Molecular Dynamics (MD) Simulation of DNA Dodecamer with 5-hydroxy-6-cytosinyl Radical

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(Received, September 5, 1994)
(Revision received, January 19, 1996)
(Accepted, January 22, 1996)

DNA/dodecamer/molecular dynamics/AMBER 4.0

Molecular dynamics (MD) simulation of DNA dodecamer d(CGCGAATTC*GCG)₂ with a primary radiation damage represented by 5-hydroxy-6-cytosinyl radical (C*) in position 9 was performed with AMBER 4.0 force field with periodic boundary conditions for the solvent. The temperature, potential energy of the system, energetic contributions from groups and RMS deviation from original positions were examined throughout the course of the simulation up to 140 ps. The stabilized structure (after 100 ps) was distorted and bent near the damaged site, which is similar to that observed in the MD of DNA with thymine glycol (Miaskiewicz, K. et al (1994) Radiat. Protection Dosimetry, 52, 149-153). The results suggest that a small and local damage in DNA may cause a large and global conformational change in DNA. Water molecules form two layers at distance 2.5 Å and 5.5 Å around the DNA. The MD simulation is a new approach to study radiation damages in molecular level.

INTRODUCTION

Events that lead from the initial interaction of ionizing particles with molecules of living cells to biophysical and biochemical processes at the cellular level is of fundamental relevance for understanding the mechanisms of radiation damage. One of the most important subcellular organelles of a cell is the DNA (deoxyribonucleic acid) macromolecule as a target of ionizing radiation. Lesions induced in DNA, such as base and sugar modifications, crosslinks and strand breaks, are relevant events that lead to destruction of DNA itself, mutations, or conformational

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changes that may affect regulation of gene expression. These lesions may be produced by direct and indirect radiation actions. The interactions of active water radicals (OH, H, eaq) produced by deposition of radiation energy in the environment of DNA, with bases and sugars often lead to strand breaks, which could be considered as lethal lesions in DNA\(^1\). Fraction of indirect action by OH is experimentally estimated as much as about 70% for yield of strand breaks and reproductive cell death in vitro for low LET radiation\(^2\). Important evidence for sugar radicals formation by base radicals are shown by esr and laser pulse experiments\(^3,4\). In these experiments the laser pulse produces only base radical cations, because the sugar moiety is not directly affected by laser light. The result was that, high yields of strand breaks were observed and demonstrated the involvement of base radicals in strand break formation through the intramolecular process of H-abstraction from the sugar. These studies emphasize the importance of initial base damage in connection to strand breaks. However, they were observed in single stranded poly (U), poly (C) and poly (A), as well as in single stranded DNA. There is no direct experimental examination that more important double stranded DNA undergoes the same mechanism. And these experiments are difficult so far\(^5\).

Theoretical computational simulations is a method to study this as an alternative approach. The computational simulation, however, has not been fully established either to study this specific problem: specification of base damages and subsequent intramolecular process leading to strand breaks. At present, even though, this method has been applied to study only conformational and structural changes in double stranded DNA, these results would provide insights of intramolecular mechanisms and the development of this method would make it possible to study this specific problem in future.

An interesting trial of this method was to see the initial consequence of initial base damage, the change in conformational properties of the damaged DNA. For the first time this was demonstrated by the molecular dynamics (MD) simulation of a dodecamer of DNA d(CGCGAATTCGCG\(_2\)) with the thymine in position seven replaced by a 5-hydroxy-6-thyminyl radical\(^6\). This damaged DNA showed a specific kink at the damaged site.

In this paper we exemplify another pyrimidine damage of DNA, 5-hydroxy-6-cytosinyl radical (C\(^*\)) at position 9 of the DNA dodecamer, and discuss the method itself and its usefulness in radiation biology.

**METHODS**

The primary damage of DNA was represented by a 5-hydroxy-6-cytosinyl radical. Experiments\(^6\) indicate that formation of pyrimidine radicals by OH addition is kinetically controlled. This is in agreement with quantum chemical calculations\(^7\), which show that the relative energies of the radicals with OH group on C6 are lower than those with OH on C5. For our computational simulation we have chosen the axial position of OH in the pyrimidine ring. The structure of the 5-hydroxy-6-cytosinyl radical, shown in Figure 1, was obtained by quantum mechanical structure optimization: bond length, bond angles and atomic charges.

Energy minimization and molecular dynamics simulation of the dodecamer...
The steps forward the MD are as follows:

1. The crystallographic coordinates of the duplex dodecamer \( d(CGCGAATTC*GCG)_2 \) were taken from Brookhaven Protein Data Bank (PDB).

2. To neutralize the charges of phosphates, 22 \( Na^+ \) counterions were placed initially at the positions bisecting the O-P-O angle, at a distance of 5.0 Å from the phosphorus atom.
3. The cytosine in position 9, (C9), was replaced with 5-hydroxy-6-cytosinyl radical representing the lesion.

4. The DNA and counterions were placed in a box of TIP3P “Monte Carlo equilibrated” water molecules with EDIT module of AMBER 4.0. This procedure placed 1751 water molecules around DNA. In subsequent minimizations, heating and dynamic simulation, periodic boundary conditions with constant volume were applied on the box.

5. The system of the dodecamer incorporating with the damaged cytosine, counterions and the water molecules was optimized in two steps. First, the geometry of the duplex dodecamer and counterions was kept frozen and only the potential energy of the surrounding water molecules was minimized. This was followed by a full minimization of the potential energy of the entire system.

6. After minimization the structure was heated to 300°K over a period of 10 ps. The initial velocities of atoms for the heating run were taken from a Maxwellian distribution.

7. To equilibrate the density of the whole system (DNA + counterions + water), a constant pressure MD run was performed for 4 ps after heating. The density stabilized at 1.003 g/cm$^3$ and the final size of the box including DNA, counterions and water molecules, was determined as $48.2 \times 37.6 \times 36.1 \, \text{A}^3$.

8. The MD simulation with constant volume and constant temperature at 300 K was continued for 140 ps. Constant temperature was regulated by separate time coupling of the solute and solvent which in effect kept their temperatures around 300 K. The time segment was set equal to 1 fs to solve Newtonian equations for all atoms. The structure output data, coordinates and velocities of all atoms, were stored every 0.2 ps in files for further analysis. ANAL module of AMBER 4.0 and the program CURVES were used for analysis.

All calculations were performed on a Hewlet Packard computer, models 715/33 and 735/75. One picosecond of MD simulation required approximately 1.5 or 0.5 h of CPU time, respectively.

**RESULTS AND DISCUSSION**

The potential energy of the system decreased rapidly during the first 20 ps. It oscillated largely till 80 ps and then became more stable with a smaller fluctuation toward lower energy. After 100 ps the energy stabilized around $-17.35 \pm 0.06 \times 10^3 \text{ kcal/mole}$ (see Figure 2). This stabilization process can be further seen in components of the total energy, from the DNA, the water ensemble, and counterions (CIO) (see Figure 3). It is noted that the energy of water is about 10 times larger than that of DNA and CIO. The energies of DNA and CIO show small variations and remain stable throughout this stable region (after 100 ps) around a value of $-1.84 \pm 0.03 \times 10^3 \text{ kcal/mole}$ and $-1.02 \pm 0.02 \times 10^3 \text{ kcal/mole}$, respectively. The energy of water, however, shows larger fluctuations, $(-14.33 \pm 0.08 \times 10^3 \text{ kcal/mole})$, that contribute significantly to variations in the total energy. Much larger fluctuations in the energy of water are observed (period from 20 to 40 ps) before the system is stabilized. It suggests that water molecule is major component in stabilization of the system compared to DNA and CIO.
Figure 2. Variations of total potential energy of the system with time.

- Energy of DNA
- Energy of water
- Energy of Na+

Time [ps]
Temperature of the system stabilizes around 30 ps at the value of 308.11 ± 0.88 K and remains stable throughout the simulation. The temperature of the solute is somewhat lower, 305 K than that of the solvent 309 K. The temperature is adjusted by scaling velocities of atoms with respect to the regulated temperature at every picosecond.

Root mean square (RMS) deviation of DNA, from its original positions increases rapidly from the beginning of MD to 95 ps indicating that conformation of the system undergoes major changes in this period to accommodate 5-hydroxy-6-cytosinyl radical to the DNA. After 100 ps the RMS deviation of DNA stabilizes around the value of 6.40 ± 0.24 Å. (see Figure 4). According to RMS, there are considerable changes in structure during first 100 ps, that correspond to the earlier sharp drop of the total potential energy (see Figure 2). These initial 100 ps could be considered as an equilibration period. The structure stabilized and reached a "converged" conformation after 100 ps. This structure was analyzed.

The program CURVES analyzes the DNA structure in the interval from 100 ps to 140 ps. Structures of simulated dodecamer in the period after 100 ps are shown in Figure 5. As can be seen from these structures, there is a considerable distortion of DNA as a whole and a large

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**Figure 4.** Time evolution of RMS deviation of DNA, counterions and water molecules from their original positions.

**Figure 3.** Energy contributions of DNA, counterions and water to the total potential energy as a function of time.
bending in the helical axis. The actual bent region was not at the damaged site (C9) but at A6 and T7. Since this large bending at A6 and T7 was not observed in non-damaged DNA\textsuperscript{10}, it suggests some intramolecular interactions among C9, A6 and T7.

The present result may be compared with the other works which used the same DNA but with different type of damage (5-hydroxy-6-thyminyl radical at the position T7)\textsuperscript{5} or without damage\textsuperscript{11}). Their system had 1532 water molecules and stabilized in the period from 80 to 120 ps at RMS of around 3 Å. Our observed distortion in DNA structure is more less the same as that from their MD simulation, but somewhat larger distortion was observed which may well be that the shorter distance of the damage to the end of the DNA (in our case distance between C9 and G12, while in the case of T7 distance between T7 and G12) may have contributed to the larger distortion. This is a limitation of the MD simulation to see conformational change, such as finite length of DNA and related position of damage. It is suggested, however, that OH adduct reaction on thymine and cytosine may cause a local strain and hence result in a similar global conformational change in DNA.

We observed further that the hydrogen bonds were distorted at the damaged cytosine (C9) and this distortion affected hydrogen bonds between bases of neighboring pairs. The distortion of hydrogen bonds should be studied more carefully whether it is damage specific or not, because MD simulations of DNA even without damage show a similar distortion of hydrogen
More importantly the water molecules may play an important role to make present conformation of DNA. Figure 6 shows the number of water molecules in layers of width of 0.5 Å from DNA as a function of simulation time. At each time the minimal distance of each water molecule to any atom of DNA was calculated and then a frequency distribution of the numbers of waters in a shell 0.5 Å width was obtained. It can be seen, that there are two peaks, sharp at 2.5 Å and broad around 5.5 Å and that after 60 ps the water molecules as a whole come closer to DNA and form a tighter shell around DNA within about 10 Å. This tighter shell may contribute to the stabilization of the conformation of the DNA. In the stabilized region (after 100 ps) the positions of peaks are more less the same. This agrees with the result of the work of the same DNA sequence but without damage. The distributions of water in both cases are distance distributions of water molecules from any atom of DNA. With this data we can not see whether their distribution is specific in the case of damaged DNA. We need more detailed analysis of behavior of water (e.g. hydration and configuration of water molecules around atoms of DNA) by developing new method of analysis.

In this paper we have presented a molecular dynamics simulation of a DNA dodecamer d((CGCGAATTGCG)2 including 22 sodium counterions and 1751 water molecules, using all atom AMBER force field and with periodic boundary conditions. The results of MD visualize large conformational changes in DNA. The time analysis of the structures suggests that the DNA stabilizes after 100 ps having distorted and bent form. This time scale can be suggested...
only by the MD simulation. Water may have an important role in the stabilization of the
distorted structure. The conformational change in the DNA caused by a base damage may
provide data at molecular level for possible analysis of mechanisms of a repair enzyme. The
MD simulation is based on:
1) finer specification of radiation damage at molecular level;
2) quantum chemical determination of the structure of damaged site;
3) development of computer technology to deal with more realistic dynamic processes (longer
DNA and much water molecules, including intramolecular process) and
4) development of analysis program of conformational changes of DNA.

With improvement of these aspects the MD approach is useful for radiation biology to study
mechanisms in molecular level.

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