Base Miscoding and Strand Misalignment Errors by Mutator Klenow Polymerases with Amino Acid Substitutions at Tyrosine 766 in the O Helix of the Fingers Subdomain*

(Received for publication, September 10, 1996, and in revised form, December 4, 1996)

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A mutant derivative of Klenow fragment DNA polymerase containing serine substituted for tyrosine at residue 766 has been shown by kinetic analysis to have an increased misinsertion rate relative to wild-type Klenow fragment, but a decreased rate of extension from the resulting mispairing (Carroll, S. S., Cowart, M., and Benkovic, S. J. (1991) Biochemistry 30, 804–813). In the present study, we use an M13mp2-based fidelity assay to study the error specificity of this mutator polymerase. Despite its compromised ability to extend mispairs, the Y766S polymerase and a Y766A mutant both have elevated base substitution error rates. The magnitude of the mutator effect is mispair-specific, from no effect for some mispairs to rates elevated by 60-fold for misincorporation of TMP opposite template G. The results with the Y766S mutant are remarkably consistent with the earlier kinetic analysis of misinsertion, demonstrating that either approach can be used to identify and characterize mutator polymerases. Both the Y766S and Y766A mutant polymerases are also frameshift mutators, having elevated rates for two-base deletions and a 276-base deletion between a direct repeat sequence. However, neither mutant polymerase has an increased error rate for single-base frameshifts in repetitive sequences. This error specificity suggests that the deletions generated by the mutator polymerases are initiated by misinsertion rather than by strand slippage. When considered with recent structure-function studies of other polymerases, the data indicate that the nucleotide misinsertion and strand-slippage mechanisms for polymerization infidelity are differentially affected by changes in distinct structural elements of DNA polymerases that share similar subdomain structures.

Among the most important properties of a DNA polymerization reaction is its fidelity. Numerous studies (reviewed in Refs. 1–3) have shown that two events initiate most polymerization errors. One is the misinsertion of an incorrect nucleotide. This usually yields a base substitution mutation, but can also yield a frameshift when the misinserted nucleotide is complementary to an adjacent template base and the primer relocates to produce misaligned strands. Several steps in the reaction cycle can affect the rate of errors initiated by misinsertion, including dNTP binding, a subsequent conformational change preceding chemistry and the rate of phosphodiester bond formation. Also critical is the balance between extension of a misinserted base and its exonucleolytic removal or rearrangement. The second error-initiating event is template-primer slippage (Ref. 4, reviewed in Ref. 2), usually resulting in deletion or addition of one or more nucleotides, particularly in repetitive sequences.

One approach for understanding these two error-initiating events is to study the properties of DNA polymerases whose x-ray crystal structures have revealed interactions with the template-primer and incoming dNTPs that might influence fidelity. Studies of four polymerases (reviewed in Ref. 5) indicate that they share a similar global structure comprised of three subdomains that together form a U-shaped cleft that binds the template-primer. The base of the cleft, designated as the “palm” subdomain by analogy to a right hand, contains the carboxylate residues that are highly conserved among many polymerases and that are important for catalysis. The walls of the cleft, formed by the “fingers” and “thumb” subdomains, also contain a number of highly conserved residues that are presumed to have important functions.

Although the use of structural information to investigate which amino acid residues in polymerase subdomains influence fidelity is at an early stage of development, some information is available. Studies of two DNA polymerases have shown that amino acid changes in the thumb subdomain alter the rate of errors initiated by strand slippage. For example, the structure of the binary complex of HIV-1 reverse transcriptase bound to DNA (6) indicates interactions between α helix H in the thumb subdomain of the reverse transcriptase and base pairs in the duplex template-primer 3 to 5 base pairs from the 3′-OH terminus. This is where an extra nucleotide might reside if a strand slippage-initiated misalignment in a short homopolymeric run were to occur. HIV-1 reverse transcriptase is particularly prone to this type of error (7). Alanine scanning mutagenesis of helix H has identified two mutant reverse transcriptases that have strongly reduced DNA binding affinity, reduced processivity, and reduced fidelity for errors in homopolymeric runs that were consistent with the strand slippage model (8, 9). More recently, we have found that a mutant derivative of Klenow fragment polymerase lacking 24 amino acids that share similar subdomain structures.

* This work was supported in part by the National Institutes of Health Intramural AIDS Targeted Antiviral Program (to T. A. K.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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This paper is available on line at http://www-jbc.stanford.edu/jbc/
acids at the tip of the thumb has reduced DNA binding affinity and also has reduced fidelity for one-base addition and deletion errors in homopolymeric runs (10). These frameshift mutant mutants of HIV-1 reverse transcriptase and Klenow polymerase have relatively normal rates for base substitution errors initiated by nucleotide misinsertion.

The identity of amino acids in a polymerase domain that could influence errors initiated by misinsertion is suggested by structural and biochemical studies of Klenow fragment polymerase (reviewed in Refs. 5 and 11; also see Ref. 12 and references therein). Among these are residues in helix O of the fingers subdomain that face the cleft and are thought to contact either the incoming dNTP (Arg-754, Lys-758, and Phe-762) or the single stranded template (Tyr-766). Mutant polymerases with amino acid substitutions at these positions have substantially altered properties, including altered nucleotide discrimination (e.g. see Refs. 13 and 14). Of particular interest for base substitution fidelity are observations with mutant polymerases in which the tyrosine at residue 766 has been replaced with phenylalanine, serine, or alanine. During synthesis with mutases in which the tyrosine at residue 766 has been replaced by phenylalanine, serine, or alanine. During synthesis with these mutants, the polymerase inserted incorrect nucleotides more efficiently, but extended terminal mispairs less efficiently, than did wild-type Klenow fragment. The magnitude of these effects varied among several individual mispairs examined. In a later study (12), the Y766A mutant Klenow fragment polymerase was found to have a slightly higher degradation constant that was elevated by more than 20-fold, and a weak mutant phenotype 

These properties of the Tyr-766 mutant Klenow fragment polymerases and our interest in understanding polymerase-substrate interactions that control the rates of misinsertion and misalignment-initiated errors prompted us to address the following issues in the present study. Since stable misincorporation requires misinsertion followed by mispair extension, one objective was to determine whether the previously studied Y766S enzyme has reduced base substitution fidelity when both steps in the reaction are required. We answer this question using an M13mp2-based fidelity assay to determine the average error rates for all 12 possible mispairs, each in a variety of sequence contexts. The Tyr-766 and Y766A mutant Klenow fragment polymerases are indeed strong mutators for several base substitution errors, but not for all 12 mispairs. Given the elevated Km,DNA of the Y766A mutant and the relationship between reduced DNA binding affinity, reduced processivity, and strand slippage-initiated errors mentioned above, a second objective was to determine whether the frameshift fidelity and/or processivity of the Y766S and Y766A mutant polymerase was altered compared to wild-type Klenow fragment. The results indicate that both mutant polymerases have only moderately altered processivity and have normal error rates for frame shifts in homopolymeric runs, i.e. errors initiated by strand slippage. However, they do have elevated rates for deletion of non-reiterated nucleotides, consistent with the model wherein misinsertion is followed by rearrangement to misalign the template-primer.

EXPERIMENTAL PROCEDURES

Polymersases—Mutant Klenow fragment polymerases were constructed and purified as described (15). All contain an inactivating D424A substitution in the 3'-5' exonuclease active site (16), thus focusing on polymerase selectivity in the absence of proofreading. To simplify the descriptions, enzymes are referred to by the genotype of the polymerase domain. The "wild-type" polymerase (Tyr-766) contains a tyrosine at amino acid residue 766, while the mutants contain either a serine (Y766S), alanine (Y766A), or phenylalanine (Y766F) at this location.

Fidelity Measurements—DNA synthesis fidelity was measured as described previously (17) using an M13mp2 DNA substrate with a 407-nucleotide single-stranded gap containing the lacZ gene. Synthesis using an M13mp2-based fidelity assay to determine the lacZ gene. Synthesis reactions (30 μl) contained 20 mM HEPES (pH 7.8), 2 mM dithiothreitol, 10 mM MgCl₂, 150 ng of gapped DNA, 50 μM of dATP, dTTP, dCTP, and dGTP, and 40–70 nmol of polymerase. Reactions were incubated at 37 °C for 10 min, terminated by addition of EDTA, and analyzed for complete gap-filling synthesis by agarose gel electrophoresis. All reactions filled the gap to the extent that products migrated coincident with nicked circular duplex DNA (17). Products were introduced into Escherichia coli and these cells were plated to score M13mp2 plaques as either wild-type (dark blue) or mutant (lighter blue or colorless). DNAs of independent mutant phage were sequenced to define the polymerase error specificity.

Proteolytic Analysis—Measurements of processivity were performed using the same template sequence as for the fidelity assay. Single stranded M13mp2 DNA was primed with a 15-base oligonucleotide (lacZ 105 primer, Ref. 18), phosphorylated at the 5'-end using 10 mM MgCl₂, 150 ng of gapped DNA, 50 μM of dATP, dTTP, dCTP, and dGTP, and 40–70 nmol of polymerase. Reactions were incubated at 37 °C and aliquots were removed at 5, 15, and 30 min and mixed with an equal volume of stop dye solution (99% formamide, 5 mM EDTA, 0.1% xylene cyanole, 0.1% bromphenol blue). Under these reaction conditions and at each of the time points, the amount of reinitiation on previously extended template-primer molecules relative to the total pool of product DNA was negligible (e.g. see Ref. 18). Thus, a single cycle of processive chain elongation was analyzed. The amount of each DNA product was quantified after separation on a 12% denaturing polyacrylamide gel using DNA markers generated by dideoxy sequencing from the same template-primer. Termination probability at a specific site is given by the ratio of the number of product molecules at a given chain length divided by this number plus the number of all longer products.

RESULTS

Reduced Fidelity of Y766A and Y766S Mutant Klenow Polymerases—In order to focus on the fidelity of Klenow fragment polymerase in the absence of exonucleolytic proofreading, all proteins studied here carry the D424A mutation that inactivates 3'-5' exonuclease activity (16). To simplify the descriptions, these polymerases are referred to by the genotype of the polymerase domain. The fidelity of each polymerase was monitored during gap-filling DNA synthesis templated by the lacZ complementation sequence in M13mp2 DNA. Synthesis by the wild-type (Tyr-766) polymerase generated M13mp2 products having lacZ mutant frequencies of 28 and 48 × 10⁻⁴ (duplicate experiments), values severalfold higher than the uncopied control DNA substrate (see legend to Table I). These values are similar to an earlier study (19) and demonstrate that the exonuclease-deficient, but otherwise wild-type, Klenow fragment polymerase generates errors during a single round of DNA synthesis at readily detectable rates. The mutant frequency of the products of synthesis by the Y766F mutant derivative was similar to that obtained with the wild-type enzyme (data not shown). This is consistent with the lack of an effect of this amino acid change on kinetic parameters for correct and incorrect incorporation using oligonucleotide substrates (13). In contrast, the products of DNA synthesis by the Y766S and Y766A polymerases had lacZ mutant frequencies of 240 and 220 × 10⁻⁴, respectively, values elevated by more than 5-fold relative to the wild-type enzyme. A repeat experiment yielded values of 330 and 240 × 10⁻⁴, respectively.

Specificity of Mutators—In order to determine the specificity of the mutant phenotype of the mutant enzymes, collections of independent lacZ mutants generated by the wild-type, Y766A, and Y766S polymerases were analyzed by DNA sequence analysis. The distribution of mutants containing single-base sub-
Mutator Klenow Polymerases

TABLE I
Error frequencies by class for Klenow fragment derivatives

| Mutation          | Tyr-766 Mutants M.F. \(^a\) \((\times 10^{-4})\) | Y766S Mutants M.F. \((\times 10^{-4})\) | Y766A Mutants M.F. \((\times 10^{-4})\) |
|-------------------|--------------------------------------------------|----------------------------------------|----------------------------------------|
| Base substitutions| 67 \((\times 10^{-4})\)                           | 90 \((\times 10^{-4})\)                | 71 \((\times 10^{-4})\)                |
| 1-base frame shifts\(^b\) | 34 \((\times 10^{-4})\)                           | 7 \((\times 10^{-4})\)                | 9 \((\times 10^{-4})\)                |
| 2-base deletions  | 8 \((\times 10^{-4})\)                           | 8 \((\times 10^{-4})\)                | 11 \((\times 10^{-4})\)               |
| 276-base deletion | 0.24 \((\times 10^{-4})\)                         | 4 \((\times 10^{-4})\)                | 17 \((\times 10^{-4})\)               |
| Others\(^d\)      | 8 \((\times 10^{-4})\)                           | 2 \((\times 10^{-4})\)                | 4 \((\times 10^{-4})\)                |
| Total             | 118 \((\times 10^{-4})\)                         | 121 \((\times 10^{-4})\)              | 103 \((\times 10^{-4})\)              |
| Mutant frequency  | 28 \((\times 10^{-4})\)                          | 240 \((\times 10^{-4})\)              | 220 \((\times 10^{-4})\)              |

\(^a\) M.F. indicates mutant frequency.
\(^b\) Ratio of mutant frequencies, Tyr-766 mutant to wild-type.
\(^c\) All but one were one nucleotide deletions.
\(^d\) Others include mutants with multiple changes (see legend to Fig. 1) or large deletions.

The mutant frequency for uncopied control DNA ranged from 3.0 to \(6.7 \times 10^{-4}\).

in Table II for each of the 12 possible base-base mispairs. The rates for the first six mispairings listed in Table II are also expressed as mutant: wild-type ratios in Table III. The error rates for the wild-type Klenow fragment are similar to those observed earlier (19); the highest average error rate (55 \(\times 10^{-6}\)) is for misincorporation of dGMP opposite the 27 detectable template thymine residues where this substitution yields a mutant plaque phenotype. This is also the most common misincorporation for the Y766S and Y766A polymerases (Table II), whose average rates are elevated by 13- and 17-fold, respectively (Table III). As before (19), the wild-type polymerase is about 10-fold more accurate for the reciprocal mispair, i.e. misincorporation of dTMP opposite template guanines (4.5 \(\times 10^{-6}\)). In comparison, the Y766S and Y766A mutants are 60- and 31-fold mutators for this error (Table III). Wild-type Klenow fragment error rates for the transition mispairs involving adenine and cytosine also differ somewhat. The wild-type Klenow fragment error rate for the AdCTP mispair is 7.8 \(\times 10^{-6}\) (Table II) and the Y766S and Y766A mutants are 24- and 18-fold mutators for this error (Table III). In contrast, the wild-type Klenow fragment error rate for the reciprocal error (apparent CdATP mispairing) is higher (32 \(\times 10^{-6}\), Table II) and the Y766S and Y766A mutants are not mutators for this substitution (Table III). The higher rate with the wild-type enzyme and the lack of a mutator effect are both consistent with the possibility that many of these substitutions actually reflect “correct” incorporation of dAMP opposite template uracil resulting from cytosine deamination.

All three Klenow fragment DNA polymerases are generally more accurate for transversion errors (Table II). The small numbers of lacZ transversion mutants recovered precludes conclusions on the relative fidelity of the mutant and wild-type Klenow fragment for six transversions, but the differences between the mutant and wild-type Klenow fragment for AdGTP and AdATP mispairs are highly significant (see legend to Table II for statistical calculation).

Processivity of Mutant Polymerases—We have previously observed a correlation between processive synthesis and the fidelity of HIV-1 reverse transcriptase for errors initiated by strand-slippage (7, 22). Given this correlation and the lack of a mutator effect in the present study for one-nucleotide deletions in repetitive sequences, we examined the processivity of the Y766S and Y766A polymerases in comparison to wild-type Klenow fragment. A single cycle of processive synthesis by the wild-type Klenow fragment using the lacZ template yields products that vary in length and represent the addition of from one to over 50 nucleotides (Fig. 2A). Quantitative product analysis (Fig. 2B) reveals that the median probability of termination of processive synthesis is 0.048, i.e. the average processivity of wild-type Klenow fragment with this template is about 20
**Fig. 1.** Error spectra for Klenow polymerases. The 5'-3' sequence of the viral (+)-template strand of the lacZ gene in M13mp2 is shown from position −69 through +174, where position 1 is the first transcribed base. The nucleotide changes shown were found in individual lacZ mutants recovered from the wild-type Klenow fragment reaction (panel A), from the Y766S polymerase reaction (panel B, mutants shown below the template sequence), or from the Y766A polymerase reaction (panel B, mutants shown below the template sequence). Individual letters indicate single base substitutions and represent the new base found in the viral strand DNA. The loss of a single base is indicated by a Δ, while the addition of a single base is indicated by a Ç. When these events occur in repetitive sequences, the location of the base lost or added is unknown, so the symbol is centered within the repetitive sequence. The loss of two consecutive bases is indicated by a Ç placed above or below the 5'-most template base lost. In addition to the mutants shown, one lacZ mutant had lost a T residue at position 183–184, and several lacZ mutants were recovered from each polymerase reaction that had more than a single sequence change. Point mutations in these multiple mutants that are known from previous studies to yield a detectable mutant plaque phenotype were considered as independent polymerase errors and used in conjunction with the single mutations shown above to calculate the polymerase error rates shown in Table II. Thus the multiple mutants are listed here, with the mutations used in the calculations marked with an asterisk. Tyr-766Klenow fragment, 6 mutants: C at 236 and G at 178; G at 87 and A at 90; T at 114 and A at 174; A at 8 and T at 5, T at 75 to 78. Y766A Klenow fragment, 8 mutants: A at 85 and T at 57, A at 85 and G at 80; A at 8 and T at 5, T at 75 to 78, T at 174, A at 8 and T at 5, T at 75 to 78.
Error rates are per detectable nucleotide incorporated, and were calculated by multiplying the mutant frequency data for the duplicate determinations with each polymerases (see text) by the proportion of mutants for each class (from Fig. 1 and its legend), dividing by 0.6 to correct for expression of errors in E. coli (see Ref. 17), and dividing by the number of known detectable sites for each class of mutations (from Table I in 17). When no lacZ mutants were recovered, the value shown is a "less than or equal to" error rate. Values with an asterisk are statistically different from the wild-type polymerase error rate, with \( p \) values all <0.01, when calculated using likelihood ratio statistics (21). To make this calculation, the number of sequenced mutants was considered as a Poisson random variate. Given the small number of mutants for each category, we applied a Monte Carlo approach to likelihood ratio statistics. For each comparison, we simulated a million random mispair events from the distribution that fixed the unknown Poisson parameters at values estimated by maximum likelihood under the null hypothesis. The statistical significance level is the fraction of times the randomly generated values of the test statistic exceeded the particular value of the statistic calculated from the data.)

**DISCUSSION**

This study of Klenow fragment polymerase mutants containing amino acid changes at residue 766 in the O helix of the fingers subdomain contributes to our understanding of both the base substitution and frameshift fidelity of DNA polymerization reactions. For substitution fidelity, both misinsertion and mispair extension are required to generate a duplex, premutational intermediate containing a mispair. A previous study (13) demonstrated that a Y766S mutant of Klenow fragment polymerase has an elevated rate of misinsertion but a decreased ability to extend certain mispairs. For example, an 8-fold increase in rate for misinsertion of dGMP opposite T by the Y766S polymerase is accompanied by a 4-fold decrease in the rate of mispair extension (Table III). Despite the compromised mispair extension, in the present study the Y766S and Y766A polymerases are 13- and 17-fold mutators, respectively, for this error in the M13 assay (Table III). In fact, the change in error rates observed here for the transition mispairs during gap-filling synthesis are remarkably similar to the change in misinsertion rates observed earlier with oligonucleotide substrates. Thus, the transition mispair mutant effect of the
Y766S substitution in both assays, from greatest to least change, is G-dTTP ≅ Ad-dCTP ≅ Td-dGTP ≫ C-dATP (Table III). This remarkable concordance in mutator effects for the Y766S polymerase with two very different assays suggests that either approach will be valuable for future structure-function studies of DNA polymerase fidelity. These data, obtained in the absence of proofreading activity, also suggest that the rate of misinsertion rather than the rate of mispair extension may be the primary determinant of the transition error specificity of Klenow fragment.

The ratio of the Y766S to wild-type Klenow fragment for misinsertion determined kinetically (Table III, data from Ref. 13) versus stable misincorporation in the M13mp2-based fidelity assay (Table III) do differ for two transversion mispairs, Ad-dGTP and Ad-dATP (Table III). These differences between the two studies could result from the fact that Carroll et al. (13) examined misinsertion at either one (for Ad-dATP) or two (for Ad-dGTP) template nucleotides per mispair, while the present study provides an average error rate for either 23 or 17 template nucleotides, respectively (17). Earlier studies of Klenow fragment (24) and other DNA polymerases (25, 26) have shown that the ratio of rates of misinsertion and mispair extension are not constant for all 12 mispairs in all sequence contexts.

The Y766S and Y766A polymerases are frameshift mutators, having error rates for the loss of two nucleotides that are increased by 8- and 13-fold, respectively, relative to wild-type Klenow fragment (Table I). In most cases, the two deleted nucleotides are not in repetitive dinucleotide sequences. This error specificity, and the observation that the mutant polymerases do not have elevated error rates for one-nucleotide deletions in homopolymeric runs, suggests that neither polymerase is a mutator for errors initiated by template-primer slippage. Given that they are mutators for base substitutions, an alternative explanation for some of the two-base deletions observed here is that they may be initiated by nucleotide misinsertion. Misinsertion could be followed by primer relocation prior to further incorporation, perhaps due to the difficulty in extending the terminal mispair. If correct pairing occurred between the terminal misinsertion and the template base two nucleotides downstream, the resulting misaligned template-primer would ultimately yield the two-base deletion. This model is consistent with earlier observations in which the frameshift fidelity of Klenow fragment (27) and other DNA polymerases (28) was altered in a predictable manner by changing the template sequence and the relative concentrations of the dNTPs in the reaction. The current observations with the Y766S and Y766A mutants add additional support for the model by changing the miscoding properties of the polymerase rather than changing the template sequence or the dNTP pool.

Although the Y766S and Y766A mutants were error-prone for two-base deletions, their rates for deletion of single non-reiterated nucleotides is in fact similar to wild-type Klenow fragment. This is unexpected since the misinsertion-initiated frameshift model was originally proposed (29) to explain the origin of single non-reiterated nucleotide deletions by wild-type Klenow fragment (Ref. 27, Fig. 1A). Although an explanation for why the Y766S and Y766A mutants generated two-base deletions but not one-base deletions must await further experimentation, several possibilities exist based on the misinsertion model, which requires misinsertion, primer relocation, and extension of misaligned substrates in order to generate the completed intermediate. The observed specificity could also reflect the nature of the interaction between the side chain of residue 766 (tyrosine, serine, or alanine) and a particular template nucleotide (see below).

The error rate for deletion of 276 nucleotides by both the Y766S and Y766A polymerases is also increased relative to wild-type Klenow fragment, by 17-fold (Table I). The nucleotides lost include one of two nine-base direct repeat sequences (underlined in Fig. 3) and the intervening 267 nucleotides, suggesting a slippage mechanism between the direct repeats. Although deletions involving direct repeats have been observed among error spectra by several DNA polymerases (for review, see Ref. 30), this study reveals the first DNA polymerases containing single amino acid changes that are mutators for a large deletion error. Given that the Y766S and Y766A polymerases are not mutators for frameshifts in other repeat sequence elements (Fig. 1), we speculate that the mutator effect for this large deletion also results from misinsertion. The model involves correct synthesis through the first repeat by the Y766S (or Y766A) polymerase, followed by misinsertion of dGMP opposite template A (Fig. 3). Note that the rate for this misinsertion is elevated for the Y766S enzyme relative to wild-type Klenow fragment (13). Moreover, difficulty in extending this mispair has been observed with wild-type Klenow fragment (24). A similar circumstance with the mutator polymerase might allow disruption of the repetitive terminal nucleotides and subsequent formation of base pairs involving the newly made DNA and the downstream direct repeat. In that circumstance, the misinserted dGMP would form a correct base pair with template C, yielding a template-primer for continued synthesis that has 10 correct base pairs at the terminus, i.e. a stable deletion intermediate (Fig. 3). This idea that misinsertions may enhance formation of deletion intermediates is also supported by the observation of deletions formed between imperfect direct repeats during synthesis by a proofreading-deficient mutant of E. coli DNA polymerase II (see Fig. 7 in Ref. 20). Thus, mutations in either the polymerase or exonuclease-lytic functions of DNA polymerase genes may influence the rate of large scale genome instabilities in vivo.

The mutator effects observed here are distinctly different from earlier observations with mutants of HIV-1 reverse transcriptase (8, 9) or Klenow fragment (10). Those enzymes are mutators for frameshifts in homopolymeric runs that may be initiated by template-primer slippage, and they contain changes in the thumb subdomain thought to interact with the duplex template-primer. In contrast, Tyr-766 may interact with the single-stranded template at or close to the site of nucleotide addition (31). Biochemical data support this idea. The Y766A polymerase has reduced DNA binding affinity, but its dNTP binding affinity is only slightly lower than wild-type, consistent with the suggestion that Tyr-766 may help position
the incoming dNTP via interaction with the template-primer (12). Structural data for a complex of the closely related Taq DNA polymerase with duplex DNA shows a hydrophobic interaction between the tyrosine side chain equivalent to Tyr-766 and the template base at the blunt end of the duplex (32). The importance of this interaction in maintaining insertion fidelity is suggested by the observation that the Y766F mutation (which would preserve the hydrophobic interaction) does not have a mutator phenotype, whereas Y766A and Y766S mutations cause decreased fidelity. The structural and biochemical data thus suggest that Tyr-766 of Klenow fragment may have a function similar to that of Arg-283 in DNA polymerase β. In the crystal structure of the rat pol β-DNA/ddCTP ternary complex, the side chain of Arg-283 is in a position to interact with the template base in the polymerase active site (33). Replacement of arginine 283 with lysine, leucine, or alanine yields β polymerases with strongly reduced catalytic efficiency and base substitution fidelity (34). Thus, side chain interactions with the active site template base may be critical for efficient and accurate polymerization by two unrelated DNA polymerases.

Acknowledgments—We thank Xiaojun Chen Sun for purification of the mutant proteins used in this study, Phuong Pham and Rajendra Prasad for their critical evaluation of the manuscript, and Thomas Darden and David Umbach for statistical analysis of the data in Table II.

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