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Damage Recognition, Protein Signaling and Fidelity of Base Excision Repair
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Introduction:

A variety of environmental agents, including ionizing radiation, produce oxidative DNA damage. One of the most abundant and extensively studied forms of oxidative DNA damage is 8-oxoguanine (8oG). Its tendency to mispair with adenine during replication makes 8oG a direct source of GC to TA transversion mutations. Elaborate defense systems to protect against the mutagenic effects of 8oxoG are found in most organisms from bacteria to humans.

The mutagenic potential of 8oG lesions is believed to result from Coulomb repulsion between the oxygen atom added to the guanine bases and oxygen atoms on deoxyribose backbone, which causes 8oG to prefer a syn conformation of the glycosyl torsion angle and to form a Hoogsteen base pair with adenine. Expression of this preference occurs at DNA replication when 8oG is in the separated template strand. The anti conformation of the glycosyl torsion angle is maintained in 8oG:C base pairs and the presence of a 8oG:C base pair destabilizes duplex DNA by, at most, a few kcal/mole (1)

A part of the stability of DNA containing 8oG:C base pairs appears to be related to a change in the bending dynamics that favors bending into the major groove. Miller et al. (2) demonstrated this effect in MD simulations of the DNA oligonucleotide GGGAAACAAC TAG:CTAGTTGTTCCC, using the PARM99 force field (3), with and without guanine in the central G₁₉:C₆ base pair replaced by 8oG. Analysis of the dynamics of the native sequence showed a correlation between bending into the minor groove and shorter distances between the H8 atom on guanine and backbone oxygen atoms of the G₁₉ nucleotide, suggesting that bending in this direction will be energetically unfavorable when H8 in guanine is replaced by O8 in 8oG.

Work by Ishida (4) suggested that DNA containing 8oG:C was also stabilized by changes in the hydration of the oxidized base. Ishida observed water molecules bridging between the atoms O8 and O5' in the 8oG nucleotide during an MD simulation of the 12mer d(CGCGAATTTCGCG)₂ using the AMBER 95 force field (5). In addition to a different base sequence and force field, our MD simulations of DNA containing 8oG have different partial atomic charges on the 8oG nucleotide. In Ishida's simulation, the charge on the O8 atom of 8oG was essentially the same ours but the charge on the O4' atom the sugar moiety was 27% more negative than the standard AMBER charges for deoxyribose, which we use. Hence, Ishida's model had greater Coulomb repulsion between base and sugar in the 8oG nucleotide. Given the differences in base sequence, force-field parameters and partial charges, we were interested in determining if our MD simulations would show changes in DNA hydration due to 8oG that were similar to those reported by Ishida (4).

Methods:

Results from MD simulations of GGGAAACAAC TAG:CTAGTTGTTCCC were analyzed for the patterns of hydration near the G₁₉:C₆ base pair in the presence and

absence of 8oG. In the set of simulations (native and modified) for our earlier work (2), the oligonucleotide was solvated by 22 Na⁺ counterions and about 7000 water molecules. In a repeat of these simulations for this study, a larger box of water was used that contained about 15,000 water molecules. In both cases, coordinates of all atoms in the simulation (solute, solvent and counterions) were saved every 2 ps resulting in 1000 frames from each 2 ns trajectory file that could be analyzed for hydration patterns. These frames were systematically search for sustained contacts between DNA atoms and water molecules. Atoms separated by a distance less than or equal to their Van der Waals radii plus 0.0075 nm were considered to be in contact. Sustained contacts were defined as those surviving for at least 50 consecutive frames of a trajectory file.

For each of the 1000 frames sampled from the trajectory files of the simulations of native and damaged DNA, distances were calculated between all oxygen atoms of water molecules in the solvent and DNA atoms. If two atoms made contact as defined above, the solvent atom was added to a list belonging to the solute atom it contacted. After this first contact, a counter was incremented for that solvent atom for each successive frame in which it contacted the solute atom. Solvent atoms that had 50 or more contacts with a particular solute atom were listed along with their contact counts and corresponding solute atoms after all frames were processed. Each solute atom that experienced high contact counts with one or more solvent atoms had a profile written that showed the distances between itself and those solvent atoms over the course of the 2 ns of simulation. Solvent molecules with long contacts to DNA atoms of special interest were profiled for contacts with other solvent molecules to discover networks of water molecules.

Results:

Typical structures within the hydration layer that probably contribute to the stabilization of DNA containing 8oG are shown in Figure (1). Patterns I and II are bridging configurations of single water molecules at connect the O8 atom of the modified base to the O2P and O5' atoms of the sugar-phosphate moiety, respectively. In our earlier work (2) we noted that the presence of 8oG increased the probability of finding a counterion near the damaged base pair. Analysis of the effects of 8oG on DNA hydration revealed that these counterions assist the formations of water networks of the type illustrated by Patterns III and IV in Figure (1), where the light blue sphere shows the location of a counterion.

The 1000 frames sampled from the trajectory files of our MD simulations of GGGAACAAC TAG:CTAGTT8oGTTCCC were exhaustively searched for bridging water molecules like the one shown in Pattern II of Figure (1). In the simulation with the smaller number of water molecules reported in (2), 348 instances of Pattern II were found with a combined distance of atoms O5* and O8 in 8oG₁₉ nucleotide less than 0.6 nm from the oxygen atom of the bridging water molecule. In an exhaustive search of the corresponding simulation of the native sequence with a similar number of water molecules, 158 instances were found of water molecules in a similar orientation as Pattern II with their oxygen atoms less than 0.6 nm (combined distance) from atoms O5* and H8 in G₁₉ native nucleotide.

When the same analysis was applied to the more recent simulations with a larger number of water molecules solvating the oligonucleotides, we found 531 examples of

Pattern II with water oxygen atoms less than 0.6 nm combined distance from atoms O5*, and O8 of 8oG₁₉ compared with 188 cases of Pattern II in the native simulation with water oxygen atoms less than 0.6 nm combined distance from atoms O5*, and H8 of G₁₉. Hence we conclude that the more electronegative O8 atom in 8oG makes the occurrence of hydration Pattern II about 2.5 times more probable than a similar pattern of hydration on the native guanine nucleotide. This effect on hydration probably increases the stability of DNA containing 8oG by reducing the electrostatic repulsion between the O8 atom of the modified base and backbone oxygen atoms. Future studies will attempt to quantify the magnitude of this stabilization effect.

Literature Citations:

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Figure1. Typical patterns of hydration in residue 8oG₁₉ of the oligonucleotide GGGAACAAGTAG:CTAGTT8oGTTCCC that link atom O8 to backbone oxygen atoms

Pattern I: bridging from O8 to O2P

Pattern II: bridging from O8 to O5'

Pattern III: O8 – Na⁺ - H2O – O2P

Pattern IV: O8 – Na⁺ - H2O – O5'