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Structural and Functional Characterization of the Human Brain D-Aspartate Oxidase[1]

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D-Aspartate oxidase (DDO) cDNAs were isolated from the human brain RNA using the RT-PCR method. Two forms (DDO-1 and DDO-2) of DDO mRNA were detected. Structural analysis of the DDO cDNAs and genomic DNA showed that DDO-1 and DDO-2 are produced by alternative splicing from a single gene. A protein encoded by the DDO-1 cDNA consists of 341 amino acids, and the amino acid sequence of DDO-2 was identical to that of DDO-1 except for the absence of 59 amino acids covering residues 95-153 of DDO-1. A homogenous preparation of DDO-1 was obtained using an expression system in *Escherichia coli*. DDO-1 selectively catalyzed the oxidative deamination of D-aspartate and its *N*-methylated derivative, *N*-methyl D-aspartate; the values of K_m and k_{cat} for D-aspartate were 2.7 mM and 52.5 mol D-aspartate oxidized·s^{-1}·mol^{-1} and those for *N*-methyl D-aspartate were 6.8 mM and 37.7 mol *N*-methyl D-aspartate oxidized·s^{-1}·mol^{-1}, respectively.

Key words: D-aspartate oxidase, cDNA, expression, flavoenzyme, human brain.

D-Aspartate oxidase (DDO, EC 1.4.3.1) (the abbreviation is based on the one-letter code D for aspartate) is a flavoprotein that catalyzes the oxidative deamination of dicarboxylic D-amino acids to give the corresponding α-ketoacids and ammonia. DDO is inactive with D-amino acids that are oxidized by D-amino acid oxidase (DAO, EC 1.4.3.3), which catalyzes the same reaction with neutral and basic D-amino acids as substrates. The presence of DDO has been reported in many mammalian tissues (*1, 2*), and the spectrophotometric and kinetic properties of DDO have been mainly investigated for the enzyme purified from the bovine kidney cortex (*2, 3*). The primary structure of the bovine enzyme has been reported (*4*), but the gene has not been cloned. DDO and DAO, both of which are FAD-containing flavoenzymes, constitute a family of homologous proteins different from the one comprising the FMN-containing oxidases, such as lactate oxidase (*5*) and glycolate oxidase (*6*). Comparison of the primary structure of DDO from bovine kidney and that of DAO from porcine kidney showed 41% identity (*4*).

The *in vivo* function of DDO was considered enigmatic because D-amino acids had not been found in higher animals, with the exception of some classes of bacteria and some invertebrates (*7-9*). However, advances in detection methods for chiral amino acids have led to the discovery that free D-amino acids are present in significant quantities in mammals (*10-13*). Although the biological role of DDO is still unclear, DDO is phylogenetically conserved in mammalian central nervous system and periphery (*14-16*). Moreover, recent evidence has demonstrated that the endogenous substrate for DDO exists in the developing central nervous system of human and in rat periphery at markedly high concentrations (*17, 18*). These discoveries have raised the possibility that DDO is involved in the regulation of the central nervous system and/or the development of the brain in mammals.

As a first step to understand the catalytic mechanism and biological role of mammalian DDO, we isolated human brain DDO cDNA and developed an expression system for DDO in *Escherichia coli*. This paper reports the first cloning and characterization of the mammalian DDO cDNA and the functional expression thereof.

MATERIALS AND METHODS

pET-11d, an expression vector, was obtained from Novagen, M-MLV reverse transcriptase from GIBCO BRL, and restriction endonucleases and other DNA-modifying enzymes from Takara Shuzo. FAD was purchased from Sigma. Isopropyl-β-D(−)-thiogalactopyranoside (IPTG) was obtained from Wako Pure Chemical Industries. Phenyl Sepharose CL-4B and DEAE Sepharose CL-6B were obtained from Pharmacia. All other chemicals were of analytical grade and used as supplied.

Human brain RNA was kindly provided by Dr. Y. Misumi of Fukuoka University School of Medicine and human total DNA was extracted from whole peripheral blood cells as described previously (*19*).

cDNA was synthesized from human brain RNA using

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Abbreviations: DAO, D-amino acid oxidase; DDO, D-aspartate oxidase; EST, Expressed Sequence Tag; IPTG, isopropyl-1-thio-β-D-galactopyranoside; NMDA, *N*-methyl D-aspartate; RT-PCR, reverse transcription-polymerase chain reaction.

reverse transcriptase and oligo(dT) as a primer. The DDO cDNA was amplified by PCR using the human brain cDNA as a template and isolated by a conventional procedure. For this purpose, two primers, 5′-GACACAGCACGGATTG-CA-3′ and 5′-GAGCTGCCTGTGATCTTCTTA-3′, were synthesized, based on the sequence of two Expressed Sequence Tags (EST), GenBank accession numbers H29653 and H29569, which contain the 5′ and 3′ portions of the human DDO mRNA, respectively. After 30 cycles of denaturation (1 min, 94°C), annealing (1 min, 65°C), and elongation (30 s, 72°C), two kinds of PCR products of approximately 1.8 (DDO-1) and 1.6 kb (DDO-2), were extracted from the 1% agarose electrophoresis gel and purified. The resulting fragments were each phosphorylated with T4 polynucleotide kinase, and then inserted into the expression vector pET-11d which was linearized at the unique *Nco*I site and treated with Klenow fragment. Finally, the expression plasmids pETDDO-1.8 and pETDDO-1.6 were obtained.

E. coli BL21 (DE3) cells harboring pETDDO-1.8 or pETDDO-1.6 were grown in 72 liters of LB medium containing 50 μg/ml carbenicillin at 37°C to an absorbance of 0.6 at 600 nm. IPTG was added to a final concentration of 0.01 mM and cells were grown for additional 20 h. The cells were harvested by centrifugation and stored at −20°C until use. Purification of the enzyme was performed by a modification of the procedure described by Negri *et al.* (*2*). The cell pellets were suspended in 50 mM potassium phosphate, pH 8.0, containing 5 mM sodium potassium tartrate, 20 μM FAD and 0.3 mM EDTA and treated with 1 mg/ml lysozyme for 1 h in an ice bath. The cells were then disrupted by sonication. After centrifugation, the supernatant was brought to pH 5.2 with acetic acid and was heated at 55°C for 15 min, then cooled rapidly to 4°C in an ice bath, and the precipitate formed was removed by centrifugation. The supernatant was brought to pH 6.7 by the addition of 1 M potassium phosphate, pH 8.0, and then applied to a Phenyl Sepharose CL-4B column (3.2×12.5 cm) equilibrated with 200 mM potassium phosphate, pH 8.0, containing 5 mM sodium potassium tartrate. After washing of the column with 10 column volumes of the equilibration buffer, proteins were eluted with 5 mM potassium phosphate buffer, pH 8.0. Yellow fractions were pooled, dialyzed against 10 mM potassium phosphate, pH 8.0, containing 5 mM sodium potassium tartrate and applied to a DEAE Sepharose CL-6B column (3.2×20 cm) equilibrated with the same buffer. Elution of proteins was performed using the same buffer. Fractions containing DDO and showing a single protein band on SDS-PAGE were pooled.

DDO activity was measured by the oxygen consumption method with a Galvani-type oxygen electrode (Sensonix Japan, Tokyo). The reaction mixture (1.5 ml kept at 25°C) consisted of 50 mM sodium pyrophosphate, pH 8.3, 20 μM FAD, the substrate D-amino acid and the enzyme. The reaction was initiated by addition of the substrate. The initial velocity was calculated taking the initial oxygen concentration as 240 μM at 25°C. Enzyme concentration was determined on the basis of FAD bound to the enzyme using a molar extinction coefficient of 11.3 $mM^{-1} \cdot cm^{-1}$ at 455 nm, on the assumption that the extinction coefficient at 455 nm for free FAD does not change in the complex with the human DDO-1 protein. The reaction rate for DDO is thus expressed as $\mu mol\ O_2$ consumed $\cdot s^{-1} \cdot (mol\ FAD\ bound)^{-1}$.

Spectrophotometric measurements were performed with a Hitachi U-2000 spectrophotometer. The amino acid and cDNA sequences were determined with an Applied Biosystems gas-phase protein sequencer and an Applied Biosystems 373A DNA sequencer, respectively.

RESULTS AND DISCUSSION

Isolation of the Human DDO cDNA—Since the nucleotide sequence of the DDO cDNA or genomic DNA has not been reported, we searched the Expressed Sequence Tags (EST) in the EST database (dbEST), a division of GenBank, for the DDO mRNA sequence, and found two EST clones which contain the 5′ and 3′ portions of the human brain DDO mRNA, respectively. Using a primer synthesized based on the sequence of the EST clone and human brain RNA, we cloned the human DDO cDNA by the RT-PCR method. Interestingly, two types of DDO cDNA (DDO-1 and DDO-2) were amplified. We sequenced a part of the DDO-1 cDNA including the complete coding region and 174 bp downstream from the termination codon TAG. The amino acid sequence of DDO-1 was determined by alignment with that of bovine kidney DDO previously reported (*4*). Sequence analysis revealed that DDO-1 consists of 341 amino acid residues, whose sequence was homologous with that of bovine kidney DDO, except for the three extra amino acid residues (SNL) attached to the C-terminus of human DDO-1 (Fig. 1). The amino acid sequence identity between human DDO-1 and bovine DDO was 86.4%. In several peroxisomal enzymes, the C-terminal sequence (SKL- or SHL-motif) is known to be a minimal peroxisome-targeting signal (*20*), suggesting that the SNL sequence of human DDO-1, in which the lysine (or histidine) residue is substituted by asparagine, has the same function. In the case of bovine DDO, it is probable that such a tripeptide has been proteolytically lost during the purification step. On the other hand, DDO-2 encoded a 282-amino acid peptide whose sequence was identical (including the assumed peroxisome-targeting tripeptide) to that of DDO-1 except for the absence of 59 amino acids covering from residue 95 to residue 153 of DDO-1 (Fig. 1).

So far, very little is known about the catalytic mechanism of DDO. Recently, we determined the X-ray crystallographic structure of porcine kidney DAO for the complex form with the substrate analog, benzoate, and proposed several amino acid residues including Tyr224, Tyr228, and Arg283, to be involved in catalysis (*21, 22*), although the catalytic role of Tyr224 is not entirely clear at present. The carboxylate group of the benzoate makes a salt-bridge with the guanidino group of Arg283 and a hydrogen bond with the hydroxyl group of Tyr228. The phenol ring of Tyr224 is located just above the benzene ring of benzoate, implying the importance of this residue in catalysis. According to the alignment of the amino acid sequences of human brain DDO-1, bovine kidney DDO (*4*) and porcine kidney DAO (*23*) shown in Fig. 2, Tyr228 and Arg283 are conserved in all the enzymes. These findings support our notion that DDO-1 shares common catalytic features with DAO. Although Tyr224 can not be found in human DDO-1 or bovine DDO, a functionally equivalent residue may be present around the position corresponding to Tyr224.

As described above, the difference between the two types (DDO-1 and DDO-2) of DDO mRNA is limited to a small

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   1 ATGGACACAGCACGGATTGCAGTTGTCGGGGCAGGTGTGGTGGGGCTCTCCACGGCTGTGTGCATCTCCAAACTGGTGCCCCGATGCTCC
     M  D  T  A  R  I  A  V  V  G  A  G  V  V  G  L  S  T  A  V  C  I  S  K  L  V  P  R  C  S   30

  91 GTTACCATCATTTCAGACAAGTTTACTCCAGATACCACCAGTGATGTGGCAGCCGGAATGCTTATTCCTCACACTTATCCAGATACACCC
     V  T  I  I  S  D  K  F  T  P  D  T  T  S  D  V  A  A  G  M  L  I  P  H  T  Y  P  D  T  P   60

 181 ATTCACACGCAGAAGCAGTGGTTCAGAGAAACCTTTAATCACCTCTTTGCAATTGCCAATTCTGCAGAAGCTGGAGATGCTGGTGTTCAT
     I  H  T  Q  K  Q  W  F  R  E  T  F  N  H  L  F  A  I  A  N  S  A  D  A  G  D  A  G  V  H   90

 271 TTGGTATCAGGTTGGCAGATATTTCAGAGCACTCCGACTGAAGAAGTGCCATTCTGGGCTGACGTGGTTCTGGGATTTCGAAAGATGACT
     L  V  S  G  L  Q  I  F  Q  S  T  P  T  E  E  V  P  F  W  A  D  V  V  L  G  F  R  K  M  T  120

 361 GAGGCTGAGCTGAAGAAATTCCCCCAGTATGTGTTTGGTCAGGCTTTTACAACCCTGAAATGTGAATGCCCTGCCTACCTCCCGTGGTTG
     E  A  E  L  K  K  F  P  Q  Y  V  F  G  Q  A  F  T  T  L  K  C  E  C  P  A  Y  L  P  W  L  150

 451 GAGAAAAGGATAAAGGGAAGTGGAGGCTGGACACTCACTCGGCGAATAGAAGACCTGTGGGAACTTCATCCGTCCTTTGACATCGTGGTC
     E  K  R  I  K  G  S  G  G  W  T  L  T  R  R  I  E  D  L  W  E  L  H  P  S  F  D  I  V  V  180

 541 AACTGTTCAGGCCTTGGAAGCAGACAGCTTGCAGGAGACTCAAAGATTTTCCCTGTAAGGGGCCAAGTCCTCCAAGTTCAGGCTCCCTGG
     N  C  S  G  L  G  S  R  Q  L  A  G  D  S  K  I  F  P  V  R  G  Q  V  L  Q  V  Q  A  P  W  210

 631 GTGGAGCATTTTATCCGAGATGGCAGTGGGCTGACATATATTTATCCTGGTACATCCCATGTAACCCTAGGTGGAACTAGGCAAAAAGGG
     V  E  H  F  I  R  D  G  S  G  L  T  Y  I  Y  P  G  T  S  H  V  T  L  G  G  T  R  Q  K  G  240

 721 GACTGGAATCTGTCCCCGGATGCAGAAAATAGCAGAGAGATTCTTTCCCGATGCTGTGCTCTGGAGCCCTCCCTCCACGGAGCCTGCAAC
     D  W  N  L  S  P  D  A  E  N  S  R  E  I  L  S  R  C  C  A  L  E  P  S  L  H  G  A  C  N  270

 811 ATCAGGGAGAAGGTGGGCTTGAGGCCCTACAGGCCAGGCGTGCGACTGCAGACAGAGCTCCTTGCGCGAGATGGACAGAGGCTGCCTGTA
     I  R  E  K  V  G  L  R  P  Y  R  P  G  V  R  L  Q  T  E  L  L  A  R  D  G  Q  R  L  P  V  300

 901 GTCCACCACTATGGCCATGGGAGTGGGGGCATCTCAGTGCACTGGGGCACTGCTCTGGAGGCCGCCAGGCTGGTGAGCGAGTGTGTCCAT
     V  H  H  Y  G  H  G  S  G  G  I  S  V  H  W  G  T  A  L  E  A  A  R  L  V  S  E  C  V  H  330

 991 GCCCTCAGGACCCCCATTCCCAAGTCAAACCTGTAGatgacataaaatgacagcaaagagactgagagactgttgatcaaagcacagaac
     A  L  R  T  P  I  P  K  S  N  L  ***

1081 aggttcaaataacttttccactgcatgaaagtttaattagacatttctttgttttcaacattagaagtggtgtaacatgtaagctgagca

1171 cggtagcatgcctatagtcccagctacttg
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Fig. 1. **Nucleotide and deduced amino acid sequences of the human DDO cDNA.** The predicted amino acid sequence is shown below the nucleotide triplets. The underline indicates the nucleotide sequence (nucleotides 282–458) specific for the DDO-1 cDNA, which is absent in the DDO-2 sequence. The termination TGA codon (nucleotides 1024–1026) is indicated by asterisks.

region, suggesting that they are produced by alternative splicing from a single gene. To test this hypothesis, we isolated the human genomic DNA specific for DDO-1 and the surrounding region, by means of the PCR method. For this purpose, we synthesized two primers, one corresponding to the sequence of DDO-1 cDNA from 190 to 207 (5′-CAGAAGCAGTGGTTCAGA-3′), and the other corresponding to the anti-sense strand sequence from 352 to 369 (5′-CACAGCCTCAGTCATCTT-3′). The amplified DNA region spans approximately 4 kb, and contains one intron and two exons (shown as exon n and $n+1$, in Fig. 3). Comparison of the nucleotide sequences between the DDO cDNAs and the DDO gene revealed that the exon $n+1$ codes the specific sequence for DDO-1, but not DDO-2. This finding strongly suggests that the exons n, $n+1$, and $n+2$ are utilized to generate the DDO-1 mRNA, and the exons n and $n+2$ to generate the DDO-2 mRNA.

As the presence of enzyme corresponding to DDO-2 has not been reported thus far, it would be interesting to examine the enzyme properties and physiological role of human DDO-2 in comparison with those of DDO-1.

Expression and Purification of Human DDO—To obtain homogeneous preparations of human DDO-1 and DDO-2, we developed an expression system of each type of DDO in *E. coli* cells as described under "MATERIALS AND METHODS." To find optimal conditions for the expression, we cultured *E. coli* BL21(DE3) harboring pETDDO-1.8 or pETDDO-1.6 with IPTG at various concentrations. With IPTG at a concentration of 10 μM, DDO-1 was most efficiently produced in a soluble form. Judging from the SDS-PAGE analysis of the extract from cells cultivated under the optimum conditions, the expressed protein of DDO-1 corresponded to about 10% of the total soluble proteins. In the case of DDO-2, however, most of the expressed protein was accumulated in an insoluble form. We are at present searching for optimal conditions for the expression of DDO-2 in a soluble form.

Recombinant DDO-1 expressed in *E. coli* cells carrying pETDDO-1.8 was purified by a three-step procedure as described under "MATERIALS AND METHODS." Using this purification procedure, about 200 mg of DDO-1 was obtained from 72 liters of IPTG-induced culture. The DDO-1 preparation obtained was homogeneous as judged by SDS-PAGE, which showed a single protein band corresponding to the molecular mass of 37 kDa (Fig. 4). The N-terminal amino acid sequence of the purified protein was identical to that predicted from the nucleotide sequence of DDO-1 cDNA up to residue 20 (data not shown). As shown in Fig. 5, the absorption spectra of purified DDO-1 and its complex with tartrate, the competitive inhibitor for bovine DDO (*2*), were practically identical to those of bovine kidney DDO (*2*).

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                10        20        30        40        50
hDDO-1:MDTARIAVVGAGVVGLSTAVCI---SKLV-PRCSVTIISDKFTPDTTSDVAAGM
bDDO  :***V*********M********---**M*-*G**I*V*******E*********
pDAO  :*---*VV*I****I*****L**HERYHS*LQPLDVKVYA*R***F**T*****-
                  10        20        30        40        50

              60        70        80        90        100
hDDO-1:LIPHTYPDTPIHTQ-KQWFRETFNHLFAIANSADAGDAGVHLVSGLQIFQS
bDDO  :***P*******QK*-****R***D*****V***E*E****I****W*****
pDAO  :*WQP-*TSE*SNP*EAN*NQQ***Y*L*HIG*PN*ANM*LTP***YNL*--
                60        70        80        90

             110       120       130       140       150
hDDO-1:TPTEEVPFWADVVLGFRKMTEAELKKFPQYVFGQAFTTLKCECPAYLPWL
bDDO  :I******Y***********KD*******H***H********G*******
pDAO  :REAVPD*Y*K*M******L*PR**DM**D*RY*WFN*S*IL*GRK**Q**
      100       110       120       130       140

             160       170       180       190       200
hDDO-1:EKRIKGSGGWTLTRRIEDLWE-LHPSFDIVVNCSGLGSRQLAGDSKIFPVR
bDDO  :Q**V**N**LI**********-****************************
pDAO  :TE*LTER*VKFFL*KV*SFEEVARGGA*VII**T*VWAGV*QPDPLLQ*GR
      150       160       170       180       190

             210           220           230       240       250
hDDO-1:GQVLQVQAPWVEHFI--RD-GSG---LTYIYPGTSHVTLGGTRQKGDWNLSPDAEN
bDDO  :****K******K***---**-S**---*******I*N*******************I
pDAO  :**IIK*D***LKN**ITH*LER*IYNSP**I**LQA******F*V*N**EINNIQD
     200       210       220       230       240       250

             260       270       280       290       300
hDDO-1:SREILSRCCALEPSLHGACNIREKVGLRPYRPGVRLQTELLARDGQRLPV
bDDO  :*K**************R**YDL**R******T******EK****Q*SR****
pDAO  :HNT*WEG**R***T*KD*KIVG*YT*F**V**Q***ER*Q*RFGSSNTE*
         260       270       280       290       300

             310       320           330       340
hDDO-1:VHHYGHGSGGISVHWGTALEAARL---VSECVHALRTPIPKSNL
bDDO  :***********AM********T**---*N***QV****A**
pDAO  :I*N****GY*LTI***C***V*K*FGK*L*ERNL*TM*-*-*H*
         310       320       330       340
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Fig. 2. **Comparison among the primary structures of human brain DDO-1, bovine DDO, and porcine DAO.** hDDO-1, human brain DDO-1; bDDO, bovine kidney DDO (*4*); pDAO, porcine kidney DAO (*23*). Asterisks indicate that corresponding amino acid residues are identical in all these proteins. Dashes represent arbitrary gaps inserted to maximize homology, and one dash corresponds to one amino acid. The upper line of the numbering system refers to the human DDO-1 sequence, and the lower line to the porcine DAO sequence. The amino acid residues in boxes indicate the active site residues of porcine DAO proposed on the basis of the X-ray crystallographic studies (*21, 22*).

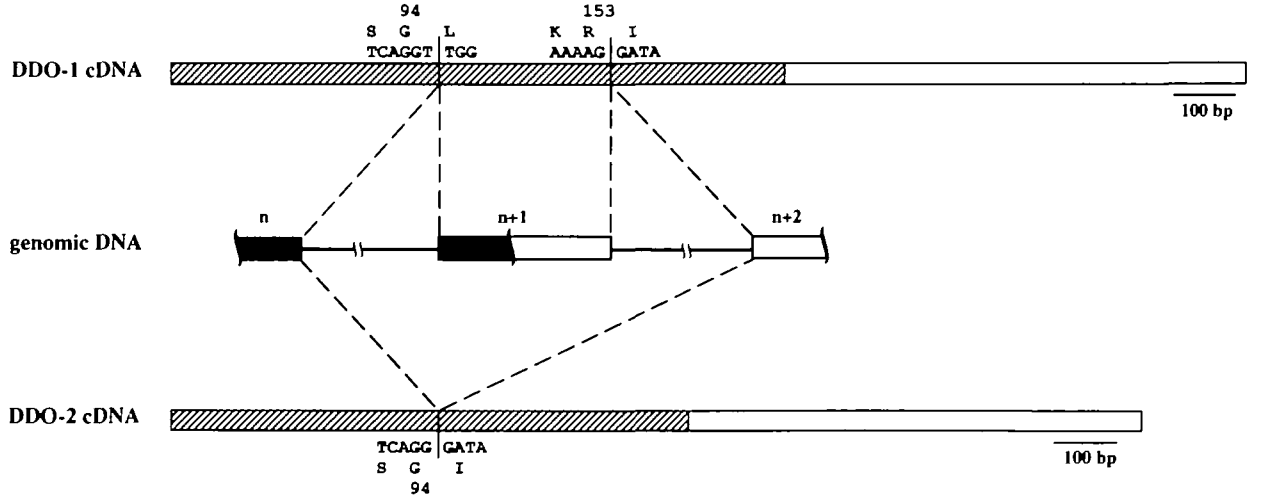

Fig. 3. **Postulated mechanism of alternative splicing yielding two forms of DDO mRNA.** In the genomic DNA, exons are boxed (*n*, *n*+1, and *n*+2). Only the filled regions (3′ portion of the exon *n* and 5′ portion of the exon *n*+1) were analyzed. The introns are shown by the solid and interrupted lines. In the cDNA, the hatched and open boxes indicate coding and 3′ untranslated regions, respectively. The positions (in cDNA) of introns are indicated by vertical lines, the nucleotide sequences of the 3′ and 5′ portions of each exon are indicated and the amino acid residues that correspond to introns are numbered.

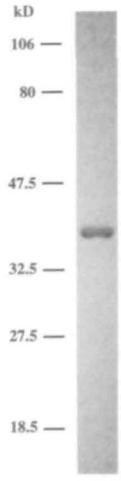

Fig. 4. **SDS-PAGE of recombinant human DDO-1.** Approximately 1 μg of the purified enzyme was analyzed by SDS-PAGE with 12.5% gel. The values of molecular-mass standards in kDa are shown to the left side.

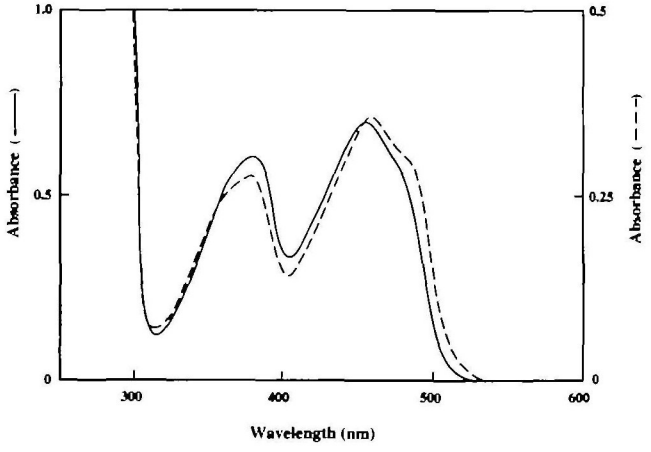

Fig. 5. **Absorption spectra of the human DDO-1.** ——, enzyme (61.1 μmol) in the free form in 10 mM potassium phosphate, pH 8.0; · ·, enzyme (31.8 μmol) complexed with tartrate in the same buffer containing 5 mM sodium potassium tartrate. In the case of DDO-1/tartrate complex, the enzyme concentration was determined on the assumption that the extinction coefficient (11.3 mM^{-1}) at 455 nm for free FAD does not change in the complex formation.

Enzymatic Properties of Human DDO-1—D-Aspartate oxidase activity was measured to confirm the catalytic activity of expressed human DDO-1 (Fig. 6). The apparent kinetic parameters were determined for two substrates. D-Aspartate and its *N*-methylated derivative, *N*-methyl-D-aspartate (NMDA), were good substrates for human DDO-1, and the values of K_m were 2.7 mM for D-aspartate and 6.8 mM for NMDA at pH 8.3. On the other hand, D-glutamate turned out to be a poor substrate for DDO-1. The measured velocity did not tend to saturate even when high concentrations of D-glutamate (greater than 10 mM) were used. Due to the low affinity of human DDO-1 for D-glutamate, the K_m and k_{cat} values could not be determined. The Lineweaver-Burk plots for D-aspartate and NMDA gave the k_{cat} values of 52.5 mol D-aspartate oxidized·s^{-1}·mol^{-1} and 37.7 mol NMDA oxidized·s^{-1}·mol^{-1},

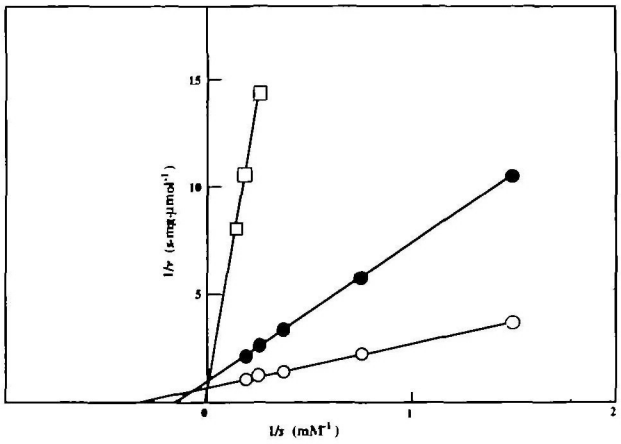

Fig. 6. **Lineweaver-Burk plots of DDO-1 for dicarboxylic D-amino acids.** Activity was measured by the oxygen consumption method as described under "MATERIALS AND METHODS," using 2.38 μg of enzyme and various concentrations of the following substrates, D-aspartate (open circles), NMDA (closed circles), and D-glutamate (open squares).

respectively. The former value is in reasonable agreement with the value of 26.0 mol of D-aspartate oxidized·s^{-1}·mol^{-1} reported for the bovine enzyme (*2*), though the temperature and pH were different from those we employed.

In conclusion, we have reported the presence of two forms (DDO-1 and DDO-2) of human DDO mRNA generated by alternative splicing. The structural and enzymatic properties of DDO-1 were very similar to those of the bovine kidney enzyme previously reported (*2, 4*). However, the occurrence of the enzyme corresponding to DDO-2 has not been identified in mammals. Although the enzymatic properties of DDO-2 are unclear at present, further studies, including the development of expression and purification systems, are needed to reach an understanding of the function as well as the physiological role of DDO-2.

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