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

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Abstract

Translesion synthesis past an oxidized abasic site, 2-deoxyribonolactone, in *E. coli* results in high levels of dG incorporation and is dependent upon Pol V. Kinetic experiments performed here affirm that Pol V preferentially incorporates dG opposite L. Pol V discriminates between dG and dA based upon 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 dA incorporation opposite AP is ascribed to the "A-rule."^{1, 2} The A-rule was also invoked to explain *in vitro* studies on L.³ Recent investigations on the replicative bypass of various abasic lesions in *E. 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 Pol V played a pivotal role in dG incorporation 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.

Since the 5'-dC or -T in mutagenicity studies on 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**, 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}

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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>.

the primer containing dA, but 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 greater preference for incorporating dG opposite L in comparison to AP. Importantly, preferential dG incorporation 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

AP	abasic site
L	2-deoxyribonolactone
Pol V	DNA polymerase V
$K_{D,App}$	apparent K_D

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Figure 1. Nucleotide discrimination (dATP versus dGTP) by Pol V for translesion synthesis opposite AP and L. A. $K_{D,App}$. B. k_{Pol} .

Scheme 1.

Scheme 2.

Table 1Kinetic constants for translesion synthesis opposite AP and L by Pol V. ^a

Template	Lesion	5'-Adj. dN	dNTP	K _{D,app} (μM)	k _{Pol} (min ⁻¹)	k _{Pol} /K _{D,app} (× 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 the average ± std. dev. of at least two experiments, each consisting of 3 replicates.

Table 2Kinetic constants for extension past AP and L by Pol V.^a

Template	Base Pair	$K_{D,app}$ (μ M)	k_{Pol} (min^{-1})	$k_{Pol}/K_{D,app}$ ($\times 10^{-4} \mu\text{M}^{-1}\cdot\text{min}^{-1}$)
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

^aValues presented are the average $\pm$ std. dev. of at least two experiments, each consisting of 3 replicates.