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Collaborative Effects of CuZnSOD and Human AP Endonuclease against Oxidative Stress

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Abstract

The defenses against free radical damage include specialized repair enzymes that correct oxidative damages in DNA, and detoxification systems such as superoxide dismutases. These defenses may be coordinated genetically as global responses. We hypothesized that the expression of the SOD and the DNA repair genes would inhibit DNA damage under oxidative stress. Therefore, the protection of E. coli mutants deficient in SOD and DNA repair genes (sod^- xth^- and nfo^-) was demonstrated by transforming the mutant strain with a plasmid pYK9 which encoded *Photobacterium leiognathi* CuZnSOD and human AP endonuclease. The results show that survival rates were increased in sod^+ xth^- nfo^+ cells compared to sod^- xth^- ap^+, sod^-xth^- ap^-, and sod^+ xth^- ap^- cells under oxidative stress generated from 0.1 mM paraquat or 3 mM H_2O_2. The data suggested that, at least, SOD and DNA repair enzymes may have collaborate protection and repair of the damaged DNA. Additionally, both enzymes are required for protection against free radicals.

Key words: Superoxide dismutase, DNA repair enzyme, sod gene, Apl endonuclease, xth nfo mutants

Introduction

Reactive oxygen intermediates are toxic in aerobically metabolizing cells due to the formation of intermediate products resulting from univalent reduction of molecular oxygen, including the superoxide radical ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($HO^{\bullet}$). These partially reduced oxygen intermediates differ substantially in their interaction and can cause extensive cellular damage such as nucleic acid strand scission [1, 2], modification of polypeptides [3, 4] and lipid peroxidation [5-7]. Antioxidant enzymes can, however, minimize this toxic damage to a limited degree. Superoxide dismutase (SOD) [8], catalase [9] and glutathione peroxidase [10] as well as small molecules such as vitamin E [11] are mainly responsible for the primary defense against oxidative damage. Defense against free radicals can therefore be viewed as a compendium of enzymes that function to protect the cells against oxidative stress [12].

Increasing evidence indicates that oxygen free radicals are capable of inducing oxidative DNA

damage such as the formation of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodGuo) derived from predominant reaction with guanine residues [13 and 14], while $HO^{\bullet -}$ produces DNA strand breaks and sites of base loss in high yields. Hydroxyl radicals may also attack all four bases in DNA molecules [15].

In particular, single-strand breaks of DNA with 3'-blocked termini are a major class of DNA lesion produced by free radical pathways caused by ionizing radiation or other sources of oxygen radicals [16]. It has long been known that AP site (apurinic/pyrimidinic site) and 3'-blocked single-strand breaks are generally repaired by cellular DNA repair systems. Ap sites induced by free radicals cause the cessation of DNA replication and increases the rate of mutagenesis in living organisms [17]. Therefore, these lesions should inevitably be repaired for overall genomic function and for the cells to survive against unfavorable environmental conditions. Repair of these damages is thought to be mostly initiated by AP endonuclease/DNA 3' repair diesterase [18-20]. In *E. coli*, there are two discrete families: AP endonucleases, Exo III and Endo IV, respectively [21]. These AP endonucleases have evolved not only to repair AP sites, but also to correct distinct DNA lesions. Recent studies [22] have cloned the human cDNA (APE) that encodes the main nuclear AP endonuclease. These studies found that expression of the active human enzyme in AP endonuclease-deficient *E. coli* conferred significant resistance to killing by the DNA-alkylating agent methyl methanesulfonate. *E. coli* xth (Exo III) nfo (Endo IV) double or single mutants [23, 24] were isolated respectively and were studied on DNA repair models [25, 26]. Also Agnez *et al.* (1996) [27] demonstrated that *E. coli* exonuclease III and endonuclease IV are involved in the repair of singlet oxygen-induced DNA damage. It has been demonstrated that endonuclease IV is an inducible DNA repair-enzyme and that its induction can be mediated via the production of superoxide radicals [28]. However, in *E. coli*, genetic relationship among sod and AP endonucleases remain obscure and there has been no conformation of the regulatory role of oxidative stress. A greater understanding of the relationship between the sod gene and AP endonucleases demands greater knowledge of the defense system. Therefore, we decided to evaluate the collaborative function of sod and AP endonuclease in protecting mutants of *E. coli* lacking sod^- xth^- nfo^- against oxidative stress.

Materials and Methods

Chemicals

Bacterial Plasmids

Growth Conditions and Response to Oxidative Stress

Results

Protective Response to Oxidative Stress

We screened the protective effects of sod and AP endonuclease gene products against the oxidative-damaging agents, hydrogen peroxide and paraquat, in *E. coli*. The growth rate and the

survival in the presence of 3mM H_2O_2 or 0.1 mM PQ was tested in the different transformants that carried different mutations in sod and AP endonuclease genes (Fig.2). QC1467 (pYK9) was compared to each QC1467, QC779 (), BW528 (-) and wild type GC4468 under and 1mM treatment [33]. The QC 1467 (sod^- ape^-) mutant was more sensitive to PQ and H_2O_2, but was least sensitive when both the sod and ape genes were present (QC1467/pYK9). Mutants containing sod^- ape^+ (QC779) or sod^+ ape (BW528) were more sensitive to PQ and H_2O_2 than when both genes were present (QC1467/pYK9). However, sod mutants cells were more sensitive than ape mutant cells. All cells were more sensitive to H_2O_2 than to PQ. This protective effect was also observed when the growth rate of the mutants was compared in LB medium. These results imply that AP endonuclease may eliminate the DNA damage caused by reactive oxygen species. However, this effect did not reach that of the wild type.

Expression of SOD and AP endonuclease in sodAB, xth and nfo mutants

To evaluate the collaborative function of AP endonuclease and SOD in the presence of PQ, both enzymes were assessed using polyacrylamide gel electrophoresis of crude cell free extracts for the presence of SOD activity or the AP protein. Both enzymes were slightly increased by PQ treatment compared with that of non-treatment control. These patterns were similar to the single SOD or AP deficient cells in the same environment (data not shown). Thus, it is suggested that both enzymes may play a collaborative role. SOD expression was further assessed by other free radical generators, and by H_2O_2 at different concentrations (data not shown).

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and Others