SHORT COMMUNICATION

Involvement of *Escherichia coli* exonuclease III and endonuclease IV in the repair of singlet oxygen-induced DNA damage

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Singlet molecular oxygen (1O2) has been implicated in several biological processes that may lead to genetic damage. The relevance of various repair pathways in plasmid inactivation mediated by 1O2 was investigated. Plasmid treated with 1O2, chemically generated, was transfected into *Escherichia coli* strains deficient in genes implicated in the DNA repair of oxidative damage. The ability to transform bacteria is significantly reduced in the double mutant xth, fnp, deficient in both exonuclease III and endonuclease IV, although it was similar to wild-type cells in single mutants. The products of these two genes are able to cleave DNA damaged by 1O2 and to remove DNA polymerization blocks from 3'-termini generated either directly by 1O2 treatment or after the action of the formamidopyrimidine-DNA-N-glycosylase (Fpg protein). The results indicate that the exonuclease III and endonuclease IV participate in the excision of lethal lesions induced in DNA by 1O2.

Singlet molecular oxygen (1O2) is a reactive excited form of molecular oxygen which is generated in several biological systems, including enzymatic reactions, lipid peroxidation, the immune system and photosensitizers plus light (for reviews, see 1, 2). DNA is one of the main targets of 1O2 and the abundance of oxidative DNA damage poses two biological problems: (i) blocking of DNA synthesis, which is lethal, and (ii) miscoding, which is premutagenic. It has been demonstrated that 1O2 reacts preferentially with guanine residues either as free nucleosides (3) or as components of the DNA molecule (2), yielding a variety of DNA lesions selectively at guanine sites. These include DNA cleavage (4), alkali- and piperidine-labile sites (including abasic sites) (5), cyanuric acid (3), 2,6-diamino-4-oxo-5-formamidopyrimidine (FapyG), a guanine derivative with an open imidazole ring (6) and 7,8-dihydro-8-oxodeoxyguanine (8-oxodG) (7). Quantification of 8-oxodG in DNA exposed to photosensitized methylene blue, which also generates 1O2, has shown that this oxidized base is one of the main types of damage, as it occurs more frequently than strand breaks (8) or FapyG (6).

The ability to repair 1O2-induced DNA lesions has been investigated in some detail in *Escherichia coli*. The repair of oxidized bases in DNA is mostly mediated by the base excision repair pathway, the first step being catalyzed by DNA glycosylases (9,10). The main role of formamidopyrimidine-DNA-N-glycosylase (Fpg protein) is as the glycosylase which excises 8-oxodG as part of the GO repair system (11). The repair reaction proceeds by the incision of the abasic site, generating a gap which is a substrate for repair synthesis and ligation. The nucleotide excision repair pathway, mediated by the UvrABC complex, may also participate in the repair of oxidized bases when the DNA glycosylases are inactivated or saturated (12, 13). The importance of this protein complex in vivo was demonstrated by the transfection of plasmid DNA treated with photosensitized methylene blue into *E. coli* mutants defective in excision repair activities (14). It was found that plasmid inactivation was more significant in bacteria deficient in both the fpg and uvrA genes when compared to wild-type or fpg or uvrA single mutants. The data indicate that the repair pathways mediated by the Fpg protein and UvrABC endonuclease have an important role on the repair of lethal damage induced by 1O2. These two pathways can complement each other by restoring damaged plasmid activity in the single mutants.

In this work, the repair of 1O2-damaged DNA in bacteria was investigated by transfection of plasmid molecules into various repair-deficient bacterial hosts. The ability to reanimate the damaged molecules was evaluated by measuring transformation efficiency. The experiments indicate that, in addition to Fpg protein and UvrABC endonuclease, exonuclease III and endonuclease IV have a role in the repair of 1O2-induced damage.

Plasmid DNA was exposed to the water-soluble endoperoxide of 3,3'-((1,4-naphthylidene)dipropionate (NDPO3), a clean and pure source of 1O2 (15). The plasmid pAC189 (6.4 kb) is a derivative of pZ189 (16): it carries the chloramphenicol acetyl transferase gene under control of the trp promoter. This construction avoids the growth of satellite colonies that are common in plasmids with ampicillin resistance. After treatment (17), the electrophoretic mobility of the vector was analysed, as it monitors the extent of DNA single-strand breaks (SSBs) induced by 1O2 (18). The damaged pAC189 DNA was then transfected into different *E. coli* strains defective for DNA repair genes and the number of transformed clones scored. The relative ability to reanimate the damaged plasmids may reveal a role for the studied genes in the repair of 1O2-induced lesions.

Figure 1(A) shows that the transfection efficiencies of NDPO3-treated plasmid were nearly the same in either the uvrA or fpg single mutant as in the wild-type cells. However, the transfection efficiency of 1O2-damaged plasmid was greatly reduced in the uvrAfpg double mutant. This reduction in plasmid viability in the double mutant was dose dependent. These data confirm previous observations employing other in vitro systems such as pBR322 and M13mp18 DNA treated with photosensitized methylene blue (14,19), indicating that base excision repair and the nucleotide excision repair pathways mediated by Fpg protein and UvrABC endonuclease respect-
enzymes on 8-oxodG and FapyG (6) implicates these lesions as the major DNA lesion induced by 'O\textsubscript{2} (26). The action of these pathways suggests that they are involved in the metabolism of detectable amount of lesions in transformation assays (data not shown).

The cooperative action between Fpg and UvrABC repair pathways suggests that they are involved in the metabolism of the same lesions induced by 'O\textsubscript{2}. The action of these enzymes on 8-oxodG and FapyG (6) implicates these lesions as responsible, to some extent, for the lethal effects on 'O\textsubscript{2}-damaged DNA in uvrA\textsubscript{fg} bacteria, but does not exclude the contribution of other lesions. This assumption is confirmed by the loss of transfection efficiency in xth, nfo E. coli strain. As the major DNA lesion induced by 'O\textsubscript{2}, 8-oxodG is not a substrate for exonuclease III or endonuclease IV and would be eliminated by Fpg glycosylase and/or UvrABC pathways, the need for these two endonucleases must be otherwise explained. In fact, these enzymes cleave 'O\textsubscript{2}-damaged DNA in sites other than Fpg protein (data not shown). Thus, it seems that 'O\textsubscript{2} treatment of DNA may induce some other lesions that reduce plasmid survival in the absence of bacterial repair pathways controlled by the xth and nfo products. With regard to the main activity of these proteins, apurinic/apyrimidinic (AP) sites appear as the most suitable candidates for the effects described here, as they are also found in DNA damaged by 'O\textsubscript{2} (5,20). However, it cannot be excluded that other lesions induced by 'O\textsubscript{2}, different from AP sites, are also repaired by these enzymes, since both Fpg protein and UvrABC endonuclease are able to act on AP sites. These experiments indicate that, in addition to Fpg protein and UvrABC endonuclease, exonuclease III and endonuclease IV play a role in the repair of 'O\textsubscript{2}-induced damage.

Exonuclease III and endonuclease IV have also been described as capable of removing 3' termini that block DNA polymerase activity from DNA damaged by bacterial treatment with hydrogen peroxide (21). This activity was assayed for DNA exposed to 'O\textsubscript{2}. DNA treated with NDPO\textsubscript{2} was incubated with DNA polymerase I (Klenow fragment) in the presence of a radioactive precursor ([\(\alpha\)-\textsuperscript{32}P]dCTP). After electrophoretic separation of the different forms of DNA, the incorporated radioactivity was detected in form II DNA by autoradiography and revealed the presence of free 3'-OH DNA termini that are recognized as primers for DNA synthesis by the polymerase. The same analysis were performed in damaged DNA treated with different endonucleases, with treatment conditions as described (21,22). Results from one set of experiments are illustrated in Figure 2. The results clearly show that SSBs induced by 'O\textsubscript{2} have 3' ends that have polymerization blocks inhibiting primer activity (lane 7). Also, the termini generated by Fpg treatment do not prime DNA synthesis by E. coli DNA polymerase (lane 8). On the other hand, 'O\textsubscript{2}-damaged DNA treated with exonuclease III or endonuclease IV becomes a weak substrate for the incorporation of radioactive precursors (lanes 11 and 12). A better substrate for DNA polymerase action is found after exonuclease III and endonuclease IV treatment of DNA pre-incised with Fpg protein (lanes 9 and 10). The data confirm that DNA cleavage by exonuclease III and endonuclease IV generates 3'-OH extremities which may act directly as primers for DNA polymerization. These enzymes also remove 3' polymerase blocks from DNA cleaved by Fpg. It should be noted that although experiments with untreated DNA also lead to DNA synthesis (lanes 1–6), the amount of radioactive incorporation is much lower than that observed for 'O\textsubscript{2}-damaged DNA.

During repair processes, some abnormal bases or sugar moieties can be generated, as secondary lesions, requiring complementary repair. Homogeneous Fpg protein of E. coli also catalyses the nicking of the phosphodiester bond on both the 3' and 5' sides of abasic sites in the DNA, due to its associated AP lyase activity, leaving a gap bordered by 5'- and 3'-phosphoryl groups (23). The residual 3'-termini are not primers for DNA synthesis. Exonuclease III as well as...
endonuclease IV are also 3' to 5' exo nucleases for double-stranded DNA, removing any blocking groups from DNA 3' termini [21].

The data shown in Figure 2 confirm that the SSBs induced directly by \( \mathrm{O}_2 \) or after Fpg cleavage do not generate 3'-OH extremities. This indicates that DNA SSBs produced by 8-oxo attacks on deoxynucleosines have 3' terminal deoxyribose fragments or simple 3' phosphates, as with other oxidative agents. For DNA cleaved by Fpg protein, the presence of 3'-phosphate termini is expected considering the associated AP lyase activity. The stimulation of DNA primer activity from \( \mathrm{O}_2 \)-damaged DNA simultaneously treated with Fpg protein and exonuclease III or endonuclease IV provides good evidence that they may participate in the repair pathway initiated by the former enzyme.

In conclusion, although 8-oxodG is probably the most frequent lesion induced in DNA by 8-oxo, other lesions that correspond to a minor fraction of damage, such as AP sites, may play an important role when damaged DNA is replicated inside the cells. Exonuclease III and endonuclease IV participate directly, or after Fpg cleavage, in the repair of these lesions. The absence of DNA repair pathways that require these enzymes causes 8-oxo-induced plasmid inactivation. The biological consequences of remaining lesions, such as mutagenesis, are being investigated, in order to establish their relative importance in damaged DNA.

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References


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