Molecular dynamics study on DNA damage by tritium disintegration

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Using molecular dynamics (MD) simulation, we simulate the structural change of a telomeric DNA by β-decay of substituted tritium to helium-3. The configuration of the telomeric DNA is obtained by removing TRF2 protein from the TRF2-Dbd-DNA complex (Protein Data Bank ID is 3SJM). We assume that hydrogens (H) of guanines in the telomeric DNA are replaced to helium-3. Since this replacement of the H atoms to the ^{3}He atoms changes the charge distribution significantly, the charge distribution used in the MD simulation for the modified guanine is obtained by the density functional theory calculations. We adopt, as the MD simulation, NAnoscale Molecular Dynamics (NAMD) code with CHARMM36 force field using Langevin thermostat and Nosé-Hoover Langevin piston to control the temperature and pressure of the system, respectively. Moreover, changing both the number of replaced guanine N and the temperature of the system T, we calculate the root mean square deviation RMSD to quantify the dependence of the durability of the telomeric DNA on the β-decays. From the MD simulation, it is found that as N or T becomes larger, the RMSD of the DNA becomes also larger. Namely, it denotes that as the intensity of the β-decays becomes larger or as the temperature is increased, the DNA structure becomes more fragile.

1. Introduction

A future nuclear fusion power plant will use tritium and deuterium as fuels. Tritium disintegrates to helium-3 (^{3}He) with emissions of a low energy ($\leq$ 18.6 keV) β-electron (β-ray) and an antineutrino at a half-life of 12.323 years.[1)] It is one of unavoidable problems for the

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realization of a nuclear fusion reactor to elucidate quantitatively biological effects of tritium. The tritium (T) transfer from foods containing Organic Bound Tritium (OBT) or tritiated water (HTO) to DNA was measured using oryzias latipes eggs[2] and *E. coli*.[3] In the latter work, it was reported that the T transfer from OBT to DNA is larger than from HTO to DNA. Thus, tritium just adjacent or in the cell nucleus can damage their DNA.[1] The United Nations Scientific Committee on the Effects of Atomic Radiation has conducted an independent review of the scientific literature relating to the biological effects of tritium.[4] However, it is still difficult to quantify cancer risks of low and/or protracted radiation exposures.[5]

Towards better physical understanding of DNA damages by tritium, we have examined double strand breaks (DSBs) of giant DNA molecules using a single molecule observation (SMO) technique.[6–9] In our previous experimental work,[9] the genome DNA of bacteriophage T4 GT7 was immersed in sterilized tritiated water (5.2 MBq/cm^3) and non-sterilized tritiated water (4.2 MBq/cm^3), and then the length of DNA molecules was measured using the SMO technique. Acceleration of DSBs by tritium was significant solely in the sterilized water, and it was suggested that the effects of tritium were far weaker than those of microorganisms (*e.g.*, bacteria) and impurities in water even at the tritium concentration as high as 5.2 MBq/cm^3. However, detailed mechanisms underlying the cleavage of DNA sugar phosphate backbone by tritium have not been clear yet. In particular, separation of effects of β-ray irradiation (direct and indirect actions) from those of bond cleavage by tritium decay to inert ^{3}He is difficult even if we used SMO method.

From these viewpoints, we have decided[10] to adopt molecular dynamics (MD) simulation[11,12] to elucidate the mechanism how β-decays of substituted tritium damage DNA. Based on the above computational strategy,[10] we studied structural change of polyethylene by β-decay of substituted tritium instead of DNA in our previous MD simulation.[13] From this simulation of polyethylene,[13] it was found that the more the number of removed hydrogen (H) atoms is, the higher the potential energy is and the lower the value of the global-orientational-order-parameter and the average number of consecutive *trans* bonds become. Thus, we probed that the MD simulation is powerful to reveal the mechanism of the damage on molecules by β-decay of substituted tritium. Here, we emphasize that the MD simulation on DNA for the $\beta-$decay is our original attempt though there are a lot of MD simulations for biopolymeres.[14–19] In this paper, using the MD simulation which was developed in our polyethylene study,[13] we predict the structural changes of tritium-substituted DNA by β-decays of tritium to ^{3}He.

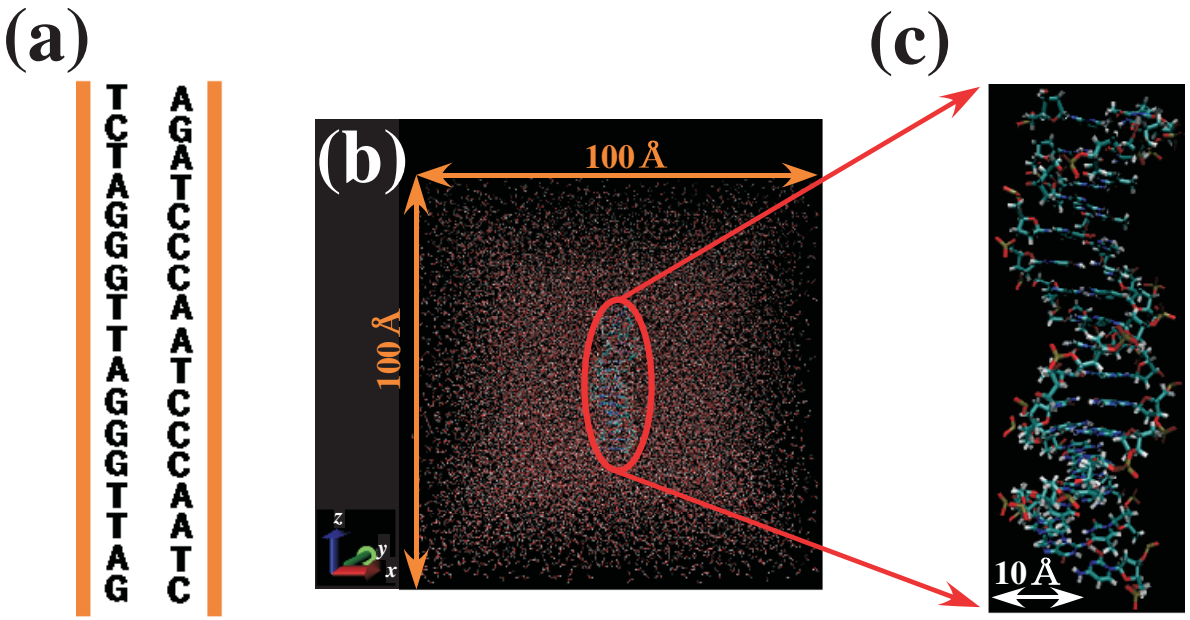

Fig. 1. (a) The base sequence of the DNA. (b) A telomere structure of human DNA with 71 000 water molecules, 121 Na^+ and 89 Cl^-. The DNA is composed of 1078 atoms. The initial simulation box is 100 Å × 100 Å × 100 Å. (c) The original configuration of the telomeric DNA.

2. MD simulation model

We employ a telomere structure of the human DNA shown in Fig. 1 to reveal the mechanism of the damage on biological molecules by tritium. The reason why we choose telomeric DNA as the simulation model is as follows: The shortening of telomeres replication in human cells has an important role in cellular senescence.[20] Therefore, we consider that the damage of human cell by tritium depends on the damage of the telomeric DNA. The structure of the telomeric DNA is obtained by removing TRF2 protein from the TRF2-Dbd-DNA complex, PDB ID of which is 3SJM.[21] The telomeric DNA has 17 base pairs, $d(TCTAGGGTTAGGGTTAG)_2$, which consists of 1078 atoms as shown in Fig. 1 (a). Here we define the positions of all the atoms in the telomeric DNA as $\boldsymbol{r}_i^{\mathrm{PDB}}$, where $i = 1, 2, \cdots, 1078$. We add 121 sodium ions (Na^+) and 89 chlorine ions (Cl^-) into the simulation box to neutralize the electric charge of the DNA. Moreover, 71 000 water molecules are also added as a solvent. The initial size of the simulation box is 100 Å × 100 Å × 100 Å. The pressure of the system is set to 1 atm. We apply the periodic boundary conditions to the x, y, and z-directions and set the temperature of the system T to 290 K or 310 K. At the same temperatures, we are making the experiment for DSBs of DNA using SMO technique is under construction in vitro. In the near future, it is possible to compare the simulation results with the experimental results.

First, we make the steady state of the telomeric DNA structure in waters at each temperature T by the following method. We run MD simulation from the "original" telomeric DNA structure (Fig. 1) just as is provided in PDB until the simulation system composed of the telomeric DNA and waters reaches the steady state by NAnoscale Molecular Dynamics (NAMD) code.[22] The NAMD was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign. The simulation time to stabilize the DNA structure is 2.0 ns or 0.3 ns for 290 K or 310 K, respectively. In the MD simulation, we adopt CHARMM36 force field[23] in NAMD and Langevin thermostat algorithm[24] to control the temperature of the system. The pressure of the system is set to 1 atom by the Nosé-Hoover Langevin piston method.[25,26] The time step of MD simulation is 1 fs.

Next, we express the disintegration effect of β-decay of tritium to ^{3}He with the replacement of two H atoms in each guanine (G) of the steady telomeric DNA to two ^{3}He atoms as in Fig. 2. Here we comment the reason why we choose only G to analyze the $\beta-$decay effect. The hydrogen bonds compose the structure of the DNA double helix. Because the binding energy of hydrogen bond is smaller than that of the covalent bonds in DNA, it is expected that hydrogen bond is more sensitive for β-decay than covalent bond. Therefore, we pay attention to the influence only on the hydrogen bond by β-decay. Moreover, because there are three hydrogen bonds between G and cytosine (C), and two hydrogen bonds between adenine (A) and thymine (T), G-C pairs is more dominant than A-T pairs to compose the DNA double helix structure around 310K.

Last, we choose the replacement as Fig.2 in three hydrogen bonds in G-C pair, because it is easiest to make the initial simulation structure. It means that the replacement of hydrogen atoms in two other hydrogen bonds to helium changes the initial G structure more than in Fig.2. Hereafter we call the replaced guanine as Γ. This replacement denotes the following behavior: Tritiums ingested diffuse in a cell, and the cell, before long, becomes in the equilibrium state where H atoms in the guanine are replaced with tritium atoms each other stochastically. The replaced tritium in the guanine is disintegrated to ^{3}He by β-decay accidentally. The covalent bond interaction between N and H in G [see Fig. 2(a)] disappears as soon as the replacement of H to ^{3}He. Therefore, we set the force field parameter corresponding to the interaction between N and H as Fig.2(a) to null in the simulation. The replaced ^{3}He atoms are interacted with the other atoms by van der Waals force. We also comment that any specific constraint potential is not imposed at the ends of DNA to keep its structure.

The replacement from H to ^{3}He changes the charge distribution in guanine significantly.

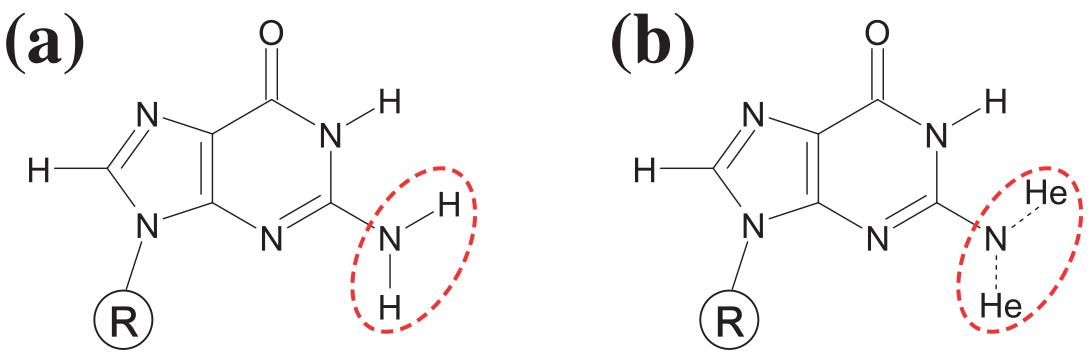

Fig. 2. (a) The original guanine (G) structure. The two hydrogen atoms of guanine are replaced by two helium-3 atoms as shown in (b). The replaced guanine is named as Γ. Though the hydrogen-nitrogen bond in the original guanine (G) is a covalent bond, the helium-3 and the nitrogen interact with each other not by a covalent bond anymore, but by a weak interaction, i.e. an intermolecular force.

Table I. Charge distribution in G and Γ. The charge distributions are calculated by DFT for the original guanine (G) and the damaged guanine (Γ). "Position" corresponds to the indices defined in Fig. 3. In both cases, the total charges were fixed to zero. We use the electric charge e ($e = 1.602176634 \times 10^{-19}$ C) as a unit of charge.

Position in G or Γ	Atom	Charge in G [e]	Charge in Γ [e]
1	N	-0.605	-0.585
2	C	0.239	0.266
3	H	0.211	0.221
4	N	-0.493	-0.483
5	C	-0.037	0.024
6	C	0.693	0.713
7	O	-0.605	-0.546
8	N	-0.685	-0.717
9	H	0.419	0.423
10	H	0.707	0.584
11	N	-0.832	-0.258
12	H ⇒ removed.	0.389	(null)
13	H ⇒ removed.	0.407	(null)
14	N	-0.632	-0.437
15	C	0.395	0.346
16	C	0.428	0.440

Therefore, the charge distributions of guanine and Γ are obtained by the density functional theory (DFT) calculations using Gaussian09,[27] as in Fig. 3 and Table I. Since the ^{3}He atoms in Γ cannot make chemical bonds and soon move away from the remaining part of guanine

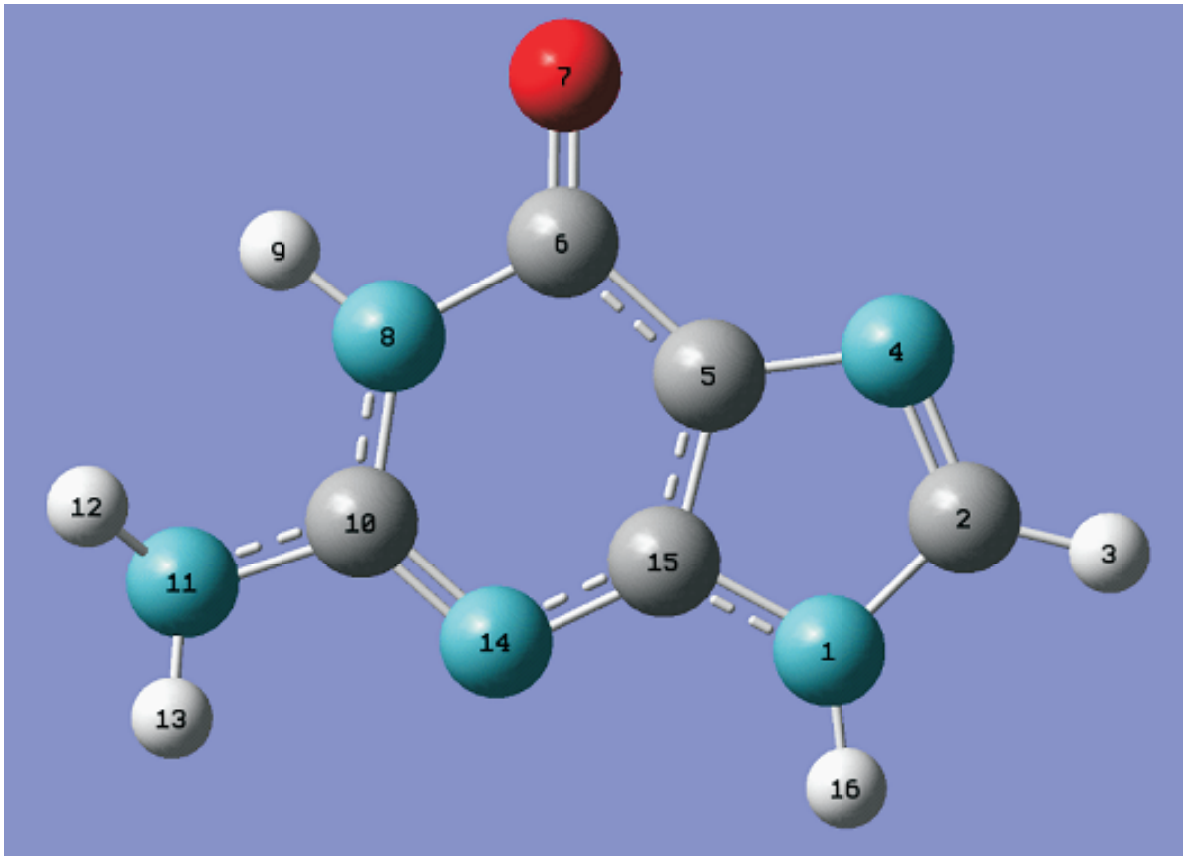

Fig. 3. Molecular structure of guanine optimized by DFT calculation. The integers denote the indices of the atoms. Red, blue, light-gray and dark-gray balls denote oxygen, nitrogen, hydrogen, and carbon, respectively. Charge distribution is given in Table I.

during the geometry optimization, we calculate the charge distribution of the remaining part of guanine without ^{3}He atoms. In the MD simulations, we use the force field of Γ, in which the electronic charges of the ^{3}He atoms are set to zero independently from their positions and those of the remaining atoms are set to the values calculated by DFT. We comment here on the charge of ^{3}He atom which is generated by β-decay of T as

$$\mathrm{T} \rightarrow {}^{3}_{1}\mathrm{He}^{+} + \mathrm{e}^{-} + \bar{\nu}_{\mathrm{e}}, \tag{1}$$

where e and $\bar{\nu}_{\mathrm{e}}$ denote electron and electron antineutrino, respectively. In this simulation, we assume that the positive charge of ^{3}He is neutralized by the surroundings, i.e. negative ions or electrons in solvents in a sufficiently short time. B3LYP exchange-correlation functional[28,29] and cc-pVDZ[30] basis-set are used in the DFT calculations. The charge distributions are calculated by the natural population analysis with the NBO program[31] in Gaussian09.

There are eight guanines in one telomeric DNA molecule as shown in Fig. 1(a). We replace N guanines in eight Γ bases as Fig. 4. Thus, we set up the initial configuration of the telomeric

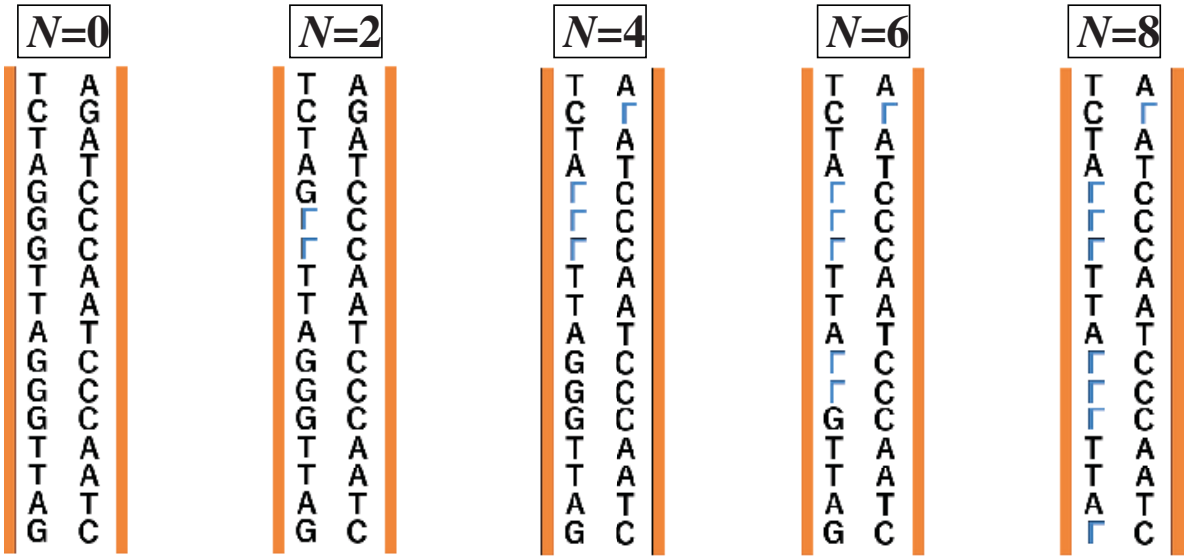

Fig. 4. N guanines (G) in the original telomeric DNA is replaced with N replaced guanines (Γ). Two hydrogen atoms in each Γ are replaced with two helium-3 atoms as Fig. 2.

DNA damaged by tritium. Figure 5 shows five initial configurations, i.e. $N = 0, 2, 4, 6$ and 8, for the DNA where G bases are replaced by Γ bases.

Finally, we run the MD simulation from the above initial configurations by NAMD with CHARMM36 potential and Langevin thermostat algorithm for each T and N. The pressure is set to 1 atm by the Nosé-Hoover Langevin piston method. By finding the N dependence of the telomeric DNA at two different temperatures, i.e. $T = 290$ K or 310 K, it is possible to obtain the durability of the DNA against the tritium β-decay.

3. MD Simulation Results and Discussions

3.1 Structural changes of DNA

We perform the MD simulation using the initial configuration Fig. 5 at the temperature T. Using the visualization software VMD,[32] the motions of all atoms can be visualized. In Fig. 6, we pick up the snapshots of DNA structure at t= 0.3 ns, 0.6 ns and 0.9 ns at $T = 310$ K in two cases ($N = 0$ and 8) chosen from all simulation data. Because the initial configuration is in the steady state for $N = 0$ which corresponds to the non-damaged DNA by β-decay of tritium, the DNA structure hardly changes as shown in Fig. 6(a). In the case that $N = 8$ which corresponds to the most damaged DNA by β-decay in our simulations, the spatial gap between two DNA chains becomes larger when time passes as in Fig. 6(b). This simulation results are consistent with the intuitive consideration for the tritium effect of DNA. The quantitative analysis of the DNA durability is shown in the next subsection.

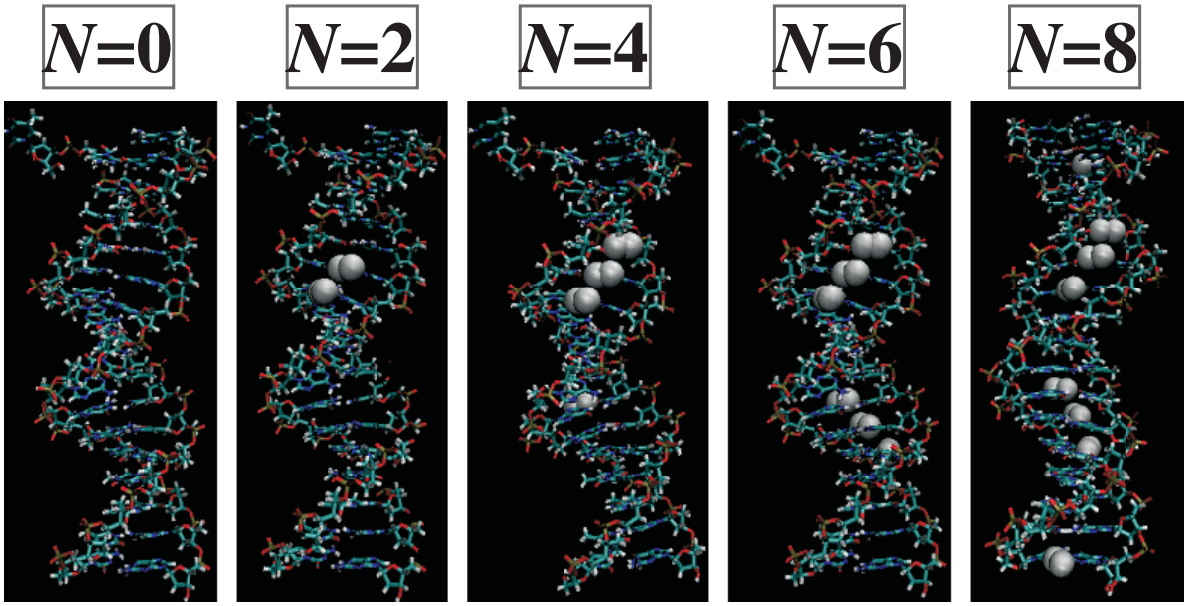

Fig. 5. Initial molecular structures of the telomeric DNA for each N. The $N = 0$ structure was obtained by performing the MD simulation using NAMD code with CHARMM36 potential, Langevin thermostat algorithm and the Nosé-Hoover Langevin piston method until the structure becomes stable. The $2N$ hydrogen atoms are replaced to helium-3 atoms (gray balls) . The charge distribution in a guanine of the telomeric DNA is also caused by loss of hydrogen atoms. The charge distribution in the replaced guanine Γ is given by DFT code as in Table I. Using these initial structures, we perform the MD simulation and obtain the structure transformation of DNA. These snapshots corresponds to the case of $T = 310$ K.

3.2 Root mean square deviation of DNA

The root mean square deviation RMSD is calculated to quantify the time dependence of the DNA structure. It is defined as

$$\mathrm{RMSD}(t) := \sqrt{\frac{1}{n_{\mathrm{D}}^0}\sum_{i=1}^{n_{\mathrm{D}}^0}\left|\boldsymbol{r}_i(t) - \boldsymbol{r}_i^{\mathrm{PDB}}\right|^2}\,, \tag{2}$$

where n_{D}^0 is the number of the atoms composing the DNA without H and He atoms and $n_{\mathrm{D}}^0 = 691$. The position of the i-th atom in the DNA at time t is defined by $\boldsymbol{r}_i(t)$. The RMSD is the deviation from the reference position. In our analysis, we adopt the DNA structure obtained from the PDB $\boldsymbol{r}_i^{\mathrm{PDB}}$ as the reference position.

In Fig. 7, we plot the time dependence of RMSD at $T =$ (a) 290 K and (b) 310 K. At each T, we also plot the RMSD for $N = 0, 2, 4, 6$ and 8. The plotted data are obtained by averaging five simulations for each (T, N). The error bars at the final time correspond to the deviation of these five simulations. The difference among five simulation-conditions is merely a random number sequence, which is necessary to generate random forces in Langevin thermostat.

From all figures, it is found that the RMSD becomes larger as N is increases. It is interpreted as that the DNA structure becomes fragile as $\beta-$decay progresses. Moreover, as the temperature of the system is increased, the DNA moves more actively.

4. Conclusions

The structure change of tritium-substituted telomeric DNA in water by β-decays of tritium to helium-3 has been studied using the all-atom MD simulations which was based on our previous study of polyethylene.[13)] In the present study, we calculate the root mean square deviation RMSD of the DNA atoms, while changing the number of replaced helium-3 pairs N to quantify the dependence of the durability of the telomeric DNA on the β-decays at T = 290K and 310 K. We have found that as more hydrogen atoms are removed, the RMSD of the DNA becomes larger. Namely, it denotes that as the intensity of the β-decays becomes stronger, the DNA structure becomes more fragile. This dependence is more evident, as the temperature is increased. Thus, we have proved that it is possible to quantify the durability of a biological molecule, i.e. DNA by the MD simulation.

Acknowledgment

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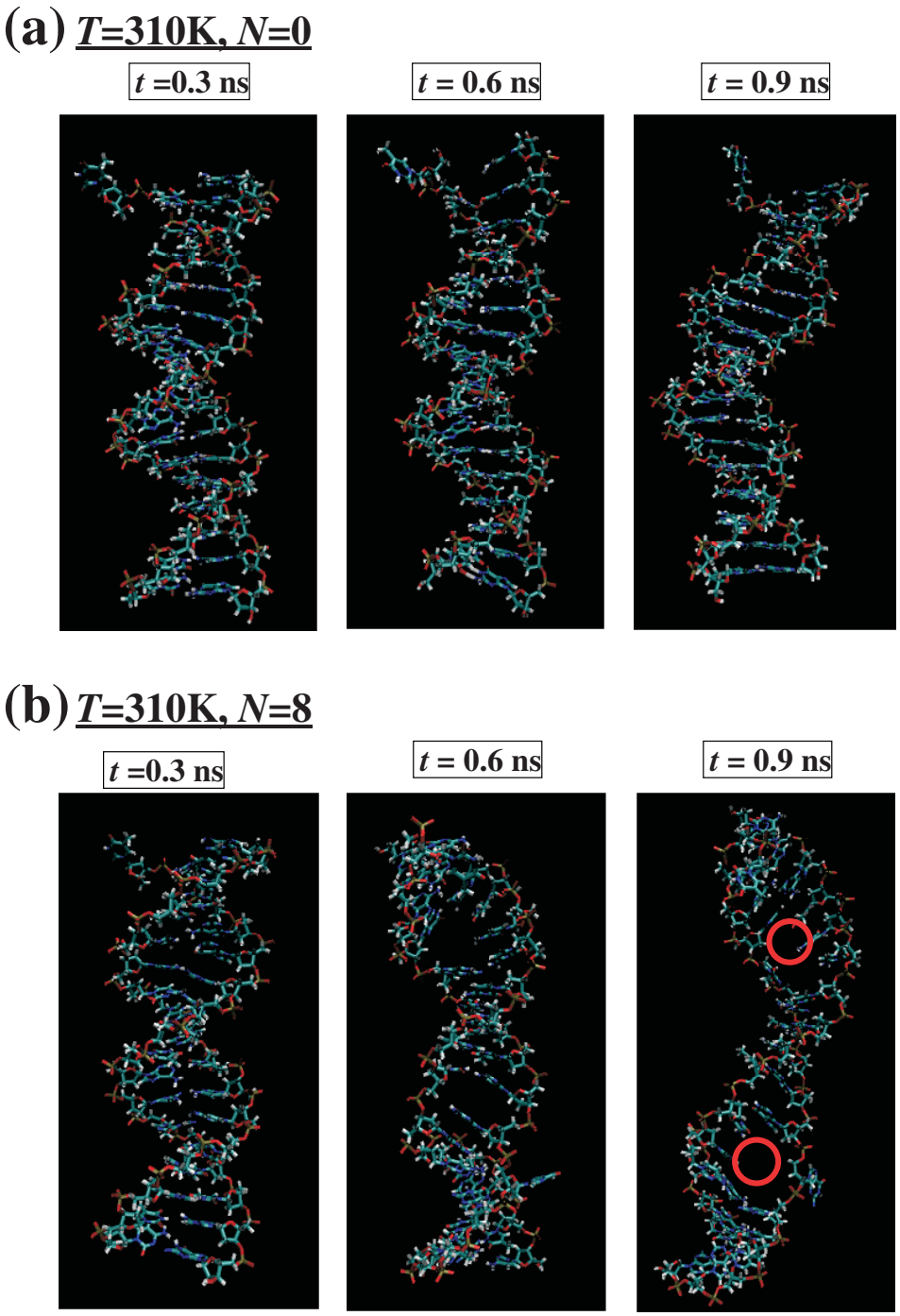

Fig. 6. Time dependences of the DNA structures. (a) The structure of the non-substituted DNA, i.e. $N = 0$ case, has barely changed from the steady state $t = 0.3$ ns. (b) For $N = 8$, the length between DNA chains becomes larger as time passes. Wide gaps between chains (the region denoted by circles) are found clearly at $t = 0.9$ ns. Some helium-3 atoms have flown away from the inside of DNA. The temperature is fixed to $T = 310$ K in both N.

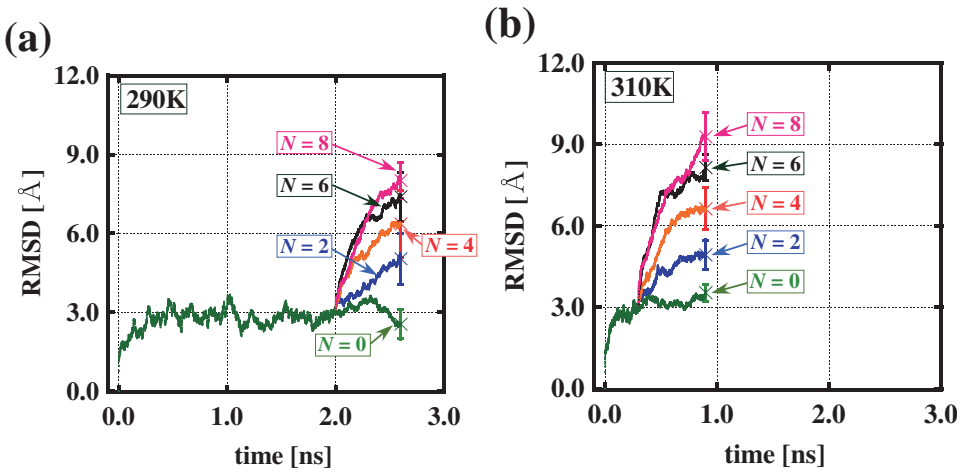

Fig. 7. Root mean square deviation of DNA at 290 K or 310 K. 2N hydrogen atoms are replaced with helium-3 atoms. The simulation time to stabilize the DNA structure for $N = 0$ structure is 2.0 ns or 0.3 ns for T = (a) 290 K or (b) 310 K, respectively. After the stabilization, we replace the N G bases to N Γ bases in the telomeric DNA. Then we run the MD simulation more for 0.6 ns at all temperatures.

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