Molecular Insights into DNA Polymerase Deterrents for Ribonucleotide Insertion

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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL METHODS

Quantum calculations (unconstrained) were performed on the system (a) depicted in **supplemental Fig. S3** using Gaussian09 at the B3LYP/aug-cc-pvtz level. Mol 1 in the top right (an alcohol) represents the OH group attached to the C2′ position. Mol 2 in the middle models the carbonyl group of Tyr-271 and amide of Phe-272. Mol 3 at the bottom (an aldehyde) represents the carbonyl group of the Gly-268. The calculations were extended for the systems comprised of (b) Mol A + Mol B, (c) Mol B + Mol C, (d) Mol A alone, (e) Mol B alone, (f) Mol C alone to investigate the concept of amide bond resonance potentially acting on the system. Also, the calculations were repeated for systems (a), (b), and (c) using constrained O–O distance between Mol A and Mol B and N–O distance between Mol B and Mol C at their crystallographic values. The charges calculated using the CHELPG procedure and the inter-atomic distances are given in **supplemental Table S2**.

SUPPLEMENTAL RESULTS

Molecular Modeling—The molecular dynamics simulations described in the text indicate that with standard force field charges, the distance between rCTP(O2′) and Tyr-271(O) would increase significantly (~0.3 Å) to a stable distance relative to that observed in the crystallographic structure (*i.e.,* 2.54 Å). However, the observed short separation between these oxygens suggests that one of them could be negatively charged. To test this idea, the force field employed above was modified by changing the charge from -0.5679e (the standard charge in the force field) to -1.0e for the Tyr-271 carbonyl oxygen. With this enhanced charge on this single atom, the MD simulation with the rCTP

indicated that the rCTP(O2′)–Tyr-271(O) distance decreased from an average distance of 2.83 Å to 2.58 Å (**supplemental Fig. S2**). Thus, if the carbonyl of Tyr-271 is more negative, the hydrogen bond decreases in length in the direction of the experimental observation, but the local interaction energy is very favorable.

Two small complexes were constructed to follow charge flow in the ribonucleotide system quantum mechanically (**supplemental Fig. S3**). The first system provides for a hydrogen bond between methyl alcohol and the carbonyl of an amide bond blocked at both ends by methyl groups. In an-all atom optimization, we find the system reaches a minimum energy with a distance between the alcohol oxygen and carbonyl oxygen of 2.84 Å. In the second calculation, a third molecule (acetaldehyde) is added to the system arranged so that this molecule's carbonyl interacts with the amide of the two body model with a hydrogen bond (**supplemental Fig. S3**). This second three body system models the ribose/Tyr-271/Gly-268 network observed in the crystallographic structure. Complete all-atom optimization shows a small decrease in the alcohol and carbonyl oxygen distance of only 0.02 Å with the addition of the third body interaction. In crystallographic structures, Gly-268 and Tyr-271 are near the carboxyl-terminus of a short α-helix where the helix dipole points at the carbonyl of Tyr-271. A calculation of atomic charge on the carbonyl oxygen for the two and three body systems shows a small increase (~0.020e) in the negative charge for the three-body system relative to the two-body system. This result is consistent with the Pauling concept of amide bond resonance and also consistent with a recent quantum mechanical study of the Pauling concept. The main conclusion, however, from the model quantum mechanical calculations, is that the decrease in the hydrogen bond distance and the increase in the carbonyl oxygen charge, while in the right direction,

are not large enough to account for the short rCTP(O2′)/Tyr-271(O) distance observed experimentally.

Finally, a calculation was performed on system A+B (**supplemental Fig. S3**) in which the distance of the carbonyl oxygen of molecule B and the hydroxyl oxygen of molecule A was systematically shortened from an unconstrained value of 2.84 Å (**supplemental Table S2**) to 2.53 Å, the crystallographic value of the interaction of interest. The only constraint was this distance. The cost of energy to reduce this bond was ~2.2 kcal/mol.

TABLE S1

Kinetic summary for insertion/misinsertion of nucleotides with modified sugars

Assays were performed as described under "Experimental Procedures." The DNA substrate was single-nucleotide gapped DNA with a templating guanine. The results represent the mean (S.E.) of at least three independent determinations.

Incoming Nucleotide	Enzyme	k_{cat}	K_m	k_{cat}/K_m
		10^{-2} (s^{-1})	*μM*	*10^{-3} (1/s–μM)*
			(dG–**rC**) dG[a]	
dCTP	WT[b]	80 (10)	0.8 (0.2)	1000 (300)
	Y271A	110 (10)	1.3 (0.1)	900 (100)
rCTP	WT	5.3 (0.7)	200 (20)	0.27 (0.04)
	Y271A	24 (2)	70 (20)	3 (1)
dTTP	WT	2.0 (0.7)	1800 (300)	0.011 (0.004)
	Y271A	2.07 (0.09)	570 (20)	0.036 (0.002)
			(**rG**-dC) dG	
dCTP	WT	8.6 (0.7)	0.75 (0.03)	120 (10)
	Y271A	8.8 (0.9)	1.2 (0.2)	70 (10)
rCTP	WT	0.25 (0.03)	560 (50)	0.0045 (0.0007)
	Y271A	0.86 (0.09)	60 (4)	0.14 (0.02)
dTTP	WT	0.11 (0.01)	1100 (200)	0.0010 (0.0002)
	Y271A	0.29 (0.02)	1030 (80)	0.0028 (0.0003)
			(**rG-rC**) dG	
dCTP	WT	27 (3)	0.62 (0.03)	440 (50)
	Y271A	35 (4)	1.9 (0.1)	180 (20)
rCTP	WT	0.90 (0.06)	610 (70)	0.015 (0.002)
	Y271A	3.9 (0.7)	160 (20)	0.24 (0.05)
dTTP	WT	0.24 (0.02)	690 (40)	0.0035 (0.0004)
	Y271A	1.1 (0.2)	1100 (200)	0.010 (0.003)
			(dG-dC) **rG**	
dCTP	WT	59 (1)	5.3 (0.8)	110 (20)
	Y271A	60 (10)	8.1 (0.7)	70 (10)
rCTP	WT	0.51 (0.06)	810 (80)	0.006 (0.001)
	Y271A	2.2 (0.5)	350 (20)	0.06 (0.01)
dTTP	WT	0.081 (0.009)	880 (80)	0.0009 (0.0001)
	Y271A	0.40 (0.02)	1400 (200)	0.0029 (0.0004)

[a] The sequence headings refers to the primer terminus and templating base: (template-primer) templating base. The base with the ribose sugar (rN) is also highlighted in bold type.

[b] WT, wild-type enzyme.

TABLE S2
Charges and distances for quantum systems

(a) Chelpg charges from unconstrained calculation

	Mol A+B+C	Mol A+B	Mol B+C	Mol A or B or C alone
CH3	0.183	0.182		0.240
O	-0.651	-0.636		-0.612
H	0.460	0.452		0.372
CH3	-0.078	-0.097	-0.105	-0.114
O	-0.632	-0.613	-0.603	-0.583
C	0.713	0.754	0.699	0.731
N	-0.445	-0.534	-0.398	-0.509
H	0.236	0.290	0.230	0.282
CH3	0.159	0.202	0.127	0.194
O	-0.463		-0.458	-0.497
C	0.550		0.538	0.575
H	-0.028		-0.027	-0.057
CH3	-0.004		-0.003	-0.021

(b) Chelpg charges from constrained calculation (the distances of O-O between Mol A and Mol B and N-O between Mol B and Mol C are constrained to their X-ray distances). Note that the values for individual Mol A, Mol B, and Mol C are same as the ones in Table (a).

	Mol A+B+C	Mol A+B	Mol B+C	Mol A or B or C alone
CH3	0.167	0.163		0.240
O	-0.662	-0.618		-0.612
H	0.469	0.437		0.372
CH3	-0.075	-0.097	-0.106	-0.114
O	-0.625	-0.601	-0.605	-0.583
C	0.697	0.753	0.687	0.731
N	-0.421	-0.526	-0.378	-0.509
H	0.235	0.293	0.215	0.282
CH3	0.148	0.196	0.118	0.194
O	-0.464		-0.447	-0.497
C	0.562		0.546	0.575
H	-0.028		-0.024	-0.057
CH3	-0.003		-0.006	-0.021

(c) Inter-atomic distances (Å) from unconstrained calculations. Molecule labels are in parentheses in the column 1.

	Mol A+B+C	Mol A+B	Mol B+C	Mol A or B or C alone
C(A)-O(A)	1.41	1.42		1.42
O(A)-H(A)	0.97	0.97		0.96
O(A)-O(B)	2.82	2.84		
H(A)-O(B)	1.84	1.87		
O(B)-C(B)	1.23	1.23	1.22	1.22
C(B)-N(B)	1.35	1.35	1.36	1.36
N(B)-H(B)	1.01	1.00	1.01	1.00
N(B)-O(C)	3.08		3.10	
H(B)-O(C)	2.07		2.10	
O(C)-C(C)	1.21		1.21	1.21

(d) Inter-atomic distances (Å) from constrained calculations. Molecule labels are in parenthesis in the column 1.

	Mol A+B+C	Mol A+B	Mol B+C	Mol A or B or C alone
C(A)-O(A)	1.41	1.41		1.42
O(A)-H(A)	0.98	0.97		0.96
O(A)-O(B)	2.53	2.53		
H(A)-O(B)	1.56	1.57		
O(B)-C(B)	1.23	1.23	1.22	1.22
C(B)-N(B)	1.34	1.35	1.36	1.36
N(B)-H(B)	1.01	1.00	1.01	1.00
N(B)-O(C)	2.86		2.86	
H(B)-O(C)	1.85		1.85	
O(C)-C(C)	1.21		1.21	1.21

Charges on CH3 groups are combined.

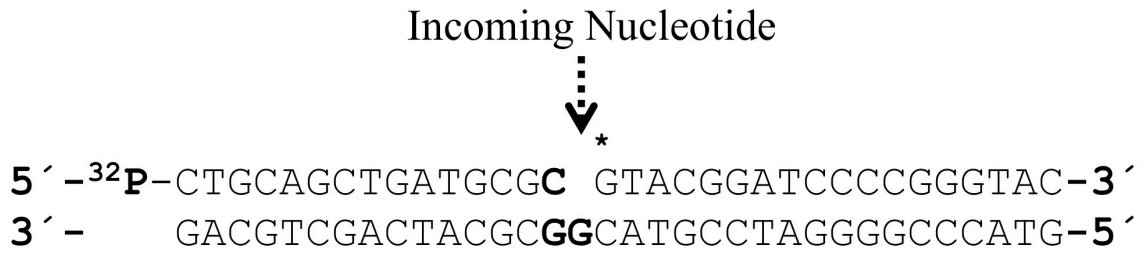

Figure S1**. Oligonucleotide sequences used in this study.** The single-nucleotide gapped DNA substrate was constructed as described previously (*1*). The primer terminus was a G-C (template-primer) base pair, and guanine was the coding templating base. As noted in **supplemental Table S1**, the sugars at the template-primer base pair or the coding template sugar were modified. The downstream primer was synthesized with a 5´-phosphate (*), and the upstream primer was 5´-labeled with [γ-^{32}P] ATP.

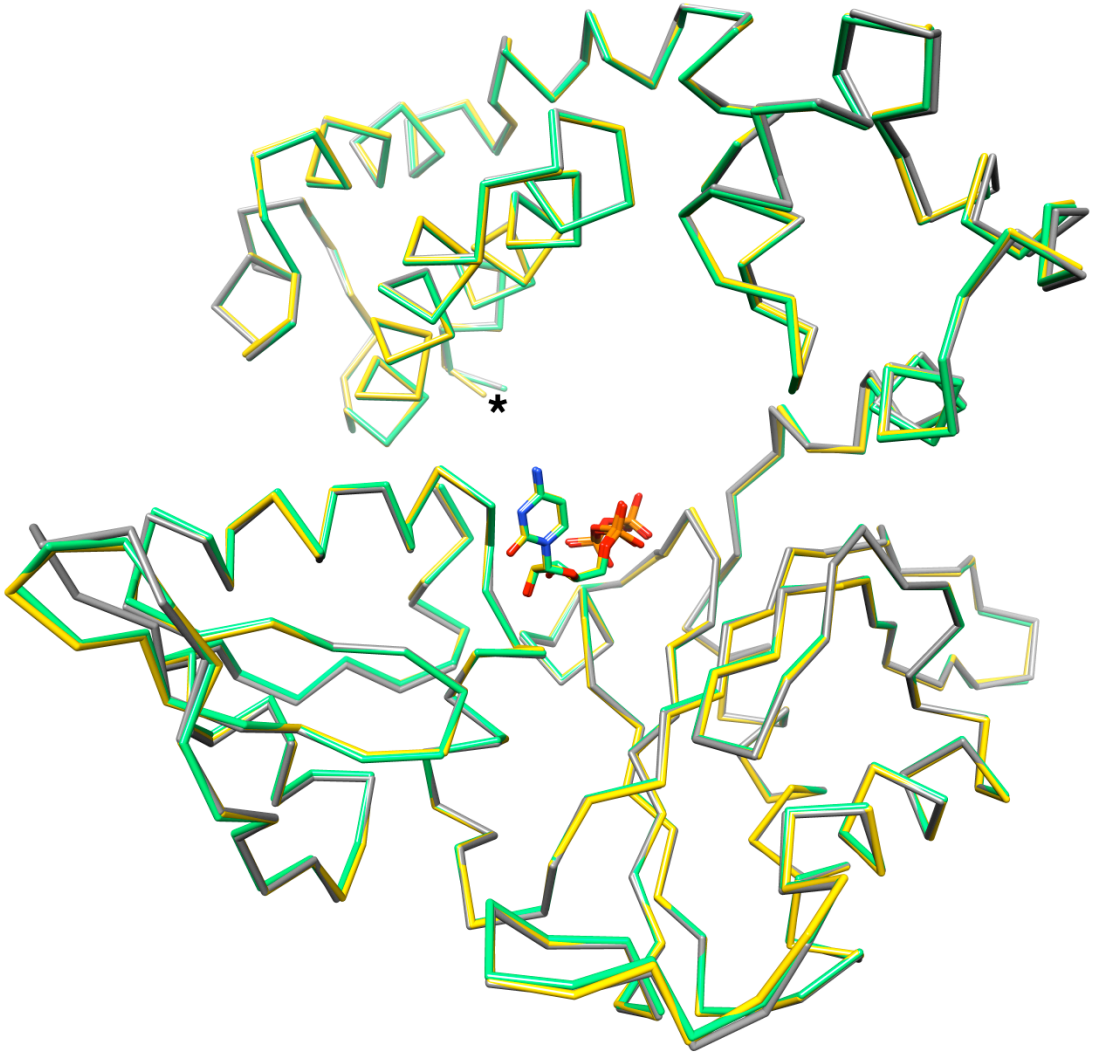

Figure S2. **Closed conformation of the ternary substrate complexes with an active site rCTP.** Stick representation of the wild-type pol β backbone (Cα) of the ternary substrate complex with ddCTP (PDB ID 2FMP, gray) or rCTP (green) incoming nucleotides superimposed with that for rCTP bound to the Y271A mutant (yellow). The rmsd for all Cαs relative to wild-type enzyme/dCTP is 0.35 and 0.27 Å for wild-type and Y271A enzymes/rCTP, respectively. Only the incoming rCTP is shown, but the DNA and incoming ddCTP for the wild-type structure is omitted for clarity. The amino–terminus is indicated with an asterisk.

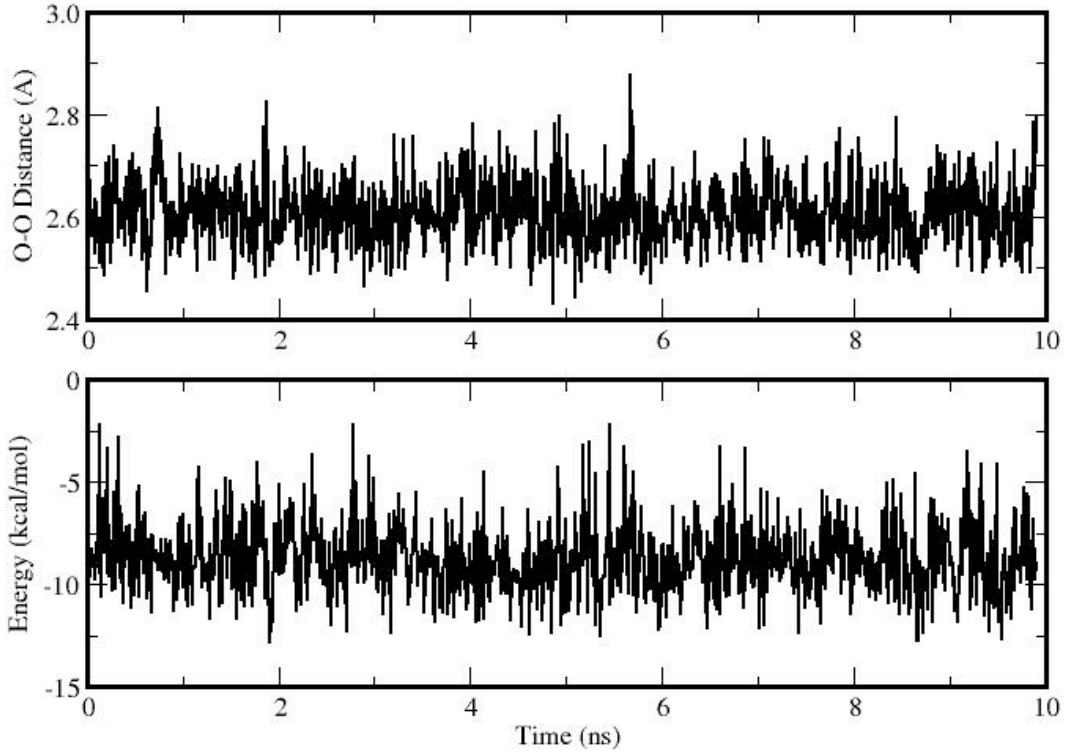

Figure S3**. Interaction of the carbonyl of Tyr-271 with a modified charge and an incoming ribonucleotide**. MD simulations were performed as described in "Experimental Procedures" and the final unconstrained runs (10 ns) were carried out with time steps of 1 fs. The charge on Tyr-271(O) was set at -1.0e rather than the standard -0.5679e. The average rCTP(O2′)/Tyr-271(O) distance and interaction energy are 2.58 ± 0.25 Å and –8.69 ± 1.92 kcal/mol.

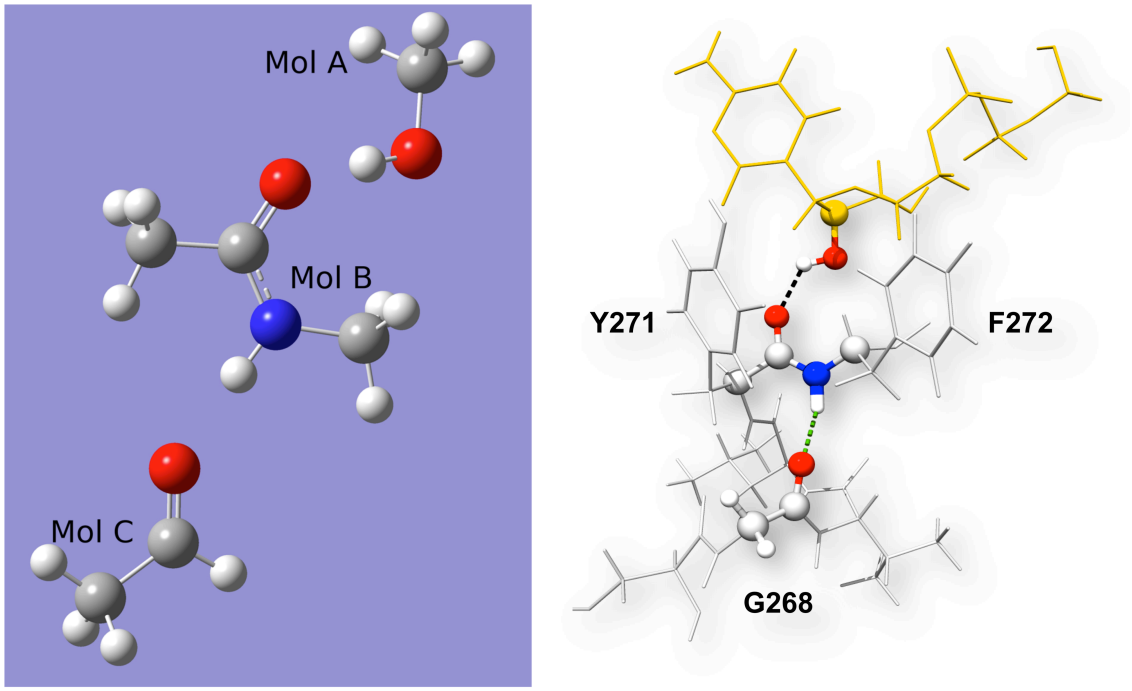

Figure S4**. System set-up for quantum calculations.** The model system illustrated in the left panel was set-up to mimic key atoms/bonds (ball-and-stick) observed in the wild-type pol β structure with an incoming rCTP (yellow) to probe the interaction between rCTP(O2′) and Tyr-271(O) (black dotted line). Methyl alcohol (Mol A, top right) mimics the hydroxyl attached to C2′ of an incoming ribonucleotide and the carbonyl of an amide bond blocked at both ends by methyl groups (Mol B, middle) represents the carbonyl group of Tyr-271 and the amide of Phe-272. The acetaldehyde (Mol C, bottom left) represents the carbonyl group of Gly-268.

REFERENCES

1. Beard, W. A., Shock, D.D., and Wilson, S.H. (2004) Influence of DNA structure on DNA polymerase β active site function: Extension of mutagenic DNA intermediates, *J. Biol. Chem. 279*, 31921-31929.