Published in final edited form as:

FEBS Lett. 2007 February 20; 581(4): 775–780.

Pol β catalytic efficiency mirrors the Asn279-dCTP H-bonding strength

Václav Martínek^{a,b}, Urban Bren^{a,d,#}, Myron F. Goodman^c, Arie Warshel^c, and Jan Florián^{a,d,*}

a Department of Chemistry, Loyola University Chicago, Chicago, IL 60626

b Department of Biochemistry, Faculty of Science, Charles University, Albertov 2030, 12840 Prague, Czech Republic

c Department of Chemistry, University of Southern California, Los Angeles, CA 90089

d Institute of Physics, Faculty of Mathematics and Physics, Charles University, 12116 Prague, Czech Republic

Abstract

Ternary complexes composed of wild type DNA polymerase β and pol β mutants bound to DNA and dCTP substrates were studied by molecular dynamics (MD) simulations. The occurrences of contact configurations (CC) of structurally important atom pairs were sampled along the MD trajectories, and converted into free-energy differences, ΔG_{CC} . ΔG_{CC} values were correlated with the experimental binding and catalytic free energies for the WT pol β and its Arg183Ala, Tyr271Ala, Asp276Val, Lys280Gly, Arg283Ala, and Glu295Ala mutants. The correlation coefficients show that the strength of the H-bond between dCTP and Asn279 is a strong predictor of the mutation-induced changes in the catalytic efficiency of pol β . This finding is consistent with the view that enzyme preorganization plays a major role in controlling DNA polymerase specific activity.

Keywords

DNA polymerase β ; enzyme preorganization; near attack conformers; molecular dynamics; site mutagenesis; dNTP; free energy

1. Introduction

DNA polymerase β (pol β), a member of the X family of DNA polymerases, plays a prominent role in human base-excision repair [1,2]. Pol β is upregulated in some types of adenocarcinomas [3]. Mutations in pol β are associated with colon and lung tumors and esophageal cancer [4]. Pol β is a relatively small (39 kDa) template-dependent DNA polymerase lacking proofreading, which makes it an attractive system for in vitro biochemical and *in silico* studies of the replication fidelity.

DNA polymerases enhance DNA synthesis by many orders of magnitude compared to the corresponding reaction in water [5]. Besides achieving huge rate enhancements, DNA polymerases are capable of selecting the right dNTP substrate so that Watson–Crick base pairs

* Send correspondence to Jan Florián, Department of Chemistry, Loyola University Chicago, Chicago, IL 60626. Email: jfloria@luc.edu.

#Permanent address: National Institute of Chemistry, Hajdrihova 19, SI-1001 Ljubljana, Slovenia

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

are preferentially formed. The resulting fidelity of DNA synthesis is several orders of magnitude larger than would be expected just from the energetics of Watson–Crick base pairing at the end of DNA duplex in water [6,7].

The control of the catalytic power and fidelity of DNA polymerases involves contributions from the protein, DNA residues and Mg^{2+} ions. To advance our understanding of these complex effects and to improve the predictive power of theoretical approaches, we have examined the correlation of several free-energy based theoretical descriptors with the set of free energies derived from experimental data. The observed mutation-induced changes in the apparent dissociation constant for dNTP (K_d), the polymerization rate constant at saturating concentration of dNTP (k_{pol}) and the catalytic efficiency (k_{pol}/K_d) of pol β were extracted from literature [8–11] and transformed to the corresponding free energies in kcal/mol (Figure 1) using relations,

$$\log k_{\text{pol}} = \log(RT / N_A h) - \Delta g_{\text{cat}}^{\ddagger} / 2.303 RT = \log(RT / N_A h) - (\Delta g^{\ddagger} - \Delta G_{\text{bind}}) / 2.303 R \quad (1)$$

$$\log(K_d) = \Delta G_{\text{bind}} / 2.303 RT \quad (2)$$

$$\log(k_{\text{pol}} / K_d) = \log(RT / N_A h) - \Delta g^{\ddagger} / 2.303 RT \quad (3)$$

where ΔG_{bind} , $\Delta g_{\text{cat}}^{\ddagger}$, $\Delta g^{\ddagger}$, N_A , h , R and T are dNTP binding free energy, activation free energy of the chemical step, apparent reaction activation free energy, Avogadro constant, Planck's constant, the universal gas-constant, and the thermodynamic temperature, respectively [12, 13].

The structural and energetic properties of the transition state (TS) region of the free-energy surface are needed to calculate $\Delta g^{\ddagger}$. However, TS properties are difficult to determine accurately either experimentally or theoretically. Although crystal structures of the ground state DNA polymerase-substrate complexes are available [14], they do not define the relevant TS. To circumvent the “challenging” task of attempting to compute a valid TS, we assume that if a specific property, for example a hydrogen bond, stabilizes the TS, then the same bond also lowers the free energy of the ground state enzyme-substrate complex. This assumption is best satisfied for properly preorganized binding sites where the reactant state has assumed a configuration close to that needed to stabilize the TS. [15]. In this paper, we provide an analysis of ground state parameters that correlate well with the effects of specific amino acid replacements in pol β on experimentally observed catalytic efficiencies and specificities. This analysis can be viewed as a computationally effective alternative to approaches that use TS binding as a theoretical descriptor to study pol β mutation effects [16]. As a general goal, we propose to ascertain a series of ground state parameters that exhibit strong correlations between selected amino acid replacements and intrinsic polymerase properties. Here we specifically show that the strength of the Asn279-dCTP H-bond exhibits a strong correlation with the catalytic efficiency of pol β .

2. Methods

MD simulations

MD trajectories were generated using the AMBER force field [17] implemented in the program Q [18]. The charges and vdW radii for the triphosphate moiety of dCTP⁴⁻ and for Mg^{2+} ions were identical as those used in our previous simulations [5,19,20]. All production trajectories generated constant-temperature ensembles at 310K. The trajectory for each simulated system was followed for 8 ns using an integration stepsize of 2 fs. The simulated complexes were immersed in a sphere (24 Å radius) of TIP3P water molecules subjected to the surface constraint

all atom solvent (SCAAS) spherical boundary conditions [18,21]. These constraints were designed to mimic infinite aqueous solution. DNA and protein atoms protruding beyond the 24 Å sphere boundaries were restrained to their coordinates in the crystal structure using harmonic restraints. Nonbonding interactions of these atoms were turned off. Nonbonding interactions between non-substrate atoms inside the simulation sphere were subjected to a 10 Å cutoff. The local-reaction field method (LRF) [22] was used to treat long-range electrostatic interactions for distances beyond a 10 Å cutoff. The electrostatic free energies calculated using SCAAS and LRF algorithms were shown to be insensitive to the system size [23–25]. In our experience, the use of the SCAAS+LRF methods allows significantly faster convergence of electrostatic energies in proteins than alternative periodic boundary condition methods [25]. For dCTP substrate and Mg^{2+} atoms, non-bonding interactions were explicitly evaluated for all distances. The SHAKE algorithm was used for bonds involving hydrogen atoms. The structure and trajectory analyses were carried out using the program VMD 1.8.5 [26]. The presence of contact configurations (CC) was evaluated for a set of 80000 configurations of the simulated system. These configurations were sampled every 0.1 ps along each of the seven 8 ns MD trajectories generated on different simulated systems.

Initial structure preparation

The simulations were initiated using the high-resolution crystal structure of the human DNA polymerase β -DNA-ddCTP ternary complex (PDB code 2FMP) [27]. All the crystallographic water molecules were replaced by TIP3P waters generated using the Qprep [18] program by immersing the simulation system into the sphere of bulk water molecules. Those water molecules that were not sterically overlapping with the atoms present in the crystal structure were retained in the starting structures for the MD simulations. The origin of the simulation sphere was placed at the coordinates of the C4' atom of ddCTP in the crystal structure. The 3'-terminal dideoxynucleotide of the primer strand and ddCTP were modified by adding the 3'-OH group. MD simulations of the WT complex were carried out with the following selection of the charged amino acid residues: ARG (40, 102, 149, 152, 182, 183, 243, 254, 258, 283, 299, 328, 333), ASP (145, 190, 192, 226, 256, 276, 321, 332), LYS (27, 48, 141, 148, 234, 280, 317, 331), GLU (147, 154, 186, 295, 316, 329, 335), HIS (135). This selection, along with dCTP⁴⁻, 2Mg²⁺, and 7 DNA phosphates resulted in the zero total charge inside the simulation sphere. In general, this selection of the charged residues is consistent with Glu, Asp, Lys, Arg, and phosphate groups that lie closer than 19 Å from the center of the simulation sphere having charges based on their pK_a constants in water. All residues further than 19 Å from the center of the simulation sphere were overall electroneutral. The initial structure for six mutant complexes was generated by manually changing residues Arg183, Tyr271, Arg283, and Glu295 to Ala, Asp276 to Val, and Lys280 to Gly. The total charge of the simulated system was 0 for WT and Y271A, -1 for R183A, K280G and R283A, and +1 for D276V and E295A mutants. By this design, all differences between 7 simulated systems are directly structurally connected to the replacement of a single protein residue, thus providing a consistent platform for the correlation analysis.

The structure of all the simulated system was relaxed by the two 30000- and 20000-step MD simulations at 5K that used a stepsize of 0.005 and 0.01 fs, respectively. In these simulations, 200 kcal/mol harmonic positional restraints were applied on the positions of all WT protein and DNA heavy atoms. No positional restraints were used in further simulations, which included a sequence of four 20000- and one 50000-step simulations with increasing stepsize (up to 1 fs) at 5K, a gradual heating of the simulated system from 5 K to 298 K in a series of eight MD simulations with 0.5 or 1 fs stepsize and 200 ps total simulation time, and a 140 ps simulation at 310K using a 2 fs stepsize.

3. Results

The experimental kinetic data for pol β mutants shown in Figure 2 are presented in Table 1. Because the mutational effects depend on the identity of the dNTP-template base pair, only the data for dCTP as substrate and G as template were included in Table 1 and in the correlation analysis. In order to achieve consistency, the mutational effects reported in Table 1 were normalized to average values of the WT pol β .

Calculated structural properties were obtained by ground-state molecular dynamics (MD) simulations of WT dNTP•DNA•pol β ternary complex, and six mutant complexes that were generated by Arg183Ala, Tyr271Ala, Asp276Val, Lys280Gly, Arg283Ala, and Glu295Ala substitutions (Figure 2). These properties included distances between the nucleophile (O3' atom) and P $_{\alpha}$ of the substrate, hydrogen of the 3'OH group of the primer and the O5' bridging oxygen of dCTP, the catalytic Mg²⁺ and all its ligands, the nitrogen atom of Asn279 and the O2 oxygen of the incoming dCTP base, hydrogen of the 3'OH group of the substrate and one of the non-bridging oxygens of β -phosphate of the same molecule, the length of the central H-bond between the templating G and dCTP nucleotides, and hydrogen of the OH group of the Ser180 and one of the non-bridging oxygens of γ -phosphate of the dCTP molecule (Figure 3). Of the three hydrogen bonds between the templating G and dCTP, we limited our correlation analysis to the central hydrogen bond, because we expected the correlation behavior of all three H-bonds to be similar. For each atom pair we defined a cutoff distance and determined the number of sampled configurations with a distance less than the cutoff (N_{CC}). Using the ratio of N_{CC} to the number of configurations with distances longer than the cutoff distance ($N_{total} - N_{CC}$) we calculated the free energies needed to form these contact configurations (ΔG_{CC}) at T = 310K,

$$\Delta G_{CC}(\text{kcal/mol}) = -2.303RT \log(N_{CC}/(N_{total} - N_{CC})). \quad (4)$$

We have correlated these free energies with the experimentally observed $\Delta g^{\ddagger}_{cat}$, ΔG_{bind} and $\Delta g^{\ddagger}$ (eq. 1–3) for the WT and six mutant enzymes. For each class of atom pairs, the cutoff distance was adjusted to obtain the best correlation between ΔG_{CC} and $\Delta g^{\ddagger}$. Since more frequent occurrences of short interatomic distances lead to lower ΔG_{CC} values, the magnitude of ΔG_{CC} represents a measure of the relative stability or strength of the given bond in the mutant proteins. The calculated ΔG_{CC} and correlation coefficients are presented in Table 2.

The only statistically significant correlation was observed between experimental $\Delta g^{\ddagger}$, $\Delta g^{\ddagger}_{cat}$ and ΔG_{bind} (eq. 1–3, Figure 1) and the strength of the H-bond between the amino group of Asn279 and O2 of the incoming dCTP base (Figure 4). This finding supports a hypothesis that the Asn279 residue plays significant role not only in substrate binding stabilization ($R = 0.72$), but is also a key factor affecting $\Delta g^{\ddagger}_{cat}$ ($R = 0.74$) – Table 2. Although there is no cross-correlation between *experimental* ΔG_{bind} and $\Delta g^{\ddagger}_{cat}$ ($R = 0.18$), the resulting $\Delta g^{\ddagger}$ exhibits a synergic effect resulting in the highest correlation coefficient ($R = 0.88$) with the stability of the H-bond connecting Asn279 and dCTP (Figure 4). The optimal cut-off for the correlation between $\Delta g^{\ddagger}$ and Asn279-dCTP H-bond stability was found to be 3.9 Å (Figure 5). This distance provides high correlation while yielding statistically significant number of configurations in both N_{CC} and $N_{total} - N_{CC}$ populations and is also consistent with the H-bonding limit distance of 3.9 Å between the proton donor and acceptor atoms determined by the statistical analysis of H-bond distances in protein crystal structures [28]. However, any selection of the cutoff distance in the structurally reasonable range of 3.6 – 4.0 Å also results in a high correlation coefficient (Figure 5).

The correlation tendency ($R = 0.63$) was obtained between the strength of the central H-bond of the G•dCTP base pair and the experimental ΔG_{bind} . However, the correlations of this H-

bond with $\Delta g^\ddagger$ are weaker ($R = 0.57$) because of the absence of any significant correlation with $\Delta g^\ddagger_{cat}$ ($R = 0.18$). Similarly, the observed correlation tendency for an intramolecular H-bond between O3'H group of the deoxyribose and β -phosphate and $\Delta g^\ddagger_{cat}$ ($R = 0.70$) is weakened for $\Delta g^\ddagger$ ($R = 0.69$) because its correlation with ΔG_{bind} is small ($R = 0.47$). Mutations that destabilize this intramolecular H-bond tend to have lower $\Delta g^\ddagger_{cat}$ and higher frequency of H-bonding contacts between 3'OH of dCTP and the backbone carbonyl group of Phe272.

4. Discussion

Our previous theoretical and computational analysis [16] have shown that the observed mutation effects on $\Delta g^\ddagger$ correlate well with the binding free energy of the TS calculated using the linear response approximation (LRA) method. In this analysis, the two Mg^{2+} active site metals were considered to be a part of the protein rather than the TS, and the assumed TS structure corresponded to the last microscopic chemical step, i.e. to the TS for breaking the P-O bond (with the pyrophosphate as the leaving group). The present work reflects a “bioinformatics” approach and explores the correlation between structural parameters of the ground state for the catalytic reaction of pol β and the observed effects of mutations of near active site amino acid residues. Among various structural parameters, including the abundance of near attack conformers (NACs) [29], the only significant correlation was observed for H-bonding between the base moiety of dCTP and the amino group of Asn279. The lack of a significant correlation for the NACs and contact configurations other than Asn279-dCTP may indicate that these structural parameters are not directly involved in the rate-determining chemical step, that they are coupled in a complicated non-additive way with other structural parameters, or that their ΔG_{CC} values are not sufficiently sensitive to point mutations. While the absence of a correlation might be expected given the complexity of factors determining $\Delta g^\ddagger$, $\Delta g^\ddagger_{cat}$ and ΔG_{bind} it is notable that ΔG_{CC} for Asn279-dCTP is well-correlated. This finding is consistent with important role of Asn279 in the pol β active site organization revealed by previous structural, kinetic and computational studies (see e.g. ref. [1,9,19,30–32] and references therein).

It is not surprising that amino acid mutations that weaken the Asn279-dCTP interaction also significantly increase ΔG_{bind} , but that this same effect is linked to an increase in $\Delta g^\ddagger_{cat}$. Therefore, mutations that destabilize the Asn279-dCTP H-bond cause a significant decrease in pol β catalytic efficiency (Figure 1). Given the nature of correlation analysis one can ask, which is the cause and which the consequence? Based on experimental data showing that the highest point on the overall free energy surface corresponds to a chemical transformation rather than conformational change [33–35], the following points can be made.

The Asn279 residue plays a major role in the stabilization of dCTP binding opposite the correct template (G). Therefore, a mutation-induced disruption of the dCTP - Asn279 interaction is expected to increase the free energy of both the ground (denoted ES in Figure 1) and transition ($ES^\ddagger$ in Figure 1) states by the same amount relative to their energies in WT enzyme. However, the correlation data indicate that the mutation-induced disruption of the Asn279-dCTP hydrogen bond destabilizes the rate-limiting transition state more than the ground state. This effect is fully consistent with a previous preorganization analysis applied to pol β [16,19]. That is, the electrostatic preorganization in the nucleotide base binding site is determined by the template and its localized surroundings. A reduction in preorganization of the binding site leads to a transfer of allosteric information to the catalytic site and a reduction of preorganization in this site, which, in turn, reduces the effect on catalysis (Figure 6). As is the case with most preorganization effects [36,37] they are already present in the ground state since the ground state must be preorganized beforehand so as to reduce the reorganization energy needed to reach the TS.

The analysis of Xiang et al [16] contains an estimate of the effects of Asn279 on the TS binding using the protein-residue interaction matrix generated using the PDL/S-LRA method. This analysis shows that eliminating the charge on the atoms of Asn279 alters TS binding free energy by 2 ± 1 kcal/mol, which is significant. The interaction matrix also reveals that the interaction between Asn279 and TS is strongly coupled with the interactions of the TS with Arg283, Asp276, and the cytosine base of the dCTP substrate. Thus, a strong interaction between cytosine and Asn279 is present in both the ground state - as evidenced by large observed Asn279Ala mutation effects on dNTP binding [9,30,31] and by simulations presented here - and in the TS [16]. Unfortunately, it is impossible to directly correlate $\Delta G_{CC}(\text{Asn279})$ with k_{cat}/K_m observed for the Asn279 mutants [9,30,31] because $\Delta G_{CC}(\text{Asn279})$ becomes undefined when the Asn279 side chain is removed from the protein.

Nevertheless, we show here that the alteration of the strength of the H-bond between cytosine and Asn279 by mutations of other protein residues is linearly related to changes of $\Delta g^\ddagger$ caused by the same mutations. This relationship may seem surprising because Asn279 does not directly interact with the α -phosphate of dCTP or primer. However, this relationship is consistent with the fact that the *experimental* $\Delta g^\ddagger$ correlates more strongly with the *experimental* ΔG_{bind} ($R = 0.92$) than with the *experimental* $\Delta g^\ddagger_{cat}$ ($R = 0.55$) (Table 1). That is, the strength of the Asn279-cytosine interaction in the ground state is important for the catalytic efficiency of mutant pol β because the mutants evaluated here alter the substrate binding more than the activation barrier for the chemical step. This central observation is consistent with preorganization-based predictions [19], namely that pol β may have evolved to achieve a rapid rate of correct substrate incorporation by optimizing the substrate binding free energy to a greater extent than by lowering $\Delta g^\ddagger_{cat}$.

Our definition of ΔG_{CC} for Asn279 (Table 2) can be used for dCTP and dTTP substrates, which contain the O2 atom as H-bond acceptor. This definition can be generalized to predict mutation effects for pol β catalyzed insertion of dATP and dGTP substrates by choosing their N3 atom, which is situated in a similar minor-groove position as O2 of dTTP or dCTP, as the new H-bond acceptor. The correlation coefficient for this general predictor of mutation effects could be further refined if angular parameters of Asn279-dNTP hydrogen bond were considered along with the H-bond distance [28] in the definition of ΔG_{CC} for Asn279. If this generalization could be established, we would then have an efficient theoretical tool for predicting pol β replication fidelity. The formation of a mispair can be treated in the framework of our model as the mutation of one residue of the protein/DNA complex. Additional systematic presteady state kinetic studies of pol β mutants are needed to refine this ΔG_{CC} function for all dNTPs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by NIH grants 1U19CA105010 and R37GM21422.

References

1. Beard WA, Wilson SH. Structure and mechanism of DNA polymerase β . Chem Rev 2006;106:361–82. [PubMed: 16464010]
2. Showalter AK, Lamarche BJ, Bakhtina M, Su MI, Tang KH, Tsai MD. Mechanistic Comparison of High-Fidelity and Error-Prone DNA Polymerases and Ligases Involved in DNA Repair. Chem Rev 2006;106:340–360. [PubMed: 16464009]
3. Srivastava DK, Husain I, Arteaga CL, Wilson SH. DNA polymerase beta expression differences in selected human tumors and cell lines. Carcinogenesis 1999;20:1049–1054. [PubMed: 10357787]

4. Dobashi Y, Kubota Y, Shuin T, Torigoe S, Yao M, Hosaka M. Polymorphisms in the human DNA polymerase beta gene. *Human Genetics* 1995;95:389–390. [PubMed: 7705833]
5. Florián J, Goodman MF, Warshel A. Computer Simulation of The Chemical Catalysis of DNA Polymerases: Discriminating Between Alternative Nucleotide Insertion Mechanisms for T7 DNA Polymerase. *J Am Chem Soc* 2003;125:8163–8177. [PubMed: 12837086]
6. Petruska J, Goodman MF, Boosalis MS, Sowers LC, Cheong CI, Tinoco J. Comparison between DNA melting thermodynamics and DNA polymerase fidelity. *Proc Natl Acad Sci USA* 1988;85:6252–6256. [PubMed: 3413095]
7. Bren U, Martinek V, Florián J. Free Energy Simulations of Uncatalyzed DNA Replication Fidelity: Structure and Stability of T-G and dTTP-G Terminal DNA Mismatches Flanked by a Single Dangling Nucleotide. *J Phys Chem B* 2006;110:10557–10566. [PubMed: 16722767]
8. Kraynov VS, Showalter AK, Liu J, Zhong XJ, Tsai MD. DNA polymerase β : Contributions of template-positioning and dNTP triphosphate-binding residues to catalysis and fidelity. *Biochemistry* 2000;39:16008–16015. [PubMed: 11123928]
9. Kraynov VS, Werneburg BG, Zhong X, Lee H, Ahn J, Tsai MD. DNA polymerase β : analysis of the contributions of tyrosine-271 and asparagine-279 to substrate specificity and fidelity of DNA replication by pre-steady-state kinetics. *Biochem J* 1997;323:103–111. [PubMed: 9173867]
10. Ahn J, Werneburg BG, Tsai MD. DNA polymerase β : Structure–fidelity relationship from pre-steady-state kinetic analyses of all possible correct and incorrect base pairs for wild type and R283A mutant. *Biochemistry* 1997;36:1100–1107. [PubMed: 9033400]
11. Berg BJV, Beard WA, Wilson SH. DNA structure and aspartate 276 influence nucleotide binding to human DNA polymerase beta - Implication for the identity of the rate-limiting conformational change. *J Biol Chem* 2001;276:3408–3416. [PubMed: 11024043]
12. Eyring H. The activated complex and the absolute rate of chemical reactions. *Chem Rev* 1935;17:65–77.
13. Warshel, A. *Computer Modeling of Chemical Reactions in Enzymes and Solutions*. John Wiley & Sons; New York: 1991.
14. Doublié S, Sawaya MR, Ellenberger T. An open and closed case for all polymerases. *Structure* 1999;7:R31–R35. [PubMed: 10368292]
15. Warshel A. Energetics of Enzyme Catalysis. *Proc Natl Acad Sci USA* 1978;75:5250. [PubMed: 281676]
16. Xiang Y, Oelschlaeger P, Florián J, Goodman MF, Warshel A. Simulating the Effect of DNA Polymerase Mutations on Transition State Energetics and Fidelity: Evaluating Amino Acid Group Contribution and Allosteric Coupling for Ionized Residues in Human Pol β . *Biochemistry* 2006;45:7036–7048. [PubMed: 16752894]
17. Cornell WD, et al. A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J Am Chem Soc* 1995;117:5179–5197.
18. Marelius J, Kolmodin K, Feierberg I, Åqvist J. Q: A molecular dynamics program for free energy calculations and empirical valence bond simulations in biomolecular systems. *J Mol Graphics and Modeling* 1999;16:213–225.
19. Florián J, Goodman MF, Warshel A. Theoretical investigation of the binding free energies and key substrate-recognition components of the replication fidelity of human DNA polymerase β . *J Phys Chem B* 2002;106:5739–5753.
20. Bren U, Martinek V, Florián J. Decomposition of the solvation free energy of deoxyribonucleoside triphosphates using the free energy perturbation method. *J Phys Chem B* 2006;110:12782–12788. [PubMed: 16800613]
21. King G, Warshel A. A surface constrained all-atom solvent model for effective simulations of polar solutions. *J Chem Phys* 1989;91:3647–3661.
22. Lee FS, Warshel A. A local reaction field method for fast evaluation of long-range electrostatic interactions in molecular simulations. *J Chem Phys* 1992;97:3100–3107.
23. Alden RG, Parson WW, Chu ZT, Warshel A. Calculations of electrostatic energies in photosynthetic reaction centers. *J Am Chem Soc* 1995;117:12284–12298.
24. Sham YY, Warshel A. The surface constraint all atom model provides size independent results in calculations of hydration free energies. *J Chem Phys* 1998;109:7940–7944.

25. Warshel A, Sharma PK, Kato M, Parson WW. Modeling electrostatic effects in proteins. *Biochimica et Biophysica Acta* 2006;1764:1647–1676. [PubMed: 17049320]
26. Humphrey W, Dalke A, Schulten K. VMD - Visual Molecular Dynamics. *J Molec Graphics* 1996;14.1:33–38.
27. Batra VK, Beard WA, Shock DD, Krahn JM, Pedersen LC, Wilson SH. Magnesium-Induced Assembly of a DNA Polymerase Catalytic Complex. *Structure* 2006;14:1–10. [PubMed: 16407058]
28. McDonald IK, Thornton JM. Satisfying hydrogen bonding potential in proteins. *J Mol Biol* 1994;238:777–793. [PubMed: 8182748]
29. Bruice TC. A view at the millennium: The efficiency of enzymatic catalysis. *Acc Chem Res* 2002;35:139–148. [PubMed: 11900517]
30. Beard WA, et al. Enzyme-DNA interactions required for efficient nucleotide incorporation and discrimination in human DNA polymerase β . *J Biol Chem* 1996;271:12141–12144. [PubMed: 8647805]
31. Lavrik OI, et al. dNTP binding to HIV-1 reverse transcriptase and mammalian DNA polymerase β as revealed by affinity labeling with a photoreactive dNTP analog. *J Biol Chem* 1996;271:21891–21897. [PubMed: 8702991]
32. Radhakrishnan R, Arora K, Wang Y, Beard WA, Wilson SH, Schlick T. Regulation of DNA repair fidelity by molecular checkpoints: Gates in DNA polymerase β substrate selection. *Biochemistry* 2006;45:15142–15156. [PubMed: 17176036]
33. Showalter AK, Tsai MD. A reexamination of the nucleotide incorporation fidelity of DNA polymerases. *Biochemistry* 2002;41:10571–10576. [PubMed: 12186540]
34. Bakhtina M, Lee S, Wang Y, Dunlap C, Lamarche B, Tsai MD. Use of Viscogens, dNTPRS, and Rhodium(III) as Probes in Stopped-Flow Experiments To Obtain New Evidence for the Mechanism of Catalysis by DNA Polymerase. *J Am Chem Soc* 2005;127:5177–5187.
35. Sucato CA, et al. Modifying the β - γ leaving-group bridging oxygen alters nucleotide incorporation efficiency, fidelity and catalytic mechanism of DNA polymerase β . *Biochemistry* 2007;46:461–471. [PubMed: 17209556]
36. Warshel A. Electrostatic Origin of the Catalytic Power of Enzymes and the Role of Preorganized Active Sites. *J Biol Chem* 1998;273:27035–27038. [PubMed: 9765214]
37. Cannon WR, Benkovic SJ. Solvation, reorganization energy, and biological catalysis. *J Biol Chem* 1998;273:26257–26260. [PubMed: 9756847]
38. Beard WA, Shock DD, Yang XP, DeLauder SF, Wilson SH. Loss of DNA polymerase beta stacking interactions with templating purines, but not pyrimidines, alters catalytic efficiency and fidelity. *J Biol Chem* 2002;277:8235–8242. [PubMed: 11756435]
39. Warshel A, Florián J. Computer simulations of enzyme catalysis: Finding out what has been optimized by evolution. *Proc Natl Acad Sci U S A* 1998;95:5950–5955. [PubMed: 9600897]

Abbreviations

WT	wild type
MD	molecular dynamics
CC	contact configurations
dNTP	deoxyribonucleoside triphosphate
pol β	DNA polymerase β
TS	

transition state

Figure 1.

Schematic free energy profile for the extension of the primer DNA strand by a single nucleotide. E and S denote, respectively, DNA polymerase-DNA complex and free dNTP substrate in solution. ES represents the ternary complex (ground state) and $ES^{\ddagger}$ denotes the rate-limiting transition state. The $ES^{\ddagger}$ state can be stabilized either by lowering free energy of the ground state while keeping $\Delta g^{\ddagger}_{\text{cat}}$ unchanged, or by decreasing $\Delta g^{\ddagger}_{\text{cat}}$. [39]

Figure 2.

The active site of pol β including dCTP⁴⁻ substrate, the ‘catalytic’ (Mg_A) and ‘structural’ (Mg_B) Mg²⁺ ions and their Asp ligands, DNA template and primer nucleotides. The amino acid residues mutated in this study are denoted by red labels.

Figure 3.
Detailed view of the pol β active site. dCTP substrate, Asn279 and Asp256 residues are rendered in the stick representation. Distances that were used for the correlation study are indicated by green or purple lines.

Figure 4. Correlation between Asn279 – dCTP H-bond stability and apparent reaction activation free energy. Error bars were estimated as the difference between ΔG_{cc} calculated from the first and second half the simulation trajectory.

Figure 5.

Effect of the cut-off distance on the correlation between ΔG_{CC} for the Asn279 – dCTP H-bond and experimental $\Delta g^\ddagger$ (points indicated by blue dots and values on the left vertical axis). The numbers of conformations in a population that corresponds to distances larger than the cut-off distance are shown as orange triangles corresponding to numerical values on the right vertical axis. The vertical dashed line indicates the cut-off value used in Table 2 and Figure 4.

Figure 6.

Schematic model of the coupling between the electrostatic preorganization of the binding and TS sites mediated by Asn279. In WT pol β (left), the electrostatic preorganization of both the binding site (Asn279 and dipole A) and TS site (dipole B) is induced by the interaction with the protein residue (indicated by a gray sphere with a + sign). In a mutant protein (right), Asn279 and dipoles A and B relax to a more stable configuration, which is less favorable for binding to both the ground state and TS. Negative charge developing on the α -phosphate of the dCTP substrate in TS is indicated by a δ^- sign.

Table 1

Experimental data used for correlations

	k_{pol} (1/s)	$\Delta G_{cat}^\ddagger$ (kcal/mol)	K_D (μ M)	ΔG_{bind} (kcal/mol)	k_{pol}/K_D ($s^{-1}M^{-1}$)	$\Delta G^\ddagger$ (kcal/mol)	Reference
WT ^a	6.23	17.4	4.31	-7.8	2214000	9.6	[8–11,38]
R183A	1.29	18.5	13.39	-7.1	147600	11.5	[8]
Y271A	1.06	18.5	0.90	-8.8	1801588	9.8	[9]
D276V	3.92	17.7	0.46	-9.2	12987151	8.5	[11]
K280G	9.34	17.2	116.44	-6.1	123098	11.4	[38] ^b
R283A	0.55	19.0	85.25	-5.8	9862	13.0	[10]
E295A	1.00	18.6	22.70	-6.8	67091	12.1	[8]

^a Experimental data were normalized to average values of wild type human DNA polymerase β

^b Steady state kinetic data

Table 2
Calculated data and correlation coefficients between the calculated and observed free energies

Property	Cut-off (Å)	ΔG_{CC} (kcal/mol) ^a							correlation coefficient ^b		
		WT	R183A	Y271A	D276V	K280G	R283A	E295A	ΔG_{bind}	$\Delta G_{cat}^{\ddagger}$	$\Delta G^{\ddagger}$
PA-O3'	3.1	3.2	3.1	3.2	3.5	3.2	3.4	3.6	0.02	0.26	0.09
H3T-O5'(dCTP)	2.5	-3.1	-2.8	-2.5	-3.0	-2.4	-3.2	-3.0	0.07	0.32	0.13
Mg-O3'	2.5	-2.9	-2.4	-2.7	-3.0	-2.8	-3.0	-2.9	0.00	0.14	0.10
Mg-O1A(dCTP)	3.0	-3.3	-3.8	-3.6	-2.9	-3.6	-3.3	-3.2	0.30	0.12	0.33
Mg-OD2(D256)	2.15	-3.2	-3.3	-3.0	-3.3	-3.0	-3.0	-2.8	0.44	0.47	0.60
Mg-OD2(D190)	2.15	-3.8	-3.6	-3.7	-3.6	-3.7	-3.7	-3.7	0.13	0.11	0.09
Mg-OD1(D192)	2.15	-2.7	-2.6	-2.6	-2.8	-2.7	-2.7	-2.7	0.34	0.42	0.47
Mg-H ₂ O	2.3	-2.5	-2.8	-2.7	-2.5	-3.0	-2.4	-2.8	0.27	0.25	0.21
ND2(N279)-O2 (dCTP)	3.9	-4.2	-3.1	-4.6	-5.2	-4.6	-2.5	-3.4	0.72	0.74	0.88
O1B(dCTP)-H3T (dCTP)	3.5	-2.9	-3.5	-3.2	-2.9	-2.9	-2.7	-2.8	0.47	0.70	0.69
N3(dCTP)-H1 (dG _{temp})	2.25	-3.4	-3.2	-3.1	-3.3	-2.9	-2.5	-3.4	0.63	0.23	0.57
O ₄ (S180)-O3P _γ (dCTP)	3.2	-3.4	-3.0	-3.1	-3.3	-3.1	-3.3	-3.4	0.03	0.04	0.06

^aEq. 4.

^bCorrelation coefficients (R) for the linear correlations between ΔG_{CC} and experimental ΔG_{bind} , $\Delta G_{cat}^{\ddagger}$ or $\Delta G^{\ddagger}$ listed in Table 1.