The primary structure of bovine monoamine oxidase type A

Comparison with peptide sequences of bovine monoamine oxidase type B and other flavoenzymes

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We have isolated cDNA clones believed to encompass the full-length coding sequences for a subunit of bovine monoamine oxidase type A (MAO-A). The clones code for an apoprotein of 527 amino acid residues corresponding to a molecular mass of 59806 Da. The inferred protein sequences show an overall similarity of 68 % with partial amino acid sequences of bovine type B MAO (about 41 % of the total sequence), as well as a greater similarity (greater than 90 %) with some regions including that for the published sequence of the flavin-binding region. Sequence comparisons indicate that these two forms of MAO are encoded by distinct genes. Comparison of this sequence with other flavoenzymes showed similarity with regions associated with non-covalent flavin-binding sites. Analysis of mRNAs coding for MAO enzymes showed a heterogeneity of transcripts consistent with several different forms of monoamine oxidase.

INTRODUCTION

Monoamine oxidase (MAO, EC 1.4.3.4) catalyses the oxidative deamination of dietary and neurotransmitter amines such as dopamine (2,4-dihydroxyphenethylamine), 5-hydroxytryptamine and noradrenaline (Glover & Sandler, 1986). Two isoenzymic forms (type A and B) have been identified by pharmacological (Johnston, 1968), biochemical (Houslay & Tipton, 1974) and immunological (Denney *et al.*, 1982) criteria, and the isoenzymes have discrete neuronal localizations (Westland *et al.*, 1985; Levitt *et al.*, 1982). The two activities are produced by two similar but non-identical enzymes. Both enzymes consist of a flavin-containing active-site subunit and possibly an associated subunit of similar molecular mass (Minamiura & Yasunobu, 1978). The flavin-containing subunits of MAO-A and MAO-B for several mammalian enzymes have been compared by labelling with a radioactive covalent inhibitor pargyline, and differences in apparent molecular masses have been shown by denaturing polyacrylamide-gel electrophoresis (Brown *et al.*, 1980; Cawthon & Breakefield, 1979). Limited proteolysis and peptide mapping also revealed differences (Brown *et al.*, 1980; Cawthon & Breakefield, 1979). However, the cofactor FAD is covalently linked to the cysteine residue of the same pentapeptide (Ser-Gly-Gly-Cys-Tyr) for both MAO-A and MAO-B in a number of mammalian tissues (Nagy & Salach, 1981; Yu, 1981).

MAO has been implicated in several disease states. MAO-B metabolizes the compound 1,2,3,6-tetrahydro-1-methyl-4-phenylpyridine to its toxic metabolites in an idiopathic form of Parkinson's disease (Markey *et al.*,

1984). Lower than normal levels of activity have also been described in patients with various psychiatric disorders (Fowler *et al.*, 1982; Murphy, 1984). Inhibitors of MAO have antidepressant properties (Murphy, 1985). As an initial approach towards understanding the structure and function of MAO-A and MAO-B, we report here the isolation of a cDNA clone for bovine MAO-A, and compare the deduced protein sequence with that of bovine MAO-B.

MATERIALS AND METHODS

Purification and sequencing of MAO-B

MAO-B was purified from bovine liver by a procedure involving phospholipase treatment and Triton X-100 extraction (Salach, 1979). The enzyme was determined to be 99 % pure on the basis of specific activity and migration on electrophoresis in SDS/polyacrylamide gels. The enzyme was carboxymethylated and digested with trypsin in the presence of urea (Williams *et al.*, 1985). Peptides were resolved by reversed-phase h.p.l.c. on a μ Bondapak C₁₈ column with a linear gradient of acetonitrile in 10 mM-potassium phosphate buffer, pH 2.55. Peptides from isolated peaks were evaluated for purity on the basis of amino acid composition. Two peptides were immobilized on aminopolystyrene and their sequences were determined on a Sequemat Mini-15 solid-phase Sequenator (Hemmings *et al.*, 1984).

Cloning of MAO cDNA

Polyadenylated RNA from bovine adrenal medulla was purified by the procedure of LoMedico & Saunders

Abbreviation used: MAO, monoamine oxidase.

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(1976). A cDNA library was constructed in plasmid pBR322 (Saadat *et al.*, 1987). The 47-mer oligonucleotide used for screening the library was produced with an Applied Biosystems 380A synthesizer by the phosphoramidite method and purified by polyacrylamide-gel electrophoresis. A 100 ng portion of the oligonucleotide was labelled with [γ - 32 P]ATP (7000 Ci/mmol; New England Nuclear) by using T4 polynucleotide kinase (Boehringer). A total of approx. 14000 different colonies were screened. About 7000 colonies were transferred to each 20 cm $\times$ 20 cm nitrocellulose filter and lysed by the procedure of Grunstein & Hogness (1975), and the filters were prehybridized at 37 °C for 4 h in hybridization buffer [6 $\times$ SSC (1 $\times$ SSC: 0.15 M-NaCl/0.015 M-sodium citrate buffer, pH 7.0), 5 $\times$ Denhardt's solution (1 $\times$ Denhardt's solution: 0.02 % Ficoll/0.02 % polyvinylpyrrolidone/0.02 % bovine serum albumin; Denhardt, 1966), 50 mM-sodium phosphate buffer, pH 6.5, 100 μ g of boiled herring DNA/ml, 20 % (v/v) deionized formamide and 0.1 g of dextran sulphate (500000 Da)/ml]. The filters were then hybridized at 37 °C for 12 h with the oligonucleotide (100000 c.p.m./ml) in hybridization buffer. The nitrocellulose filters were washed at 37 °C for 30 min in 1 $\times$ SSC/0.1 % SDS with several changes of buffer and exposed overnight with an intensifying screen at -70 °C. Positive colonies were picked and rescreened under the same conditions.

The single strongest-hybridizing clone (G1) was picked and a 0.5 kb *Pst*I fragment was inserted into the *Pst*I site of the pGEM2 vector (Promega Biotec). An RNA probe was transcribed from the SP6 promoter of the pGEM2 vector and used to screen a larger bovine-adrenal-medulla cDNA library (Iacangelo *et al.*, 1986) by the procedure of Maniatis *et al.* (1982). The hybridization solution contained 20 % formamide, 6 $\times$ SCC, 5 $\times$ Denhardt's solution, 0.1 % SDS, 0.2 mg of denatured salmon sperm DNA/ml, 0.2 mg of yeast tRNA/ml and 1 mM-EDTA. Incubation was at 50 °C for 48 h. The filters were washed in 2 $\times$ SSC/0.1 % SDS three times, 10 min each, at room temperature, and then washed in 0.5 $\times$ SSC/0.1 % SDS at 50 °C twice, 30 min each.

Sequencing of MAO-A cDNA

Restriction fragments from clones G1 and 34-3A were subcloned into bacteriophages M13mp18 and M13mp19, and DNA sequences were determined by the dideoxy method with [γ - 35 S]thio[dATP] (Williams *et al.*, 1986; Reed *et al.*, 1986). Exonuclease III treatment was used to generate further clones for sequence analysis (Henikoff, 1984). Both strands of DNA were sequenced from independent subclones.

Northern blots

Bovine tissues were frozen in liquid N₂ within minutes of death. Tissues were pulverized, and RNA was extracted in guanidinium thiocyanate and centrifuged through a CsCl cushion (Chirgwin, 1979). Polyadenylated RNA was isolated by using oligo(dT)-cellulose columns (Maniatis *et al.*, 1982). RNA samples and RNA standards (Bethesda Research Laboratories) were resolved by electrophoresis in formaldehyde gels, standards were located under u.v. light after staining with ethidium bromide and samples were blotted on to Nytran filters (Schleicher and Schuell). Filters were baked under vacuum at 80 °C for 2 h. Prehybridization and hybridization were carried out at 50 °C (Melton *et al.*,

1984); blots were washed successively in 0.2 $\times$ SSC and 0.1 % SDS at 50 °C and exposed to X-Omat AR (Kodak) film for 1-3 days.

MAO assays

Bovine tissues were frozen immediately after death and homogenates were prepared in 10 mM-potassium phosphate buffer, pH 7.4, as described previously (Pintar *et al.*, 1983). Activity was measured with 30 μ M-[G- 3 H]-tryptamine hydrochloride (1 Ci/mmol; Amersham-Searle); reactions were carried out at 37 °C for 30 min, and products were extracted into toluene as described previously (Edelstein & Breakefield, 1986). To determine the relative proportions of type A and B activities, homogenates were preincubated with serial dilutions of clorgyline (1 pM to 1 mM) at 37 °C for 30 min before assay. Curves of inhibition were monophasic or biphasic. Type A activity was designated as that inhibited at 0.1 μ M-clorgyline, type B at 1 mM-clorgyline.

Sequence comparison

The deduced amino acid sequence was compared with the protein database by using the Dayhoff mutational data matrix.

RESULTS

Cloning of MAO cDNA

A cDNA library (Saadat *et al.*, 1987) from bovine adrenal medulla was screened with a synthetic 47-mer oligonucleotide, the sequence of which was derived from part of the amino acid sequence for a peptide of bovine liver MAO-B (FPSVWNPITYLDHNNL, peptide 3 in Fig. 2). The oligonucleotide was synthesized without degenerations by using the most common codon usage for the appropriate amino acids (Grantham *et al.*, 1980). A single strongly hybridizing colony from approx. 14000 bovine-adrenal-medulla cDNA clones was obtained. This initial clone (G1) contained an insert of approx. 500 bp and was sequenced. The region of the clone complementary to the oligonucleotide coded for a protein sequence identical with that of the corresponding bovine liver MAO peptide (type B) except for three mismatches. This clone was used to screen another bovine-adrenal-medulla cDNA library (Iacangelo *et al.*, 1986) in order to gain further sequence information. A total of eight positives were obtained from about 200000 colonies. They contained either a 2.7 kb or a 1.2 kb insert. Restriction mapping showed that the 1.2 kb clones were a subset of the 2.7 kb clones. One of the 2.7 kb clones, 34-3A, was used for further study.

Nucleotide sequence and inferred amino acid sequence

The combined nucleotide sequence of clones G1 and 34-3A is shown in Fig. 1. It appears that clone G1 was fortuitously derived by oligo(dT) priming of an internal poly(A) tract in the mRNA (nucleotide residues 511-516) during construction of the library, so that the two clones overlap by approx. 100 nucleotides and together code for a cDNA of 3.0-3.1 kb long [not including the poly(A) tail]. There is a single long open reading frame with two possible methionine initiation codons. The first in-frame ATG codon corresponds more closely to the consensus start sequence reported by Kozak (1984). Further evidence that this is in fact the initiation codon is obtained by comparison of this sequence with the nucleotide and

			10			20			30			40			50			60					
1	CACGC	CGG	GAG	AAC	CGC	AAC	CAG	AGC	ATG	GAG	AGT	CTG	CAG	AAG	ACG	AGT	GAC	CGC	GGC	CAG			
									Met	Glu	Ser	Leu	Gln	Lys	Thr	Ser	Asp	Ala	Gly	Gln			
13	ATG	TTC	70	GAC	GTA	GTC	80	ATA	GGA	GGC	GGC	ATC	TCA	GGA	TTA	TCT	110	GCT	CGC	AAA	CTC	TTA	
	Met	Phe	Asp	Val	Val	Val	Val	Ile	Gly	Gly	Gly	Ile	Ser	Gly	Leu	Ser	Ala	Ala	Lys	Leu	Leu		
33	GCC	GAA	130	CAT	GAA	GTT	140	AAT	GTT	TTG	GTT	TAA	GAA	GCC	CGG	GAG	AGA	170	GTT	GGA	GGA	AGA	ACA
	Ala	Glu	His	Glu	Val	Val	Asn	Val	Val	Leu	Val	Leu	Glu	Ala	Arg	Glu	Arg	Val	Gly	Gly	Arg	Thr	
53	TAC	ACA	GTG	AGG	AAT	GAA	CAT	GTT	GAC	TAC	GTA	GAT	GTT	GGC	GGT	230	GCT	TAT	GTA	GGC	CCG	CGG	
	Tyr	Thr	Val	Arg	Asn	Glu	His	Val	Asp	Tyr	Val	Asp	Val	Gly	Gly	Ala	Tyr	Val	Gly	Pro			
73	ACC	CAG	AAT	AGG	ATC	TTA	CGG	TTA	TCT	AAG	CAA	CTG	GGT	TTA	GAA	ACT	290	TAC	AAA	GTG	AAT		
	Thr	Gln	Asn	Arg	Ile	Leu	Arg	Leu	Ser	Lys	Lys	Gln	Leu	Gly	Leu	Thr	Thr	Lys	Val	Asn	Asn		
93	GTA	AAC	GAG	CGT	CTT	GTT	320	CAT	TAT	GTC	AAG	GGA	AAA	ACT	TAT	CCA	350	TTT	CGG	GGT	GCC	TTC	
	Val	Asn	Glu	Arg	Leu	Val	Val	His	Tyr	Val	Lys	Gly	Lys	Thr	Tyr	Pro	Phe	Arg	Gly	Ala	Phe		
113	CCT	CCA	GTA	TGG	AAT	380	ATT	GCT	TAT	CTG	GAT	TAC	AAC	AAC	CTC	410	TGG	CGG	ACC	ATG	GAT		
	Pro	Pro	Val	Trp	Asn	Pro	Ile	Ala	Tyr	Leu	Asp	Tyr	Asn	Asn	Leu	Trp	Arg	Thr	Met	Asp			
133	AAC	ATG	GGA	AAA	GAG	440	CCA	GCT	GAT	GCA	CCA	TGG	GAG	GCC	CCA	470	GCA	GTG	GAA	TGG			
	Asn	Met	Gly	Lys	Glu	Ile	Pro	Ala	Asp	Ala	Pro	Trp	Glu	Ala	Pro	His	Ala	Val	Glu	Trp			
153	GAT	AAG	ATG	ACT	ATG	500	GAT	CTC	ATC	GAA	AAA	ATC	TGC	TGG	ACA	530	AAG	ACT	GCT	CGG	CAG		
	Asp	Lys	Met	Thr	Met	Lys	Asp	Leu	Ile	Glu	Lys	Ile	Cys	Trp	Thr	Lys	Thr	Ala	Arg	Gln			
173	TTT	GCA	TCT	CTC	TTT	560	AAT	ATC	AAT	GTG	ACC	TCT	GAA	CCC	CAT	590	GAG	GTG	TCT	GCC	CTG		
	Phe	Ala	Ser	Leu	Phe	Val	Asn	Ile	Asn	Val	Thr	Ser	Glu	Pro	His	Glu	Val	Ser	Ala	Leu			
193	TGG	TTC	TTG	TGG	TAC	620	AAA	CAA	TGT	GGG	GGC	ACA	ACT	CGG	ATA	650	TTC	TCC	ATT	ACC	AAC		
	Trp	Phe	Leu	Trp	Tyr	Val	Lys	Gln	Cys	Gly	Gly	Thr	Thr	Arg	Ile	Phe	Ser	Ile	Thr	Asn			
213	GGT	GGC	CAG	GAA	CGG	680	TTT	GTA	GGT	GGA	TCT	GGT	CAA	GTA	AGC	710	GAA	CGG	ATA	ATG	CAA		
	Gly	Gly	Gln	Glu	Arg	Lys	Phe	Val	Gly	Gly	Ser	Gly	Gln	Val	Ser	Glu	Arg	Ile	Met	Gln			
233	CTC	CTA	GGG	GAC	AGG	740	AAA	CTG	AGG	AGC	CCT	GTC	ACC	TAT	GTT	770	GAC	CAG	TCA	AGT	GAA		
	Leu	Leu	Gly	Asp	Arg	Val	Lys	Leu	Arg	Ser	Pro	Val	Thr	Tyr	Val	Asp	Gln	Ser	Ser	Glu			
253	AAC	ATC	ACC	GTA	GAG	800	CTG	AAT	CGT	GAA	CTT	TAT	GAG	TGC	CGG	830	TAT	GTG	ATT	AGT	GCC		
	Asn	Ile	Thr	Val	Glu	Thr	Leu	Asn	Arg	Glu	Leu	Tyr	Glu	Cys	Arg	Tyr	Val	Ile	Ser	Ala			
273	ATC	CCA	CCA	ACT	TTG	860	GCC	AAG	ATA	CAC	TTT	AGA	CCA	GAG	CTT	890	CCA	TCA	GAG	CGA	AAC		
	Ile	Pro	Pro	Thr	Leu	Thr	Ala	Lys	Ile	His	Phe	Arg	Pro	Glu	Leu	Pro	Ser	Glu	Arg	Asn			
293	CAG	CTG	ATA	CAG	CGT	920	CCA	ATG	GGG	GCT	GTC	ATT	AAG	TGC	ATG	950	TAT	TAC	AAG	GAG			
	Gln	Leu	Ile	Gln	Arg	Leu	Leu	Met	Gly	Ala	Val	Ile	Lys	Cys	Met	Met	Tyr	Tyr	Lys	Glu			
313	GCC	TTT	TGG	AAG	AAA	980	GAT	TAC	TGT	GGC	TGT	ATG	ATC	ATC	GAA	1010	GAT	GAG	GAA	GCT	CCA		
	Ala	Phe	Trp	Lys	Lys	Lys	Asp	Tyr	Cys	Gly	Cys	Met	Ile	Ile	Glu	Asp	Glu	Glu	Ala	Pro			
333	ATT	TCA	ATA	ACA	CTG	1040	GAT	ACC	AAG	CCA	GAT	GGA	TCA	CTG	CCT	1070	GCT	ATC	ATG	GGC	TTC		
	Ile	Ser	Ile	Thr	Leu	Asp	Asp	Thr	Lys	Pro	Asp	Gly	Ser	Leu	Pro	Ala	Ile	Met	Gly	Phe			
353	ATC	CTC	GCT	CGA	AAA	1100	GCT	GAC	CGC	CTC	GCT	AAA	GTG	CAT	AAA	1130	ATG	AGG	AAA	AGA	AAA		
	Ile	Leu	Ala	Arg	Lys	Ala	Asp	Arg	Leu	Ala	Lys	Val	His	Lys	Asp	Ile	Arg	Lys	Arg	Lys			
373	ATC	TGT	GAA	CGG	TAC	1160	AAA	GTG	CTA	GGA	TCT	CAA	GAA	GCT	TTA	1190	CAT	CCA	GTG	CAC	TAT		
	Ile	Cys	Glu	Arg	Tyr	Ala	Lys	Val	Leu	Gly	Ser	Gln	Glu	Ala	Leu	His	Pro	Val	His	Tyr			
393	GAA	GAG	AAG	AAC	TGG	1220	CAG	GAG	CAG	TAC	TCC	GGA	GGC	TGC	TAC	1250	ACT	GCC	TAC	TTC	CCC		
	Glu	Glu	Lys	Asn	Trp	Cys	Gln	Glu	Gln	Tyr	Ser	Gly	Gly	Cys	Tyr	Thr	Ala	Tyr	Phe	Pro			
413	CCG	GGG	ATC	ATG	ACT	1280	CAA	TAC	GGA	AGG	GTT	ATT	CGC	CAA	CCT	1310	GTA	GGC	AGG	ATT	TAC	TTT	
	Pro	Gly	Ile	Met	Thr	Gln	Tyr	Gly	Arg	Val	Ile	Arg	Gln	Pro	Val	Gly	Arg	Ile	Tyr	Phe			
433	GCT	GGC	ACG	GAA	ACT	1340	ACA	CAA	TGG	AGC	GGT	TAT	ATG	GAA	GGA	1370	GTA	GAG	GCT	GGT			
	Ala	Gly	Thr	Glu	Thr	Ala	Thr	Gln	Trp	Ser	Gly	Tyr	Met	Glu	Gly	Ala	Val	Glu	Ala	Gly			
453	GAA	CGG	GCA	GCT	AGA	1400	GTT	TTG	AAT	GCT	TTG	GGG	AAG	CTG	TCT	1430	GCC	AAA	GAT	ATC	TGG		
	Glu	Arg	Ala	Ala	Arg	Glu	Val	Leu	Asn	Ala	Leu	Gly	Lys	Leu	Ser	Ala	Lys	Asp	Ile	Trp			
473	ATC	CAA	GAA	CCT	GAA	1460	GAT	GTT	CCA	GCT	GTA	GAG	ATC	ACC	CCC	1490	AGC	TTC	TGG	GAG			
	Ile	Gln	Glu	Pro	Glu	Ala	Glu	Asp	Val	Pro	Ala	Val	Glu	Ile	Thr	Pro	Ser	Phe	Trp	Glu			
493	AGA	AAC	CTG	CCT	TCC	1520	GTA	TCA	GGC	CTG	CTG	AAG	ATT	GTT	GGG	1550	TTT	TCC	ACA	TCG	ATA	ACT	
	Arg	Asn	Leu	Pro	Ser	Val	Ser	Val	Leu	Leu	Lys	Ile	Val	Gly	Phe	Ser	Thr	Ser	Ile	Thr			
513	GCC	CTG	TGG	TTT	GTG	1580	TAC	AGA	TTC	AGG	CTG	CTA	AGC	CGA	TCC	1610	TGA	AGT	TTT	GGC	TTC		
	Ala	Leu	Trp	Phe	Val	Met	Tyr	Arg	Phe	Arg	Leu	Leu	Ser	Arg	Ser								
	ATG	CTT	TTT	GCT	TAT	1640	TCC	CCG	GTA	TCA	TCA	AAA	GCA	AAT	TGC	1670	TGA	CCC	GTA	GAA	CAG		
	CCG	TGC	AAT	CAG	ATC	1700	TTT	AAG	TGA	ACT	GAA	TTT	AAC	CCC	TCA	1730	GTT	TAA	TCT	CAG	TGT		
	CAT	GAC	CTC	CTT	AAT	1760	TTC	CAC	GAA	GAT	CAA	GCT	CCA	TCT	TGT	1790	GAT	TTG	TCT	TA			

Fig. 1. Nucleotide and predicted amino acid sequences of bovine MAO-A cDNA

Sequences similar to those of peptides of bovine liver MAO-B are underlined. The sequence of the covalent flavin-binding pentapeptide is double underlined (Nagy & Salach, 1981; Yu, 1981).

Peptide	Residues	Sequence
1	31-45	L L H D S G L N V I V L E A R * * A E H E V * * L * * * * *
2	62-79	Y V D L G G S Y V G P T Q N H I L R * * * V * * A * * * * * R * * *
3	110-129	G S F P S V W N P I T Y L D H N N L W R * A * * P * Y * * * A * * * Y * * * * *
4	268-288	Y V I S A V P P V L G G K I H F N P P L P * * * * I * * T L T A * * * * R * E * *
5	298-305	V P L G S V I K L * M * A * * *
6a		R I V Y Y K E P F W
6b		P I M Y Y K E P F W K
6c		Y K E P F W I P P P M ? K
6d	306-332	C M M * * * * A * * K K K D Y * G C * * * Q D Q Q * *
7	336-356	A L D D T K P D G S Y P A I L G F K L A R T * * * * * * * L * * * M * * I * * *
8	372-395	K L C D L Y A K V L G S Q E A L H P V H Y E E K * I * E R * * * * * * * * * * * * * *
9	396-413	N W C E E Q Y S G G C Y T A Y F P (R) * * * Q * * * * * * * * * * * * P
10	430-441	I Y F A G T E T A T (H)(K) * * * * * * * * Q W
11	466-494	I P E D E I W L P E P E S V D V P A K P I S T S S M M M P L S A K D * * I Q * * * A E * * * * V E * T P * F W E R N

Fig. 2. Comparison of peptide sequences of bovine liver MAO-B peptides (upper sequences) with the predicted amino acid sequence of MAO-A deduced from the cDNA sequence (lower sequence)

Asterisks indicate identical amino acids; question marks or parentheses indicate unknown or uncertain amino acids in protein sequence. Data were from the present paper (peptides 3 and 8) or from Chen & Weyler (1988). Peptides have been combined to give longer continuous sequences. Where protein sequence data indicated heterogeneity in sequence, all sequences found were included.

inferred protein sequence of the corresponding human cDNA (Hsu *et al.*, 1988; Bach *et al.*, 1988). The inferred protein sequences are conserved starting from this first methionine codon (50 % for next 12 amino acid residues, 78 % for corresponding nucleotides); however, nucleotide residues preceding this are considerably less similar (42 %), suggesting that the sequence upstream of position 27 represents the 5' untranslated region. Taking this codon at position 27 as the start codon, the predicted size of the protein is 527 amino acid residues corresponding to a molecular mass of 59806 Da for the apoenzyme or 60600 Da including the FAD cofactor. This molecular mass is consistent with values determined biochemically (Brown *et al.*, 1980; Cawthon & Breakefield, 1979).

Comparison of the predicted protein sequence of this clone with that obtained from protein sequences of tryptic peptides of bovine liver MAO-B is shown in Fig. 2. None of the peptides was completely identical with the predicted protein sequence, although an overall degree of similarity of 68 % was obtained. It seemed possible that the isolated cDNA codes in fact for MAO-A as bovine adrenal medulla contains approximately equal amounts of both MAO-A and MAO-B activities (our data) [chromaffin cells have been reported to express type B MAO and fibroblasts together with associated cells to express type A (Youdim *et al.*, 1984)]. The deduced amino acid sequence shows greater similarity to the amino acid sequence of human placental MAO-A than

to that of bovine MAO-B [81.5 % over 163 residues (Bach *et al.*, 1988; S. Chen & W. Weyler, unpublished work)], supporting this hypothesis.

MAO mRNAs

The amount and type of MAO activity was measured in homogenates of bovine brain cortex and liver. RNA was extracted from the same tissue samples, and the sizes of mRNAs encoding MAO sequences were determined by hybridizing a subclone of 34-3A (containing coding sequences in the region of the FAD-attachment site, nucleotide residues 905-1470) to Northern blots (Fig. 3).

Specific activity in bovine liver was 2578 pmol/min per mg of protein, 98 % type B, and in brain cortex was 268 pmol/min per mg of protein, 90 % type A and 10 % type B. On Northern blots a predominant band of 5.3 kb (slightly larger than 28 S RNA) was observed in brain cortex. Another dense band of 2.0 kb was noted in cortex as well as fainter bands of 6.3, 3.0 and 2.8 kb in decreasing order of density. A relatively intense band of 2.8 kb was seen in liver as well as a fainter band of 5.3 kb.

The differently sized transcripts are believed to correspond to mRNAs of MAO that have differing degrees of similarity to the MAO-A clone used, and may correspond to products of differential splicing, transcription initiation or polyadenylation from one or more MAO genes.

Fig. 3. Northern-blot analysis of MAO-A

Total (lane 1, 10 μ g) or polyadenylated RNA (lane 3, 10 μ g) from bovine brain cortex and total (lane 2, 10 μ g) or polyadenylated RNA (lane 4, 10 μ g) from bovine liver were hybridized with a subclone of 34-3A (nucleotide residues 905–1470). Sizes were calculated by comparison with RNA standards (Bethesda Research Laboratories).

Sequence comparison with other proteins

A comparison of the MAO-A protein sequence with the sequence data library showed significant sequence similarity to several flavin-containing enzymes (Weyler *et al.*, 1987). A region of the *N*-terminus of the predicted protein contains many of the features associated with a flavin-binding site. This is illustrated in Fig. 4, with the residues important in prediction of an ADP-binding $\beta\alpha\beta$ -fold boxed (Wierenga *et al.*, 1986); several other FAD-binding enzymes are aligned with MAO-A to illustrate the sequence conservation. The predictive

sequence fingerprint is the presence of a basic or hydrophilic residue in the first position, conservative substitution of small hydrophobic residues in the first β -fold (positions 2 and 4) as well as invariant glycine residues (positions 6, 8 and 11), small hydrophobic residues at positions 15, 18, 25 and 27 and an acid residue at position 29. The only divergence from this fingerprint is the substitution of an aspartic acid residue in the first position for the MAO-A sequence. There is another shorter region of similarity with other flavoenzymes, illustrated in Fig. 5. This sequence is known to correspond to the FMN phosphate-binding residues (indicated with asterisks) of NADPH-cytochrome *P*-450 reductase and the bacterial flavodoxins; cytochrome *P*-450 is also homologous in this region. There is also a covalent flavin-binding site at position 406, known from the direct sequence data (Nagy & Salach, 1981; Yu, 1981).

DISCUSSION

We present here nucleotide and deduced amino acid sequences for a subunit of bovine MAO-A, inferred from clones isolated from bovine adrenal-medulla cDNA libraries. Although the cDNA clone was initially selected by an oligonucleotide probe based on the sequence of a peptide of bovine MAO-B, parts of the deduced amino acid sequence, were similar to but not identical with other peptide sequences of bovine MAO-B (Fig. 2). Evidence that this clone codes for a subunit of MAO-A rather than MAO-B was obtained from comparison with human MAO-A peptide sequences (87% similarity with human MAO-A; 68% similarity with bovine MAO-B).

Comparison of the deduced amino acid sequence for bovine MAO-A with that for bovine MAO-B showed regions of almost complete identity together with regions of partial identity and conservative substitution; since all the peptides showed at least one mismatch, this indicates that the two enzyme activities are the products of similar but different genes, and do not arise from alternative splicing of a single gene. The covalent flavin-binding site identified by direct sequence data (Nagy & Salach, 1981; Yu, 1981) is located at position 406 about 120 residues from the *C*-terminus. This region (residues 377–411) is identical in both bovine MAO-A and bovine MAO-B and in human MAO-A (Hsu *et al.*, 1988; Bach *et al.*, 1988), and may form part of a recognition domain

Enzyme	Cofactor		β	α	β
MAO-A (bovine)	Covalent FAD	15–45	D V V V I G G I S G L S A A K L L A E H E V N V L V L E A R		
FRDHase-A (<i>Escherichia coli</i>)	Covalent FAD	8–38	D L A I V G A G G A G L R A A I A A A Q A N P N A K I A L I S		
SDHase-A (<i>Bacillus subtilis</i>)	Covalent FAD	6–36	S I I V V G G G L A G L M A T I K A A E S G M A V K L F S I V		
LPDHase (pig heart)	Non-covalent FAD	6–36	D V T V I G S G P G G Y V A A I K A A Q L G F K T V C I E K N		
MRase (<i>Pseudomonas aeruginosa</i>)	Non-covalent FAD	100–130	Q V A V I G S G G A A M A A A L K A V E Q G A Q V T L I E R G		
GRase (human)	Non-covalent FAD	22–52	D V L V I G G S G G L A S A R R A A E L G A R A A V V E S H		
GRase (human)	NADP	189–225	R S V I V G A G Y I A V E M A G I L S A L G S K T S L M I R H		

Fig. 4. Comparison of sequences involved in the FAD-binding fold for MAO-A and other flavoenzymes

Key: FRDHase-A, fumarate reductase A (Cole, 1982); SDHase-A, succinate dehydrogenase A (Phillips *et al.*, 1987); LPDHase, lipoamide dehydrogenase (Williams *et al.*, 1982); MRase, mercuric reductase (Brown *et al.*, 1983); GRase, glutathione reductase (Krauth-Siegel *et al.*, 1982).

Enzyme		Sequence
MAO-A (bovine)	430-447	I Y F A G T E T A T Q W S G Y M E G
Cyt P-450 (rat)	295-312	L F F A G T E T S S T T L R Y G F L
Cyt P-450 Rase (rat)	83-99	V F Y * G S Q T G T A E E F A N R L
FDX (<i>Desulfovibrio vulgaris</i>)	6-22	I V Y * G S T T G N T E Y T A E T I
FDX (<i>Megasphaera elsdenii</i>)	4-20	I V Y * W S G T G N T E A M A N E I

Fig. 5. Sequence similarities between MAO-A, cytochrome P-450 reductase and cytochrome P-450

Key: Cyt P-450, phenobarbital-inducible cytochrome P-450 (Fujii-Kuriyama *et al.*, 1982); Cyt P-450 Rase, NADPH-cytochrome P-450 reductase (Porter & Kaspar, 1986); FDX, ferredoxin-NADP⁺ reductase (Tanaka *et al.*, 1974, 1977). Asterisks refer to residues referred to in text.

necessary for the enzymic attachment of the flavin cofactor.

A search of the protein and nucleic acid databases revealed that the inferred protein sequences showed similarities to those of other flavoenzymes. A region near the *N*-terminus contained many of the features associated with a flavin-binding site (Fig. 4). These include the triplet of glycine residues thought to be important in formation of the Rossman nucleotide-binding fold (discussed in Wierenga *et al.*, 1986). Another smaller region near the covalent FAD-binding site (residue 406) is similar to a region of cytochrome P-450 reductase (Fig. 5) involved in binding of the FMN phosphate residues in this enzyme (Porter & Kaspar, 1986). Unexpectedly, however a non-flavoprotein cytochrome P-450 was also similar in this region, so that the significance of this similarity has yet to be clarified.

In order that *N*-terminal flavin-binding site can interact with the covalently bound FAD cofactor (residue 406), the protein must fold so that the *N*- and *C*-termini are close together. Alternatively, two protein subunits with their associated flavin cofactors could be arranged in a parallel configuration such that the covalent flavin of each subunit is associated with the *N*-terminal flavin-binding region of the other subunit.

MAO is an integral mitochondrial outer-membrane protein (Greenawalt, 1972) and is to our knowledge the first mammalian mitochondrial outer-membrane protein to be cloned. The predicted sequence of MAO-A should therefore contain those amino acid motifs that target the protein to the mitochondria and anchor it in the lipid bilayer. The sequences responsible for targeting and anchoring a yeast mitochondrial outer-membrane protein have been identified, all the information being contained within the *N*-terminal 41 amino acid residues (Hase *et al.*, 1984). Deletion of this entire segment, which consisted of a stretch of 28 uncharged amino acid residues flanked at both ends by basic residues, resulted in a failure of membrane insertion. However, several smaller deletions showed that targeting was mediated by a shorter region including the 11 *N*-terminal acid residues whereas the permanent membrane anchor required the presence of the whole sequence. Although the sequence of MAO-A contains several hydrophobic regions, none of these is longer than 17 uncharged amino acid residues in length. An α -helical hydrophobic domain must be at least 21 residues in length to span the membrane (Eisenberg, 1984). The possibility exists that a shorter non-helical

domain could be responsible for targeting the insertion of MAO-A to the mitochondrial outer membranes, but it is difficult to predict such sequences by computer algorithms (discussed in Eisenberg, 1984). An alternative intriguing speculation is that MAO-A is anchored in the mitochondrial outer membrane by a different mechanism. An increasingly large number of proteins are now known to be covalently bound to lipid, and for several cell-surface proteins these covalently bound complex phospholipids anchor the protein to the cell membrane (reviewed in Sefton & Buss, 1987). Treatment of cells with phospholipase C releases these proteins. Earlier reports in the literature have suggested that preparations of purified monoamine oxidases contained significant amounts of phospholipids [e.g. bovine liver MAO, 7 mol of phospholipid/mol of 100 000 Da protein (Gomes *et al.*, 1969); rat liver MAO, 36 mol of phospholipid/mol of 130 000 Da protein (Baker & Hemsworth, 1978)]. Indeed, treatment with phospholipase C of purified rat liver MAO released 95-96% of the associated phospholipids (Baker & Hemsworth, 1978). The isolation of cDNA clones for MAO-A now enables this problem to be addressed directly. The ability of truncated or altered proteins, created by the transfection of cDNA clones mutagenized *in vitro* into mammalian cells, to be correctly inserted in to the mitochondrial outer membrane will identify the mechanism of attachment and domains important for targeting.

Isolation of this clone also allows us to determine the role that MAO has in the many disease states in which it has been implicated and further to compare the structure of MAO-A with that of MAO-B. The rational design of novel inhibitors of the two enzymes will also be facilitated.

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