ABSTRACT

To protect cells from oxidative DNA damage and mutagenesis, organisms possess multiple glycosylases to recognize the damaged bases and to initiate the Base Excision Repair (BER) pathway. Three DNA glycosylases have been identified in mammals that are homologous to the *Escherichia coli* Fpg and Nei proteins, Neil1, Neil2 and Neil3. Neil1 and Neil2 in human and mouse have been well characterized while the properties of the Neil3 protein remain to be elucidated. In this study, we report the characterization of *Mus musculus* (house mouse) Neil3 (MmuNeil3) as an active DNA glycosylase both *in vitro* and *in vivo*. In duplex DNA, MmuNeil3 recognizes the oxidized purines, spiroiminodihydantoin (Sp), guanidinohydantoin (Gh), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG) and 4,6-diamino- 5-formamidopyrimidine (FapyA), but not 8-oxo-7,8-dihydroguanine (8-oxoG). Interestingly, MmuNeil3 prefers lesions in single stranded DNA and in bubble structures. In contrast to other members of the family that use the N-terminal proline as the nucleophile, MmuNeil3 forms a Schiff base intermediate via its N-terminal valine. We expressed the glycosylase domain of MmuNeil3 (MmuNeil3Δ324) in an *Escherichia coli* triple mutant lacking Fpg, Nei and MutY glycosylase activities and showed that MmuNeil3 greatly reduced both the spontaneous mutation frequency and the level of FapyG in the DNA, suggesting that Neil3 plays a role in repairing FapyG *in vivo*.