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Cloning and Sequence Analysis of cDNAs Encoding Mammalian Mitochondrial Malate Dehydrogenase†

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ABSTRACT: A cDNA clone, named ppmMDH-1 and covering a part of the porcine mitochondrial malate dehydrogenase (mMDH; L-malate:NAD^+ oxidoreductase, EC 1.1.1.37) mRNA, was isolated from a porcine liver cDNA library with a mixture of 24 oligodeoxyribonucleotides as a probe. The sequences of the probe were deduced from the known sequence of porcine mMDH amino acid residues 288–293. ppmMDH-1 covered the coding region for porcine mMDH amino acid residues 17–314 and the 3′ untranslated region. Subsequently, mouse mMDH cDNA clones were isolated from a mouse liver cDNA library with the ppmMDH-1 cDNA as a probe. One of the clones, named pmmMDH-1 and containing a cDNA insert of about 1350 base pairs, was selected for sequence analysis, and the primary structure of the mouse precursor form of mMDH (pre-mMDH) was deduced from its cDNA sequence. The sequenced coding regions for the porcine and mouse mMDH mRNAs showed about 85% homology. When the deduced amino acid sequence of the mouse pre-mMDH was compared with that of the porcine mMDH, they shared a 95% homology, and the mouse pre-mMDH yielded a leader sequence consisting of 24 amino acid residues and a mature mMDH, consisting of 314 amino acid residues. The leader sequence contained three basic amino acid residues, no acidic residues, and no hydrophobic amino acid stretch. The mouse mMDH leader sequence was compared with those of three other rodent mitochondrial matrix proteins.

Malate dehydrogenase (MDH;[1] L-malate:NAD^+ oxidoreductase, EC 1.1.1.37), an NAD^+-dependent dehydrogenase, occurs in two isozymic forms in animal tissues, one in the cytosol and the other in the mitochondria (Edwards & Hopkinson, 1977; Delbrück et al., 1959). These isoenzymes are composed of about 300 amino acid residues. The amino acid sequence has been available only for porcine heart mitochondrial MDH (mMDH) (Birktoft et al., 1982). The two isoenzymes, in cooperation with aspartate aminotransferase (AspAT) isoenzymes, seem to play a pivotal role in the malate–aspartate shuttle operative in a metabolic coordination between cytosol and mitochondria, in various mammalian tissues (Williamson et al., 1973).

We initiated studies on the molecular basis of the regulation of cellular levels of mitochondrial and cytosolic isoenzymes in the malate–aspartate shuttle. The mouse seems to be the most suitable species for such studies. We recently isolated mouse mitochondrial AspAT (mAspAT) and cytosolic AspAT (cAspAT) cDNAs, using the cloned porcine mAspAT and cAspAT cDNAs as probes (Joh et al., 1985; Obaru et al., 1986). In ongoing work on this project, we attempted to isolate mouse mMDH cDNAs. Because there is no available amino acid sequence data for the mouse mMDH, we first isolated porcine mMDH cDNAs, and subsequently, using one of the cloned porcine mMDH cDNAs as a probe, we isolated mouse mMDH cDNAs.

Mouse mMDH is encoded by a nuclear gene assigned to chromosome 5 (Roderick & Davisson, 1984) and represents one of the typical enzymes synthesized as larger precursors on free ribosomes in the cytosol, transported through the

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[1] Abbreviations: MDH, malate dehydrogenase; mMDH, mitochondrial MDH; pre-mMDH, precursor form of mMDH; cMDH, cytosolic MDH; AspAT, aspartate aminotransferase; mAspAT, mitochondrial AspAT; pre-mAspAT, precursor form of mAspAT; cAspAT, cytosolic AspAT; SSC, standard saline citrate; SDS, sodium dodecyl sulfate; ppmMDH, plasmid carrying porcine mMDH cDNA; pmmMDH, plasmid carrying mouse mMDH cDNA; kb, kilobase pairs; bp, base pairs.

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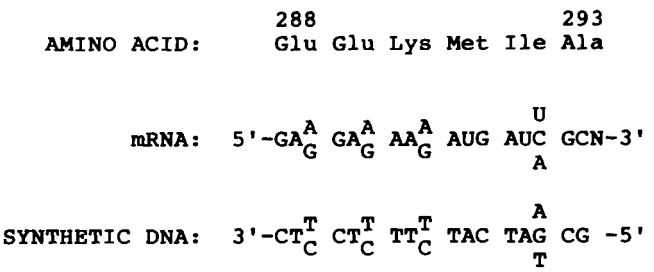

FIGURE 1: Sequences of the synthetic oligodeoxyribonucleotides used for probing cloned cDNAs for mMDH. The partial amino acid sequence of the porcine mMDH (Birktoft et al., 1982) (top line), the corresponding codons (middle line), and all possible complementary sequences of the 17 base long oligodeoxyribonucleotides that were synthesized for use as probes (bottom line) are shown. N, all four possible nucleotides.

mitochondrial membranes, and processed to mature forms in the mitochondrial matrix (Schatz & Butow, 1983; Aziz et al., 1981). Several in vitro studies indicated that mMDH was synthesized as a higher molecular weight precursor (pre-mMDH) in the cytosol of rat liver and was converted to the mature form with a molecular weight 1500–2000 less than that of the precursor protein (Aziz et al., 1981; Chien & Freeman, 1984). However, the primary structure of the possible amino-terminal "leader sequence" of mMDH has not yet been described.

We report herein the nucleotide sequences of a part of the porcine mMDH mRNA and the entire coding region of the mouse pre-mMDH mRNA. We compared the deduced amino acid sequence of the mouse pre-mMDH with that of the porcine mature mMDH and found that the mouse pre-mMDH contains a leader sequence consisting of 24 amino acid residues. The structure of mouse mMDH leader sequence was compared with those of the three other nuclear-coded rodent mitochondrial matrix proteins.

Experimental Procedures

Materials. Materials were obtained from the following sources: T4 polynucleotide kinase from Takara Shuzo (Kyoto, Japan); *Escherichia coli* DNA polymerase I from New England Biolabs; restriction enzymes from Takara Shuzo, New England Biolabs, and Boehringer Mannheim; calf intestinal alkaline phosphatase from Boehringer Mannheim; [γ-^{32}P]ATP (7000 Ci/mmol; 1 Ci = 37 GBq) and [α-^{32}P]dCTP (3000 Ci/mmol) from New England Nuclear and Amersham, respectively. Construction of cDNA libraries on the poly(A+) RNAs extracted from porcine and mouse livers were as described (Joh et al., 1985; Wakasugi et al., 1985).

Synthesis of Oligodeoxyribonucleotides. A mixture of the 24 oligodeoxyribonucleotides (17 bases long) was synthesized against the known porcine mMDH amino acid sequence (Figure 1), by the solid-phase phosphotriester technique (Wallace et al., 1979). The mixture of 24 oligodeoxyribonucleotides was radioactively labeled with T4 polynucleotide kinase and [γ-^{32}P]ATP to a specific activity of 7×10^8 cpm/μg of DNA for use as a hybridization probe.

Isolation of Porcine mMDH cDNA Clones. The porcine liver cDNA library (Joh et al., 1985) was plated on nitrocellulose filters on nutrient agar plates containing 50 μg/mL ampicillin, and the preparation was incubated overnight. The plasmids were amplified with 200 μg/mL chloramphenicol, and the filters were prepared for hybridization, as described by Hanahan and Meselson (1983). The filters were hybridized at 42 °C with a ^{32}P-labeled mixed oligodeoxyribonucleotide probe, as described by Wallace et al. (1979). The hybridization mixture contained 4× SSC, 10× Denhardt's solution (Maniatis et al., 1982), 20 μg of poly(A+) RNA/mL, 100 μg of denatured *E. coli* DNA/mL, and 0.03 μg of 5′ end labeled mixed oligodeoxyribonucleotide probe/mL with the specific activity mentioned above. The filters were washed 3 times for 5 min with 4× SSC containing 0.1% SDS at room temperature and then twice for 30 min with the same solution at 42 °C. Plasmid DNAs were isolated from candidate clones by the alkaline lysis method (Maniatis et al., 1982). One of the plasmids was named ppmMDH-1 and was sequenced by the method of Maxam and Gilbert (1980).

Isolation of Mouse mMDH cDNA Clones. The 472 base pair *Pst*I/*Xho*I fragment was excised from the ppmMDH-1, nick translated, and labeled with [α-^{32}P]dCTP. The mouse liver cDNA library (Wakasugi et al., 1985) was screened by colony hybridization, with the above-described 472-bp fragment as a probe. Colony hybridization was carried out essentially as described by Wakasugi et al. (1985), with the following exceptions: the hybridization solution contained 50% formamide, and the final filter washing was performed at 56 °C. A plasmid named pmmMDH-1 was selected for DNA sequence analysis. The nucleotide and the deduced amino acid sequences were analyzed by the microcomputer programs GENIAS and PRINAS, respectively, purchased from Mitsui Knowledge Industry (Tokyo, Japan).

Northern Blot Analysis of Mouse mMDH mRNA. Total RNAs were extracted from four different tissues of mice by the guanidine–cesium chloride procedure (Maniatis et al., 1982). Northern blot analysis was performed as described by Thomas (1983). RNA samples were denatured, fractionated on formamide–agarose gel, and transferred onto a nitrocellulose filter. Three kinds of DNA fragments were prepared for hybridization probes: (1) a 646-bp *Pst*I/*Bam*HI fragment excised from the pmmMDH-1 cDNA insert, as a pre-mMDH probe; (2) a 733-bp *Pst*I/*Pvu*II fragment excised from the mouse prealbumin (=transthyretin) cDNA clone pmPA1 (Wakasugi et al., 1985), as a prealbumin probe; (3) a 1.3-kb *Bam*HI/*Bam*HI fragment excised from the clone carrying mouse ribosomal RNA genes, p6.6-(1) (Tiemeier et al., 1977), as a 28S ribosomal RNA probe. The recombinant plasmid p6.6-(1) was a generous gift from Dr. P. Leder, Harvard Medical School, through Dr. M. Muramatsu, University of Tokyo, Japan. Each probe was labeled with [α-^{32}P]dCTP to a specific activity of around 1×10^8 cpm/μg of DNA.

Results

Isolation and Characterization of Porcine and Mouse mMDH cDNA Clones. A porcine liver cDNA library was screened for the porcine mMDH cDNA sequences by hybridization with a mixture of the ^{32}P-labeled synthetic oligodeoxyribonucleotides (see Experimental Procedures). Four out of 1×10^6 clones screened showed strong positive hybridization signals. Recombinant plasmids extracted from these four clones were digested with various restriction enzymes and electrophoresed in agarose gels to determine the size (Figure 2a; ppmMDH-1 to ppmMDH-4). One of the plasmids, named ppmMDH-1, contained a cDNA insert of about 2.0 kb in length. As shown in the following section, ppmMDH-1 apparently covered a coding region for 298 amino acid residues from the carboxyl-terminal end of the porcine mMDH and was used as a probe for screening mouse mMDH cDNAs from a mouse liver cDNA library.

To isolate mouse cDNA clones covering the entire pre-mMDH mRNA sequence, we screened a mouse liver cDNA library, using the 472-bp *Pst*I/*Xho*I fragment of the ppmMDH-1, as a probe (Figure 2a). Of the 1.6×10^6 clones screened, four positive clones were isolated (Figure 2b;

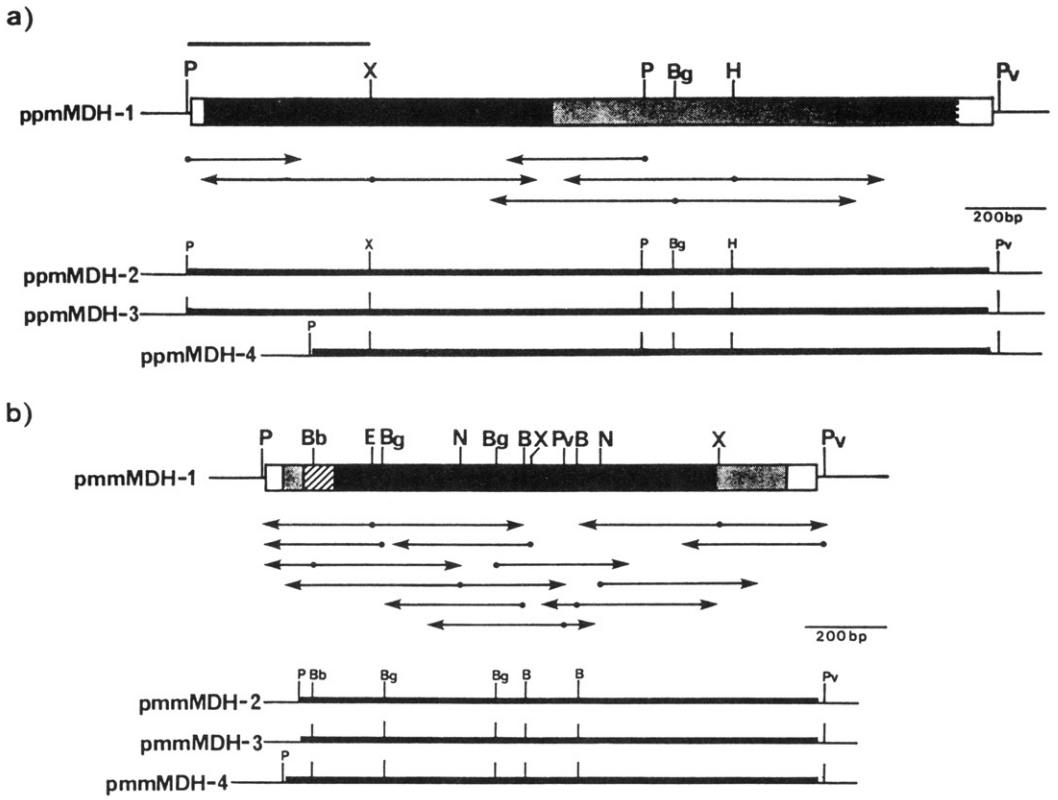

FIGURE 2: Restriction maps and sequencing strategy. (a) Restriction maps of the four clones carrying the porcine mMDH cDNAs, ppmMDH-1 to ppmMDH-4, and (b) those of the four clones carrying the mouse mMDH cDNAs, pmmMDH-1 to pmmMDH-4. Black and hatched regions encode the mature protein and the leader sequence, respectively. The stipplings indicate the 5′ and 3′ untranslated regions. The open boxes represent the G-C tailing at the 5′ end and the poly(A) tract at the 3′ end of the cDNAs, and the thin lines represent the vector DNAs. The sequencing strategy is summarized beneath the maps, with solid circles indicating positions of 5′ end labeling and horizontal arrows indicating the direction and extent of sequencing from a given labeled end. A horizontal line above the map of ppmMDH-1 indicates the 472-bp *Pst*I/*Xho*I fragment used as hybridization probe to isolate mouse cDNA clones. B, *Bam*HI; Bb, *Bbe*I; Bg, *Bgl*II; E, *Eco*RV; H, *Hin*dIII; N, *Nco*I; P, *Pst*I; Pv, *Pvu*II; X, *Xho*I.

pmmMDH-1 to pmmMDH-4). The longest recombinant plasmid, named pmmMDH-1, contained a cDNA insert of about 1.3 kb in length. This clone was subjected to further nucleotide sequence analysis.

Nucleotide Sequence Analysis. The cDNA inserts of ppmMDH-1 and pmmMDH-1 were analyzed with a series of restriction enzymes. Figure 2 shows the restriction maps and sequencing strategies. The nucleotide sequences of these two cDNA inserts, determined by the chemical degradation method, are summarized in Figure 3.

The ppmMDH-1 cDNA insert was partially sequenced, and the sequenced region contained the coding sequence covering porcine mMDH amino acid residues 17–314 (Birktoft et al., 1982) and a part of the 3′ untranslated region. The 3′ untranslated region is apparently longer than 815 nucleotides.

The nucleotide sequence of the pmmMDH-1 cDNA insert is also shown in Figure 3. The entire mouse mMDH mRNA sequence was obtained from both the message and complementary strands (Figures 2 and 3). The pmmMDH-1 cDNA insert contained 1313 nucleotides including the length of the poly(A) tail and was preceded by a poly(dG) segment of 39 residues at the 5′-terminus. This indicates that pmmMDH-1 contains DNA covering all of the 3′ untranslated region of the mouse mMDH mRNA. We assumed that the first methionine codon (ATG) is located at nucleotide positions −72 to −70 of the pmmMDH-1 (Figure 3). The pmmMDH-1 cDNA contained 46 nucleotides in the 5′ untranslated region, 1014 nucleotides in the coding region, and 184 nucleotides in the 3′ untranslated region preceding the poly(A) tail. With this information, we could analyze most of the structure of mouse mMDH mRNA. In the 5′ untranslated region, we found 8-bp direct repeats at nucleotide positions −113 to −106 and −100 to −93 and 4-bp direct repeats at nucleotide positions −88 to −85 and −84 to −81 (Figure 3). The 3′ untranslated region contained in the expected position, 28 nucleotides upstream from the poly(A) tail, hexanucleotide A-G-U-A-A-A, which is homologous to the consensus polyadenylation signal sequence, A-A-U-A-A-A. This region also contained one unusual block of $(AGC)_6$ at nucleotide positions 961–978 and 5-bp direct repeats at nucleotide positions 1116–1120 and 1122–1126 (Figure 3).

A comparison of the coding regions of mouse and porcine mMDH mRNAs revealed a homology of 85%, and that of the 3′ untranslated regions revealed the presence of several highly conserved regions bounded by nonhomologous regions. For example, the following three segments are highly conserved between these two mRNAs: mouse nucleotide positions 980–1003, 1042–1051, and 1066–1074 and porcine nucleotide positions 971–996, 1041–1050, and 1071–1079 (Figure 3).

Predicted Amino Acid Sequence of Porcine and Mouse mMDHs. The amino acid sequence of a part of the porcine mMDH and that of the complete mouse pre-mMDH were deduced from the nucleotide sequence analyses (Figure 3). The predicted amino acid sequence of the mouse pre-mMDH, from Ala-1 to Lys-314, showed 95% homology with that determined for the porcine mMDH (Birktoft et al., 1982). Among the 314 amino acid residues of the porcine mMDH, 15 are replaced by different amino acids in the mouse mMDH (Figure 3). Eight out of the 15 substituted amino acids are caused by a single base change in their codons, and 3 out of the 15 substitutions are of conservative exchanges, such as Val/Ile/Leu and Thr/Ser (Figure 3).

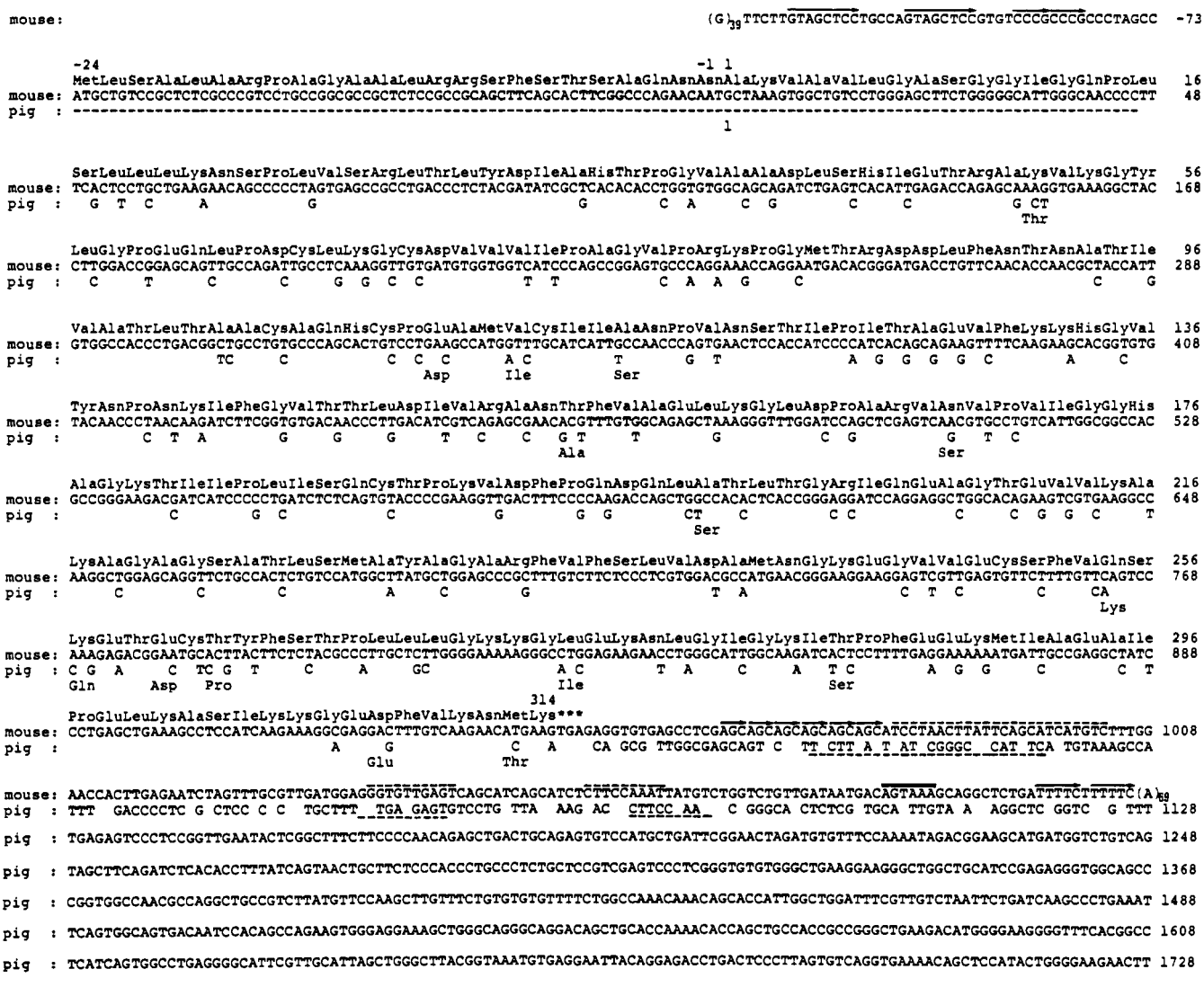

FIGURE 3: Primary structures of porcine and mouse mMDH cDNAs and their deduced amino acid sequences. The predicted amino acid sequences are shown above the nucleotide sequence for the mouse mMDH and beneath the nucleotide sequence for the porcine mMDH. The porcine mMDH nucleotide and amino acid sequences are displayed only where they differ from the mouse sequences. Dashes indicate the nucleotides that are not covered by the ppmMDH-1 cDNA insert. For both the amino acid and nucleotide positions, positive numbers start at the first residue of the mature protein. Horizontal arrows above the sequence indicate direct repeats. Three highly conserved segments in the 3′ untranslated regions of the mouse and porcine mMDH mRNAs are indicated by broken lines above the mouse and under the porcine sequences, respectively. A putative polyadenylation signal in the 3′ untranslated region is overlined. (***) stop codon.

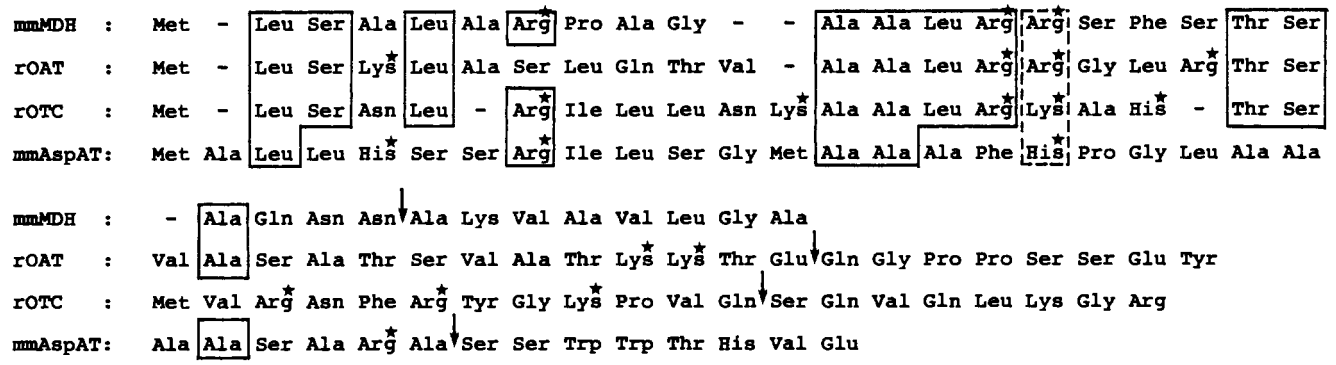

FIGURE 4: Comparison of predicted amino acid leader sequences of mouse mMDH (row mmMDH) and three rodent nuclear-coded mitochondrial proteins: rat ornithine aminotransferase (row rOAT) Mueckler & Pitot, 1985); rat ornithine transcarbamylase (row rOTC) (Takiguchi et al., 1984); mouse mitochondrial aspartate aminotransferase (row mmAspAT) (Obaru et al., 1986). The amino acids in solid-line boxes indicate common residues in at least three of the four sequences; those in dashed-line boxes are chemically homologous but not identical. Arrows indicate the cleavage sites between leader sequences and mature proteins. Dashes represent arbitrary gaps inserted to maximize homology, and one dash corresponds to one amino acid.

This comparison revealed that the mouse pre-mMDH sequence contains the amino-terminal segment of 24 amino acids (Figures 3 and 4). This size seems to be consistent with a molecular weight of about 2000 estimated for the rat mMDH precursor amino-terminal segment (Aziz et al., 1981; Chien & Freeman, 1984). The leader sequence contained three basic

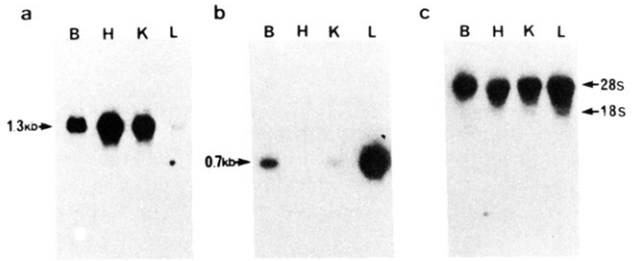

FIGURE 5: Northern blot analysis of mouse pre-mMDH mRNA. Total RNAs were extracted from mouse brain (B), heart (H), kidney (K), and liver (L), and 10 μg each of these RNAs was subjected to blotting analysis, with the nick-translated DNA fragments as probes (see Experimental Procedures). Probes used were as follows: (panel a) pre-mMDH probe, (panel b) prealbumin probe, and (panel c) 28S ribosomal RNA probe. Panel a was exposed to Fuji RX films with a Du Pont Lightning-Plus intensifying screen for 12 h at −80 °C, and panels b and c were exposed for 7 and 2 h, respectively, under the same conditions. 28S and 18S ribosomal RNAs were run separately as size markers.

amino acid residues, no acidic residues, and no hydrophobic amino acid stretch (Figures 3 and 4).

Codon Usage. Codon utilization for porcine and mouse mMDHs is not random and exhibits a preference for codons encoding with G or C. For example, in the mouse mMDH, while 24 valines end in G or C, 5 end in A or T; similarly for leucine, 26 codons end in G or C and 5 end in A or T. Such a nonrandom codon usage has been observed in genes from other animals (Wain-Hobson et al., 1981). There are apparently several exceptions. For example, while 19 alanines end in G or C, 20 end in A or T; similarly for proline, 9 codons end in G or C and 12 end in A or T.

Northern Blot Analysis of Mouse Pre-mMDH mRNA. The size of the mouse pre-mMDH mRNA was estimated by Northern blot hybridization of the total RNAs from four different tissues of mice, with the nick-translated pmmMDH-1 cDNA insert as a probe (Figure 3). As shown in Figure 5 (panel a), one major band for the pre-mMDH mRNA was detected in all these RNAs examined. The levels of the mRNAs were high in heart, brain, and kidney but relatively low in liver. Although the mouse pre-mMDH mRNA levels were different among these tissues, sizes of the mRNAs were approximately equal and were estimated to be about 1.3 kb (Figure 5, panel a). This size supports the idea that the pmmMDH-1 cDNA insert covers almost the full length of the mouse pre-mMDH mRNA.

As one internal control for this experiment, the level and size of mouse prealbumin mRNA were estimated by Northern blot hybridization of these four total RNA samples, and the results shown in Figure 5 (panel b) were exactly the same as those reported previously (Wakasugi et al., 1986). In contrast to the pre-mMDH mRNA, the prealbumin mRNA was abundant in liver and brain, barely detectable in kidney, but not detectable in heart (compare panels a and b of Figure 5). As another internal control, we estimated the level and size of the 28S ribosomal RNA and confirmed that they are approximately equal among the tissues examined (Figure 5, panel c). Altogether, these results suggest that the level of the 1.3-kb mouse pre-mMDH mRNA is regulated in a tissue-specific manner.

Discussion

We determined a part of the coding sequence of the porcine mMDH mRNA and the entire coding sequence of the mouse pre-mMDH mRNA from respective cDNA clones (Figure 3). The predicted amino acid sequence of porcine mMDH, from Ser-17 to Lys-314, was identical with that previously determined for the porcine heart mMDH, by direct amino acid sequence analysis (Birktoft et al., 1982), except for one amino acid, at position 313 (Figure 3). By the amino acid sequence analysis, the amino acid assignment for this position was reported to be Met (Birktoft et al., 1982), which should be coded by ATG. Our nucleotide sequence data indicated that this is Thr, which is coded by ACG (Figure 3). Accordingly, this difference is created by the transition from T to C in this region of the porcine mMDH gene and is probably due to polymorphism rather than error in sequencing.

As far as the available sequence data are concerned, it is apparent that, between the porcine and the mouse mMDH mRNAs, the length and sequence of the coding regions are highly homologous, while those of the 3′ untranslated regions are distinctly dissimilar (Figure 3); the 3′ untranslated region of the porcine mMDH mRNA is more than 620 nucleotides longer than that of the mouse mRNA.

In the mouse pre-mMDH mRNA, we noted the following characteristic features. First, there is no typical consensus polyadenylation signal sequence in the 3′ untranslated region (Figure 3). Instead, we found a similar hexanucleotide, AGUAAA, at the expected position. Recently, Wickens and Stephenson (1984) derived, from a computer-assisted comparison of the 3′-terminal sequences of 134 mRNAs from vertebrates, the following consensus sequence: $A_{98}A_{91}U_{100}A_{99}A_{99}A_{98}$, where the subscripts indicate the percentage of mRNAs containing that base. The variation AGUAAA, present in the 3′-terminal sequence of the mouse pre-mMDH mRNA, was not observed among these 134 mRNAs. However, from the location of this hexanucleotide, we presume that this sequence functions as a signal for poly(A) addition or for the termination of transcription (Wickens & Stephenson, 1984).

Second, there are several direct repeat structures. Two pairs of direct repeats are present in the 5′ untranslated region, and one unusual block of $(AGC)_6$ and 5-bp direct repeats are present in the 3′ untranslated region (Figure 3). More work has to be done to assign roles for these repeated structures.

Mouse mMDH is one of the nuclear-coded mitochondrial proteins and is localized in the mitochondrial matrix. A precursor–mature protein relationship has been demonstrated for the rat mMDH (Aziz et al., 1981; Chien & Freeman, 1984; Chien et al., 1984). The pre-mMDH was reported to have a molecular weight 1500–2000 larger than that of the monomeric form of the mature protein (Aziz et al., 1981; Chien & Freeman, 1984). The predicted pre-mMDH amino-terminal leader sequence of 24 amino acid residues is consistent with this estimation.

Among the nuclear-coded rodent mitochondrial matrix proteins, primary structures of the leader sequences of the rat ornithine aminotransferase (Mueckler & Pitot, 1985), rat ornithine transcarbamylase (Takiguchi et al., 1984), and mouse mAspAT (Obaru et al., 1986) are available. Figure 4 compares the primary structures of the leader sequences of these four proteins. While they are apparently different in length and amino acid sequence, a common feature is the presence of several basic amino acid residues and the absence of acidic residues. There is no distinct sequence homology among them, but there are two relatively conserved regions, one at the amino-terminal region and the other at the central portion of the leader sequences (Figure 4). The hydrophobic profile around the leader sequence of mouse mMDH showed the presence of two hydrophobic peaks: one at the amino-terminal region and the other right after the cleavage site. It also revealed the presence of two hydrophilic regions: one between

the amino-terminal region and the central portion of the leader sequence and the other just before the cleavage site. Similar hydrophobic profiles can be commonly observed around the cleavage sites of these four rodent mitochondrial proteins.

Prediction of the secondary structure for the mMDH leader sequence with Robson parameters (Robson & Suzuki, 1976) revealed the following propensities; one stretch of α-helix at amino acid residues from −13 to −24, a reverse turn region at amino acid residues −8 and −9, and a random coil structure at amino acid residues −1 to −7. Similarly, the presence of a stretch of α-helix in the amino-terminal region and a random coil structure around the cleavage site is also predicted for the leader sequences of precursors to the other three rodent enzymes (Figure 4). Several gene fusion experiments have suggested that a leader sequence contains the information for intramitochondrial sorting of mitochondrial protein (van Loon et al., 1986; Hurt et al., 1985). As described above, leader sequences for these four nuclear-coded and matrix-targeted enzyme precursors show partially common features. However, they are significantly different in size and amino acid sequence. Further study has to be done to determine whether or not the structural difference might determine the specificity of the processing mechanism, including site recognition and proteolysis.

By Northern blot analysis of the total RNAs extracted from four different tissues of mice, we found that the levels of pre-mMDH mRNA differed widely among the tissues (Figure 5). Availability of complete cDNA sequences for mouse pre-mAspAT, cAspAT, pre-mMDH, and cMDH will facilitate isolation of the corresponding genomic genes. Structural analysis of the genomic DNAs, as well as the estimation of levels of respective mRNAs in mouse organs, under various experimental conditions, should lead to elucidation of the regulation mechanism of isoenzymes participating in the metabolically important functions.

Added in Proof

After this paper had been submitted, a report appeared dealing with the isolation and nucleotide sequence of the precursor form of rat mMDH (Grant et al., 1986). The length of the mature protein and that of amino-terminal leader sequence are identical with those of the mouse pre-mMDH, as herein described, and the homology of the amino acid sequence between these two pre-mMDHs is about 95%.

Acknowledgments

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Registry No. mMDH, 9001-64-3; mMDH (mouse precursor reduced), 107407-70-5; pre-mMDH, 99332-64-6; mMDH (mouse reduced), 107407-69-2; DNA (mouse malate dehydrogenase messenger RNA complementary), 107407-68-1.

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