Biochem. J. (1989) 260, 857–862 (Printed in Great Britain)

The second subunit of methanol dehydrogenase of *Methylobacterium extorquens* AM1

David N. NUNN,* Darren DAY and Christopher ANTHONY†
Department of Biochemistry, University of Southampton, Southampton SO9 3TU, U.K.

The nucleotide and deduced amino acid sequence of a novel small (β) subunit of methanol dehydrogenase of *Methylobacterium extorquens* AM1 (previously *Pseudomonas* AM1) has been determined. Work with the whole protein has shown that is has an $\alpha_2\beta_2$ configuration.

INTRODUCTION

Methanol dehydrogenase (MDH) is a soluble periplasmic quinoprotein dehydrogenase whose primary electron acceptor is a specific novel *c*-type cytochrome called cytochrome c_L (Anthony, 1982, 1986). Most MDHs consist of identical dimers of about 60 kDa, each subunit carrying one non-covalently bound pyrrolo-quinoline quinone (PQQ) prosthetic group (Anthony, 1982). It has been suggested, however, that what was previously assumed to be a small contaminating protein might be a subunit of the enzyme, only dissociating under extreme conditions (Ohta & Tobari, 1981; Janssen *et al.*, 1987; Elliott & Anthony, 1988).

It has previously been shown that the structural genes for MDH (*moxF*) and cytochrome c_L (*moxG*) map very closely on the chromosome of *Methylobacterium extorquens* AM1 (previously *Pseudomonas* AM1) (Nunn & Lidstrom, 1986*a*,*b*). In order to study this putative *moxFG* operon in more detail, a system for expressing these genes in *Escherichia coli* has been developed which uses a dual plasmid bacteriophage T7 RNA polymerase/promoter. A DNA fragment containing *moxFG* was subcloned into the T7 expression vector, and the polypeptides encoded by this region of DNA subsequently expressed and labelled (Anderson & Lidstrom, 1988); the conclusions from this work are as follows. Four genes were identified and tentatively designated *moxF* (coding for a polypeptide of 60 kDa), *moxJ* (30 kDa), *moxG* (20 kDa) and *moxI* 12 kDa), the sequence of genes being in the order given (*FJGI*). The identity of the polypeptide coded by *moxJ* could not be determined, but the polypeptide encoded by *moxI* reacted with antibody raised to MDH and its relative mobility on SDS/PAGE corresponded to that of the MDH-associated peptide (Anderson & Lidstrom, 1988).

We have recently sequenced the *moxG* gene coding for cytochrome c_L in *M. extorquens* AM1 (Nunn & Anthony, 1988*a*). The present paper describes the nucleotide sequence of the adjacent *moxI* gene and shows that the predicted amino acid sequence corresponds to that determined for the MDH-associated peptide after its dissociation from the holo-MDH. Our results confirm that this is a second subunit of MDH and demonstrate that MDH exists in an $\alpha_2\beta_2$ conformation. A preliminary report of the DNA sequence of *moxI* has been published previously (Nunn & Anthony, 1988*b*).

METHODS

DNA sequencing

All the methods for construction of plasmids, generation of nested deletions and DNA sequencing were exactly as described previously (Nunn & Anthony, 1988*a*,*b*). As previously, plasmids were selected for sequencing so that each length of DNA overlapped others by at least 50 bp and so that the sequence could be fully determined in both senses of the DNA. The gene coding for the small (β) subunit of MDH described in the present paper was on the same 2.2 kb fragment as that containing the gene for cytochrome c_L; the whole sequence has been submitted to the European Molecular Biology Laboratory Sequence Data Library (EMBL) under the accession no. XO7856. Translation and analyses of DNA and DNA-derived protein sequences were done using the PC/Gene computer program marketed by Genofit S.A., Geneva, Switzerland.

Purification of MDH

M. extorquens AM1 was grown on methanol, harvested, broken by ultrasonication, and soluble protein fractions prepared as previously described (O'Keeffe & Anthony, 1980*b*). The soluble protein fraction from about 15 g wet weight of bacteria was passed down a column (24 mm × 130 mm) of DEAE-Sepharose Fast Flow (Pharmacia) equilibrated in 20 mM-Tris/HCl (pH 8.0). The MDH did not absorb and was immediately transferred to a hydroxyapatite column (16 mm × 100 mm) equilibrated in the same Tris buffer. The column was washed with 25 mM-phosphate buffer, pH 7.0, and MDH was eluted with 90 mM-phosphate buffer, pH 7.0. The active fractions were pooled, concentrated on a 50 kDa cut-off membrane in an Amicon concentrating cell, and purified by gel filtration on a Pharmacia Superose-12 column equilibrated in 20 mM-Mes buffer, pH 5.5. Active fractions were stored at −20 °C. When pure MDH was required for sequencing, it was purified by cation-exchange chromatography on a 1 ml Pharmacia Mono-S column equilibrated in 20 mM-Mes buffer, pH 5.5.

Abbreviations used: MDH, methanol dehydrogenase; PTH, phenylthiohydantoin; PITC, phenyl isothiocyanate; OPA, *o*-phthaldialdehyde.

* Present address: Department of Microbiology, School of Medicine, University of Washington, Seattle, WA 98195, U.S.A.
† To whom correspondence and reprint requests should be sent.

After the column had been washed with the same buffer, MDH was eluted with this buffer containing 100 mM-NaCl.

SDS/polyacrylamide-gel electrophoresis

This was done at pH 8.3 using the SDS/Tris/glycine system and protein was stained with Coomassie Brilliant Blue R250 as described by Weber & Osborn (1975), with a final acrylamide concentration of 15%. The proportion of each protein in electrophoresed samples was determined by peak integration using a Chromoscan-3 gel scanner (Joyce–Loebel). Molecular-mass standards were the BDH Kit no. 44264-2L (M_r 12300–78000) and Pharmacia polypeptide molecular-mass kit (M_r 1695–17201).

Protein sequencing

Protein sequencing was done using an Applied Biosystems 470A 'gas phase' (pulsed liquid) protein sequencer coupled to a model 120 phenylthiohydantoin (PTH)-derivative analyser.

For analysis of the undissociated MDH, fractions were taken direct from the Mono-S column and applied to the sequencer (100–300 pmol of MDH). When two cycles of treatment with phenyl isothiocyanate (PITC) were used before the cleavage reaction, the *N*-terminal amino acids of both subunits reacted equally. When only one cycle of PITC treatment was used, however, the *N*-terminal amino acid of the small (β) subunit reacted incompletely. This resulted in a 'staggered' sequencing in which each cycle of the Edman degradation gave all of the amino acid derivative of the α-chain and a mixture of two adjacent amino acid derivatives from the β-chain. This permitted a simple identification of which amino acids were derived from the two chains and permitted co-sequencing of the first 14 residues. Sequencing beyond this was achieved by sequencing the whole MDH after two PITC treatment cycles for 14 cycles of automated Edman degradation. This was followed by treatment with *o*-phthaldialdehyde (OPA) as described in the Applied Biosystems instruction manual. This blocked all *N*-terminal residues, except for prolyl-, to Edman degradation, thus allowing the further sequencing up to 28 residues of the β-chain; this was possible because the OPA reagent did not react with the proline at position 15 on the β-chain. By using a second sample, the whole MDH was treated with OPA after 16 cycles of the automated Edman degradation. Unexpectedly, there was a marked decrease in yield of the β-chain after this reaction, and so the chains could be distinguished, and the α-chain readily sequenced up to residue 26.

For analysis of the α- and β-subunits separately, these were first separated by gel filtration. MDH from the Mono-S purification step was concentrated with an Amicon Centricon-30 concentrator to 200 μl, which contained 3–4 mg of protein. This was incubated at 80 °C for 20 min in 2.0% SDS in 50 mM-Tris/HCl, pH 8.0, followed by gel filtration on a Pharmacia Superose-12 column equilibrated in 50 mM-Tris/HCl, pH 8.0, containing 0.1 M-NaCl and 0.2% SDS. The elution profile was very similar to that shown in Fig. 2. The fractions containing the large (α) and small (β) subunits were collected separately, precipitated with acetone (90%, v/v, final concn.), and washed with water. The precipitate was resuspended in water and directly applied to the sequencer, using a sufficient amount to provide 100–300 pmol of protein.

RESULTS AND DISCUSSION

DNA sequence of the *moxI* gene

The DNA sequence of the *moxI* gene is shown in Fig. 1 together with the deduced amino acid sequence. The first triplet (TGA) of the sequence presented in Fig. 1 is the termination triplet of the adjacent gene (*moxG*), which codes for cytochrome c_L (Nunn & Anthony, 1988*a*). For comparison with possibly related organisms that might be used for future molecular biology of methylotrophs and related bacteria, the codon usage is presented in Table 1. As in other bacterial genes, rare codons such as AGA, AGG and ATA are avoided, and there is a clear bias in this organism against the A/T ending in triplets: about 91% of codons terminate in G or C. A similar high proportion of codons terminating in C or G has been noted in the large subunit of MDH sequenced in a closely related methylotroph (Machlin & Hanson, 1988) and in structural genes of *Rhodobacter*

```
                                                    1422-TGATCCTTCGGGGTTTCGGGCCGGGCTCGAA
1153  CCGGCGCGATCGAGGGGGCGGGATCGCCGCTCGCGACAAGTCCCAAGAAGAGCAAGTCC

1512  CAT AAA GTG GAG GAA ACC ATG AAG ACC ACT CTC ATC GCC GCC GCC
 -22                          MET Lys Thr Thr Leu Ile Ala Ala Ala

1557  ATC GTC GCC CTG TCC GGC CTC GCC GCC CCG GCG CTC GCC TAT GAC
 -13  Ile Val Ala Leu Ser Gly Leu Ala Ala Pro Ala Leu Ala Tyr Asp

1602  GGC ACC AAG TGC AAG GCC GCG GGC AAT TGC TGG GAG CCG AAG CCC
   3  Gly Thr Lys Cys Lys Ala Ala Gly Asn Cys Trp Glu Pro Lys Pro

1647  GGC TTC CCC GAG AAG ATC GCC GGC TCC AAG TAC GAT CCC AAG CAC
  18  Gly Phe Pro Glu Lys Ile Ala Gly Ser Lys Tyr Asp Pro Lys His

1692  GAT CCC AAG GAG CTG AAC AAG CAG GCC GAT TCC ATC AAG CAG ATG
  33  Asp Pro Lys Glu Leu Asn Lys Gln Ala Asp Ser Ile Lys Gln Met

1737  GAA GAG CGC AAC AAG AAG CGT GTC GAG AAC TTC AAG AAG ACC GGC
  48  Glu Glu Arg Asn Lys Lys Arg Val Glu Asn Phe Lys Lys Thr Gly

1782  AAG TTC GAA TAC GAC GTC GCC AAG ATC TCG GCG AAC TGATCCGCTGAG
  63  Lys Phe Glu Tyr Asp Val Ala Lys Ile Ser Ala Asn

1830  GCTCCGCCTCTCCCCTTCGGATCTCGGACCTGTCCGGGATCCGTCCTGACCTGCCGGATC

1890  GGGCAGGCCCGATCCGGAGCGGGAGGGGCGGACACCGCGTGAGGGCGCTCCCAACGACAA

1950  GAAAAAGGCCAACCGCCGAATGAACACCTTGCGCCCCGGCGACGCCATGCTCACCGACTG

2010  GCGGGACGCGGCCGCGGCGTTTCGAGCGCGAGATCGCCAAGGCCGTCGTCGGTCAGGACCG

2070  GGCGATCCGCCTGCTGACGATCGCGATTTTCGCCCGCGGCCACGTCATGCTCGAAGGCGA

2130  TGTCGGCGTCGGCAAGACCACGCTGCTGCGCGCGGTCGCCCCCGGCCTCGGCGGCGCCTA

2190  CGAGCGCGTCGAGGGCACCGTC
```

Fig. 1. Nucleotide sequence and deduced amino acid sequence of the gene for the small subunit of methanol dehydrogenase (*moxI*)

The *moxI* gene coding region extends from 1531 to 1817. The numbering system is that used in describing the complete 2.2 kb fragment the sequence of which has been submitted to the EMBL (accession no. X07856). The first triplet (TGA) of the sequence presented above is the termination triplet of the adjacent gene (*MoxG*) which codes for cytochrome c_L (Nunn & Anthony, 1988*a*). The following are underlined: a ribosome-binding site (bases 1515–1527); a potential hairpin loop (transcription terminator) (bases 2011–2032); and the *N*-terminal region as sequenced by direct protein sequencing. The deduced signal peptide (amino acids −12 to −1) is presented in italics.

Table 1. Codon usage in the genes for small subunit of MDH (*moxI*) and for cytochrome c_L (*moxG*) of *Methylobacterium extorquens* compared with usage in *Rhodobacter* and *Paracoccus*

Column 1 is usage for the β-subunit of MDH of *M. extorquens* (present work); column 2 is usage for cytochrome c_L of *M. extorquens* (Nunn & Anthony, 1988*a*); column 3 is usage for the gene for subunit II of cytochrome oxidase of *Paracoccus denitrificans* (Steinrucke *et al.*, 1987); column 4 is usage for the *pet* operon coding for the cytochrome bc_1 complex of *Rhodobacter capsulatus* (Davison & Daldal, 1987).

Column...	1	2	3	4		1	2	3	4
TIT Phe	0	0	1	3	TCT Ser	0	0	0	2
TTC Phe	3	5	12	48	TCC Ser	3	4	3	6
TTA Leu	0	0	0	0	TCA Ser	0	0	0	0
TTG Leu	0	0	0	0	TCG Ser	1	2	5	24
CIT Leu	0	2	2	10	CCT Pro	0	0	0	0
CTC Leu	3	10	1	18	CCC Pro	4	4	6	8
CTA Leu	0	1	0	0	CCA Pro	0	0	0	0
CTG Leu	2	7	24	48	CCG Pro	2	8	14	49
ATT Ile	0	2	1	2	ACT Thr	0	0	0	1
ATC Ile	5	5	18	50	ACC Thr	3	8	8	39
ATA Ile	0	0	0	0	ACA Thr	0	0	0	0
ATG MET	2	5	7	33	ACG Thr	0	8	4	9
CTT Val	0	0	2	4	GCT Ala	0	0	0	0
GTC Val	3	3	14	35	GCC Ala	11	11	21	50
GTA Val	0	1	0	0	GCA Ala	0	1	2	3
GTG Val	0	5	17	37	GCG Ala	3	3	17	37
TAT Tyr	1	1	0	9	TGT Cys	0	0	0	0
TAC Tyr	2	5	4	28	TGC Cys	2	4	4	8
TAA ---	0	0	—	—	TGA ---	0	0	0	3
TAG ---	0	0	—	—	TGG Trp	1	3	7	27
CAT His	0	0	2	4	CGT Arg	1	0	1	4
CAC His	1	4	4	19	CGC Arg	1	4	6	25
CAA Gln	0	2	2	1	CGA Arg	0	0	0	0
CAG Gln	2	7	10	15	CGG Arg	0	1	1	5
AAT Asn	1	3	2	1	AGT Ser	0	1	0	0
AAC Asn	4	8	7	29	AGC Ser	0	0	5	5
AAA Lys	0	0	3	6	AGA Arg	0	0	0	0
AAG Lys	16	13	8	29	AGG Arg	0	0	0	1
GAT Asp	3	1	4	14	GGT Gly	0	0	0	8
GAC Asp	2	10	12	37	GGC Gly	6	20	11	66
GAA Glu	2	2	3	20	GGA Gly	0	1	1	1
GAG Glu	5	9	11	15	GGG Gly	0	3	3	8

capsulatus (Davidson & Daldal, 1987) and *Paracoccus denitrificans* (Harms *et al.*, 1987; Kurowski & Ludwig, 1987; Steinrucke *et al.*, 1987) (Table 1). Together with other data, this has led Steinrucke *et al.* (1988) to suggest that these two organisms are in the same (α-3) division of the Eubacterial Kingdom (Woese, 1987); this division may well include, therefore, the pink facultative methylotrophs such as *Methylobacterium extorquens*. This suggestion is further supported by observations on the signal peptides of the three genera (Nunn & Anthony, 1988*a*).

The DNA sequence between the end of the protein-coding region and the end of the 2.2 kb fragment is presented in Fig. 1 in order to illustrate the possible presence of a hairpin loop (bases 2011–2032) which may be involved in termination of translation.

Protein sequence of the small (β) subunit of MDH

The chosen reading frame for *moxI* is the same as that for the cytochrome c_L gene (*moxG*), which is encoded upstream on the same 2.2 kb fragment (Nunn & Anthony, 1988*a*).

The deduced signal peptide is shown in italics in Fig. 1. It starts with a methionine residue, coded by ATG, which is 10 bp downstream from a typical ribosome-binding site (the Shine–Dalgarno sequence underlined in Fig. 1). Possession of a signal sequence is consistent with the known periplasmic location of methanol dehydrogenase (Alefounder & Ferguson, 1981; Anthony, 1988; Ferguson, 1988). The signal peptide is typical of Gram-negative bacteria, having a positively charged residue (lysine) near the *N*-terminus which is probably involved in binding to the negatively charged outer phospholipids of the periplasmic membrane. This is followed by a stretch of hydrophobic residues, essential for transport of the protein across the membrane, and the peptide terminates in the peptidase recognition and cleavage site which has previously been shown to be very similar to that of the signal peptide of cytochrome c_L of this organism and the cytochrome c_2 of *Rhodobacter sphaeroides* (Nunn & Anthony, 1988*a*). In order to determine whether or not this 'specific' peptide cleavage site precludes the processing of these proteins in *E. coli*, it will be necessary for their transcriptional promoters to be recognized; for the only protein investigated so far (the MDH α-subunit of *Methylobacterium organophilum*), this was not the case (Machlin & Hanson, 1988).

The M_r of the deduced mature protein (excluding signal peptide), namely 8463, is consistent with the value of about 9000 for the small protein always observed on SDS/polyacrylamide-gel electrophoresis (Elliott & Anthony, 1988); it also corresponds (approximately) to the size of the small protein coded for by the fragment of DNA sequenced in this work and used in a coupled *in vivo* T7 RNA polymerase/promoter gene-expression system from *Escherichia coli* (Anderson & Lidstrom, 1988). In this system the small protein was detected using antibodies raised to pure native MDH; it did not react with antibodies to the large subunit alone (obtained by electroelution of electrophoresed MDH). It should be noted that it is not possible to separate the small protein from MDH by conventional gel-filtration or ion-exchange chromatography, even in the presence of high concentrations of salt (e.g. 1.0 M-NaCl).

That the (deduced) protein coded by the *moxI* gene corresponds to that of the small MDH-associated protein was demonstrated by direct protein sequencing after its separation from the large subunit by gel filtration in SDS on a Superose-12 column; this provided the sequence of the first ten amino acid residues. It was also possible to determine the sequences of the next 18 residues by using whole undissociated enzyme by modifying the parameters used in sequencing (see the Methods section). The protein sequence as determined by these methods (shown underlined on Fig. 1) corresponded perfectly with the deduced amino acid sequence. This sequence shows no similarity to that of any protein in the EMBL databases.

The deduced amino acid sequence gives the amino acid composition (residues/molecule) as follows: Ala, 6; Arg, 2; Asn, 5; Asp, 5; Cys, 2; Gln, 2; Glu, 7; Gly, 5; His, 1; Ile, 3; Leu, 1; Lys, 15; Met, 1; Phe, 3; Pro, 5; Ser, 3; Thr, 2; Trp, 1; Tyr, 3; Val, 2.

Primary sequence of the large (α) subunit of MDH

The DNA sequence of the large (α) subunit of MDH from *M. extorquens* is not available. For this reason, our

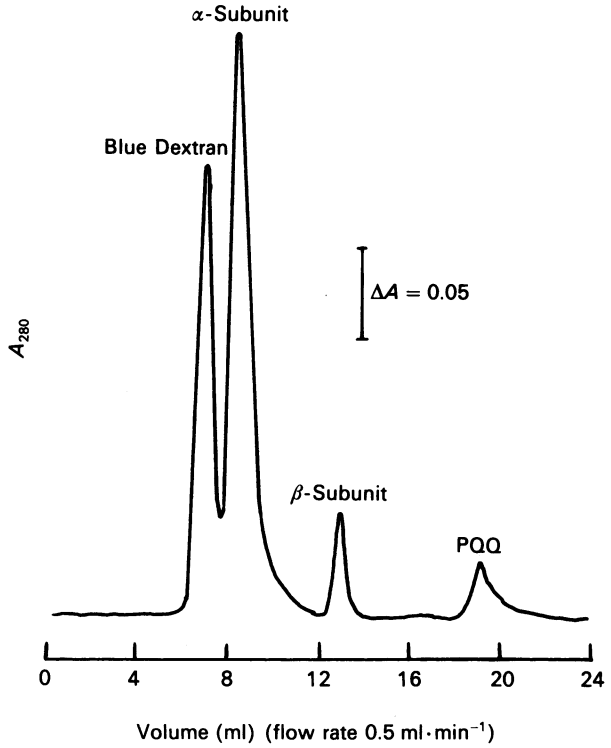

Fig. 2. Dissociation of MDH into its prosthetic group (PQQ) and α- and β-subunits

Pure MDH was separated into its component parts by gel filtration on a Pharmacia Superose-12 column in 6 M-guanidinium chloride in 50 mM-Tris/HCl, pH 8.0, as described in the Methods section. In the example shown in the Figure, Blue Dextran was included for information, but this was omitted when the fractions were to be collected or measured.

available information on the protein sequence of the α-subunit is presented here.

The sequence of the first five amino acids of the *N*-terminal region of the large (α) subunit MDH was determined after its separation from the whole enzyme by gel filtration in SDS as described in Fig. 2. The sequence of 24 of the first 26 residues was also determined using the whole enzyme as described in the Methods section.

This sequence is presented below, together with the *N*-terminal sequences of the large subunit of MDHs determined for the closely related pink facultative methylotroph *Methylobacterium organophilum* XX (Machlin & Hanson, 1988) and from the facultative autotroph *Paracoccus denitrificans* (Harms *et al.*, 1987). There is clearly a very high degree of sequence similarity in this region of the α-chain of MDH:

	1	2	3	4	5	6	7	8	9	10	11	12	13
M. extorquens AM1	Asn	Asp	Lys	Leu	Val	Glu	Leu	Ser	Lys	Ser	Asp	Asp	Asn–
M. organophilum XX	Asn	Asp	Lys	Leu	Val	Glu	Leu	Ser	Lys	Ser	Asp	Asp	Asn–
P. denitrificans	Asn	Asp	Gln	Leu	Val	Glu	Leu	Ala	Lys	Gly	Pro	Ala	Asn–
	14	15	16	17	18	19	20	21	22	23	24	25	26
M. extorquens AM1	–Trp	Val	Met	Pro	Gly	Lys	Asn	Tyr	Asp	Ser	Xaa	Xaa	Phe–
M. organophilum XX	–Trp	Val	Met	Pro	Gly	Lys	Asn	Tyr	Asp	Ser	Asn	Asn	Tyr–
P. denitrificans	–Trp	Val	Met	Thr	Gly	Arg	Asp	Tyr	Asn	Ala	Gln	Asn	Tyr–

Subunit structure of MDH

The results described above are all consistent with the conclusion that MDH consists of an α-subunit of 62 kDa and a β-subunit, tightly bound to it, of about 8.5 kDa, the genes for these two subunits (*moxF and moxI*) being very close on the bacterial chromosome. They are separated by *moxJ* (coding for a 30 kDa protein of unknown function) and *moxG*, which codes for the specific electron acceptor for MDH (cytochrome c_L).

The only methods found so far for separating the two subunits have involved the use of SDS or high concentrations of guanidinium chloride. After SDS/15% (w/v)-polyacrylamide-gel electrophoresis and staining with Coomassie Blue R250, the molar ratio of the two subunits was estimated by peak integration of the scans taken with a Joyce–Loebl gel scanner. The average ratio (α/β) was 1:1.23 (for five runs with two different preparations of MDH; range 1:1.17–1.36).

Fig. 2 shows the separation of the two subunits and the prosthetic group (PQQ) by gel filtration on a Pharmacia Superose-12 column in 6 M-guanidinium chloride in 50 mM-Tris/HCl, pH 8.0. Integration of the A_{280} peak heights indicated that the subunits exist in the molar ratio (α/β) 1:0.87 (average of four runs of two separate preparations; range 1:0.76–0.95).

As described above, it was possible to determine part of the protein sequence of both subunits using the whole MDH. This procedure enabled us to determine the ratios of the two subunits (see Raftery *et al.*, 1980; Miller *et al.*, 1988). The molar ratio (α/β) of the subunits, based on the amounts of the first ten PTH-derivatized amino acids, was 1:1.24. This method is based on calibration of the sequencer with standard derivatized amino acids. An alternative method, which limits the number of assumptions related to this method, involved comparison of the amounts of asparagine and lysine in the α-chain with the amounts of these amino acids in the β-chain (thus in effect using an internal standard). The molar ratio (α/β) determined by this method was 1:1.18 (using asparagine) and 1:1.23 (using lysine).

In conclusion, as determined by the three methods used here, the molar ratio (α/β) was approx. 1:1 (1:1.25; 1:0.87 and 1:1.12). If the native M_r is about 120000 as measured by gel filtration (O'Keeffe & Anthony, 1980*a*), then the methanol dehydrogenase must exist in an $\alpha_2\beta_2$ conformation. If the molecular masses of the two subunits are assumed to be 60 kDa and 8.5 kDa then the total M_r is 137000, a value that is clearly greater than 120000. This discrepancy is even greater if it is assumed that the M_r of the α-subunit is about 66000 as indicated by the consideration of the gene-derived sequence of the α-subunits of MDH from the closely related methylotroph *M. organophilum* XX (Machlin & Hanson, 1988). The expected value of 149000 (i.e. 2×66000 plus 2×7500) is, however, very close to the value for relative molecular mass of 146000 first measured for MDH of

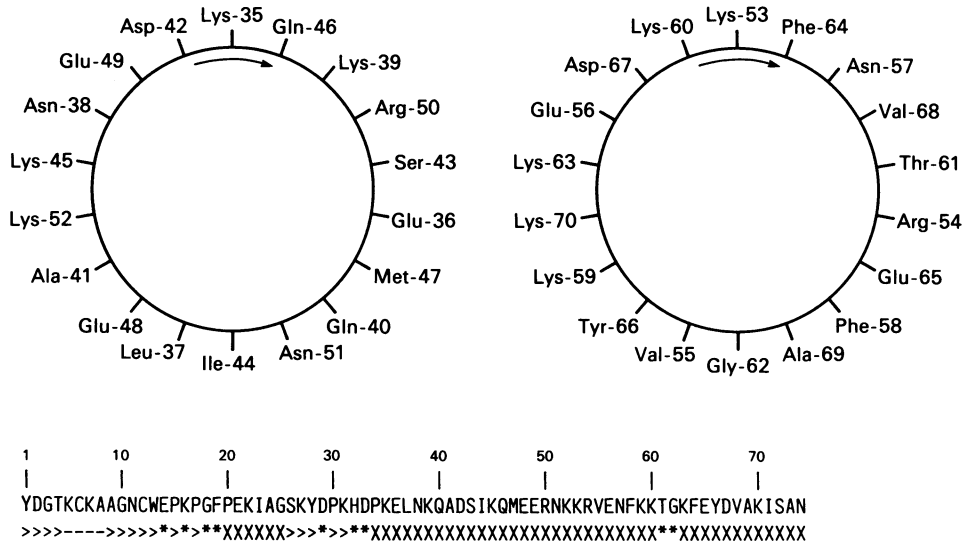

Fig. 3. Predicted secondary structure of the β-subunit

The semi-graphical representation of the secondary structure as predicted by the method of Garnier *et al.* (1978) is given: X, helical conformation; >, turn conformation; −, extended conformation (β-strand); *, random-coil conformation. The potential helical region is presented as two helical wheels in order to demonstrate the amphipathic potential of this region and to illustrate the asymmetrical distribution of lysine residues; this asymmetric distribution would, of course, still occur if there were more than two helices between residues 35 and 70.

M. extorquens using the more direct method of analytical centrifugation (Anthony & Zatman, 1967).

General discussion

The results presented above demonstrate that MDH consists of two types of subunit arranged in an $\alpha_2\beta_2$ configuration. The reason that the small β-subunit has been overlooked for so many years is probably because it gives a weakly staining band on SDS/polyacrylamide-gel electrophoresis and because small proteins are often run off the gels along with the dye front when gels with lower concentrations of acrylamide (7–10%) are used.

If the α-subunit has any specific definable function it is clearly of interest to consider whether this involves binding the substrate, activator, electron acceptor (cytochrome c_L) or prosthetic group. In this context the exceptionally high proportion of lysine residues in the β-subunit and their position in the predicted secondary structure of the subunit is likely to be relevant. The secondary structure [predicted by the method of Garnier *et al.* (1978)] is given in Fig. 3. The β-chain has two distinguishable regions. The conformation of the first 34 amino acids is predominantly non-helical, and the remainder of the chain is able to adopt a helical conformation. The representation of the potential helical region in Fig. 3 shows that this sequence is able to form amphipathic helices, with lysine residues constituting a well-defined region on one side, and hydrophobic regions on the other. It is not possible to determine the number of separate helices that might be adopted, but it is clearly likely to be at least two.

We thank Lawrence Hunt, and the Protein Sequencing Unit of the Institute of Biomolecular Sciences at the University of Southampton, for help with the protein sequencing. This research was supported by the Science and Engineering Research Council (U.K.) and the National Institutes of Health (U.S.A.).

REFERENCES

Alefounder, P. R. & Ferguson, S. J. (1981) Biochem. Biophys. Res. Commun. **98**, 778–784
Anderson, D. J. & Lidstrom, M. E. (1988) J. Bacteriol. **170**, 2254–2262
Anthony, C. (1982) The Biochemistry of Methylotrophs, Academic Press, London
Anthony, C. (1986) Adv. Microbial Physiol. **27**, 113–210
Anthony, C. (1988) in Bacterial Energy Transduction (Anthony, C., ed.), pp. 293–316, Academic Press, London
Anthony, C. & Zatman, L. J. (1967) Biochem. J. **104**, 953–959
Davidson, E. & Daldal, F. (1987) J. Mol. Biol. **195**, 13–24
Elliott, J. E. & Anthony, C. (1988) Biochem. J. **134**, 369–371
Ferguson, S. J. (1988) in Bacterial Energy Transduction (Anthony, C., ed.), pp. 151–182. Academic Press, London
Garnier, J., Osguthorpe, D. J. & Robson, B. (1978) J. Mol. Biol. **120**, 97–120
Harms, N., de Vries, G. E., Maurer, K., Hoogendijk, J. & Stouthamer, A. H. (1987) J. Bacteriol. **169**, 3969–3975
Janssen, D. B., Kenning, S. & Witholt, B. (1987) J. Gen. Microbiol. **133**, 85–92
Kurowski, B. & Ludwig, B. (1987) J. Biol. Chem. **262**, 13805–13811
Machlin, S. M. & Hanson, R. S. (1988) J. Bacteriol. **170**, 4739–4747
Miller, M. J., Hermodson, M. & Gennis, R. B. (1988) J. Biol. Chem. **263**, 5235–5240
Nunn, D. N. & Anthony, C. (1988*a*) Biochem. J. **256**, 673–676
Nunn, D. N. & Anthony, C. (1988*b*) Nucleic Acids Res. **16**, 7722
Nunn, D. N. & Lidstrom, M. E. (1986*a*) J. Bacteriol. **166**, 581–590
Nunn, D. N. & Lidstrom, M. E. (1986*b*) J. Bacteriol. **166**, 591–597
Ohta, S. & Tobari, J. (1981) J. Biochem. (Tokyo) **90**, 215–224
O'Keeffe, D. T. & Anthony, C. (1980*a*) Biochem. J. **192**, 411–419
O'Keeffe, D. T. & Anthony, C. (1980*b*) Biochem. J. **192**, 481–484

Raftery, M. A., Hunkapiller, M. W., Strader, C. D. & Hood, L. E. (1980) Science **208**, 1454–1456
Steinrucke, P., Steffens, G. C. M., Panskus, G., Buse, G. & Ludwig, B. (1987) Eur. J. Biochem. **167**, 431–439
Steinrucke, P., Gerhus, E. & Ludwig, B. (1988) Proc. Eur. Bioenerg. Conf. 5th 142
Weber, K. & Osborn, M. (1975) Proteins 3rd Ed. **1**, 179–223
Woese, C. R. (1987) Microbiol. Rev. **51**, 221–271

Received 10 November 1988/5 April 1989; accepted 13 April 1989