent cells of *E. coli* were prepared by the method described by Inoue et al. (28). Transformants containing plasmid pUC19 and its derivatives were grown in broth supplemented with 100 μg of ampicillin per ml.

Isolation of genomic DNA. DNA was prepared by a modification of two existing procedures (40, 48). One gram of frozen *M. barkeri* cell paste was resuspended in 10 ml of 1% sodium dodecyl sulfate (SDS)–45 mM EDTA–45 mM ammonium bicarbonate buffer (pH 8.0). The suspension was then passed through a French press at 500 lb/in^2 and then DNA was extracted by standard methods (49).

Cloning and sequencing techniques. Standard molecular technique protocols were employed as described by Sambrook et al. (49), with *E. coli* DH5α as the host strain and pUC19 as the vector. Degenerate oligonucleotide probes were constructed with the N-terminal sequences of both subunits of the 480-kDa CoM methylase (55). Probe 30A [GA(A/G)ATGAA(A/G)GA(A/G)AA(A/G)GA] and probe 30B [GA(C/T)GA(A/G)GG(A/G/C/T)AT(A/C/T)ATGAT] complemented DNA encoding the N terminus of the β subunit, and probe 40B [GA(C/T)TA(C/T)GT(A/G/C/T)CC(A/G/C/T)CC(A/G/C/T)GC] complemented DNA corresponding to the N terminus of the α subunit. Plasmid DNA for sequencing was prepared by the alkaline-lysis miniprep method described by Kovalenko et al. (31). Nested deletions were made with exonuclease III (25). Sequenase version 2.0 (United States Biochemicals Corp., Cleveland, Ohio) and [α-^{35}S]dATP (1,000 Ci/mmol; Amersham Life Science Inc., Arlington Heights, Ill.) were used for sequencing reactions. Both strands of DNA corresponding to the coding region of the 480-kDa CoM methylase were sequenced.

RNA isolation and Northern hybridizations. RNA was prepared and Northern blotting was performed by modifications of the techniques described by Hennigan and Reeve (27) and Pihl et al. (45). *M. barkeri* was grown to the mid-log phase and harvested by filtering through Whatman no. 1 filter paper. The collected cells were immediately resuspended in RNase-free distilled water and passed through a French press at 20,000 lb/in^2 directly into acid phenol-chloroform (Ambion Inc., Austin, Tex.). RNA preparations were stored as an ethanol precipitate at −70°C. The molecular mass of the transcript was estimated by using RNA size standards (Gibco-BRL, Grand Island, N.Y.).

Determination of transcript start site. The 5′ end of the *mts* transcript was determined with an S1 nuclease mapping procedure (49). An M13 ladder generated by dideoxy sequencing with −40 primer (United States Biochemicals) was used to determine the size of the protected fragment. In control experiments where *E. coli* tRNA was used instead of *M. barkeri* RNA, no protected fragment was detected.

Homology searches. BLAST searches (1) were run with the nonredundant database maintained at the NIH National Center for Biotechnology Information or the PRODOM database of protein families extracted from SWISSPROT (52). Searches of the SWISSPROT database were performed by use of the MPsrch program by S. Sturrock and J. Collins that employs the Smith-Waterman algorithm (51) on the BLITZ server maintained at EMBL. Multiple alignments of sequences were made with CLUSTALW (57) on the World Wide Web server maintained at the Baylor College of Medicine. All of these programs employed the BLOSUM60 or BLOSUM62 substitution matrix (26). Similarities of sequences in the alignments were calculated by summing the percentage of aligned residues with a positive score in the substitution matrix with the percentage of aligned identical residues.

Nucleotide sequence accession number. The sequence described in the present article is deposited in GenBank under accession number U36337.

RESULTS

Organization of the genes encoding the 480-kDa CoM methylase. Restriction digests of *M. barkeri* genomic DNA were probed with oligonucleotides designed to complement DNA sequence encoding the N terminus of either the α subunit or the β subunit of the 480-kDa CoM methylase. A 4.7-kb *Eco*RI-*Hin*dIII fragment (hybridizing with the β-specific probes) and a 3.7-kb *Pst*I fragment (hybridizing with the α-specific probes) were identified and cloned into pUC19. Subclones were then generated from nested deletions produced by exonuclease digestion of the original clones and sequenced. The sequences of the originally cloned fragments were found to overlap, and the merged sequence contained two open reading frames which began with the N-terminal sequences of the α or β subunit of the CoM methylase (Fig. 1).

The gene encoding the α subunit of the CoM methylase (*mtsA*) is 5′ to the gene encoding the β subunit (*mtsB*). The two genes overlap, since the TGA stop codon of *mtsA* extends 4 nucleotides into the reading frame of *mtsB*. A ribosome binding site complementary to the 3′ region of the 16S rRNA of *M. barkeri* (GenBank accession number M59144) is found 9 nucleotides upstream of the start codon of *mtsA*. A similar sequence is found 10 nucleotides upstream of the initiation codon of *mtsB*. Following *mtsB* are three polypyrimidine sequences (designated T1 through T3) which could be involved in termination of transcription (47, 63).

Two separate groups of direct repeats were noted upstream of the initiation codon of *mtsA* (Fig. 1). The first group extends from −159 to −200 and consists of three repeats of a 14-nucleotide sequence (ACTCTCTGGTGTGA) without intervening sequence. The second group extends from −407 to −464 and consists of two directly repeating 25-nucleotide sequences (ATAATAATTAGAAAACAAGAAAAAA) separated by 6 nucleotides.

Regulated cotranscription of *mtsA* and *mtsB*. Total RNA isolated from the organism grown to the late log phase on either acetate, methanol, or trimethylamine was probed with DNA complementary to the mRNA encoding MtsA (a synthetic oligonucleotide complementary to nucleotides extending from positions +42 to +65 and a *Hin*dIII-*Pst*I restriction fragment extending from positions +280 to +926) or MtsB (a *Pst*I-*Sal*I restriction fragment extending from positions +926 to +1269). All three probes identified a fragment of approximately 1.9 kb in the total RNA isolated from cells grown on acetate (Fig. 2). The minimal size of a transcript extending from the start codon of *mtsA* and ending at the stop codon of *mtsB* would be 1,926 nucleotides. This suggests the two genes are transcribed as a single unit.

Equivalent amounts of RNA isolated from cells grown on trimethylamine or methanol showed little or no signal when hybridized with the same probes which hybridized with the 1.9-kb transcript present in cells grown on acetate. Transcription of the 480-kDa CoM methylase is therefore a regulated event.

Potential promoter sites of the *mts* transcript. The methanogen consensus BoxA sequence, TTTA(A/T)ATA (47), has several close matches upstream of *mtsA*, which are designated

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-517 cttttgaagatgataataaataaaaatataaaattaaacacataaaaaacaaaacataataattag

-451 aaaacaagaaaaaacaaaatataataattagaaaacaagaaaaaatgtataaagagtagaaaaata

-385 tgaggattcgtttttttcttaacggaaaaatctgggaattacctgtgtatgttcacaatacagtttt

-319 gatgtgaatttttccatatacctgtaatatagacttttgatattattttaaacttaacttttttga

-253 atgcgcgcttataggatcgttttatactctgtttaagcagaagcaagccttttactctctggtgtg

-187 aactctctggtgtgaactctctggtgtgaaatttgagggtcttgggaaaaataaagtggttgttga
                    A3                                          A2
-121 acacattcgtgcaatgaaattttaaaacatatataaatttttccgtatttttagagatatatatatt
                          A1                                +1    S/D
-55  tttccttattatgctaactgcatttagttaaatatccaaatattcatattaataaAtaaaaggaga

              M  V  S  E  M  T  P  T  R  R  V  M  A  A  V  L  G  G  R
+12  catatatcatggtaagtgaaatgactccaacccgaagagttatggcagccgtgcttggaggcaggg
      V  D  Y  V  P  P  A  N  P  L  A  Q  T  T  T  E  L  M  Q  I  C  N
+78  ttgattacgtacccccccgctaatcccctcgctcagactacaacagaactgatgcagatatgcaatg
      A  S  W  P  K  A  H  F  D  S  K  M  M  A  D  L  A  A  A  P  Y  E
+144 cttcctggccaaaagcccactttgacagcaagatgatggcagaccttgcagccgctccctatgaaa
      I  C  G  I  E  A  A  R  P  Q  F  D  I  S  L  E  A  E  V  L  G  C
+210 tttgcggcatagaggctgcacgtccccagtttgatatttctcttgaagcggaggttttgggatgca
      K  L  D  W  N  K  P  D  R  P  P  V  T  G  P  A  Y  T  D  P  A  D
+276 agcttgattggaacaaaccggaccgacctcccgtaacaggtcctgcttataccgatcccgccgaca
      I  T  W  P  D  N  L  E  E  A  G  R  I  P  V  V  L  G  A  I  E  E
+342 taacctggcctgacaatctggaagaagccggaagaatcccggtagtgctcggagcaatcgaggaac
      L  R  K  R  Y  D  G  M  L  P  V  I  P  V  L  T  S  P  F  T  V  A
+408 tgaggaagcggtacgatggtatgcttcctgtcataccagtcctgacatctccttttacagtagctg
      G  H  I  A  G  V  E  N  L  V  R  W  T  K  T  D  P  E  K  A  H  A
+474 gccacatagcaggggttgaaaacctggtaagatggacaaagaccgatcccgaaaaagcccatgctt
      F  I  E  A  A  T  D  F  V  I  A  Y  G  K  L  Q  T  A  Y  G  A  H
+540 ttattgaggcagcaactgatttcgtgatagcttacggaaagttacagaccgcttacggggcacata
      I  L  F  P  A  D  P  S  A  S  G  D  L  I  S  G  E  T  Y  K  E  F
+606 tacttttccggcagatccatcagcctcaggagacctgatttctgggaaacttacaaagaattcg
      V  L  P  A  H  K  R  M  A  K  E  I  S  C  P  L  I  L  H  I  C  G
+672 tacttcctgcccacaaaaggatggcaaaggaaatcagctgccctttgatcctgcacatctgcggag
      D  T  S  K  L  L  P  Y  I  K  Q  S  G  I  D  C  F  S  F  D  A  V
+738 ataccagcaaacttctgccttacataaagcagagtggtatcgattgtttctccttgatgccgtgc
      P  V  W  Y  C  R  Q  V  M  G  N  E  M  S  I  L  G  S  L  D  V  I
+804 cagtctggtactgccgtcaggtgatgggcaacgagatgtccattcttggaagcctggacgttatag
      D  L  M  P  N  G  T  P  E  Q  V  Y  N  R  T  R  E  C  I  L  Q  G
+870 accttatgccaaacggcacccctgaacaggtatataatagaactcgagaatgcatcctgcagggag
      A  D  I  V  G  T  A  C  D  V  S  F  G  T  S  L  E  N  L  R  A  Y
+936 ctgatattgttgggactgcatgcgacgtttcattcggtacttcccttgaaaacctcagggcctatg
      V  R  A  C  K  E  T  P  I  P  K  Y  D  D  V  E  D  I  I  R  Q  I
+1002 tcagggcctgcaaggagactccgattccgaaatatgatgatgtggaagacatcatcaggcagatcg
      G  V  G  I  G  R  N  M  K  E  N  V  L  G  G  M  Q  K  *
                                                S/D
+1068 gagtcggtattggaagaaacatgaaggaaaatgtcctgggaggatgcagaaatgatcaggcacat
        D  L  A  V  Q  N  I  L  E  M  K  E  K  E  P  A  K  F  K  R  L  I
+1134 tgaccttgcagttaagaatattttagagatgaaagaaaaagagcctgcaaaatttaaaagactcat
        D  E  G  I  M  I  G  L  G  V  D  L  E  D  G  N  K  E  V  T  T  V
+1200 tgacgaagggatcatgatcggccttgggtagatcttgaagacggaaataaagaagtaactactgt
        D  Q  I  K  N  Q  N  R  P  K  D  P  E  Y  A  S  V  A  E  A  V  I
+1266 cgaccagataaagaatcagaacagaccgaaagatcccgaatacgcatccgtagcagaagctgtgat
        E  G  N  N  A  E  T  V  K  L  I  S  A  L  L  E  K  G  K  D  P  T
+1332 agaaggaaataatgcagaaacagtaaaactcatatctgccttacttgaaaaaggaaaagatcctac
        D  L  V  L  N  A  L  M  P  G  I  Q  T  V  C  E  L  Y  D  I  G  E
+1398 agatttggttttgaacgcccttatgcccggaatccagaccgtgtgtgagctttacgatattgggga
        S  Y  V  P  E  I  L  L  A  N  E  A  L  M  K  G  V  E  L  C  Q  K
+1464 gagctatgtgccggaaatcttgctggcaaacgaagctcttatgaaaggagttgaactctgccagaa
        K  K  G  E  V  P  S  Q  G  K  V  V  S  L  V  I  V  G  D  L  H  D
+1530 aaagaaaggagaagtcccatctcagggaaaagtggtatcccttgttattgtaggagacctgcatga
        I  G  K  N  I  V  A  A  I  L  R  A  N  G  F  E  V  I  D  L  G  R
+1596 tataggaaagaatattgtagccgctattctcagagcaaatggcttcgaagtaatcgaccttggaag
        D  V  T  V  E  A  A  V  E  A  V  K  S  T  K  A  N  L  V  T  G  T
+1662 agatgtaactgtggaagcagctgtcgaagccgtaaagtcaacaaaagctaatcttgtcaccggaac
        T  L  M  S  T  T  K  G  G  L  K  A  L  A  N  A  L  E  P  E  G  V
+1728 aacccttatgagcacaaccaaaggaggcctcaaagccctcgcaaatgctctggaacccgaggggt
        P  L  A  C  G  G  A  A  V  D  R  R  F  V  D  T  F  G  N  S  V  Y
+1794 ccctctggcctgcggaggggctgctgtggacaggcgtttcgttgataccttggggaactcggtata
        G  R  T  P  L  D  A  V  K  I  A  K  E  I  C  E  G  K  S  W  E  E
+1860 tggaagaacaccacttgatgcggtaaaaatcgcaaaggagatatgtgaaggaaagagctgggaaga
        A  R  N  E  L  Y  *                T1          T2          T3
+1926 agcccgcaacgaacttttattgattctactttttcttcctgttttttttattttttgtgttcgagattt

+1992 atctctctttcatttagtgaattttaaaatatggaaaacaatttctgtaaaatgaataaagatcag

+2058 acagagacaataaagatcagaataagataataaagattagaaatagacaataaaaaccagaa
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FIG. 1. Nucleic acid sequence of *mtsA* and *mtsB* and the predicted amino acid sequence of the α and β subunits of the 480-kDa CoM methylase. Nucleotides in the sequence are numbered relative to the 5′ end of the transcript determined by S1 nuclease mapping. Arrows are drawn below two separate sets of direct repeats. A1, A2, and A3 indicate putative BoxA sequences. Stop codons are indicated by an asterisk. T1, T2, and T3 indicate sequences which may signal transcriptional termination. S/D over a boldface sequence indicates a ribosome binding site.

A1 to A3 in Fig. 1. To determine which of these might direct transcription of *mts*, we identified the 5′ end of the *mts* transcript in cells grown on acetate. Attempts to use primer extension were unsuccessful, possibly because the 5′ region of the *mts* transcript possesses a high potential for secondary structure. Therefore, S1 nuclease analysis was employed to map the start site of the transcript. Total RNA isolated from cells grown on acetate was denatured in the presence of a radiolabelled *Bss*HII-*Hin*dIII fragment (nt −246 to +279) and allowed to anneal overnight. The major protected DNA fragment identified following S1 nuclease digestion was 279 bases

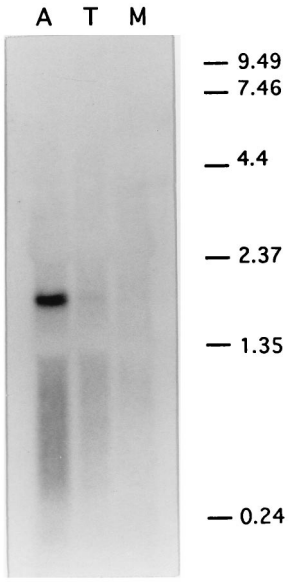

FIG. 2. Detection of *mts* transcript in RNA extracted from cells grown on acetate (A), trimethylamine (T), or methanol (M). Numbers on the right indicate positions and kilobase sizes of RNA standards in the gel following electrophoresis. The Northern blot shown was probed with an oligonucleotide complementary to the 5′ region of the transcript (+42 to +65). Similar results were obtained with the other probes mentioned in the text.

in length (Fig. 3). A few faint bands were also detected above and below the major protected fragment. This indicated that the stable form of the transcript begins 6 nucleotides before the ribosome binding site indicated in Fig. 1. This is 25 nucleotides from the center of the putative BoxA sequence A1, which is closest to the translational start site.

Homology of MtsB to the cobalamin binding sequence exemplified by methionine synthase. A BLAST search of the nonredundant database maintained at the National Center for

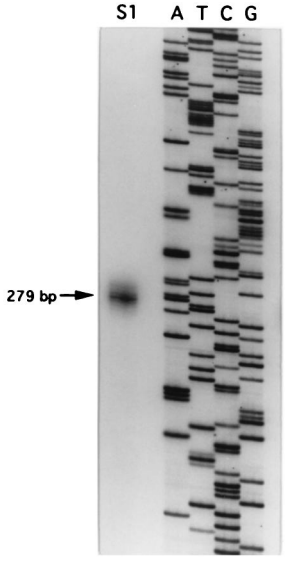

FIG. 3. Determination of the 5′ end of the *mts* transcript. A 525-bp fragment was denatured and hybridized to total cellular RNA, and then single-stranded nucleic acid was digested with S1 nuclease (S1). An M13 DNA sequencing ladder was used to estimate the size of the protected fragment (ATCG).

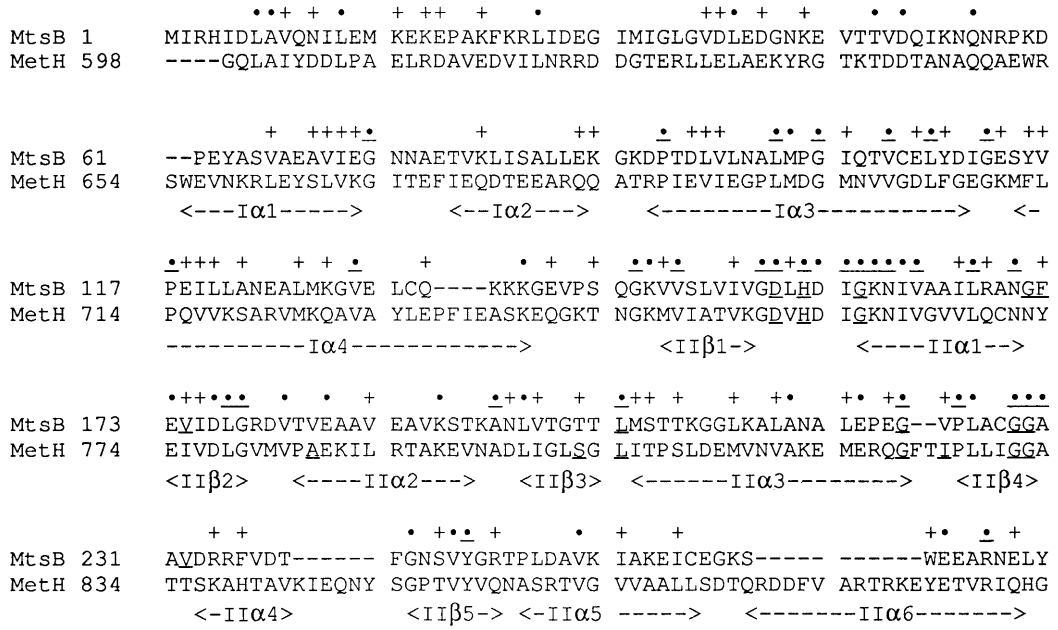

FIG. 4. Sequence homology of MtsB (β subunit of the 480-kDa CoM methylase) to *E. coli* methionine synthase. Symbols: •, identity between MetH and MtsB; +, alignment of MetH and MtsB residues with positive scores from the BLOSUM62 matrix used to produce the alignment; •, alignment of residues that are also identical in the methionine synthases from *C. elegans* and *M. leprae*. Underlined residues (e.g., X) indicate positions that are also identical in alignments of methionine synthases, MtsB, clostridial glutamate mutase, and human methylmalonyl-CoA mutase. The spans of residues designated Iα1 and IIβ4, etc., indicate α helices or β sheets in domain I or II of the cobalamin binding fragment of MetH (11).

Biotechnology Information indicated homology of the β subunit of the 480-kDa CoM methylase to cobalamin-dependent methionine synthase from *E. coli* (MetH) (2), *Caenorhabditis elegans* (GenBank accession number Z46828), and *Mycobacterium leprae* (GenBank accession number U00017). Homology was also indicated by a search of the SWISSPROT database using the EMBL BLITZ server. This search produced an alignment of MetH (positions 688 to 838) with MtsB (positions 92 to 236), with a score of 185. The number of sequences in the database which would randomly give this score when paired with MtsB over this length was predicted to be 6×10^{-23}.

The region of homology is within a tryptic fragment of MetH which binds cobalamin and whose three-dimensional structure was recently determined (11). A CLUSTALW alignment of this region is presented in Fig. 4. The alignment was generated by use of the sequences of MtsB and all the methionine synthases mentioned above, but only the MetH and MtsB sequences are shown. The region of homology extends over both domains of the MetH cobalamin binding fragment, beginning with the α helix in MetH, designated Iα3, and continuing through to β sheet IIβ4 (16). Several residues have been identified as invariant in the binding of cobalamin by MetH (11) as well as glutamate mutase component S (37) and methylmalonyl-CoA mutase (11). These amino acids are conserved in the alignment of MetH with MtsB. The only exception was the substitution of serine at positions 804 and 810 in MetH with threonine in MtsB.

The sequence GD(L/V)HDIGKNIV is found in both MetH and MtsB. In MetH, this sequence is found at the junction of β sheet IIβ1 and α helix IIα1 of the cobalamin binding fragment, the histidyl residue serving as the lower axial ligand of the central cobalt ion of cobalamin.

Homology of MtsA to MT2-A and -M. The sequences of the MT2-A and MT2-M isozymes have been determined from *M. barkeri* NIH (35) and *M. barkeri* Fusaro (22). BLAST homology searches against the nonredundant composite database maintained at the National Center for Biotechnology Information indicated very strong similarity of the α subunit of the 480-kDa CoM methylase with nearly the entire length of both MT2 isozymes. The MT2-A (from strain NIH) and α subunit alignment had a score of 168 with a $P(4)$ value of 3.6×10^{-50}, while that of MT2-M (from strain NIH) with the α subunit had a score of 191 with a $P(3)$ value of 1.7×10^{-44}.

An alignment of all three sequences by the CLUSTALW program is presented in Fig. 5. The α subunit shows an overall similarity of 49.7% with MT2-A, with 31.2% of aligned residues being identical. The alignment of the α subunit with MT2-M showed an overall similarity of 50.6%, with 26.5% identity. In comparison, the MT2-A and MT2-M isozymes were 48.8% similar to each other, with 36.4% identical residues at aligned positions. The high similarity of the α subunit to both MT2-A and MT2-M indicates that this protein is a new member of the MT2 family of methylcobamide:CoM methyltransferases.

One notable area conserved among all three proteins was the sequence (V/I)LHICG, which has been suggested to be involved in zinc binding by MT2-A and MT2-M (35).

Similarity of MtsA to UROD. It has been noted that the sequences of MT2-A and MT2-M could be aligned with that of uroporphyrinogen decarboxylase (UROD) from *E. coli* (22). Searches using the BLITZ server of the SWISSPROT database with the MtsA sequence as the query sequence demonstrated that this is a general property of members of the MT2 family. BLITZ searches produced statistically significant alignments of all three MT2 homologs with UROD from *Synechococcus* sp. (30), *E. coli* (42), *Bacillus subtilis* (21), *Saccharomyces cerevisiae* (17), and *Rhodococcus* sp. (SWISSPROT P42503). For example, the BLITZ alignment of positions 9 to 328 of the MT2-M sequence with the *Synechococcus* UROD sequence indicated 60% similarity and 35% identity in this region. The *Synecho-*

FIG. 5. Sequence homology of MtsA (α subunit of the 480-kDa CoM methylase) with MT2-A and MT2-M. Identities (•) and positive residue pairings (+) in the BLOSUM62 matrix are indicated. The symbols refer only to the alignments between the MT2 sequence above or below the sequence of MtsA. The MT2 isozyme sequences are from *M. barkeri* NIH (GenBank accession numbers U38918 and U38919) (35).

coccus UROD sequence was aligned with positions 85 to 336 of the MT2-A sequence, with 58% similarity and 39% identity. The program aligned positions 52 to 292 of the MtsA sequence with the *E. coli* UROD sequence and found 63% similarity over this region, with 29% identical residues.

BLAST searches of the PRODOM database of consensus sequences derived from the aligned protein families further confirmed the relationship between the MT2 family of polypeptides and the UROD proteins. The UROD consensus sequence was aligned with four segments of the MtsA sequence, extending from positions 67 to 281 with a score of 82 and a $P(4)$ value of 3.8×10^{-10}. The consensus sequence had 25% identity and an overall similarity of 46% when aligned by CLUSTALW with this same region of the MtsA sequence. The overall similarity of the UROD enzymes to the MT2 family of proteins allows an alignment of the sequences of these two sets of proteins (Fig. 6). Notable are tyrosine, phenylalanine, glycine, and proline residues conserved among all UROD and MT2 polypeptide sequences.

DISCUSSION

The 480-kDa methylcobalamin:CoM methyltransferase is a regulated protein which can constitute more than 1% of the soluble protein in cells grown on acetate (33). This is not surprising for a protein involved in a major catabolic reaction such as the methylation of CoM (55). The region 5′ of the *mts* operon has features which may reflect the strong and regulated expression of the protein. We could identify three putative methanogen BoxA sequences preceding *mtsA*. While multiple promoters are often observed preceding highly expressed genes, only one of these appears functional during transcription of *mts* in acetate-grown cells. The center of the proximal BoxA sequence A1 is within the expected distance of 27 ± 4 nucleotides from the transcriptional start (47, 63). A1 would not be predicted to be a strong promoter, since the placement of a purine in the first position would decrease transcription initiation (24, 44). However, the 480-kDa protein is transcriptionally regulated, and a weak promoter such as this might

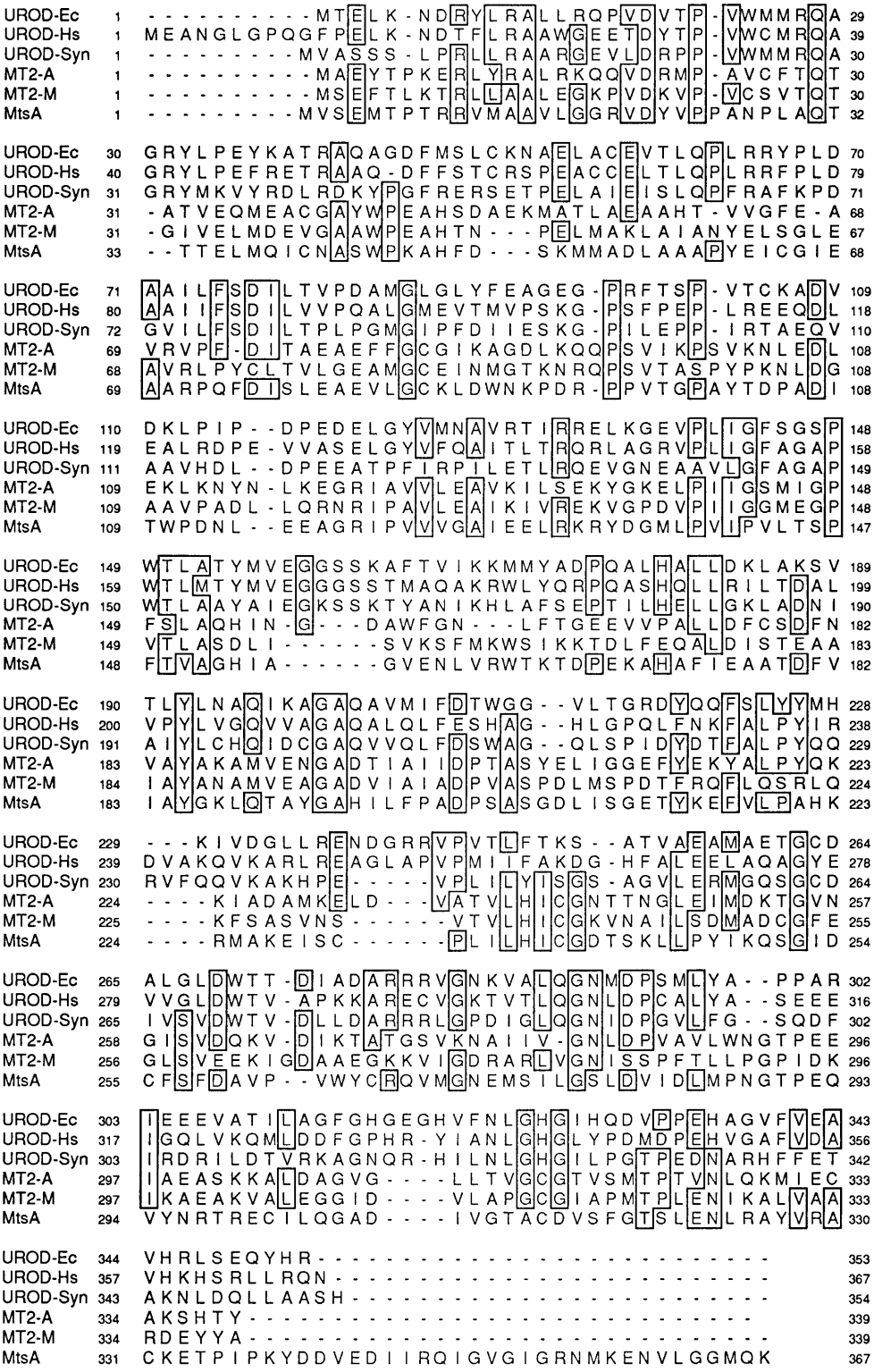

FIG. 6. Sequence alignment of the α subunit of the 480-kDa corrinoid CoM methylase (MtsA), MT2-A, and MT2-M isozymes with URODs from *E. coli* (Ec), human (Hs), and *Synechococcus* species (Syn).

require an activation factor produced in response to a physiological signal to initiate strong transcription of the gene. It is also possible that the other putative promoter sequences direct transcription but that the transcript is processed and we are simply observing the most stable form of the *mts* transcript.

Another interesting feature of the region 5′ of the promoter region is the presence of two separate groups of directly repeating sequences. Direct repeats have been implicated in regulation of transcription both in *Eucarya* and *Bacteria* (34, 38). Direct repeats have been noted previously in *Methanosarcina*

species (41, 50). It is possible that the repeating sequences might be involved in the regulated transcription of the *mts* operon. Only one function for direct repeats has been demonstrated in *Methanosarcina* species. The 3′ ends of four different transcripts of the *pta* operon were mapped to the beginnings of four consecutive direct repeats (50). The function of the repeating sequences upstream of the *mts* operon is not as clear, since no open reading frame terminates within or near either set of repeating sequences.

The 480-kDa protein carries out CoM methylation with either free methylcobalamin or the endogenously bound corrinoid cofactor (55). The role of the β subunit in the latter reaction appears to be tight binding of the corrinoid cofactor, since this subunit possesses the corrinoid binding motif identified in methionine synthase, glutamate mutase, and methylmalonyl-CoA mutase (11, 37). This is the first identification of a known corrin binding site in a methanogenic protein. The homology of the corrinoid binding site is much higher between methionine synthase and MtsB than between either methyltransferase and the mutases. This is not due to the interaction of the 480-kDa protein with MetH substrates. Methionine synthase catalyzes the methylation of homocysteine with methyltetrahydrofolate, but the 480-kDa protein is not methylated by methyltetrahydrosarcinapterin or demethylated by homocysteine. The α subunit, not the β subunit, methylates the thiol of CoM (55). Rather, the homology between MtsB and MetH seems to reflect the binding of methylated cobamides. For example, the two longest helices of domain I in the MetH cobalamin binding fragment (designated Iα3 and Iα4) are conserved between MetH and the 480-kDa CoM methylase but are lacking in the adenosylcobalamin-binding mutases (11).

The sequence corresponding to the loop between the first β sheet (IIβ1) and α helix (IIα1) of MetH is almost identically conserved in MtsB. Crystallography and electron paramagnetic resonance spectroscopy have shown that in MetH, the histidyl residue of this loop serves as the α ligand of the central cobalt ion of the bound cobalamin (11). The base of the corrinoid cofactor is no longer ligated to cobalt and is sequestered in a hydrophobic pocket. The strong sequence homology between MtsB and the MetH IIβ1-IIα1 loop, as well as the extension of sequence homology to the residues which line the benzimidazole binding pocket, suggests that binding of corrinoid occurs by a similar mechanism in MtsB. We could not detect homology between other sequenced methanogen corrinoid proteins (18, 39, 53) and MetH.

The isolated α subunit of the 480-kDa protein carries out the methylation of CoM with free methylcobalamin as well as with the corrinoid ligated to the β subunit (55). The primary sequence of the α subunit revealed that this methylcobalamin: CoM methyltransferase is homologous to MT2-A and MT2-M. The α subunit of the 480-kDa protein, MT2-A, and MT2-M can therefore now be classified as members of a single family of homologous methyl-cobamide:CoM methyltransferases.

The tight association of this novel MT2 homolog, the α subunit, with the corrinoid-ligating β subunit is unlike either MT2-A or MT2-M, which are both isolated as monomeric polypeptides without a bound corrinoid polypeptide (19). MT2-A is encoded on a monocistronic transcript (22), while the α and β subunits of the 480-kDa protein are cotranscribed. An explanation for these differences may lie in the need for MT2-M and MT2-A to bind with several different corrinoid proteins during methanogenesis from different substrates. MT2-M interacts with a 122-kDa corrinoid protein (MT1) during methanogenesis from methanol (59). MT2-A associates with a 29-kDa corrinoid protein during methanogenesis from monomethylamine (8). Both MT2-A and MT2-M can interact with a 26-kDa corrinoid protein during methanogenesis from trimethylamine (12). MT2-A or MT2-M must have higher dissociation constants with these different corrinoid proteins than that of the α subunit with the β subunit of the 480-kDa corrinoid protein. Apparently, dissociation of this novel MT2 homolog, MtsA, from the interactive corrinoid protein, MtsB, is neither thermodynamically nor physiologically favored.

MT2 homologs bind corrinoid since they mediate CoM methylation with methylcobamides. The similarity of all three members of the MT2 family to UROD may have revealed a second corrinoid binding motif in methanogens. The substrate of UROD, uroporphyrinogen III, is a tetrapyrrole ring, although it lacks a nucleoside such as that found in corrinoid cofactors. Long segments of each of the MT2 homolog sequences can be aligned with their most-similar UROD sequence with approximately 30% identity. Protein domains with this level of identity often adopt similar folding structures (43). The two protein families may have maintained similar structures to bind their respective tetrapyrrole substrates.

The 480-kDa corrinoid protein thus appears to be composed of two proteins, both of which bind the corrinoid cofactor by two different mechanisms. The possession of the signature residues of the MetH corrinoid binding motif (11) by MtsB suggests that the β subunit binds the lower face of the cofactor, with a histidyl residue serving as α ligand to the cobalt, and the benzimidazole bound in a hydrophobic pocket. The α subunit may therefore interact primarily with the upper face or edges of the corrin ring, possibly in a manner similar to tetrapyrrole binding by URODs.

As an MT2 homolog and its associated corrinoid protein, MtsA and MtsB provide a model for the interaction of corrinoid in other MT2 homologs and their associated corrinoid proteins. Since MT2-A, MT2-M, and MtsA are homologous, it seems likely that the corrinoid proteins which associate with them (8, 12, 59) will also be homologous and have the MetH corrinoid binding motif. Recently, it was found that the corrinoid protein involved in monomethylamine metabolism does possess this same motif (7).

Since the 480-kDa CoM methylase consists of a polypeptide with a strong corrinoid binding motif tightly bound to a novel member of the MT2 family, one would predict its participation in the transfer of methyl groups from a methylotrophic methanogenic substrate to CoM, by analogy to the metabolic roles of MT2-A and MT2-M. Recent results from our laboratory have demonstrated that the purified 480-kDa protein is responsible for the methylthiol:CoM methylase activity which is induced when *M. barkeri* is cultured on acetate (54).

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