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Minireview

DNA polymerase structure-based insight on the mutagenic properties of 8-oxoguanine

William A. Beard, Vinod K. Batra, Samuel H. Wilson*

Laboratory of Structural Biology, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC 27709, USA

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ABSTRACT

An aerobic environment burdens DNA polymerase substrates with oxidized substrates (DNA and nucleotide pools). A major promutagenic lesion resulting from oxidative stress is 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxoG). Guanine oxidation alters the hydrogen bonding properties of the base and glycosidic-preference of the nucleotide. The favored glycosidic *syn*-conformation exposes the Hoogsteen edge of the base for hydrogen bonding with adenine during DNA synthesis. The cell has recognized the threat of this lesion and has evolved an intricate surveillance system to provide DNA polymerases with unmodified substrates. Failure to do so leads to transversion mutations. Since the mutagenic properties of the base are dictated by the *anti–syn*-conformation of the nucleotide, the molecular interactions of 8-oxoG in the confines of the DNA polymerase active site are expected to influence its coding potential. Recent structural characterization of DNA polymerases from several families with this lesion in the nascent base pair binding pocket has provided insight to the mutagenic properties of this modified nucleotide. These structures reveal that flexibility around the template-binding pocket can permit 8-oxoG to assume an *anti*- or *syn*-conformation and code for cytosine or adenine incorporation, respectively. In contrast, the binding pocket for the incoming nucleotide does not have this flexibility so that 8-oxodGTP insertion opposite cytosine is strongly discouraged.

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1. Introduction

Oxidative stress leads to the production of multiple toxic chemicals that can threaten the integrity of genomic DNA, ultimately leading to mutations and chromosome instability. These events are known to be adverse for cells and are considered to be extremely important in the balance between human health and disease, especially regarding chronic conditions and diseases such as cancer and aging [1]. Organisms throughout nature maintain elaborate and diverse mechanisms for protecting themselves against the adverse genotoxic consequences of oxidative stress. A major lesion found in DNA and dNTP pools exposed to reactive oxygen species (ROS) is the promutagenic lesion 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxoG or Go). Elegant biochemical and genetic

* Corresponding author. Tel.: +1 919 541 4701; fax: +1 919 541 4724.
E-mail address: wilson5@niehs.nih.gov (S.H. Wilson).

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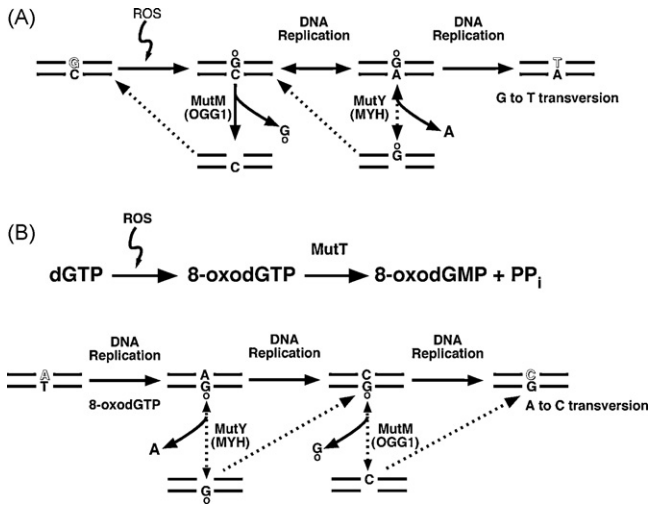

Fig. 1. DNA repair and replication of 8-oxoG: the GO system. (A) The repair of the oxidized base, 8-oxoG, in DNA is initiated by a DNA damage-specific glycosylase. *E. coli* MutM (human homolog, OGG1) removes 8-oxoG paired with cytosine to purify the genome of this oxidized base. During DNA replication, unrepaired 8-oxoG can code for dCMP or dAMP. To remove misinserted adenines residues, *E. coli* MutY (human homolog, MYH) initiates BER by removing the inappropriate adenine. DNA polymerase β gap-filling DNA synthesis (dashed lines) will result in a DNA substrate for MutM (8-oxoG–C) or MutY (8-oxoG–A). Replication of the unrepaired adenine-containing strand results in a G to T transversion. (B) *E. coli* MutT (human homolog, MTH1) is an 8-oxodGTPase that cleanses the dNTP pools of this oxidized nucleotide. Failure to remove 8-oxoG that has been misinserted opposite A results in an A to C tranversion. As above, pol β gap-filling DNA synthesis (dashed lines) will result in a DNA substrate for MutM (8-oxoG–C) or MutY (8-oxoG–A).

studies in *E. coli* and yeast defined key elements of a multifaceted cellular defense system to reduce 8-oxoG mutagenesis and is often referred to as the "GO system" (Fig. 1). Importantly, the conserved nature of this system underscores the deleterious nature of this lesion [2–7].

The early genetic and biochemical studies of cellular mutagenesis associated with oxidative stress led to the discovery in 1983 by Nishimura and associates of the oxidized deoxynucleotide called 8-oxoG [7]. This early work led to an understanding of the special coding property of 8-oxoG when acting as a substrate (template DNA or incoming nucleotide) for the replicative and repair DNA polymerases. Thus, oxidative stress leads to production of 8-oxodGTP in the dNTP pool and 8-oxoG in DNA. Since 8-oxoG is found to base pair with adenine in addition to cytosine, an explanation for the mutagenic consequences of 8-oxoG becomes apparent. The discovery of three *E. coli* genes, known as MutM, MutY and MutT, that comprised the original GO system, along with the subsequent characterization of their gene products, were instrumental in sparking the development of a research field focused on the repair of simple base lesions, base excision repair (BER). Finally, the BER process and components of the GO system are conserved from microorganisms to higher organisms, including humans [6,7]. Knowledge of the impact of oxidative stress on human health and, in particular, the pathobiology of chronic disease such as cancer [8], is continuing to emerge, but its importance may be even greater than we currently appreciate.

Because the mutagenic effects of 8-oxoG are mediated by the action of DNA polymerases, structural studies of DNA polymerases with 8-oxoG provide insight into DNA polymerase substrate specificity (fidelity and mutagenesis) and mechanism. Similarly, studies of the GO system link our understanding of BER to mutagenesis and human health. These linkages have enhanced enthusiasm for medical research on DNA polymerase mechanism and BER, and both areas have enjoyed considerable growth in recent years.

2. Mutagenesis and the GO system

2.1. Oxidative DNA damage

DNA is under continual threat of attack by ROS generated during aerobic respiration. In the initial step, oxygen radicals generated by oxidative stress produce the 8-oxoG lesion in DNA. Features of the *E. coli* GO system as it relates to the repair of 8-oxoG in DNA are illustrated in Fig. 1A. This scheme depicts a model where the oxidized base is removed by a damage-specific DNA glycosylase (*E. coli* MutM, human OGG1) thereby initiating BER. If 8-oxoG escapes repair and a replicative DNA polymerase misinserts adenine opposite 8-oxoG, an alternate BER pathway is initiated by a DNA glycosylase (*E. coli* MutY, human MYH) that will remove the inappropriate adenine. DNA polymerase (pol) β gap-filling DNA synthesis will result in a DNA substrate for OGG1 (8-oxoG–C) or MYH (8-oxoG–A). Thus, pol β can maintain the lesion-containing mutagenic base pair by inserting A during BER (as illustrated by the double-headed arrow in Fig. 1A). Therefore, BER in general and DNA synthesis by pol β, are viewed as important modulators in the mammalian GO system. Replication of the unrepaired adenine-containing strand results in a G to T transversion.

2.2. 8-OxodGTPase (MutT)

Features of another aspect of the *E. coli* GO system are summarized in Fig. 1B. This scheme depicts a model for the role of 8-oxodGTP in generating an A to C transversion mutation as a function of DNA replication and BER. *E. coli* MutT (human homolog, MTH1) is an 8-oxodGTPase that is responsible for cleansing the dNTP pool of this mutagenic nucleotide. Cellular events that modulate the activity of the MutT enzyme, leading to increased levels of 8-oxodGTP, are known to have a large effect in boosting mutagenesis in *E. coli* [9]. Genetic studies in *E. coli* suggest that 8-oxodGTP misincorporation is significant even in the presence of MutT [10]. If 8-oxodGTP is utilized during DNA replication, it has a high probability of being inserted opposite adenine [11]. Once 8-oxodGTP is incorporated opposite adenine, BER cannot correct this aberrant base pair, but could be involved in the stable fixation of the A to C tranversion mutation (Fig. 1B).

3. Base pairing properties of 8-oxoG

At physiological pH, the major tautomeric form of 8-oxoG has a carbonyl group at C8 and is protonated on N7 [12,13]. Thus, guanine oxidation results in altering the hydrogen bonding capacity of its Hoogsteen edge. Whereas the unmodified deoxyguanine glycosidic torsion angle preference is *anti*, 8-oxoG favors a *syn*-conformation that can form a Hoogsteen base pair with adenine (Fig. 2). The altered glycosidic torsion angle preference is due to steric repulsion between O8 and deoxyribose [14]. Structural characterization of duplex DNA containing 8-oxoG indicates that the glycosidic torsion angle preference is determined by its base pairing partner; being *anti* with a complementary cytosine [15,16] and *syn* when base-paired with adenine [17,18].

Although the 8-oxoG (*syn*)—A (*anti*) base pair does not exhibit Watson-Crick hydrogen bonding, this mispair is well-accommodated within duplex DNA [18]. Likewise, the hydrogen bond acceptors positioned in the DNA minor groove resemble those for a T–A base pair where the carbonyl at C8 of 8-oxoG is predicted to be positioned where the carbonyl of C2 of thymidine would be located [19]. Thus, polymerase-dependent minor groove hydrogen bond scanning would not identify this mutagenic base pair as a substrate for proofreading exonucleases.