Biomolecular Simulation of Base Excision Repair and Protein Signaling

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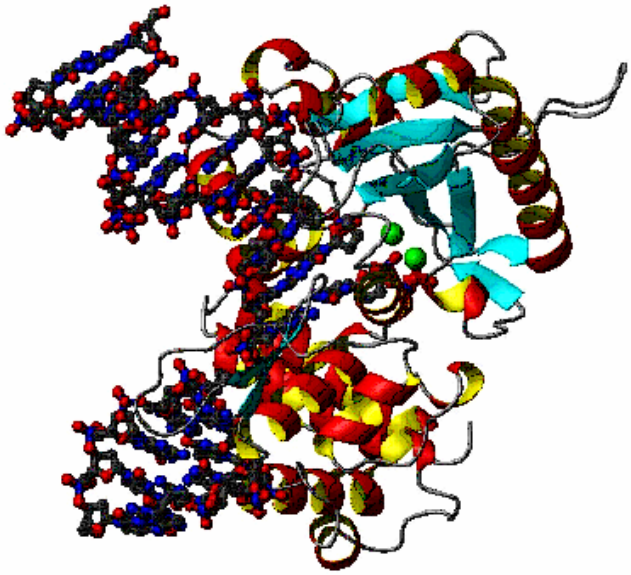
Environmental factors including ionizing radiation contribute to continuous damage of cellular DNA, in addition to endogenous sources. The damage resulting from oxidative stress and ionizing radiation is primarily in the form of oxidized bases, single strand breaks, and loss of bases. These are the targets of the Base Excision Repair (BER) mechanism enzymes, including polymerase-β. Failure to correctly and timely repair these damaged DNA sites can result in cell death, carcinogenesis, or genetic diseases. Resulting mutations in cell signal transduction enzymes can lead to uncontrolled cell proliferation or differentiation. For example, mutations in Ras, the molecular switch in several growth-factor signaling pathways, have been found in about 30% of human tumors. These signaling pathways often involve a chain of protein kinases that activate or deactivate each other through phosphorylation reactions, eventually controlling the activation of transcription factors in the cell nucleus.

Fidelity of Base Excision Repair Protein Human Polymerase β

A detailed understanding of the mechanism by which fidelity is assured by polymerase β (pol β) in the gap-filling DNA repair process is expected to require a complete and accurate description of the pol β active site in its active conformation just prior to the chemical nucleotidyl transfer step. Thus far, crystal structures obtained for pol β–gapped DNA–dNTP complexes have been incomplete due to intentional structural alterations necessary to prevent the chemical step in order to obtain a stable structure from the mid point of the pol β catalytic cycle. Using the methods of molecular dynamics (MD) and quantum chemical calculation, we have been able to more fully characterize the active conformation of the pol β active site.

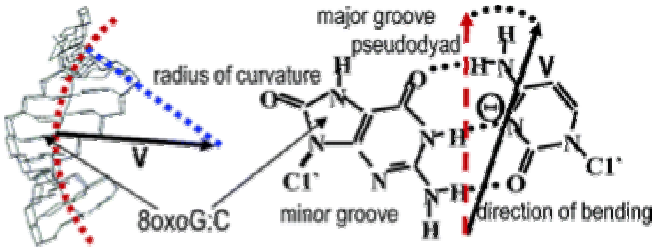


Closed conformation of the pol β (as ribbons) - gapped DNA (as balls and sticks) – dCTP ternary complex. dCTP substrate and magnesium ions (in green) present in the active site can be seen in center of the picture.

Effects of 8-oxoguanine on DNA Bending

Molecular dynamics (MD) simulations were carried out on the fully solvated and cation neutralized DNA oligonucleotide

GGGAA***C***AACTAG:CTAGTT***G***TTCCC in its native form and with guanine in the central G_{19}:C_6 base pair replaced by 8-oxoguanine (8oxoG). The direction and magnitude of global bending were assessed by a technique used previously to analyze simulations of DNA containing a thymine dimer. The presence of 8oxoG did not greatly affect the magnitude of DNA bending; however, bending directions that compress the major groove and expand the minor groove were significantly more probable when 8oxoG replaced G_{19}. Crystal structures of glycosylases bound to damaged-DNA substrates consistently show bending that expands the minor groove. We conclude that changes in bending dynamics that facilitate this expansion are part of the mechanism by which 8oxoG is recognized by the base excision repair pathway.



Simulation of Bulges in DNA and RNA

Aim of the ongoing project is to characterize the structure and dynamics of chemically modified DNA due to oxidative stress or irradiation. During the present first phase of the project molecular dynamics simulations have been performed on a 12 base pair B-DNA including water molecules and ions. The simulations have been performed for approximately 2 ns simulation time. The equilibrated simulation will serve as a reference system for simulations that include chemically modified nucleotide-bases. In addition, the equilibrated system will also be used to induce structural DNA deformations that are known to occur during DNA repair processes such as minor groove opening and extra-helical base flipping. Molecular dynamics simulation on single unmatched extra bases (bulges) located at the center of otherwise double-stranded DNA and RNA have been initiated. Such non-helical structures occur frequently in RNA and also in DNA as a result of DNA modification and damage. The simulations on a single adenine and a single uridine bulge in RNA have now reached a duration of ~1.5 ns. However, due to the increased flexibility of the central bulge nucleotide compared to regular nucleic acids the simulations are not yet fully equilibrated.

Protein Kinase A

A molecular dynamics simulation on the apo form of protein kinase A has been carried out, currently to about 3 ns. Snapshots from about 1 ns of this trajectory have been used for ligand docking experiments. We used Autodock 3.05 to dock balanol to these snapshots. The averaged binding energy is -12 +/- 2 kcal/mol. This value is quite close to the experimental values of -10/-11 kcal/mol. For the scoring function used in Autodock 3.05, the electrostatic interactions contribute about -2 kcal/mol to the binding energy. Although these results are consistent with those obtained by using a PB/SA (Poisson-Boltzmann/Solvent-accessible Area) model employed earlier, they differ quantitatively. The PB/SA model gave significantly more favorable binding energy (-27 kcal/mol) and the electrostatics contributions (-0.9 kcal/mol) accounted for a smaller fraction of the total binding energy. However, the results from Autodock agree better with a semi-empirical PB/SA model in which a few adjustable parameters were introduced to scale the results of the PB/SA model to fit existing experimental data. The semiempirical model gave a hydrophobic contribution of -16 kcal/mol, an electrostatics contribution of -1 kcal/mol, and other contributions accounted to about 2 kcal/mol.
Further study will include other derivatives of balanol and other ligands as planned. Additional analyses will also be carried out to characterize the dynamics of the apo protein and their influence on molecular recognition.