

Plant Gene Register

cDNA for Catalase from Etiolated Mung Bean (*Vigna radiata*) Hypocotyls¹

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Catalase (H₂O₂:H₂O₂ oxidoreductase, EC 1.11.1.6) is present in a variety of organisms and has been thought to play a role in scavenging toxic H₂O₂ that is produced in many oxidative reactions and environmental stresses (Scandalios, 1990). The enzyme localizes in microbodies. In plants, microbodies are categorized in three classes according to their apparent function: glyoxysome, leaf peroxisome, and non-specialized microbodies (Beever, 1979). Glyoxysomal and leaf peroxisomal catalases have been shown to degrade H₂O₂ generated by flavin-containing oxidases. Nonspecialized microbodies are found in etiolated or nongreen tissues, but the role of catalase present in this class of microbodies is not well understood.

cDNAs for catalase have been cloned from many plants. Although etiolated tissues generally contain abundant amounts of catalase, catalases in such tissues have not been studied in detail. During the course of our study of the auxin action on ethylene production, we noticed that hypocotyls of etiolated mung bean (*Vigna radiata*) seedlings contain a large amount of catalase that is believed to be present in nonspecialized microbodies. We purified the enzyme, raised antibodies, and isolated a cDNA clone (VRCAT1) by immunoscreening.

The cDNA of 1824 bp contained an open reading frame that encodes a protein of 492 amino acid residues. Sequence similarities to other plant catalases were higher than animal and yeast catalase: e.g. to cotton, 91% (Ni et al., 1990); to maize 1, 82% (Redinbaugh et al., 1988); to rat, 41% (Furuta et al., 1986); and to *Candida tropicalis*, 35% (Okada et al., 1987). His⁶⁵ and Asn¹³⁸, which are assigned as the catalytic center amino acids in other catalases, and Tyr³⁴⁸, which is involved in binding to heme, are present at almost identical relative positions to other catalases (Murthy et al., 1981). Like all other catalases characterized so far, mung bean catalase contains two internal potential microbody-targeting signals; Ser⁸³-His-Leu and Ser⁴⁸⁴-His-Leu. The mRNA for catalase was 2.1 kb in length and is abundantly expressed in growing hypocotyls.

Table 1. Characteristics of a cDNA for the catalase from mung bean

Organism:	<i>Vigna radiata</i> Wilczek.
Location on Chromosome:	Unknown.
Function:	Catalase; catalyzing the dismutation of H ₂ O ₂ into oxygen and water.
Clone Type; Designation:	Full-length cDNA, VRCAT1.
Sources:	cDNA library in pTTQ18 (Amersham) for polyadenylated RNA from etiolated hypocotyls of 3-d-old mung bean (Mori et al., 1991).
Method of Isolation:	Library was screened by the antibodies raised in rabbit against mung bean catalase.
Identification:	Three internal amino acid sequences are present in the deduced amino acid sequence.
Sequencing Strategy:	Unidirectional deletion subcloning and complete dideoxy sequencing of both strands.
Feature of cDNA Structure:	An open reading frame, 1476 bp; 5' untranslated region, 53 bp; 3' untranslated region, 294 bp.
Expression:	The mRNA of 2.1 kb was abundantly present in total RNA isolated from etiolated hypocotyl.
Codon Usage:	TCC(S) and CGC(R) not used. Arg with a high bias to AG(A/G).
(G+C) content:	43.5% overall, 45.3% in the coding region.
Structural Features of Protein:	Open reading frame, 492 amino acids; calculated M _r , 56,841; isoelectric point, 6.84. The catalytic active site, His ⁶⁵ and Asn ¹³⁸ . The proximal heme binding residue, Tyr ⁴⁸⁴ . Two internal potential microbody-targeting signals, Ser ⁸³ -His-Leu and Ser ⁴⁸⁴ -His-Leu.
Antibodies:	Not available.
Subcellular Location of Protein Product:	Microbody.

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