JOURNAL OF BACTERIOLOGY, Apr. 1990, p. 1828–1833
0021-9193/90/041828-06$02.00/0

Vol. 172, No. 4

Identification of the *mcrD* Gene Product and Its Association with Component C of Methyl Coenzyme M Reductase in *Methanococcus vannielii*

BRUCE A. SHERF† AND JOHN N. REEVE*

Department of Microbiology, The Ohio State University, Columbus, Ohio 43210

Received 3 November 1989/Accepted 12 January 1990

A *mcrD-lacZ* gene fusion has been constructed and expressed under *lacP* control in *Escherichia coli*. Antibodies raised against the product of this gene fusion have been used in Western blotting (immunoblotting) to demonstrate the gene product of *mcrD* (gp*mcrD*) in *Methanococcus vannielii*. The α, β, and γ subunit polypeptides of component C of methyl coenzyme M reductase (MR) were coprecipitated with gp*mcrD* when bound by antibodies raised either against MR or against gp*mcrD-lacZ*. This association of MR and gp*mcrD* did not withstand polyacrylamide gel electrophoresis under nondenaturing conditions.

Component C of methyl coenzyme M reductase (MR) catalyzes the terminal reaction in methanogenesis, releasing methane (2, 8, 9, 11, 19). MR, which constitutes approximately 10% of the cellular protein, has been purified from several methanogens and has been shown in every case to contain three different polypeptide subunits which form a holoenzyme with the organization $\alpha_2\beta_2\gamma_2$ (2, 8, 9). The genes encoding the α, β, and γ subunits, *mcrA*, *mcrB*, and *mcrG*, respectively, have been cloned and sequenced from five different methanogens (3, 4, 7, 12, 22) and have been found in every case to be part of a cluster of tightly linked, cotranscribed genes, arranged *mcrBDCGA* and designated the *mcr* operon. All extant *mcr* operons appear to have evolved from a common ancestor (12, 22, 23). The extent of divergence of the *mcr* sequences correlates well with current methanogen phylogenetics as determined by differences in ribosomal RNA sequences (11). The *mcrD* and *mcrC* genes are conserved and found at the same location in all five sequenced *mcr* operons (12, 22), arguing for important and conserved functions for the gene products of *mcrD* (gp*mcrD*) and *mcrC* (gp*mcrC*), respectively. These polypeptides do not, however, copurify with the MR holoenzyme, so their functions, indeed their existence, in methanogens has remained a matter of speculation. The *mcrD* gene of *Methanococcus vannielii* was expressed when cloned in *Escherichia coli* (7), and by using antibodies raised against the product of a *mcrD-lacZ* fusion (gp*mcrD-lacZ*), synthesized in *E. coli*, we have now demonstrated gp*mcrD* in *M. vannielii* and have investigated its association with MR holoenzyme.

MATERIALS AND METHODS

Bacterial strains, plasmids, and phage. Subcloning and expression of *mcrD* and *mcrD-lacZ* gene fusions used *E. coli* JM101 Δ(*lac-pro*) *supE thi*(F′ *traD36 proAB lacI*^{q} *lacZ*) (16) and *E. coli* DS410 *minA minB rpsL* (18). Bacteriophage M13mp19 and derivatives of this phage were grown on JM101. Plasmids pET1300 (7) and pSKS106 (5) were the sources of the *mcrD* and *lacZ* genes, respectively. Plasmid pSKS106ΔR is identical to pSKS106 except that an *Eco*RI site at position 9.9 kilobases on the parental plasmid (5) was deleted by *Eco*RI digestion, S1 nuclease digestion, and religation. Construction of plasmids pMRD107 and pD′Lac, by subcloning and site-specific mutagenesis (24), is described in Results.

Growth conditions and media. *E. coli* strains were grown aerobically at 37°C by using Luria-Bertani medium (10 g of tryptone [Difco Laboratories, Detroit, Mich.] per liter, 5 g of yeast extract [Difco] per liter, 10 g of NaCl per liter), solidified when necessary by addition of 15 g of Bacto-Agar per liter. Ampicillin (100 μg/ml) was added as required. Lactose induction medium (LI) was 0.5× Luria-Bertani medium containing 0.8% (vol/vol) glycerol and 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). Lactose repression medium was 0.5× Luria-Bertani medium containing 0.4% (wt/vol) glucose.

M. vannielii was grown at 37°C with a pressurized atmosphere of 20% CO_2–80% H_2 (40 lb/in^2) in ER medium (10) lacking vitamins and cleared before inoculation by passage through Whatman 934-AH glass microfiber filters. Cysteine was omitted from the reducing agent to obtain the ER-cys medium used in the [^{3}S]Trans in vivo labeling protocol (see below).

Labeling of *M. vannielii* proteins in vivo. Cells from 8 ml of an exponentially growing culture (A_{600}, ~0.5) were pelleted anaerobically, washed once with ER-cys, and resuspended in 300 μl of ER-cys containing 360 μCi of [^{35}S]Trans (a mixture of [^{35}S]methionine and [^{35}S]cysteine; ICN Biochemicals Inc., Costa Mesa, Calif.) in a 25-ml glass tube (Bellco Glass, Inc., Vineland, N.J.). The tube was capped, pressurized to 15 lb/in^2 by using 20% CO_2–80% H_2, and incubated at 37°C for 4 h. The labeled cells so obtained were pelleted by using an Eppendorf Microfuge; they were washed with ER-cys, repelleted, frozen and thawed twice, suspended in 300 μl of sonication buffer (50 mM potassium phosphate, pH 6.8, containing 8 μg of DNase I per ml and 8 μg of RNase A per ml), removed from the anaerobic chamber, and sonicated twice for 1 s (Sonifier cell disrupter; Branson Sonic Power Co., Danbury, Conn.). The resulting lysate was incubated at 0°C for 10 min and then frozen and stored at −70°C.

Labeling of proteins in *E. coli* minicells. Plasmids were introduced into *E. coli* DS410, a minicell-producing strain (18), by transformation and selection for the vector-encoded

* Corresponding author.
† Present address: Biotechnology Center, The Ohio State University, Columbus, OH 43210.

ampicillin resistance. Minicell preparation, [^{35}S]methionine labeling and analysis of plasmid-encoded polypeptides synthesized in minicells by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and autoradiography have been described previously (18).

Preparation of lysates of *E. coli*. Expression of the *mcrD-lacZ* gene fusion in *E. coli* JM101 containing pD'Lac was induced by 4 h of growth at 37°C in LI containing 100 μg of ampicillin per ml. Cells were removed from suspension by centrifugation (2,000 × *g*, 5 min, 4°C), suspended at a 75× concentration in sonication buffer containing 4 mg of lysozyme per ml, frozen and thawed twice, sonicated on ice for 10 s, and incubated at 20°C for 5 min. The resulting lysate was cleared by centrifugation (4,000 × *g*, 5 min, 4°C), dialyzed for 24 h at 4°C against dialysis buffer [2.28 g of K_2HPO_4, 5.85 g of NaCl, 0.37 g of EDTA, 0.19 g of ethylene glycol-*bis*-(β-aminoethyl ether) *N,N,N',N'*-tetraacetic acid, 0.1 g of 1,10-phenanthroline; pH 8.0] and used immediately or stored frozen at −70°C.

Preparation and fractionation of lysates of *M. vannielii*. Cells from exponentially growing cultures were pelleted anaerobically by centrifugation, and the pellets were frozen and thawed and resuspended in 1/100 the original culture volume of sonication buffer. The lysate obtained after two 1-s exposures to sonication and a 10-min incubation at 0°C was cleared by centrifugation (2,000 × *g*, 5 min, 4°C) and used immediately or stored frozen at −70°C.

Cytoplasmic (S-120 supernatant) and particulate (S-120 pellet) fractions were separated by centrifugation for 2 h at 4°C in an airfuge (Beckman Instruments, Inc., Fullerton, Calif.) at 120,000 × *g*. The S-120 pellet was washed with 50 mM potassium phosphate buffer (pH 6.8) by vigorous, repeated passages through the needle of a 50-μl Hamilton syringe and then repelleted by using the same conditions in the airfuge.

Gel electrophoresis. Proteins were separated by SDS-PAGE and native PAGE by using the discontinuous buffer system described by Laemmli (13). Exponential 11 to 14% (wt/vol) polyacrylamide gradient gels were used for SDS-PAGE analyses of cell lysates, and a 7.5% (wt/vol) polyacrylamide gel was used preparatively to obtain the 135-kilodalton gp*mcrD-lacZ*. Exponential 5 to 15% (wt/vol) polyacrylamide gradient gels were used in the absence of SDS to separate nondenatured protein complexes. Proteins in gels were either stained with Coomassie brilliant blue or transferred to nitrocellulose filters (Schleicher & Schuell, Inc., Keene, N.H.) for analysis by Western blotting.

Electrophoretic separation of DNA molecules used 0.8% (wt/vol) agarose gels as described by Maniatis et al. (14).

Preparation of gp*mcrD-lacZ*. (i) Electroelution from gels. The region of the 7.5% polyacrylamide gel that contained gp*mcrD-lacZ* was excised, macerated, and placed in a glass tube (0.5-cm internal diameter) sealed at one end by a plug of 2% (wt/vol) agarose containing 0.1% (wt/vol) SDS. The tube was fitted with a length of dialysis tubing, and electrophoresis at 90 V for 16 h transferred gp*mcrD-lacZ* from the macerated gel into solution inside the dialysis tubing. The contents of the dialysis tubing were dialyzed for 12 h at room temperature against 6 liters of dialysis buffer containing 20% (vol/vol) isopropanol and then for 24 h at 4°C against 6 liters of phosphate-buffered saline (8 g of NaCl, 0.2 g of KCl, 0.2 g of KH_2PO_4, 1.15 g of Na_2HPO_4 per liter of H_2O [pH 7.4]). The dialyzed solution containing gp*mcrD-lacZ* was concentrated fivefold by rotary evaporation and stored frozen at −70°C.

(ii) Affinity chromatography. Dialyzed lysates of extracts of *E. coli* JM101(pD'Lac) induced for gp*mcrD-lacZ* synthesis by growth in LI medium were subjected to *p*-aminobenzyl-β-D-thiogalactopyranoside (ABTG)-Sepharose affinity chromatography (Sigma Chemical Co., St. Louis, Mo.) exactly as described by Ullmann (20). The gp*mcrD-lacZ* bound to ABTG-Sepharose and was released, without detectable contaminating proteins, by elution with 100 mM sodium borate buffer at pH 9.5.

Preparation of rabbit antisera. Rabbit antiserum containing antibodies raised against the MR holoenzyme ($\alpha_2\beta_2\gamma_2$) was prepared as previously described (7). Anti-gp*mcrD-lacZ* antibodies were raised by vaccinating New Zealand White rabbits with 50 μg of gp*mcrD-lacZ*, electroeluting from gels and emulsifying in 1 ml of complete Freund adjuvant, and boosting 8 and 16 weeks later by injections of 50 μg of gp*mcrD-lacZ* eluted from the ABTG-Sepharose column and emulsified in 1 ml of incomplete Freund adjuvant. Cell-free antiserum was obtained by clotting (20 min, 37°C), centrifugation (8,000 × *g*, 15 min, 4°C), and filtration through 0.45-μm filters (Schleicher & Schuell).

Anti-β-galactosidase antibodies were purchased from Promega Biotec (Madison, Wis.).

Western blot (immunoblot) analyses. Proteins were transferred by electrophoresis for 1 h from either SDS- or native polyacrylamide gels onto nitrocellulose filters by using 80 V at 4°C in EB solution (3.07 g of Tris base, 14.6 g of glycine, 1 g of SDS, 200 ml of CH_3OH, 800 ml of H_2O). Native gels were immersed in SDS-PAGE running buffer (13) for 30 min at room temperature and 68°C for 5 min before electroblotting.

The nitrocellulose filters were washed with water, air dried, placed in blotting buffer for 1 h (20 mM Tris hydrochloride, 0.5 M NaCl, 0.05% NaN_3, 3% gelatin [pH 7.5]), rinsed, and transferred to and incubated for 2 h in blotting buffer containing 0.1% Tween 20, 1% gelatin, and the primary antibody. The location(s) of bound antibodies was detected by using goat anti-rabbit antibodies conjugated to alkaline phosphatase as described by Connolly and Gilmore (6).

Antibody precipitation of radioactively labeled proteins. Dilutions of antiserum were mixed and incubated at 4°C for 16 h with portions of the S-120 supernatant fraction obtained from *M. vannielii* cells labeled by incorporation of [^{35}S]Trans. Sepharose CL4B-protein A beads were added, and incubation was continued for 2 h at 4°C. The pellet obtained following centrifugation in an Eppendorf Microfuge for 15 min at 4°C was washed twice with 1 M NaCl–40 mM Tris hydrochloride, pH 7.5, dissolved in SDS-PAGE sample buffer and subjected to SDS-PAGE (13). Following electrophoresis, the locations of labeled proteins were visualized by autoradiography and antigens were detected by Western blotting.

RESULTS

Construction and expression of the *mcrD-lacZ* gene fusion. The *mcrD* gene of *M. vannielii* was subcloned from pET1300 (7) into pUC19 to obtain pMRD107 and from pMRD107 into M13mp19 to obtain M13-D (Fig. 1). An A to G transition was introduced into *mcrD* by site-specific mutagenesis (24) at position −13 relative to the 3′ terminus of the gp*mcrD* coding sequence by using the mutagenic oligonucleotide (31-mer) primer shown in Fig. 1. This mutation created an *Eco*RI site in *mcrD* without changing the *mcrD*-encoded amino acid [GAA (glu) → GAG (glu)]. Replicative DNA from phage M13-DR1, which carries the novel *Eco*RI site,

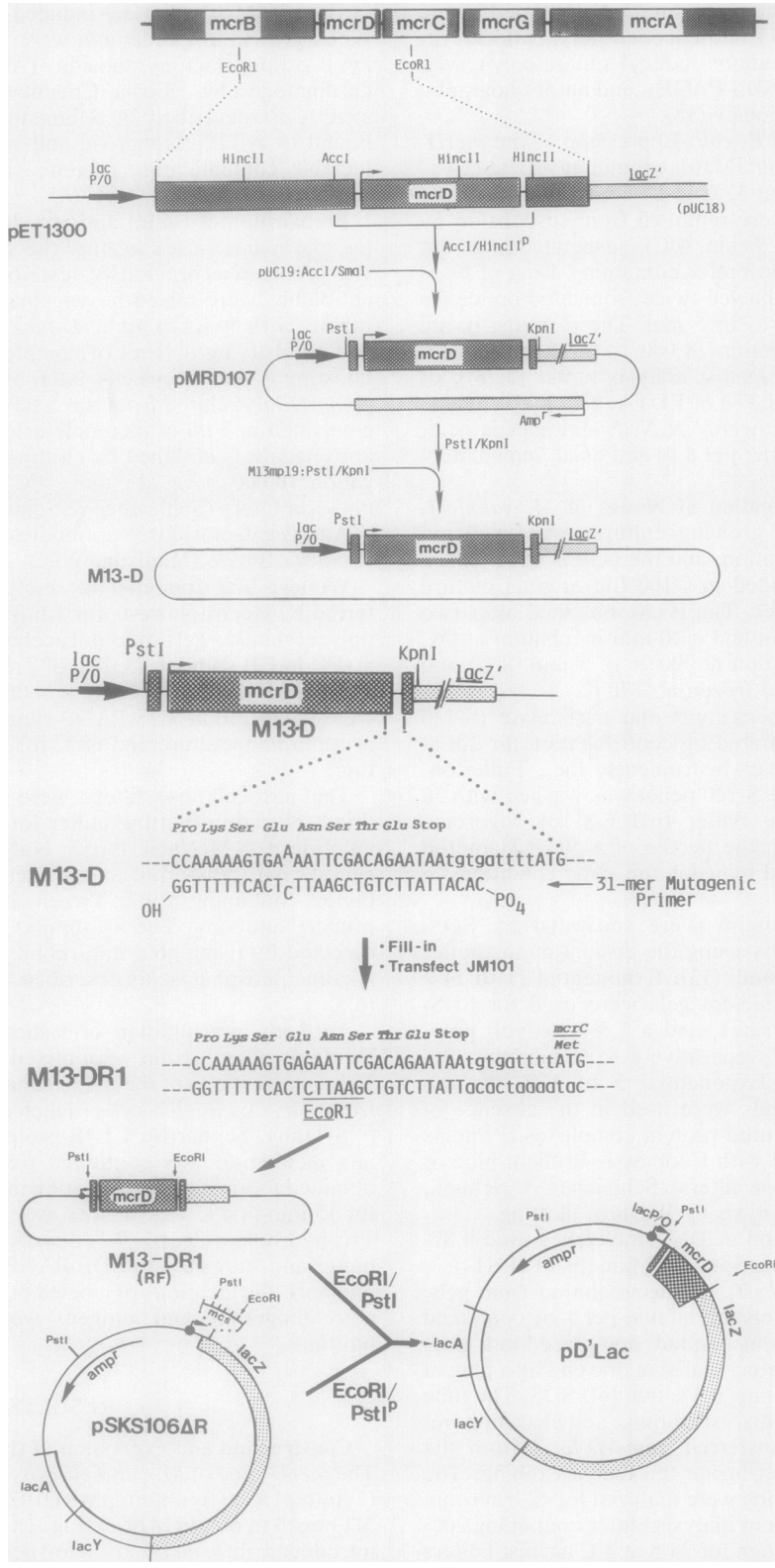

FIG. 1. Construction of the *mcrD-lacZ* gene fusion. The organization and sequence of the *mcrBDCGA* operon in *M. vannielii* and isolation of pET1300 were described previously (7). A fragment of DNA containing the intact *mcrD* gene was isolated from pET1300 by *Acc*I digestion and partial digestion with *Hin*cII, indicated as *Hin*cII^P, and cloned into *Acc*I-*Sma*I-digested pUC19 to produce pMRD107. Digestion with *Pst*I and *Kpn*I and ligation into *Pst*I-*Kpn*I-digested M13mp19 replicative DNA resulted in M13-D. The oligonucleotide shown (31-mer) was synthesized in vitro and used in site-specific mutagenesis (24) to isolate a derivative of M13-D, designated M13-DR1, which contained a new *Eco*RI site. The replicative form of M13-DR1 DNA was digested with *Eco*RI and *Pst*I, and the resulting *mcrD*-containing fragment was cloned into pSKS106ΔR, linearized by a partial *Pst*I digestion (*Pst*I^P) and *Eco*RI digestion to produce pD'Lac.

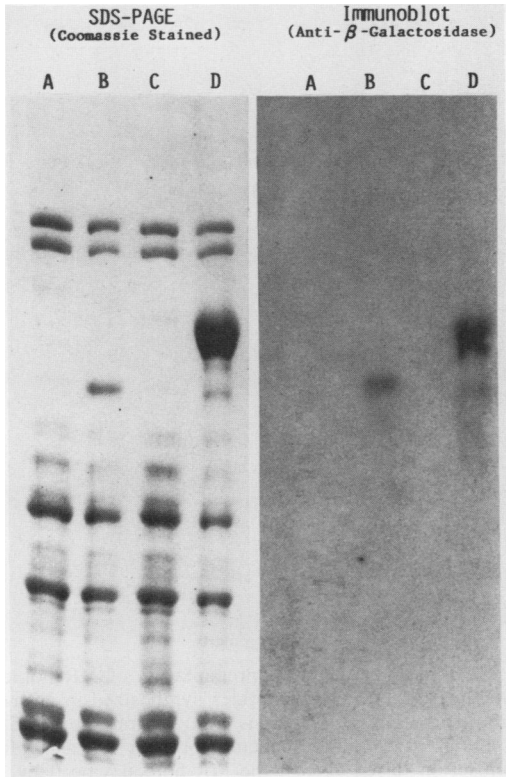

FIG. 2. Expression of the *mcrD-lacZ* gene fusion in *E. coli*. The polypeptides synthesized in *E. coli* JM101(pSKS106ΔR) grown in lactose repression and LI media are shown, separated by SDS-PAGE, in lanes A and B, respectively. The polypeptides synthesized in *E. coli* JM101(pD'Lac) grown in lactose repression and LI media are shown in lanes C and D, respectively. The right-hand panel is a Western blot, using anti-β-galactosidase antibodies, of the material shown stained with Coomassie brilliant blue in the left-hand panel.

was digested with *Eco*RI and *Pst*I, and the *mcrD*-containing fragment of DNA so obtained was ligated to DNA of plasmid pSKS106ΔR which had been linearized by partial digestion with *Pst*I and then cleaved by *Eco*RI digestion. Plasmid pD'Lac obtained by this construction (Fig. 1) contains an in-frame *mcrD-lacZ* gene fusion lacking only 2 codons from the carboxyl terminus of *mcrD* and 6 codons from the amino terminus of *lacZ*. The encoded gp*mcrD-lacZ*, containing 1,158 amino acid residues and with a calculated molecular mass of ~135 kilodaltons, was synthesized in large amounts in *E. coli* JM101(pD'Lac) grown in the presence of isopropyl-β-D-thiogalactopyranoside (Fig. 2). Synthesis of gp*mcrD-lacZ* did not occur in the presence of glucose or absence of isopropyl-β-D-thiogalactopyranoside. Western blots demonstrated β-galactosidase epitopes on gp*mcrD-lacZ* (Fig. 2) which facilitated its subsequent purification by ABTG-affinity chromatography (20).

Demonstration of anti-gp*mcrD* antibodies in the antiserum raised against gp*mcrD-lacZ*. Extracts of *E. coli* DS410 cells, either plasmid-free or containing plasmid pUC19 or pMRD107, were analyzed by Western blotting by using the rabbit antiserum raised against gp*mcrD-lacZ*. All three *E. coli* extracts bound antibodies, presumably due to the presence of anti-*E. coli* antibodies in this rabbit antiserum; however, there were also antibodies present that bound to a protein found only in the *E. coli* DS410(pMRD107) extract. This protein had an electrophoretic mobility identical to that

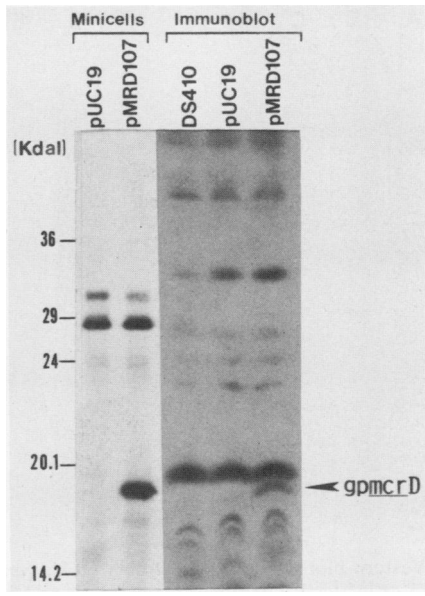

FIG. 3. Detection of gp*mcrD* synthesized in *E. coli*. Radioactively labeled polypeptides synthesized in *E. coli* minicells containing pUC19 or pMRD107 (see Fig. 1) incubated in the presence of [^{35}S]methionine (18) were separated by SDS-PAGE and detected by autoradiography. The band identified as gp*mcrD* has the electrophoretic mobility predicted for a polypeptide with the calculated molecular mass (18,000 kilodaltons) of gp*mcrD* and comigrates with the polypeptide previously identified as being gp*mcrD* synthesized in minicells containing pET1300 (7). Polypeptides in plasmid-free *E. coli* DS410 cells (labeled DS410) and in *E. coli* DS410 cells containing pUC19 or pMRD107 (labeled pUC19 and pMRD107) were separated by SDS-PAGE and probed in a Western blot to demonstrate anti-gp*mcrD* antibodies in the rabbit antiserum (diluted 1:1,000) raised against gp*mcrD-lacZ*.

of radioactively labeled gp*mcrD* synthesized in *E. coli* DS410 minicells containing pMRD107 (Fig. 3).

Detection of gp*mcrD* in *M. vannielii* and its association with MR holoenzyme. The rabbit antibodies raised against the MR holoenzyme and against gp*mcrD-lacZ* were used in Western blotting to identify the α, β, and γ subunits of MR and gp*mcrD* in fractions of *M. vannielii* cells separated by SDS-PAGE and native PAGE (Fig. 4). The separated α, β, and γ subunits and gp*mcrD* were readily detected in the S-120 supernatants by Western blotting after SDS denaturation and electrophoresis, but gp*mcrD* could not be detected by Western blotting of native gels. The MR holoenzyme formed a visible yellow band on nondenaturing gels which bound antibodies raised against the MR holoenzyme but did not bind antibodies raised against gp*mcrD-lacZ* (Fig. 4). Elution, SDS denaturation, and SDS-PAGE resolved this band into the α, β, and γ subunits, but Western blotting of this material showed no evidence for gp*mcrD*. Analysis of the material in the S-120 pellets, by SDS-PAGE and by Western blotting, demonstrated that this particulate material contained only trace amounts of MR holoenzyme and no detectable gp*mcrD*.

An association of the MR holoenzyme and gp*mcrD* in vivo that did not withstand native gel electrophoresis was investigated by using antibody-antigen coprecipitation. Antibodies raised against the MR holoenzyme were used to precipitate proteins from [^{35}S]Trans-labeled extracts of *M. vannielii* cells, and the material precipitated was visualized by autoradiography after SDS-PAGE. The α, β, and γ

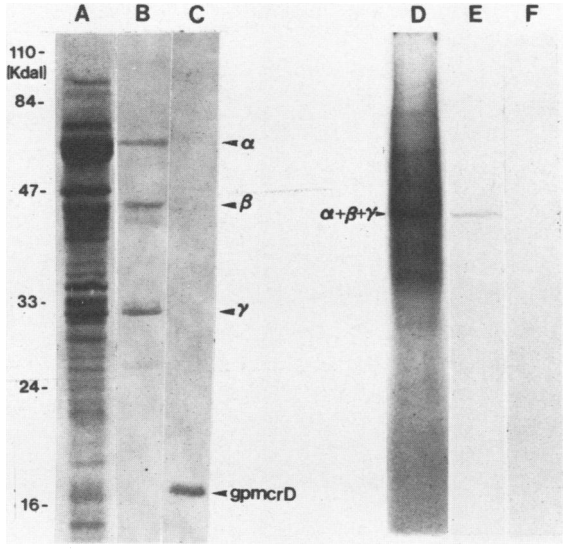

FIG. 4. Western blot analyses of extracts of *M. vannielii* cells. Proteins (40 μg) in the S-120 supernatant fraction of *M. vannielii* cells, separated by SDS-PAGE or native PAGE and stained with Coomassie brilliant blue, are shown in lanes A and D, respectively. The material in lanes A and D was probed by Western blotting by using the antiserum (diluted 1:20,000) raised against the MR holoenzyme (lanes B and E) and the antiserum (diluted 1:1,000) raised against gp*mcrD-lacZ* (lanes C and F). The antiserum raised against the MR holoenzyme did not contain anti-gp*mcrD* antibodies, nor did the antiserum raised against gp*mcrD-lacZ* contain antibodies that bound to the α, β, or γ subunits of MR. Nevertheless, the antiserum raised against the MR holoenzyme did coprecipitate gp*mcrD* (Fig. 5), and the antiserum raised against gp*mcrD-lacZ* did coprecipitate the α, β, and γ subunits (result not shown) from S-120 supernatants.

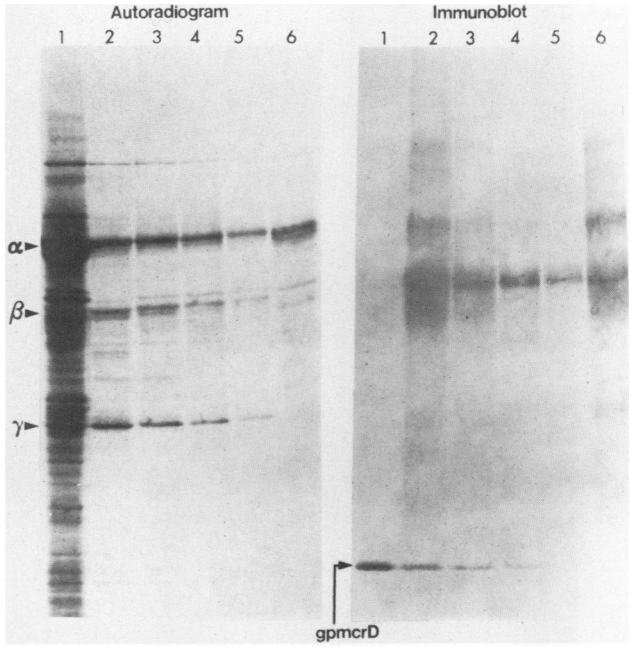

FIG. 5. Antibody-mediated coprecipitation of MR holoenzyme and gp*mcrD*. ^{35}S-labeled polypeptides synthesized in *M. vannielii* grown in the presence of [^{35}S]Trans were separated by SDS-PAGE (lane 1) or were precipitated by incubation with increasing dilutions of the antiserum raised against the MR holoenzyme (lane 2, 20% antiserum; lane 3, 5% antiserum; lane 4, 1.25% antiserum; lane 5, 0.3% antiserum) or with preimmune serum (lane 6, 20% control serum), and the material precipitated was separated by SDS-PAGE. The left-hand panel is an autoradiogram of the resulting gel, and the right-hand panel is a Western blot of the same gel probed with the antiserum (diluted 1:1,000) raised against gp*mcrD-lacZ*.

subunits of MR were clearly visible in the antibody-precipitated material, but very little gp*mcrD* was detectable by autoradiography (Fig. 5). Probing this gel by Western blotting by using anti-gp*mcrD-lacZ* antibodies did, however, demonstrate gp*mcrD* in the material precipitated by the anti-MR antibodies (Fig. 5).

DISCUSSION

Although *mcrD* genes have been cloned and sequenced from five methanogens (3, 4, 7, 12, 22), the results reported here are the first demonstration of a gp*mcrD* in a methanogen. The primary sequences predicted for the different gp*mcrD*s contain only limited regions with conserved amino acid residues (12, 22, 23), but nevertheless they do all appear to have evolved from a common ancestor and are therefore likely to have a common function. The challenge is to determine that function. MR purified on the basis of enzyme activity does not contain gp*mcrD* (2, 8, 9), nor is gp*mcrD* present in the MR holoenzyme isolated by electrophoresis through native gels (Fig. 4), but as suggested by the antibody-mediated coprecipitation (Fig. 5), an association of gp*mcrD* and MR in vivo is still a possibility. If this coprecipitation accurately reflects an in vivo association, then the ratio of gp*mcrD* to MR holoenzyme in these complexes appears to be low. It is, however, also possible that most of the gp*mcrD*-MR complexes dissociate during cell lysis or that the coprecipitation results from nonspecific entrapment of small amounts of gp*mcrD* within the large amounts of MR present in, and precipitated from, *M. vannielii* S-120 supernatants by the anti-MR antibodies. In situ localizations indicate that MR holoenzyme is located primarily at the cell periphery in *Methanococcus voltae* (17) and in methanogen strain Göl (15) but that in *Methanobacterium thermoautotrophicum* the subcellular location of MR is growth rate dependent (1, 17). We found both MR holoenzyme and gp*mcrD* almost exclusively in the S-120 supernatant fraction of *M. vannielii*, indicating that if MR is associated with the cell membrane in *M. vannielii*, this association does not survive, in a particulate form, cell lysis by freezing and thawing. Complex subcellular structures containing the α, β, and γ subunit MR have been described (15, 21), and a role for gp*mcrD* in the construction or as a component of these "methanoreductosomes" would still be consistent with the results reported here.

ACKNOWLEDGMENTS

We thank C. Daniels, J. Krzycki, and past and present members of the Reeve laboratory for their help and intellectual input in the development of this work.

Support for this research was provided by the Department of Energy grant DE-FG02-87ER13731A001.

LITERATURE CITED

1. **Aldrich, H. C., D. B. Beimborn, M. Bokranz, and P. Schonheit.** 1987. Immunocytochemical localization of methyl-coenzyme M reductase in *Methanobacterium thermoautotrophicum*. Arch. Microbiol. **147**:190–194.
2. **Ankel-Fuchs, D., R. Huster, E. Morschel, S. P. J. Albracht, and R. K. Thauer.** 1986. Structure and function of methyl-coenzyme M reductase and of factor F_{430} in methanogenic bacteria. Syst.

Appl. Microbiol. **7**:383–387.
3. **Bokranz, M., G. Baumner, R. Allmansberger, D. Ankel-Fuchs, and A. Klein.** 1988. Cloning and characterization of the methyl coenzyme M reductase genes from *Methanobacterium thermoautotrophicum*. J. Bacteriol. **170**:568–577.
4. **Bokranz, M., and A. Klein.** 1987. Nucleotide sequence of the methyl coenzyme M reductase gene cluster from *Methanosarcina barkeri*. Nucleic Acids Res. **15**:4350–4351.
5. **Casadaban, M., A. Martinez-Arias, S. Shapiro, and J. Choi.** 1983. β-Galactosidase gene fusions for analyzing gene expression in *Escherichia coli* and yeast. Methods Enzymol. **100**: 293–308.
6. **Connolly, T., and R. Gilmore.** 1989. The signal recognition particle receptor mediates the GTP-dependent displacement of SRP from the signal sequence of the nascent polypeptide. Cell **57**:599–610.
7. **Cram, D. S., B. A. Sherf, R. T. Libby, R. J. Mattaliano, K. L. Ramachandran, and J. N. Reeve.** 1987. Structure and expression of the genes, *mcrBDCGA*, which encode the subunits of component C of methyl coenzyme M reductase in *Methanococcus vannielii*. Proc. Natl. Acad. Sci. USA **84**:3992–3996.
8. **Ellefson, W. L., and R. S. Wolfe.** 1980. Role of component C in the methyl reductase system of *Methanobacterium*. J. Biol. Chem. **255**:8388–8389.
9. **Hartzell, P. L., and R. S. Wolfe.** 1986. Comparative studies of component C from the methyl reductase system of different methanogens. Syst. Appl. Microbiol. **7**:376–382.
10. **Hook, L., R. Corder, P. T. Hamilton, J. I. Frea, and J. N. Reeve.** 1984. Development of a plating system for genetic exchange studies in methanogens using a modified ultra-low oxygen chamber. *In* W. R. Strohl and O. H. Tuovinen (ed.), Microbial chemoautotrophy. Ohio State University Press, Columbus, Ohio.
11. **Jones, W. J., D. P. Nagle, and W. B. Whitman.** 1987. Methanogens and the diversity of archaebacteria. Microbiol. Rev. **51**: 135–171.
12. **Klein, A., R. Allmansberger, M. Bokranz, S. Knaub, B. Muller, and E. Muth.** 1988. Comparative analysis of genes encoding methyl coenzyme M reductase in methanogenic bacteria. Mol. Gen. Genet. **213**:409–420.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
14. **Maniatis, T., E. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
15. **Mayer, F., M. Rohde, M. Salzmann, A. Jussofie, and G. Gottschalk.** 1988. The methanoreductosome: a high-molecular-weight enzyme complex in the methanogenic bacterium Göl that contains components of the methylreductase system. J. Bacteriol. **170**:1438–1444.
16. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101**:70–78.
17. **Ossmer, R., T. Mund, P. L. Hartzell, U. Konheiser, G. W. Kohring, A. Klein, R. S. Wolfe, G. Gottschalk, and F. Mayer.** 1986. Immunocytochemical localization of component C of the methylreductase system in *Methanococcus voltae* and *Methanobacterium thermoautotrophicum*. Proc. Natl. Acad. Sci. USA **83**:5789–5792.
18. **Reeve, J. N.** 1979. Use of minicells for bacteriophage directed polypeptide synthesis. Methods Enzymol. **68**:493–503.
19. **Rouvière, P., and R. S. Wolfe.** 1988. Novel biochemistry of methanogenesis. J. Biol. Chem. **263**:7913–7916.
20. **Ullmann, A.** 1984. One-step purification of hybrid proteins which have β-galactosidase activity. Gene **29**:27–31.
21. **Wackett, L. P., E. A. Hartwieg, J. A. King, W. H. Orme-Johnson, and C. T. Walsh.** 1987. Electron microscopy of nickel-containing methanogenic enzymes: methyl reductase and F_{420}-reducing hydrogenase. J. Bacteriol. **169**:718–727.
22. **Weil, C. F., D. S. Cram, B. A. Sherf, and J. N. Reeve.** 1988. Structure and comparative analysis of the genes encoding component C of methyl coenzyme M reductase in the extremely thermophilic archaebacterium *Methanothermus fervidus*. J. Bacteriol. **170**:4718–4726.
23. **Weil, C. F., B. A. Sherf, and J. N. Reeve.** 1989. A comparison of the methyl reductase genes and gene products. Can. J. Microbiol. **35**:101–108.
24. **Zoller, M., and M. Smith.** 1983. Oligonucleotide-directed mutagenesis of DNA fragments cloned in M13 vectors. Methods Enzymol. **100**:468–500.