

Mouse D-amino-acid oxidase gene: restriction fragment length polymorphism among mouse strains

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Summary. Genomic DNA was extracted from mice of 15 strains (A/J, AKR, BALB/c, C3H/He, C57BL/6, CBA/J, CD-1, CF#1, DBA/2, ddY/DAO⁺, ddY/DAO⁻, ICR, NC, NZB and NZW) for the examination of the difference in the structure of the D-amino-acid oxidase gene among the mouse strains. The DNAs were digested with restriction endonucleases and analyzed by Southern hybridization using D-amino-acid oxidase cDNA as a probe. The 15 strains showed the same hybridization patterns in the *EcoRV*, *BamHI* or *BglII* digestion. In the *EcoRI* digestion, the DBA/2 strain showed a different hybridization pattern from the other 14 strains. In the *PvuII* and *XbaI* digestion, C3H/He, CBA/J, ddY/DAO⁺ and NC strains were different from the other 11 strains. In the *PstI* and *HindIII* digestion, restriction fragment length polymorphisms were observed, and the 15 strains were classified into four groups according to their hybridization patterns. These results indicate that the 15 strains of mice carry a structurally similar D-amino-acid oxidase gene, but there is a variation in its inside sequence among the groups of the strains.

Keywords: D-Amino-acid oxidase – Southern hybridization – Mouse strains – RFLP – Gene structure

Introduction

D-Amino-acid oxidase (EC 1.4.3.3) catalyzes oxidative deamination of D-amino acids (stereoisomers of naturally occurring L-amino acids) to the corresponding 2-oxo acids (Krebs, 1935). This enzyme has been conserved through evolution: it is present in yeasts, molds, mollusks, insects, fish, amphibians, reptiles, birds and mammals (Meister, 1965). Almost all higher animals have this enzyme in the kidney, liver and brain. In spite of its widespread occurrence, however, the physiological role of this enzyme is not

clear because D-amino acids are rare in eukaryotes (Meister, 1965). It has been demonstrated that this enzyme is involved in the metabolism of D-amino acids derived from intestinal bacteria (Konno and Yasumura, 1992; Konno et al., 1993). However, the presence of a large quantity of D-amino-acid oxidase with high activity in the kidney and liver, combined with its presence in the brain suggest that this enzyme still has some other functions.

D-Amino-acid oxidase is a single polypeptide and has flavin adenine dinucleotide as a coenzyme. It consists of 347 amino acid residues in the human (Momoi et al., 1990), pig (Ronchi et al., 1982; Fukui et al., 1987), and rabbit (Momoi et al., 1988), whereas it consists of 345 amino acid residues in the mouse (Tada et al., 1990). The 25th and 173rd residues present in the human, pig and rabbit enzymes are missing in the mouse enzyme. However, the homology in the amino acid sequences is high and more than 80% of the amino acids are the same among these mammalian enzymes (Momoi et al., 1990).

Nucleotide sequences of D-amino-acid oxidase cDNA have been determined in the human (Momoi et al., 1990), pig (Fukui et al., 1987), rabbit (Momoi et al., 1988) and mouse (Tada et al., 1990). Mouse D-amino-acid oxidase cDNA consists of 68 nucleotides of the 5'-untranslated region, 1,035 nucleotides of the coding region, 544 nucleotides of the 3'-untranslated region, and the poly(A) tail (Tada et al., 1990). The gene structure of D-amino-acid oxidase is known only in humans. It spans 20 kilobase pairs (kb) and is separated into 11 exons (Fukui and Miyake, 1992).

For the elucidation of the physiological role of D-amino-acid oxidase, it is important to know the structure of the gene encoding this enzyme and the regulatory mechanism of its expression. The mouse is one of the most useful animals for this purpose because its genetic background is well known. In our previous study, Southern hybridization using a D-amino-acid oxidase cDNA probe showed a presence of a restriction fragment length polymorphism (RFLP) in *Hind*III-digested DNA among mouse strains (Konno et al., 1995). In this study, therefore, we extended the Southern hybridization analysis using various restriction endonucleases to examine the difference in the gene structure among mouse strains.

Materials and methods

Mice

A/J Slc, AKR/N SLC and Slc: ICR mice were obtained from Japan SLC (Hamamatsu). BALB/cAnN mice were purchased from Doken (Shimodate). C3H/HeN Jcl, C57BL/6N Jcl, CBA/J Jcl, DBA/2J Jcl and NC/Jic mice were from CLEA Japan (Tokyo). Crj: CD-1, NZB/NCrj and NZW/NCrj mice were obtained from Japan Charles River (Yokohama). ddY/DAO⁺ and ddY/DAO⁻ mice were maintained in our laboratory. The kidneys of CF#1 mice were kindly supplied by Dr. T. Mizutani (The Institute of Physical and Chemical Research (RIKEN), Wako).

Probe

Construction of plasmid p_nDAO(ddY/DAO⁺) carrying D-amino-acid oxidase cDNA was described in our previous paper (Konno et al., 1995). The plasmid was digested with *Bam*HI and *Xho*I and the digest was electrophoresed on a 1% low-melting-temperature agarose gel. A gel slice containing the D-amino-acid oxidase cDNA insert was cut out under ultraviolet illumination. The DNA fragment was purified by the standard method (Sambrook et al., 1989).

Southern hybridization

High molecular genomic DNA was extracted from the mouse livers or kidneys of the 15 strains according to the standard method (Sambrook et al., 1989). The DNAs (10 µg) were digested to completion with 20 units of restriction endonucleases (Nippon Gene, Tokyo; Takara, Otsu). The digests were electrophoresed on 0.9% agarose gels. The DNA fragments were transferred to nylon membranes (GeneScreen Plus, NEN Research Products, Boston, MA) according to the method of Southern (1975).

D-Amino-acid oxidase cDNA (25 ng) was labeled with [α -³²P]dCTP (~110 TBq/mmol, Amersham, Buckinghamshire) by the method of Feinberg and Vogelstein (1983). Hybridization was carried out as described before (Konno et al., 1995). After washing, the membranes were exposed to an imaging plate and analyzed using an image analyzer (Model BAS-2000II, Fuji Photo Film, Tokyo).

Results

Genomic DNA was extracted from mice of 15 strains (A/J, AKR, BALB/c, C3H/He, C57BL/6, CBA/J, CD-1, CF#1, DBA/2, ddY/DAO⁺, ddY/DAO⁻, ICR, NC, NZB and NZW) and digested with nine endonucleases which recognize six nucleotide pairs. The DNA fragments were separated on agarose gels and transferred to nylon membranes. They were hybridized with a D-amino-acid oxidase cDNA probe containing the entire coding region.

Digestion of the DNAs with *Ava*I, *Sal*I, *Sma*I or *Xho*I did not produce small fragments which could be separated on 0.9% agarose gels, so hybridization was not successful.

When the DNAs were digested with *Eco*RV, all the 15 strains gave one hybridizing band of about 20.4 kb (Fig. 1). When the DNAs were digested with *Bam*HI, all the 15 strains gave three hybridizing bands of about 7.3, 6.7 and 4.2 kb (Fig. 2). Similarly, when the DNAs were digested with *Bgl*II, all the strains produced four hybridizing bands of 13.1, 9.5, 4.0 and 1.1 kb (Fig. 3). No variation was observed among the 15 strains in these digestions.

When the DNAs were digested with *Eco*RI, the DNAs of 14 strains gave two hybridizing bands of about 14.8 and 7.4 kb. The DBA/2 strain was an exception. It gave only one band of about 17.4 kb (Fig. 4).

The 15 strains were separated into two groups by the hybridization patterns of the *Pvu*II-digested DNAs (Fig. 5). *Pvu*II digestion produced three hybridizing bands of about 10.0, 8.8 and 3.0 kb in the A/J, AKR, BALB/c, C57BL/6, CD-1, CF#1, DBA/2, ddY/DAO⁻, ICR, NZB and NZW strains. However, it produced four bands of 10.0, 5.6, 3.0 and 1.9 kb in the C3H/He,

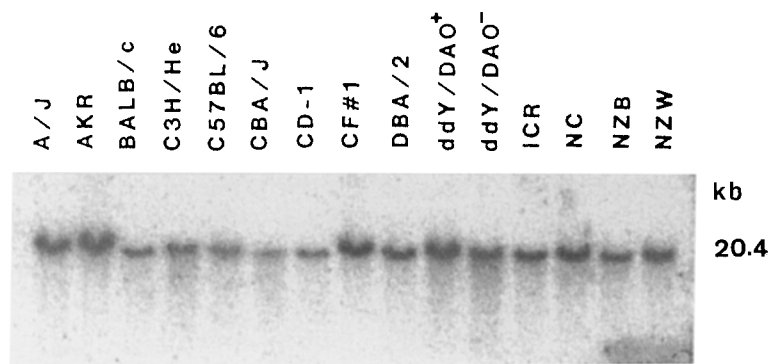


Fig. 1. *EcoRV*-digested mouse genomic DNA hybridized with the D-amino-acid oxidase cDNA probe. Genomic DNAs extracted from mice of 15 strains were digested with *EcoRV*, electrophoresed, transferred to a membrane, and hybridized with the cDNA probe containing the entire coding region

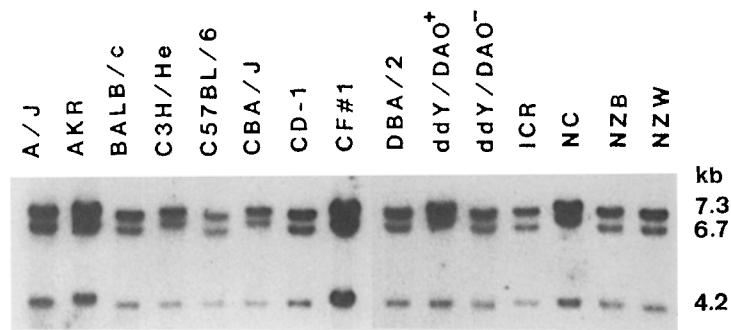


Fig. 2. *Bam*HI-digested mouse genomic DNA hybridized with the D-amino-acid oxidase cDNA probe. Genomic DNAs extracted from mice of 15 strains were digested with *Bam*HI, electrophoresed, transferred to a membrane, and hybridized with the cDNA probe containing the entire coding region

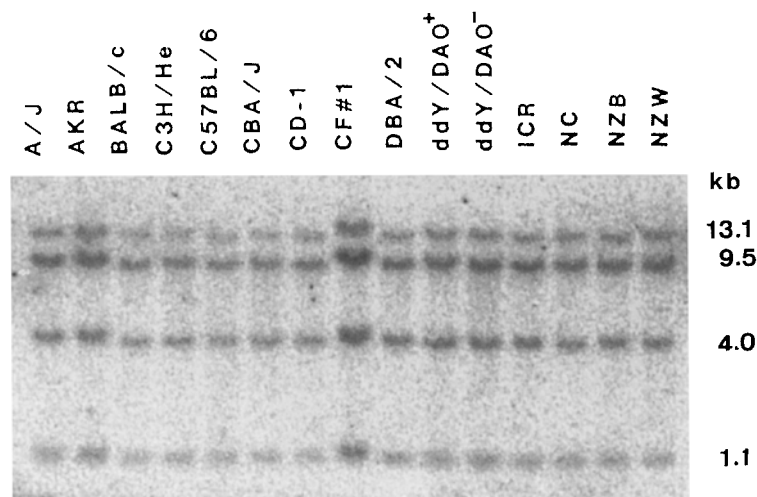


Fig. 3. *Bgl*II-digested mouse genomic DNA hybridized with the D-amino-acid oxidase cDNA probe. Genomic DNAs extracted from mice of 15 strains were digested with *Bgl*II, electrophoresed, transferred to a membrane, and hybridized with the cDNA probe containing the entire coding region

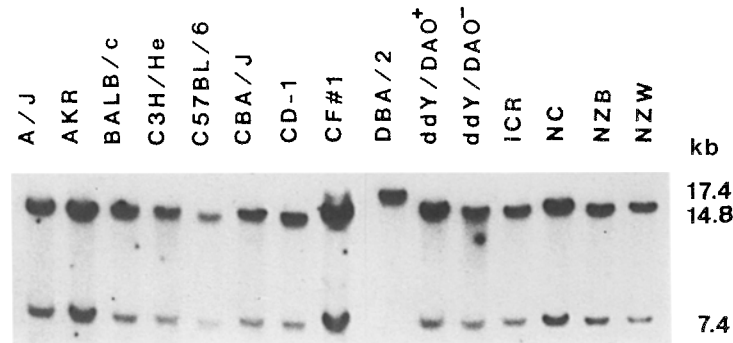


Fig. 4. *Eco*RI-digested mouse genomic DNA hybridized with the D-amino-acid oxidase cDNA probe. Genomic DNAs extracted from mice of 15 strains were digested with *Eco*RI, electrophoresed, transferred to a membrane, and hybridized with the cDNA probe containing the entire coding region

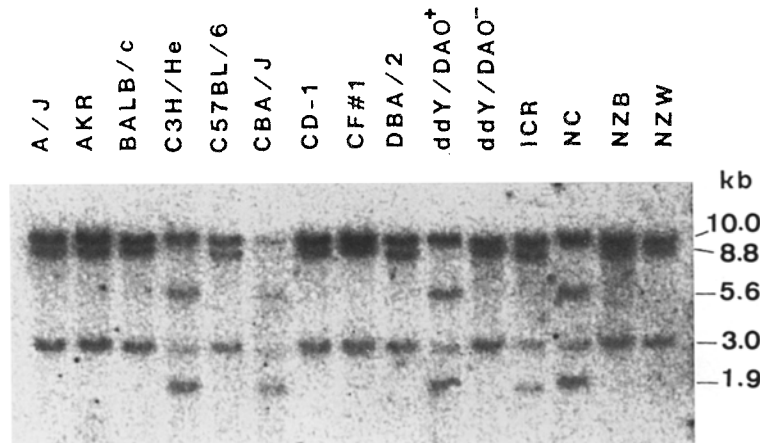


Fig. 5. *Pvu*II-digested mouse genomic DNA hybridized with the D-amino-acid oxidase cDNA probe. Genomic DNAs extracted from mice of 15 strains were digested with *Pvu*II, electrophoresed, transferred to a membrane, and hybridized with the cDNA probe containing the entire coding region

CBA/J, ddY/DAO⁺ and NC strains. The 8.8-kb band was missing but, instead, two bands of 5.6 and 1.9 kb were present in these four strains. The ICR mouse had a hybridizing band of about 1.7 kb but this band was not always observed. Quite similar patterns were observed in the *Xba*I-digested DNAs (Fig. 6). The *Xba*I digestion produced four hybridizing bands in every strain. The 10.9-, 1.2- and 1.0-kb bands of the four bands were common in all the strains. In addition to these, however, the four strains (C3H/He, CBA/J, ddY/DAO⁺ and NC) had a 4.2-kb band whereas the other 11 strains possessed a little smaller 3.8-kb band. In these digestions, the C3H/He, CBA/J, ddY/DAO⁺ and NC strains were distinguished from the other 11 strains.

When the DNAs were digested with *Pst*I, a restriction fragment length polymorphism (RFLP) was observed (Fig. 7). The A/J, AKR, C57BL/6, ICR

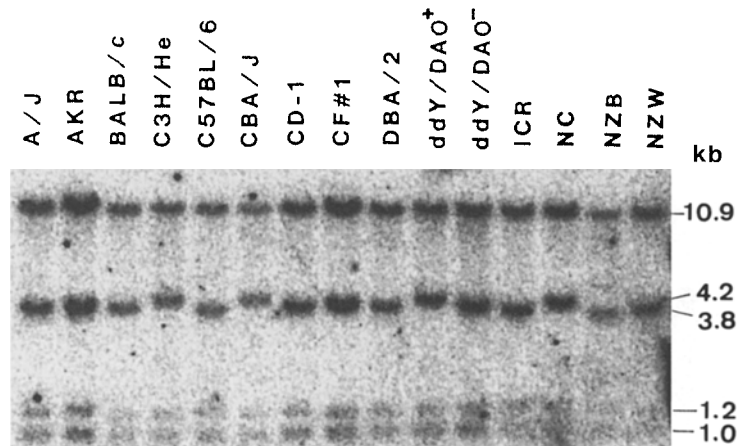


Fig. 6. *Xba*I-digested mouse genomic DNA hybridized with the D-amino-acid oxidase cDNA probe. Genomic DNAs extracted from mice of 15 strains were digested with *Xba*I, electrophoresed, transferred to a membrane, and hybridized with the cDNA probe containing the entire coding region

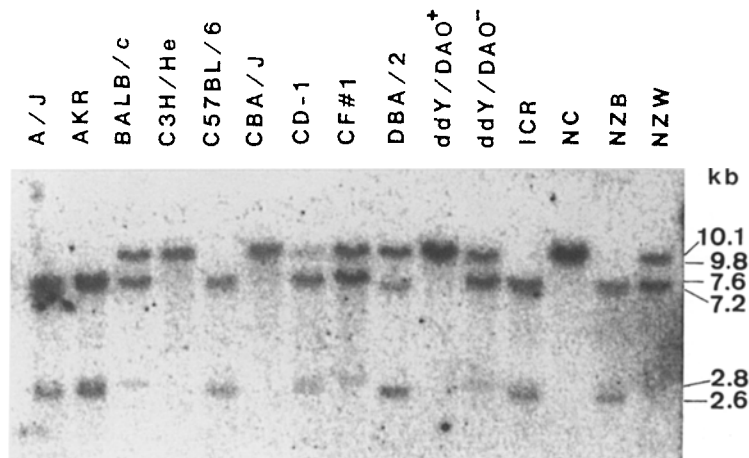


Fig. 7. *Pst*I-digested mouse genomic DNA hybridized with the D-amino-acid oxidase cDNA probe. Genomic DNAs extracted from mice of 15 strains were digested with *Pst*I, electrophoresed, transferred to a membrane, and hybridized with the cDNA probe containing the entire coding region. The 7.2-kb and 2.8-kb bands are fairly faint and are not clearly reproduced in the picture

and NZB strains had four hybridizing bands of about 7.6, 7.2, 2.8 and 2.6kb. The BALB/c, CF#1, ddY/DAO⁻ and NZW strains had three bands of about 9.8, 7.6 and 2.8kb. The C3H/He, CBA/J, ddY/DAO⁺ and NC strains had two bands of about 10.1 and 9.8kb. The CD-1 strain had five bands of about 9.8, 7.6, 7.2, 2.8 and 2.6kb. The DBA/2 strain had three bands of about 10.1, 7.2 and 2.6kb. The 7.2-kb and 2.8-kb bands observed in these strains were fairly faint compared with other bands. The *Hind*III digestion of the DNAs gave quite similar patterns (Fig. 8). The A/J, AKR, C57BL/6, DBA/2, ICR and NZB strains had three hybridizing bands of about 12.0, 9.9 and 6.2kb. The

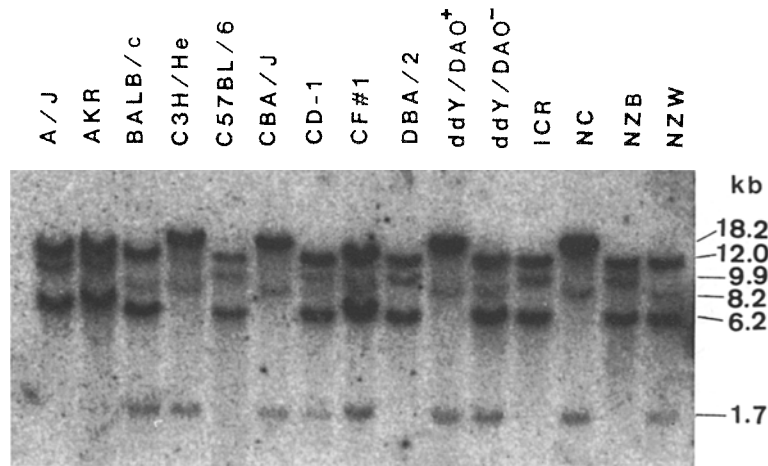


Fig. 8. *Hind*III-digested mouse genomic DNA hybridized with the D-amino-acid oxidase cDNA probe. Genomic DNAs extracted from mice of 15 strains were digested with *Hind*III, electrophoresed, transferred to a membrane, and hybridized with the cDNA probe containing the entire coding region

Table 1. Classification of mouse strains based on the Southern hybridization patterns to the D-amino-acid oxidase cDNA probe¹

Restriction endonuclease					Strain
<i>Eco</i> RI	<i>Pvu</i> II	<i>Xba</i> I	<i>Pst</i> I	<i>Hind</i> III	
A	C	E	G	K	A/J, AKR, C57BL/6, ICR, NZB
A	C	E	H	L	BALB/c, CF#1, ddY/DAO ⁻ , NZW
A	C	E	G + H	K + L	CD-1
A	D	F	I	M	C3H/He, CBA/J, ddY/DAO ⁺ , NC
B	C	E	J	K	DBA/2

¹ Mouse genomic DNA was digested with the restriction endonucleases, electrophoresed, transferred to the membranes and hybridized with the D-amino-acid oxidase cDNA probe. The sizes of the hybridizing fragments are as follows. *A* 14.8 and 7.4kb; *B* 17.4kb; *C* 10.0, 8.8 and 3.0; *D* 10.0, 5.6, 3.0 and 1.9kb; *E* 10.9, 3.8, 1.2 and 1.0kb; *F* 10.9, 4.2, 1.2 and 1.0kb; *G* 7.6, 7.2, 2.8 and 2.6kb; *H* 9.8, 7.6 and 2.8kb; *I* 10.1, and 9.8kb; *J* 10.1, 7.2 and 2.6kb; *K* 12.0, 9.9 and 6.2kb; *L* 12.0, 8.2, 6.2 and 1.7kb; *M* 18.2, 8.2 and 1.7kb.

BALB/c, CF#1, ddY/DAO⁻ and NZW mice had four bands of about 12.0, 8.2, 6.2 and 1.7kb. The C3H/He, CBA/J, ddY/DAO⁺ and NC strains had three bands of about 18.2, 8.2 and 1.7kb. The CD-1 strain had five bands of about 12.0, 9.9, 8.2, 6.2 and 1.7kb. These results were the same as those observed in our previous study (Konno et al., 1995).

According to these hybridization patterns, the 15 strains were classified into four groups (Table 1); the A/J, AKR, C57BL/6, ICR and NZB strains made the first group, the BALB/c, CF#1, ddY/DAO⁻ and NZW strains made the second group, and the C3H/He, CBA/J, ddY/DAO⁺ and NC strains made the third group. The DBA/2 and CD-1 strains were not classified into any of these groups.

These results indicate that the 15 strains of mice carry a structurally similar D-amino-acid oxidase gene, but there is a variation in its inside sequence among the groups of the strains.

Discussion

The structure of mouse D-amino-acid oxidase cDNA has been already determined (Tada et al., 1990) and is shown in Fig. 9. No cleavage site of *Ava*I, *Bam*HI, *Eco*RI, *Sal*I, *Sma*I or *Xho*I is present in the cDNA. One *Eco*RV cleavage site is present at the 3' end (nucleotide 1,575) of the cDNA. One *Bgl*II cleavage site is present at the 5' end (nucleotide 96) of the coding region. Two *Xba*I cleavage sites are present: one is in the central part (nucleotide 412) and the other is at the 3' end (nucleotide 994) of the coding region. One cleavage site of *Hind*III, *Pst*I and *Pvu*II is present at nucleotide 470, 582 and 724, respectively, in the 3' half of the cDNA. The digestion of genomic DNAs of 15 mouse strains with these restriction endonucleases and subsequent Southern hybridization with a D-amino-acid oxidase cDNA probe containing the entire coding region yielded quite consistent results. The *Eco*RV digestion produced only one hybridizing fragment in the 15 strains (Fig. 1). The *Bam*HI digestion produced three hybridizing fragments but the hybridization patterns were the same in the 15 strains (Fig. 2). The *Eco*RI digestion produced two hybridizing fragments in the 14 strains and only one fragment in the DBA/2 strain (Fig. 3). The *Bgl*II digestion produced four hybridizing fragments but the hybridization patterns were the same in the 15 strains (Fig. 4). In contrast to the almost identical restriction patterns above, both *Pvu*II and *Xba*I digestion produced hybridization patterns in the four strains (C3H/He, CBA/J, ddY/DAO⁺ and NC) different from the other 11 strains (Figs. 5 and 6). In addition, both *Hind*III and *Pst*I digestion produced restriction fragment length polymorphisms among the mouse strains (Figs. 7 and 8). These results indicate that the D-amino-acid oxidase gene is quite similar in the whole structure but there is a variation among the strains in the sequence which encodes the central part of D-amino-acid oxidase.

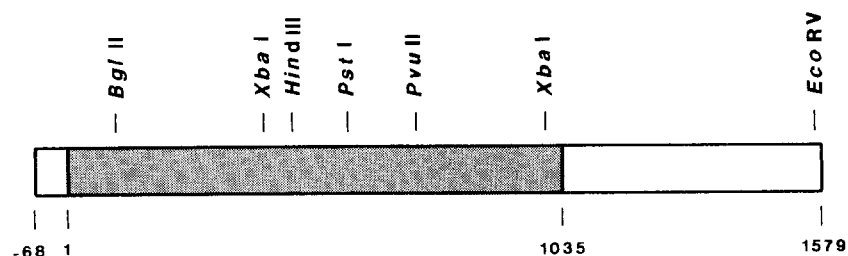


Fig. 9. Structure of mouse D-amino-acid oxidase cDNA. The open boxes show the 5'- and 3'-noncoding regions. The solid box shows the coding region. Base A of the initiation codon is numbered 1 and the bases are numbered upstream and downstream. Cleavage sites of restriction endonucleases are shown above the bar. The structure of the D-amino-acid oxidase cDNA is based on the nucleotide sequence determined in the BALB/c mice by Tada et al. (1990)

Fifteen mouse strains are classified into four groups according to the Southern hybridization patterns with the D-amino-acid oxidase cDNA probe (Table 1). The first group consists of the A/J, AKR, C57BL/6, ICR and NZB strains, the second group consists of the BALB/c, CF#1, ddY/DAO⁻ and NZW strains, and the third group consists of the C3H/He, CBA/J, ddY/DAO⁺ and NC strains. The DBA/2 and CD-1 strains are different from these three groups in the hybridization patterns. At present, no clear relationship is detected between this classification and the genealogy of the 15 mouse strains. The NC strain, derived from Japanese fancy mice, is genealogically different from other mouse strains of European origin but it has similar hybridization patterns with the C3H/He, CBA/J and ddY/DAO⁺ strains. The NZB and NZW strains have been maintained in New Zealand for a long time but they showed different hybridization patterns from one another. The ddY/DAO⁺ and ddY/DAO⁻ strains also showed quite different hybridization patterns from one another (Figs. 5–8), though they were established from the same colony of the ddY strain (Konno and Yasumura, 1983). However, these results agree well with those obtained by Fukui et al. (1991). They have shown that a population of the ddY mice carries two alleles at the D-amino-acid oxidase locus. This is because two types of hybridization patterns are observed among the mice when DNAs of these mice are digested with *Pst*I or *Hind*III and hybridized with a D-amino-acid oxidase cDNA probe. Heterogeneity of the ddY strain is due to the fact that this strain has been maintained as a closed colony.

The hybridization patterns of the *Hind*III- and *Pst*I-digested DNA of the CD-1 strain were quite different from those of other strains. In *Hind*III digestion, the A/J-group strains produced three hybridizing fragments of about 12.0, 9.9 and 6.2kb. The BALB/c-group strains gave four hybridizing fragments of about 12.0, 8.2, 6.2 and 1.7kb. However, the CD-1 strain gave five hybridizing fragments of about 12.0, 9.9, 8.2, 6.2 and 1.7kb (Table 1). In our previous experiment, we observed that the *Hind*III digestion of the CD-1 DNA produced five hybridizing fragments, which was considered to be due to incomplete digestion (Konno et al., 1995). However, further investigation indicated it was not true because the prolonged digestion of the CD-1 DNA or digestion with an excess amount of the enzyme did not change the hybridization patterns. The *Pst*I digestion yielded quite similar results. When the DNAs were digested with *Pst*I, the A/J-group strains produced four hybridizing fragments of about 7.6, 7.2, 2.8 and 2.6kb. The BALB/c-group strains gave three hybridizing fragments of about 9.8, 7.6 and 2.8kb. However, the CD-1 strain produced five hybridizing fragments of about 9.8, 7.6, 7.2, 2.8 and 2.6kb (Table 1). These results are most easily explained if we consider that the CD-1 mouse was a heterozygote carrying both the A/J-type and BALB/c-type allele at the D-amino-acid oxidase locus. This possibility is high because the CD-1 strain is not an inbred strain but is maintained as a closed colony.

The present experiments show that all the 15 strains examined have a D-amino-acid oxidase gene similar in the whole structure though there is a internal variation among the strains. Since polymerase chain reaction using

RNA extracted from the kidney could amplify D-amino-acid oxidase cDNA in all these strains (Konno et al., 1995), this gene is properly transcribed into mRNA in this organ. Conservation and expression of the gene suggest that D-amino-acid oxidase has some important functions, though its precise role is not well established yet.

The structure of the mouse D-amino-acid oxidase gene is not yet known. The present experiments show that a 20.4-kb *EcoRV* fragment covers the entire coding region (Fig. 1). This size of the gene is not different from that of the human D-amino-acid oxidase gene. It is shown to span 20 kb comprising 11 exons (Fukui and Miyake, 1992). Mouse D-amino-acid oxidase locus has already been mapped on chromosome 5 (Hilgers and Arends, 1985; Konno et al., 1989). Restriction fragment length polymorphisms in this gene would be useful for gene mapping, linkage analysis and genome analysis.

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