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Review Article

Repair of 8-oxo-7,8-dihydroguanine in prokaryotic and eukaryotic cells: Properties and biological roles of the Fpg and OGG1 DNA *N*-glycosylases☆

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ABSTRACT

Oxidatively damaged DNA results from the attack of sugar and base moieties by reactive oxygen species (ROS), which are formed as byproducts of normal cell metabolism and during exposure to endogenous or exogenous chemical or physical agents. Guanine, having the lowest redox potential, is the DNA base the most susceptible to oxidation, yielding products such as 8-oxo-7,8-dihydroguanine (8-oxoG) and 2–6-diamino-4-hydroxy-5-formamidopyrimidine (FapyG). In DNA, 8-oxoG was shown to be mutagenic yielding GC to TA transversions upon incorporation of dAMP opposite this lesion by replicative DNA polymerases. In prokaryotic and eukaryotic cells, 8-oxoG is primarily repaired by the base excision repair pathway (BER) initiated by a DNA *N*-glycosylase, Fpg and OGG1, respectively. In *Escherichia coli*, Fpg cooperates with MutY and MutT to prevent 8-oxoG-induced mutations, the "GO-repair system". In *Saccharomyces cerevisiae*, OGG1 cooperates with nucleotide excision repair (NER), mismatch repair (MMR), post-replication repair (PRR) and DNA polymerase η to prevent mutagenesis. Human and mouse cells mobilize all these pathways using OGG1, MUTYH (MutY-homolog also known as MYH), MTH1 (MutT-homolog also known as NUDT1), NER, MMR, NEILs and DNA polymerases η and ƛ, to prevent 8-oxoG-induced mutations. In fact, mice deficient in both OGG1 and MUTYH develop cancer in different organs at adult age, which points to the critical impact of 8-oxoG repair on genetic stability in mammals. In this review, we will focus on Fpg and OGG1 proteins, their biochemical and structural properties as well as their biological roles. Other DNA *N*-glycosylases able to release 8-oxoG from damaged DNA in various organisms will be discussed. Finally, we will report on the role of OGG1 in human disease and the possible use of 8-oxoG DNA *N*-glycosylases as therapeutic targets.

1. Origin and biological consequences of 8-oxo-7,8-dihydroguanine

Oxidatively damaged DNA results from the attack of sugar and base moieties by reactive oxygen species (ROS), which are formed as byproducts of normal cell metabolism (about 1% of O_2 is converted into $O_2^{\bullet -}$) and during exposure to endogenous or exogenous chemical or physical agents such as menadione, γ- or UV-radiation. ROS such as superoxide anion radical ($O_2^{\bullet -}$), hydroxyl radical (*OH), hydrogen peroxide (H_2O_2) or singlet oxygen ($^1O_2$) damage DNA either directly or after metal-catalyzed transformation in the cell. Oxidatively damaged DNA includes a multiplicity of DNA base lesions, DNA sugar lesions, DNA strand breaks with blocked ends and [DNA-protein]-cross-links [1–4]. Among the four DNA bases, Guanine, having the lowest redox potential, is the most susceptible to oxidation, yielding different products in DNA such as 8-oxo-7,8-dihydroguanine (8-oxoG) and 2–6-diamino-4-hydroxy-5-formamidopyrimidines (FapyG) (Fig. 1a) [5]. In addition, 8-oxoG is subject to further oxidation yielding spiroimidohydantoin (Sp) and guanidinohydantoin (Gh) [2,6].

Importantly, 8-oxoG in DNA was shown to be mutagenic yielding nearly exclusively base pair substitutions and mostly GC to TA transversions upon incorporation of dAMP opposite this lesion by replicative DNA polymerases δ and ε [7–9] (Fig. 1b). In prokaryotic and eukaryotic cells, 8-oxoG is primarily released from DNA by the base excision repair pathway (BER) initiated by a DNA *N*-glycosylase (Fig. 2). The Fpg protein from *Escherichia coli* (also known as MutM) and the OGG1 protein of *Saccharomyces cerevisiae* are the archetypes of 8-oxoG DNA *N*-glycosylases in bacteria and eukaryotes, respectively. Genes encoding Fpg (*fpg*) from *E. coli* and OGG1 (*OGG1*) from *S. cerevisiae* were first cloned in our laboratory [10,11]. The impact of 8-

☆ This article is one of a series of papers on the subject of oxidative DNA damage & repair that have been published as a special issue of Free Radical Biology & Medicine to commemorate the Nobel Prize won by Prof. Tomas Lindahl. A detailed introduction and synopsis of all the articles in the special issue can be found in the following paper by Cadet & Davies [288].

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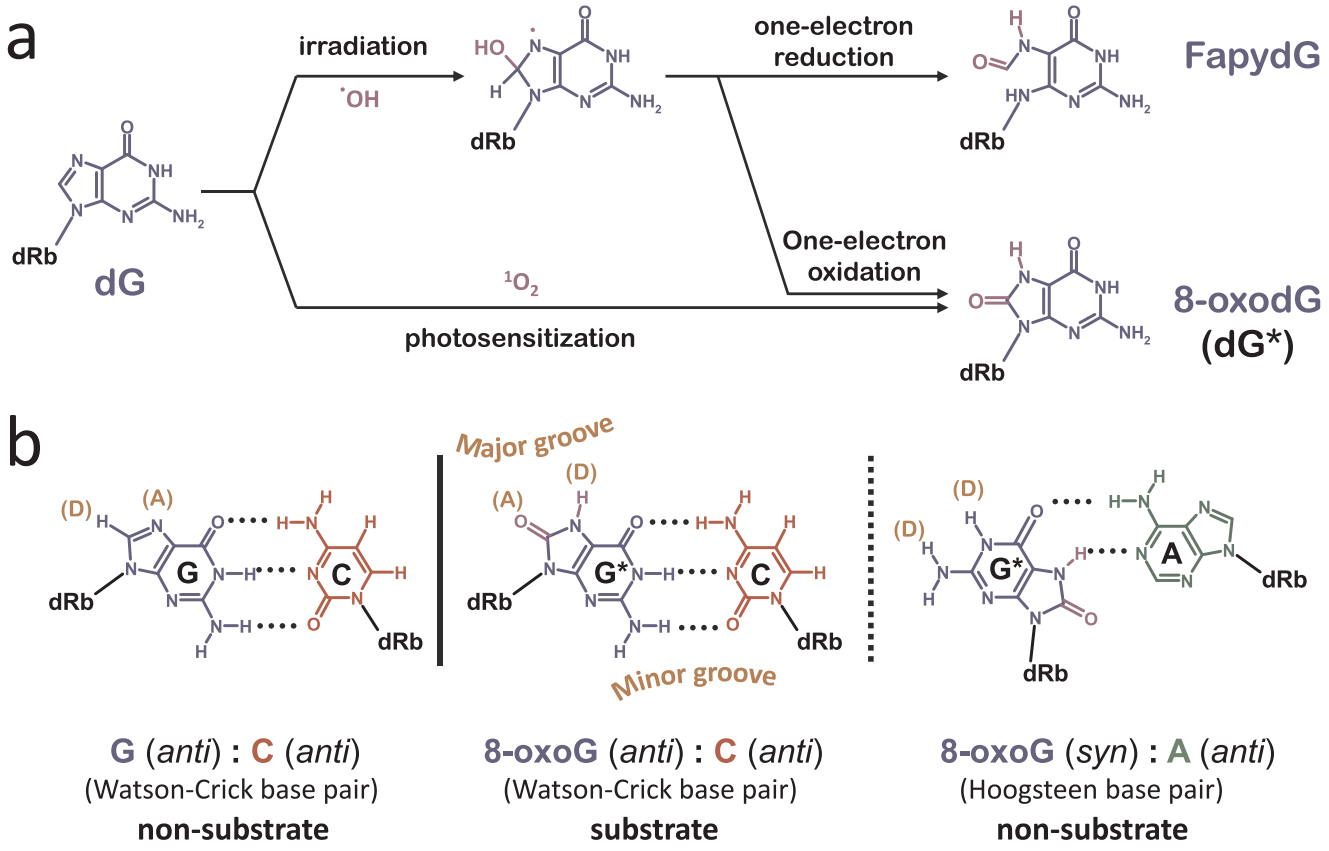

Fig. 1. Formation and structural determinants of 8-oxo-7,8-dihydroguanine. (a) Two ways for the oxidation of a guanine into 8-oxo-7,8-dihydroguanine. *OH : hydroxyl radical. $^1O_2$: singlet oxygen. (b) Base pairing properties of 8-oxo-7,8-dihydroguanine. (D) and (A) are for hydrogen donor and acceptor, respectively.

oxoG on genetic stability was assessed by the spontaneous and specific (GC to TA) mutator phenotype displayed by Fpg-deficient cells in *E. coli* and OGG1-deficient cells in *S. cerevisiae*, respectively [12,13]. In *E. coli*, Fpg cooperates with MutY, an Adenine DNA *N*-glycosylase and MutT, an 8-oxodGTPase, to form the "GO-repair system" (for reviews see; [14–16]). In *S. cerevisiae*, in the absence of MutY-homolog, OGG1 cooperates with mismatch repair (MMR), post-replication repair (PRR) mediated by RAD6-RAD18 and DNA polymerase η to prevent mutations, the GO-repair network (for reviews see; [17,18]). Finally, mammalian cells mobilize multiple DNA repair pathways to prevent mutations due to 8-oxoG in DNA and nucleotide pools [8,19,20]. The biological impact of 8-oxoG in mice was revealed by targeting DNA repair genes, yielding *Ogg1*$^{-/-}$ *and Mutyh*$^{-/-}$ knockout mice. Indeed, inactivation of both OGG1 and MUTYH results in early death due to tumor development in different organs [20]. In human, molecular and epidemiological studies also confirm the impact of 8-oxoG on genetic stability and cancer predisposition. In this review, we summarize current knowledge about 8-oxoG repair and biological consequences, focussing on the 8-oxoG DNA *N*-glycosylases Fpg and OGG1.

2. Base excision repair of 8-oxo-7,8-dihydroguanine

2.1. 8-8-oxoG-DNA N-glycosylases from the Fpg/Nei structural superfamily

2.1.1. Fpg, the bacterial 8-oxoG-DNA N-glycosylase

Fpg was initially identified in *E. coli* as a DNA *N*-glycosylase removing 2,6-diamino-4-hydroxy-5-N-methylformamidopyrimidine (*N7*-MeFapyG), hence its name Formamidopyrimidine-DNA *N*-glycosylase (FapyG-DNA glycosylase or Fpg) [21,22]. Based on this activity, the *fpg* gene was cloned, sequenced and the protein purified [10]. The hallmarks of the Fpg/Nei superfamily extracted from primary structure alignments are the highly conserved N-terminus starting by the catalytic P1 and E2, and the Zinc-finger motif at the C-terminus (-C-X_2-C-X_{16}-C-X_2-C- in Fpg and Nei from *E. coli*) (Fig. 3a). Site directed mutagenesis of cysteines from Fpg has clearly identified those involved in Zn^{2+} coordination and the role of the zinc finger (ZnF) motif in the binding of 8-oxoG-containing DNA [23]. The purified Fpg protein is endowed with a DNA *N*-glycosylase activity, an AP lyase activity catalyzing β,δ-elimination and a 5′-dRP-lyase activity [24,25].

We first used the technique of gas chromatography/mass spectrometry (GC-MS) to identify and quantitate pyrimidine- and purine-derived lesions released by Fpg from DNA exposed to γ-irradiation [26]. Thirteen oxidative DNA base products were detected in γ-irradiated calf thymus DNA. GS-MS analysis revealed that Fpg efficiently excised 8-oxoG, FapyG, and FapyA (4,6-diamino-5-formamidopyrimidine), whereas 8-oxoA (8-oxo-7,8-dihydroadenine) was poorly released (Table 1) [26]. In contrast, none of the nine pyrimidine-derived lesions was excised at a detectable rate by the Fpg protein [26]. Afterwards, G C-MS was used to analyze the substrate specificity of a number of DNA *N*-glycosylases acting on oxidatively damaged DNA. Short oligodeoxyribonucleotides (ODNs) containing a single lesion at a defined position were also used to investigate the substrate specificity of DNA *N*-glycosylases, allowing the authors to explore the impact of the DNA base opposite the lesion upon enzyme activity (Table 1). Monomodified ODNs also allowed the study of lesions non-identified by GC/MS such as Sp and Gh [27]. The Fpg protein efficiently excises 8-oxoG in duplex ODNs with Cytosine (C), Thymine (T) or Guanine (G) opposite the lesion [23,28]. In contrast, Fpg excises 8-oxoG opposite A at very slow rate, which is beneficial for the cell, preventing mutation fixation and facilitating the action of MutY [15]. In addition, Fpg excises Sp and Gh opposite G or C, and, although at a reduced rate, opposite Adenine (A) [27,29]. This result suggests that the specificity of Fpg for the opposite base is relaxed with any lesions other than 8-oxoG. Although both technologies (GC-MS and ODNs) generally generate coherent results,