

Abstract

During infection, *Mycobacterium tuberculosis* bacteria are engulfed by macrophages as part of the host's innate immune response. This response generates oxidative stress on the mycobacteria, causing DNA damage and mutagenesis. Thus, DNA repair pathways are essential in the maintenance of genome integrity and cell survival. A previous study conducted in *Mycobacterium smegmatis*, a non-pathogenic close relative of *M. tuberculosis*, indicated a novel interplay between the endonuclease III (Nth) DNA glycosylase and two homologues of the endonuclease VIII (NeiI and NeiII) DNA glycosylases of the base excision repair pathway, in controlling DNA damage under oxidative stress conditions. A previous study indicated that the combinatorial loss of the *nth* and *neiI* genes has a larger impact on *M. smegmatis* survival under oxidative stress compared to when the *nth* and *neiII* genes were simultaneously lost. Bioinformatic analysis of NeiI and NeiII DNA glycosylases show that the two enzymes differ by three amino acid residues in the DNA intercalation loop that is responsible for the catalysis of binding damaged DNA to the DNA glycosylase. The NeiI enzyme has GLY residues whilst the NeiII homologue has the KME residues. In *E. coli*, mutation of the intercalation loop of the DNA-binding protein (SSB) resulted in a 100-fold decrease in DNA binding efficiency in the mutant compared to the parental strain. Hence, in this study we attempted to assess whether the GLY residues of the NeiI protein are indeed responsible for DNA glycosylase activity. Recombinant vectors containing the *neiI* gene with the GLY loop and another with the GLY loop changed to a GAF loop were genetically engineered and electroporated into the double deletion mutant lacking the *neiI* and *nth* genes ($\Delta nI\Delta nth::nI$ and $\Delta nI\Delta nth::\Delta GAF$). Growth kinetics of these strains indicate a similar growth pattern for the wildtype, $\Delta nI\Delta nth::nI$ and $\Delta nI\Delta nth::\Delta GAF$ strains, and no significant difference in growth between the strains was noted. The resulting mutants were then phenotypically characterized together with the respective parental strains to assess if the GLY loop is responsible for the increased DNA glycosylase activity of NeiI. Deletion of the *nth* gene combined with the *neiI* gene led to an increase in spontaneous mutagenesis in response to rifampicin when compared to the wildtype mc²155 strain. However, the increased spontaneous mutations were also observed in the $\Delta nI\Delta nth::nI$ and $\Delta nI\Delta nth::\Delta GAF$ strains when compared to the parental strain. Thus, alteration of the GLY residues to GAF residues of the NeiI endonuclease did not significantly impact the genome integrity of the NeiI endonuclease.