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SHORT COMMUNICATION

Cloning and DNA Sequencing of Cytosolic Cu/Zn Superoxide Dismutase Gene from Chinese Cabbage

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A cDNA clone for the cytosolic Cu/Zn superoxide dismutase (Cu/Zn SOD) from Chinese cabbage (*Brassica campestris* ssp. *pekinensis*) was isolated and its DNA sequence was determined. The cDNA clone contains a complete coding sequence which encodes a protein of 152 amino acids and a 3'-untranslated region including a poly A signal. The deduced amino acid sequence shows that it is highly homologous to the Cu/Zn SODs from other plants (60~90%). The lack of a putative chloroplast targeting transit peptide indicates that the clone represents a cytosolic form of Cu/Zn SOD. Genomic Southern hybridization suggests that cytosolic Cu/Zn SOD genes are present in 1 or 2 copies per genome.

Keywords: cytosolic Cu/Zn SOD sequence, chinese cabbage

Superoxide dismutase (SOD: EC 1.15.1.1) plays an important role in removing superoxide which is generated during normal aerobic metabolic processes as well as stress conditions (Scandalios, 1993: Bowler *et al.*, 1992). Superoxide itself and superoxide-derived hydroxyl radicals (OH·) are known to initiate various cellular damages, such as DNA breakage or membrane degradation (Bowler *et al.*, 1992). Particularly, hydroxyl radicals are considered to be very potent and are mainly responsible for cellular damages. It is well established that SOD is a major defensive mean to protect cells from many environmental stresses. SOD catalyses the dismutation of superoxide ($O^{2-}\cdot$), producing hydrogen peroxide and dioxygen. The reaction requires metal cofactors, and SODs are classified by the metal cofactor requirements: Mn SOD, Fe SOD and Cu/Zn SOD. In plants, Mn SOD is primarily located in mitochondria and Cu/Zn SOD in cytosol and chloroplast. Fe SOD is also present in certain plants, unlike Mn SOD and Cu/Zn SOD which are present in all plants (Duke *et al.*, 1985). In general, the chloroplastic Cu/Zn SOD protects photosynthesis-related damages, and Mn SOD is mainly involved in removing superoxides produced during the aerobic respiration. Cytosolic Cu/Zn SOD is suggested to play a role in many cytosolic processes which have not been clearly characterized (Mittler *et al.*, 1994).

Recently several experiments have been directed to improve stress-resistance via the transgenic plant system overexpressing SODs in chloroplasts (Bowler *et al.*, 1991: Sen Gupta *et al.*, 1993: Van Camp *et al.*, 1994). Transgenic tobacco carrying overexpressed Mn SOD in chloroplasts became more resistant to photoinhibition and paraquat, a herbicide which artificially generates superoxide through the photosystem I (Bowler *et al.*, 1991). These results suggest that SOD can be effectively used to scarvenge superoxides and protect plants from the various environmental adversities. However, there have been no attempts to overexpress SODs in cytosol where many non-photosynthetic reactions occur. It is reasonable to expect that transgenic plants with overexpressed SODs in cytosol would provide them better resistance during the non-photosynthetic environmental stresses, such as prolonged darkness or infection. Toward this objective, we isolated a cDNA clone which encodes a cytosolic Cu/Zn SOD in Chinese cabbage and the entire 0.8 kb cDNA was sequenced. The coding sequence will be used tobacco plants.

MATERIALS AND METHODS

DNA Sequencing

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A positive clone from the Chinese Cabbage cDNA library (λZAP, Strategene) containing the suspected cytosolic Cu/Zn SOD sequence (outcome of the random cDNA sequencing programme of Chinese cabbage, the Korean Agricultural Science-Technology Institute, Suwon, Korea) was used as a starting material. Partial DNA sequencing of the cDNA clone and its homology search indicated that this clone was very homologous to the *Arabidopsis* Cu/Zn SOD (Hindges *et al.*, 1992). To confirm the clone, the 0.8 kb cDNA insert (*Eco*RI/*Xho*I, Fig. 1) was subcloned into pGem-5zf(+) (Strategene) for DNA sequencing. A properly located *Nco*I site within the insert was used to generate 0.24 kb *Eco*RI/*Nco*I and 0.56 kb *Nco*I/*Xho*I fragments, fragments were used for DNA sequencing (Fig. 1). DNA sequencing was done by the dideoxy-chain termination method using the SequenaseII kit (USB).

Plant DNA Isolation and Southern Hybridization

Fresh leaves (2g) were ground to powder using a prechilled mortar and pestle. The extraction buffer contained 1% cetyltrimethylammonium bromide, 0.1 M Tris-HCl (pH 7.5), 10 mM EDTA, 0.7 M NaCl and 1% β-mercaptoethanol. Total plant DNA extraction was essentially done according to Naga, *et al.* (Nagao *et al.*, 1981). Two μg of the extracted DNA were digested with 10 units of *Bam*HI, *Hind*III and *Pst*I. Digested DNA was subjected to agarose gel electrophoresis (0.8%). The separated DNA fragments were transferred into a nylon membrane (BM), and hybridized with the DNA probe. The 0.8 kb *Eco*RI/*Xho*I fragment was labelled with the DIG-labelling system (BM) and the hybridization process were carried out in a stringent condition (60°C).

RESULTS AND DISCUSSION

The DNA sequence of the cytosolic Cu/Zn SOD clone from Chinese cabbage and derived amino acid sequence were determined (Fig. 2). It contains an open reading frame beginning with the ATG initiation codon and ending with the TAA termination codon. It encodes a protein of 152 amino acids, and its calculated molecular mass is approximately 15 kDa. There is the consensus sequence of the translation initiation site (AACTATGG) (Heidecker *et al.*, 1986), well conserved in most dicot plants, and the polyA tail with a preceding AATAA polyadenylation signal. The amino acid sequence was compared to both cytosolic and chloroplastic Cu/Zn SOD from Arabidopsis thaliana (Ahindges *et al.*, 1992). It also showed significant homologies to those from maize (78%) (Cannon *et al.*, 1987), cabbage (86%) (Steffens *et al.*, 1986), tobacco (80%) (Tsang *et al.*, 1991). It is known that cytosolic Cu/Zn SOD also has high homology to chloroplastic Cu/Zn SOD. Our cytosolic Cu/Zn SOD showed 55% homology to the chloroplastic Cu/Zn SOD from tomato in which only the sequence from mature Cu/Zn SOD was used for the comparison (Kardish *et al.*, 1994). This Chinese cabbage clone apparently does not have a transit peptide sequence which is essential for targeting to chloroplast. The transit peptide usually contains many basic, hydroxylated, and hydrophobic amino acids. Taken together, the clone we sequenced is apparently a cytosolic Cu/Zn SOD.

Boxed regions in Fig. 3 represent the conserved sequences throughout the Cu/Zn SODs from different plants, most of them are suggested to be crit-

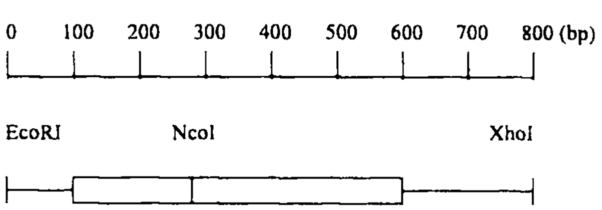

Fig. 1. Restriction map of the cDNA encoding the cytosolic Cu/Zn SOD from *B. campestris* ssp. *Pekinensis* and DNA sequencing strategy. Open box represents the coding sequence of the clone.

Fig. 2. The nucleotide and deduced amino acid sequences of the cDNA encoding the cytosolic Cu/Zn SOD from *B. cmpestris* ssp. *Pekinensis.*

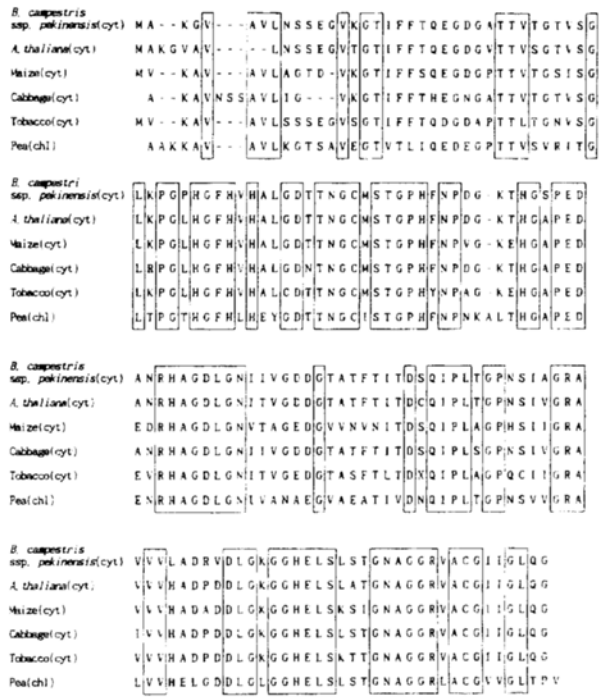

Fig. 3. Comparisons of the deduced amino acid sequences of the *B. cmpestris* ssp. *Pekinensis* Cu/Zn SOD with the sequences from other species (References in the text).

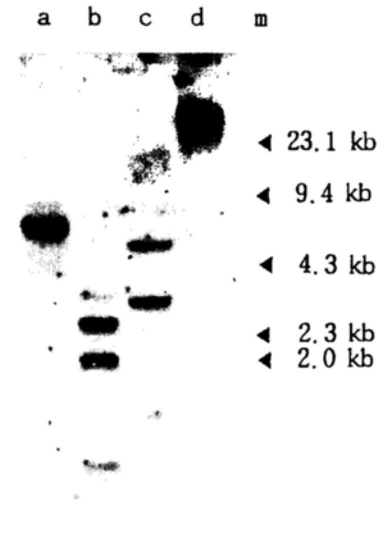

Fig. 4. Southern hybridization of total leaf DNA probed with the 0.8 kb Cu/Zn SOD cDNA. Lanes a: digested with *Bam*HI, b: *Hind*III, c : *Pst*I, d: undigested, m: size markers.

ical for the catalysis. The histidines (positions 45, 47, 62, 70, 79) and aspartic acid (position 82) residues in these conserved regions are suggested to be particularly important for the interactions with Cu and Zn atoms, and they are well conserved in this clone (Scioli *et al.*, 1988). The two cysteine residues (positions 56 and 145) are suggested to form the only disulfide bond which is important for the overall Cu/Zn SOD polypeptide structure, since other Cu/Zn SODs also contain these cysteine residues at the equivalent locations. Chloroplastic Cu/Zn SOD gene from *Arabidopsis* was suggested to present in one or two copies in the nuclear genome. In order to determine the copy number of the Chinese cabbage clone, a genomic Southern hybridization was carried out in high stringency condition (Fig. 4). *Bam*HI digestion of the total leaf DNA produced a single strong band, whereas *Eco*RI and *Pst*I digestions produced two major bands with one or two minor bands. Since these restriction enzyme sites were not present in the coding sequences, it would be expected that there is only one single major band if there is only one copy per genome. The discrepancies may explain that there are *Eco*RI and *Pst*I sites in the putative intron regions, generation the two major bands. With the uncertainty described, it appears that there is likely one or two cytosolic Cu/Zn SOD copies per genome of Chinese cabbage.

Superoxide generated in cytosol would react with hydrogen peroxide to produce hydroxyl radicals which could damage many cytosolic enzymes or organelle membranes. Therefore presence of SOD in cytosol must be important for various cytosolic functions. Recently drought stress induced the cytosolic SOD activities as well as other reactive scarvenge enzymes, such as ascorbate peroxidase and catalase (Mittler *et al.*, 1994). Currently, the Cu/Zn SOD gene from Chinese cabbage is being transformed into tobacco to observe enhanced stress-resistance.

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