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Recognition of the oxidized lesions spiroiminodihydantoin and guanidinohydantoin in DNA by the mammalian base excision repair glycosylases NEIL1 and NEIL2

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Abstract

8-Oxoguanine (8-oxoG) is an unstable mutagenic DNA lesion that is prone to further oxidation. High valent metals such as Cr(V) and Ir(IV) readily oxidize 8-oxoG to form guanidinohydantoin (Gh), its isomer iminoallantoin (Ia), and spiroiminodihydantoin (Sp). When present in DNA, these lesions show enhanced base misincorporation over the parent 8-oxoG lesion leading to G → T and G → C transversion mutations and polymerase arrest. These findings suggested that further oxidized lesions of 8-oxoG are more mutagenic and toxic than 8-oxoG itself. Repair of oxidatively damaged bases, including Sp and Gh/Ia, are initiated by the base excision repair (BER) system that involves the DNA glycosylases Fpg, Nei, and Nth in *E. coli*. Mammalian homologs of two of these BER enzymes, OGG1 and NTH1, have little or no affinity for Gh/Ia and Sp. Herein we report that two recently identified mammalian glycosylases, NEIL1 and NEIL2, showed a high affinity for recognition and cleavage of DNA containing Gh/Ia and Sp lesions. NEIL1 and NEIL2 recognized both of these lesions in single-stranded DNA and catalyzed the removal of the lesions through a β- and δ-elimination mechanism. NEIL1 and NEIL2 also recognized and excised the Gh/Ia lesion opposite all four natural bases in double-stranded DNA. NEIL1 was able to excise the Sp lesion opposite the four natural bases in double-stranded DNA, however, NEIL2 showed little cleavage activity against the Sp lesion in duplex DNA although DNA trapping studies show recognition and binding of NEIL2 to this lesion. This work suggests that NEIL1 and NEIL2 are essential in the recognition of further oxidized lesions arising from 8-oxoG and implies that these BER glycosylases may play an important role in the repair of DNA damage induced by carcinogenic metals.

Keywords: NEIL1; NEIL2; DNA oxidation; Spiroiminodihydantoin; Guanidinohydantoin; DNA repair; Chromium

1. Introduction

Oxidative damage, occurring from reactive oxygen species or xenobiotics such as metals, is implicated in a variety of disease states such as cancer, rheumatoid arthritis, cardiovascular disease, and aging [1,2]. Oxidation of the nucleic acid guanine to 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxoG) is one of the most widely studied lesions in cellular systems. 8-OxoG has been shown to produce high levels of G:C → T:A transversion mutations in mammalian and bacterial cells [3–5]. Interestingly, the redox potential of 8-

Abbreviations: 8-oxoG, 7,8-dihydro-8-oxo-2′-deoxyguanosine; Gh, guanidinohydantoin; Ia, iminoallantoin; Sp, spiroiminodihydantoin; 5-OHU, 5-hydroxyuracil; TG, thymine glycol; nt, nucleotide; bp, base pair; Fpg, Fapy glycosylase; Nei, endonuclease VIII; OGG1, 8-oxoguanine DNA glycosylase I; NTH1, endonuclease III homolog I; NEIL, Nei-like; BER, base excision repair; Cr(V)-salen, *N*,*N*′-ethylenebis(salicylideneanimato)oxochromium(V)

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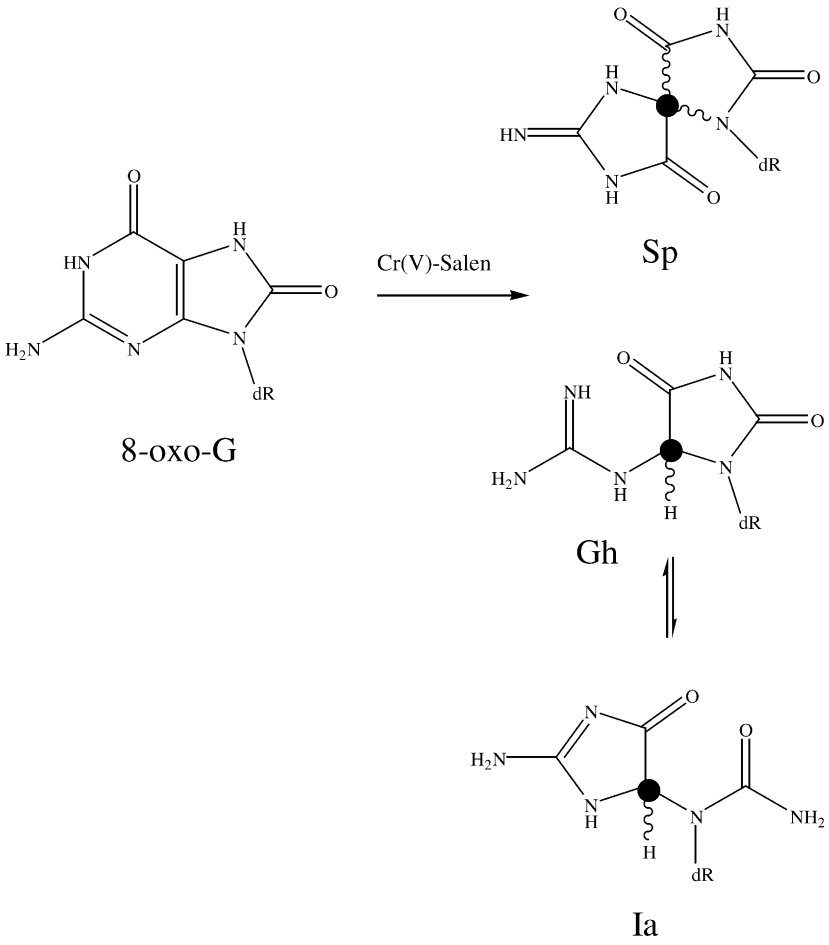

Fig. 1. Oxidation of 8-oxoG by Cr(V)-salen produces the lesions guanidinohydantoin (Gh), and spiroiminodihydantoin (Sp). Previous studies have shown that Gh equilibrates with an isomeric form, iminoallantoin (Ia). The highlighted atom in the structure denotes the sp3 carbon.

oxoG makes this lesion prone to degradation into further oxidized products. Studies on electron transfer through DNA have shown that oxidative "hole migration" can occur over long distances [6–8] and oxidative events will occur at sites of lowest redox potential [8], such as an 8-oxoG lesion. The reaction of 8-oxoG containing oligonucleotides with a high valent Cr(V)-salen complex lead to the site-specific oxidation of 8-oxoG to further oxidized lesions [9], specifically the isomeric pair guanidinohydantoin/iminoallantoin (Gh/Ia) and spiroiminodihydantoin (Sp) (Fig. 1). These two lesions, Gh/Ia and Sp, lead to a higher rate of G → T and G → C transversion mutations than that observed with 8-oxoG alone [10–12].

Oxidative damage to individual DNA bases is repaired via the base excision repair (BER) pathway which recognizes and removes damaged bases from DNA [13]. MutM/Fpg removes the 8-oxoG lesion when it is paired opposite a cytosine while MutY removes the mismatched adenine from an 8-oxoG:A or a G:A pair to provide a second chance for Fpg recognition and repair [14]. Until recently only two mammalian repair enzymes, 8-oxoguanine-DNA glycosylase (OGG1) and endonuclease III homolog 1 (NTH1), were known to recognize and cleave oxidized guanine lesions from DNA [15]. Surprisingly, Nth1 knockout mice remain healthy [16] and while $Ogg1^{-/-}$ mice show increased mutation rates in some tissues, they have no associated increase in the incidence of cancer [17,18]. These findings suggested that an additional BER enzyme system exists that can recognize oxidized guanine residues.

A new set of BER enzymes have been identified that are mammalian homologs of the *E. coli* MutM/Nei (endonuclease VIII) family [19–21]. These mammalian homologs of Nei were designated the "Nei-like" or, "NEIL" family of glycosylases. The NEIL1 gene maps to the 15q22 chromosome in humans and loss of heterozygosity at this site is observed in over 70% of small cell lung carcinoma [22]. Thus far the identified substrates of NEIL1 and NEIL2 have consisted mainly of oxidized pyrimidines such as 5-hydroxyuracil (5-OHU), the formamido-pyrimidines, (FapyG and FapyA), and thymine glycol [19–21,23]. More recently, the human NEIL enzymes have been shown to recognize 5-OHU and 8-oxoG in single-stranded bubble structures of DNA [15].

In this paper, we report the identification of two additional oxidized lesions that are readily recognized and