

## NOTES

# The Small-Subunit Polypeptide of Methylamine Dehydrogenase from *Methylobacterium extorquens* AM1 Has an Unusual Leader Sequence

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**The nucleotide sequence for the N-terminal region of the small subunit of methylamine dehydrogenase from *Methylobacterium extorquens* AM1 has revealed a leader sequence that is unusual in both its length and composition. Gene fusions to *lacZ* and *phoA* show that this leader sequence does not function in *Escherichia coli* but does function in *M. extorquens* AM1.**

Methylamine dehydrogenase (MADH; EC 1.4.99.3) is a periplasmic protein found in many gram-negative methylotrophic and autotrophic bacteria, in which it functions during growth on methylamine as the sole source of carbon and/or nitrogen (8). MADH from *Methylobacterium extorquens* AM1 consists of two large and two small subunits with molecular weights of 40,000 and 14,000 (20). Recent data have shown that MADH has a new type of redox cofactor—a cross-linked ditryptophan derivative designated tryptophan tryptophylquinone (5, 15). This cofactor is apparently synthesized by posttranslational modification of two tryptophan residues in each of the small subunit polypeptides, a process which must involve both oxidative and cross-linking reactions. The biochemical reactions involved and their temporal sequence with regard to the secretion process are unknown, but it seems likely that novel features may be involved.

In a previous paper (5), we described the cloning and sequencing of the gene (*mauA*) for the structural portion of the MADH small subunit of *M. extorquens* AM1. The region encoding the leader peptide portion of this polypeptide has now been sequenced. The DNA manipulations involved were done as described by Maniatis et al. (14), and DNA sequencing was performed by the dideoxy chain termination method (19) with Sequenase and Taquenase sequencing kits according to instructions of the manufacturer (U.S. Biochemical Corp., Cleveland, Ohio). The plasmids used are noted in Table 1, and all *Escherichia coli* DH5 $\alpha$  (F<sup>-</sup> *recA1 lacZYA* r<sup>-</sup> m<sup>+</sup> *thi-1 gyrA66 supE44 endA1  $\phi$ 80lacZ $\Delta$ M15*) and *E. coli* CC118 (F<sup>-</sup> *araD139 lacX74 phoA galE galK thi-1 rpsE rpoB recA1*) strains containing plasmids were grown on L medium (16) with the addition of the appropriate antibiotics (100  $\mu$ g of ampicillin or kanamycin per ml or 20  $\mu$ g of tetracycline per ml). Plasmid pAYC152.1 was used for initial sequencing (Fig. 1 and Table 1) (6), and the sequence data are shown in Fig. 2. An open reading frame encoding the proposed leader peptide that coincides in frame with the known amino acid sequence of MauA was identified (5). This open reading frame has two possible translational start sites, but the first contains a better ribosome-binding sequence (GAGG) than the second (GAG). If the start occurs from the

first methionine codon, a polypeptide consisting of 186 amino acids with a molecular weight of 20,084 is synthesized. If the second methionine codon is used as a start site, a polypeptide consisting of 173 amino acids with a molecular weight of 18,545 is synthesized. The sizes of the predicted leader sequences are 57 and 44 amino acids, respectively, which are surprisingly large. In addition, both proposed leader sequences have unusual compositions. This caused concern that the sequence might have been generated artifactually by rearrangements occurring during the subcloning either of the original 5.2-kb *Bam*HI-*Hind*III insert in pAYC147 (5) or of the insert in pAYC152.1. Therefore, sequence data were generated independently from plasmid pAYC142, a clone containing *mauA* that was isolated directly from an independent partial clone library (5). An oligonucleotide (5'-GTTGTCCTGCGGCTTCCACTT-3') complementary to the region encoding the mature portion of the polypeptide was synthesized and used as a primer for sequencing of pAYC142. The sequence obtained was identical to the sequence shown in Fig. 2.

To obtain further evidence that the start codons identified were translated, translational fusions of the *mauA* open reading frame with *lacZ* were generated (Fig. 1). These were constructed from plasmid pAYC150 (Table 1). Plasmid pAYC150a (Fig. 1) was generated by deleting an *Acc*I-*Acc*I fragment of pAYC150, treating the *Acc*I recessed ends with Klenow fragment, and religating. This leads to the loss of approximately one-third of the *mauA* downstream sequence and brings in frame the sequences of this portion of *mauA* (*mauA'*) and the part of *lacZ* in pUC19 (*lacZ'*) (5, 26).  $\beta$ -Galactosidase assays were carried out in cell extracts as described by Miller (16). *E. coli* DH5 $\alpha$  cells containing the appropriate plasmids were grown in L broth with the appropriate antibiotics, and the *lac* promoter was activated by supplementing liquid medium with 40  $\mu$ g of isopropyl- $\beta$ -D-thiogalactopyranoside (IPTG) per ml (16). Protein concentrations were determined by published methods (13).  $\beta$ -Galactosidase activity (18.5 nmol min<sup>-1</sup> mg of protein<sup>-1</sup>) was detected in *E. coli* strains containing pAYC150a. Two deletion derivatives of pAYC150a were created to confirm that the activity observed was due to the presence of the appropriate upstream region. pAYC150c was obtained by deletion

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TABLE 1. Plasmids used in this study

Plasmid	Genotype	Source or reference
pRK310	Tc <sup>r</sup> <i>lacZ</i> <i>mob</i> <sup>+</sup>	9
pRK2013	Km <sup>r</sup> Tra <sup>+</sup>	9
pUC18, pUC19	Ap <sup>r</sup> <i>lacZ'</i>	Pharmacia
pTZ19TT	Ap <sup>r</sup> ( <i>cat-lacZ'</i> )	2
pCM335	Tc <sup>r</sup> Km <sup>r</sup> ( <i>TnphoA</i> ) ( <i>bla-phoA</i> )	Colin Manoil
pAYC142	Ap <sup>r</sup> 7.3-kb <i>Bam</i> HI insert of <i>M. extorquens</i> AM1 chromosome, containing <i>mau</i> genes in pUC7	3
pAYC147	Ap <sup>r</sup> 5.2-kb <i>Bam</i> HI- <i>Hind</i> III insert of <i>M. extorquens</i> AM1 chromosome containing <i>mau</i> genes in pTZ19TT	3
pAYC152.1	Ap <sup>r</sup> 0.7-kb <i>Xho</i> I- <i>Bcl</i> II insert of <i>M. extorquens</i> AM1 chromosome, containing <i>mauA</i> in pTZ19TT	6
pAYC150	Ap <sup>r</sup> 1.3-kb <i>Xho</i> I- <i>Sal</i> I insert of <i>M. extorquens</i> AM1 chromosome, containing <i>mauA</i> , <i>mauC</i> in pUC19	This study
pAYC150a	Ap <sup>r</sup> ( <i>mauA'</i> - <i>lacZ'</i> )	This study
pAYC150c	Ap <sup>r</sup> $\Delta$ 1( <i>mauA'</i> - <i>lacZ'</i> )	This study
pAYC150d	Ap <sup>r</sup> $\Delta$ 2( <i>mauA'</i> - <i>lacZ'</i> )	This study
pAYC62	Ap <sup>r</sup> Km <sup>r</sup> ( <i>lacZ'</i> - <i>phoA</i> )	This study
pAYC157	Ap <sup>r</sup> Km <sup>r</sup> ( <i>mauA'</i> - <i>phoA</i> )	This study
pAYC158	Tc <sup>r</sup> ( <i>mauA'</i> - <i>phoA</i> ) <i>mob</i> <sup>+</sup>	This study
pAYC159	Tc <sup>r</sup> ( <i>lacZ'</i> - <i>phoA</i> ) <i>mob</i> <sup>+</sup>	This study
pAYC162	Tc <sup>r</sup> ( <i>bla-phoA</i> ) <i>mob</i> <sup>+</sup>	This study

of two nucleotides of the recessed ends of *Sac*II in pAYC150a. This deletion should lead to a frameshift for both possible start codons and the loss of expression of the *mauA'*-*lacZ'* fusion. The observed  $\beta$ -galactosidase activity in cells with this plasmid was 2.1 nmol min<sup>-1</sup> mg of pro-

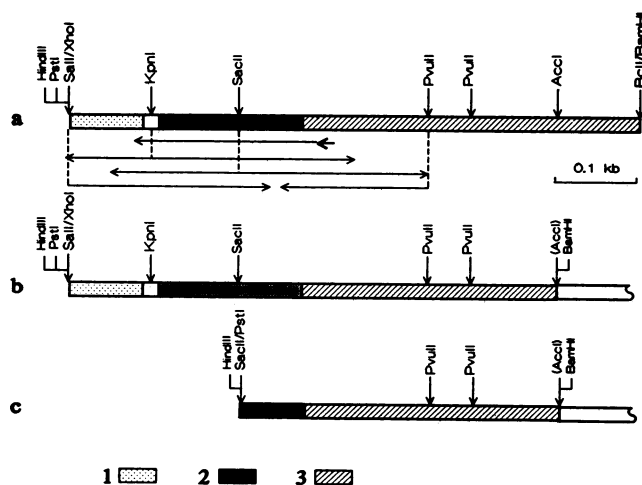


FIG. 1. Map of the insertions of the plasmids pAYC152.1 (a), pAYC150a (b), and pAYC150d (c). Boxes: 1, sequence of the upstream open reading frame (*mauD*); 2, sequence of the leader peptide of the MADH small subunit; 3, sequence of the mature part of the MADH small subunit. Sequencing strategy is shown in thin arrows on the map of pAYC152.1. Solid arrow designates the place of hybridization of the probe with pAYC142 for direct sequencing of the leader. Open boxes on the right portions of pAYC150a and pAYC150d designate sequence of *lacZ* from pUC19.

1	CTCGAGGCGGACAAGAGCGGTTTCGCTCCATCCAGCAGTTCATGACGAGCC LeuGluAlaAspLysSerGlyPheAlaSerIleGlnGlnPheMetThrSer
53	GGAAGCACAGCCAGCAGCCAAAGCCGCTAATCGCCCGGTCTCCGGACGGT ArgLysHisSerHisAspAlaLysAlaAla
105	ACCTGCATCAGCACCCGATAAACTGACGAGGAACGATGCTCGGAAAATCC METLeuGlyLysSer
157	CAATTCGACGATCTCTTCGAGAAGATGTCGCCGCAAGGTGGCGGGCATACCA GlnPheAspAspLeuPheGluLysMETSerArgLysValAlaGlyHisThr
209	GCCGCCGCGGCTTCATCGGGCGCGTCGGCACGGCGGTGGCGGGCGTCGCGCT SerArgArgGlyPheIleGlyArgValGlyThrAlaValAlaGlyValAlaLeu
261	AGTGCCTCTGCTGCCGTCGATCGCCGTGGCGCGTCAGCCGCGCAATGCT ValProLeuLeuProValAspArgArgGlyArgValSerArgAlaAsnAla
313	GCCGAG *AlaGlu

FIG. 2. Nucleotide and amino acid sequence of the leader peptide of the MADH small subunit and an upstream open reading frame (presumably *mauD*) from *M. extorquens* AM1. The numbers refer to the nucleotide (upper) sequence. The underlined codons for the MADH small-subunit leader sequence are two possible start methionine triplets; the underlined codon for the upstream open reading frame is the terminator triplet; and the double-underlined nucleotides are possible ribosome-binding sites. \*, signal peptidase cleavage site. Arrows above the nucleotide sequence indicate inverted repeats.

tein<sup>-1</sup>, about 10% of the level found with pAYC150a. A second deletion derivative, pAYC150d, was obtained by deletion of the *Pst*I-*Sac*II fragment in pAYC150a (Fig. 1). This deletes both putative translational start sites for the *mauA'*-*lacZ'* fusion, and this region contains no other putative translational start sites (Fig. 2) (5). A significant drop in  $\beta$ -galactosidase activity was observed in cells containing this plasmid (1.3 nmol min<sup>-1</sup> mg of protein<sup>-1</sup>). Negative controls (no plasmid) gave an activity of 0.016 nmol min<sup>-1</sup> mg of protein<sup>-1</sup>, and positive controls (pTZ19TT) gave an activity of 10.6 nmol min<sup>-1</sup> mg of protein<sup>-1</sup>. These data suggest that translation of *mauA* in *E. coli* does occur from at least one of the putative start codons. T7 expression experiments (6) have shown that polypeptides with apparent molecular masses of 22 and 20.6 kDa are produced from *mauA*. These are similar to the predicted polypeptides of 20 and 18.5 kDa, suggesting that both start sites are utilized under these conditions. The larger polypeptide predominates, which is consistent with the poor ribosome-binding site and the presence of an inverted repeat structure at the second potential start site (Fig. 2).

The unusual features of the *mauA* leader sequence noted above raise the question of whether it is active in *E. coli* and *M. extorquens* AM1. The *mauA* leader sequence is apparently active in *M. extorquens* AM1, since MADH is a periplasmic enzyme (8) and the N-terminal sequence of the small subunit as purified from *M. extorquens* AM1 shows that the mature polypeptide begins just after the proposed cleavage site (12). Several observations suggest that this leader sequence functions poorly in the *E. coli* secretion system. In T7 expression experiments with subclones containing only *mauA*, the unprocessed forms of the MauA polypeptide are expressed in *E. coli* but only trace amounts of the polypeptide that appears to be the processed form can be detected (6). In addition, the data presented here show that  $\beta$ -galactosidase activity in fusions of *lacZ'* to the *mauA* leader sequence was detectable at levels that were even higher than that of the pTZ19TT positive control. Since  $\beta$ -galactosidase is normally inactive when fused to a functional leader sequence, this suggests that little secretion and processing of this fusion protein occurred in *E. coli*. To

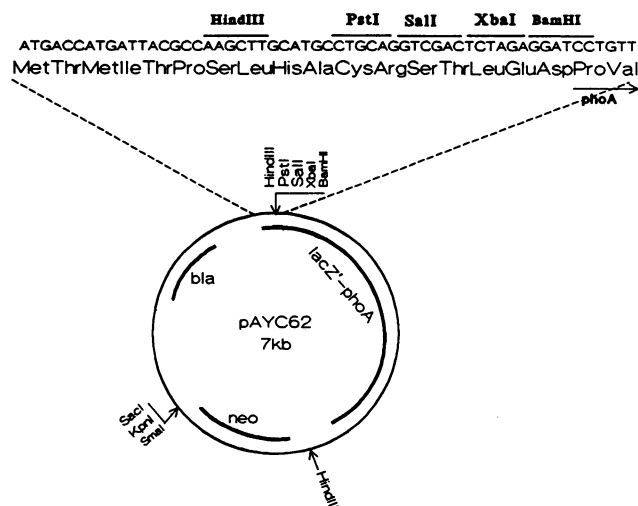


FIG. 3. Map of the plasmid pAYC62. Above the map, DNA (upper) and protein (lower) sequences are shown in the place of the *lacZ'*-*phoA* fusion. Arrow indicates the beginning of the structural part of *phoA*.

obtain further evidence concerning the ability of the *mauA* leader sequence to function in *E. coli* and *M. extorquens* AM1, a fusion was constructed between *mauA* and the gene for alkaline phosphatase (*phoA*). Plasmid pCM335, which contains *TnphoA* inserted into the *bla* gene of pBR322, was used as a donor of the *phoA* gene (*bla-phoA* fusion). The *TnphoA* in pCM335 has been modified so that it has an additional *Bam*HI site directly upstream of *phoA*. The *Bam*HI-*Sma*I fragment of pCM335 carrying *phoA* and *neo* was recloned into pUC19 to produce plasmid pAYC62 (Fig. 3), which has a *lacZ'*-*phoA* fusion. The *Hind*III-*Bam*HI fragment of pAYC150a was then recloned into pAYC62, producing plasmid pAYC157, which contains a *mauA'*-*phoA* hybrid translationally fused at the same position as the *mauA-lacZ'* fusions described earlier. In order to study expression of these fusion proteins in *M. extorquens* AM1, all of the gene fusions were recloned into the broad-host-range vector pRK310. *Hind*III fragments from pAYC157, pAYC62, and pCM335 were inserted into the unique *Hind*III site of pRK310 in an orientation such that all fusion proteins were transcribed from the *lac* promoter. Broad-host-range derivatives (pAYC158, pAYC159, and pAYC162; Table 1) were introduced into *M. extorquens* AM1 by triparental matings by using the helper plasmid pRK2013 as described previously (9). In *E. coli* strains carrying pAYC157 or pAYC158 (containing the *mauA'*-*phoA* fusion), colonies on X-phos medium (L medium containing 0.04 mg of 5-bromo-4-chloro-indolylphosphate per ml, 5 mM phosphate buffer [pH 7.0], and 0.02 mg of tetracycline per ml) were white. However, *M. extorquens* AM1 harboring pAYC158 gave blue colonies on X-phos medium. Alkaline phosphatase activity was measured in *E. coli* CC118 and *M. extorquens* AM1 in whole cells according to published procedures (25). *E. coli* cells were grown for 24 h at 37°C in L broth supplemented with 0.04 mg of IPTG per ml, 5 mM phosphate buffer (pH 7.0), and 0.05 mg of tetracycline per ml. *M. extorquens* AM1 cells were grown for 96 h at 30°C in Hypho medium (9) containing 0.02 mg of tetracycline per ml. Specific activity was expressed in nmol of *p*-nitrophenol released per min per unit of optical density at 600 nm of cells.

TABLE 2. Activities of alkaline phosphatase in *E. coli* CC118 and *M. extorquens* AM1 strains containing different plasmids

Plasmid	Fusion	Activity <sup>a</sup> in:		
		<i>E. coli</i>	<i>M. extorquens</i> grown on methanol	<i>M. extorquens</i> grown on methylamine
None		0	0	0
pAYC158	<i>mauA-phoA</i>	0	$4.21 \times 10^{-5}$	$4.26 \times 10^{-5}$
pAYC159	<i>lacZ-phoA</i>	0	0	0
pAYC162	<i>bla-phoA</i>	$1.1 \times 10^{-3}$	$13.16 \times 10^{-5}$	$3.56 \times 10^{-5}$

<sup>a</sup> nmol min<sup>-1</sup> per unit of optical density at 600 nm of cells. In the case of pAYC162 in *E. coli*, this corresponds to 78 nmol min<sup>-1</sup> mg of protein<sup>-1</sup>.

No alkaline phosphatase activity was detectable in *E. coli* strains with plasmid pAYC62 (the negative control) or pAYC157, but strains with the positive control plasmid, pCM335, had an activity of  $5 \times 10^{-3}$  nmol per min per unit of optical density at 600 nm of cells. For the broad-host-range plasmids, no activity was detected in strains with plasmids pAYC159 (the negative control) or pAYC158, but strains with pAYC162 (the positive control) did show activity (Table 2). In *M. extorquens* AM1, activity was observed in strains containing plasmids pAYC158 and pAYC162, but not pAYC159. The alkaline phosphatase activity in *E. coli*

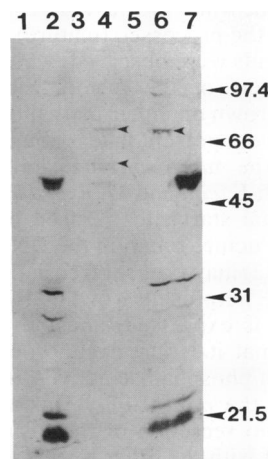


FIG. 4. Immunoblot of extracts from the following strains: 1, plasmid-free *E. coli* DH5 $\alpha$  grown in L medium containing 10 mM phosphate buffer, pH 7.0 (conditions that repress the chromosomal *phoA* gene); 2, *E. coli* DH5 $\alpha$  containing pAYC159 grown in L medium containing 10 mM phosphate buffer, pH 7.0; 3, *M. extorquens* AM1 containing pAYC158 grown in Hypho medium in the presence of methanol (1%) and methylamine (0.2%); 4, *M. extorquens* AM1 containing pAYC158 grown in Hypho medium in the presence of methanol (1%); 5, plasmid-free *M. extorquens* AM1 grown in Hypho medium in the presence of methanol (1%); 6, *E. coli* DH5 $\alpha$  containing pAYC158 grown in L medium containing 10 mM phosphate buffer, pH 7.0; and 7, plasmid-free *E. coli* DH5 $\alpha$  grown in L medium (conditions that derepress the chromosomal *phoA* gene). Rabbit antiphosphatase antibodies (5 Prime-3 Prime, Inc., West Chester, Pa.) were used as primary antibodies. Upper arrows indicate the bands for the premature form of the *mauA-phoA* fusion protein. Lower arrow indicates the band ascribed to the mature form of the *mauA-phoA* fusion. Standards are indicated on the right, in kilodaltons. Lanes with *E. coli* contained 10  $\mu$ g of protein, while lanes with *M. extorquens* AM1 contained 4  $\mu$ g of protein, except lane 3, which contained 2  $\mu$ g of protein.

strains was one to two orders of magnitude higher than in *M. extorquens* AM1 strains. This could be due to differences in *M. extorquens* AM1, such as weak transcription of the *lac* promoter, weak translation of the fusion, instability of the fusion, or inefficiency of the whole cell assay.

Immunoblotting experiments with antibody to alkaline phosphatase were conducted to analyze the *mauA'*-*phoA* fusion proteins expressed in *E. coli* and *M. extorquens* AM1 (Fig. 4). In all *E. coli* strains expressing either plasmid-borne or chromosomal *phoA* or *phoA* fusions, a series of small polypeptides that are presumably breakdown products of *PhoA* or *PhoA* fusions were observed. In addition, in *E. coli* strains containing either a derepressed chromosomal *phoA* or a plasmid-borne *lacZ'*-*phoA* fusion, a dark band corresponding in size to the expected polypeptide was observed (49.5 and 51 kDa, respectively). In *E. coli* cells containing the *mauA'*-*phoA* fusion, only one other major band was observed, with an apparent molecular mass of 70.5 kDa. This matches well the molecular mass of the unprocessed (premature) *mauA'*-*phoA* fusion protein (67 kDa) predicted from the nucleotide sequence, if the first of the two possible translation start sites is used. No trace of a polypeptide of the correct molecular mass for the processed form of the *mauA'*-*phoA* protein (59.4 kDa) was detected. The large amount of the degradation products compared to the full-sized polypeptides suggests these fusions are unstable in *E. coli*. In *M. extorquens* AM1, four polypeptides that cross-reacted with the *PhoA* antibody were observed. The uppermost band (70.5 kDa) corresponded in size to the premature *mauA'*-*phoA* fusion, while the next largest (57.5 kDa) corresponded in size to the processed (mature) fusion. Two other slightly smaller bands were observed, which might represent degradation products of the fusion. Similar bands were observed in cells grown on either methanol or methanol plus methylamine but were present in less abundance in the latter case. The size of the *mauA'*-*phoA* fusion proteins detected suggest that in both *E. coli* and *M. extorquens* AM1, the first of the two potential start sites is used preferentially. The putative hairpin structure found in the DNA sequence of the leader region (Fig. 2) may prevent translation initiation at the second start site. The presence of this fusion protein in *E. coli* shows that it is expressed, and the size of the fusion protein suggests that it is largely unprocessed. Therefore, the lack of alkaline phosphatase activity observed in *E. coli* strains containing the *mauA'*-*phoA* fusion is apparently a result of little or no secretion of the fusion protein. These data are consistent with the other results suggesting that the *MauA* leader sequence functions poorly in *E. coli*.

The data presented above suggest that the MADH small subunit contains a leader sequence that is not well recognized in *E. coli*. Although this sequence has some features in common with known leader sequences, it also has some very uncommon aspects (Fig. 5) (4, 11, 23). In common with other leader sequences, it contains positive charges in the N-terminal region and a hydrophobic core consisting of 16 amino acids. At the C-terminal end, it has the sequence Ala-Asn-Ala, a beta-turn (predicted by the method of Chou and Fasman) (7) near the known site of cleavage and no net charge in the first five amino acids of the mature polypeptide (5). The hydrophobic core of the leader is predicted by the method of Rao and Argos (18) to produce a transmembrane helix. However, in contrast to other leaders, it is unusually long. Most prokaryotic leader sequences are 20 to 28 amino acids (4, 23), although longer sequences are known: for example, the *Desulfovibrio vulgaris* hydrogenase small subunit (34 amino acids) (24), *Staphylococcus aureus* protein A

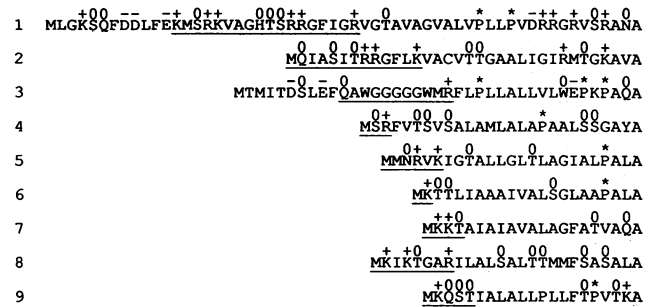


FIG. 5. Comparison of nine leader peptides. 1, Small subunit of MADH from *M. extorquens* AM1; 2, small subunit of hydrogenase from *Desulfovibrio vulgaris* (24); 3, fusion of  $\beta$ -galactosidase and proinsulin (21); 4, large subunit of methanol dehydrogenase from *M. extorquens* AM1 (1); 5, cytochrome  $c_L$  from *M. extorquens* AM1 (17); 6, small subunit of methanol dehydrogenase from *M. extorquens* AM1 (17); 7, *OmpA* protein from *E. coli* (11); 8, maltose-binding protein from *E. coli* (11); 9, alkaline phosphatase from *E. coli* (11). Positively charged N-terminal regions of signal peptides are underlined. +, -, and 0 above amino acids denote positively charged, negatively charged, and polar amino acids, respectively. An asterisk above an amino acid denotes a helix-breaking proline residue.

(36 amino acids) (23), and an embedded leader sequence constructed by fusing the proinsulin gene to the N-terminal portion of *lacZ* (39 amino acids; Fig. 5) (21). To our knowledge, no sequences with as many as 57 amino acids have been reported. In addition, 13 mostly hydrophilic amino acids precede the positively charged amino acids at the N-terminal portion. Secondary structure analysis by the method of Garnier et al. (10) predicts that the first 19 N-terminal amino acids of the leader form an alpha helix structure following initiation of transport of the MADH small subunit across the membrane. Finally, the leader contains a large net positive charge in the C-terminal portion. This is especially surprising, since it has been shown that in *E. coli* addition of charged amino acids by mutation in this region of common leader peptides leads to considerable slowing of transport and leader peptidase processing (4). One known leader sequence that does have positive charges at the C-terminus, that of the *D. vulgaris* hydrogenase small subunit (Fig. 5), also functions very poorly in *E. coli* (22).

At this time, the natural function of this uncommon leader structure is unknown. *M. extorquens* AM1 genes for several periplasmic proteins have been sequenced, and in all cases, leader sequences are present that have normal structures (Fig. 5) (1, 17). When these are expressed in *E. coli*, substantial processing occurs (2, 6). Data from T7 expression experiments and immunoblotting experiments also suggest that the MADH large subunit has a leader sequence that is capable of being processed in *E. coli* cells (6). Therefore, it appears that this structure is unusual in *M. extorquens* AM1, even within the MADH system. The other unusual leader sequence that has been mentioned, that of the small subunit of the hydrogenase from *D. vulgaris* (8, 24), has been proposed to take part in a novel secretion pathway. The large subunit of this hydrogenase does not have a recognizable leader sequence, and evidence has been obtained that the secretion of the large subunit is dependent upon the presence of the small subunit, perhaps through a novel mechanism involving the unusual leader peptide (22). In MADH, this is not the case, since the large subunit appears to have a normal leader peptide. However, it may be

possible that the uncommon structure of the MADH small-subunit leader also reflects a novel transport mechanism, which could be connected with the unique structure of the MADH cofactor. The leader may be functioning somehow in the protection of the cofactor during transport through the inner membrane. Alternatively, it is possible that the role of this structure is to prevent cotranslational secretion of the small-subunit polypeptide, causing some temporary delay in transport important for cofactor synthesis. Future work will be required to address these unanswered questions.

**Nucleotide sequence accession number.** The nucleotide sequence presented in this article has been deposited in the GenBank data base under accession number M57963.

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