THE 8-OXO-7,8-DIHYDRO-2′-dGTP BEHAVIOR IN ACTIVE SITE OF HUMAN DNA POLYMERASE β: STRUCTURAL INVESTIGATION *IN SILICO*

A. Yu. Nyporko

Taras Shevchenko National University of Kyiv, 64/13, Volodymyrska St., Kyiv 01601, Ukraine,
dfnalex@gmail.com

The oxidized bases in the composition of DNA as well as DNA precursors (desoxy-nucleotide triphosphates, dNTPs) appearing in living cell as a result of oxidative stress are the one of major sources of genomic instability. Among oxidized forms of nitrogenous bases, the 8-oxo-7,8-dihydro-2-deoxyguanine (8odG, 8-oxo-dG) is the most ubiquitous. This compound has a high mutagenic potential due to its ability to preferably interact with adenine instead of cytosine. In particular, the 8odG in the composition of the incoming nucleotide triphosphate (8-oxo-GTP) is able to immediately incorporate into the growing DNA chain and, thus, to cause the invert replacement dA → dC because it is possible to pair with the incoming dCTP as well as dATP in the next round of DNA replication.

The efficiency of 8oxo-dG incorporation in growing DNA clearly depends on the nature of appropriate DNA polymerases. One of the most sensitive to 8-oxo-dGTP is the eukaryotic DNA polymerase β (pol β). The binding of 8-oxo-dGTP in the active center of pol β can result in two different molecular events. First of them is the incorporation of 8oxoguanine into a growing DNA chain, the other is a discrimination of 8-oxo-dGTP from the active center. While effects of incorporation of this modified guanine in DNA are well studied, the immediate consequences of 8-oxo-dGTP discrimination are still unclear.

The behavior of 8-oxo-dGTP molecule in the area of the active site of human DNA polymerase β was investigated using molecular dynamics (MD) calculation. The studied system were prepared on the basis of the X-ray derived structure of ternary complex “human DNA polymerase β: DNA: 8-oxo-dGTP” at 2.0 Å resolution deposited in RCSB Protein Data Bank (access code is 3MBY). Molecule of 8-oxo-dGTP in pol β active site is paired with adenine of the template strand. All MD computations were performed with GROMACS software version 4.6.5 using CHARMM27 force field with implemented CMAP. The

topology of 8-oxo-dGTP was performed via web-based tool SwissParam. To simulate intracellular conditions all furthel calculations were carried out in water solution containing 0.15M NaCl. Four productive MD trajectories were calculated for 100 ns time interval at 300 K. Fluctuations of 8-oxo-dGTP structure in free and bound states were evaluated using the *rho* method with *g_rms* modules of the GROMACS software. All energy parameters of the investigated system and its components were calculated with *g_energy* module. The dynamics of hydrogen bonds was calculated using the *g_hbond* module.

The principle phenomenon revealed as investigation results is existence of two cardinally different models of behavior inherent to 8-oxo-dGTP molecule. In two cases the ligand molecules loses the connections with template dA and starts to migrate inside of enzyme space (migrate trajectories). In the other two cases 8-oxo-dGTP stably stays in DNA polymerase active site, “keeps in touch” with template nucleotide and maintains the hydrogen bonds with it (stable trajectories).

The spatial structure of 8-oxo-dGTP in stable trajectories appears to be sufficiently rigid despite the presence of number of bonds around which the free rotation is possible, and its conformational energy is characterized by high stability over the time of studied MD. Average values of energy (-10229.7 and -10227.1 kJ/mol) are practically the same for both cases. Amino acid microenvironment of 8-oxo-dGTP also practically doesn’t change over the studied MD interval. Thus, stable variants of 8-oxo-dGTP behavior evidently correspond to case of the further incorporation modified 8-oxo-dG into growing DNA strand.

The behavior of 8-oxo-dGTP molecule in migrate trajectories is significantly more complicated. The 8-oxo-dGTP loses the H-bonds with template dA6 (at 11 and 6.5 ns of MD in first and second case respectively) and starts to migrate in DNA polymerase space. The 8-oxo-dGTP spatial structure regularly exhibits much more flexibility in comparison to itself behavior in stable trajectories that reflects in corresponded values of individual atomic fluctuations. However, contrary to the expectations the general levels of conformational energy of 8-oxo-dGTP as well as energy fluctuation patterns in both migratory trajectories are completely time stable. The average values of conformational energy are -9938.6 and -10018.6 kJ/mol for trajectories 1 and 2 respectively that is slightly more than corresponded

values for stable trajectories. The 8-oxo-dGTP movement pathways of don't coincide each other that is confirmed by differences of their conformational spaces and amino acid microenvironment. It seems to be the most important that 8-oxo-dGTP not only doesn't leave the enzyme space but directly prevent transition of DNA polymerase from closed to open conformation as well as the further binding of incoming dNTP. This observation lets a possibility to consider it as natural inhibitor of DNA pol β activity and possible intracellular regulator which mediates the direct transition of the cell from normal state to programmed cell death omitting the malignancy stage.

Acknowledgements: Author thanks the Extreme Science and Engineering Discovery Environment (XSEDE) for the award allocation number TG-DMR110088.