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Molecular Cloning and Sequence Analysis of cDNAs Encoding Porcine Kidney D-Amino Acid Oxidase†

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ABSTRACT: Complementary DNAs encoding D-amino acid oxidase (EC 1.4.3.3, DAO), one of the principal and characteristic enzymes of the peroxisomes of porcine kidney, have been isolated from the porcine kidney cDNA library by hybridization with synthetic oligonucleotide probes corresponding to the partial amino acid sequences. Analysis of the nucleotide sequences of two clones revealed a complete 3211-nucleotide sequence with a 5′-terminal untranslated region of 198 nucleotides, 1041 nucleotides of an open reading frame that encoded 347 amino acids, and a 3′-terminal untranslated region of 1972 nucleotides. The deduced amino acid sequence was completely identical with the reported sequence of the mature enzyme [Ronchi, S., Minchiotti, L., Galliano, M., Curti, B., Swenson, R. P., Williams, C. H. J., & Massey, V. (1982) *J. Biol. Chem. 257*, 8824-8834]. These results indicate that the primary translation product does not contain a signal peptide at its amino-terminal region for its translocation into peroxisomes. RNA blot hybridization analysis suggests that porcine kidney D-amino acid oxidase is encoded by three mRNAs that differ in size: 3.3, 2.7, and 1.5 kilobases. Comparison of the sequences of the two cDNA clones revealed that multiple polyadenylation signal sequences (ATTAAA and AACAAA) are present in the 3′-untranslated region, making the different mRNA species. The efficiency of 3′ processing of the RNA was quite different between the two signal sequences ATTAAA and AACAAA. Southern blot analysis showed the presence of a unique gene for D-amino acid oxidase in the porcine genome.

Flavoenzymes catalyze a variety of reactions by transferring one or two electrons between chemically diverse donor and acceptor molecules. D-Amino acid oxidase (EC 1.4.3.3, DAO)[1] is one of the representative flavoproteins with flavin adenine dinucleotide (FAD) as the prosthetic group that catalyzes the oxidative deamination of D-amino acids. Since the initial characterization (Krebs, 1935) and crystallization (Kubo et al., 1958; Massey et al., 1961; Yagi et al., 1962) of this enzyme, many investigations have been made to clarify the physicochemical properties and reaction mechanism of the enzyme. Systematic studies of DAO activity in various tissues revealed its existence in liver, kidney proximal tubules, certain parts of brain (Dunn & Perkoff, 1963), and granules of neutrophilic leukocytes (Cline & Lehrer, 1969). However, D-amino acids have not been found in mammalian proteins, and they do not appear to be intermediates in normal metabolism. Several lines of evidence indicating the function of the enzyme were reported (Hamilton et al., 1979; Nakajima et al., 1981), but the biological significance of this enzyme still remains to be elucidated.

Intracellular localization of DAO is reported to be in the special organelle, i.e. peroxisome (de Duve & Baukhuin, 1966), and DAO is one of the principal and characteristic enzymes of the peroxisomes of porcine kidney. This organelle has a

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[1] Abbreviations: DAO, D-amino acid oxidase; bp, base pair; kb, kilobase; FAD, flavin adenine dinucleotide; PAS, periodic acid-Schiff.

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single membrane, metabolizing hydrogen peroxide and catalyzing the β-oxidation of fatty acids. Our previous study has demonstrated that DAO is synthesized on free ribosomes, and both the in vivo and in vitro synthesized DAO have the same size as that of the mature enzyme, indicating a posttranslational transfer of DAO into peroxisomes without any proteolytic modification (Fukui et al., 1986). Studies on the biosynthesis of other peroxisomal enzymes such as catalase, the enzymes catalyzing fatty acid β-oxidation, and urate oxidase agreed with our result (Goldman & Blobel, 1978; Miura et al., 1984), but not in the case of 3-ketoacyl-CoA thiolase. cDNA cloning analysis revealed that catalase and the enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme have no terminal peptide extension as a signal for translocation into peroxisomes (Osumi et al., 1985; Furuta et al., 1986).

We have started molecular cloning of the cDNA for DAO with the following aims: (1) to facilitate the elucidation of the biological function of DAO; (2) to study the regulation of the biosynthesis of this enzyme; (3) to approach the translocation mechanism of DAO into peroxisomes at the molecular level in order to obtain some clues for the analysis of the biogenesis of this organelle. In this paper, we describe the molecular cloning and the complete nucleotide sequence of the cDNA encoding porcine kidney DAO. In addition, the expression in the tissue and the genomic organization of the DAO gene are also presented.

Experimental Procedures

Materials. Reagents were obtained as follows: [γ-^{32}P]ATP (sp act. 3000–4000 Ci/mmol) and [α-^{32}P]TTP and [α-^{32}P]-dGTP (sp act. 3000 Ci/mmol) from New England Nuclear/Du Pont; [α-^{32}P]dCTP (sp act. 410 Ci/mmol) from Amersham, U.K.; avian myeloblastosis virus reverse transcriptase from Midwest Bio-Products; oligo(dT)–cellulose (type 7), oligo(dA)–cellulose (type 7), *Escherichia coli* ribonuclease H, *E. coli* DNA polymerase I, *E. coli* DNA ligase, the Klenow fragment of *E. coli* DNA polymerase I, terminal deoxynucleotidyl transferase, T4 polynucleotide kinase, and Okayama-Berg vector strain kit (pSV7186 and pSV1932) from Pharmacia, Sweden; restriction endonuclease from Toyobo Co., Japan; nitrocellulose filter from Advantec, Japan, and Schleicher & Schuell, West Germany; M13 mp18 and mp19 RF-DNA from Takara Shuzo Co., Japan; agarose type I from Sigma; guanidinium thiocyanate from Fluka, Switzerland; RNasin from Promega-Biotec; sequencing reagents from Takara Shuzo and Toyobo Co., Japan.

Oligonucleotide Synthesis. The two amino acid sequences corresponding to residues 106–111 (Tyr-Trp-Lys-Asp-Met-Val) and 66–71 (Asn-Trp-Asn-Gln-Gln-Thr) of DAO (Ronchi et al., 1982) were chosen for the synthesis of oligonucleotide probes (Figure 1). Mixtures of all possible oligonucleotides complementary to mRNA were synthesized by the phosphoramidite method (Matteucci & Caruthers, 1981) with an automated DNA synthesizer (Applied Biosystems, Model 380A). Full-length oligonucleotides were purified by high-performance liquid chromatography on a reverse-phase (C_{18}) column. The synthetic probes were phosphorylated at the 5′ end by transfer from [γ-^{32}P]ATP with T4 polynucleotide kinase. Unincorporated nucleotides were removed by chromatography over a DEAE-cellulose column.

RNA and DNA Preparation. High molecular weight DNA was obtained from porcine kidney as described (Yaoita & Honjo, 1980). Total RNA was extracted from a porcine kidney with guanidinium thiocyanate (Chirgwin et al., 1979), and poly(A+) RNA was isolated by subjecting the total RNA to oligo(dT)–cellulose column chromatography (Aviv & Leder, 1972).

cDNA Library Construction and Isolation of cDNA for DAO. Vector primer and oligo(dG)-tailed linker for cDNA library construction were prepared from pSV7186 and pSV1932, respectively, according to the original procedure described by Okayama and Berg (1982). A porcine kidney cDNA library was then constructed with the purified mRNA. *E. coli* HB101 was transformed and selected for ampicillin resistance. About 500 000 transformants derived from the cDNA library were screened by hybridization (Hanahan & Meselson, 1980) with the mixture of oligonucleotide probes as described in Figure 1. Hybridization and filter washing conditions were as described (Wallace et al., 1979). Twenty clones gave positive hybridization with both probes I and II, and two clones (pDAO-10, pDAO-13), which apparently carry the largest insert, were selected for further sequence analysis. Cloning procedures were carried out under P2 conditions according to the Japanese guidelines for recombinant DNA research.

DNA Sequence Determination. cDNA clones were digested with a variety of restriction enzymes to give convenient DNA fragments for subcloning into M13 phage vectors mp18 and mp19 (Yanisch-Perron et al., 1985). The nucleotide sequences were determined by the dideoxy chain-termination method of Sanger et al. (1977).

Northern and Southern Blot Analysis. For RNA blot analysis, RNA (5 μg of poly(A+) RNA per lane) was denatured with glyoxal and dimethyl sulfoxide, separated on a 1.5% agarose gel, and transferred to a nitrocellulose filter according to Thomas et al. (1980). For DNA blot analysis, high molecular weight DNA from porcine kidney was digested with restriction enzymes, electrophoresed on a 0.7% agarose gel (2 μg per lane), and transferred to a nitrocellulose filter according to Southern (1975). The filter was hybridized with DNA fragments derived from a cDNA insert that was labeled by nick translation with [α-^{32}P]dCTP to obtain a specific activity of 150–300 cpm/pg (Rigby et al., 1977). Hybridization and filter washing conditions were as described (Honjo et al., 1979). When the mixture of synthetic oligonucleotides was used as probes, the hybridization conditions were the same as those of library screening.

Results

Isolation of cDNA Clones for DAO. The approach used to clone cDNA for DAO was to screen a library of cDNA clones by hybridization with synthetic oligonucleotide probes that had been designed on the basis of known amino acid sequence data. A cDNA library was constructed with the elaborated plasmid vector of Okayama and Berg (1982) by the use of poly(A+) RNA extracted from a porcine kidney cortex. Two stretches of amino acid sequence from the published data (Ronchi et al., 1982) were chosen for the oligonucleotide synthesis as shown in Figure 1. The cDNA library was screened by hybridization with two oligonucleotide probes containing all possible cDNA sequences for the selected amino acid sequences. Twenty hybridization-positive clones for both probes I and II were identified from approximately 500 000 transformants. Upon restriction enzyme analysis, these clones were found to share common restriction sites. Two clones (pDAO-10 and pDAO-13), which apparently harbored the largest cDNA insert, were selected for further analysis and subjected to nucleotide sequence determination.

Nucleotide Sequence Analysis. The restriction endonuclease map and outline of the strategy used to determine the whole nucleotide sequence of the cDNA insert in clone pDAO-10

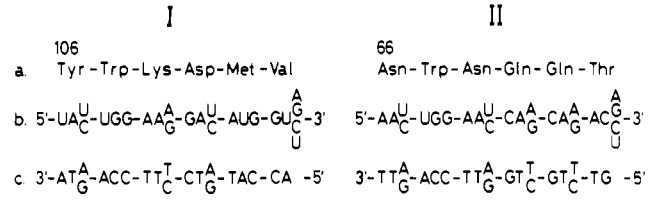

FIGURE 1: Synthetic oligonucleotides used as hybridization probes. Two portions of the amino acid sequence of DAO (I and II) were chosen for the synthesis of oligonucleotide probes: (a) amino acid sequence of DAO; (b) predicted mRNA sequences; (c) 17-mer oligonucleotide probes complementary to possible mRNAs.

as well as the partial sequence of the insert in pDAO-13 are shown in Figure 2. Comparison of restriction endonuclease sites reveals complete homology between the two clones except the limited region of the 5′ end. Figure 3 shows the 3211-nucleotide sequence encoding the porcine DAO, determined with clones pDAO-10 and pDAO-13. This sequence is flanked by a poly(A) tract at the 3′ end. Determination of the 5′-terminal sequence of pDAO-13 cDNA detected a 109-nucleotide deletion and a 5′-directed 54-nucleotide extention compared with that of pDAO-10, whereas the other nucleotide sequences analyzed were identical with each other.

As shown in Figure 3, pDAO-10 starts at nucleotide 55, and pDAO-13 lacks nucleotides from 81 to 189 (109 nucleotides). The first ATG codon is located at nucleotides 99–101. However, the TGA termination codon (nucleotides 114–116) appears in-frame 12 nucleotides downstream, and this reading frame codes only 5 amino acid residues. The second ATG codon exists at nucleotides 199–201 and appears downstream from the in-frame terminator TGA (nucleotides 82–84). This ATG is preceded by sequences that fulfill the Kozak (1981) criteria for initiation codon. The open reading frame is followed by the TGA termination codon at nucleotides 1240–1242. The 1041-nucleotide reading frame codes for the 347 amino acids, and the deduced amino acid sequence was in complete agreement with the reported sequence by Ronchi et al. (1982). One ambiguous amino acid residue in the reported sequence was Asx at position 192. This residue was assigned to be Asp on the basis of the nucleotide sequence determination. To confirm the N-terminal amino acid, DAO was purified from porcine kidney according to Curti et al. (1973). The N-terminal amino acid sequence (32 residues) of the purified enzyme preparation, determined by a gas-phase amino acid sequenator (Hewick et al., 1981), starts with methionine as does the deduced sequence and provides the same result (data not shown). Therefore, the ATG codon at nucleotides 199–201 is concluded to be the initiator of translation.

Taken altogether, the complete cDNA sequence is composed of a 5′-untranslated region of 198 nucleotides, a coding region of 1041 nucleotides for 347 amino acids, and a 3′-terminal untranslated region of 1972 nucleotides. These total 3211 nucleotides covered almost the full length of the mRNA sequence on the basis of the RNA blot analysis (see below). It should be also noted that the sequence difference between pDAO-10 and pDAO-13 exists only within the untranslated region, leaving the coding sequences identical with each other.

The 3′-untranslated regions contain 1972 and 1400 nucleotides in clones pDAO-10 and pDAO-13, respectively. The polyadenylation signal, ATTAAA (nucleotides 3178–3183), is present 29 residues upstream of the poly(A) tail in clone pDAO-10. In the case of clone pDAO-13, the sequence AACAAA (nucleotides 2628–2633) located 11 nucleotides before the site of poly(A) addition is presumed to be the polyadenylation signal.

There are two potential asparagine-linked glycosylation sites conforming to the consensus sequence of Asn-X-Ser/Thr (Marshall, 1974) at amino acid positions 134 and 180. However, our preliminary analysis indicated the absence of a carbohydrate moiety associated with the mature enzyme preparation as judged by the PAS staining (data not shown).

Codon choices for the DAO mRNA have been assigned (Table I), according to the amino acid sequence shown in Figure 3. There is a preferential use of certain codons for some amino acids by DAO mRNA. Compared with the mammalian codon usage pattern surveyed by Ikemura (1985), the UCU codon was used for Ser in preference to AGC, and for Ala, GCU and GCA were more often used than GCC. In addition, an apparent preference for G or C at the third

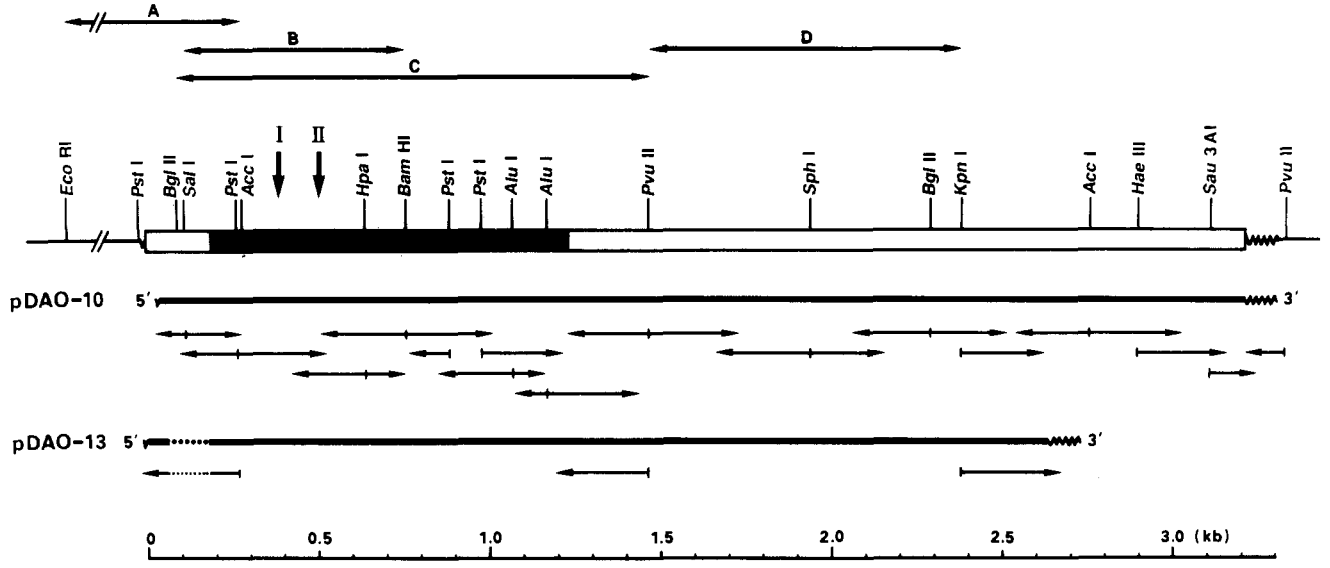

FIGURE 2: Restriction endonuclease map and sequencing strategy for the cDNA inserts. The map displays only the relevant restriction endonuclease sites. A solid line represents the vector DNA; a zigzag line represents the oligo(dG)/(dC) or poly(dA)/(dT) tail; a solid box represents the coding region for DAO; an open box represents the untranslated region. The cDNA inserts are shown by solid bars. The broken bar in pDAO-13 indicates the deleted region. The direction and extent of the sequence determination are indicated by horizontal arrows under each insert used. Vertical arrows above the restriction map show the positions of the oligonucleotide probes used for screening of the cDNA library. Fragments A–D show the probes used for Southern and Northern blot hybridization experiments (Figures 4 and 5).

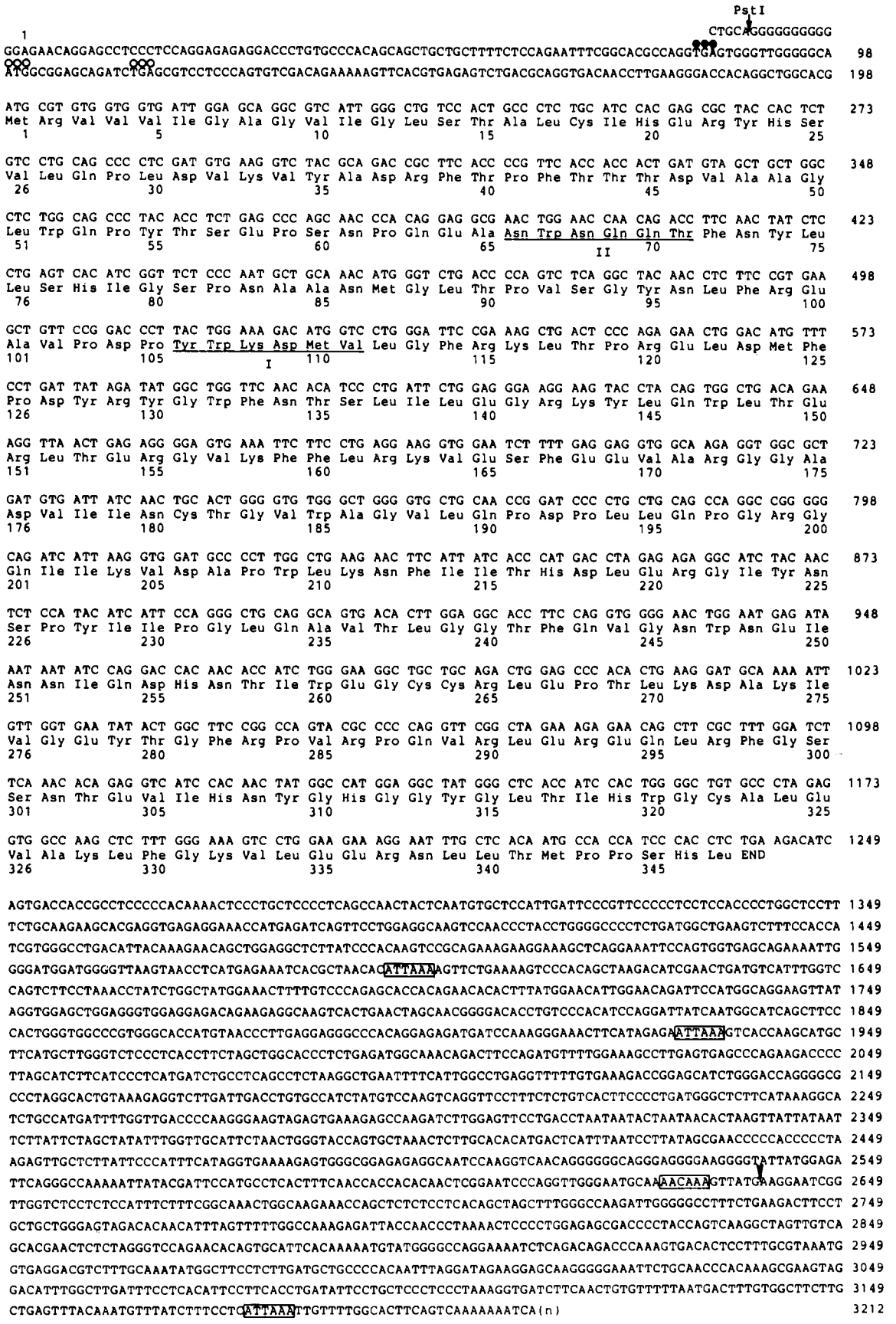

FIGURE 3: Nucleotide and corresponding amino acid sequences of porcine kidney D-amino acid oxidase. Nucleotides are numbered in the 5′ to 3′ direction, beginning with the first nucleotide of the cDNA insert preceded by the oligo(dG)/(dC) linker. The nucleotide sequence was deduced by combining the sequences of the cDNA inserts of clones pDAO-10 and pDAO-13. In the 5′-untranslated region, the nucleotide residues 1–54 are absent in pDAO-10, and pDAO-13 has a deletion between 81 and 189. The deduced amino acid residues are indicated below the nucleotide triplets. Four possible polyadenylation signals, ATTAAA and AACAAA, are boxed. The sequences that correspond to the two synthetic oligonucleotide probes (I and II) are underlined, respectively. An upstream in-frame stop codon at nucleotides 82–84 is indicated by closed circles. A small open reading frame in the 5′-untranslated region that could encode only five amino acids is shown by open circles at the ATG and TGA codons. In the 3′-untranslated region, pDAO-13 is included up to the position indicated by the arrowhead.

Table I: Codon Usage in Porcine Kidney DAO mRNA and Amino Acid Composition Predicted from the Nucleotide Sequence[a]

amino acid	residues	codon	no.	amino acid	residues	codon	no.
Leu	36	UUA	1	Ser	13	UCU	6
		UUG	1			UCC	3
		CUU	2			UCA	2
		CUC	9			UCG	0
		CUA	4			AGU	1
		CUG	19			AGC	1
Arg	21	CGU	2	Thr	22	ACU	6
		CGC	4			ACC	10
		CGA	1			ACA	6
		CGG	3			ACG	0
		AGA	6	Ile	20	AUU	8
		AGG	5			AUC	11
Pro	22	CCU	3			AUA	1
		CCC	8	Asn	19	AAU	5
		CCA	8			AAC	14
		CCG	3	Phe	15	UUU	4
Gln	14	CAA	2			UUC	11
		CAG	12	Tyr	14	UAU	6
Lys	12	AAA	4			UAC	8
		AAG	8	Glu	22	GAA	10
Ala	17	GCU	6			GAG	12
		GCC	4	Cys	5	UGU	1
		GCA	6			UGC	4
		GCG	1	His	9	CAU	2
Val	26	GUU	3			CAC	7
		GUC	7	Asp	13	GAU	7
		GUA	2			GAC	6
		GUG	14	Met	5	AUG	5
Gly	32	GGU	4	Trp	10	UGG	10
		GGC	13				
		GGA	7				
		GGG	8				

[a] The amino acid of the porcine kidney DAO was predicted solely from the nucleotide sequence of the mRNA. Numbers next to codons indicate the numbers of amino acids using particular codons.

position was observed; 101 codons (29%) terminate in G, 120 (35%) in C, 66 (19%) in U, and 60 (17%) in A.

Presence of Three DAO mRNAs in Porcine Kidney. To determine the size of the DAO mRNA, Northern blot hybridization experiments were carried out with the cDNA insert of pDAO-10 and a synthetic oligonucleotide as hybridization probes (Figure 4). The oligonucleotide or cDNA fragment covering the protein-coding region of DAO detected three bands, the mobilities of which corresponded to sizes of approximately 3.3, 2.7, and 1.5 kb (Figure 4, lanes a and b). D-Amino acid oxidase with 347 amino acid residues requires at least 1041 bases for coding sequence. Therefore, these three species of mRNA are large enough to share a common sequence for the DAO coding region. The nucleotide sequences deduced from the two clones pDAO-10 and pDAO-13 differ in the length of the 3′-untranslated region (Figure 3). The size difference of the 3.3- and 2.7-kb bands, estimated from their mobilities on the electrophoretic gel, was consistent with the difference in the length of the 3′-untranslated region between the clones pDAO-10 and pDAO-13. These results indicate that cDNAs of pDAO-10 and pDAO-13 correspond to the largest and the middle-size mRNA, respectively. The cDNA corresponding to 1.5-kb mRNA has not been identified. However, when the cDNA fragment derived from the 3′-untranslated region (*Pvu*II–*Kpn*I) was used for hybridization, the band corresponding to 1.5 kb became weak (Figure 4, lane c). It is suggested, therefore, that a large portion of the 3′-untranslated region should be absent in the 1.5-kb mRNA.

The hybridization signals of the larger and the smaller mRNA were significantly more intense than that of the middle-size mRNA. This suggests that the efficiencies of their expression are considerably different.

Genomic Organization of DAO Gene. Southern blot analysis of genomic DNA was used to examine the copy number of

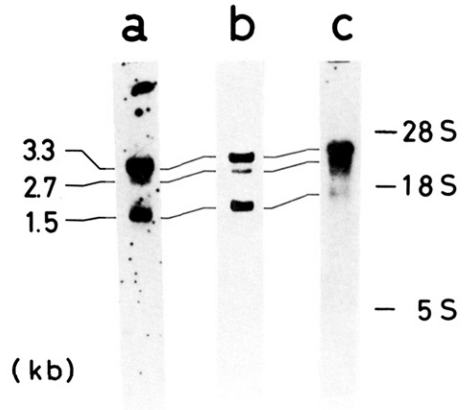

FIGURE 4: Northern hybridization analysis of porcine D-amino acid oxidase mRNAs. Five micrograms of poly(A+) RNA per lane was fractionated by electrophoresis on a 0.7% agarose gel and transferred to a nitrocellulose filter. Blots were hybridized with nick-translated cDNA fragments (Figure 2) or 5′-end labeled oligonucleotide probe (Figure 3), followed by washing and autoradiography. Hybridization probes were as follows: (lane a) synthetic oligonucleotide (17-mer) (I in Figure 2); (lane b) *Bgl*II–*Pvu*II fragment (1367 bp) (C in Figure 2); (lane c) *Pvu*II–*Kpn*I fragment (913 bp) (D in Figure 2). The size markers used were porcine rRNAs.

the DAO gene in the porcine genome. When high molecular weight DNA was digested with *Hind*III and hybridized with the 0.65-kb fragment (*Sal*I–*Bam*HI; Figure 2, probe B) corresponding to the N-terminal half of the coding sequence or the 1.4-kb fragment (*Bgl*II–*Pvu*II; Figure 2, probe C) covering the whole protein-coding region, a sharp single 1.8-kb band was observed for both probes (Figure 5, lanes a and b). No other cross-hybridizing band was detected under stringent conditions. These results suggest that the DAO gene exists as a single copy in the porcine genome.

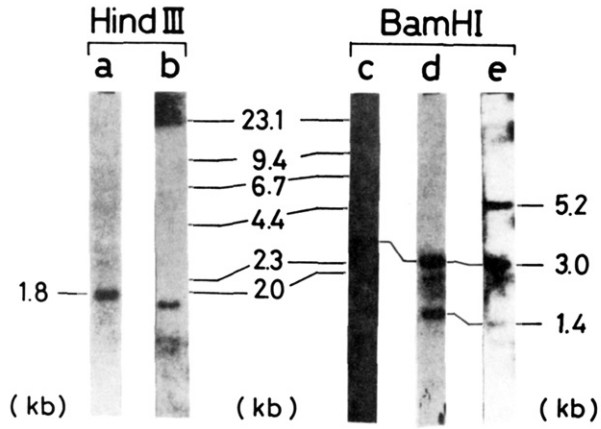

FIGURE 5: Southern hybridization analysis. High molecular weight DNA from porcine kidney (2 μg/gel lane) was digested with *Hind*III (lanes a and b) and *Bam*HI (lanes c–e). The following hybridization probes were used: (lanes a and d) *Sal*I–*Bam*HI fragment (641 bp) (B in figure 2); (lanes b and e) *Bgl*II–*Pvu*II fragment (1367 bp) (C in Figure 2); (lane c) *Eco*RI–*Acc*I fragment (557 bp) (A in Figure 2).

However, the Southern hybridization pattern seen with *Bam*HI digestion was rather complex (Figure 5, lanes c and d), because of the presence of a recognition site for this enzyme within the cDNA. We therefore examined *Bam*HI-digested DNA with three different probes (Figure 2, probes A–C). Probe A corresponding to the 5′-untranslated region as well as the extremity of the N-terminal coding region recognized a single 3.0-kb band (Figure 5, lane c). This confirms that we are dealing with a single gene. The whole coding region (probe C) detected three bands: 5.2, 3.0, and 1.4 kb (Figure 5, lane d). The N-terminal half of the coding region (probe B) recognized two bands of 3.0 and 1.4 kb. These patterns could be most readily explained by the presence of multiple introns in the DAO gene, and the arrangement of the three *Bam*HI fragments in the porcine genome is presumed to be in the following order: 3.0, 1.4, and 5.2 kb from 5′ to 3′.

DISCUSSION

Biogenesis of DAO. The assignment of the initiation site of translation was based on the fact that ATG at nucleotides 199–201 was preceded by the in-frame stop codon TGA at 82–84 and followed by 1041 nucleotides of an open reading frame that encoded 347 amino acids. In addition, this ATG is preceded by sequences that fulfill the Kozak (1981) criteria for initiation codon. The predicted amino acid sequence starting with methionine was confirmed by the N-terminal amino acid sequence determination of the mature enzyme for 32 residues. The reported sequence for DAO was also in complete agreement with the deduced sequence shown in Figure 3, and the ambiguous residue Asx was concluded to be Asp. The molecular weight of the deduced sequence was calculated to be 39 335, which is also in a good agreement with the value determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (Fukui et al., 1986). These results demonstrate directly the absence of a cleavable presequence at the amino terminal of the primary translation product of DAO mRNA, indicating the posttranslational translocation of DAO into peroxisomes without any proteolytic modification. It would seem reasonable to speculate the presence of a recognizable signal for translocation to peroxisomes within the polypeptide. Plots of the relative hydrophobicity at each amino acid position in DAO were made according to the Kyte–Doolittle method (Kyte & Doolittle, 1982) and revealed a highly hydrophobic region, which encompasses the first 17 amino acids of the protein (data not shown). This sequence might function as a signal for DAO to associate with peroxisomal membrane, but a more thorough analysis and survey of other peroxisomal proteins are necessary. Two potential asparagine-linked glycosylation sites were observed within the coding sequence, although the mature enzyme showed the absence of a carbohydrate moiety. These results also support the idea that DAO is synthesized on free ribosomes and transported through the cytosol into peroxisomes.

Nucleotide Sequence of cDNA Clones. The 5′-untranslated region of pDAO-13 lacks 109 nucleotides compared with pDAO-10. This could be caused by the secondary structure of the relatively long 5′-untranslated region of DAO mRNA or simply by the cloning artifact for sequencing. However, this result is of interest in relation to mRNA processing, since the site of deletion coincided with the consensus sequences of donor and acceptor for splicing (Breathnach & Chambon, 1981). In addition, the branch site sequence (Wallace & Edmonds, 1983) was found at nucleotides 157–161. We have to wait for the genomic sequence analysis to clarify these possibilities. The analysis of the 5′ upstream region of the DAO gene will also provide information for the mechanism of regulation governing the biosynthesis of DAO.

Northern and Southern Hybridization. Our Southern analysis revealed that DAO was coded by a single gene in the porcine genome. This conclusion is consistent with the genetic studies by Konno and Yasumura (1984), showing that both the brain and kidney DAO were coded by the same gene in the mouse. On the other hand, the analysis of the expression of this gene showed the presence of three DAO mRNAs in the pig kidney. Therefore, we postulated that the multiple DAO mRNAs arise from a single gene by utilization of four possible polyadenylation signals, which are found in the 3′-untranslated region as indicated by boxes in Figure 3. Among these signals, it was shown from this study that the third and the last signals were utilized by 2.7- and 3.3-kb mRNAs. The utilization of the first ATTAAA signal by 1.5-kb mRNA is inferred from its size. However, the mRNA that utilizes the second ATTAAA signal was not found in the Northern blot. These results are not unusual, since the generation of multiple mRNAs from a single gene has been reported for several mRNAs, such as IL-2 receptor (Leonard et al., 1984; Nikaido et al., 1984) and prekininogen mRNAs (Kageyama et al., 1984). But the biological significance of this phenomenon remains unclear. Moreover, Northern hybridization signals of the larger and the smaller mRNAs were more intense than that of the medium mRNA (Figure 4, lane b), suggesting that the sequence of ATTAAA is used more effectively than that of AACAAA as a polyadenylation signal for the generation of the DAO mRNAs. A sequence of AACAAA was reported to be recognized as a polyadenylation signal in human coagulation factor XI cDNA (Fujikawa et al., 1986). Point mutation studies by Wickens and Stefenson (1984) showed that the AACAAA signal yielded a 3′ end of RNA at a very much reduced level. This study clearly demonstrates the difference in efficiencies for 3′ processing of the RNA between the polyadenylation signals in the physiological system. In this respect it would be of great interest to examine the tissue-specific alternative expression of the different DAO mRNAs, since the same gene can produce two or more mRNAs in different cell types or at different stages of differentiation. This study opens the way to a detailed analysis of the regulation of the biosynthesis of this enzyme and biogenesis of peroxisomes. Furthermore, molecular genetic studies with the DNA probe would lead us to the elucidation of the biological function of DAO.

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Registry No. DNA (rat pancreas cationic trypsinogen messenger RNA complementary), 108007-28-9; trypsinogen, 9002-08-8; trypsin, 9002-07-7; pretrypsinogen, 81989-96-0; trypsinogen (rat pancreas cationic precursor reduced), 108007-29-0; trypsinogen (rat pancreas cationic reduced), 108007-30-3.

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