Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 4934–4938, July 1988
Neurobiology

cDNA cloning of human liver monoamine oxidase A and B: Molecular basis of differences in enzymatic properties

(brain/neurotransmitters/amine catabolism)

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Communicated by Erminio Costa, March 10, 1988 (received for review December 17, 1987)

ABSTRACT **The monoamine oxidases play a vital role in the metabolism of biogenic amines in the central nervous system and in peripheral tissues. Using oligonucleotide probes derived from three sequenced peptide fragments, we have isolated cDNA clones that encode the A and B forms of monoamine oxidase and have determined the nucleotide sequences of these cDNAs. Comparison of the deduced amino acid sequences shows that the A and B forms have subunit molecular weights of 59,700 and 58,800, respectively, and have 70% sequence identity. Both sequences contain the pentapeptide Ser-Gly-Gly-Cys-Tyr, in which the obligatory cofactor FAD is covalently bound to cysteine. Based on differences in primary amino acid sequences and RNA gel blot analysis of mRNAs, the A and B forms of monoamine oxidase appear to be derived from separate genes.**

Monoamine oxidases A and B [MAO A and MAO B, respectively; amine:oxygen oxidoreductase (deaminating) (flavin-containing), EC 1.4.3.4] in the central nervous system and in peripheral tissues catalyze the oxidative deamination of neuroactive and vasoactive amines (1) and the oxidation of xenobiotics, including the parkinsonism-producing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (2, 3). These enzymes, which are integral proteins of the outer mitochondrial membrane (4), are distinguished by differences in substrate preference (5), inhibitor specificity (6), tissue and cell distribution (7), and immunological properties (8, 9). MAO A preferentially oxidizes the biogenic amine serotonin and is inactivated irreversibly by the acetylenic inhibitor clorgyline. MAO B preferentially oxidizes phenylethylamine and benzylamine and is inactivated by the irreversible inhibitors pargyline and deprenyl. The level of MAO activity in almost all human tissues consists of a mixture of both forms of the enzyme, but placental tissue contains predominantly MAO A (10), whereas platelets and lymphocytes express only MAO B (11, 12). MAO A and B from several tissue sources and species appear to consist of two subunits with approximate molecular masses of 60 kDa (13, 14). One subunit has an essential covalently bound FAD (14, 15), coupled by facile binding or by an uncharacterized enzyme (16). Studies of [^{3}H]pargyline-labeled enzyme resolved in NaDodSO$_4$/PAGE have shown that the FAD-containing subunit of human MAO A from placenta and MAO B from platelets have molecular masses of ≈63 kDa and ≈60 kDa, respectively (17). A comparison of highly purified human placental MAO A and human liver MAO B revealed that the A form of the enzyme is larger by ≈2 kDa (18). Sequence studies of fragments obtained by digestion with chymotrypsin and trypsin have identified a pentapeptide fragment, Ser-Gly-Gly-Cys(FAD)-Tyr, that is identical in the A and B forms (15, 19). Peptide maps obtained from proteinase digestion of [^{3}H]pargyline-labeled crude or partially purified MAO A and B suggest that these enzymes differ in their amino acid sequences (17, 20). Furthermore, differences in degrees of photo-dependent inactivation of these two enzymes suggest the existence of conformational or structural differences in their active sites (21–23).

To clarify the molecular basis of structural and functional differences between these important enzymes, we have isolated and characterized cloned cDNAs¶ encoding these proteins. The nucleotide and deduced amino acid sequences for human liver MAO A and B show that these two proteins are derived from separate genes.

MATERIALS AND METHODS

Construction and Screening of the Human Liver cDNA Library. A λgt10 library was constructed from poly(A)$^+$ mRNA isolated from human liver (24). The phage library contained 2 × 10^6 individual clones of which 5 × 10^5 clones were subjected to hybridization under moderately stringent conditions of 20% (vol/vol) formamide/5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate) at 37°C with ^{32}P-labeled synthetic oligonucleotides. When screening for MAO A cDNA clones, a synthetic oligonucleotide probe and a *Bam*HI fragment of MAO B cDNA were used for hybridization (50% formamide/5× SSC at 37°C). Oligonucleotide probes were designed from the amino acid sequences of human MAO A and B fragments generated by trypsin digestion. HL-60 (5′ GAGATCCTGCATGCCATGGGCAAGATCCCTGAGGACGAGATCTGGCAGTCTGAG 3′) corresponded to the amino acid sequence Glu-Ile-Leu-His-Ala-Met-Gly-Lys-Ile-Pro-Glu-Asp-Glu-Ile-Trp-Gln-Ser-Glu and HL-45 (5′ GAGAATGTGCTGGTGGAGACCCTGAACCATGAGATGTATGAGGCCAAG 3′) to Glu-Asn-Val-Leu-Val-Glu-Thr-Leu-Asn-His-Gln-Met-Tyr-Glu-Ala-Lys. Both represented MAO B-specific oligonucleotides. HP-99 (5′ GATGTGCCTGCTGTGGAGATCACCCACACCTTCTGGGA 3′), which coded for the peptide fragment Asp-Val-Pro-Ala-Val-Glu-Ile-Thr-His-Thr-Phe-Trp-Glu, represented a MAO A-specific oligonucleotide.

Phage DNA was prepared from double-positive clones (HL-45 and HL-60 for MAO B; HP-99 and *Bam*HI fragment of MAO B cDNA for MAO A), and the cDNA inserts were excised by digestion with *Eco*RI and subcloned into M13

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Abbreviations: MAO A, monoamine oxidase A; MAO B, monoamine oxidase B.

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¶The sequences reported in this paper are being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession nos. J03792 for MAO A and J03793 for MAO B).

phage vectors (25). DNA was sequenced by the chain-termination technique (26).

Protein Purification and Amino Acid Sequencing. Human liver MAO A was purified as described by immunoaffinity chromatography with MAO B-1C2, a monoclonal antibody that recognizes human MAO B but not MAO A (8). Human placental MAO A was purified by the method of Wyler and Salach (18). The preparations of MAO A and B were judged to be >95% pure when resolved on $NaDodSO_4$/PAGE.

Purified proteins were dissolved in 8 M urea and digested with trypsin (trypsin/protein, 1:25 ratio by weight) at 37°C for 24 hr (0.1 M NH_4HCO_3, pH 8.0). The resulting peptides were separated by reverse-phase HPLC on a Aquapore C_8 column (1 mm × 25 cm). The purified peptides were sequenced by automated Edman degradation on a gas-phase protein sequencer (Applied Biosystems).

RNA Gel Blot Analysis. Poly(A)$^+$ mRNA was prepared from human brain frontal cortex and placenta as described by Chirgwin *et al.* (27). Poly(A)$^+$ mRNAs were subjected to electrophoresis on a 1% agarose/formaldehyde gel and transferred to a nylon membrane as described (28). The RNA blot was hybridized with ^{32}P-labeled cDNA probes. The probes were labeled by the random-primer procedure described by Feinberg and Vogelstein (29).

Materials and Reagents. DNA-modifying enzymes were obtained from Boehringer Mannheim or New England Biolabs, packaging extracts were from Promega Biotec (Madison, WI), and blotting materials were from Schleicher & Schuell. Oligonucleotides were synthesized on a Applied Biosystems DNA synthesizer model 380B.

RESULTS

Molecular Cloning of Human MAO B. A human liver cDNA library was screened with two oligonucleotide probes (HL-45 and HL-60) derived from peptide sequences of human liver MAO B. Five clones were identified that hybridized to both probes. The longest cDNA insert of ≈2.5 kilobases (kb) (clone hMAO B-1) was sequenced. The DNA sequence and the deduced amino acid sequence of this cDNA clone are shown in Fig. 1. The hMAO B-1 cDNA is 2498 nucleotides long, including 858 nucleotides of untranslated sequence at the 3′ end after the TAA termination codon. A poly(A) tail begins 14 nucleotides after the consensus poly(A) sequence AATAAA (30), which is located at position 2472. The open reading frame with the putative ATG initiation codon at position 78 codes for a polypeptide of 520 amino acids with a molecular mass of 58.8 kDa. Both peptide sequences used for the design of the oligonucleotide probes, and the five other peptide sequences (see Fig. 1 legend) not used in the cloning strategy were found to align with the deduced protein sequence.

Examination of the nucleotide sequence at the 5′ end suggests that the first ATG represents the initiation codon for MAO B. However, evidence for this assignment is not conclusive because the reading frame preceding this ATG contains no termination codon. Further screening of another (randomly primed) cDNA library gave cDNA clones for MAO B with only a 40-base-pair extension at the 5′ end that also lacked an in-frame stop codon.

Molecular Cloning of Human MAO A. A cDNA clone that encoded MAO A was isolated from the same liver cDNA library by using a *Bam*HI fragment of hMAO B-1 cDNA and a synthetic oligonucleotide (HP-99) derived from a peptide fragment of human placental MAO A. Hybridization of the oligonucleotide probe was performed under stringent conditions (20% formamide/5× SSC at 37°C) to exclude hybridization of HP-99 to human liver MAO B. Digestion of the double-positive clones with *Eco*RI showed that the longest insert had a length of ≈2.0 kb (clone hMAO A-7). The cDNA sequence and the deduced amino acid sequence of this insert are shown in Fig. 2. DNA sequencing revealed an open reading frame specifying a polypeptide with a molecular mass of 59.7 kDa. The peptide sequence represented by the

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   1 CTGGCAGGCAGGACTGGGATCGAGGCCCAGAAAACGGAGCAGCGGGCACCAGGGAGGCCT
  61 GGAACGGGGCGAGCGCCATGAGCAACAAATGCGACGTGGTCGTGGTGGGGGGCGGCATCT
                M  S  N  K  C  D  V  V  V  V  G  G  G  I
 121 CAGGTATGGCAGCAGCCAAACTTCTGCATGACTCTGGACTGAATGTGGTTGTTCTGGAAG
      S  G  M  A  A  A  K  L  L  H  D  S  G  L  N  V  V  V  L  E
 181 CCCGGGACCGTGTGGGAGGCAGGACTTACACTCTTAGGAACCAAAAGGTTAAATATGTGG
      A  R  D  R  V  G  G  R  T  Y  T  L  R  N  Q  K  V  K  Y  V
 241 ACCTTGGAGGATCCTATGTTGGACCAACCCAGAATCGTATCTTGAGATTAGCCAAGGAGC
      D  L  G  G  S  Y  V  G  P  T  Q  N  R  I  L  R  L  A  K  E
 301 TAGGATTGGAGACCTACAAAGTGAATGAGGTTGAGCGTCTGATCCACCATGTAAAGGGCA
      L  G  L  E  T  Y  K  V  N  E  V  E  R  L  I  H  H  V  K  G
 361 AATCATACCCCTTCAGGGGGCCATTCCCACCTGTATGGAATCCAATTACCTACTTAGATC
      K  S  Y  P  F  R  G  P  F  P  P  V  W  N  P  I  T  Y  L  D
 421 ATAACAACTTTTGGAGGACAATGGATGACATGGGGCGAGAGATTCCGAGTGATGCCCCAT
      H  N  N  F  W  R  T  M  D  D  M  G  R  E  I  P  S  D  A  P
 481 GGAAGGCTCCCCTTGCAGAAGAGTGGGACAACATGACAATGAAGGAGCTACTGGACAAGC
      W  K  A  P  L  A  E  E  W  D  N  M  T  M  K  E  L  L  D  K
 541 TCTGCTGGACTGAATCTGCAAAGCAGCTTGCCACTCTCTTTGTGAACCTGTGTGTCACTG
      L  C  W  T  E  S  A  K  Q  L  A  T  L  F  V  N  L  C  V  T
 601 CAGAGACCCATGAGGTCTCTGCTCTCTGGTTCCTGTGGTATGTGAAGCAGTGTGGAGGCA
      A  E  T  H  E  V  S  A  L  W  F  L  W  Y  V  K  Q  C  G  G
 661 CAACAAGAATCATCTCGACAACAAATGGAGGACAGGAGAGGAAATTTGTGGGCGGATCTG
      T  T  R  I  I  S  T  T  N  G  G  Q  E  R  K  F  V  G  G  S
 721 GTCAAGTGAGTGAGCGGATAATGGACCTCCTTGGAGACCGAGTGAAGCTGGAGAGGCCTG
      G  Q  V  S  E  R  I  M  D  L  L  G  D  R  V  K  L  E  R  P
 781 TGATCTACATTGACCAGACAAGAGAAAATGTCCTTGTGGAGACCCTAAACCATGAGATGT
      V  I  Y  I  D  Q  T  R  E  N  V  L  V  E  T  L  N  H  E  M
 841 ATGAGGCTAAATATGTGATTAGTGCTATTCCTCCTACTCTGGGCATGAAGATTCACTTCA
      Y  E  A  K  Y  V  I  S  A  I  P  P  T  L  G  M  K  I  H  F
 901 ATCCCCCTCTGCCAATGATGAGAAACCAGATGATCACTCGTGTGCCTTTGGGTTCAGTCA
      N  P  P  L  P  M  M  R  N  Q  M  I  T  R  V  P  L  G  S  V
 961 TCAAGTGTATAGTTTATTATAAAGAGCCTTTCTGGAGGAAAAAAGGATTACTGTGGAACCA
      I  K  C  I  V  Y  Y  K  E  P  F  W  R  K  K  D  Y  C  G  T
1021 TGATTATTGATGGAGAAGAAGCTCCAGTTGCCTACACGTTGGATGATACCAAACCTGAAG
      M  I  I  D  G  E  E  A  P  V  A  Y  T  L  D  D  T  K  P  E
1081 GCAACTATGCTGCCATAATGGGATTTATCCTGGCCCACAAAGCCAGAAAACTGGCACGTC
      G  N  Y  A  A  I  M  G  F  I  L  A  H  K  A  R  K  L  A  R
1141 TTACCAAAGAGGAAAGGTTGAAGAAACTTTGTGAACTCTATGCCAAGGTTCTGGGTTCCC
      L  T  K  E  E  R  L  K  K  L  C  E  L  Y  A  K  V  L  G  S
1201 TAGAAGCTCTGGAGCCAGTGCATTATGAAGAAAAGAACTGGTGTGAGGAGCAGTACTCTG
      L  E  A  L  E  P  V  H  Y  E  E  K  N  W  C  E  E  Q  Y  S
                                                          ****
1261 GGGGCTGCTACACAACTTATTTCCCCCCTGGGATCCTGACTCAATATGGAAGGGTTCTAC
      G  G  C  Y  T  T  Y  F  P  P  G  I  L  T  Q  Y  G  R  V  L
           **********
1321 GCCAGCCAGTGGACAGGATTTACTTTGCAGGCACCGAGACTGCCACACACTGGAGCGGCT
      R  Q  P  V  D  R  I  Y  F  A  G  T  E  T  A  T  H  W  S  G
1381 ACATGGAGGGGGCTGTAGAGGCCGGGGAGAGAGCAGCCCGAGAGATCCTGCATGCCATGG
      Y  M  E  G  A  V  E  A  G  E  R  A  A  R  E  I  L  H  A  M
1441 GGAAGATTCCAGAGGATGAAATCTGGCAGTCAGAACCAGAGTCTGTGGATGTCCCTGCAC
      G  K  I  P  E  D  E  I  W  Q  S  E  P  E  S  V  D  V  P  A
1501 AGCCCATCACCACCACCTTTTTGGAGAGACATTTGCCCTCCGTGCCAGGCCTGCTCAGGC
      Q  P  I  T  T  T  F  L  E  R  H  L  P  S  V  P  G  L  L  R
1561 TGATTGGATTGACCACCATCTTTTCAGCAACGGCTCTTGGCTTCCTGGCCCACAAAAGGG
      L  I  G  L  T  T  I  F  S  A  T  A  L  G  F  L  A  H  K  R
1621 GGCTACTTGTGAGAGTCTAAAGAGAGAGGGTGTCTGTAATCACACTCTCTTCTTACTGTA
      G  L  L  V  R  V  *
1681 TTTGGGATATGAGTTTGGGGAAAGAGTTGCAAGTAAAGTTCCATGAAGACAAATAGTGTG
1741 GAGTGAGGCGGGGGAGCATGAAGATAAATCCAACTCTGACTGTAAAATACAATGGTATCT
1801 CTTTCTCCGTTGTGGCCCCTGCTTAGTGTCCCTTACCTGGCTTAGCGTTCTGTTTCACCA
1861 GTTTCCAAGTTTATTGCCCTCAAATCTTTAGAATAGTTAAATTGGCTTGTTTAAGGTTCT
1921 TGCTGCCCCACAACACACCTTGCCCATGCACAGGATGAATTTTTTCCTACCATTATGGCT
1981 TTGTGCTTGTTCTTCCTCTTACCTGTATAGCCTCACTTCCCTAGTTCTTTGCATTCGTCC
2041 TTAGGTACTGTATTGTTACAGCTGAAAGACAGTAAAGACCATTTAGTCCTCACCTTCTGT
2101 TTTAGAGTTGAGCAAACTGAAGCCCACAGAGGTGGAACTTAATTACCTAAGAGCCACAAT
2161 AAGCCACTGGTATCTGGGGGACTAGAACACAAATAATTGCTTTTCCCACCTCTTTGGATG
2221 TTTTCCCCAATTATCCTCCTTCACTCCCTGTCATAGTTACCGATGGTGTCCCGTTGTGTG
2281 GGTTTACTCTGTGCTAAGTTGTCTTACACTTCTCAAATGCTACTCAGTATATAGCCTTAA
2341 CTCTTACTGTTTTGTGCGGTGTGTCTCCAGCTGATTTTAACTTTTTGATGGTAGAAATT
2401 TTATCTCTTCTTCCTTTTGTATCCTCCATTGTATCTTCATACAAAGGACAGTACACACTT
2461 GGGTAATTAAAAATAAAAGTTGATTGACCATAAAAAAA
```

FIG. 1. Nucleotide sequence of human liver MAO B and the deduced amino acid sequence. Numbering of nucleotides is shown on the left side. The nucleotide sequences that correspond to the hybridization probes HL-45 and HL-60, respectively, are underlined. The pentapeptide containing the FAD-binding site is identified by asterisks. Amino acid sequences of five additional tryptic peptides of MAO B are residues 74–82, 102–118, 229–242, 422–425, and 458–477. The single-letter amino acid code is used.

```
1     CATAGAAGGGTCCTTCCCACCCTTTGCCGTCCCCACTCCTGTGCCTACGACCCAGGAGCG
61    TGTCAGCCAAATCATGGAGAATCAAGAGAAGGCGAGTATCGCGGGCCACATGTTCGACGT
                M  E  N  Q  E  K  A  S  I  A  G  H  M  F  D  V
121   AGTCGTGATCGGAGGTGGCATTTCAGGACTATCTGCTGCCAAACTCTTGACTGAATATGG
        V  V  I  G  G  G  I  S  G  L  S  A  A  K  L  L  T  E  Y  G
181   CGTTAGTGTTTTGGTTTTAGAAGCTCGGGACAGGGTTGGAGGAAGAACATATACTATAAG
        V  S  V  L  V  L  E  A  R  D  R  V  G  G  R  T  Y  T  I  R
241   GAATGAGCATGTTGATTACGTAGATGTTGGTGGAGCTTATGTGGGACCAACCCAAAACAG
        N  E  H  V  D  Y  V  D  V  G  G  A  Y  V  G  P  T  Q  N  R
301   AATCTTACGCTTGTCTAAGGAGCTGGGCATAGAGACTTACAAAGTGAATGTCAGTGAGCG
        I  L  R  L  S  K  E  L  G  I  E  T  Y  K  V  N  V  S  E  R
361   TCTCGTTCAATATGTCAAGGGGAAAACATATCCATTTCGGGGCGCCTTTCCACCAGTATG
        L  V  Q  Y  V  K  G  K  T  Y  P  F  R  G  A  F  P  P  V  W
421   GAATCCCATTGCATATTTGGATTACAATAATCTGTGGAGGACAATAGATAACATGGGGAA
        N  P  I  A  Y  L  D  Y  N  N  L  W  R  T  I  D  N  M  G  K
481   GGAGATTCCAACTGATGCACCCTGGGAGGCTCAACATGCTGACAAATGGGACAAAATGAC
        E  I  P  T  D  A  P  W  E  A  Q  H  A  D  K  W  D  K  M  T
541   CATGAAAGAGCTCATTGACAAAATCTGCTGGACAAAGACTGCTAGGCGGTTTGCTTATCT
        M  K  E  L  I  D  K  I  C  W  T  K  T  A  R  R  F  A  Y  L
601   TTTTGTCAATATCAATGTGACCTCTGAGCCTCACGAAGTGTCTGCCCTGTGGTTCTTGTG
        F  V  N  I  N  V  T  S  E  P  H  E  V  S  A  L  W  F  L  W
661   GTATGTGAAGCAGTGCGGGGGCACCACTCGGATATTCTCTGTCACCAATGGTGGCCAGGA
        Y  V  K  Q  C  G  G  T  T  R  I  F  S  V  T  N  G  G  Q  E
721   ACGGAAGTTTGTAGGTGGATCTGGTCAAGTGAGCGAACGGATAATGGACCTCCTCGGAGA
        R  K  F  V  G  G  S  G  Q  V  S  E  R  I  M  D  L  L  G  D
781   CCAAGTGAAGCTGAACCATCCTGTCACTCACGTTGACCAGTCAAGTGACAACATCATCAT
        Q  V  K  L  N  H  P  V  T  H  V  D  Q  S  S  D  N  I  I  I
841   AGAGACGCTGAACCATGAACATTATGAGTGCAAATACGTAATTAATGCGATCCCTCCGAC
        E  T  L  N  H  E  H  Y  E  C  K  Y  V  I  N  A  I  P  P  T
901   CTTGACTGCCAAGATTCACTTCAGACCAGAGCTTCCAGCAGAGAGAAACCAGTTAATTCA
        L  T  A  K  I  H  F  R  P  E  L  P  A  E  R  N  Q  L  I  Q
961   GCGGCTTCCAATGGGAGCTGTCATTAAGTGCATGATGTATTACAAGGAGGCCTTCTGGAA
        R  L  P  M  G  A  V  I  K  C  M  M  Y  Y  K  E  A  F  W  K
1021  GAAGAAGGATTACTGTGGCTGCATGATCATTGAAGATGAAGATGCTCCAATTTCAATAAC
        K  K  D  Y  C  G  C  M  I  I  E  D  E  D  A  P  I  S  I  T
1081  CTTGGATGACACCAAGCCTGATGGGTCACTGCCTGCCATCATGGGCTTCATTCTTGCCCG
        L  D  D  T  K  P  D  G  S  L  P  A  I  M  G  F  I  L  A  R
1141  GAAAGCTGATCGACTTGCTAAGCTACATAAGGAAATAAGGAAGAAGAAAATCTGTGAGCT
        K  A  D  R  L  A  K  L  H  K  E  I  R  K  K  K  I  C  E  L
1201  CTATGCCAAAGTGCTGGGATCCCAAGAAGCTTTACATCCAGTGCATTATGAAGAGAAGAA
        Y  A  K  V  L  G  S  Q  E  A  L  H  P  V  H  Y  E  E  K  N
1261  CTGGTGTGAGGAGCAGTACTCTGGGGGCTGCTACACGGCCTACTTCCCTCCTGGGATCAT
        W  C  E  E  Q  Y  S  G  G  C  Y  T  A  Y  F  P  P  G  I  M
                                ***************
1321  GACTCAATATGGAAGGGTGATTCGTCAACCCGTGGGCAGGATTTTCTTTGCGGGCACAGA
        T  Q  Y  G  R  V  I  R  Q  P  V  G  R  I  F  F  A  G  T  E
1381  GACTGCCACAAAGTGGAGCGGCTACATGGAAGGGGCAGTTGAGGCTGGAGAACGAGCAGC
        T  A  T  K  W  S  G  Y  M  E  G  A  V  E  A  G  E  R  A  A
1441  TAGGGAGGTCTTAAATGGTCTCGGGAAGGTGACCGAGAAAGATATCTGGGTACAAGAACC
        R  E  V  L  N  G  L  G  K  V  T  E  K  D  I  W  V  Q  E  P
1501  TGAATCAAAGGACGTTCCAGCGGTAGAAATCACCCACACCTTCTGGGAAAGGAACCTGCC
        E  S  K  D  V  P  A  V  E  I  T  H  T  F  W  E  R  N  L  P
1561  CTCTGTTTCTGGCCTGCTGAAGATCATTGGATTTTCCACATCAGTAACTGCCCTGGGGTT
        S  V  S  G  L  L  K  I  I  G  F  S  T  S  V  T  A  L  G  F
1621  TGTGCTGTACAAATACAAGCTCCTGCCACGGTCTTGAAGTTCTGTTCTTATGCTCTCTGC
        V  L  Y  K  Y  K  L  L  P  R  S  *
1681  TCACTGGTTTTCAATACCACCAAGAGGAAAAATATTGACAAGTTTAAAGGCTGTGTCATT
1741  GGGCCATGTTTAAGTGTACTGGATTTAACTACCTTTGGCTTAATTCCAATCATTGTTAAA
1801  GTAAAAACAATTCAAAGAATCACCTAATTAATTTCAGTAGATCAAGCTCCATCTTATTTG
1861  TCAGTGTAGATCACTCATGTTAATTGATAGAATAAAGCCTTGTGATCATTTCTGAAATTC
1921  ACAAGTAACGTGTATGTGCTCATCAGAACAAAAAAAAA
```

FIG. 2. Nucleotide sequence of human liver MAO A and the deduced amino acid sequence. Numbering of nucleotides is shown on the left side. The nucleotide sequence that corresponds to the oligonucleotide probe HP-99 is underlined. The pentapeptide containing the FAD-binding site is identified by asterisks. Amino acid sequences of three additional tryptic peptides of MAO A are residues 137–151, 282–291, and 380–394. The single-letter amino acid code is used.

oligonucleotide probe HP-99 was found close to the 3′ end of the coding region and was identical to the deduced sequence for MAO A. The 3′-untranslated region is 301 nucleotides long, including a poly(A) signaling sequence 53 nucleotides upstream from the poly(A) tail. As found for the hMAO B-1 cDNA clone, no in-frame stop codon was observed preceding the putative start codon. Additional extensive screening of the human liver cDNA library (2×10^6 independent clones) did not reveal a clone with an in-frame stop codon.

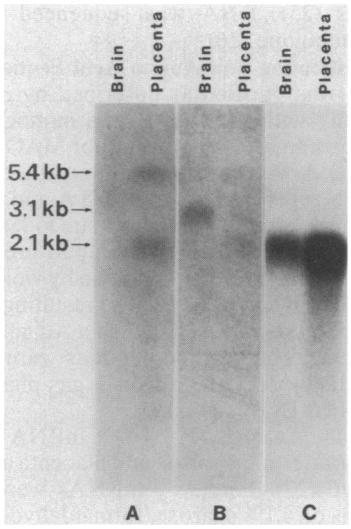

FIG. 3. RNA gel blot analysis. Approximately 5 and 8 μg of poly(A)$^+$ mRNA from human brain frontal cortex and human placenta, respectively, were electrophoresed on a 1% agarose/formaldehyde gel and blotted into a nylon membrane. The same blot was hybridized in sequence with ^{32}P-labeled probes for MAO A (blot A), MAO B (blot B), and a human β-actin pseudogene (blot C). The hybridization was carried out in 50% formamide/5× SSC at 37°C, and the blot was washed in 0.1× SSC at 60°C. The faint 2.1- and 5.4-kb bands in blot B are the remainders of the radioactive bands shown in blot A, since the same blot was used without stripping. Other experiments showed that these two probes do not cross-hybridize under our experimental conditions. Blots A and B were exposed 1 week whereas blot C was exposed 4 hr.

RNA Gel Blot Analysis of MAO Transcripts. Using the hMAO A-7 cDNA (2.0 kb) as a probe, transcripts of 2.1 and

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                10        20        30        40        50        60
 1 MENQEKASIAGHMFDVVVIGGGISGLSAAKLLTEYGVSVLVLEARDRVGGRTYTIRNEHV
                **** ******  *****   *  * ************** ** *
 1          MSNKCDVVVVGGGISGMAAAKLLHDSGLNVVVLEARDRVGGRTYTLRNQKV

                                                                  .
61 DYVDVGGAYVGPTQNRILRLSKELGIETYKVNVSERLVQYVKGKTYPFRGAFPPVWNPIA
   *** ** ************** **** ******  ***   **** ***** ********
52 KYVDLGGSYVGPTQNRILRLAKELGLETYKVNEVERLIHHVKGKSYPFRGPFPPVWNPIT

                                                                  .
121 YLDYNNLWRTIDNMGKEIPTDAPWEAQHADKWDKMTMKELIDKICWTKTARRFAYLFVNI
    *** ** *** * ** *** **** * *  *  ** ****** ** ***  *   * ****
112 YLDHNNFWRTMDDMGREIPSDAPWKAPLAEEWDNMTMKELLDKLCWTESAKQLATLFVNL
                                    ΔΔΔ
    ΔΔΔ
                                                                  .
181 NVTSEPHEVSALWFLWYVKQCGGTTRIFSVTNGGQERKFVGGSGQVSERIMDLLGDQVKL
    ** * ********************** * ************************** ***
172 CVTAETHEVSALWFLWYVKQCGGTTRIISTTNGGQERKFVGGSGQVSERIMDLLGDRVKL

                                                                  .
241 NHPVTHVDQSSDNIIIETLNHEHYECKYVINAIPPTLTAKIHFRPELPAERNQLIQRLPM
    **  **   *  ****** ** **** ******  **** * **  *** * * * * *
232 ERPVIYIDQTRENVLVETLNHEMYEAKYVISAIPPTLGMKIHFNPPLPMMRNQMITRVPL

                                                                  .
301 GAVIKCMMYYKEAFWKKKDYCGCMIIEDEDAPISITLDDTKPDGSLPAIMGFILARKADR
    * ****  **** ** ****** ***  * **   ******* *   ******** **
292 GSVIKCIVYYKEPFWRKKDYCGTMIIDGEEAPVAYTLDDTKPEGNYAAIMGFILAHKARK

                                                                  .
361 LAKLHKEIRKKKICELYAKVLGSQEALHPVHYEEKNWCEEQYSGGCYTAYFPPGIMTQYG
    ** * ** * ** ********** *** ********************** ****** ****
352 LARLTKEERLKKLCELYAKVLGSLEALEPVHYEEKNWCEEQYSGGCYTTYFPPGILTQYG

                                                                  .
421 RVIRQPVGRIFFAGTETATKWSGYMEGAVEAGERAAREVLNGLGKVTEKDIWVQEPESKD
    ** **** ** ******** ******************** *   **  *  **  **** *
412 RVLRQPVDRIYFAGTETATHWSGYMEGAVEAGERAAREILHAMGKIPEDEIWQSEPESVD

                                                                  .
481 VPAVEITHTFWERNLPSVSGLLKIIGFST--SVTALGFVLYKYKLLPRS
    *** ** ** ** **** *** **  *  * ***** *  ** *
472 VPAQPITTTFLERHLPSVPGLLRLIGLTTIFSATALGFLAHKRGLLVRV.
```

FIG. 4. Comparison of the deduced amino acid sequences of human liver MAO A and B. The numbering of amino acids is shown on the left side. The asterisks indicate positions occupied by identical amino acids. The potential glycosylation sites are indicated by triangles. Dashes indicate spaces that were introduced to allow for maximum alignment of identical amino acids. The single-letter amino acid code is used.

5.4 kb were detected in human placenta (Fig. 3, lane A). The size of the mRNA for MAO A (2.1 kb) is consistent with the length of the cDNA insert, suggesting that a full-length cDNA clone of MAO A has been obtained. The nature of the longer transcript (5.4 kb) that hybridized with the hMAO A-7 cDNA is not known. It is possible that this transcript is a MAO A mRNA species containing a longer 5′- or 3′-untranslated sequence or that it represents a transcript related, but not identical, to MAO A mRNA. No significant amount of MAO A transcript was detected in human brain frontal cortex under our hybridization conditions. This finding is consistent with immunocytochemical analysis that showed that the concentration of MAO A is much lower than that of MAO B in this tissue (7, 31). Using the hMAO B-1 cDNA (2.5 kb) as a probe for hybridization, we detected a 3.1-kb mRNA in frontal cortex of human brain. The size of the mRNA for MAO B is larger than the cDNA obtained, suggesting that the transcript contains a long 5′-untranslated region. Relatively low levels of RNA transcripts were observed in placenta when hMAO B-1 cDNA was used as the probe (Fig. 3, lane B). This result is consistent with catalytic activity assays since predominantly MAO A activity has been found in human placenta (10).

When the same RNA blot was hybridized with ^{32}P-labeled actin pseudogene, the radioactivity observed (Fig. 3, lane C) was much stronger than that observed with cDNA probes for either MAO A (Fig. 3, lane A) or MAO B (Fig. 3, lane B). This result indicates that the levels of MAO transcripts were relatively low in these tissues.

Comparison Between Human Liver MAO A and B. Comparison of the amino acid sequences of MAO A and MAO B indicate that these proteins are ≈70% identical (Fig. 4). In contrast, the 5′- and the 3′-noncoding regions show little sequence similarity. The regions of amino acid sequence identity appear randomly distributed over the entire coding region. The overall sequence similarity of these enzymes is also demonstrated by the hydropathy plots of MAO A and B (Fig. 5). The hydropathy patterns are nearly identical, with small but significant differences between amino acids 270 and 290. Examination of the hydropathy plot reveals seven hydrophobic regions (residues 15–30, 110–130, 170–200, 265–270, 295–315, 400–450, and 485–C termini) in each polypeptide that could be responsible for anchoring the protein to the outer mitochondrial membrane. Potential *N*-glycosylation sites were identified in both enzymes, but they are located in dissimilar regions. The potential *N*-glycosylation sites in human liver MAO A and B are specified by the sequence Asn-Val-Thr at positions 181 to 183 and the sequence Asn-Met-Thr at positions 145 to 147, respectively.

DISCUSSION

By using specific oligonucleotide probes, we have isolated full-length cDNA clones that encode the FAD-containing subunits of human liver MAO A and B. The cDNA clones were discriminated by an oligonucleotide (HP-99) that was derived from a peptide generated by tryptic digestion of human placental MAO A. Under the hybridization conditions used, this probe hybridized only to a clone assigned as a MAO A cDNA clone. No stable hybrids were formed with the cDNA assumed to code for MAO B, indicating that those clones were identified correctly. Furthermore, the amino acid sequences of five additional tryptic peptides obtained from MAO B (residues 74–82, 102–118, 229–242, 422–425, and 458–477) and three additional tryptic peptides from MAO A (residues 137–151, 282–291, and 380–394) were identical and specific to those found in the deduced sequences of these two enzymes. Additional evidence that MAO B has been successfully cloned comes from preliminary studies in which this cDNA clone was expressed in *Escherichia coli* as a fusion protein. This protein could be detected with the

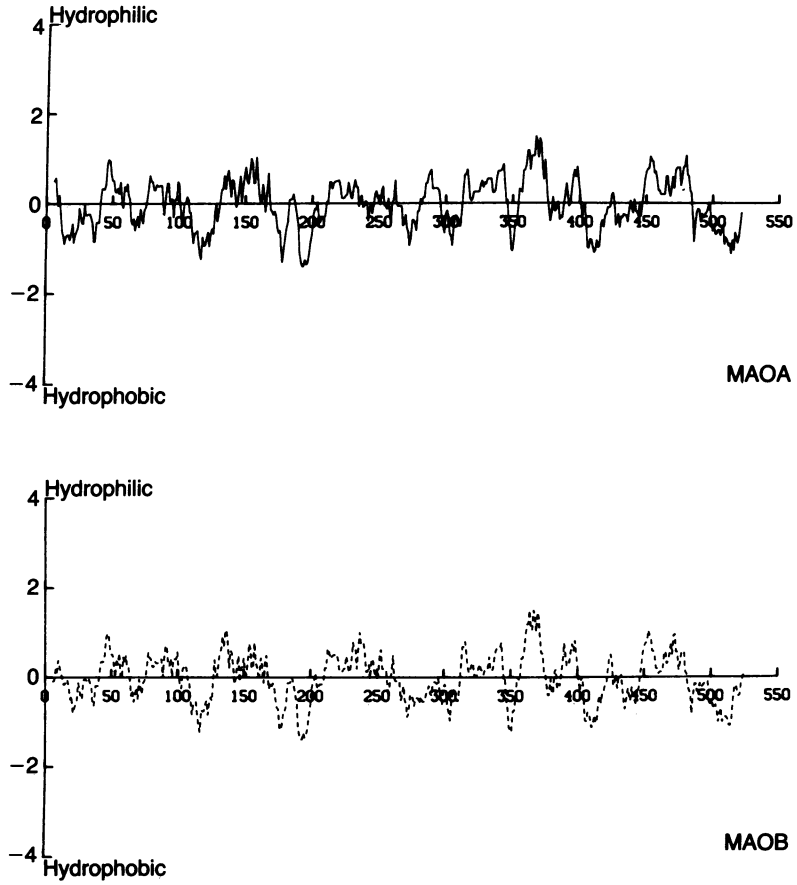

FIG. 5. Hydropathy plots of human liver MAO A and B. The numbers along the *y*-axis represent relative values for hydrophobicity. The numbers along the *x*-axis correspond to the amino acid number.

specific monoclonal antibody MAO B-1C2 in an ELISA and by immunoblot analysis (data not shown).

The deduced polypeptide encoded by hMAO B-1 cDNA has a molecular mass of 58.8 kDa, whereas the protein encoded by hMAO A-7 cDNA is slightly larger (59.7 kDa). These results agree with the apparent subunit molecular weights of these proteins from $NaDodSO_4$/PAGE (18). It has been shown (14) that catalytically active MAO A and B have molecular masses of ≈120 kDa. Whether the two subunits within each enzyme are identical or have small differences that are indistinguishable on $NaDodSO_4$/PAGE is unknown. The fact that the deduced amino acid sequences for MAO A or B contain all of the precise tryptic peptide sequences obtained from purified placental MAO A or liver MAO B, respectively, suggests, but does not prove, that the two subunits within each enzyme are identical.

Comparison of amino acid sequences between human liver MAO A and B reveals a high degree of sequence identity between these two enzymes, suggesting that the genes for MAO A and B are derived from a common progenitor gene. However, examination of their sequences show that these enzymes have clearly distinguishable primary structures. The differences in the restriction patterns of both cloned cDNAs (data not shown) and in the predicted primary structures of these enzymes rule out the hypothesis that the different enzymatic properties of MAO A and B are due to post-transcriptional modifications of the same primary transcript or post-translational modifications of their products. These results support the interpretation that MAO A and B are products of different genes.

Conclusive identification of the N-terminal amino acids for MAO A and B remains to be established because open reading frames precede the putative start codons for these enzymes and because the N-terminal amino acids appear to be blocked (data not shown). However, assignment of the start codon (AUG) is supported by the Kozak sequence rules (32) and the calculated molecular masses of the deduced proteins (59.7 kDa and 58.8 kDa for MAO A and B, respectively).

The FAD-binding site is located in the C-terminal region of both proteins in equivalent positions. Furthermore, an AMP-binding site near the N-terminal end of these molecules (amino acid residues 15–29 and 6–20 in MAO A and B, respectively) corresponds to a region that shows extensive sequence identity in several other flavoproteins, including lipoamide dehydrogenase, glutathione reductase, thioredoxin reductase, D-amino acid oxidase, *p*-hydroxybenzoate hydroxylase, and lactic dehydrogenase (33).

Examination of the primary structures of MAO A and B does not support the hypothesis that these proteins are extensively glycosylated. Only one potential *N*-glycosylation site exists in each protein, with each site in a different relevant position. Furthermore, it appears unlikely that MAO A is glycosylated *in vivo* because the potential *N*-glycosylation site is located within a hydrophobic region.

Immunocytochemical studies of primate brain with monoclonal antibodies that specifically recognize MAO A or B demonstrate that MAO A and B are localized in catecholaminergic and serotonergic neurons, respectively, and in some glial cells and terminal elements throughout this tissue (7, 31). Our finding that MAO A and B are derived from different genes suggests that the differential expression of these enzymes in the central nervous system and in peripheral tissues (7, 31, 34) may be regulated independently by tissue-specific factors. Isolation of two distinct MAO A and B cDNAs provides a basis for examining the possible differential physiological function of these proteins in the central nervous system.

We thank Kenneth Williams, Ph.D., and Kathy Stone at the Yale University Protein Chemistry Facility and Minnie McMillan, Ph.D., and Lynn Williams at the University of Southern California Cancer Center Microchemical Core Laboratory for performing amino acid sequencing studies. We also thank Dr. Roberto Bayardo (Medical Examiner, Austin, TX) for providing human autopsy liver and Dr. Clyde Dorr (Brackenridge Hospital, Austin, TX) for human placenta. We thank the Alzheimer Disease Research Center, University of Southern California, for providing post-mortem human brain. We thank Nutan Patel, Ph.D. (University of Texas Medical Branch, Austin, TX), for aid in preparing human liver MAO B. We also acknowledge the expert technical assistance of Heike Steininger and Bettina Crusius. This work was generously supported by Grants MH 39085, MH 37020, and MH 42729 from the National Institute of Mental Health and by Grant NS 24932 from the National Institutes of Health and a grant from the Ministry of Science and Technology, Federal Republic of Germany, to P.H.S.

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