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Hydrogen Bonding Contributes to the Selectivity of Nucleotide Incorporation Opposite an Oxidized Abasic Lesion

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Hydrogen Bonding Contributes to the Selectivity of Nucleotide Incorporation Opposite an Oxidized Abasic Lesion

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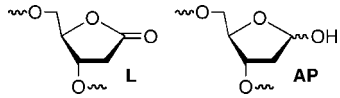
The ability of DNA polymerases to maintain the integrity of the genome by reading a template and deciding which nucleotide to incorporate even after it has been structurally altered is vital.[1–3] Mechanistic, synthetic, and structural chemistries have been used to study how nucleotide incorporation by DNA polymerases is affected by size, shape, π-stacking, and hydrogen bonds.[4–7] The complexity of the problem is increased by the existence of multiple families of polymerases with differing chemical roles and structures.[1] We are interested in determining how DNA polymerases determine which nucleotide to incorporate opposite the genotoxic family of abasic lesions (e.g., AP, L). Because they lack a Watson−Crick base to direct nucleotide incorporation opposite them, AP sites have been categorized as noninstructive lesions.[8,9] The "A-rule" has been invoked to rationalize why dA is predominantly incorporated opposite AP sites in *Escherichia coli*. However, recent studies on oxidized abasic sites, such as 2-deoxyibonolactone (L), revealed that polymerase bypass of each lesion is distinctive.[10,11] Herein we describe the utilization of abasic site analogues that indicate that hydrogen bond formation between the incoming nucleotide and 2-deoxyribonolactone (L) plays a role in its bypass.

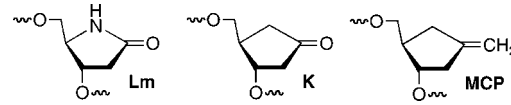


Abasic sites (AP) arise spontaneously but are also formed as intermediates during base excision repair and when DNA is damaged. Oxidized abasic lesions (e.g., L) are produced in significant quantities when DNA is exposed to agents such as hydroxyl radical (OH•) and some antitumor agents.[12,13] Unlike AP, DNA polymerase bypass of L in *E. coli* does not adhere to the A-rule.[10] High levels of dG incorporation opposite L indicated that it is instructive despite lacking a Watson−Crick base. In trying to determine why increased levels of dG were incorporated opposite L, we focused on the presence of a hydrogen bond acceptor at C1 in L compared to a hydroxyl group in AP. Consequently, we prepared oligonucleotides containing AP, L, and a variety of analogues designed to test the influence of the C1 carbonyl group on dG incorporation opposite an abasic lesion.[14–16]

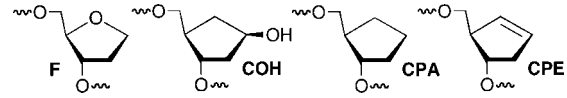
The lactam (Lm) and ketone (K) probes were designed to test whether the presence of a carbonyl group at C1 was sufficient to direct dG incorporation opposite L (Scheme 1). The methylenecyclopentane (MCP) was intended to approximate the overall shape and size of 2-deoxyribonolactone. The tetrahydrofuran (F) is a common chemically stable AP analogue that serves as a suitable model for the abasic site's bypass in *E. coli*.[17] In addition to F, we examined three other molecules as models of an AP site (Scheme 2).

Single-stranded genomes containing one of the nine abasic sites or thymidine in two sequence contexts (5′-TXG, 5′-CXG; X = abasic site) were prepared via reported methods.[18,19] The genomes were used to transfect *E. coli* in which an SOS response was induced, resulting in the up regulation of bypass polymerases (Pol II, Pol IV, and Pol V). Bypass efficiencies of the abasic sites were determined by comparing the number of colonies produced on media plates by cells transfected with genome containing an abasic site to those from cells transfected with plasmid containing thymidine in place of the lesion. The bypass efficiencies ranged from ~9 to 17%.[19] The data reveal that the abasic lesions inhibit polymerases and are consistent with previous reports for abasic lesions in SOS-induced cells.[17] No bypass was observed in cells lacking Pol II, Pol IV, and Pol V, indicating that one or more were necessary for reading through the abasic sites.

Scheme 1. 2-Deoxyribonolactone (L) Analogues



Scheme 2. Abasic Site (AP) Analogues



The nucleotide incorporation frequencies opposite the abasic sites were determined quantitatively using the restriction endonuclease and postlabeling (REAP) assay.[18] In the REAP assay, the progeny produced by replicating the genome containing the lesion of interest are recovered, and through a series of enzymatic manipulations, the nucleotide incorporated opposite the lesion is ultimately determined by quantifying the radiolabeled nucleotides that are separated by thin layer chromatography. In addition, the REAP assay enables one to measure the relative amounts of full-length replication products and frameshift products (e.g., single nucleotide deletions) in a single experiment.

When nucleotide incorporation opposite the nine abasic sites was analyzed, dA and dG accounted for >85% of the events in both sequence contexts (Figures 1 and 2). Bypass of AP and its analogues (Scheme 2) adhered to the A-rule when they were present in the 5′-TXG (Figure 1A) or 5′-CXG (Figure 1B) sequence. In each instance, dA was incorporated opposite the abasic site >77%

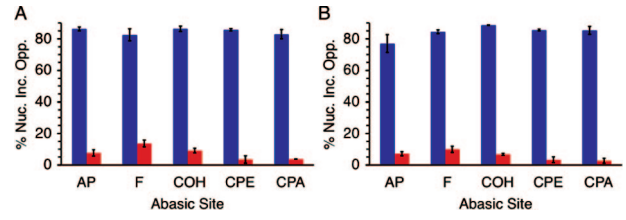


Figure 1. REAP analysis of nucleotide incorporation opposite AP and its analogues (A) 5′-TXG sequence and (B) 5′-CXG sequence. dA, blue; dG, red. The data are a minimum of two independent experiments in triplicate.

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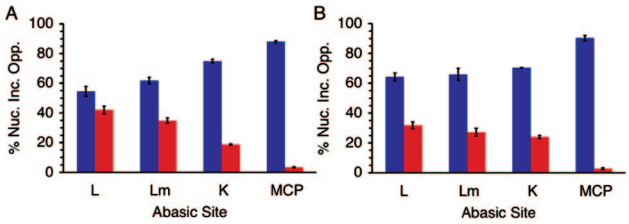

Figure 2. REAP analysis of nucleotide incorporation opposite L and its analogues (A) 5′-TXG sequence and (B) 5′-CXG sequence. dA, blue; dG, red. The data are a minimum of three independent experiments.

of the time and in only one instance (5′-TFG, 13.7%, Figure 1A) did dG incorporation account for more than 10% of the bypass events.

Significantly higher levels of dG were incorporated opposite 2-deoxyribonolactone (L) and its carbonyl containing analogues (Figure 2). The differing levels of dG incorporation suggest that other structural properties in addition to the carbonyl group contribute to determining nucleotide incorporation opposite L, Lm, and K. In addition, L and Lm give rise to significant amounts of single nucleotide deletions, but all three carbonyl containing analogues produce larger amounts of deletions in the 5′-CXG than 5′-TXG sequence.[19] When a carbonyl containing abasic site was flanked by dC on the 5′-side, dG incorporation (Figure 2A) opposite L and Lm was lower than in the 5′-TXG context. The flanking sequence effect on dG incorporation was consistent with the previously proposed nucleotide incorporation−misalignment mechanism to explain L bypass in noninduced cells.[10] The variation in single nucleotide deletions and the levels of dG incorporation opposite L, Lm, and K indicate that the oxygen atom in the ring and the ring's conformation may play modest roles in bypass. In contrast, replication of genomes containing the methylenecyclopentane (MCP) in which dG incorporation was less than 5% emphasizes the importance of the hydrogen bond acceptor in the increased dG incorporation.

The identity of the DNA polymerase(s) responsible for bypassing L, Lm, and K was determined by carrying out experiments in *E. coli* in which a single bypass polymerase was deleted.[19] Deleting Pol II or Pol IV had little effect on nucleotide, bypass efficiency, or ratio of full-length to single nucleotide deletion products. However, modest increases in dG incorporation opposite the abasic sites were observed. The effect of Pol V on L, Lm, and K bypass was more pronounced. Although L and Lm bypass was of comparable efficiency as in wild-type cells, the bypass efficiency past K was <1% in Pol V deficient *E. coli* and was not investigated further. Bypass of L and Lm in Pol V deficient *E. coli* produced single nucleotide deletion products exclusively, which REAP analyses indicate result from skipping over the abasic site. In contrast, bypass of L and Lm when Pol V was present produced between ∼5 and ∼37% of single nucleotide deletions depending upon the flanking sequence. These data indicate that, although the carbonyl containing abasic sites clearly provide different instructions to Pol V regarding translesion nucleotide incorporation than do AP and F, the polymerase's role in producing full-length bypass products from templates containing the abasic site is comparable.[17]

Having established that the carbonyl group in 2-deoxyribonolactone (L) plays a central role in directing a bypass polymerase to incorporate dG opposite it, we sought to model such an interaction. Hydrogen bonds were observed between L and the N1 and N2 hydrogens of 9-methylguanine (MeG, Figure 3A). A DNA−Pol V cocrystal structure is unavailable. Hence, we examined the viability of such an interaction during L bypass by

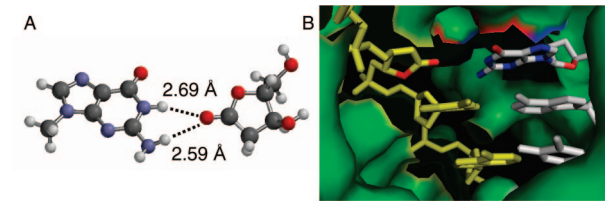

Figure 3. (A) Molecular modeling of L:MeG using constraints based upon the respective distances in a related DNA crystal structure.[19] (B) Model of the Dpo4 active site containing the L:ddGDP pair based on the crystal structure (PDB ID: 1JX4).

substituting L and ddGDP (using the coordinates from Figure 3A) for T and ddADP in a cocrystal structure of a different Y family polymerase, Dpo4 (Figure 3B). The distance between the C1 carbonyl oxygen of L and N1 (3.58 Å) and N2 nitrogens (3.48 Å) of ddGDP in this static picture was ∼0.6 Å greater than a typical hydrogen bond. However, the corresponding protons and oxygen were within van der Waals contact with one another. This simplistic modeling suggests that, in the dynamic situation of polymerase extension, an instructive interaction between dG and the carbonyl of 2-deoxyribonolactone is possible.

Pol V is a Y family DNA polymerase that is responsible for bypassing a variety of DNA lesions in *E. coli*. The Y family DNA polymerases have flexible active sites that enable them to accept a variety of DNA lesions.[2] The extent that hydrogen bonding between a DNA template and an incoming nucleotide contributes to these polymerases' nucleotide incorporation selectivity is an ongoing discussion.[4,6] These experiments indicate that hydrogen bonding contributes to determining nucleotide incorporation opposite a DNA lesion by Pol V.

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Supporting Information Available: All experimental procedures, MS of oligonucleotides, and cellular analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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