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Plant Gene Register

The Primary Leaf Catalase Gene from *Nicotiana tabacum* and *Nicotiana sylvestris*[1]

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Large amounts of hydrogen peroxide are generated in C_3 plants by the glycolate oxidase reaction during the photorespiratory pathway of photosynthesis (Zelitch, 1992), and catalase (EC 1.11.1.6) in peroxisomes of leaves functions in the decomposition of hydrogen peroxide. The major tobacco leaf catalase, CAT-1, reaches a maximum level about 15 d postgermination, and a minor isozyme, CAT-3, with enhanced peroxidatic activity is also present (Havir and McHale, 1987). A tobacco mutant has been identified with more catalase protein than wild type and an increased catalase activity of about 40% (Zelitch et al., 1991). The mutant has increased net photosynthesis when photorespiration is rapid (Zelitch, 1992). Additional interest in the role of catalase arises from recent reports of its function in a plant defense mechanism against a broad range of pathogens (Chen et al., 1993) and in resistance to chilling-induced stress in maize seedlings (Prassad et al., 1994).

To enable us to produce transgenic plants with elevated and depressed levels of leaf catalase, we have isolated and characterized a full-length cDNA clone (1.9 kb) from *Nicotiana tabacum* ($2n = 48$) and a partial cDNA (1.4 kb) from *Nicotiana sylvestris* ($2n = 24$), one of the ancestral diploids of *N. tabacum* (Table I). The *N. sylvestris* clone (383 residues) lacks about 0.4 kb from the 5′ coding end (Zelitch et al., 1991). The full-length leaf catalase cDNA from *N. tabacum* (492 residues) has 28 untranslated bases at the 5′ end followed by 1476 bases in the open reading frame.

The predicted amino acid sequence of this full-length *N. tabacum* catalase was compared to the predicted sequences of catalases from *N. tabacum* (489 residues) (Chen et al., 1993), *N. sylvestris* (partial cDNA) (this report), *Arabidopsis* leaf (492 residues) (Chevalier et al., 1992), cottonseed (492 residues) (Ni et al., 1990), and maize seedling CAT-2 (529 residues) (Redinbaugh et al., 1988). All plant catalases have a high degree of homology and near-perfect homology in the region between 53 and 150 bp. Notably the His residues at positions 65 and 79 are conserved. This is the region of the heme-binding site, analogous to His^{74} in beef liver catalase (Reid et al., 1981). Our *N. tabacum* catalase clone differs from the *N. tabacum* sequence (Chen et al., 1993) and our *N. sylvestris* clone at position 540 bp, where an A occurs rather than a G. This base change generates a conservative Arg-to-Lys alteration in the predicted proteins. There are three base pair mismatches at the end of the open reading frame between our full-length *N. tabacum* clone and the partial *N. tabacum* clone (Chen et al., 1993), at positions 1434, 1435, and 1437. The corresponding amino acid changes are from Ser and Tyr in our clones to Cys and Ser. All of the above plant catalases encode the proposed Ser-Arg-Leu consensus

Table I. *Characteristics of partial N. sylvestris leaf catalase cDNA and full-length N. tabacum leaf catalase cDNA*

Organism:
Nicotiana sylvestris light-grown mature leaf; 3-week old tobacco leaves (*Nicotiana tabacum*, cv Petit Havana SR1) light grown in tissue culture.

Gene Function:
Breakdown of hydrogen peroxide produced in leaf peroxisomes by oxidation of glycolate during photorespiration.

Source:
Partial cDNA (1.4 kb, 383 residues) from a *N. sylvestris* leaf-specific λgt11 library (Zelitch et al., 1991) by screening with cottonseed catalase cDNA subunit 1 as a probe (Ni et al., 1990). Full-length cDNA from *N. tabacum* (1.9 kb, 492 residues) from leaf-specific library in λZAP II (Stratagene). To enable us to obtain a full-length clone, the library was first probed with the *N. sylvestris* cDNA and then a second time with a mixture of fragments from the 5′ ends of cottonseed cDNA (−48 to +150 bp) (Ni et al., 1990) and *Arabidopsis* leaf catalase cDNA (−54 to +275 bp) (Chevalier et al., 1992).

Sequencing:
cDNA fragments sequenced by dideoxy chain termination on both strands using Sequenase (United States Biochemical) by overlapping subclones and specific primer initiation.

Features of cDNAs:
The *N. sylvestris* cDNA has 1149 bp in the open reading frame and a 254-bp 3′ untranslated region. The *N. tabacum* cDNA has 28 untranslated bases at the 5′ end preceding the open reading frame, 1476 bp, and then a 387-bp 3′ untranslated region.

Antibodies:
Not available.

Subcellular Location of Protein Product:
Peroxisomes.

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sequence six amino acids from the carboxyl end of the predicted proteins that is believed to be a peroxisomal targeting sequence (Gonzalez, 1991).

The full-length *N. tabacum* catalase cDNA has a calculated protein mol wt of 56,820. This predicted mol wt is closer to the 55,300 ± 750, the subunit M_r found for CAT-1, than the 53,300 ± 850 found for CAT-3 by SDS-PAGE (Havir and McHale, 1990). The calculated isoelectric point from our clone is 6.64, whereas on chromatofocusing the CAT-1 isozymes show five peaks with isoelectric points ranging from 7.8 to 6.8, and CAT-3 has a value of 6.0 (E. Havir, personal communication). Thus, the protein predicted by the full-length *N. tabacum* clone closely resembles CAT-1, the primary leaf catalase isozyme.

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The EMBL/GenBank/DDBJ accession numbers for the sequences reported in this article are U07626 for the *N. sylvestris* clone and U07627 for the *N. tabacum* clone.

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