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Original article

Molecular characterization of a cDNA encoding copper/zinc superoxide dismutase from cultured cells of *Manihot esculenta*

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Abstract

Superoxide dismutase (SOD) cDNA, *mSOD2*, encoding cytosolic copper/zinc SOD (CuZnSOD) cDNA was isolated from suspension-cultured cells of cassava (*Manihot esculenta* Crantz) by cDNA library screening, and its expression was investigated in relation to environmental stress. *mSOD2* is 774 bp in length with an open reading frame (ORF) of 152 amino acids, corresponding to a protein of predicted molecular mass 15 kDa and a p*I* of 5.22. One copy of the *mSOD2* gene was found to be present in the cassava genome by Southern analysis using an *mSOD2* cDNA-specific probe. Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis revealed diverse expression patterns for the mSOD2 gene in various tissues of intact cassava plants, at various stages of the growth in suspension cultures, and in the leaf tissues exposed to different stresses. The *mSOD2* gene was highly expressed in suspension-cultured cells and in the stems of intact plants. However, it was expressed at low levels in leaves and roots. During suspension cell growth, the *mSOD2* transcript progressively increased during culture. Moreover, the *mSOD2* gene in excised cassava leaves responded to various stresses in different ways. In particular, it was highly induced in leaf tissue by several abiotic stresses, including high temperature (37 °C), chilling (4 °C), methyl viologen (MV) exposure, and wounding treatment. These results indicate that the *mSOD2* gene is involved in the antioxidative process triggered by oxidative stress induced by environmental change.
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Keywords: Cultured cells; Environmental stress; *Manihot esculenta*; Oxidative stress; Superoxide dismutase

1. Introduction

Superoxide dismutase (SOD; EC 1.15.1.1) is a ubiquitous enzyme, which catalyzes the dismutation of the superoxide radical into hydrogen peroxide and molecular oxygen ($2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$), and plays a central role in protecting against oxidative stress. Three types of SOD have been characterized based on the nature of the metal co-factor present at the catalytic site, i.e. copper/zinc (CuZnSOD), iron (FeSOD), or manganese (MnSOD) SODs. CuZnSOD is generally found in the cytosol and chloroplasts, MnSOD in mitochondria, whereas FeSOD is present within the chloroplasts of some plants [3].

Unlike most other organisms, plants contain multiple SOD isozymes. The first plant SOD gene was cloned from maize [7]. However, the molecular cloning of SODs in cultured plant cells has been reported only in the liverwort [23] and cassava [16], whereas the sequences of many SODs have been cloned in intact plants, e.g. tobacco [5], tomato [20], maize [6], rice [11,21], and aspen [1]. Moreover, nine SOD isoenzymes have been described in maize [4], and seven in *Arabidopsis* [14].

Plant cells growing in culture are subjected to high oxidative stress, relative to whole plants, which suggests that they

Abbreviations: CuZnSOD, copper/zinc superoxide dismutase; DAS, d after subculture; HAT, h after treatment; MV, methyl viologen; ORF, open reading frame; POD, peroxidase.

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offer good models for the study of antioxidation mechanisms and the production of antioxidants. In previous studies, we established an efficient production system for SOD and peroxidase (POD) using cell cultures of cassava (*Manihot esculenta*) and sweetpotato (*Ipomoea batatas*) [15,24], respectively, and isolated and characterized the POD genes from cultured cells of sweetpotato [10,12,13,19]. One SOD cDNA, *mSOD1* was isolated and characterized from cell cultures of cassava [16]. Moreover, *mSOD1* was found to be strongly expressed in cultured cells as compared with the intact tissues of cassava, and notably was found to be strongly induced by stresses such as high temperature and stress-related compounds [16]. Analyses of cassava SOD isozymes have revealed seven SOD genes in cassava. Moreover, several recent reports have suggested that multiple genes encoding isoforms of SOD exist in most plants [22]. Native gel analysis suggests that there are at least seven SOD isozymes in the cultured cells of cassava [24], thus the physiological functions of several SOD genes remain to be clarified. Until quite recently, little was known about the expressions or responses of individual SOD genes to different stressors. In this paper, we report upon the molecular characterization of a new SOD cDNA (*mSOD2*), which was isolated from a cell suspension culture of cassava and upon its expression due to various stresses.

2. Results and discussion

2.1. Cloning and analysis of a cytosolic CuZnSOD cDNA

To isolate the cDNA clone encoding CuZnSOD, a cDNA library was constructed from suspension-cultured cells of cassava and screened with a probe produced by PCR using degenerate oligonucleotides [16]. A positive clone containing the largest insert was designated *mSOD2*, and selected for further analysis.

The *mSOD2* cDNA was 774 bp in length with an open reading frame (ORF) of 456 bp, corresponding to 152 amino acids encoding a protein with a calculated molecular mass of approximately 15.1 kDa and a calculated p*I* of 5.22 (GenBank Accession number AY642137). *mSOD2* cDNA contained the putative polyadenylation signal AATAAT, 41 bp downstream from the stop codon (Fig. 1). No clear signal peptide sequences were detected, indicating that mSOD2 cDNA probably is located in the cytosol.

The deduced amino acid sequence showed considerable homology to the CuZnSODs of other plants, as shown in Table 1. Its deduced amino acid sequence showed higher identities (81–90%) with the sequences of cytosolic CuZnSODs of other plant species rather than chloroplast (63–70%) CuZnSODs. The residues required for coordinating copper (His-45, -47, -62, and -119) and zinc (His-62, -70, and -79 and Asp-82), as well as the two cysteines (C-56 and C-145) that form a single disulfide bond, were conserved as they are in all reported CuZnSOD sequences.

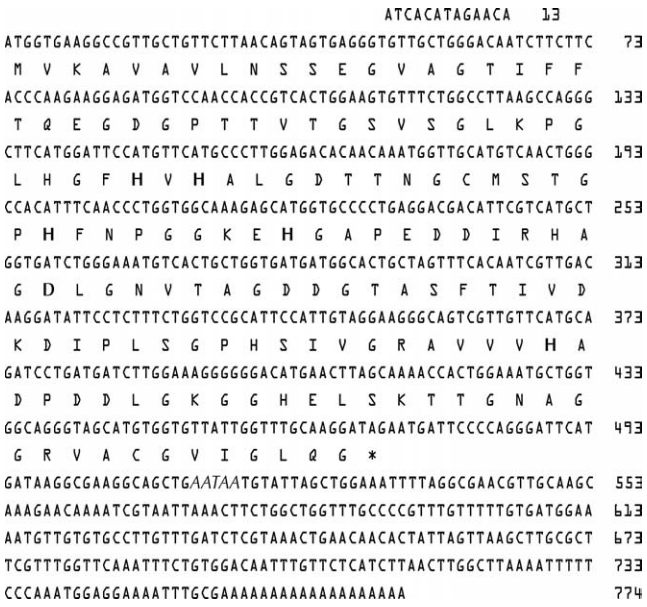

Fig. 1. Nucleotide and deduced amino acid sequence of a CuZnSOD cDNA, *mSOD2*, isolated from suspension-cultured cells of cassava (*M. esculenta*). The deduced amino acid sequence is shown in single-letter code below the nucleotide sequence. Numbers to the right refer to nucleotides. Bold letters represent putative copper/zinc binding sites. The asterisk denotes the translation stop signal. Possible polyadenylation signals are italics.

Table 1
Comparisons of amino acid sequence homology among plant CuZnSODs

	mSOD2	mSOD1	cyt-SOD1	cyt-SOD2	cp-SOD	CSD3	CSD2
CSD1	84	82	86	87	60	61	66
CSD2	63	65	66	65	45	61	
CSD3	62	65	62	62	56		
Cp-SOD	85	84	42	58			
Cyt-OD2	88	89	91				
Cyt-OD1	85	84					
mSOD1	87						

Amino acid sequences of the cytosolic CuZnSODs, mSOD1 (Acc. No. AF170297), and mSOD2 (Acc. No. AY642137) from cassava [16], CSD1 (Acc. No. X60935), CSD2 (Acc. No. AF061519), and CSD3 (Acc. No. AF061520) from *Arabidopsis* [14], and cyt-SOD1 (Acc. No. AJ278669), cyt-SOD2 (Acc. No. AF016892), and cp-SOD (Acc. No. AJ278668) from *Poplus* [1] were deduced from cDNA sequences.

2.2. Genomic organization of mSOD2

In order to determine the copy number of the *mSOD2* gene, the genomic DNA (15 μg) of cassava leaves was digested with *Eco*RI, *Hin*dII, or *Hin*dIII and hybridized under stringent conditions using the ^{32}P-labeled 3′-untranslated region of *mSOD2* as a probe. Digestion with *Eco*RI, *Hin*dII, or *Hin*dIII generated single bands of 2.2, 2.8, and 1.6 kb, respectively (Fig. 2), which strongly suggests that cassava contains a single *mSOD2* gene. A similar result was also reported for the *mSOD1* gene in cassava [16]. Cytosolic CuZnSOD genes also exist as single copies in the maize genome [6], *Nicotiana plumbaginifolia* SOD is also encoded by a single gene [9], whereas most plants contain multiple genes encoding SOD isoforms.

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