SUPPORTING INFORMATION

Implications of Active Site Constraints on Varied DNA Polymerase Selectivity

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Sequences. DNA substrates were PAGE purified.

Primer template complexes:

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5′-GTG GTG CGA AAT TTC TGA CAG ACA                    primer 24nt
3′-CAC CAC GCT TTA AAG ACT GTC TGT X CT GTC TGC GTG   template 36nt
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"correct" insertion: **X** = A; misinsertion: **X** = G, C, or T, respectively.

Protein expression and purification. Recombinant heterodimeric wild type and M184V mutant HIV-1 RTs were expressed in *E. coli* and purified as described before.[1,2] Enzyme concentrations were routinely determined using an extinction coefficient at 280 nm of 260450 $M^{-1}cm^{-1}$.

Primer extension assays. Primer-template substrates (vide supra) were annealed by mixing 5'-^{32}P labelled primer (primer strands were labelled with [γ-^{32}P]ATP using T4 polynucleotide kinase (*Stratagene*) according to the procedure recommended by the supplier) with twice the amount of template in reaction buffer (see below). The mixture was heated to 95°C for 5 min and subsequently allowed to cool to room temperature over 1 h. Reactions were started by addition of a preincubated DNA/enzyme mixture (5 µl) to an equal volume of dNTP (concentrations indicated in the Figure legends, PCR grade, *Roche*) solution in reaction buffer and incubated at 37°C. Reactions were performed in 50 mM Tris-HCl pH 8.0, 10 mM MgCl, and 50 mM KCl. 50 nM primer and 7 nM enzyme were used for insertion assays and 14 nM enzyme for misinsertion assays, respectively. After incubation (10 min in insertion assays; 5 min in misinsertion assays) the reactions were quenched by addition of 30 µl of gel loading buffer (80% formamide, 20 mM EDTA) and subsequently heated to 95°C for 5 min. Reactions were analysed by 14% polyacrylamide gel electrophoresis containing 8M urea, transferred to filter paper, dried under vacuum, and visualized by autoradiography.

Steady-state kinetics assays. The steady-state kinetic data were derived from single nucleotide insertion assays as described above except that the concentration of the nucleotides (at least seven different nucleotide concentrations were used in each experiment), the enzyme concentration, and the reaction time were adjusted for the different reactions to allow 20% or less primer extension ensuring single completed hit conditions according to published procedures.[3,4] The reactions were applied to 14% denaturing polyacrylamide gel electrophoresis, and the data were quantified by PhosphorImager analysis. Relative velocity v was measured as ratio of extended product (I_{ext}) to unextended primer (I_{prim}) using the following equation: $v = I_{ext}/I_{prim}\, t$, where t represents the reaction time, and normalized for the lowest enzyme concentration used. The apparent K_M and V_{max} values were obtained from Hanes-Woolf plots as described by Goodman.[3,4]

Table 1. Steady-state kinetic data obtained for nucleotide insertion and misinsertion using unmodified or modified thymidine triphosphates. The data presented are averages of experiments performed twice or in triple.

		HIV-1 RT (wt)			M184V		
T^RTP	Template[a]	V_{max} [min^{-1}]	K_M [µM]	V_{max}/K_M [M^{-1} min^{-1}]	V_{max} [min^{-1}]	K_M [µM]	V_{max}/K_M [M^{-1} min^{-1}]
H	**A**	0.051±0.001	0.050±0.002	1 020 000	0.039±0.003	0.047±0.002	830 000
Me	**A**	0.051±0.009	0.034±0.012	1 500 000	0.054±0.006	0.036±0.012	1 500 000
Et	**A**	0.048±0.006	0.40±0.05	120 000	0.066±0.006	1.8±0.3	36 000
*i*Pr	**A**	0.039	19.7±0.4	2 000	0.013±0.001	48.6±1	270
H	**G**	0.040±0.003	11.5±3.0	3500	0.036±0.001	51±6	706
Me	**G**	0.030±0.001	14.8±1.4	2030	0.0066±0.001	176±24	38
H	**C**	0.015±0.004	44.1±10.3	340	0.017±0.001	45±4	378
Me	**C**	0.014±0.001	182±28	77	0.0028±0.0004	355±43	8
H	**T**	0.016±0.001	55±1.3	290	0.009±0.001	29±5	310
Me	**T**	0.0069±0.0008	160±7	43	0.004±0.0003	368±52	11

[a] Primer/template substrates used:

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5′-GTG GTG CGA AAT TTC TGA CAG ACA                    primer (24nt)
3′-CAC CAC GCT TTA AAG ACT GTC TGT A CT GTC TGC GTG   template A
3′-CAC CAC GCT TTA AAG ACT GTC TGT G CT GTC TGC GTG   template G
3′-CAC CAC GCT TTA AAG ACT GTC TGT C CT GTC TGC GTG   template C
3′-CAC CAC GCT TTA AAG ACT GTC TGT T CT GTC TGC GTG   template T
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References

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(2) R. Krebs, U. Immendörfer, S.H. Thrall, B.M. Wöhrl, R.S. Goody, *Biochemistry* **1997**, *26*, 10292–110300.
(3) S. Creighton, L. B. Bloom, M. F. Goodman, *Methods Enzymol.* **1995**, *262*, 232–256.
(4) M. S. Boosalis, J. Petruska, M. F. Goodman, *J. Biol. Chem.* **1987**, *262*, 14689–14696.