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Base-excision repair of oxidative DNA damage by DNA glycosylases

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Abstract

Oxidative damage to DNA caused by free radicals and other oxidants generate base and sugar damage, strand breaks, clustered sites, tandem lesions and DNA–protein cross-links. Oxidative DNA damage is mainly repaired by base-excision repair in living cells with the involvement of DNA glycosylases in the first step and other enzymes in subsequent steps. DNA glycosylases remove modified bases from DNA, generating an apurinic/apyrimidinic (AP) site. Some of these enzymes that remove oxidatively modified DNA bases also possess AP-lyase activity to cleave DNA at AP sites. DNA glycosylases possess varying substrate specificities, and some of them exhibit cross-activity for removal of both pyrimidine- and purine-derived lesions. Most studies on substrate specificities and excision kinetics of DNA glycosylases were performed using oligonucleotides with a single modified base incorporated at a specific position. Other studies used high-molecular weight DNA containing multiple pyrimidine- and purine-derived lesions. In this case, substrate specificities and excision kinetics were found to be different from those observed with oligonucleotides. This paper reviews substrate specificities and excision kinetics of DNA glycosylases for removal of pyrimidine- and purine-derived lesions in high-molecular weight DNA.
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Keywords: Base-excision repair; DNA glycosylases; Free radicals; Gas chromatography/mass spectrometry; Modified DNA bases; Oxidative DNA damage

1. Introduction

Oxidative damage to DNA is caused by reactive oxygen-derived species including free radicals, most notably the highly reactive hydroxyl radical. In living cells, these species are formed by normal cellular metabolism and by exogenous sources such as ionizing radiations [1]. Oxidative DNA damage includes modified bases and sugars, DNA–protein cross-links, single-and double-strand breaks, base-free sites, tandem lesions such as 8,5′-cyclopurine-2′-deoxyribonucleosides and clustered damaged sites (reviewed in [2–5]). Oxidative DNA damage is repaired in living cells by base-excision repair (BER) mechanisms, and also, to a lesser extent, by nucleotide-

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excision repair (NER) (reviewed in [6–8]). Modified bases are subject to BER, whereas tandem lesions such as 8,5′-cyclopurine 2′-deoxynucleosides are likely to be repaired by NER because of the presence of a covalent bond between the sugar and base moieties of the same nucleoside [9–12]. Unless repaired, oxidative DNA damage may cause detrimental biological consequences in living cells such as cell death and mutations that may increase the genomic instability and thus enhance the risk of the development of cancer and other diseases. There is evidence that such diseases can be prevented not only by avoiding exposure to DNA-damaging agents, but also by modulating defense mechanisms of living organisms such as DNA repair. Thus understanding DNA repair mechanisms is of utmost importance.

BER is highly conserved from bacteria to humans. It is a multiprotein pathway, which is different from NER and mismatch repair in that the substrate specificity depends on diverse DNA glycosylases rather than a multiprotein complex. The first step of BER is the removal of a modified base from DNA by a DNA glycosylase that hydrolyzes the *N*-glycosidic bond between the modified base and the sugar moiety, releasing the modified base and generating an apurinic/apyrimidinic (AP) site. Some DNA glycosylases also possess an associated AP-lyase activity that hydrolyzes the 3′-phosphodiester bond of the AP site by a β- or β-δ-elimination mechanism generating 3′ α,β-unsaturated aldehyde and 5′-phosphate products. Generally, the lyase action is associated with DNA glycosylases specific for oxidatively modified DNA bases, and not with those that, e.g., remove alkylated bases. DNA glycosylase reactions are followed by two subpathways named short patch and long patch that process the AP sites by AP endonucleases, polymerases and ligases. It is thought that short patch is initiated by DNA glycosylases, whereas AP sites resulting from spontaneous hydrolysis of bases are repaired by long patch (reviewed in [6,8]). Numerous prokaryotic and eukaryotic DNA glycosylases have been isolated and purified. Their substrate specificities have been determined using various types of substrates. The crystal structure of numerous DNA glycosylases has been solved [13]. Mechanisms of action of DNA glycosylases supported by biochemical and structural data have been proposed (reviewed in [6,14]).

The determination of substrate specificities of DNA glycosylases has been performed in general using oligonucleotides with a single modified base incorporated at a specific position. The use of such oligonucleotides and analytical techniques limited to analysis of only one product permitted the investigation of excision of one modified base at a time. Almost two decades ago, we proposed a different concept for the investigation of substrate specificities of DNA glycosylases [15], with the use of gas chromatography/mass spectrometry (GC/MS) and damaged DNA substrates containing numerous modified bases instead of oligonucleotides with a single lesion. Since then, substrate specificities and excision kinetics of numerous DNA glycosylases have extensively been investigated. This paper reviews these studies.

2. Substrate specificities and excision kinetics of DNA glycosylases

Oxidative damage to DNA by free radicals such as hydroxyl radical generated by ionizing radiation or by metal ion-catalyzed reactions of superoxide radical and hydrogen peroxide causes formation of a plethora of products from all DNA bases. Fig. 1 illustrates the major base products in DNA including 8,5′-cyclopurine 2′-deoxynucleosides, which represent a concomitant damage to both the base and sugar moieties of the same nucleoside. These products can concurrently be identified and quantified in a given DNA sample by GC/MS with the isotope-dilution mode (reviewed in [16]). This ability of GC/MS permits the identification and quantification of lesions that are excised or are not excised from damaged DNA by a given DNA glycosylase. Excision rates are also measured simultaneously. Experiments include the preparation of damaged DNA samples in the first step followed by treatment with the DNA glycosylase, and then precipitation of DNA with ethanol. Supernatant and pellet fractions are separated and analyzed by GC/MS [17]. Fig. 2 illustrates the reaction scheme. Supernatant fractions are analyzed without hydrolysis because they contain putative modified bases, which are released by the DNA glycosylase. As controls, DNA samples are incubated without the enzyme or with the heat-deactivated enzyme. The results of a typical experiment are shown in Fig. 3.