CHLOROPLAST-LOCALIZED DNA ENDONUCLEASE UNDER PARAQUST TREATMENT; A POTENTIAL NOVEL DNA ENDONUCLEASE INVOLVED IN RESPONSES TO OXIDATIVE STRESSES

Woo Sung Lee
Department of Biology, Sung Kyun Kwan University, Suwon, Korea

A chloroplast-localized endonuclease was induced in tobacco leaves stressed oxidatively with methyl viologen(MV, also known as paraquat) under illumination. The endonuclease was able to cleave chloroplast, plasmid and single-stranded DNA, where the cleavage was estimated by DNA smear on agarose gel electrophoresis. The enzyme was sensitive to the endonuclease-specific inhibitors, surintricarboxylic acid and $ZnSO_4$. According to an activity SDS/PAGE, a single 22kD endonuclease polypeptide was identified only in the chloroplasts from MV-stressed leaves, but not in the nucleus. The induction was sensitive to cycloheximide, an inhibitor of the cytoplasmic protein synthesis. These results strongly suggested that the endonuclease was synthesized in the cytoplasm and subsequently transported into the chloroplasts. The induction of the endonuclease occurred clearly ahead of that of chloroplastic superoxide dismutase(SOD), suggesting that the endonuclease plays an active role at an early stage of response, prior to SOD-mediated antioxidation, to oxidative stress. A significant protection from the degradation of chloroplast DNA (cpDNA) was observed in transgenic tobacco plants (pAL100) overexpressing the gene encoding manganase superoxide dismutase (MnSOD) from pea in their chloroplasts. In contrast, induction and activity of the endonuclease occurred similarly in both pAL100 and untransformed plants. These results suggested that both active oxygens and the endonuclease independently participated in the observed degradation. The potential roles of the endonuclease are discussed.