

DNA Synthesis Efficiency and Fidelity Mechanisms

The burden of high accuracy synthesis in DNA replication and repair falls in large part on DNA polymerases which incorporate nucleotides to the primer strand complementary to the template bases. Since mutations stemming from polymerase errors can result in permanent genomic change that may lead to human diseases like colon, skin, or lung cancer and premature aging, understanding fidelity mechanisms is of great scientific and biomedical importance.

The fidelity of DNA polymerases refers to their ability to choose correct nucleotides from a pool of structurally similar molecules. Across the many known polymerases, fidelity varies from one to nearly one-million base errors per one-million nucleotides synthesized. The preference for correct rather than incorrect nucleotides depends on the environment and possibly plasticity of the binding pocket and the thermodynamic and dynamic considerations associated with the process of nucleotide incorporation.

In this work we focus on pol beta, for which based on a large body of structural and enzyme kinetics measurements, it is established that the process of nucleotide incorporation is accompanied by a large conformational change in the enzyme structure followed by "chemistry", which involves the catalysis of the phosphoryl transfer reaction.

Recently, we reported our results of the application of the transition path sampling (TPS) methodology to the closing conformational change before chemistry [1,2], in which we identified a complex orchestration of subtle conformational changes that steers the enzyme toward the reaction-competent state and found the slowest conformational step to be Arg258's rotation of order 20 +/- 3 kT; this value is close to, but less than (by a few kT) the overall rate-limiting step measured experimentally (kpol).

In this work we report an analogous TPS application to an incorrect (G:A) nascent basepair and suggest thermodynamic as well as geometric differences from (G:C) in the coordination of ligands to the catalytic magnesium ion and in the positioning of the O3' oxygen of the DNA primer base to P_{alpha} of the incoming nucleotide. We also report our results for the mechanisms associated with the catalytic process using a mixed quantum mechanics/molecular mechanics (QM/MM) methodology. Using the QM/MM Hamiltonian we considered the active site of a solvated pol beta system with correct (G:C) and incorrect (G:A) base pairing. The quantum region consisted of the Mg²⁺ ions, the conserved aspartates 190, 192, 256, incoming nucleotide, terminal primer of DNA, Ser 180, Arg 183, and water molecules within hydrogen bonding distance of the QM atoms. These 86 'quantum atoms' were treated using a B3LYP density functional and 6-311G basis set. The molecular mechanical region consisted of the rest of the protein, DNA, Na⁺, Cl⁻, and solvent molecules up to three solvation shells adjoining the protein/DNA/dNTP complex. Wave function optimizations in the QM region were performed according to a density functional formalism, and geometry optimizations of the whole system were performed using the

Adopted Basis Newton Raphson method implemented in CHARMM.

Evolution of key active-site distances and energies in the geometry optimization procedure with the QM/MM Hamiltonian indicates that these key distances differ from those predicted based on the classical CHARMM~27 force-field alone, and that suggests that the chemical reaction step may still involve an activated process with a large free energy barrier both for G:C as well as G:A systems. In particular, for the G:C system, the reactive O3'-P_alpha distance and the Catalytic Mg2+ - O3' distances are larger than those expected for the transition state. These non-ideal distances may be a result of the O3' group at a protonated state and the Catalytic Mg2+ coordinated by three water molecules. Thus, the primary activated event in the chemical step may involve the deprotonation of the O3' group of the terminal DNA primer. Our geometry is consistent with this conclusion. Moreover, based on our geometry, the base that likely abstracts this proton is Asp 256, because the Odelta1 and Odelta2 oxygen atoms of Asp190 and Asp192 are closely coordinating the two Mg2+ ions, while only the Odelta1 atom of Asp256 is involved with the Catalytic Mg2+ ion. Furthermore, there is a network of hydrogen bonds through two mediating water molecules separating the Odelta2 atom of Asp256 and the O3' atom, strongly implicating a concerted proton transfer mechanism for the deprotonation of the O3' group. This concerted proton transfer hypothesis will be tested in our subsequent QM/MM/TPS study of the nucleotideincorporation process.

Our conclusions point to a rate-limiting chemical step for both the G:C and G:A systems whith the latter posing a much larger barrier for catalysis. Ongoing work in aimed at quantifying these barriers using free energy calculations involving the QM/MM Hamiltonian.

[1] R. Radhakrishnan, T. Schlick, Proc. Nat. Acad. Sci. USA, 101, 5970 (2004).

[2] R. Radhakrishnan, T. Schlick, (2004) J. Chem. Phys., 121, 2436.