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Flipping Back of Extrahelical Guanine after Methyl Repair

Rajendra Prasad Koirala and Narayan Prasad Adhikari*

Central Department of Physics, Tribhuvan University, Nepal

Email: narayan.adhikari@cdp.tu.edu.np

ABSTRACT

Methylation at O6 atom of guanine is a type of DNA damage which can cause a cancer. This damage at O6 atom of guanine in a DNA can be transferred to SG atom of cysteine in O6-alkylguanine-DNA alkyltransferase (AGT). Flipping out of methylated guanine from its base stack is essential to give off the methyl adduct (CH_3) to AGT. AGT receives the methyl adduct at cysteine leaving guanine demethylated, but still in flipped out orientation. The repair mechanism of DNA would be completed only when the extrahelically flipped guanine returns back into the base stack, which is considered as the final step of the DNA repair mechanism. Here, the intrahelical flipping mechanism of repaired guanine has been studied. The work is further extended to examine the stability of hydrogen bonds between guanine and its pair partner cytosine. The overall result shows that intrahelical rotation of repaired guanine is possible and the base pairing is stable as the ordinary hydrogen bonding.

1. Introduction

Genetic integrity is constantly threatened by several types of damages in DNA like alkylation, deletion or crosslinking of nucleotides, structural changes etc. Many endogenous (chemicals in metabolisms) and exogenous (like ultravoilets and other external agencies) factors are responsible in such DNA insult [1,2]. The aberrations in DNA nucleotide have both positive and negative impacts in body mechanism. Alkylation, specifically methylation, in DNA can regulate the gene expression and suppression, but excessive expression and suppression can harm the body [3-5]. Besides these, there are many harmful effects of DNA methylation. The methylation at O6 point of guanine base in a DNA can cause the cancer [6,7].

A protein, O6-alkylguanine-DNA alkyltransferase (AGT), can be employed to

remove the methyl adduct from DNA nucleotide [8,9]. It detects the damage, interaction at methylated vicinity and repairs the methyl damage. AGT binds via minor groove of DNA mediated by several nucleotide-residue pairs in DNA and AGT. The cysteine at 145th position of AGT receives the methyl adduct as a suicidal reaction [10]. Then, the DNA gets free from damage. The activity of AGT is then degraded and lost the binding capacity. The methylated cysteine thus deactivated and does not harm the body [11].

The extrahelical flipping of methylated guanine is one of the essential condition to repair the methyl damage from DNA nucleotide [12]. When AGT detects the damaged region in DNA, the sidechain of arginine at 128th position of AGT intercalates between the base strands and forms hydrogen bond with cytosine at the 20th position [8,13]. Moreover, O6 atom at guanine leaves its pair partner N4 of cytosine. These events weaken the hydrogen bonding of methylated guanine with its partner. Then, the methylated guanine rotates out from the backbone and approaches at the binding cavity of AGT. Thus, the methyl transfer is possible [9,14].

During the extrahelical condition, the methylated guanine is inserted into the AGT active site and nearer to the methyl receiver CYS145. DNA and AGT form a stable complex mediating several amino acids, out of them, CYS145, HIS146, GLU172, TYR114, LYS165 and SER159 contributes as the major contributing amino acids [15]. CYS145 is then converted to cysteine thiolate anion. Then, abstraction of TYR114 proton by N3 site of O6 point of methylated guanine would occur and finally the methyl transfer takes place [15,16].

Several studies have been carried out regarding the recognition of methylation damage, interaction with AGT, extrahelical flipping of nucleotide and transfer of methyl adduct from DNA [17-19]. As far as our best knowledge, the intrahelical flipping of repaired nucleotide has not been studied yet. To deal such process, we have taken a molecular system containing extrahelical guanine base in the double stranded

DNA. Then, the flipping in mechanism of guanine and its stability after the base pair between this guanine and its base partner cytosine have been studied. We believe that this study conveys the complete repairing cycle of DNA methylation damage at nucleotide.

2.Material and methods
2.1 System setup

A DNA-protein complex was taken from protein data bank with PDB ID 1T38 [20]. The original structure of the complex contains a double stranded DNA with methylation at O6 position of seventh residue guanine and a DNA repair protein, O6-alkylguanine alkyltransferase (AGT). In the complex, AGT was at centre of mass (COM) distance 24.8 Å far from the DNA. To set the DNA and AGT at non-interacting condition, the DNA is shifted manually along negative x direction with COM distance 42.8 Å. The 145th residue of AGT, serine is mutated to cysteine (S145C) by using CHARMM-GUI online web server [21]. The methyl adduct (CH_3) was removed from guanine and then modelled the methylated cysteine in AGT. The complex was solvated in water with periodic boundary condition (PBC) box of dimension $(128 \times 64 \times 72)$ Å^3 and then added Na$^+$ and Cl$^-$ ions in order to neutralize the entire system.

2.2 Molecular Dynamics Simulations

All atom molecular dynamics were carried by using NAnoscale Molecular Dynamics (NAMD) simulation package [22]. The simulation was executed by employing the CHARMM36m force field [23]. Newly designed force field is used for the modified structure of cysteine. The Particle Mesh Edward (PME) was used to treat the long-range interactions with a 12.0 Å non-bonded cutoff. To remove the steric hindrance and undesired position of atoms, the prepared system was executed for energy minimization run for 10,000 steps [24-27]. The output structure of the molecular system was then used

as the input of equilibration run. The equilibration run was carried out for 2 ns for maintaining at 1 bar pressure and 300 K temperature. Finally, production run was propagated for 10 ns by using Langevin dynamics with a damping constant of 1 ps^{-1} under NPT conditions with time step of 2 fs.

To examine the stability of hydrogen bonding between targeted guanine (GUA7) and its pair partner (CYT20), we also prepared a new molecular system picking out only DNA system from the output structure of above 10 ns simulation run and molecular dynamics simulation was performed. The procedure of system set up is similar to the aforementioned process. The system was solvated in water of PBC box size $(67 \times 67 \times 67)$ Å^3 and neutralized by adding Na$^+$ and Cl$^-$ ions. Then, energy minimization was performed for 10,000 steps and equilibration run was executed for 2 ns at pressure 1 bar and temperature 300 K. The production run was propagated for 100 ns under NPT condition.

Visual Molecular Dynamics (VMD) [28] and pymol [29] is used to visualize the molecular structure and to evaluate the various physical parameters during the analysis of outcomes of simulation. Xmgrace program is used to plot the graphs.

3. Results and Discussion

The molecular dynamics simulations have been carried out to examine intrahelical flipping back of methyl-free guanine and to evaluate the stability of hydrogen bonding between GUA7 and CYT20 in double stranded DNA. The formation of hydrogen bonding, variation of distance between the atoms to form the ordinary hydrogen bonding and radial distributions of interacting atoms in residues GUA7 and CYT20 have been investigated from the outcomes of MD simulations.

3.1 Orientations and Hydrogen bonding

The interaction between repaired DNA and methylated AGT is weaker than that of the pairs of methylated DNA and normal AGT [9,18]. Considering the degrading activity of AGT after methylation, we have translated DNA from AGT along negative x direction maintaining the COM distance at 42.8 Å so that there is no significant interaction between them. In the beginning of the MD simulation, the flipped out guanine (GUA7) was oriented extrahelically towards the active cavity of AGT as shown in Fig. 1 (a).
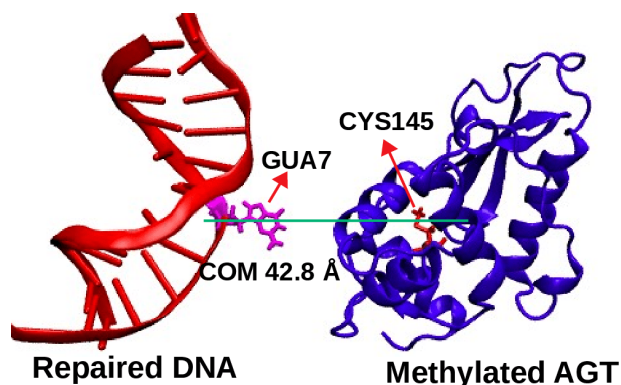

Fig. 1: The structure of repaired DNA and methylated AGT complex. The center of mass (COM) distance between the DNA and AGT are set 42.8 Å so that there is no significant interaction between these molecules. The methylated cysteine is shown by arrowhead.

In the first step, we investigated the activity of flipped out nucleotide, GUA7, during 10 ns simulation time. Upto the 5 ns simulation time scale, we have observed no any rotation of GUA7 from its base stack, oscillated extrahelical region. After 5 ns, the flipped out nucleotide attempted to rotate inside the backbone. The rotation was observed significant during the 6 ns to 7 ns time interval and finally came back to form the ordinary pair between 9 ns and 10 ns time scale as shown in Fig. 2 (a).

We observed the hydrogen bonding pattern during the 10 ns simulation time. Of course, hydrogen bonding play crucial role in the formation of stable structure of protein and DNA, and their complex [30-32]. Hydrogen bonding contributes in the formation of secondary and higher order proteins. Likewise, it

has important role in the formation of convoluted structure of DNA. Specifically in DNA, guanine forms three hydrogen bonds with cytosine (G ≡ C) and adenine forms two hydrogen bonds with thymine (A = T) [33,34]. In this study, the guanine was at the flipping out condition so that it was disconnected from the hydrogen bonding with its pair partner cytosine. We have investigated whether the flipped out guanine could rotate back intrahelically and form the ordinary hydrogen bonds with cytosine or not. For this, 10 ns simulation run was carried out under NPT condition.

Till the 5 ns simulation run, GUA7 has not been returned back into the base stack and so no hydrogen bond has been detected between GUA7 and its pair partner CYT20. After 5 ns, the GUA7 was found gradually rotated back intrahelically. The flipping back is relatively

(a)

(b)
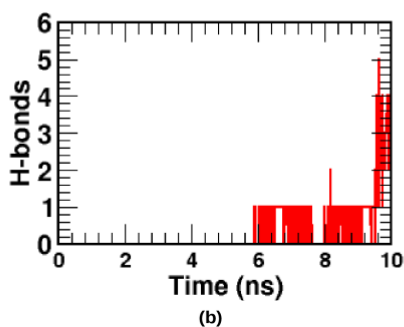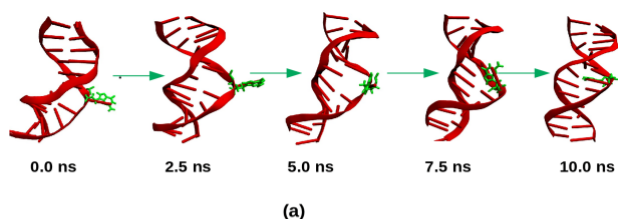
faster in 6 ns to 7 ns time scale. Finally, pairing was completed before the 10 ns as shown in Fig. 2 (a) and (b).

Fig. 2: The intrahelical flipping of GUA7 during the 10 ns simulation time scale (a) five snapshots of simulation trajectories (0.0 ns, 2.5 ns, 5.0 ns, 7.5 ns and 10.0 ns) (ii) the formation of hydrogen bonds, the bonds started after the 5 ns time scale and all three ordinary hydrogen bonds form in between 9 ns and 10 ns.

3.2 Distance and distribution of targeted nucleotides

The distance and distribution of atoms in hydrogen bonding pair partners GUA7(N1)-CYT20(N3), GUA7(N2)-CYT20(O2), GUA7(O6)-CYT20(N4) have been estimated to investigate the flipping mechanism of targeted guanine. The variation of distance between the hydrogen bonding pairs is shown in Fig. 3 (a) and the corresponding distributions are shown in Fig. 3 (b). Regarding the distance analysis, the atoms pair, GUA7(O6)-CYT20(N4) came first within 3.5 Å and formed stable hydrogen bond than that of other two pairs, GUA7(N1)-CYT20(N3) and GUA7(N2)-CYT20(O2). After 9 ns time scale, remaining two pairs also rearranged within 3.5 Å to form the hydrogen bonding. Finally, all three pairs lie within 3.5 Å. The distribution of such atom pairs also supported the rearrangements of ordinary hydrogen bonding pairs as shown in Fig. 3 (b).
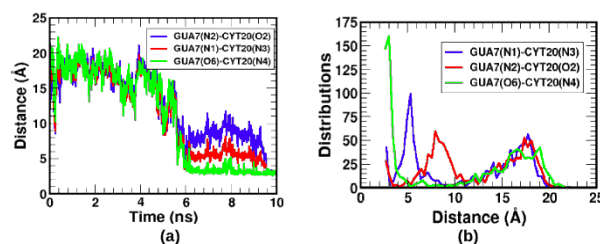

Fig. 3: Variation in (a) distance and (b) distribution, of hydrogen bonding pairs in GUA7 and CYT20 during 10 ns simulation.

The distance and distribution curves in Fig. 3 (a) and (b) also shows that the nucleotide remains relatively long time either intrahelical or extrahelical condition. The distribution curve shows that the probability of remaining the nucleotide between the distances 14 Å to 10 Å, i.e., rotational state, is very low so that distribution is also very small. Distribution between 14 Å to 20 Å shows the extrahelical and the distribution within 10 Å shows the intrahelical conditions.

3.3 Stability of hydrogen bonds

The sustainable hydrogen bonding pairs between pair partners GUA7(N1)-CYT20(N3), GUA7(N2)-CYT20(O2), GUA7(O6)-CYT20(N4) are very important to be an ordinary double stranded DNA. If the hydrogen bonding between these pairs are not stable, the coding in

newly synthesized protein or DNA strands can be erroneous. We observed the stability of such three hydrogen bonds in order to evaluate the correct repairing mechanism.

To inspect the stability of hydrogen bonding pairs, we prepared a molecular system picking only DNA from the output of above 10 ns simulation run. The system was then extended to 100 ns simulation under NPT condition. The correct bonding of GUA7 and CYT20 is shown in Fig. 4 (a). In the beginning of the simulation, the number of hydrogen bonds was observed fluctuating to gain the correct orientation of the pair partner within 50 ns. After 50 ns time scale, all three hydrogen bonds are continuously acting to keep the pair partners in contact as shown in Fig. 4 (b). This concludes that all three hydrogen bonds recover correctly after flipping back of guanine intrahelically.
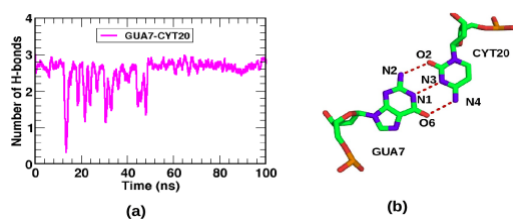

Fig. 4: Evaluation of ordinary hydrogen bonds between GUA7 and CYT20 (a) Three stable bonds are observed after 50 ns time scale, eventhough there are some fluctuations before 50 ns (b) the scheme of hydrogen bonds after intrahelical flipping of GUA7.

Conclusions

Molecular dynamics simulation has been carried out to examine the intrahelical flipping of repaired guanine after methyl transfer. We have performed 10 ns simulation run to investigate the flipping in mechanism of guanine from its base stack and 100 ns simulation is propagated to evaluate the stability of hydrogen bonds between the repaired GUA7 and its pair partner CYT20. The result is analyzed from the aspect of distance variation and distribution of hydrogen bonding pair partners. After

transferring the methyl adduct to AGT, the unpaired GUA7 flipped back intrahelically and pairs with CYT20. This could be achieved within 10 ns and all three ordinary bonds GUA7(N1)-CYT20(N3), GUA7(N2)-CYT20(O2), GUA7(O6)-CYT20(N4). All three hydrogen bonds are acted constantly till the end of 100 ns simulation. The intrahelical flipping back is supported by the distance and distribution of atom that form the ordinary hydrogen bonds between GUA7 and CYT20.

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