Mutation Research 771 (2017) 99–127

Contents lists available at ScienceDirect

Mutation Research/Reviews in Mutation Research

journal homepage: www.elsevier.com/locate/reviewsmr
Community address: www.elsevier.com/locate/mutres

Review

Repair of oxidatively induced DNA damage by DNA glycosylases: Mechanisms of action, substrate specificities and excision kinetics

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ARTICLE INFO

Article history:
Received 17 November 2016
Available online 16 February 2017

Keywords:
Oxidatively induced DNA damage
DNA repair
DNA glycosylases
Substrate specificities
Excision kinetics

ABSTRACT

Endogenous and exogenous reactive species cause oxidatively induced DNA damage in living organisms by a variety of mechanisms. As a result, a plethora of mutagenic and/or cytotoxic products are formed in cellular DNA. This type of DNA damage is repaired by base excision repair, although nucleotide excision repair also plays a limited role. DNA glycosylases remove modified DNA bases from DNA by hydrolyzing the glycosidic bond leaving behind an apurinic/apyrimidinic (AP) site. Some of them also possess an accompanying AP-lyase activity that cleaves the sugar-phosphate chain of DNA. Since the first discovery of a DNA glycosylase, many studies have elucidated the mechanisms of action, substrate specificities and excision kinetics of these enzymes present in all living organisms. For this purpose, most studies used single- or double-stranded oligodeoxynucleotides with a single DNA lesion embedded at a defined position. High-molecular weight DNA with multiple base lesions has been used in other studies with the advantage of the simultaneous investigation of many DNA base lesions as substrates. Differences between the substrate specificities and excision kinetics of DNA glycosylases have been found when these two different substrates were used. Some DNA glycosylases possess varying substrate specificities for either purine-derived lesions or pyrimidine-derived lesions, whereas others exhibit cross-activity for both types of lesions. Laboratory animals with knockouts of the genes of DNA glycosylases have also been used to provide unequivocal evidence for the substrates, which had previously been found in *in vitro* studies, to be the actual substrates *in vivo* as well. On the basis of the knowledge gained from the past studies, efforts are being made to discover small molecule inhibitors of DNA glycosylases that may be used as potential drugs in cancer therapy.

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Abbreviations: $O_2^{\bullet}$, superoxide radical; $^{\bullet}OH$, hydroxyl radical; $NO^{\bullet}$, nitric oxide; e_{aq}^{-}, hydrated electron; $H^{\bullet}$, hydrogen atom; *k*, reaction rate constant; 8-OH-Gua, 8-hydroxyguanine (also called 8-oxo-Gua); Sp, spiroiminohydantoin; Gh, 5-guanidinohydantoin; cdA, 8,5′-cyclo-2′-deoxyadenosine; cdG, 8,5′-cyclo-2′-deoxyguanosine; AP site, apurinic/apyrimidinic site; BER, base excision repair; NER, nucleotide excision repair; 5-MeCyt, 5-methylcytosine; 5′-dRP residue, 2-deoxyribose phosphate residue; Pol β, DNA polymerase β; APE1, apurinic/apyrimidinic endonuclease 1; PARP1, Poly(ADP)ribose polymerase 1; UDG, *E. coli* uracil DNA glycosylase; SMUG1, single-strand selective monofunctional uracil DNA glycosylase; TDG, thymine DNA glycosylase; MBD4, methyl CpG binding domain protein 4; Fpg, formamidopyrimidine glycosylase; Nei, endonuclease VIII; H2TH, helix-two turn-helix; Nth, endonuclease III; PNKP, polynucleotide kinase phosphatase; GC–MS, gas chromatography-mass spectrometry; GC–MS/MS, gas chromatography-tandem mass spectrometry; 5-OHMeUra, 5-hydroxymethyluracil; 5-OH-Ura, 5-hydroxyuracil; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; Me-FapyGua, 2,6-diamino-4-hydroxy-*N*7-methyl-5-formamidopyrimidine; 2-OH-Ade, 2-hydroxyadenine; FapyAde, 4,6-diamino-5-formamidopyrimidine; 5-OH-Cyt, 5-hydroxycytosine; 8-OH-Ade, 8-hydroxyadenine; *Dr*Fpg, *Deinococcus radiodurans* Fpg; 5,6-diOH-Cyt, 5,6-dihydroxycytosine; 5,6-diOH-Ura, 5,6-dihydroxyuracil; 5-OH-6-HThy, 5-hydroxy-6-hydrothymine; 5-OH-6-HUra, 5-hydroxy-6-hydrouracil; Thy glycol, thymine glycol; 5,6-diHThy, 5,6-dihydrothymine; 5,6-diHUra, 5,6-dihydrouracil; 5-OH-5-MeHyd, 5-hydroxy-5-methylhydantoin; k_{cat}/K_M, specificity constant; OGG1, 8-oxoguanine DNA glycosylase; yOGG1, *Saccharomyces cerevisiae* OGG1; hOGG1, human OGG1; dOGG1, *Drosophila melanogaster* OGG1; dS3 protein, *Drosophila* ribosomal protein S3; *At*OGG1, *Arabidopsis thaliana* OGG1; hNTH1, human NTH1; hNEIL1, human NEIL1; mNEIL1, mouse NEIL1; MmuNeil3, mouse Neil3; *Cal*Fpg, *Candida albicans* Fpg; *Ath*Fpg, *Arabidopsis thaliana* Fpg T4-pdg T4-pyrimidine dimer glycosylase; cv-pdg, Chlorella virus pyrimidine dimer glycosylase; *Mv*Nei, *Acanthamoeba polyphaga mimivirus* (Mimivirus) Nei; *Mtu*Fpg, *Mycobacterium tuberculosis* Fpg; *Mtu*Nth, *Mycobacterium tuberculosis* Mth; *Mtu*Nei, *Mycobacterium tuberculosis* Nei.

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http://dx.doi.org/10.1016/j.mrrev.2017.02.001
1383-5742/Published by Elsevier B.V.

1. Introduction

Oxygen metabolism and exogenous sources such as ionizing radiations, UV radiation, redox cycling drugs, carcinogenic compounds, and environmental toxins generate reactive species including free radicals in aerobic organisms [1]. Reactions of such species cause damage to biological molecules including DNA, proteins and lipids, and thus can lead to increased genetic instability, inflammation, proliferation, reduction of antioxidants, cell death, apoptosis and angiogenesis [1–3]. Many of these effects are hallmarks of cancer and predispose individuals to different types of this disease [2–6]. Reactive species can derive from oxygen as well as from nitrogen as free radicals or non-radicals. Four-electron reduction of the oxygen molecule done in stages generates superoxide radical ($O_2^{\bullet -}$), non-radical H_2O_2, hydroxyl radical ($^\bullet$OH) and water in the electron-transport chain [1]. Superoxide radical and H_2O_2 possess very low or intermediate reactivity unless they are converted into $^\bullet$OH by reactions with transition metal ions. Other free radicals such as hydroperoxyl radical ($HO_2^\bullet$) (protonated $O_2^\bullet$), peroxyl radical ($RO_2^\bullet$) and alkoxyl radical ($RO^\bullet$) possess low reactivity. Under physiological conditions, traces of $HO_2^\bullet$ exist in equilibrium with $O_2^\bullet$, which exhibits essentially no reactivity toward DNA components [1]. The singlet oxygens ($^1\Delta gO_2$ and $^1\Sigma g^+O_2$), which are formed from oxygen by an input of energy, are not free radicals, but possess reactivity toward biological molecules. However, $^1\Sigma g^+O_2$ rapidly decays to $^1\Delta gO_2$ and, thus, the latter mainly plays a role in biological systems [1]. Nitric oxide ($NO^\bullet$) is also a free radical with low reactivity. Nevertheless, its reaction with $O_2^\bullet$ is diffusion-controlled [7], yielding fairly unreactive non-radical peroxynitrite (ONOO) whose protonated form peroxynitrous acid (ONOOH) can undergo homolytic fission to yield $^\bullet$OH and $NO_2^\bullet$, which are generated close to each other and can recombine to yield NO_3 and H^+; therefore, the contribution of $^\bullet$OH to the biological damage done by ONOOH (if any) may be small [1]. In reactions with cellular water, ionizing radiations also generate $^\bullet$OH and, in addition, H atom ($H^\bullet$) (also a free radical), hydrated electron (e_{aq}^-), H_2O_2 and H_2 [1,8]. Hydroxyl radical is the most reactive free radical and readily reacts with most biological molecules such as DNA and proteins in living cells [1,8]. In these reactions, a plethora of products from DNA are formed that lead to various biological consequences. These lesions are repaired in living organisms by numerous mechanisms. This article reviews mechanisms of action, substrate specificities and excision kinetics of DNA glycosylases, which are responsible for removal of DNA base lesions in the first step of the base excision repair (BER) mechanism.

2. Oxidatively induced DNA damage

Hydroxyl radical reacts with DNA constituents at or near diffusion-controlled reaction rates [1,8–10]. The second-order rate constants (k) of its reactions with DNA bases amount to 4×10^9 dm^3 mol^{-1} s^{-1} to 9×10^9 dm^3 mol^{-1} s^{-1}. However, the sugar moiety of DNA (2-deoxyribose) and the methyl group of thymine react with $^\bullet$OH by H atom ($H^\bullet$)-abstraction at slower rates of $\approx 2 \times 10^9$ dm^3 mol^{-1} s^{-1}. Addition of $^\bullet$OH to purines generates C4-OH–, C5-OH– and C8-OH–adduct radicals, although the C5-OH–adduct radical of adenine is formed to a lesser extent along with the C2-OH–adduct radical [8,10–14]. Oxidation or reduction reactions of these intermediate adduct radicals in the absence of oxygen generates final products of purines. Oxygen reacts with the OH–adduct radicals of purines at different rates from $k \leq 10^6$ dm^3 mol^{-1} s^{-1} to $k = 4 \times 10^9$ dm^3 mol^{-1} s^{-1} [14,15]. Subsequent reactions of thus-formed peroxyl radicals lead to final products. The yields of the products substantially depend on the presence or absence of oxygen (reviewed in [10]). Among the purine-derived products, 8-hydroxyguanine (8-OH-Gua) (also called 8-oxo-Gua) is readily oxidized by various oxidizing agents due to its low reduction potential (0.74 V) compared to that of guanine (1.29 V) [16], giving rise to the formation of spiroiminohydantoin (Sp) and 5-guanidinohydantoin (Gh) [17–23]. Furthermore, 8-OH-Gua reacts with singlet oxygen, generating oxaluric acid and parabanic