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Review

Recent advances in the structural mechanisms of DNA glycosylases

Sonja C. Brooks, Suraj Adhikary, Emily H. Rubinson, Brandt F. Eichman *

Department of Biological Sciences and Center for Structural Biology, Vanderbilt University, Nashville, TN 37232, USA

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ABSTRACT

DNA glycosylases safeguard the genome by locating and excising a diverse array of aberrant nucleobases created from oxidation, alkylation, and deamination of DNA. Since the discovery 28 years ago that these enzymes employ a base flipping mechanism to trap their substrates, six different protein architectures have been identified to perform the same basic task. Work over the past several years has unraveled details for how the various DNA glycosylases survey DNA, detect damage within the duplex, select for the correct modification, and catalyze base excision. Here, we provide a broad overview of these latest advances in glycosylase mechanisms gleaned from structural enzymology, highlighting features common to all glycosylases as well as key differences that define their particular substrate specificities.

1. Introduction

The integrity of the chemical structure of DNA and its interactions with replication and transcription machinery is important for the faithful transmission and interpretation of genetic information. Oxidation, alkylation, and deamination of the nucleobases by a number of endogenous and exogenous agents create aberrant nucleobases (Fig. 1) that alter normal cell progression, cause mutations and genomic instability, and can lead to a number of diseases including cancer [reviewed in 1]. Many of these lesions are removed by the base excision repair (BER) pathway [2], which is initiated by a DNA glycosylase specialized for a particular type of chemical damage. Upon locating a particular lesion within the DNA, glycosylases catalyze the excision of the nucleobase from the phosphoribose backbone by cleaving the *N*-glycosidic bond, generating an apurinic/apyrimidinic (AP) site (Fig. 2). Monofunctional glycosylases catalyze only base excision, whereas bifunctional glycosylases also contain a lyase activity that cleaves the backbone immediately 3′ to the AP site. The resulting single-stranded and nicked AP sites are processed by AP endonuclease 1 (APE1), which hydrolyzes the phosphodiester bond 5′ to the AP site. This generates a 3′ hydroxyl substrate for replacement synthesis by DNA polymerase β, followed by sealing of the resulting nick by DNA ligase.

Since the glycosylases are the first line of defense against a vast array of DNA damage, they have been the subject of a large body of work to understand their mechanisms of action and cellular roles [3–12]. The first crystal structures of DNA glycosylases were reported in 1992 for bacteriophage T4 Endonuclease V (EndoV) and *Escherichia coli* (*E. coli*) Endonuclease III (EndoIII), which remove pyrimidine dimers and oxidized pyrimidines, respectively [13,14]. Soon thereafter, DNA or inhibitor-bound structures of EndoV and uracil DNA glycosylase (UDG) established that these enzymes use a base-flipping mechanism to gain access to modified nucleobases in DNA [15–19]. Subsequent studies established that glycosylases fall into one of six structural superfamilies (Fig. 3). Despite their divergent architectures, these proteins, with the exception of the ALK family (see Section 3.3) [12], have evolved the base-flipping strategy to correctly identify and orient their substrates for catalysis. Recognition of the target modification likely proceeds in several steps, in which the protein probes the stability of the base pairs through processive interrogation of the DNA duplex, followed by extrusion of the aberrant nucleobase into a specific active site pocket on the enzyme [9,20]. The enzyme-substrate complex is stabilized by nucleobase contacts within the active site and a pair of side chains that plug the gap in the DNA left by the extrahelical nucleotide and wedge into the DNA base stack on the opposite strand [3–12].

In this review, we focus on the most recent advances toward understanding the mechanisms by which each class of DNA glycosylase locates, selects, and removes its target lesions. A growing number of structures and mechanistic studies of glycosylases specific for oxidized nucleobases (Section 2), alkylation damage (Section 3), and cytosine deamination products (Section 4) have elucidated many of the structural determinants of substrate specificity and have provided new insights into catalysis of *N*-glycosidic bond cleavage. Some aspects of substrate selection and excision are common across different structural classes or substrate specificities, while others are specific to a given enzyme. Our goal in this review, therefore, is to provide a broad overview of the structural mechanisms for the entire

* Corresponding author. Tel.: +1 615 936 5233; fax: +1 615 936 2211.
E-mail address: brandt.eichman@vanderbilt.edu (B.F. Eichman).

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repertoire of DNA glycosylases in order to highlight key similarities and differences between each structural class. We note that the roles of DNA glycosylases in the cell and in the context of BER have been the subject of recent reviews, and thus we focus our discussion on the structural enzymology.

2. Oxidative damage

DNA bases undergo oxidative damage from chemical oxidants, free radicals and reactive oxygen species (ROS) produced from cellular respiration, inflammatory responses, and ionizing radiation [21–23]. Oxidized bases are often used as biomarkers for oxidative stress and cancer [22,24]. Guanines are especially susceptible to oxidation, leading to a number of lesions that are substrates for BER (Fig. 1A) [25]. Attack of a hydroxyl radical at the C8 position of guanine produces 7,8-dihydro-8-hydroxyguanine (8-OHG), which tautomerizes to 8-oxo-7,8-dihydroguanine (8oxoG), or the ring-opened 2,6-diamino-5-formamido-4-hydroxy-pyrimidine (FapyG), two of the most abundant oxidative DNA adducts [26,27]. 8oxoG is a particularly insidious lesion because of its dual coding potential by replicative polymerases, leading to G→T transversion mutations likely as a result of its ability to form both 8oxoG(syn)•A(anti) and 8oxoG(anti)•C(anti) base pairs [22,23,28–30]. Oxidation of guanine and 8oxoG also produces a variety of ring-opened purines in addition to FapyG, including hydantoin lesions, spiroiminodihydantoin (Sp), guanidinohydantoin (Gh), and its isomer iminoallantoin (Ia) (Fig. 1A) [31–33]. Fapy lesions inhibit DNA polymerases and are potentially mutagenic [34]. Hydantoin lesions have been suggested to lead to an increase in G→T and G→C transversions and stall the replication machinery [31,32,35,36]. In addition to purines, reaction of hydroxyl radicals at positions 5 or 6 of thymine produces 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol, Tg), a cytotoxic lesion that distorts the DNA duplex and can inhibit replication [26,37]. Other potentially harmful pyrimidines include dihydrothymine (DHT), dihydrouracil (DHU), 5-hydroxyuracil (5-OHU), 5-hydroxycytosine (5-OHC), 5-hydroxymethyluracil (5hmU), and 5-formyluracil (5fU) [38–43].

DNA glycosylases that remove oxidative DNA damage can be categorized on the basis of their preferences for purine or pyrimidine lesions and their structural folds (Table 1). Oxidized purines, including 8oxoG and FapyG, are removed from DNA by 8oxoG DNA glycosylase (OGG1) in eukaryotes and MutM (also known as FapyG DNA glycosylase, Fpg) in bacteria [recently reviewed in 23]. Oxidized pyrimidines are removed by endonuclease III (EndoIII, or Nth) and endonuclease VIII (Endo VIII, or Nei), and their eukaryotic orthologs, NTH1 and NEIL1 (Nei-like1), respectively. Despite their different substrates, OGG1 and EndoIII/Nth adopt a common architecture characteristic of the Helix–hairpin–Helix (HhH) superfamily of DNA glycosylases [44]. MutM/Fpg and EndoVIII/Nei are also structurally similar, with helix-two turn-helix (H2TH) and antiparallel β-hairpin zinc finger motifs, and they share a common bifunctional catalytic mechanism involving both base excision and AP lyase activities [45–49].

2.1. 8oxoG repair

Eukaryotic OGG1 and bacterial MutM/Fpg preferentially catalyze removal of 8oxoG paired with C [50,51]. Both enzymes are bifunctional in

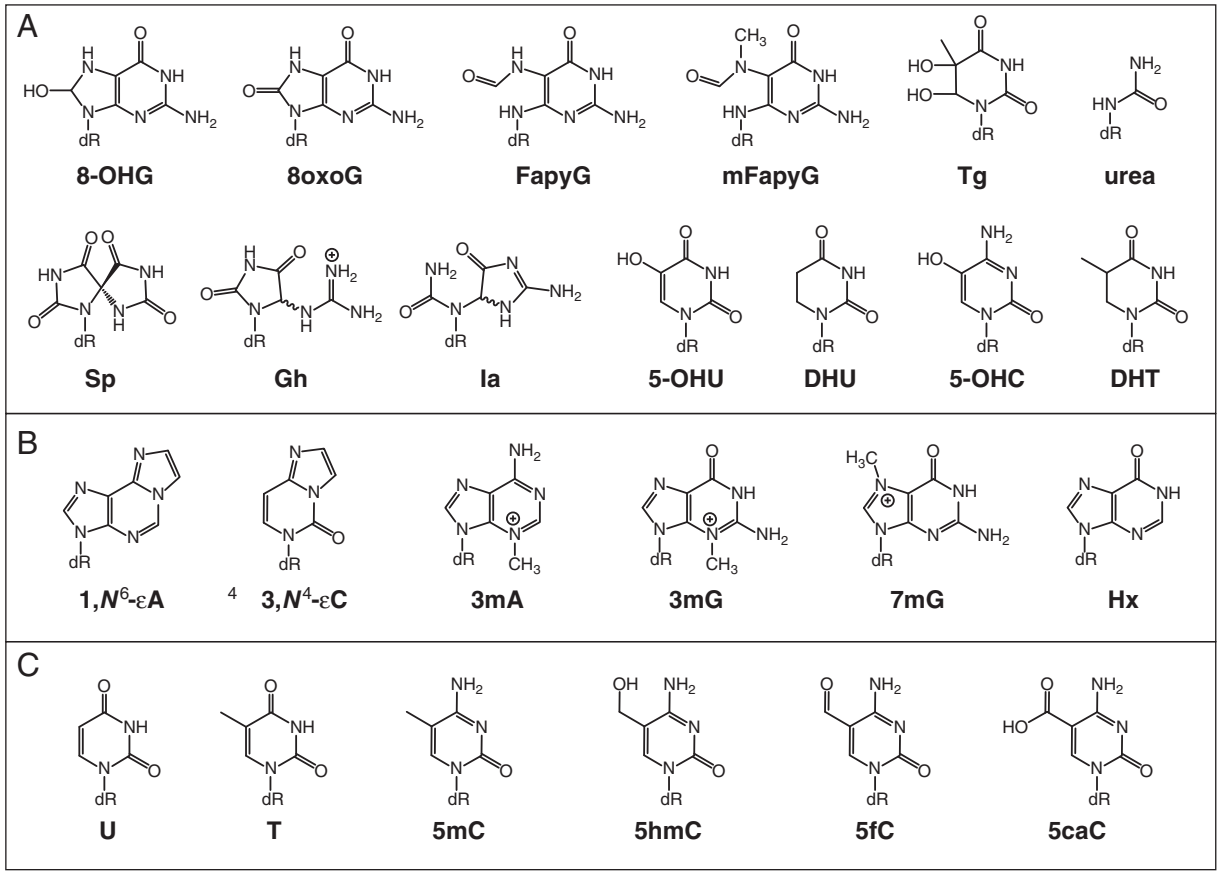

Fig. 1. Common DNA lesions referenced in this review. (A) Oxidized nucleobases. 8-OHG, 7,8-dihydro-8-hydroxyguanine; 8oxoG, 8-oxo-7,8-dihydroguanine; FapyG, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; mFapyG, *N*7-methylFapyG; Tg, thymine glycol; Sp, spiroiminodihydantoin; Gh, guanidinohydantoin; Ia, iminoallantion; 5-OHU, 5-hydroxyuracil; DHU, dihydrouracil; 5-OHC, 5-hydroxycytosine; DHT, dihydrothymine. (B) Alkylated nucleobases. εA, 1,N^6-ethenoadenine; εC, 3,N^4-ethenocytosine; 3mA, *N*3-methyladenine; 3mG, *N*3-methylguanine; 7mG, *N*7-methylguanine; Hx, hypoxanthine. (C) Nucleobases repaired by the UDG/TDG family of DNA glycosylases. U, uracil; T, thymine; 5mC, 5-methylcytosine; 5hmC, 5-hydroxymethylcytosine; 5fC, 5-formylcytosine; 5caC, 5-carboxylcytosine.