A DNA Polymerase with Specificity for Five Base Pairs

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The X family DNA polymerase from African swine fever virus (Pol X) has recently been characterized as the smallest known nucleotidyl transferase and has been suggested to play a role in DNA repair analogous to that of its mammalian sequence homologue, DNA polymerase β (Pol β).[1] In this study an in-depth kinetic analysis of Pol X, including catalytic efficiency and fidelity measurements for all possible base pairs, demonstrates that Pol X is the least faithful, or most error-prone, of all polymerases studied to date, with a specific preference for five base pairs including the four Watson−Crick base pairs plus one mismatched pair.

Our conclusion that Pol X is the least faithful polymerase is based on pre-steady-state kinetics, using model DNA substrates (Figure 1). We have first measured single turnover (with enzyme in excess of DNA substrate) saturation kinetics for all 16 possible base pairs in single-gapped DNA substrates. It has previously been shown that Pol X is a processive enzyme only when acting on gapped substrate,[1] and our observation that burst kinetics are observed only with gapped DNA (data not shown) confirm this and suggest that gapped DNA is likely to be the enzyme's natural substrate—as is the case for Pol β.

Single turnover experiments allow direct determination of the principal kinetic parameters k_{pol} (the pseudo-first-order catalytic rate constant) and $K_{d,app}$ (the apparent equilibrium constant for dissociation of nucleotide triphosphate from the enzyme·DNA complex) of nucleotide incorporation. The ratio k_{pol}/K_d is the definition of substrate specificity (it is also termed "catalytic efficiency"), and thus comparison of this value for correct and incorrect incorporations gives a quantitative measurement of the fidelity for a polymerase. The results shown in Table 1 indicate an activity which is incompatible with a repair function.

The enzyme has relatively low catalytic efficiency, on average 1/5000th that of Pol β (an enzyme known to function in base excision repair, or BER[2]) for correct base-pair incorporations.[3] More strikingly, Pol X has exceptionally low fidelities, ranging from 7700 for the C:C base pair to 1.9 for the G:G base pair. As the fidelity—defined as $[(k_{pol}/K_{d,app})_{cor} + (k_{pol}/K_{d,app})_{inc}]/(k_{pol}/K_{d,app})_{inc}$ where the subscripts "cor" and "inc" refer to the correct and incorrect incorporation, respectively—is the inverse of the error frequency, this indicates that the enzyme has no substrate specificity for a correct base pair (G:C) relative to the corresponding incorrect base pair (G:G). While the entire fidelity spectrum for Pol X is remarkably low, this absence of discrimination between the G:C and G:G base pairs represents the lowest nucleotide incorporation specificity ever observed for a template-directed nucleotide polymerase. Human Pol η, an enzyme recently determined to be the most error-prone polymerase,[4] is at least 10 times more faithful than Pol X in this instance. As illustrated in Figure 2, Pol X appears to catalyze formation of five base pairs (the four Watson−Crick pairs plus G:G) with comparable efficiency, selecting against the other 11 base pairs with fidelities ranging from modest to very low. Another important characteristic illustrated in Figure 2 is that, while the catalytic efficiency of Pol X is generally suppressed relative to that of Pol β, formation of the G:G mismatch is enhanced by nearly 8-fold. Thus, the mutagenicity of Pol X is the product of an impaired ability to form correct base pairs coupled with an enhanced ability to form the select mispair.

A

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5′-GCCTCGCAGCCGTCCAACCAACTCA CCTCGATCCAATGCCGTCC-3′
3′-CGGAGCGTCGGCAGGTTGGTTGAGTXGGAGCTAGGTTACGGCAGG-5′
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B

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5′-GCCTCGCAGCCGTCCAACCAACTCA
3′-CGGAGCGTCGGCAGGTTGGTTGAGTXGGAGCTAGGTTACGGCAGG-5′
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Figure 1. Schematic representation of gapped (A) and nongapped (B) DNA substrate. The difference between the two is the presence or absence of a downstream oligonucleotide, which is phosphorylated at the 5′-terminus.

Table 1. Kinetic Parameters of Pol X

base:pair[a]	k_{pol} (s^{-1})	$K_{d,app}$ (μM)	$k_{pol}/K_{d,app}$ ($M^{-1}\cdot s^{-1}$)	fidelity
A:T	0.77	890	860	n.a.
T:A	0.70	420	1700	n.a.
G:C	0.19	260	730	n.a.
C:G	0.53	230	2300	n.a.
A:A	5.4×10^{-4}	45	12	75
A:C	0.014	20000	0.70	1240
A:G	5.9×10^{-4}	20	30	30
C:A	0.0019	55	35	65
C:C	2.6×10^{-4}	860	0.30	7700
C:T	0.014	600	25	95
G:A	4.4×10^{-4}	80	5.5	140
G:G	0.029	35	830	1.9
G:T	0.023	460	50	16
T:C	0.0037	3400	1.1	1500
T:G	0.0036	50	70	25
T:T	0.050	1200	43	40

[a] In the base-pair notation X:Y, X refers to the templating base, and Y refers to the incoming nucleotide.

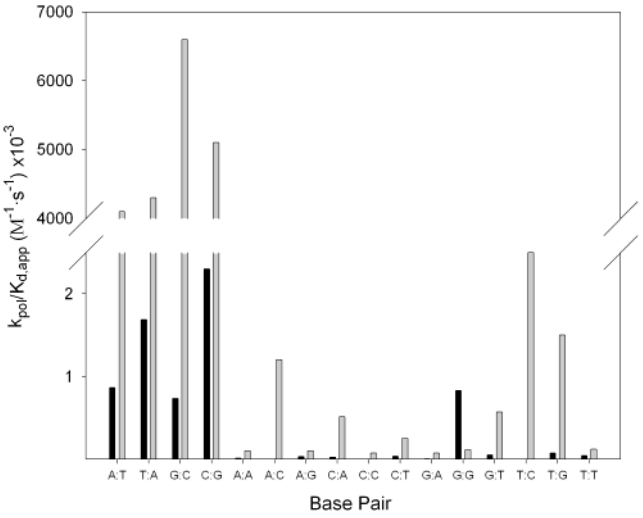

Figure 2. Catalytic efficiencies of all 16 possible base-pair incorporations into gapped DNA substrate for Pol X (black) and Pol β (gray). Note the discontinuous *y*-axis.

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(1) Oliveros, M.; Yáñez, R. J.; Salas, M. L.; Salas, J.; Viñuela, E.; Blanco, L. *J. Biol. Chem.* **1997**, *272*, 30899−30910.
(2) Sobol, R. W.; Horton, J. K.; Kuhn, R.; Gu, H.; Singhal, R. K.; Prasad, R.; Rajewsky, K.; Wilson, S. H. *Nature* **1996**, *379*, 183−186.
(3) Ahn, J.; Kraynov, V. S.; Zhong, X.; Werneburg, B. G.; Tsai, M.-D. *Biochem. J.* **1998**, *331*, 79−87.
(4) Matsuda, T.; Bebenek, K.; Masutani, C.; Hanaoka, F.; Kunkel, T. A. *Nature* **2000**, *404*, 1011−1013.

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Two interesting trends in the kinetic data point out novel features of Pol X catalysis which lead to an ultralow fidelity and five base-pair specificity. First, Pol X is the only polymerase demonstrated to bind incorrect nucleotides into the Michaelis complex with greater affinity than that with which it binds correct nucleotides. The most tightly bound incoming nucleotides are dGTP in the A:G and G:G mispairs (20 and 35 μM respectively, compared to 890 and 260 μM for the corresponding "correct" nucleotides). Second, comparison of different "types" of base-pair incorporations reveals a pattern apparently geared toward selective mutation. For the four Watson−Crick incorporations, C:G is the most efficiently catalyzed and G:C is the least. Likewise, within the four groups of mispairs (where mispairs are grouped by the template base) the X:G mispair is the most efficient and the X:C mispair the least efficient in each applicable case. In other words, the enzyme appears to enhance incorporation of dGTP and to suppress incorporation of dCTP regardless of the identity of the templating nucleotide. This suggests that Pol X has developed an active site tailored to favor dGTP incorporation and disfavor dCTP incorporation, resulting in an exceptionally low fidelity for the G:G mispair. The fact that nearly all incorrect base pairs have very low fidelity suggests that a general fidelity relaxation may be a necessary byproduct of altered substrate specificity via divergent evolution from more faithful polymerases (i.e., Pol β).

An important piece of evidence supporting Pol β's role in DNA repair was the observation that catalytic efficiency and fidelity of the enzyme are both enhanced by gapped DNA,[3] the type of substrate that a polymerase would be required to process in the BER pathway. Pol X on the other hand, while receiving a boost in catalytic efficiency, becomes more mutagenic when operating on gapped DNA (Figure 3). The comparison of gapped and nongapped DNA as acceptors for correct (G:C) and incorrect (G:G) incorporations by Pol X (Figure 3A) shows that catalytic efficiency for the mispair improves ~5-fold when the enzyme is operating on gapped DNA, while the efficiency for the correct base pair improves only marginally. The observation that the presumptive natural substrate for Pol X enhances its mutagenic ability suggests that the enzyme has evolved for mutagenesis via error-prone filling of single nucleotide gaps.

One possible explanation for the unusual properties of Pol X (ultralow fidelity coupled with low catalytic efficiency) is that the enzyme is involved in strategic DNA mutagenesis aimed at conferring hypervariability upon the virus. It cannot be completely ruled out that Pol X associates with some additional cellular factor in vivo, resulting in enhancements in activity and perhaps fidelity. But since there is no known (to our knowledge) example of an adjunct polypeptide conferring fidelity increases of the magnitude required to make Pol X behave like a repair polymerase, this line of reasoning seems unlikely to account for the unique properties of Pol X in vitro. In our view, a dedicated DNA mutase would necessarily have low catalytic efficiency. A DNA mutator that combined exceptionally low fidelity with high catalytic efficiency would likely scramble the genome to such an extent that the organism would become nonviable. The African swine fever virus is known to be hypervariable[5] and one previous analysis has suggested that its rapid genomic variation results largely from

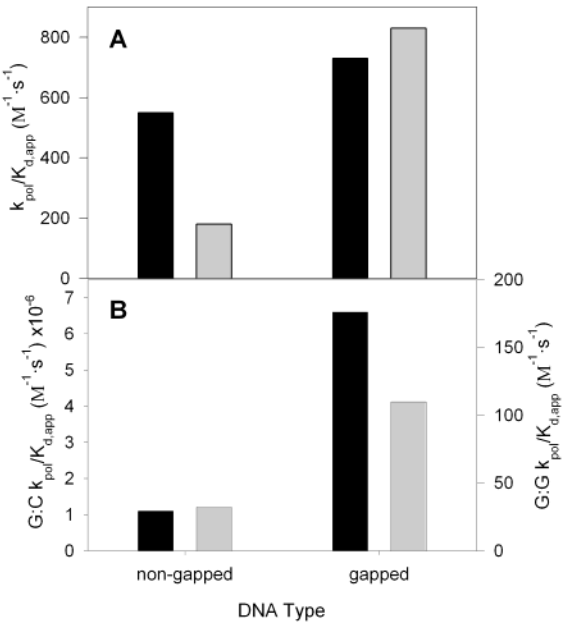

Figure 3. Catalytic efficiencies of G:C (black) and G:G (gray) base-pair formation into both gapped and nongapped substrate for Pol X (A) and Pol β (B). Note that separate *y*-axes are required in (B) for the G:C and G:G incorporations, due to the high fidelity of Pol β. The values in panel A, from left-to-right, are 550, 180, 730, and 830 $\mu M^{-1}\cdot s^{-1}$. Those in panel B are 1.1 × 10^6, 32, 6.6 × 10^6, and 110 $\mu M^{-1}\cdot s^{-1}$.

an abundance of point mutations.[6] This rapid mutability could be a reflection at the viral level of the mutagenic activity described here at the molecular level.

The concept of a mutase specific to a particular incorrect base pair is new and may lead to the discovery of mutases with different specificity. From the perspective of mechanistic enzymology, this property requires an active site with specific recognition of the G:G mispair, rather than one that simply has relaxed selectivity against all mismatches. Since Watson−Crick base pairing alone has been suggested to impart an inherent fidelity of 10−100,[7] this work raises the provocative question of how Pol X manages a specificity for "five correct base pairs". Whether this is based on hydrogen bonding,[8] shape complementarity,[9] or both, Pol X must accommodate the bulky G:G pair which would not appear to fit well in either scheme. Further mechanistic and structural studies of Pol X will likely shed light on these issues.

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Supporting Information Available: Experimental details of enzyme and substrate preparation and of kinetic analysis (PDF). This information is free of charge via the Internet at http://pubs.acs.org.

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(5) García-Barreno, B.; Sanz, A.; Nogal, M. L.; Viñuela, E.; Enjuanes, L. *J. Virol.* **1986**, *58*, 385−392.
(6) Dixon, L. K.; Wilkinson, P. J. *J. Gen. Virol.* **1988**, *69*, 2981−2993.
(7) Loeb, L. A.; Kunkel, T. A. *Annu. Rev. Biochem.* **1982**, *51*, 429−457.
(8) Petruska, J.; Sowers, L. C.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1559−1562.
(9) Matray, T. J.; Kool, E. T. *Nature* **1999**, *399*, 704−708.