Molecular Cloning and Chromosomal Localization of a Human Gene Encoding D-Amino-acid Oxidase*

Kiyoshi Fukui and Yoshihiro Miyake‡

From the Department of Biochemistry, National Cardiovascular Center Research Institute, 5-7-1, Fujishiro-dai, Suita, Osaka 565, Japan

Genomic clones covering the entire sequence of the gene encoding human D-amino-acid oxidase (DAO) (EC 1.4.3.3), one of the principal and characteristic flavoenzymes of peroxisomes, were isolated from human placental genomic libraries with the aid of a previously cloned cDNA for human DAO as a probe. Nucleotide sequence analysis revealed that the gene, present as a single copy in the human genome, comprises 11 exons and spans 20 kilobase pairs. The protein sequences containing the catalytically important residues, Tyr-228 and His-307, are coded for by separate exons. Heterologous transcription initiation sites were identified by primer extension analysis, and the sequence of the 5'-flanking region of the DAO gene was found to show some features common to other mammalian genes, such as those of glucocorticoid and the cAMP-responsive element. An additional noteworthy feature is the presence of promoter-like sequences in the first intron of the gene. In addition, two sequences of alternating pyrimidine and purine nucleotides, (CA)\textsubscript{20} and (CA)\textsubscript{17}, are also present in the first intron. Such sequences may play a role in the expression of the DAO gene in human tissues. With the use of genomic DNAs prepared from human and Chinese hamster somatic hybrid cells as templates for the polymerase chain reaction, the gene for DAO was localized to human chromosome 12.

D-Amino-acid oxidase (DAO)\textsuperscript{1} (EC 1.4.3.3) is a flavoenzyme with FAD as its prosthetic group that catalyzes the oxidative deamination of a wide range of D-amino acids. Since the initial characterization of this enzyme (1), its physicochemical properties and reaction mechanism have been well documented (2). Systematic studies on the DAO activity in various mammalian tissues revealed its existence in the liver, kidney proximal tubules (3), and certain parts of the brain (4). This enzyme was reported to be intracellularly localized in peroxisomes (5), and DAO is regarded as a characteristic marker enzyme of the peroxisomes in porcine kidney. The enzyme is also known to be significantly decreased in patients with a subtype of Zellweger syndrome, a disorder of peroxisome formation (6). It has been assumed that the physiological role of DAO is related to the function of proximal tubules in the kidney and metabolism in peroxisomes, although D-amino acids do not appear to be involved in normal mammalian metabolism. Several approaches have been taken to obtain clues as to the physiological significance of this enzyme (7, 8); however, the biological function of DAO has remained undetermined.

Previously, we determined the primary structures of the porcine (9), human (10), rabbit (11), and mouse (12) DAO mRNAs by molecular cloning and sequence analysis of the respective cloned cDNAs. Chemical modification studies on DAO revealed several candidate amino acid residues for the active center of this enzyme (reviewed in Ref. 13). Moreover, D-propargylglycine, a suicide substrate, inactivates DAO on dynamic affinity labeling (14, 15), resulting in a great loss of activity and cofactor modification at tyrosine 228 and histidine 307 of the primary structure of the porcine enzyme (16). The availability of recombinant wild-type and mutant enzymes synthesized in vitro (17–19) and also produced in Escherichia coli (13, 20) allowed us to re-examine the proposed active site of DAO, and we have been able to identify tyrosine 228 and histidine 307 as the most important residues for the catalytic function of porcine kidney DAO. Despite extensive studies on the enzymatic characteristics of DAO, little is known about the regulation of DAO expression, especially the mechanism underlying its induction or suppression at the molecular level, which appears to provide the molecular basis of the physiological function of DAO. In a functionally differentiated cell line, LLC-PR\textsubscript{K}+, derived from porcine kidney proximal tubules, DAO activity was induced when a confluent monolayer was formed, and active biosynthesis of DAO was observed (21). In addition, in the ddY mouse strain, DAO expression was suggested to be controlled at the mRNA transcriptional level (22).

In this study, to examine the structural organization of the human DAO gene and the regulation of its expression in a search for some clues as to the physiological function of DAO, we have isolated the human DAO gene from genomic libraries using cDNA as a probe. We have sequenced all of the coding and flanking regions of the human DAO gene and determined the transcription initiation site by primer extension analysis. In addition, we demonstrate that the gene for DAO is on human chromosome 12 through polymerase chain reaction analysis of human and Chinese hamster somatic hybrid cell lines.


**EXPERIMENTAL PROCEDURES**

**Materials—Reagents were obtained as follows:** [y-32P]ATP (specific activity, 111 TBq/mmol) from Du Pont/New England Nuclear; [α-32P]dCTP (specific activity, –111 TBq/mmol) from Amersham International; restriction endonucleases from Toyobo Co. and Takara Shuzou; T4 polynucleotide kinase, a random primer DNA labeling kit, a DNA ligation kit, and E. coli JM109 competent cells from Takara Shuzou; avian myeloblastosis virus reverse transcriptase from Midwest Bio-Products; an in vitro packaging kit from Stratagene; human kidney total RNA and a XENBL-3 SP6/TT human genomic library containing Sau3AI partial digests of human placental DNA from Clontech Laboratories; nick-translased filters (pore size, 0.45 μm) from Advantec and Schleicher & Schuell; agarose (type I) from Sigma; pGEM7Zf(+) and pGEM3Zf(+) plasmids from Promega Biotec; AgWESAB phage DNA EcoRI arms from Bethesda Research Laboratories; a double-stranded nested deletion kit including exonuclease III from Pharmacia LKB Biotechnology Inc.; a somatic cell hybrid panel from Bios Co.; a PCR reagent kit and Taq DNA polymerase from Perkin-Elmer Cetus Instruments; a Taq dye primer cycle sequencing kit and a Taq dye-labeled diodeoxyxucleotide terminator cycle sequencing kit from Applied Biosystems, Inc.; and an imaging plate for an image analyzer from Fuji Photo Film. Oligonucleotides were synthesized with an automated DNA synthesizer (Applied Biosystems, Inc., Model 380A). All other chemicals used were of analytical grade and purchased from Sigma, Nakarai Tesque, or Wako Pure Chemical Co.

**Southern Blot Hybridization Analysis of Human DAO Gene—**High molecular weight DNA from human placenta was digested with EcoRI or BamHI, electrophoresed on a 0.7% agarose gel (3 to 10 μg/lane), and then transblotted to nitrocellulose membranes according to the manufacturer's instructions (20). The filter was hybridized with the ApaLI-PvuII fragment (1.4 kb) of human DAO cDNA (10) and then radiolabeled by the random hexamer primed method of Feinberg and Vogelstein (24). The hybridization conditions were as described previously (25), and several wash conditions were used to change the stringency: 0.1 x SSC (1 x SSC = 0.15 M sodium chloride, 0.015 M trisodium citrate, pH 7.0) at 65 °C, 2 x SSC at 65 °C, and 2 x SSC at 55 °C.

**Construction and Screening of Phage Genomic Libraries—**Complete EcoRI digests of human genomic DNA were size-fractionated by 0.5% agarose gel electrophoresis, DNA from the fractions containing ~12-kb fragments was ligated into the EcoRI site of bacteriophage λgtWESAB, and then the resulting recombinant phage DNA was packaged in vitro. Uncloned libraries were screened by the plaque hybridization method of Benton and Davis (26) using [32P]-labeled human DAO cDNA (10) containing the entire coding sequence (ApaLI-PvuII fragment, 1.4 kb) as a probe. Another XEMBL-3 SP6/TT human genomic library containing Sau3AI partial digests of human placental DNA was also screened by the same procedure as described above, except for the probe used. In this case, we used the 5'-portion of a cDNA fragment (EcoRI-BamHI fragments was generated by digestion with exonuclease III (27). Double-stranded DNA templates prepared from mutant plasmids were sequenced by the diodeoxyxucleotide chain termination method (28) using fluorescently tagged M13 universal or reverse sequencing primers. Enzymatic extension reactions for DNA sequencing were performed with Taq DNA polymerase and a DNA thermal cycler (Perkin-Elmer Cetus Instruments, Model PJ 2000) according to the manufacturer's recommendations. This thermal cycling reaction, based on the linear polymerase chain reaction, consists of steps programmed in the step-cycle mode, with 15 cycles at 95 °C for 30 s, 55 °C for 30 s, and 70 °C for 1 min and with another 15 cycles at 95 °C for 15 s, 55 °C for 15 s, and 72 °C for 1 min, using ~1.2-1.5 μg of double-stranded plasmid DNA as the template. The sequencing reaction products were analyzed automatically with a DNA sequencer (Applied Biosystems, Inc., Model 373A). Sequencing was performed with overlapping clones throughout, and synthetic oligonucleotides based on the previously determined sequences were synthesized and used as primers to facilitate sequencing. The 12-kb EcoRI fragment of clone XHDAO-1 was also isolated and digested with Sall. The 7- and 5-kb fragments thus produced were then subcloned into the pGEM7Zf(+) vector. Synthetic oligonucleotides specific for several of the exons were used as primers for the thermal cycling sequencing reactions with dye-labeled diodeoxyxucleotide terminators, and the reaction products were analyzed as described above. The nucleotide sequences of the exons were determined on both strands. The assembly of DNA sequences and homology searches of the GenBank database (release 69, September 1991) and EMBL (release 28, September 1991) data bases were performed with a computer program developed by Software Development Co. (Version 7.0).

**Primer Extension Analysis—**Synthetic oligonucleotides of 30 bp complementary to the 3'-end of exon U (5'-CGGAAGTACCAACCTAGAGACACGACA-3') were labeled with [y-32P]ATP and T4 polynucleotide kinase and used as primer. The labeled primer was then hybridized to 50 μg of total RNA isolated from human kidney for 12 h at 45 °C in 20 μl of a solution containing 50% formamide, 40 mM PIPES, pH 6.4, 0.4 μM NaCl, and 1 mM EDTA. The RNA/DNA hybrids were precipitated with ethanol and then denatured by heating. Primers to the primer extension reaction were avian myeloblastosis virus reverse transcriptase. The extended cDNAs were electrophoresed on a 6% polyacrylamide gel containing 7 M urea along with diodeoxy-DNA sequence samples prepared by the modified linear polymerase chain reaction method with the use of Taq DNA polymerase and a radiolabeled primer identical to that used for the primer extension described above. The gel was dried under reduced pressure, exposed to a photostimulatable phosphor imaging plate, and then analyzed with an image analyzer (Fuji Photo Film, Model BAS 2000).

**Enzymatic Amplification of Genomic DNA Sequences for Chromosomal Localization of Human DAO Gene—**Target sequences were amplified in a 100-μl reaction mixture containing 50 ng of genomic DNA, 50 nM KCl, 10 mM Tris-HCl, pH 8.5, 1.5 mM MgCl₂, 0.001% (w/v) gelatin, a 200 μM concentration of each deoxyribonucleotide triphosphate (dATP, dCTP, dTTP, and dGTP), a 1 μM concentration of each 21-mer primer, and 2.5 units of Taq DNA polymerase (29). The samples were overlaid with 100 μl of mineral oil to prevent evaporation, placed in a preheated DNA thermal cycler at 94 °C and then subjected to 30 cycles consisting of a 1-min denaturation period at 94 °C, a 2-min annealing period at 55 °C, and a 3-min period at 72 °C for extension of the annexed primers. Following amplification, the reaction products were electrophoresed on a 1.5% agarose gel and then analyzed by Southern blot hybridization as described above. Synthetic oligonucleotides (21-mer) corresponding to the middle portion of the target sequence were labeled at the 5'-ends with T4 polynucleotide kinase and used as probe.

**RESULTS**

**Blotting Analysis of Chromosomal DNA—**To determine the organization and structure of the human DAO gene, we first carried out Southern blot analysis of the chromosomal DNA from human placenta. As shown in Fig. 1A, a simple pattern was obtained when a cDNA fragment for human kidney DAO (ApaLI-PvuII fragment, 1.4 kb) containing the entire coding region was used as the hybridization probe. Digestion of the DNA with EcoRI or BamHI gave rise to a single positive band (12 kb for EcoRI and 11.4 kb for BamHI). To exclude the possibility of the presence of other hybridizing bands in this analysis, the amount of DNA loaded on the gel was increased from 3 to 10 μg, and the conditions for the washing were changed to mildly stringent ones. As shown in Fig. 1B, EcoRI digestion yielded only a 12-kb positive band, even with the mildest washing conditions (lane 3; 2 x SSC, 55 °C). When an identical filter was hybridized with the 5'-portion of the cDNA as a probe, the same band was positive with EcoRI- or BamHI-digested DNA (data not shown). These observations thus indicated the presence of a single copy of the DAO gene and the absence of other closely related genes or pseudogenes in the human genome.

**Isolation of Recombinant Phage Containing DAO Gene Sequence—**Because EcoRI digestion of the total DNA gave a
were fractionated by electrophoresis on a 0.7% agarose gel, transferred pg/lane for A and 10 pg/lane for chromosomal genomic sequence. The two sequences matched clones being identified (designated as XHDAO-5). Restriction endonuclease analysis revealed that XHDAO-1 and XHDAO-3 of the DAO gene and that the 12-kb positive human DAO cDNA probe. The washing conditions were as follows: 0.1 × SSC at 65 °C (A, lanes 1 and 2; and B, lane 1), 2 × SSC at 65 °C (B, lane 2), and 2 × SSC at 55 °C (B, lane 3). Numbers are the lengths (in kilobase pairs) of the size markers (HindIII-digested fragments of λ phage DNA) and the positive EcoRI fragment.

single positive band (12 kb) on blotting analysis, we purified the 12-kb DNA fragment by agarose gel electrophoresis and cloned it into the λgtWESAB phage. About 2.2 × 10⁶ plaques were screened by the in situ procedure with the 32P-labeled cDNA fragment for human kidney DAO containing the whole coding region as a probe (ApaLI-PvuII fragment, 1.4 kb), and eight positive independent phage clones were identified. Restriction endonuclease analysis of these clones revealed the presence of only one group of recombinant phages (designated as λHDAO-1). Partial nucleotide sequence analysis and Southern blot hybridization analysis using an oligonucleotide probe corresponding to the 3'-end of the coding sequence, however, indicated that this clone (λHDAO-1) contained only the 3'-portion of the DAO gene and that the 12-kb positive band detected on Southern blot analysis (Fig. 1) represented two DNA fragments of identical size. Therefore, we screened another human placental genomic DNA library constructed with DNA partially digested with Sau3AI and cloned it into the λEMBL-3 SP6/T7 phage. About 2 × 10⁶ plaques were screened by the same procedure as described above using the 5'-portion of the cDNA fragment (EcoRI-HincII fragment, 0.59 kb) as a probe, with two independent, but identical phage clones being identified (designated as λHDAO-5). Restriction endonuclease analysis revealed that λHDAO-1 and λHDAO-5 overlapped each other and contained the same 2-kb EcoRI-BamHI fragment, as shown in Fig. 2. The inserts were separately cloned into the pGEM3Zf(+) and pGEM7Z(+) vectors for sequence analysis.

Structure of Human DAO Gene—The human DAO cDNA sequence previously determined (10) was aligned with the chromosomal genomic sequence. The two sequences matched well, but the 5'-untranslated region of the cDNA was found to be interrupted at 9 bp upstream of the translation initiation codon. Therefore, we sequenced all the 5'-upstream region and found an mRNA-coding segment corresponding only to the 5'-untranslated region (designated as exon U) at −5 kb upstream of the translation initiation site. The nucleotide sequences of the chromosomal genomic exons and the cDNA showed perfect agreement. Thus, the DAO gene, spanning ~20 kb of the human genome, consists of 10 exons for the protein-coding region and the 3'-untranslated region and another one for the 5'-untranslated region. All 11 exons are in the two DNA inserts, and the gene contains 10 introns. The organization of the DAO gene and a partial restriction map are presented in Fig. 2.

The transcription initiation site of the DAO gene was mapped by primer extension of human kidney total RNA using reverse transcriptase. The noncoding sequence was extended from the oligonucleotides corresponding to the 3'-end of exon U. As shown in Fig. 3, the transcription initiation sites of human DAO transcripts were heterologous, and four sites were determined. The total length of exon U is 134 bp, assuming that transcription begins with the first T nucleotide revealed by the primer extension assay. In addition, alignment of the sequences of the porcine and mouse cDNAs with that of human genomic DNA allows assignment of putative start sites for the porcine and mouse mRNAs, both of which reside in exon U (Fig. 4). On the basis of these assignments, the
**Fig. 4. Nucleotide sequence of 5'-flanking region of human DAO gene.** The arrows indicate the transcription initiation sites by primer extension analysis (human) and predicted by comparison of the sequences with those of cDNAs (pig and mouse). Open and closed circles indicate the 12-bp (G + C)-rich region and the glucocorticoid-responsive element-like sequence, respectively. Sequences homologous to the cyclic AMP-responsive element and steroid-dependent repressor sequences are indicated by closed stars and open diamonds, respectively. The exon U sequence is boxed. Negative numbers above the sequence indicate the distance upstream of the transcription initiation site identified by primer extension.

The analysis of the nucleotide sequence of 5'-flanking region commonly to the regulatory regions of eukaryotic genes, sequence determination for the 5'-untranslated region of the human DAO gene was extended up to nucleotide 247 upstream of the capping site determined by primer extension analysis as described above. As shown in Fig. 4, the 5'-flanking region of the human DAO gene was observed to have a high G + C content (61.94%). A TATA box sequence, which determines the specificity of mRNA synthesis initiation by RNA polymerase II, was not found in the 5'-flanking region of the DAO gene. Instead, sequence 5'-TTGCAGTCT-3' was found at positions -220 to -206 (5'-GAGCCTTCTTGCGTCT-3'). This sequence contains the 4 nucleotides determined to be the most important in binding to the glucocorticoid receptor at identical positions (underlined). Another hormone-responsive element-like sequence was present between positions -59 and -53 (5'-GTCGCAAGT-3'), which is homologous to the sterol-dependent repressor sequence present in the human low-density lipoprotein receptor and hydroxymethylglutaryl-CoA reductase genes. Furthermore, the 5'-flanking region of the human DAO gene exhibited significant homology as a whole (51.4% over 243 bp) to the human corticotropin-β-lipoprotein precursor gene (51.4% over 243 bp) to the putative regulatory region (positions -311 to -568) of the human corticotropin β-lipoprotein precursor gene (53) when the two sequences were aligned with the introduction of gaps to maximize homology (data not shown).

**Fig. 5. Exon-intron junctions of human DAO gene.** Exon-intron boundaries are indicated by vertical bars. The arrowhead and arrow indicate the transcription initiation site and the position of poly(A) attachment, respectively. The polyadenylation signal is underlined. The GT and AG consensus dinucleotides, respectively, are boxed. The glucocorticoid-responsive element-like sequence and one copy of a CAAT box sequence were found in the first intron downstream from the mapped transcription initiation site. As shown in Fig. 6, the first TATA box upstream of the translation initiation codon is at position 4997 and is preceded by a CAAT box sequence at position 4133. The second TATA box is at position 3790, and the third one at position 2770. In addition, a AP-1-binding site is at position 4997 and is preceded by a CAAT box sequence at position 4133. The second TATA box is at position 3790, and the third one at position 2770. In addition, a AP-1-binding site is at position 4997 and is preceded by a CAAT box sequence at position 4133.
FIG. 6. Nucleotide sequence of first intron of human DAO gene. Exon sequences are boxed with unbroken lines, and the sequence homologous to the portion of the 5' untranslated region of porcine kidney cDNA is boxed with a dashed line. Dinucleotide repeats of CA are underlined. CAMP-responsive element-like sequences, the AP-1 binding site, and SP-1-like sequences are indicated by closed stars, closed circles, and open circles, respectively. The TATA and CAAT boxes are indicated by open stars and open diamonds, respectively. The translation initiation codon (ATG) is shaded. Numbers indicate the distance downstream from the transcription initiation site.

Translated region of the 3.3-kb porcine kidney mRNA (positions 81-189 in Ref. 9) that was deleted in the 2.7-kb mRNA (63.5% over 104 bp). Another noteworthy feature is the presence of two sequences of alternating pyrimidine and purine nucleotides, (CA)$_n$ and (CA)$_{n+1}$ (positions 1079-1118 and 1395-1428, respectively), separated by -270 bp in the first intron.

Chromosomal Localization of Human DAO Gene—To establish the chromosomal location of the human DAO gene, we examined somatic cell hybrids as to their human chromosome contents using the polymerase chain reaction to specifically amplify human sequences, and not their Chinese hamster counterparts. DNA samples prepared from human cells, Chinese hamster cells, and 25 somatic cell hybrids were used as templates for PCR analysis. The target sequence for amplification was selected from the exon 10 sequence since this exon is the largest in size and contains the 3' untranslated region. A pair of 21-mer oligonucleotides (Fig. 7B) spanning a 338-bp fragment were synthesized, one corresponding to the 5'-end of the exon 10 coding sequence and the other to the 3'-untranslated region. Another 21-mer oligonucleotide (Fig. 7B) corresponding to the middle portion of the target sequence in exon 10 was also synthesized for use as the hybridization probe for the sensitive and specific detection of the amplified DNA fragment. Assignment of the desired PCR product to a cell line in the somatic cell panel was thus
A. PCR positive band is shown. The cDNA. The amino acids are numbered from the initiator methionine. The terminator codon (TGA) is in shaded.

B. Fig. 7. Chromosomal localization of human DAO gene by PCR analysis. A, Southern blot hybridization analysis of the PCR products amplified from somatic cell hybrids (lanes with numbers) and their parent cell lines (hamster and human). The size of the positive band is shown. B, Synthetic oligonucleotide sequences used for the PCR primers and hybridization probe. The numbers above the sequence refer to nucleotide positions in human kidney DAO cDNA. The amino acids are numbered from the initiator methionine. The terminator codon (TGA) is in shaded. The 5'-primer and probe were synthesized in the sense strand, whereas the 3'-primer was synthesized in the antisense strand.

accomplished by simple visualization of bands on autoradiography. PCR was carried out under standard conditions (29) using 50 ng of hybrid DNA, in a total volume of 100 µl. The reaction products of the amplification were electrophoresed on agarose gel and then analyzed by Southern blotting using internal oligonucleotides as hybridization probe. As shown in Fig. 7A, the human DNA was specifically amplified and detected as a band of the expected size (338 bp). No band was observed for Chinese hamster parent cell lines, confirming the absence of cross-species homology for the selected primer set. The results of Southern blot analysis of the human DNA, hamster DNA, and DNAs from 25 somatic cell hybrids are summarized in Table I. Although human chromosome 5 is very frequently retained in this hybrid panel, the results suggest strongly overall that DAO is on human chromosome 12.

DISCUSSION

We have determined the nucleotide sequence of the human DAO gene and analyzed its organization. Perfect agreement between the nucleotide sequences of the chromosomal gene exons and the cDNA sequence was observed. The human genome contains a single copy of the DAO gene, as was also the case with the porcine genome (9). We could not find any cross-hybridized DNA segment, even under nonstringent hybridization conditions. Jacobs et al. (34) previously elucidated the partial nucleotide sequence of a porcine DAO genomic clone to determine the nucleotide sequence of the mRNA. Since they could not obtain full-length cDNA for DAO, no information on the exon segment in the porcine genome corresponding to the 5'-untranslated region of the mRNA was reported; only the structures of exons 1–4 of the porcine DAO gene were described. The presence of a 5'-noncoding exon (exon U) at ~5 kb upstream of the translation initiation codon was demonstrated in the human genome. In addition, a sequence homologous to a portion of the 5'-untranslated region of the porcine DAO mRNA was found in the first intron of the human DAO gene. A corresponding sequence in porcine mRNA was present in a 3.3-kb mRNA, but absent in a 2.7-kb mRNA, which suggests that alternative splicing of this DNA segment in the porcine genome might take place and contribute in part to the heterogeneity of mRNA species in porcine kidney. Elucidation of the structure of the porcine DAO gene would be necessary to confirm this possibility. The determination of exon-intron junctions is also important since there is convincing evidence that, in a number of proteins, structural or functional units are encoded by discrete exons with splice junctions coincident with the boundaries of the protein motif. In the human DAO gene, a putative hydrophobic FAD-binding region is encoded by exon 1. The 2 chemically modifiable amino acid residues, Tyr-228 and His-307, are encoded by exons 7 and 10, respectively. These 2 residues, which are completely conserved among the four species of DAO examined, are modified by D-propargylglycine, a suicide substrate, and thus are suggested to play important roles in the catalytic function of DAO. Exon 10 exhibited several interesting structural features indicating some role in the expression of the DAO gene, such as the presence of His-307, the carboxyl-terminal tripeptide sequence of Ser-His-Leu for the translocation into peroxisomes (35), the hexanucleotide sequence ATTAAA for the polyadenylation signal, and the relatively large size of 514 bp. The organization of the DAO gene may also provide some clues as to the phylogenetic relationship of the DAO gene with other genes.

Multiple initiation sites for human DAO transcription were observed on primer extension analysis. This is consistent with common structural properties in the 5'-flanking region of the DAO gene, namely the absence of well-defined TATA and CAAT boxes. Similar heterogeneity of the transcription initiation sites was also reported for a mouse immunoglobulin C μ transcript (36). It would be interesting to determine whether or not any stimulation could induce predominant transcription from one of the four initiation sites. The 5'-flanking region of the human DAO gene was compared with the nucleotide sequence data bases to see whether or not it shared common regulatory sequences. Homology between the human DAO and human corticotropin β-lipoprotein precursor genes was significant immediately upstream of the transcription initiation site. The production of corticotropin and related peptides in the pituitary is regulated negatively by glucocorticoids and positively by corticotropin-releasing factor. A sequence homologous to a glucocorticoid-responsive element was also found in the 5'-flanking region. Moreover, there is another noteworthy DNA segment homologous to the sequence necessary for induction by cAMP. It is not known yet whether or not these regions are important in the transcriptional regulation of the DAO gene, but it is tempting to speculate that glucocorticoids and cAMP might be involved in the modulation of the gene expression of DAO. Identifica-
of an internal promoter-like region in the first intron is reminiscent of differential regulation of alternate promoters for DAO gene expression. Appropriate deletion and mutation analyses are required to provide direct evidence that any of the sequences mentioned above are important for transcription or regulation of the human DAO gene in human tissues. Particularly interesting also is the presence of alternating pyrimidine and purine nucleotides, which were reported to have the potential to form Z-DNA (37). Of note is the genetic polymorphism due to the variation in the length of the repeating unit among individuals (38). Therefore, the CA repeat turned out to be an abundant source of genetic markers. In this context, it is interesting to note that restriction enzyme length polymorphism was observed among ddY mice with the use of a hybridization probe containing the 5' untranslated region of the eDNA (39). The human DAO locus was assigned to chromosome 12 by screening a panel of Chinese hamster and human somatic cell hybrids with unique sequence primers for PCR analysis. The two sequences, (CA)_m and (CA)_n, in the human DAO gene could be used as polymorphic DNA markers on human chromosome 12. Recently, genetic mapping of a gene causing hypertension in spontaneously hypertensive rats was carried out by analysis involving polymorphic DNA markers (40, 41). Whether or not the DAO gene is linked to any genetic diseases would be interesting to determine in view of the unknown physiological function of this enzyme.

REFERENCES

1. Krebs, H. A. (1935) Biochem. J. 29, 1620-1644
2. Miura, R., and Miyake, Y. (1986) Bioorg. Chem. 18, 97-110
3. Chan, A. W. K., Perry, S. G., Burch, H. B., Fagioli, S., Alvey, T. R., and Lowry, O. H. (1979) J. Histochem. Cytochem. 27, 751-755
4. Horikawa, A., Kaji, T., Tojo, H., Yamano, T., Nogaki, M., and Maeda, T. (1979) J. Histochem. Cytochem. 27, 599-599
5. de Duve, C., and Baudhuin, P. (1966) Physiol. Rev. 46, 353-357
6. Goldfischer, S., Krebs, H. A., and de Duve, C. (1979) J. Biol. Chem. 254, 5223-5224
7. Hamilton, G. A., Buckthal, D. J., Mortensen, R. M., and Zerby, K. W. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 2625-2629
8. Nakamura, H., Ohta, M., Yamano, T., and Miyake, Y. (1981) Biochem. Biophy. Res. Commun. 109, 154-165
9. Fuku, K., Watanabe, F., Shibata, T., and Miyake, Y. (1987) Biochemistry 26, 3612-3618
10. Momoi, K., Fuku, K., Watanabe, F., and Miyake, Y. (1988) FEBS Lett. 238, 180-184
11. Momoi, K., Fuku, K., Tada, M., and Miyake, Y. (1990) J. Biochem. (Tokyo) 108, 406-413
12. Tada, M., Fuku, K., Momoi, K., and Miyake, Y. (1990) Gene (Amst.) 90, 233-297
13. Miyano, M., Fuku, K., Watanabe, F., Takahashi, S., Tada, M., Kanashiro, M., and Miyake, Y. (1991) J. Biochem. (Tokyo) 109, 171-177
14. Horikawa, A., Nishina, Y., Miyake, Y., and Yamano, T. (1976) J. Biochem. (Tokyo) 79, 57-63
15. Marquette, H., and Walsh, C. (1976) Biophys. Chem. 15, 1007-1076
16. Ronchi, S., Minichetti, L., Galliano, M., Courti, B., Spenesi, N., Williams, C. H. Jr., and Massey, V. (1982) J. Biol. Chem. 257, 8624-8634
17. Fuku, K., Momoi, K., Watanabe, F., and Miyake, Y. (1988) Biochemistry 27, 6950-6957
18. Watanabe, F., Fuku, K., Momoi, K., and Miyake, Y. (1988) FEBS Lett. 238, 269-272
19. Watanabe, F., Fuku, K., Momoi, K., and Miyake, Y. (1989) J. Biochem. (Tokyo) 106, 1024-1029
20. Watanabe, F., Fuku, K., Momoi, K., and Miyake, Y. (1989) Biochem. Biophys. Res. Commun. 168, 1422-1427
21. Fuku, K., Momoi, K., Watanabe, F., and Miyake, Y. (1986) Biochem. Biophys. Res. Commun. 141, 1222-1229
22. Miyake, Y., Fuku, K., Momoi, K., Watanabe, F., and Shibata, T. (1987) in Flavin and Flavoproteins (Edmondson, D. E., and McCormick, D. B., eds.) pp. 501-508, Walter de Gruyter & Co., Berlin
23. Southern, E. M. (1975) J. Mol. Biol. 98, 503-517
24. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6-13
25. Honjo, T., Ohtani, M., Yamawaki-Kataoka, Y., Kataoka, T., Kawakami, T., Takashashi, N., and Mano, Y. (1986) Cell 45, 589-588
26. Benton, W. D., and Davis, R. W. (1977) Science 196, 180-182
27. Herskof, S. (1984) Gene (Amst.) 28, 551-556
28. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.
Human d-Amino-acid Oxidase Gene

S. A. 74, 5463–5467
29. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988) Science 239, 487–491
30. Breathnach, R., Benoist, C., Gannon, F., and Chambon, P. (1988) Proc. Natl. Acad. Sci. U. S. A. 75, 4853–4857
31. Lerner, M. R., Boyle, J. A., Mount, S. M., Wolin, S. L., and Steitz, J. A. (1978) Nature 279, 220–224
32. Beato, M. (1989) Cell 56, 335–344
33. Beato, M., Chang, A. C. Y., and Cohen, S. N. (1982) Nature 297, 335–339
34. Jacob, H., Brokly, J., Masser, M., Lotier, R., Guillaume, J. P., Ciccarelli, E., Heinderyckx, M., Cravador, A., Biessens, B., van Elsen, A., Herzog, A., and Boilen, A. (1987) Gene (Amst.) 59, 55–61
35. Gould, S. J., Keller, G. A., and Subramani, S. (1988) J. Cell Biol. 107, 897–905
36. Lennon, G. G., and Perry, R. P. (1986) Nature 318, 475–478
37. Nordheim, A., and Rich, A. (1985) Proc. Natl. Acad. Sci. U. S. A. 80, 1821–1825
38. Weber, J. L., and May, P. E. (1989) Am. J. Hum. Genet. 44, 388–396
39. Fukui, K., Momoi, K., Watanabe, F., Tada, M., Miyano, M., and Miyake, Y. (1991) in Flavins and Flavoproteins (Curty, B., Ronchi, S., and Zazetti, G., eds) pp. 143–146, Walter de Gruyter & Co., Berlin
40. Jacob, H. J., Lindpaintner, K., Lincoin, S. E., Kusumi, K., Bunker, R. K., Muo, Y., Ganten, D., Dzau, V. J., and Lander, E. S. (1991) Cell 67, 213–224
41. Hilbert, P., Lindpaintner, K., Beckmann, J. S., Serikawa, T., Soubrier, F., Dubay, C., Cartwright, P., DeGouyon, B., Julien, C., Takahasi, S., Vincent, M., Ganten, D., Georges, M., and Lathrop, G. M. (1991) Nature 353, 521–529