Nucleotide Sequence of a cDNA Clone Encoding an Acidic Laccase from Sycamore Maple (Acer pseudoplatanus L.)

Peter L. LaFayette, Karl-Erik L. Eriksson, and Jeffrey F. D. Dean*

Department of Biochemistry and Molecular Biology, Center for Biological Resource Recovery, University of Georgia, Athens, Georgia 30602-7229

Laccases (p-diphenol:O2 oxidoreductase, EC 1.10.3.2) are members of a highly conserved class of metalloenzymes, the “blue” copper oxidases, which includes ascorbate oxidase and ceruloplasmin (Rydén and Hunt, 1993). First identified more than 100 years ago in extracts of sap from the Japanese lacquer tree (Rhus vernicifera), laccases have since been identified in fungi, insects, higher plants, and bacteria (Dean and Eriksson, 1994). Although the physiological roles played by laccases in these various organisms are for the most part poorly understood, the enzymes generally oxidize diphenol or dinaphthol metabolites with subsequent reduction of O2 to H2O. The oxidized aromatic products of these reactions often polymerize with each other or with molecules in the surrounding extracellular matrix to form chemically resilient structures that serve to protect the organism from various environmental stresses.

Plant laccases were first proposed to play a role in lignin biosynthesis after Freudenberg et al. (1958) demonstrated that a fungal laccase could oxidize coniferyl alcohol in vitro with subsequent formation of a lignin-like dehydrogenation polymer. However, studies have shown that laccases purified from R. vernicifera could not oxidize coniferyl alcohol (Nakamura, 1967) and that laccase activity could not be detected histochemically in tissue sections taken from lignifying tree stems (Harkin and Obst, 1973) led to the conclusion that peroxidases, not laccases, catalyzed the final step in lignin deposition. More recent studies have suggested that such a conclusion was likely premature (Dean and Eriksson, 1994).

Suspension-cultured cells of Acer pseudoplatanus secrete large quantities of a laccase capable of polymerizing lignin precursors (Sterjiades et al., 1992), and immunolocalization studies have shown that this enzyme is localized to the cell wall of lignifying vascular tissues in Acer stems (Driouich et al., 1992). A cDNA clone encoding this laccase was isolated through a PCR-based library screening protocol using degenerate oligonucleotide primers derived from the published N-terminal protein sequence and copper-binding domains conserved in plant ascorbic acid oxidases and fungal laccases. Clones were excised into pBluescript SK− in vivo, and cycle sequenced on both strands using a kit (TN-1000, Gold Biotechnology, St. Louis, MO) based on random insertion of a transposon harboring sequencing primer sites (Strathman et al., 1991).

Method of Identification:
- Sequence identity of the deduced amino acid sequence with that determined for the N-terminal protein sequence of purified A. pseudoplatanus laccase.
- Features of cDNA:
  - The clone was 2030 nucleotides in length and consisted of a 70-nucleotide 5′ untranslated region, a 1695-nucleotide open reading frame, and a 265-nucleotide 3′ untranslated region. The 5′ untranslated region contained a stretch of 17 A's 11 bases upstream of the translation start codon, and a polyadenylated tail was present at the 3′ end of the sequence.
- Features of Protein:
  - A single open reading frame encoded a polypeptide of 565 amino acid residues having M, 62,600. Comparison with the N-terminal sequence of the purified laccase showed that a leader peptide of 23 amino acid residues having M, 2,700 preceded the mature protein of 542 amino acid residues having M, 59,900. The sequence contained 4 copper-binding domains and 16 potential N-linked glycosylation sites.

Subcellular Location:
- Cell wall of lignifying tissues (Driouich et al., 1992).

Table 1. Characteristics of a cDNA clone encoding laccase from sycamore maple

| Organism: | Sycamore maple (Acer pseudoplatanus L.). |
| Function: | Encodes laccase (p-diphenol:O2 oxidoreductase, EC 1.10.3.2), an extracellular enzyme postulated to oxidize monolignols in the final step of lignin biosynthesis. |
| Source: | cDNA library in λUniZap XR vector constructed using poly(A) mRNA isolated from suspension-cultured A. pseudoplatanus cells. |
| Techniques: | Clones were identified through a PCR-based library screening protocol using degenerate oligonucleotide primers derived from the published N-terminal protein sequence and copper-binding domains conserved in plant ascorbic acid oxidases and fungal laccases. Clones were excised into pBluescript SK− in vivo, and cycle sequenced on both strands using a kit (TN-1000, Gold Biotechnology, St. Louis, MO) based on random insertion of a transposon harboring sequencing primer sites (Strathman et al., 1991). |

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* Corresponding author; e-mail jdeanx1@uga.cc.uga.edu; fax 1-706-542-2222.
istics of which are summarized in Table I. In a comparison with other blue copper oxidases found in GenBank (release 83.0) using BESTFIT (Genetic Computer Group Sequence Analysis software package, version 7.0), the complete *A. psuedoplatanus* laccase sequence was found to have 29% identity at the deduced amino acid level with ascorbate oxidase from cucumber (*Cucumis sativus*) and 28% identity with the laccases cloned from *Neurospora crassa* and *Agaricus bisporous*. In each case, most of the conserved amino acid residues were contained within the copper-binding domains. The cDNA was found to contain a stretch of 17 A's upstream of the predicted translation start site. The function of this sequence, if any, is unknown but could potentially be involved in poly(A)-binding protein interactions.

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