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Hamster D-amino-acid oxidase cDNA: rodents lack the 27th amino acid residue in D-amino-acid oxidase

Short Communication

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Summary. The nucleotide sequence of cDNA that encodes hamster D-amino-acid oxidase (DAO) was determined. The cDNA consisted of 1,590 nucleotides and a poly(A) tail. It had an open reading frame for a protein consisting of 346 amino acid residues. The number of the amino acid residues is the same as that of the rat DAO. However, the hamster DAO has one residue more than mouse DAO and one residue less than human, pig, rabbit, and guinea pig DAOs. Amino acid sequence of the hamster DAO was highly similar to those of mouse and rat DAOs: 89% and 88% of the amino acid residues were identical between the hamster and mouse DAOs and between the hamster and rat DAOs, respectively. The homology was slightly less between the hamster DAO and the human (81%), pig (78%), rabbit (78%), or guinea pig DAO (82%). It has been proposed that the mouse and rat DAOs lack an amino acid residue corresponding to the 25th residue of the DAOs of other mammals. However, a detailed comparison of the amino acid sequences as well as the underlying nucleotide sequences by inclusion of the hamster ones revealed that the rodent DAOs does not lack the 25th, but the 27th residue.

Keywords: D-Amino-acid oxidase – Hamster – Rodents – Amino acid sequence – Nucleotide sequence – Deletion

Introduction

D-Amino-acid oxidase (DAO) catalizes oxidative deamination of D-amino acids, stereoisomers of naturally occurring L-amino acids (Krebs, 1935). This enzyme exists in a variety of organisms: mammals, birds, reptiles, amphibians, fish, insects, mollusks, fungi, and yeasts (Meister, 1965). In higher animals, it is present mainly in the kidney, liver, and brain. Recent studies have shown that D-amino acids are present in organisms much more abundantly than they were considered in the past (see Hashimoto and Oka, 1997). For example, D-aspartic acid is present in cephalopods, chickens, rats, and humans. This D-amino acid is considered to regulate the production of some hormones (Nagata et al., 1999; D'Aniello et al., 2000). D-Serine has been detected in mammalian brain. It binds to *N*-methyl-D-aspartic acid (NMDA)-subtype glutamate receptors of neurons and enhances NMDA currents (see Snyder and Kim, 2000). Accordingly, by controlling the level of D-serine, DAO is considered to modulate the neuronal signaling (Mothet et al., 2000; Wake et al., 2001). DAO in kidney and liver, on the other hand, was shown to be involved in the metabolism of a variety of D-amino acids of internal and external origin (Konno et al., 1993; D'Aniello et al., 1993).

Both nucleotide and amino acid sequences of DAO have been determined in several organisms. In mammals, those of the human (Momoi et al., 1988), pig (Fukui et al., 1987), rabbit (Momoi et al., 1990), guinea pig (Konno et al., 1999), rat (Konno, 1998), and mouse (Tada et al., 1990) are known. Human, pig, rabbit, and guinea pig DAOs consist of 347 amino acid residues, whereas rat and mouse DAO has 346 and 345 amino acid residues, respectively. It has been believed that both rat and mouse DAOs lack an amino acid residue which corresponds to the 25th residue of other mammalian DAOs (Tada et al., 1990; Konno, 1998). In addition, mouse DAO lacks the 173rd residue

(Tada et al., 1990). These are interesting features from the point of the molecular evolution of DAO. To determine whether every rodent DAO lacks the 25th amino acid residue and whether the 173rd residue is missing only in the mouse, we examined the nucleotide sequence of cDNA encoding hamster DAO and compared its amino acid sequence with those of DAOs of aforementioned six mammals.

Materials and methods

The total RNA was extracted from the kidneys of male hamsters using an extraction kit (Isogen, Nippon Gene, Tokyo). The first strand of cDNA was synthesized using the Superscript Preamplification System (Gibco-BRL, Gaithersburg, MD). A sense primer (5′-GGTTAACTGAGAGGGGAGTGAA-3′) and an antisense primer (5′-CCATAGTTGTGGATGACCTCTG-3′) designed from the conserved region of human, pig, rabbit, and mouse DAO cDNA (Konno, 1998) were used for the amplification of a part of hamster DAO cDNA by PCR. The PCR conditions were the same as before (Konno et al., 1999). The amplified fragment was

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CAGAAACAGGAACGTGCAACCCCACACCGTTCTTCTCCAGGCACCTGGCACAAGTGGCTG      60

GTGAGATGCGTGTGGTCGTGATTGGAGCGGGAGTCATCGGACTGTCCACTGCCCTCTGCA     120
      M  R  V  V  V  I  G  A  G  V  I  G  L  S  T  A  L  C       18

TTCATGAGCGTTTCAGCCCTGTGCAGCCACTGCACATGAAGATCTACGCAGACCGCTTTA     180
I  H  E  R  F  S  P  V  Q  P  L  H  M  K  I  Y  A  D  R  F       38

CCCCATTCACCACTAGTGACGTGGCTGCGGGCTTTTGGCAGCCCTATCTCTCAGACCCCA     240
T  P  F  T  T  S  D  V  A  A  G  F  W  Q  P  Y  L  S  D  P       58

GGAACCCTCAGGAAGTGGAGTGGAATCAGCAAACCTTCGACTACCTGCTGAGCCATATCC     300
R  N  P  Q  E  V  E  W  N  Q  Q  T  F  D  Y  L  L  S  H  I       78

ATTCTCCAAATGCTGAAAAAATGGGCTTGTCCCTAATCTCAGGCTACAATCTCTTCAAAG     360
H  S  P  N  A  E  K  M  G  L  S  L  I  S  G  Y  N  L  F  K       98

AAGAAGTTCCGGACCCTTTCTGGAGGAACACAGTTCTGGGATTTCGGAAGCTGACCCCCA     420
E  E  V  P  D  P  F  W  R  N  T  V  L  G  F  R  K  L  T  P      118

GAGAGATGGACATATTCCCTGATTATGGCTACGGCTGGTTCAACACAAGCCTGACTTTAG     480
R  E  M  D  I  F  P  D  Y  G  Y  G  W  F  N  T  S  L  T  L      138

AGGGGAAGAGCTACCTGCCATGGCTGACTGAGAGGTTGACAGAGAGGGGAGTGAAGCTCT     540
E  G  K  S  Y  L  P  W  L  T  E  R  L  T  E  R  G  V  K  L      158

TCCATCGGAAGGTGGAATCTTTTGAAGAGGTGGCAAGAGGAGGCGCGGATGTGATTATCA     600
F  H  R  K  V  E  S  F  E  E  V  A  R  G  G  A  D  V  I  I      179

ACTGCACTGGGGTGTGGGCTGGAGCGCTGCAAGCAGACACCTCCCTGCAGCCCGGCCGGG     660
N  C  T  G  V  W  A  G  A  L  Q  A  D  T  S  L  Q  P  G  R      198

GCCAGATCATTCAGGTGGAGGCCCCTTGGATGAAGCATTTTATCCTCACCCATGACCCAC     720
G  Q  I  I  Q  V  E  A  P  W  M  K  H  F  I  L  T  H  D  P      218

GCCTTGGCATCTACAACTCCCCATACATCATCCCAGGGTCCAAGACAGTTACTCTCGGAG     780
R  L  G  I  Y  N  S  P  Y  I  I  P  G  S  K  T  V  T  L  G      238

GTGTCTTCCAGCTGGGGAACTGGAATGAATTAAACAGTGTCCATGACCACAACACCATTT     840
G  V  F  Q  L  G  N  W  N  E  L  N  S  V  H  D  H  N  T  I      258

GGAAAAGCTGCTGTAAACTGGAGCCCACCCTGAAAAATGCAAAAATCGTTGGGGAACTCA     900
W  K  S  C  C  K  L  E  P  T  L  K  N  A  K  I  V  G  E  L      278

CTGGCTTCCGGCCGGTCCGGCATCAGGTTCGGCTAAAAAAAAAGCAGCTTCATTTTGGAT     960
T  G  F  R  P  V  R  H  Q  V  R  L  K  K  K  Q  L  H  F  G      298

CTTCGAGTGTTGAGGTCATCCATAACTATGGTCATGGAGGCTACGGGCTCACAATTCACT    1020
S  S  S  V  E  V  I  H  N  Y  G  H  G  G  Y  G  L  T  I  H      318

GGGGTTGTGCAATGGAGGCAGCCAACCTCTTTGGGAAAATTCTAGAAGAAAAGAAGTTGT    1080
W  G  C  A  M  E  A  A  N  L  F  G  K  I  L  E  E  K  K  L      338

CCAGGATGCCTGCATCCCACCTCTAAGGACTCTAGTGACCATCACTTGCCCCTTGAGGAC    1140
S  R  M  P  A  S  H  L  *                                       346

ATCCCAGTACCCTACAGCCAATTAAGTGATAGTCTCCTTGCTGATTCTTCACTCCCCGCT    1200
CCCTGGCTTCCTTGGGCAAAGGTGTGAGGTGAGGAAATCCTGGCACTCTGCTCATCCGTC    1260
ACGGCCTGTCCTTCCCACACATCCATCTGGCAAAGCATCTGTAGGATGGATCCTGGCTGG    1320
TCCTAAGCCCATCTATATGAGACAGGCAGCATCTAGGACCATGTAGCAATCCTGTTCTCA    1380
CAACAGGATGACTGTACTAAAGCTGGTTGTACTGGGTGGCAGGGTCTGGGCTCAGTTCCC    1440
TAAGAGGTGGTCCAGAAACTTTTGCTTAGAACTCTGAAGAGTGATTCACGACACACTTGC    1500
TGTCTACCAGTAGTGACGGGAGGAGGGGGGACTCCTGCCCTCAGCACTCCATTGGTCAAT    1560
ACTATTAAAACACTCTTGGGATATCCCTA                                    1589
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Fig. 1. Nucleotide sequence of hamster DAO cDNA and deduced amino acid sequence. An asterisk indicates the termination codon. Amino acids are shown by a one-letter code

ligated to the pCR-TOPO vector (TOPO TA Cloning, Invitrogen, Carlsbad, CA) and cloned in *Escherichia coli*. The plasmid was amplified, extracted, and purified using the QIAprep Spin Miniprep Kit (Qiagen, Germany). The nucleotide sequence of the insert was determined using dye terminator chemsitry and an ABI Prism 377 DNA sequencer (PE Applied Biosysterms). From the sequence information obtained, hamster DAO-specific primers were synthesized. Using these primers, rapid amplification of 5′-cDNA end (5′-RACE) and 3′-RACE were carried out with Marathon cDNA Amplification Kit (Clontech, Polo Alto, CA). The fragments amplified by RACEs were cloned and sequenced as described above. An entire cDNA sequence encoding hamster DOA was determined by connecting these sequences.

Results and discussion

A nucleotide sequence of cDNA that encodes hamster DAO was determined. This cDNA consisted of 67 nucleotides of a 5′-noncoding region, 1,038 nucleotides of an open reading frame, 485 nucleotides of a 3′-noncoding region, and a poly(A) tail. The nucleotide and deduced amino acid sequences are shown in Fig. 1.

Hamster DAO was found to consist of 346 amino acid residues. The number of the residues is the same as that of rat DAO (Konno, 1998). However, it is one residue more than mouse DAO (Tada et al., 1990) but one residue less than human (Momoi et al., 1988), pig (Fukui et al., 1987), rabbit (Momoi et al., 1990), and guinea pig DAOs (Konno et al., 1999). Hamster DAO has a high homology with mouse and rat DAO in their amino acid sequences: 89% of the amino acids were identical between hamster and mouse DAOs, and 88% between hamster and rat DAOs. Homology in the amino acid sequences was slightly less between hamster DAO and that of human (81%), pig (78%), rabbit (78%), or guinea pig DAO (82%). These results are consistent with the taxonomic relationship of these mammals. The hamster, rat, and mouse are classified into the suborder Myomorpha in the order Rodentia. The guinea pig belongs to the same order but in a separate suborder, Cavimorpha. The human, pig, and rabbit are classified into the different orders.

It has been believed that the mouse and rat DAOs lack an amino acid residue corresponding to the 25th of DAOs of the human, pig, rabbit, and guinea pig (Tada et al., 1990; Konno, 1998). However, when the amino acid residues of seven mammalian DAOs are aligned accroding to this assumption, the mouse, rat, and hamster DAO has an entirely different amino acid residue at the 26th and 27th residues from other mammalian DAOs (Fig. 2A). The mouse, rat, and hamster DAO has Pro, while guinea pig, pig and human DAO has Val at the 26th residue. At the 27th residue,

A

	Amino acid residue						
	23	24	25	26	27	28	29
Mouse	Tyr	His	---	Pro	Thr	Gln	Pro
Rat	Tyr	His	---	Pro	Ala	Gln	Pro
Hamster	Phe	Ser	---	Pro	Val	Gln	Pro
Guinea pig	Tyr	His	Ser	Val	Leu	Gln	Gln
Rabbit	Tyr	His	Ser	Ala	Leu	Gln	Pro
Pig	Tyr	His	Ser	Val	Leu	Gln	Pro
Human	Tyr	His	Ser	Val	Leu	Gln	Pro

B

	Amino acid residue						
	23	24	25	26	27	28	29
Mouse	Tyr	His	Pro	Thr	---	Gln	Pro
Rat	Tyr	His	Pro	Ala	---	Gln	Pro
Hamster	Phe	Ser	Pro	Val	---	Gln	Pro
Guinea pig	Tyr	His	Ser	Val	Leu	Gln	Gln
Rabbit	Tyr	His	Ser	Ala	Leu	Gln	Pro
Pig	Tyr	His	Ser	Val	Leu	Gln	Pro
Human	Tyr	His	Ser	Val	Leu	Gln	Pro

Fig. 2. Amino acid sequence (23rd–29th residues) of DAO in seven mammals. **A** An alignment when mouse, rat, and hamster DAOs lack the 25th amino acid residue. **B** An alignment when they lack the 27th amino acid residue

mouse, rat, and hamster DAO has Thr, Ala, and Val, respectively, while other mammalian DAOs commonly have Leu. These are significant differences. However, if we assume that the mouse, rat, and hamster DAOs lack the 27th amino acid residue instead, the differences become much smaller. In this case, the mouse, rat, and hamster DAOs commonly have Pro while the other mammalian DAOs have Ser at the 25th residue (Fig. 2B). At the 26th residue, the rat DAO has Ala, but it is common with the rabbit DAO. The hamster DAO has Val, but it is common with the guinea pig, pig, and human DAO. The mouse DAO has a unique Thr residue at the 26th residue. As a whole, the amino acid residues can be aligned with much higher homology if we assume the mouse, rat, and hamster DAOs commonly lack the 27th residue.

Nucleotide sequences of cDNA encoding the 23–29th amino acid residues of DAO are shown in Figs. 3A and 3B. Fig. 3A shows an alignment of the nucleotide sequences according to that the mouse, rat, and hamster DAOs lack the 25th amino acid residue. In this case, the rodent DAO genes need at least three nucleotide changes at the 26th codon to encode the same amino acid (Val or Ala) as those of the other

A

	Amino acid residue						
	23	24	25	26	27	28	29
Mouse	TAC	CAC	---	CCA	ACA	CAG	CCA
Rat	TAC	CAC	---	CCA	GCC	CAA	CCT
Hamster	TTC	AGC	---	CCT	GTG	CAG	CCA
Guinea pig	TAC	CAC	TCA	GTG	CTG	CAG	CAG
Rabbit	TAC	CAC	TCG	GCC	CTG	CAG	CCT
Pig	TAC	CAC	TCT	GTC	CTG	CAG	CCC
Human	TAC	CAC	TCA	GTC	CTG	CAG	CCA

B

	Amino acid residue						
	23	24	25	26	27	28	29
Mouse	TAC	CAC	CCA	ACA	---	CAG	CCA
Rat	TAC	CAC	CCA	GCC	---	CAA	CCT
Hamster	TTC	AGC	CCT	GTG	---	CAG	CCA
Guinea pig	TAC	CAC	TCA	GTG	CTG	CAG	CAG
Rabbit	TAC	CAC	TCG	GCC	CTG	CAG	CCT
Pig	TAC	CAC	TCT	GTC	CTG	CAG	CCC
Human	TAC	CAC	TCA	GTC	CTG	CAG	CCA

Fig. 3. Nucleotide sequence of cDNA encoding 23rd–29th amino acid residues of DAO in seven mammals. **A** An alignment when mouse, rat, and hamster DAOs lack the 25th amino acid residue. **B** An alignment when they lack the 27th amino acid residue

mammals. The mouse and rat DAO also needs three nucleotide substitutions at the 27th codon to encode the Leu residue. However, if we assume that the mouse, rat, and hamster DAOs lack the 27th residue, rat and hamster only need one substitution at the 25 and 26th codon (Fig. 3B). Such ease of the nucleotide substitutions favors the hypothesis that the mouse, rat, and hamaster DAOs lack the codon for the 27th amino acid residue not one for the 25th residue.

Comparison of the amino acid and the nucleotide sequences has allowed us to construct molecular phylogenetic trees of DAO (Konno et al., 1999). Hamster DAO was found to quite nicely fit in those phylogenetic trees (see Konno et al., 1999). It indicates that DAO has evolved without drastic changes in mammals, though the enzyme has lost the 27th amino acid residue in rodent lineage, and the 173rd residue has been further lost in the lineage to the living mouse. Such unique amino acid deletion can be used as a marker for phylogenic relationship among rodents and its neighbors.

Hamsters are one of animals which are widely used for experimental researches. DAO has not been well examined in this organism. Amino acid sequence of hamster DOA and nucleotide sequence of cDNA encoding this enzyme would be useful for understanding the physiological roles of this enzyme which is not fully understood.

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