Loss of \textit{HindIII} cleavage sites in the \textit{d}-amino acid oxidase gene in some inbred strains of mice

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\textbf{Summary.} \textit{d}-Amino acid oxidase cDNA was amplified by a polymerase chain reaction using RNA extracted from the mouse kidney. When digested with \textit{HindIII}, the cDNAs of the BALB/c and ddY/DAO\textsuperscript{-} mice were cleaved into two fragments whereas the cDNA of the ddY/DAO\textsuperscript{+} mice was not. Sequencing revealed that nucleotide-471 of the cDNAs was G in the BALB/c and ddY/DAO\textsuperscript{-} mice whereas it was substituted for C in the ddY/DAO\textsuperscript{+} mice. This base substitution was the cause of the failure of the cleavage of the cDNA of the ddY/DAO\textsuperscript{+} mice.

Examination of other strains of inbred mice showed that \textit{d}-amino-acid oxidase cDNAs of A/J, AKR, C57BL/6, CD-1, CF\#1, ICR, DBA/2, NZB and NZW mice were cleaved with \textit{HindIII} into two fragments whereas those of C3H/He, CBA/J and NC mice were not. Genomic DNAs extracted from the mice of these 15 strains were digested with \textit{HindIII} and hybridized with \textit{d}-amino-acid oxidase cDNA. A 18.2-kb fragment hybridized with the probe in the C3H/He, CBA/J, ddY/DAO\textsuperscript{+} and NC mice whereas two fragments of 12 kb and 6.2 kb hybridized in the other mice. These results are consistent with those of the cDNAs, confirming the loss of the \textit{HindIII} cleavage site in the C3H/He, CBA/J, ddY/DAO\textsuperscript{+} and NC mice. The Southern hybridization revealed a loss of a different \textit{HindIII} cleavage site in the A/J, AKR, C57BL/6, DBA/2, ICR and NZB mice.

The substitution at nucleotide-471 should cause a substitution of an amino acid residue. However, this substitution did not affect catalytic activity of \textit{d}-amino acid oxidase.

\textbf{Keywords:} Amino acids – \textit{d}-Amino acid oxidase – \textit{HindIII} – RFLP – Mouse – PCR
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). It has a wide range of substrate specificity. Many D-amino acids are oxidized but L-amino acids are not oxidized at a measurable rate. Almost all higher animals have this enzyme in their kidneys, livers, and brains. However, the physiological role of this enzyme has been enigmatic because D-amino acids are rare in higher animals (Meiser, 1965). Recent investigations have shown that one of the functions of D-amino acid oxidase is the metabolism of D-amino acids derived from intestinal bacteria (Konno and Yasumura, 1992; Konno et al., 1993). The high enzyme activity and its more than adequate existence to metabolize bacterial D-amino acids suggest that this enzyme has some other functions.

D-Amino acid oxidase has been conserved through evolution: it exists in fungi, invertebrates and vertebrates (Meister, 1965). The amino acid sequences of this enzyme were determined in the pig by Ronchi et al. (1982). Since then, the nucleotide sequences of D-amino acid oxidase cDNAs have been determined in several species. This enzyme consists of 347 amino acid residues in the human (Momoi et al., 1990), pig (Fukui et al., 1987) and rabbit (Momoi et al., 1988). Strangely, the mouse enzyme consists of 345 amino acid residues. The 25th and 173rd amino acid residues existing in the other three mammals are missing in the mouse (Tada et al., 1990). However, the homology of the amino acid sequences is high and about 80% of the amino acid residues are the same among these mammals.

The nucleotide sequences of cDNA encoding D-amino acid oxidase were determined in BALB/c mice. The cDNA has a coding region of 1,035 nucleotides between a 5'-untranslated region of 68 nucleotides and a 3'-untranslated region of 544 nucleotides. A cleavage site of the restriction endonuclease HindIII is present in the middle of the coding region. The D-amino acid oxidase gene is expressed in the kidney and brain but not in the heart, liver, lung, pancreas, spleen, submandibular gland, testis or thymus of the mouse (Tada et al., 1990).

Mutant ddY/DAO- mice lacking D-amino acid oxidase activity had D-amino-acid oxidase mRNA carrying a single-base substitution at nucleotide-451. This substitution leads to a change of an amino acid residue glycine-181 to arginine. The substitution was demonstrated to be the cause of the loss of enzyme activity (Sasaki et al., 1992).

While cloning D-amino acid oxidase cDNA of normal ddY/DAO+ mice, we noticed that their cDNA was not cleaved with HindIII in contrast to the cDNAs of the BALB/c and ddY/DAO- mice. Therefore, in this study, we examined the validity of this observation and whether the loss of the HindIII cleavage site was unique to the ddY/DAO- mice.

Materials and methods

Mice

A/J Slc, AKR/N SLC and Slc:ICR mice were obtained from Japan SLC (Hamamatsu). C3H/HeN Jcl, C57BL/6N Jcl, CBA/J Jcl, DBA/2J Jcl and NC/Jic mice were purchased...