Sequence of the Sodium Ion Pump Methylmalonyl-CoA Decarboxylase from Veillonella parvula*

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The genes encoding methylmalonyl-CoA decarboxylase from Veillonella parvula were cloned on plasmids using oligonucleotides derived from N-terminal amino acid sequences as specific probes. The entire DNA sequence of the methylmalonyl-CoA decarboxylase genes together with upstream and downstream regions was determined. The genes encoding subunits α (mmdA), δ (mmdE), γ (mmdC), and β (mmdB) of the decarboxylase were clustered on the chromosome in the given order. The previously unnoted ε-chain (M, 5,888) was clearly shown to be a subunit of the decarboxylase by correspondence of the N-terminal amino acid sequence with that deduced from the DNA sequence of mmdE. The α-subunit was 60% identical with the carboxyltransferase domain of rat liver propionyl-CoA carboxylase, the β-subunit showed 61% sequence identity with the β-subunit of oxaloacetate decarboxylase from Klebsiella pneumoniae, and the biotin-containing γ-subunit was 29-39% identical with biotin-domains of other biotin enzymes. The δ-subunit of methylmalonyl-CoA decarboxylase and the γ-subunit of oxaloacetate decarboxylase did not show significant sequence homology. The gross structure of both proteins, however, was similar, consisting of a hydrophilic membrane anchor near the N terminus, a proline/alanine linker, and a remarkable accumulation of charged amino acids in the C-terminal part. The sequence of the small ε-subunit could be aligned to the C-terminal region of the δ-subunit downstream of the proline/alanine linker, where the two subunits were 47% identical. Of considerable interest for the mechanism of Na⁺ transport are the long stretches of complete sequence identity between the hydrophilic β-subunits of methylmalonyl-CoA decarboxylase and oxaloacetate decarboxylase and the presence of two conserved aspartic acid residues within putative membrane-spanning helices.

In Veillonella parvula (previously named Veillonella alcalescens) the free energy of methylmalonyl-CoA decarboxylation is conserved by conversion into an electrochemical gradient of Na⁺ ions (1, 2). The responsible methylmalonyl-CoA decarboxylase is a membrane-bound biotin-containing Na⁺ pump (3, 4). The enzyme shares a number of properties with the other members of the family of Na⁺-transporting decarboxylases: oxaloacetate decarboxylase and glutaconyl-CoA decarboxylase (1, 2). All these decarboxylases contain a peripheral membrane-bound subunit of M, 60,000-65,000 that catalyzes a carboxyl transfer from the substrate to the prosthetic biotin group. In methylmalonyl-CoA decarboxylase (3) and glutaconyl-CoA decarboxylase (5), the biotin is bound to a separate biotin carrier protein subunit of apparent M, 18,000-19,000, while oxaloacetate decarboxylase contains the biotin on the C-terminal domain of the α-subunit (6, 7). In addition, all Na⁺ transport decarboxylases contain a highly hydrophilic subunit (β) that migrates as a polypeptide of M, 32,000-33,000 on SDS gels (3, 5, 8). The true molecular weight of the β-subunit of oxaloacetate decarboxylase from Klebsiella pneumoniae and Salmonella typhimurium as derived from the DNA sequence, however, is 45,000 (7). The β-subunit is supposed to contain several membrane-spanning domains (7, 9). Its function probably is the catalysis of the decarboxylation of the carboxylated biotin carrier and coupled Na⁺ translocation across the membrane (1, 2, 8). Oxaloacetate decarboxylase contains a third subunit (γ) of M, 8,900, which probably contains one transmembrane α-helix in its N-terminal region and a more hydrophilic C-terminal part that could extend into the aqueous phase (7, 9). The fourth subunit found in methylmalonyl-CoA decarboxylase (3) and glutaconyl-CoA decarboxylase (5) (δ) could be related to the oxaloacetate decarboxylase γ-subunit. The function of this subunit is not yet known.

The sequence of the N-terminal domain of the oxaloacetate decarboxylase α-subunit revealed striking homology to the S subunit of transcarboxylase from Propionibacterium shermanii that catalyzes the same carboxyl-transfer reaction (6, 7). The C-terminal biotin-containing domain strongly resembled the biotin-containing subunits of transcarboxylase and other biotin-containing enzymes. An extended stretch of mostly alanine and proline residues in the N-terminal part of the biotin domain could provide the α-subunit with the flexibility required for the flip-flop movement of the prosthetic biotin group between the catalytic centers of the carboxyltransferase (N-terminal domain of the α-subunit) and the lyase (probably on the β-subunit). Neither the β- nor the γ-subunit of oxaloacetate decarboxylase was homologous to any known sequence (6, 7).

In our efforts to elucidate structure and function of the sodium ion transport decarboxylases, we have cloned and sequenced the genes encoding the methylmalonyl-CoA decarboxylase from V. parvula. A comparison of the deduced amino acid sequences with those of the oxaloacetate decarboxylase provides insights into potentially important amino acids, notably within the membrane-bound β-subunits.

EXPERIMENTAL PROCEDURES

Materials—V. parvula (ATCC 17745) was grown anaerobically on sodium lactate as described (4). Methylmalonyl-CoA decarboxylase
was purified from *V. parvu* by affinity chromatography on a monomeric avidin-Sepharose column (10). Restriction enzymes were from Boehringer Mannheim (Mannheim, Federal Republic of Germany). Oligonucleotides were custom-synthesized by Microsynth (Windisch, Switzerland). The plasmids pBluescript KS(+) and pBR322 and the host strain *Escherichia coli* DH5α were from laboratory stock.

**Amino Acid Sequence Analysis**—A series of methylmalonyl-CoA decarboxylase were separated by SDS-polyacrylamide gel electrophoresis according to the procedure described in Ref. 11 and electroblotted onto a hydrophobic polyvinylidene difluoride membrane (Millipore) (12). Blot staining was performed with 0.1% Coomassie Brilliant Blue G250. The filter pieces with the respective subunits were used directly for N-terminal sequence analyses using a protein sequenator (model 470/A; Applied Biosystems) with on-line phenylthiodyantoin-derivative detection using high performance liquid chromatography (model 120; Applied Biosystems).

Cyanogen bromide fragments were prepared by isolating the SDS gel pieces with the respective subunits and treatment overnight with 500 μl of 70% formic acid and 1 mg of BrCN at 25 °C. The supernatant was removed and the residual peptides were extracted from the gel pieces with treatment twice with 2×0.5 ml of 50% formic acid, 25% acetonitrile, 15% isopropanol, and 10% water for 1 h at 37 °C. The combined supernatant and wash solutions were evaporated to Speed Vac. The cyanogen bromide fragments were separated by SDS-gel electrophoresis, isolated from the gel, and sequenced.

**Southern Analyses/Restriction Map of the mmd Genes**—Chromosomal DNA of *V. parvu* was obtained as described (13). The DNA was digested with different restriction enzymes and the fragments were separated by gel electrophoresis in 0.7% agarose (20×20 cm). The DNA fragments were blotted onto a Hybond N membrane (Amer sham Corp.) and fixed on the matrix by 5 min of irradiation with UV light (320 nm). Restriction fragments were analyzed by Southern hybridization (14) with oligonucleotides 5'-GAYGAYAATGA-3' (oligonucleotide α-1), 5'-GAYGATGAYAAYATGA-3' (oligonucleotide γ-1), and 5'-GAYGCATGAYAATG-3' (oligonucleotide γ-2). Oligonucleotides α and γ-1 were deduced from the N-terminal amino acid sequences of the α- and γ-subunit, respectively, and oligonucleotide γ-2 was based on the highly conserved sequence motif Gla-Ala-Met-Lys-Met, which constitutes the biotin binding site of most known biotin kinases (15). The oligonucleotides were labeled with [γ-32P]dATP and used for hybridization as described (16). The information obtained from these experiments is summarized in the restriction map shown in Fig. 1.

**Cloning Strategy**—For cloning the *mmd* genes we used the information obtained from the restriction analyses (Fig. 1). Chromosomal DNA (100 μg) was digested completely with EcoRI, and the restriction fragments were separated by gel electrophoresis in 0.7% agarose. The desired DNA fragment that hybridized with all three oligonucleotides is about 6.2 kb long (Fig. 1). Therefore, DNA in the range of 6.0-6.5 kb was isolated from the gel by electroelution. The DNA was digested with restriction enzymes *BamHI* and *XbaI*, which do not cut inside the desired *EcoRI* fragment but will reduce the number of false *EcoRI* fragments of the same size. *EcoRI* fragments of 6.0-6.5 kb were subsequently separated from the generated smaller fragments by electrophoresis and isolated from the gel as described above. The isolated *EcoRI* fragment was further digested with *HindIII*, which cuts twice inside the *EcoRI* fragment in sufficient distance to the *EcoRI* sites so that a "pure" *HindIII*/*HindIII* fragment resulted that could be used for cloning part of the *mmd* genes. Isolated *HindIII* fragments of 2.7 kb were cloned into the pBluescript KS(+) vector and transformed into the host strain *E. coli* DH5α (16). The plasmids of 40 recombinant white colonies were isolated (17) and analyzed by hybridization against oligonucleotide γ-1, which yielded 10 positive signals. Cloning of the desired DNA fragment was verified by sequence analysis using oligonucleotide γ-1 as a primer. An amino acid sequence identical to the known N-terminal of the α-subunit was derived from the DNA sequence. The plasmid thus obtained was termed pJH1 (Fig. 1). Also cloned from the chromosomal DNA were the flanking regions of the pJH1 insert. The 2.0 kb *EcoRI/ClaI* fragment inserted into the pBluescript KS(+) and the 1.4 kb *Nhel/EcoRI* fragment (pJH40) were cloned into pBR322 (Fig. 1). Both clones were identified via colony hybridization using the pJH1 insert as a homologous probe. The pJH1 insert was DIG-labeled according to the manufacturer (Boehringer Mannheim).

**DNA Sequence Analysis**—The DNA region of the methylmalonyl-CoA decarboxylase genes was subcloned from plasmids pJH1, pJH20, and pJH40 and sequenced completely on both strands according to the dideoxynucleotide chain termination method described by Sanger et al. (18). Specific oligonucleotide primers were used to complete the sequence.

**Computer Analyses**—Computer analyses of the DNA and protein sequences were performed with the software package from the Genetics Computer Group of the University of Wisconsin (GCC version 7.1) and the PC-Gene program from IntelliGenetics (Geneva, Switzerland). The program TOP-PRED was obtained from G. von Heijne (Stockholm, Sweden).

**RESULTS**

**Cloning of the Structural Genes for Methylmalonyl-CoA Decarboxylase**—N-terminal amino acid sequence analysis of the methylmalonyl-CoA decarboxylase subunits was performed in order to synthesize specific oligonucleotide probes for Southern hybridization and cloning of the methylmalonyl-CoA decarboxylase genes. The N termini of the β- and the γ-subunits were blocked, but those of the α- and the biotin-maintaining γ-subunit should be sequenced and yielded MATVQEKKIEL for α and MKKPNVTVMGATDYVEY-NEVKAA for γ. Since the DNA sequence reported here is the first one of a *Veillonella* species, no information of the codon usage was available. Therefore, all possible codons for a given amino acid had to be considered in the design of the oligonucleotides. The oligonucleotides synthesized corresponded to the N-terminal sequence of the α-subunit (oligonucleotide α-1), to the N-terminal sequence of the γ-subunit (oligonucleotide γ-1), and to the conserved biotin binding motif of the γ-subunit (oligonucleotide γ-2) (see "Experimental Procedures"). All three oligonucleotides hybridized with the same *BamHI*, *EcoRI*, *SacI*, and *XbaI* restriction fragments of *V. parvu* chromosomal DNA, indicating that at least the genes for the α- and γ-subunits form a cluster on the chromosome. Based on the restriction map derived from the hybridization data (Fig. 1), the 6.2-kb *EcoRI* fragment was chosen for cloning the genes for the α- and γ-subunits. However, all attempts to clone this fragment via colony hybridization failed due to the high unspecific background signal obtained with the oligonucleotides. Therefore, a new strategy was developed, including an "enrichment" of the 2.7-kb *HindIII* fragment as described under "Experimental Procedures." Clone pJH1

![Figure 1](link-to-figure)

**Fig. 1. Physical map of the *V. parvu* genome region encoding methylmalonyl-CoA decarboxylase.** The upper part shows a restriction map with those enzymes used for cloning the *mmd* genes on plasmids pJH1, pJH20, and pJH40. The hybridization sites of the oligonucleotides derived from the N termini of the α- and the γ-subunit and from the conserved region of the biotinylation site (γ2-oligo) are marked by asterisks. The enlarged map in the lower part delimits the 4.68-kb DNA region sequenced and shows the cluster of the five *mmd* genes. Restriction sites are abbreviated as follows: A, *ApaI*; B, *BamHI*; C, *ClaI*; D, *EcoRI*; H, *HindIII*; K, *KpnI*; N, *NheI*; P, *PstI*; S, *SacI*, X, *XbaI*.
The nucleotide sequence of methylmalonyl-CoA decarboxylase genes of *V. parula*. The nucleotide sequence starts at the EcoRI site upstream of the *mdA* gene. The putative 370 E. coli promoters and a possible terminator are overlaid. Presumptive ribosome binding sites are underlined. Amino acids confirmed by protein sequencing of subunits or peptides are printed in bold letters. The stop codons are marked by asterisks. The lysine residue of the γ-subunit that becomes biotinylated (Lysε) is indicated by a bold asterisk.
Sequence of Methylmalonyl-CoA Decarboxylase

ORF2 was unequivocally shown to encode the δ-subunit of the methylmalonyl-CoA decarboxylase by sequencing a BrCN fragment of this subunit. The resulting sequence (Fig. 2) was identical to amino acids 87–100 of ORF2, which is therefore named mmdD.

ORF3 started at position 2377 with an ATG and stopped at position 2542 with TAA. This ORF encoded a protein of 55 amino acids with a calculated Mr of 5,888. Hitherto, such a small protein has never been detected in purified methylmalonyl-CoA decarboxylase preparations (3). However, as described below, the protein deduced from ORF3 is actually a fifth subunit of this enzyme and ORF3 is therefore named mmdE.

ORF4 started with an ATG at position 2646 and ended at position 3033 with a stop codon TAA. The deduced N-terminal amino acid sequence, as well as the presence of the biotin-binding-site motif (positions 2919–2933), clearly identified ORF4 as the structural gene for the γ-subunit, which was therefore termed mmdC. mmdC consists of 129 amino acids with a calculated Mr of 12,913. The large discrepancy of this value to the apparent Mr obtained from SDS-polyacrylamide gel electrophoresis (18,500; Ref. 3) probably results from a proline/alanine-rich sequence between residues 22 and 58. Proteins with proline/alanine linkers are known to have reduced mobilities in SDS-polyacrylamide gel electrophoresis (19).

ORF5 started at position 3106 with ATG and stopped at position 4225 with a TAA stop codon. The deduced protein consists of 373 amino acids with a calculated Mr of 38,730. Although not verified by amino acid sequence analysis, the considerable homology to the β-subunit of oxaloacetate decarboxylase from K. pneumoniae and S. typhimurium (Fig. 7) (7) definitely identified ORF4 as the gene for the β-subunit of methylmalonyl-CoA decarboxylase, and it was therefore named mmdB.

Biochemical Verification of a Fifth Subunit of Methylmalonyl-CoA Decarboxylase—As described in the previous section, DNA sequence analysis led to the discovery of a small ORF, located between mmdD and mmdC (Fig. 1). The deduced protein consists of 55 amino acids with a calculated Mr of 5,888. A protein of this size has never been observed in purified preparations of methylmalonyl-CoA decarboxylase (3), presumably because of its small size. Therefore, the decarboxylase subunits were separated on a 16.5% rather than on a 12% SDS-polyacrylamide gel. As shown in Fig. 3, a band of about 6 kDa is visible after silver staining but only if large amounts of protein are loaded on the gel. In order to verify that this band corresponds to the protein deduced from ORF3, the protein band was eluted from the gel and subjected to N-terminal amino acid sequence analysis. In the first cycle, only a weak signal was observed (typical for serine), but no signal for methionine was found. The following cycles yielded the terminal amino acid sequence, as well as the presence of the biotin-binding-site motif (positions 2919–2933), clearly identified ORF4 as the structural gene for the γ-subunit, which was therefore termed mmdC. mmdC consists of 129 amino acids with a calculated Mr of 12,913. The large discrepancy of this value to the apparent Mr obtained from SDS-polyacrylamide gel electrophoresis (18,500; Ref. 3) probably results from a proline/alanine-rich sequence between residues 22 and 58. Proteins with proline/alanine linkers are known to have reduced mobilities in SDS-polyacrylamide gel electrophoresis (19).
**Sequence of Methylmalonyl-CoA Decarboxylase**

The gel was silver-stained. The five subunits of the methylmalonyl-CoA compared are marked by an boxylase in accord with a common function of these proteins/domains, molecular masses are given in the text.

![FIG. 3](image)

**Fig. 3.** SDS-polyacrylamide gel electrophoresis of purified methylmalonyl-CoA decarboxylase from *V. parcula*. A step gradient of 5, 10, and 16.5% acrylamide was used, and after separation the gel was silver-stained. The five subunits of the methylmalonyl-CoA decarboxylase, including the hitherto undetected c-subunit, are shown in lane J. 1.9 µg of the purified protein were applied to the gel. A molecular weight standard is shown in lane M (the corresponding molecular masses are given in the ordinate).

![TABLE]

| Subunit | Molecular Mass (kDa) |
|---------|----------------------|
| A       | 31,000               |
| B       | 32,000               |
| C       | 33,000               |
| D       | 34,000               |
| E       | 35,000               |

Identical amino acid residues in all sequences and conservative exchanges by symbols for identity and for conservative exchanges are the same as in Fig. 4.

![FIG. 4](image)

**Fig. 4.** Amino acid sequence alignment of homologous regions in the methylmalonyl-CoA decarboxylase α-subunit (*VpmmdA*), in the rat propionyl-CoA carboxylase β-subunit (*Rpecb*), and in the *P. shermanii* 12 S subunit of transcarboxylase (*Pstc12*). Identical amino acid residues in all sequences are compared by an asterisk, and conservative exchanges by a dot. Gaps are indicated by lines. References for the sequences used are given in the text.

![FIG. 5](image)

**Fig. 5.** Amino acid sequence alignment of the δ- and the ε-subunit from methylmalonyl-CoA decarboxylase (*VpmmdD* and *VpmmdC*, respectively). Symbols for identity and for conservative exchanges are the same as in Fig. 4.

![FIG. 6](image)

**Fig. 6.** Comparison of the amino acid sequences of biotin enzymes in the region of the biotinylated lysin with that of the γ-subunit of methylmalonyl-CoA decarboxylase (*VpmmdC*). Symbols for identity and for conservative exchanges are the same as in Fig. 4. References are given in the text.
served amino acids are scattered over the entire C-terminal region following the conserved alanine/proline pair at position 65/66, which is required for recognition by biotin ligase (23). A remarkable sequence of the methylmalonyl-CoA decarboxylase γ-subunit is a proline/alanine linker between residues 65/66, which is required for recognition by biotin ligase (23).

The N-terminal portion of the methylmalonyl-CoA decarboxylase γ-subunit (residues 3–17) is highly homologous to the biotin-carrier protein of transcarboxylase containing 12 identical residues and 3 conservative exchanges. This region of the transcarboxylase subunit is known to be essential for binding to the 12 S carboxyltransferase subunit (25). The degree of sequence identity between the two biotin-carrier proteins, as well as between the 12 S subunit of transcarboxylase and the 12 S subunit of methylmalonyl-CoA decarboxylase, indicates a similar binding between carboxyltransferase and biotin carrier protein subunits in both complexes.

The amino acid sequences of the β-subunits from methylmalonyl-CoA decarboxylase and oxaloacetate decarboxylase are 61% identical (7) (Fig. 7). The homology extends over the entire sequence with the exception of a long deletion of 19 residues in the C-terminal position (6, 7). The biotin carrier protein of transcarboxylase of P. shermanii contains an accumulation of 9 glycine, 6 alanine, and 2 prolines out of 19 residues in this region (15), and the biotin carrier of Streptococcus mutans has 4 glutamines, 2 prolines, and 3 alanines in a stretch of 14 amino acid residues (possible Q-linker; Ref. 24) at the same location. All these amino acid sequences are supposed to serve the same purpose, i.e. to provide the protein with flexibility for movement of the prosthetic biotin group between different catalytic centers on the respective biotin-containing enzyme complexes.

**FIG. 7. Amino acid sequence alignment of the β-subunits from methylmalonyl-CoA decarboxylase (VpmmdB) and oxaloacetate decarboxylase (KpOadB) and S. typhimurium (StaodB).** Symbols for identity and for conservative exchanges are the same as in Fig. 4. References are given in the text.

and residues 12–15 of the methylmalonyl-CoA decarboxylase β-subunit are an insertion with respect to the oxaloacetate decarboxylase β-subunit. Of interest are several long stretches of amino acid sequence identity between the β-subunits of methylmalonyl-CoA decarboxylase and two different oxaloacetate decarboxylase, which indicate functionally important regions of these proteins. We have recently taken advantage of these highly conserved areas for amplifying part of the gene for the β-subunit of methylmalonyl-CoA decarboxylase from Propionigenium modestum by the polymerase chain reaction technique. The hydrophobicity plot of the β-subunit (Fig. 8) indicates a very hydrophobic protein with several putative membrane-spanning α-helices. Depending on the computer programs used, secondary structure models with 8–11 helices can be predicted. As the β-subunit secondary structure is probably conserved, the model should also fit the data derived from the oxaloacetate decarboxylase sequences (7). We therefore prefer a secondary structural model for the β-subunit with 9 transmembrane α-helices.

**FIG. 8. Hydrophathy plot of the β- and the δ-subunit from methylmalonyl-CoA decarboxylase.** The hydrophobicity value was calculated with the program TOP-PRET (29). Bars show the position of the predicted integral membrane helices.

**DISCUSSION**

**Nucleotide Sequence—**We show here that the mmdA, mmdD, mmdE, mmdC, and mmdB genes encoding subunits α, δ, ε, γ, and β, respectively, of methylmalonyl-CoA decarboxylase are clustered on the genome of V. parvula in the given order. Putative ribosome binding sites are located upstream of each gene (Fig. 2). Several putative E. coli σ70 promoter consensus sequences (consisting of the −35 and −10 regions) are detectable within 400 base pairs upstream of the mmdA gene (two of these are indicated in Fig. 2 by overlining). The upstream region also contained the 3' part of an open reading frame (residues 1–250 of the sequenced part of the genome). The derived amino acid sequence was not homologous to any protein in the database.

A palindromic sequence of 2 × 8 nucleotides, 32 base pairs downstream of the stop codon of the β-subunit, that might be functioning as a terminator is indicated in Fig. 2 by overlining. An open reading frame in this downstream region starts at position 4422 with TTG and extends to the end of the sequence. No homology of the deduced protein sequence to any known protein sequence could be found. A translation of this ORF into protein seems unlikely because more than 21% of

P. Burda, unpublished observation.
the amino acids would be aromatic. In addition, the G+C content in the region of the ORF is 10% lower (–32%) than the G+C content of the mmd genes and the ORF found upstream of the α-subunit, and TTG is a rarely used start codon in Gram-negative bacteria.

Our attempts to clone all mmd genes together in a high copy number plasmid in E. coli have not been successful, possibly because expression of methylmalonyl-CoA decarboxylase is lethal to an E. coli cell. The enzyme also catalyzes the decarboxylation of malonyl-CoA (3) and will thus interfere with fatty acid biosynthesis. It is interesting that the genes encoding methylmalonyl-CoA decarboxylase and those encoding oxaloacetate decarboxylase are clustered on the chromosome and that the genes encoding the β-subunits of both enzymes are transcribed last (7). In contrast, the gene encoding the α-subunit of gluconyl-CoA decarboxylase from Acidaminococcus fermentans is separated on the genome from the genes encoding the additional subunits of the decarboxylase (26).

The Protein—Biochemical evidence has indicated structural and functional relationships among the biotin-containing Na⁺-transporting decarboxylases oxaloacetate decarboxylase, methylmalonyl-CoA decarboxylase, and glutamyl-CoA decarboxylase (1, 2). These relationships have been refined by the complete primary structure of methylmalonyl-CoA decarboxylase reported here. All these decarboxylase complexes consist of a tightly membrane-bound β-subunit, which contains a binding site for Na⁺ (8, 27, 28) and is therefore most likely responsible for Na⁺ translocation across the membrane. The more than 60% identity of the amino acid sequences between methylmalonyl-CoA decarboxylase and oxaloacetate decarboxylase β-subunits is clear evidence for the same function of these proteins in each of these enzyme complexes. The decarboxylases also contain a peripheral α-subunit acting as carboxyltransferase. The sequences of these carboxyltransferases are homologous to those of biotin-containing carboxylases or transcarboxylase with the same substrate specificity. Transcarboxylase in fact contains two carboxyltransferases (5 and 12 S subunits) with sequence homology to the α-subunit of oxaloacetate decarboxylase and methylmalonyl-CoA decarboxylase, respectively (15). The biotin carrier protein is a distinct domain of the oxaloacetate decarboxylase α-subunit (6, 7) but occurs as a separate protein moiety in the decarboxylases acting on thioester substrates (3, 5). It is interesting that these biotin binding subunits contain alanine/proline linkers in their N-terminal region that are far more extended than putative linker sequences in the biotin carriers of carboxylases and transcarboxylase (6, 7, 15). The reason for these differences may be a requirement in the decarboxylases for a more extended movement of the prosthetic biotin group between the two catalytic centers of the carboxyltransferase and the lyase (decarboxylase). The δ-subunit of methylmalonyl-CoA decarboxylase seems to contain a hydrophobic region of 12 amino acids, and a hydrophilic region of 12 amino acids, and a hydrophilic C-terminal tail. No sequence homology of this protein was found to the γ-subunit of oxaloacetate decarboxylase (7), but the predicted secondary structures were surprisingly similar. Whether this similarity reflects a functional relationship is presently unknown. Remarkable is the presence in methylmalonyl-CoA decarboxylase of a small polypeptide (M, 5,758, ε-subunit) that shows strong sequence homology to the C-terminal portion of the δ-subunit. While this indicates an origin of this subunit by gene duplication, a correlation of the ε-subunit with function is presently not available.

Of special interest for the mechanism of Na⁺ translocation is the structure of the β-subunit. A secondary structural model is shown in Fig. 9. It is based on predictions made by hydrophobicity analysis under consideration of the positive-inside rule (29) (Fig. 8) and also takes into account arguments discussed previously for postulating the secondary structure of the β-subunit of oxaloacetate decarboxylase from K. pneumoniae and S. typhimurium (7). It is assumed that the structures of the different β-subunits were conserved during evolution. The N terminus is supposed to reach into the periplasm because it contains 2 negatively charged and no positively charged residues, while the end of the first transmembrane helix (residues 17–40) of the β-subunit of methylmalonyl-CoA decarboxylase contains an arginine and that of oxaloacetate decarboxylase contains 2 lysines side by side. These positively charged amino acids are supposed to function as stop transfer signals (30). In this first helix 12 residues are identical in the three different β-subunits and the others are conservative exchanges. It follows another highly conserved area of 19 amino acid residues containing 7 hydrophobic residues. In our model, this region was not postulated to traverse the membrane, because the putative helix would be rather short and/or would contain a charged glutamate residue. Nevertheless, the highly conserved sequence of this area indicates that it is functionally important. In the Veillonella β-subunit there then follows a large gap (Fig. 5). The second membrane helix was postulated to run from Ile83 to Ala101. A short loop of three amino acids connects helix II with helix III, which runs from Thr106 to Leu126. After a short loop the polypeptide traverses the membrane again (Ala123 to Thr153). The conserved amino acids Lys-Leu-Ala-Pro-His-(Glu) provide the linkage to helix V, which ends with Glu77. A loop with many charged residues leads to helix VI (Ile266 to Gly275), which is not as highly conserved as the other putative membrane-spanning α-helices. This region of the protein is highly hydrophobic in all the β-subunits sequenced. An aspartate in the middle of putative helix VI in oxaloacetate decarboxylase, however, is replaced by serine in the methylmalonyl-CoA decarboxylase sequence. It follows another region with many charged amino acids that is certainly the putative hydrophilic region and a more hydrophobic region that could coil into helix VII (Asn254 to Ala271). Interestingly, the putative helix VII of both oxaloacetate decarboxylases and methylmalonyl-CoA decarboxylase show the putative transmembrane regions of the polypeptide. Basic residues are boxed, and acidic residues are indicated by circles. Amino acid residues conserved in the β-subunits of oxaloacetate decarboxylase from K. pneumoniae and S. typhimurium are in bold letters. The top part represents the periplasm.

FIG. 9. Secondary structure model of the membrane-bound β-subunit of methylmalonyl-CoA decarboxylase. The boxes show the putative transmembrane regions of the polypeptide. Basic residues are boxed, and acidic residues are indicated by circles. Amino acid residues conserved in the β-subunits of oxaloacetate decarboxylase from K. pneumoniae and S. typhimurium are in bold letters. The top part represents the periplasm.
acetate decarboxylase β-subunits contains a lysine residue, which is replaced by threonine (position 268) in the methylmalonyl-CoA decarboxylase sequence. It is conceivable that the non-conserved charged amino acids in putative helices VI and VII of the oxaloacetate decarboxylase β-subunit form a salt bridge within the membrane. The next helix (VIII) is rather clearly defined by its strong hydrophobicity (Ile202 to Gly201). The following region contains one of the most highly conserved areas of the whole protein (Asn313 to Ser331), where all 19 amino acid residues are identical. This area is flanked on both sides by less conserved segments with a number of charged residues. We do not want to speculate whether the mainly hydrophobic conserved region traverses the membrane. If so, an arginine would be located within the membrane-bound part. The polypeptide chain could traverse the membrane again (helix IX) from Phe343 to leaving the short C-terminal peptide Ala-Met-Leu-Ser-Glu-His extending into the aqueous phase.

Most of the helices predicted by this model are uncharged. A conserved aspartate residue is found in the middle of putative helix II and helix IV in highly conserved areas of the protein. These aspartic acids may be important residues for Na⁺ translocation. As discussed above, the proposed model contains a number of disputable elements that must in the future be investigated by topological studies.

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