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DNA Polymerase V Kinetics Support the Instructive Nature of an Oxidized Abasic Lesion in *Escherichia coli*

John Ernest V. Bajacan and Marc M. Greenberg*

Department of Chemistry, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, United States

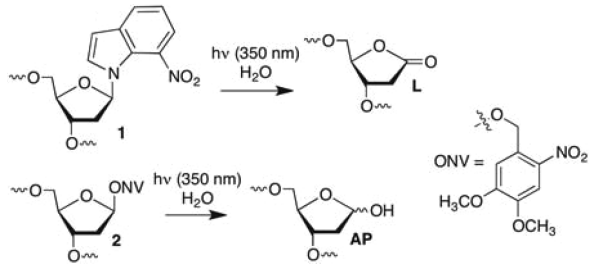
Supporting Information

ABSTRACT: Translesion synthesis past an oxidized abasic site, 2-deoxyribonolactone, in *Escherichia coli* results in high levels of dG incorporation and is dependent upon DNA polymerase V (Pol V). Kinetic experiments performed here affirm that Pol V preferentially incorporates dG opposite 2-deoxyribonolactone (L). Pol V discriminates between dG and dA on the basis of the apparent K_D, suggesting that L provides instructive structural information to the enzyme despite lacking a Watson−Crick base.

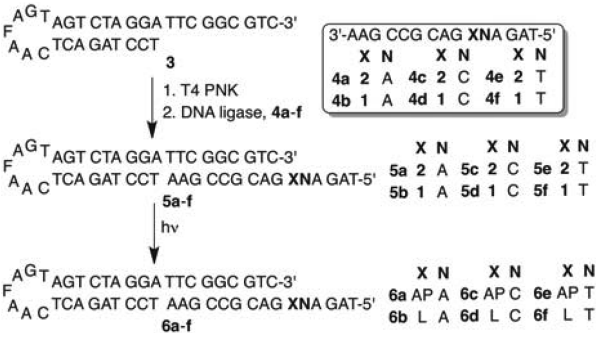
Apurinic/apyrimidinic abasic sites lack a nucleobase required for forming Watson−Crick base pairs and have been characterized as noninstructive lesions when polymerases encounter them in DNA templates during replication. Preferential incorporation of dA opposite an abasic site (AP) is ascribed to the "A-rule".[1,2] The A-rule was also invoked to explain *in vitro* studies of 2-deoxyribonolactone (L).[3] Recent investigations of the replicative bypass of various abasic lesions in *Escherichia coli* using shuttle vectors revealed that only AP abides by the A-rule.[4−6] In particular, 2-deoxyribonolactone, which is produced by several potent antitumor agents, induces as much as 42% dG incorporation opposite the lesion in *E. coli* under SOS conditions.[7] Mutagenesis studies in *E. coli* of a series of structural analogues of L led to the proposal that hydrogen bonding between an incoming dG and lactone carbonyl contributes to increased dG incorporation levels.[7] Experiments in various DNA polymerase-deficient cells suggest that DNA polymerase V (Pol V) plays a pivotal role in the incorporation of dG opposite L.[7] Herein, we report on pre-steady state kinetic experiments that probe the discrimination between dG and dA by complexes of Pol V and templates containing L or AP.

Because the 5′-dC or -T in mutagenicity studies of L could influence translesion dG incorporation via a misalignment process,[4,7] hairpin DNA templates containing a photochemical precursor to either AP or L (Scheme 1) flanked by a 5′-adjacent dA, dC, or T were prepared (**5a−f** in Scheme 2). Their syntheses were completed by ligation of an oligonucleotide containing a photochemical precursor to AP or L (**4a−f**) with **3** that contained a fluoresceinylated serinol (F) in the loop region (Scheme 2). Ligating chemically synthesized oligonucleotides containing lesion precursors (**4a−f**) facilitated purification of the modified DNA. These oligonucleotides were synthesized as previously described.[8,9] The full-length ligated hairpins were purified by gel electrophoresis. Photolysis immediately prior to kinetic experiments provided hairpins containing AP or L (**6a−f**). Hairpins used in extension experiments (**7a−d**) were prepared similarly. Pol V was isolated and its active concentration determined as previously described.[10,11]

Scheme 1



Scheme 2



Pre-steady state experiments were performed with Pol V present in a 20-fold excess over the DNA template. Product formation (in triplicate) as a function of time was fit to a single exponential {% product = $A[1 - \exp(-k_{obs}t)]$, where A is the reaction amplitude and t the time} to determine k_{obs} in the presence of a range of dATP or dGTP concentrations. Fitting k_{obs} versus [dNTP] to the hyperbolic equation $k_{obs} = (k_{Pol}[dNTP])/([dNTP] + K_{D,app})$ (where $K_{D,app}$ is the apparent K_D) provided the reaction parameters k_{Pol} and $K_{D,app}$ (Table 1).

Apparent dNTP binding ($K_{D,app}$) varied over a wider range than did k_{Pol}. Although dGTP was consistently bound more strongly than dATP in the presence of both lesions, the preference for the former was consistently greater when 2-deoxyribonolactone was present in the template (Figure 1A). Whereas L preferred dGTP over dATP by 2.4−5-fold, only a

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Table 1. Kinetic Constants for Translesion Synthesis opposite AP and L by Pol V[a]

template	lesion	5′-adjacent dN	dNTP	$K_{D,app}$ (μM)	k_{Pol} (min^{-1})	$k_{Pol}/K_{D,app}$ ($\times 10^{-4}$ μM^{-1} min^{-1})
6a	AP	A	A	899 ± 160	0.60 ± 0.18	6.5 ± 1.0
6a	AP	A	G	734 ± 263	0.23 ± 0.07	3.2 ± 0.7
6b	L	A	A	1257 ± 460	0.43 ± 0.17	3.4 ± 1.1
6b	L	A	G	523 ± 33	0.41 ± 0.12	7.8 ± 2.2
6c	AP	C	A	1120 ± 211	0.58 ± 0.20	5.1 ± 0.9
6c	AP	C	G	658 ± 83	0.23 ± 0.07	3.6 ± 1.3
6d	L	C	A	2134 ± 386	0.59 ± 0.12	2.7 ± 0.8
6d	L	C	G	883 ± 1	0.55 ± 0.09	6.2 ± 1.0
6e	AP	T	A	777 ± 36	0.45 ± 0.03	6.9 ± 2.7
6e	AP	T	G	470 ± 157	0.18 ± 0.05	3.6 ± 0.9
6f	L	T	A	976 ± 100	0.56 ± 0.09	5.7 ± 0.3
6f	L	T	G	210 ± 29	0.31 ± 0.07	15.2 ± 5.2

[a]Values presented are averages ± the standard deviation of at least two experiments, each consisting of three replicates.

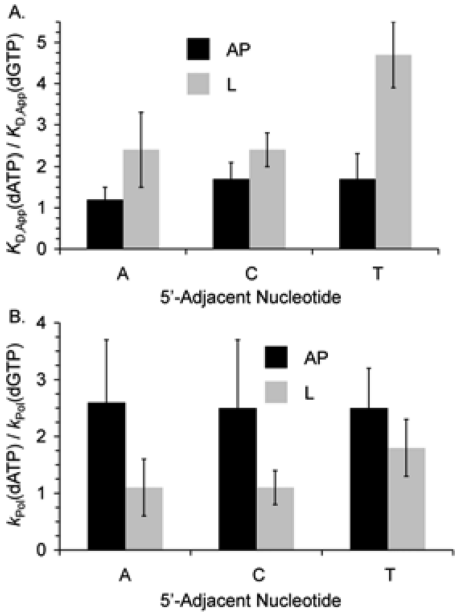
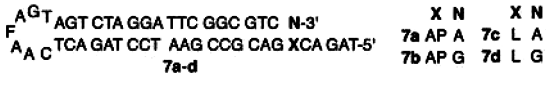


Figure 1. Nucleotide discrimination (dATP vs dGTP) by Pol V for translesion synthesis opposite AP and L: (A) K and (B) k_{Pol}.

modest dGTP binding preference was observed with AP (≤1.7-fold).

Contrary to L, selective dA incorporation opposite AP was governed predominantly by k_{Pol} (Figure 1B). All three templates containing AP incorporated dA ~2.5 times more rapidly than dG. In contrast, L-containing templates **6b** and **6d** exhibited no preference for incorporating either nucleotide. The third template (**6f**) exhibited a greater k_{Pol} for dATP, but the selectivity (1.8) was considerably lower than that exhibited by any of the AP-containing templates.



These data are consistent with steady state experiments in which incorporation of a nucleotide opposite AP is determined by differences in k_{cat} (or V_{max}) and not K_M.[12,13] However, incorporation of a nucleotide opposite 2-deoxyribonolactone is fundamentally different. Incorporation of a purine nucleotide opposite L is dictated by differences in $K_{D,app}$ but not k_{Pol}. This suggests that the Pol V–DNA complex containing L recognizes the structural differences between an incoming dGTP and dATP. The selectivity in the presence of three different 5′-adjacent nucleotides suggests that a misalignment mechanism is not responsible. Previously, molecular modeling supported the hypothesis that hydrogen bonding between the carbonyl oxygen and the guanine Watson–Crick face was possible.[7] Factors such as steric hindrance may also play a role in translesion synthesis opposite L.[14] However, AP and L are similar in size, and AP accepts very large nonnative nucleotides opposite it during translesion synthesis.[15] Furthermore, some error prone polymerases have loose active sites that accommodate nucleotides of varying size.[16] On the basis of these and previous data, it is unlikely that L distinguishes between dATP and dGTP solely by size. Therefore, we propose that hydrogen bonding contributes to the observed selectivity.[11]

The kinetic data for translesion nucleotide incorporation by Pol V correlate with the trend observed in *E. coli*. The efficiency ($k_{Pol}/K_{D,app}$) for incorporation of dG relative to dA was on average 2-fold greater opposite L, whereas AP had the equal but opposite preference (Table 1). The translesion synthesis data by themselves predict that incorporation of dG opposite L should be twice that of dA in *E. coli*, where the highest level of dG incorporation is ~40%.[7] Because the contributions of other polymerases to translesion synthesis opposite L, such as Pol IV, are negligible,[7] we probed whether extension efficiency contributes to the observed nucleotide incorporation levels in *E. coli*.

The effect of dA or dG opposite L or AP on extension was measured using templates **7a–d** (Table 2). Extension efficiency ($k_{Pol}/K_{D,app}$) by Pol V was more favorable when the lesions were opposed by dA, consistent with previous reports on AP.[17] The AP:dA pair was extended ~20-fold more efficiently than the AP:dG pair. However, the L:dA pair was extended only 9-fold faster than the L:dG pair. Greater π-stacking may inherently favor extension of the primer containing dA, but

Table 2. Kinetic Constants for Extension past AP and L by Pol V[a]

template	base pair	$K_{D,app}$ (μM)	k_{Pol} (min^{-1})	$k_{Pol}/K_{D,app}$ ($\times 10^{-4}$ μM^{-1} min^{-1})
7a	AP:dA	58 ± 19	0.86 ± 0.20	167.0 ± 56.9
7b	AP:dG	408 ± 39	0.34 ± 0.08	8.3 ± 0.7
7c	L:dA	114 ± 42	0.86 ± 0.32	96.5 ± 59.8
7d	L:dG	328 ± 113	0.36 ± 0.11	11.2 ± 3.1

[a]Values presented are averages ± the standard deviation of at least two experiments, each consisting of three replicates.

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this may be partially reduced by the more favorable pairing between L and dG. Finally, the preferred extension of primers containing dA opposite the lesion mitigates the relatively high level of translesion dG incorporation.

Overall, the kinetic experiments are consistent with the results of replication experiments in *E. coli*. Pol V shows a stronger preference for incorporating dG opposite L in comparison to AP. Importantly, preferential incorporation of dG opposite L is determined by dNTP binding ($K_{D,app}$), consistent with the hypothesis that this is the result of an instructive encounter between the dNTP and the enzyme−lactone complex.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, kinetic plots, kinetic parameters of individual experiments, and mass spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: mgreenberg@jhu.edu. Phone: (410) 516-8095. Fax: (410) 516-7044.

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Notes

The authors declare no competing financial interest.

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