Catalase (H₂O₂:H₂O₂ oxidoreductase, E.C. 1.11.1.6) is a ubiquitous antioxidant metalloenzyme located primarily within the peroxisomes of most mammalian cells. In C57BL/6 mice, liver catalase activity levels are approximately 60% of the levels found in most other mouse strains, including C3H/HeJ (1). Ganschow and Schimke (2) demonstrated using immunological techniques that equal numbers of catalase molecules were present in liver extracts derived from C57BL/6 and wild type (control) mouse strains. In contrast, these investigators demonstrated that catalase purified from C57BL/6 livers possessed approximately 60% of the catalytic activity found in catalase purified from high activity wild type mouse strains. These data thus led to the conclusion that the C57BL/6 low activity catalase variant is due to an alteration within the catalase amino acid sequence.

In order to pinpoint the molecular basis of the C57BL/6 low activity catalase, we have employed the polymerase chain reaction (PCR) method (3) to synthesize catalase cDNAs derived from C3H/HeJ- and C57BL/6-derived mRNA samples. Oligonucleotide primer sequences for PCR syntheses of catalase were based on a partial cDNA sequence of the 3'-noncoding region of mouse catalase mRNA (4) and on the 5'-noncoding sequence of rat catalase mRNA (5).

Comparative sequence analyses of PCR generated catalase cDNA clones have revealed four nucleotide differences within the coding regions of C3H/HeJ and C57BL/6 catalase mRNA. (These nucleotide differences are (HeJ—BL/6): #72 A—T, #349 G—A, #1356 T—C and #1392 T—C.) Analyses of the deduced amino acid sequences of catalase in these two strains of mice have revealed a single deduced amino acid difference between wild type C3H/HeJ and low activity C57BL/6 catalase. This difference is located at amino acid position 117, where an alanine (GCT) is replaced by a threonine (ACT) in C57BL/6 catalase (Figure 1). All other mammalian catalases that have thus far been reported (5—7) contain an alanine at this amino acid position, similar to wild type C3H/HeJ catalase. Alanine #117 (referred to as #116 in Murthy et al. (8)) is located within the heme binding site of catalase in a channel predominantly composed of hydrophobic residues (8). Substitution of alanine with the hydrophilic residue threonine at position 117 likely disrupts this hydrophobic channel and may decrease the availability of the catalytic site to substrates, thereby diminishing the catalytic efficiency of C57BL/6-derived catalase.

Figure 1. Nucleotide and deduced amino acid sequences of C3H/HeJ (wild type) mouse catalase. The alanine at amino acid position #117 (underlined) is substituted by threonine in C57BL/6 catalase.

ACKNOWLEDGEMENT

Supported by National Institutes of Health Grant HL 39631.

REFERENCES


* To whom correspondence should be addressed