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***Zea mays* L. mitochondrial Mn-superoxide dismutase: evidence in favour of potential DNA protective function**

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It is well known that a number of environmental stresses can lead to enhanced production of superoxide anion (O_2^-) radical within maize plant tissues, and plants are believed to rely on the enzyme superoxide dismutase (SOD) to detoxify this reactive oxygen species (ROS). SOD gene family in eukaryotes and prokaryotes consists of multiple genes encoding SOD isoforms. Plant cells contain almost all known SOD types which differ by their metal cofactor and subcellular location. Based on the metal cofactor used by the enzyme, SODs are classified into three groups: iron SOD (FeSOD), manganese SOD (MnSOD), and copper-zinc SOD (Cu/ZnSOD). FeSOD isoforms are located in the chloroplasts, MnSOD isoforms are in the mitochondria and the peroxisomes, and Cu-Zn SOD isoforms are in the chloroplasts, the cytosol, and the extracellular space. In this note we describe some biochemical properties of one MnSOD isoform in mitochondria. We suggest that this MnSOD isoform is involved in mitochondrial DNA protection from ROS.

Total RNA isolation from 3-day-old etiolated hybrid VIR46MV seedlings was performed by QIAGEN RNeasy Mini Kit according manufacturer's instructions. cDNA synthesis was carried out using the Promega Universal RiboClone cDNA Synthesis System. Translated sequence of cDNA of MnSOD3.1 gene flanked by *Nco*I and *Bgl*III sites was amplified using sequence-specific primers (5'-CGACCAAAGCCATGGCTCT-3' as a forward and 5'-CCGTTAAGACAGATCTAGCAAGAACA-3' as a reverse primer). Primers for reverse transcription (RT)-PCR of the SOD3.1 gene were designed using the VectorNTI5 program (Bethesda, USA). The sequence of MnSOD3.1 was obtained from GenBank database at <http://www.ncbi.nlm.nih.gov/nucleotide/> (Acc. number M33119). Obtained PCR-product was eluted from agarose gel using QIAEXII kit (QIAGEN, USA), digested by *Nco*I and *Bgl*III enzymes and ligated into pQE60 expressing vector (QIAGEN, USA). Positive clones were analyzed by PCR and subsequent sequencing on ABI PRISM 377 DNA automated sequencer as recommended by Applied Biosystems. The expression of recombinant protein was analyzed in total protein extracts in combination with superoxide dismutase activity gel assay according Garnik et al. (Russ. J. Plant Phys. 51:386-391, 2004). Elution of recombinant protein was performed on Ni-NT agarose column (QIAGEN, USA) according manufacturer's instructions.

Analysis of DNA cleavage activity of recombinant protein was performed as described by Chen et al. (Arch. Biochem. Biophys. 404:218-226, 2002) using pBlueScript KS II (+) plasmid DNA isolated by using GeneJet Plasmid purification kit (Fermentas, Lithuania). *In vitro* phosphorylation of MnSOD was conducted as described by Subota et al. (Russ. J. Plant Phys. 57:37-44, 2010).

Maize MnSOD3.1 gene was cloned in bacterial expression vector pQE60 and recombinant protein was isolated (Figure 1).

According to Fridovich (J. Biol. Chem. 272:25071-25076, 1997) and Descheneau, Newton (Int. Congr. Plant Mitochondrial Biol. P.23, 2005) MnSOD in bacteria and plants is DNA-associated protein which protects DNA against oxidative damage by reactive oxygen species. We hypothesized that to perform this function MnSOD should not possess DNA cleavage activity mediated through generation of hydroxyl radical in Fenton reaction. To test this suggestion we compared DNA cleavage activity of commercial bovine Cu-ZnSOD (Sigma, USA) and recombinant maize MnSOD. Figure 2 shows that only in the case of 5 and 10 µg of bovine CuZnSOD cleavage of plasmid DNA is observed (the quantity of linear form of plasmid DNA is increased). So, maize MnSOD does not possess DNA cleavage activity in contrast with Cu-ZnSOD.

It is known that the operation of mitochondrial electron transport chain is one of the main sources of ROS in the cell. Phosphorylation of MnSOD indicates that mitochondrial metabolism of ROS is presumably regulated (Bykova et al., FEBS Lett. 540:141-146, 2003). Figure 3 shows that maize MnSOD enzyme is a phosphorylated protein. Moreover, our analysis revealed that there are two phosphorylated MnSOD3.1 forms – native tetrameric and unusual dimeric form. Both of these forms possess superoxide dismutase activity (Figure 3).

We suggest that some not known yet properties of mitochondrial MnSOD may create background for special role of this enzyme in mitochondrial DNA protection from reactive oxygen species. This work was financially supported in part by the Integration projects SB RAS 7, 83, 98 and RFBR projects 08-04-01426, 09-04-00992.

Figure legends

Figure 1. Isolation of recombinant maize MnSOD3.1 protein. *A* - Electrophoretical analysis of bacterial total protein extracts. 1-5 – different transformed bacterial clones. *B* - Superoxide dismutase activity analysis of recombinant proteins. 1, 2 – clones. *C* - Isolation of recombinant protein from bacteria. 1- lysate, 2 – flow-through, 3 – washing, 4 –eluate.

Figure 2. Analysis of DNA cleavage activity of SOD. 1- plasmid DNA, **2-5** – plasmid DNA + maize MnSOD (1, 2.5, 5 and 10 µg, respectively), **6-9** - plasmid DNA + bovine CuZnSOD (1, 2.5, 5 and 10 µg, respectively). C – coiled DNA, L – linear DNA, SC – supercoiled DNA.

Figure 3. Phosphorylation of recombinant maize MnSOD3.1. 1 – protein kinase-containing chromatography fraction of mitochondrial proteins, 2 – phosphorylation of recombinant MnSOD3.1 by this fraction, 3 - superoxide dismutase activity analysis of phosphorylated MnSOD3.1. Narrows indicate two forms of MnSOD3.1.

Figure 1.

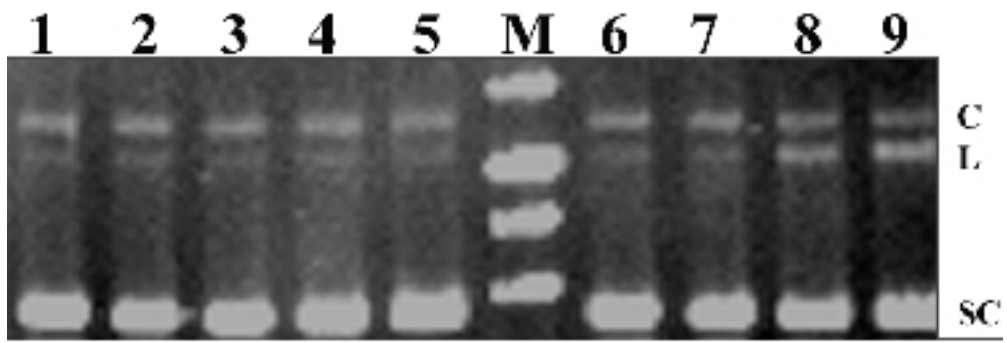


Figure 2.

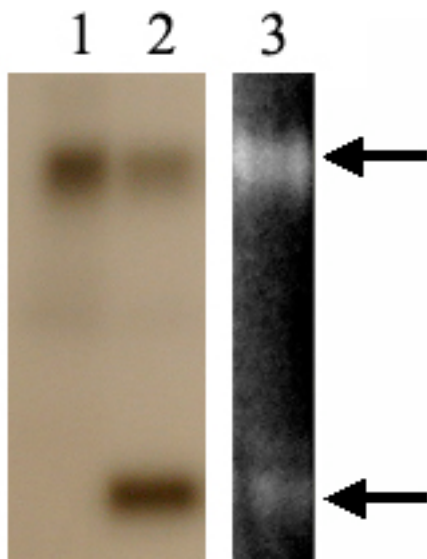


Figure 3

