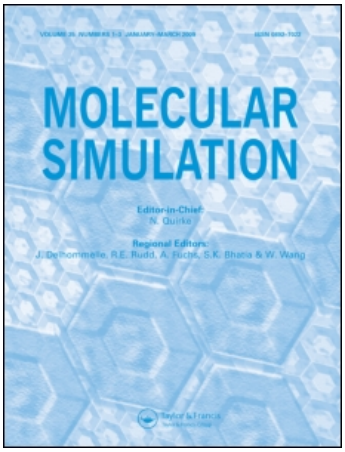

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HYDRATION AT THE TD DAMAGED SITE OF DNA AND ITS ROLE IN THE FORMATION OF COMPLEX WITH T4 ENDONUCLEASE V

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An analysis of the distribution of water around DNA surface focusing on the role of the distribution of water molecules in the proper recognition of damaged site by repair enzyme T4 Endonuclease V was performed. The native DNA dodecamer, dodecamer with the thymine dimer (TD) and complex of DNA and part of repair enzyme T4 Endonuclease V were examined throughout the 500 ps of molecular dynamics simulation. During simulation the number of water molecules close to the DNA atoms and the residence time were calculated. There is an increase in number of water molecules lying in the close vicinity to TD if compared with those lying close to two native thymines (TT). Densely populated area with water molecules around TD is one of the factors detected by enzyme during scanning process. The residence time was found higher for molecule of the complex and the six water molecules were found occupying the stabile positions between the TD and catalytic center close to atoms P, C3′ and N3. These molecules originate water mediated hydrogen bond network that contribute to the stability of complex required for the onset of repair process.

Keywords: Molecular dynamics; thymine dimer; DNA; T4 Endonuclease V; hydration

1. INTRODUCTION

The continuity of life depends upon the successful repair of damages caused by environmental radiation to DNA [1, 2]. The major damage produced by UV are thymine dimers (TD) [3, 4]. This photolesion induced in DNA by UV in sunlight are among the major causes of skin cancer [5]. An improved

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understanding of the biochemical process of the mechanisms by which repair enzyme recognizes the TD site, excises the dimers and restores the intact function of DNA will enhance the effort to prevent and cure skin cancer.

The DNA repair enzyme encoded by den V–T4 Endonuclease V–was purified and characterized [6–8]. It possesses three properties:

(a) binds to DNA molecule non-specifically and scans it in a one dimensional diffusion along the DNA [*e.g.*, 9–11];
(b) recognizes the thymine dimer site [12] and
(c) cleaves selectively the N1—C1′ bond in the 5′ thymine of the dimer and incises the C3′ phosphodiester bond of DNA at an apurinic/apyrimidinic (AP) site [13, 14].

Proper recognition of TD site is necessary for formation of DNA-enzyme complex which is the key step for the onset of the enzymatic process. Formation of DNA-enzyme complex is followed by repair process in which TD part is removed from DNA and resulting gap is refilled in DNA synthesis.

The distribution of water around individual nucleotides of DNA plays an important role in biological systems as it influences DNA bending, maintains the native structure and can also be critical for the various function, as for example enzymatic activity of proteins [*e.g.*, 15]. The conformational changes of lesioned DNA molecules have also direct effect to native hydration of DNA molecule and this specific hydration may be one of the factors that is recognized by protein [*e.g.*, 16, 17].

The aim of this paper is to present results on the study of the role of hydration and distribution of solvent molecules in specific recognition mechanism of cyclobuthane thymine dimer by the DNA repair enzyme T4 Endonuclease V. The presented study is molecular dynamics (MD) simulation of three systems, one composing of native DNA, one of DNA with the TD, and one of DNA with TD in complex with catalytic center of repair enzyme. This study may be considered as complementary to the existing X-ray crystallography and NMR data and may add to the better understanding of dynamic properties of water molecules forming hydration sites–*e.g.*, exchange of water, residence time, *etc.* Understanding of the role of hydration in the recognition mechanism of T4 Endonuclease V and in the formation of complex will not only contribute in understanding the factors that control protein-DNA interactions on the molecular level, but may also benefit the clinical effort in combating skin cancer [18].

2. METHODS

The initial structure of native DNA dodecamer d(TCGCGTTGCGCT)$_2$ was used as an idealized B-DNA molecule generated by program NUCGEN (part of AMBER 5.0 program package [19]). For simulation of TD lesioned DNA molecule, the two thymines at the positions 6 and 7 were replaced by the thymine dimer formed as a complex of two thymines joined by covalent bonds between respective C(5) and C(6) atoms (for details of formation of TD see [17]). The atomic coordinates of repair enzyme T4 Endonuclease V were obtained from the Brookhaven Protein Data Bank [20]. These 3 molecules – native DNA, DNA with TD and enzyme T4 Endonuclease V – were subjected to the 500 picosecond (ps) of MD simulation. During the simulation, the root mean square deviation (r.m.s.d.) of each molecule from the original position was calculated. Figures (1a) through (1c) show the r.m.s.d. of single molecules. It is seen that during the initial period of 200 ps molecules stabilized and remained stabile for the following 300 ps, except DNA with TD (Fig. 1(b)). There is an increase in r.m.s.d. after 400 ps, which is caused by the movement of edge residues, as no restriction was applied on their positions.

Stabile structures of enzyme and DNA with TD at 300th ps were selected for the formation of DNA-catalytic center complex. The additional 500 ps of MD was then performed for molecule of the complex. Figure (1d) shows the r.m.s.d. of complex molecule. It is seen, that this molecule stabilized quickly in first 100 ps and remained stabile throughout the performed 500 ps of MD.

Simulations were performed with program AMBER 5.0, package of programs designed for MD simulation of biomolecules [19]. The standard all atom force field for protein and nucleic acids with necessary modifications for TD was used for calculation. In simulation the periodic boundary conditions were used and the full electrostatic energy was calculated using Particle Mesh Ewald method (PME) [21]. PME is a method for calculating the full electrostatic energy of a simulation cell in a macroscopic lattice of repeating images without introducing the cut off distance. During MD simulation no restraints were applied on the movement of water molecules and position of each water molecule in the simulation cell was examined in 1 ps time steps. Protocol of MD simulation is introduced in the Appendix.

2.1. Description of Catalytic Center in the Complex

The enzyme molecule is built of 137 amino acids and has one compact domain consisting of three α-helices standing side by side (H1 – amino acids

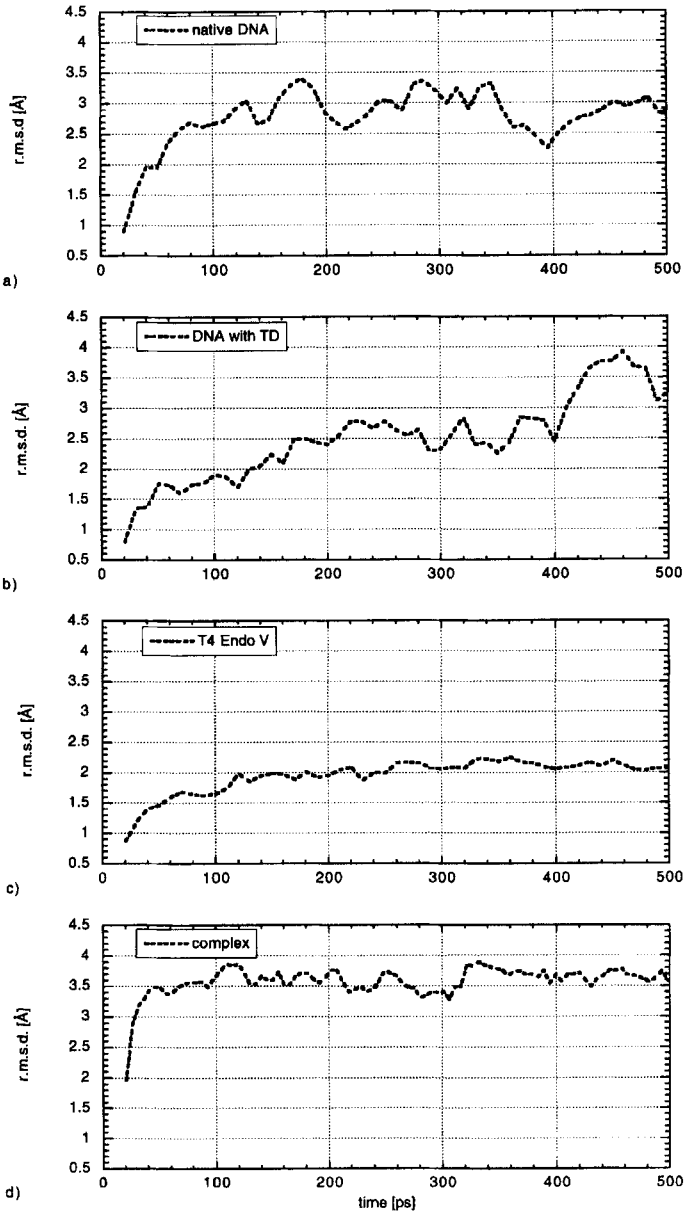

FIGURE 1 R.m.s.d. of native DNA – (a), DNA with TD – (b), repair enzyme T4 Endonuclease V – (c) and complex of DNA and part of enzyme (inclusive catalytic center) – (d). Both DNA molecules stabilized during the first 200 ps of MD and remained stabile up to 400th ps. Enzyme and molecule of the complex stabilized quickly during first 100 ps and then kept stabile with small fluctuations around respective average values.

14 through 38, H2 – 64 through 82 and H3 – 108 through 124), five reverse turns and several loops [20]. The surface is concave which may be an essential structural feature of the enzyme in order to form a close contact with bent structure of DNA near TD. This concave surface is also populated by several positively charged residues, which indicates that the electrostatic interaction between the surface and DNA may play a significant role in either the non-specific or specific binding. In the middle of them, there is amino acid Glu-23 which has been shown in previous mutagenesis studies as playing an important role in the enzymatic repair process – cleavage of N-glycosyl bond [14, 22]. Glu-23 is surrounded by amino acids Arg-3, Arg-22 and Arg-26 belonging to helix H1. Side chain of Glu-23 also forms a hydrogen bond with the backbone amino group of Arg-3. Arg-3 and Thr-2 – two polar residues at the NH_2 terminus – form several hydrogen bonds with the side chains of helices H1 and H2, and lie on the molecular surface. Considering these properties, the 8 amino acids of H1 – Glu-20, Tyr-21, Arg-22, Glu-23, Leu-24, Pro-25, Arg-26, Val-27, and 2 amino acids at NH_2 terminus – Thr-2 and Arg-3 were selected to form the selected part of enzyme used for the formation of complex DNA-catalytic center. Amino acids – Thr-2, Arg-22, Glu-23 and Arg-26, are active in the incision of thymine dimer during repair process, and together with other 6 selected amino acids – Arg-3, Glu-20, Tyr-21, Leu-24, Pro-25 and Val-27 – lie at the central part of concave site of the enzyme (Fig. 2). This part of enzyme may be easily exposed to the DNA surface and has been shown as having possible contacts with TD atoms of DNA in crystal structure of DNA in complex with T4 Endonuclease V [23].

The entire procedure of construction of complex is described in the Appendix.

3. RESULTS

Focusing on the role of water molecules in the recognition process, the number of water molecules close to the atom of solute molecule, and the residence time – period during which water molecule remains around the same position with respect to DNA atoms – were examined. As it is suggested the water may help to keep DNA and enzyme in complex together by forming hydrogen bond network between them. For the purpose of study the existing this hydrogen bond network, the water molecules between both molecules in complex were also examined. Examination was focused mainly on those molecules laying between catalytic center of enzyme and TD part of DNA.

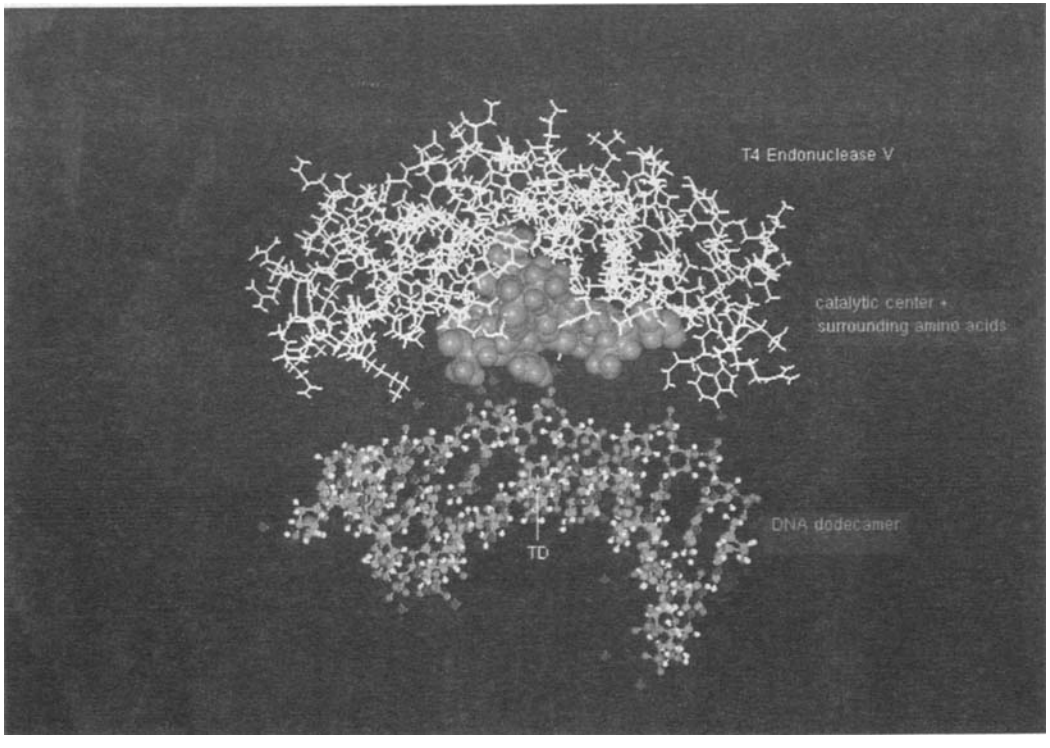

FIGURE 2 Structure of T4 Endonuclease V and DNA with TD at 300th ps of MD. The red color highlighted part of enzyme marks 10 amino acids selected for formation of complex with DNA. It includes catalytic center (Thr-2, Arg-22, Glu-23 and Arg-26) and 6 surrounding amino acids. Blue atoms scattered around DNA are sodium counterions initially located at the distance of 5 Å from phosphorus atoms. (See Color Plate I).

The water was treated as a free medium and no restrictions were applied on the movement of water molecules during MD. As a result water molecules occupied the optimal positions in respect to atoms of solute molecule taking its structure and energy conditions in system into consideration.

3.1. Hydration of DNA

Each atom of DNA, native as well with TD, was examined for the presence of close water molecules. In calculation, only positions of water oxygens were considered. Figure 3 shows the average number of water oxygens within the 3 Å distance from the atoms of DNA. It is seen that in all cases the average number calculated throughout the stabile period of MD is less than one oxygen atom per 1 solute atom per 1 ps (average number calculated throughout the stabile period of MD between 200th and 400th ps). DNA with TD has more water molecules closer to its atoms than native DNA. The most of water oxygens are close to DNA oxygens O1 and O2 bound to phosphates. If these phosphate bound DNA oxygens are not included into examination, the DNA with TD shows nearly double of water oxygens close to its atoms (0.44) compared with native DNA (0.19), see Table I. This specific water environment around the TD lesioned DNA molecule is probably one of the factors that is recognized by the repair enzyme. TD part is densily populated (more than native TT part) with water molecules close to atoms P, C1′, C2′, C3′, N1 and C2 (Figs. 4 and 5). In the case of complex molecule there is slightly lower number of closely located oxygens as the proximity of catalytic center and DNA is leaving little room for water.

3.2. Residence Time

Study of residence time can yield precise information on the location of individual water molecules as well on their exchange in and out of the DNA sites. According to crystallographic studies, the short lifetimes (less than 1 nanosecond) are typically observed on the surface of proteins and in the major grooves of DNA, while residence times longer than 1 milliseconds have so far not been observed in any protein nor protein-DNA complex [24]. MD study can provide the information on exchange of water molecules in order of picoseconds.

Residence time was calculated as a time during which water oxygen remains within 1.5 Å distance from its position taken at 200th ps. Position of each water oxygen was examined at every picosecond up to 400 ps (stabile period of r.m.s.d.). The distance 1.5 Å defines the sphere of diameter 3 Å

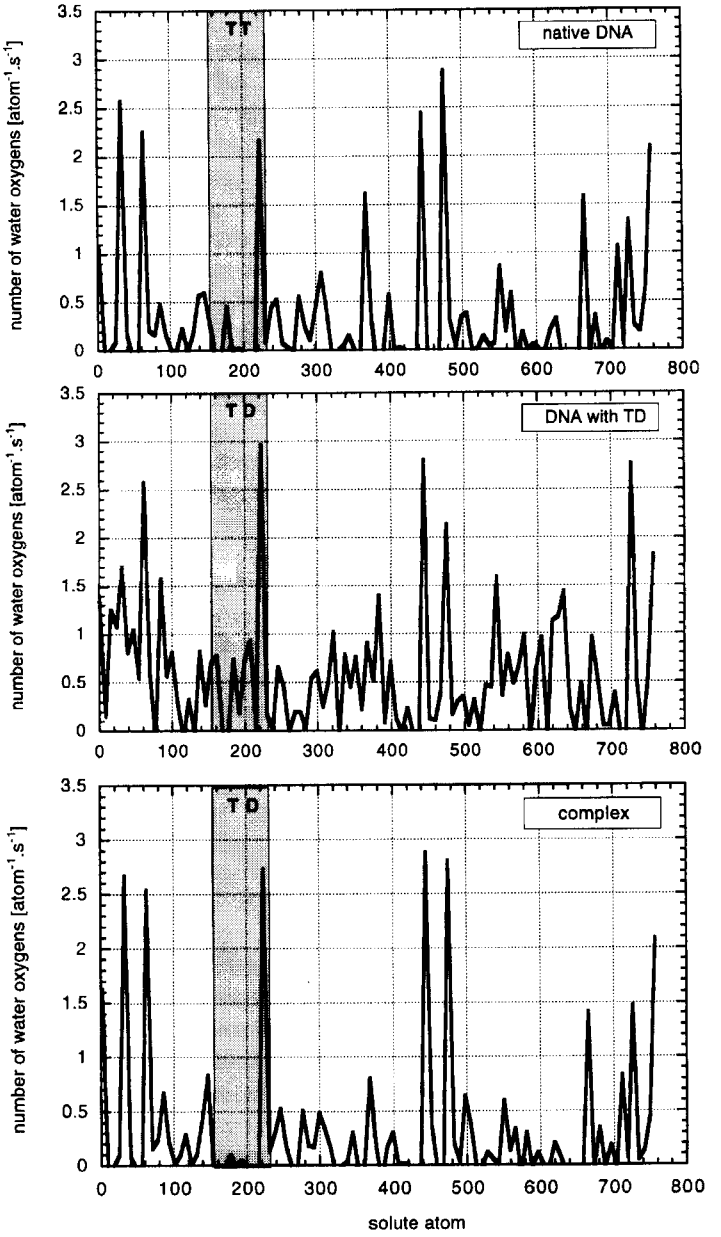

FIGURE 3 The average number of water molecules (calculated for the atom of water oxygen) located within 3 Å distance from DNA atoms. The TD part of DNA has more water molecules close to its atoms than native TT part. Less water is observed for the complex molecule where the water is pushed further from DNA surface by enzyme.

TABLE I Number of water oxygens at the positions closer than or equal to 3 Å to any atom of DNA

	No. of water oxygens within 3 Å from DNA [$atom^{-1} \cdot ps^{-1}$] (*all DNA atoms*)	*No. of water oxygens within 3 Å from DNA* [$atom^{-1} \cdot ps^{-1}$] (*except oxygens O1 and O2 bound to phosphates*)
Native DNA	0.40 ± 0.15	0.19 ± 0.06
DNA with TD	0.57 ± 0.21	0.44 ± 0.10
Complex DNA + cat.center	0.37 ± 0.12	0.16 ± 0.08

around the position at 200th ps and its value was selected to be less than the inter-nucleotide distance of 3.4 Å in B-DNA. In the stabile period between 200 and 400 ps of MD the r.m.s.d. of respective solute molecules deviates less than 0.5 Å from the average value (Fig. 1) and thus it is supposed that the water oxygen remains in the same position with respect to solute molecule and that there is no exchange with other water molecules, *i.e.*, each water molecule is the same water molecule.

In the case of DNA molecules most water molecules have residence time between 5 and 25 ps with average around 13 ps while water around complex molecule has slightly longer residence time with average around 15 ps (Tab. II and Fig. 6). Focusing on the atoms of TT and TD parts only, the higher occurrence of water molecules residing at around the same position is increasing for complex molecule where the number of water molecules having residence time longer than 30 ps is higher than that one in native DNA as well DNA with TD. This longer residence time is probably originated in the stability of complex molecule which is also reflected in its r.m.s.d. where fluctuations only less than 0.5 Å are seen (Fig. (1d)). In this stabile system the water is arranged around complex molecule and remains stabile as well.

Comparing the average residence time (13 and 15 ps respectively) and the examined period of MD simulation (200 ps), it is suggesting that most water molecules remain in the bulk solvent, with occasional short-lived contacts (calculated residence time) to the surface of DNA or complex molecule. In the view of this result, the hydration shells formed around solute molecules must be understand as shells within which the individual water molecules exchange their positions, *i.e.*, go out and in and at least move further than examined distance of 3 Å. The total number of water molecules in shell remains constant at a certain average value. Similar results was found in our previous MD study of cytosinyl radical damage in DNA, where the highest number of water oxygens was detected within 3 Å from the surface of DNA [16].

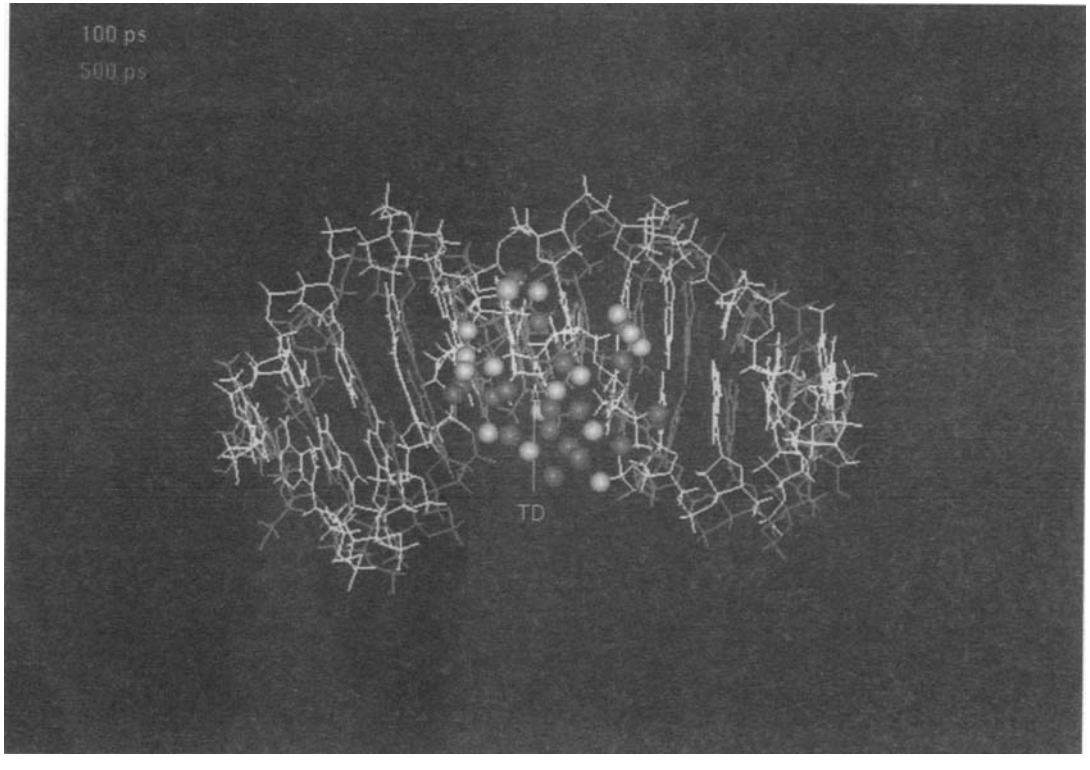

FIGURE 4 Snapshots of DNA with TD and water molecules around TD part taken at 100th ps (yellow) and 500th ps (red) of MD. (See Color Plate II).

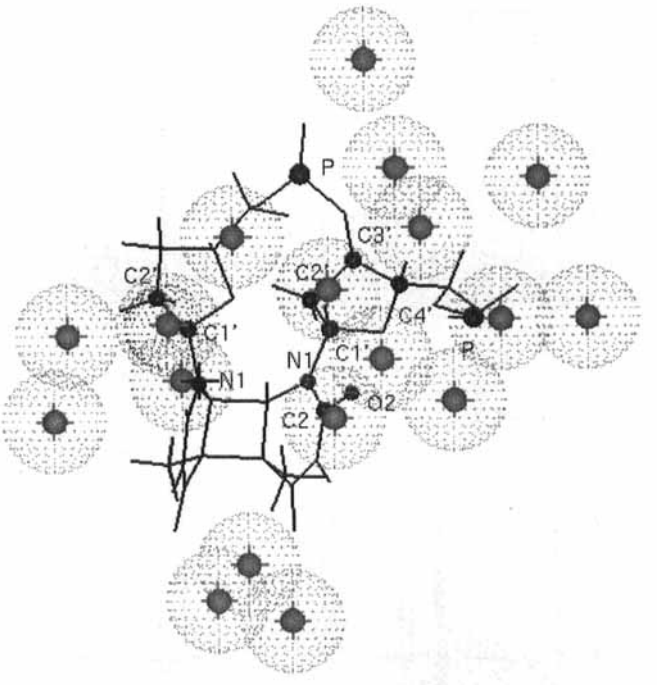

FIGURE 5 Detail view of the water molecules located around atoms of TD (situation at 500th ps). The water atoms are close to P, C1′, C2′, C3′, N1 and C2 atoms. The water molecules (red) are shown with the VDW surface. (See Color Plate III).

TABLE II Residence time of water molecules – period during which water molecule remains around the same position with respect to DNA atoms

	Residence time [ps] (all water atoms)
Native DNA	13.29 ± 3.50
DNA with TD	13.31 ± 2.54
Complex DNA + cat.center	15.19 ± 5.45

3.3. Water Located Between TD and Catalytic Center of Enzyme

To determine the role of water molecules in keeping the enzyme and DNA stabile, the water located between DNA and enzyme in complex was examined. Detail examination of the situation between catalytic center and TD part of DNA shows that there are 6 water molecules located at the positions

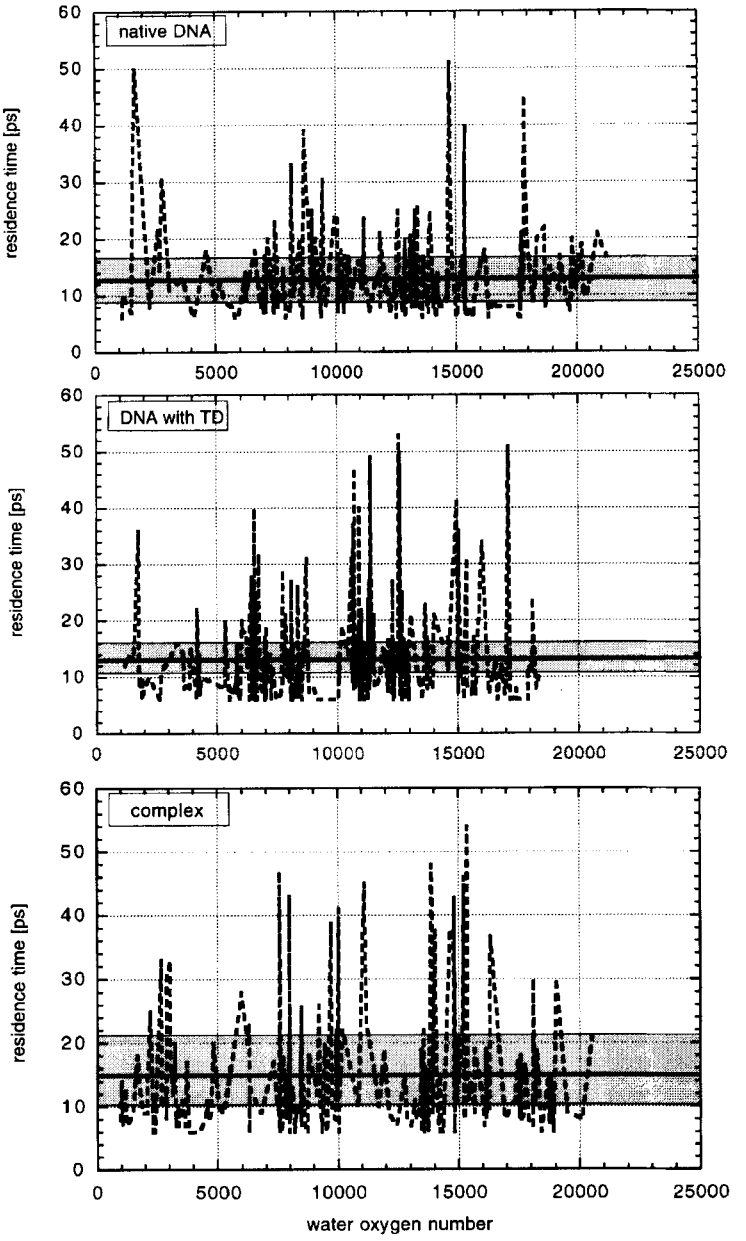

FIGURE 6 Residence time of simulated molecules. Highlighted is mean value with standard deviation (Tab. II). Water molecules around complex have longer average residence time than those around DNAs.

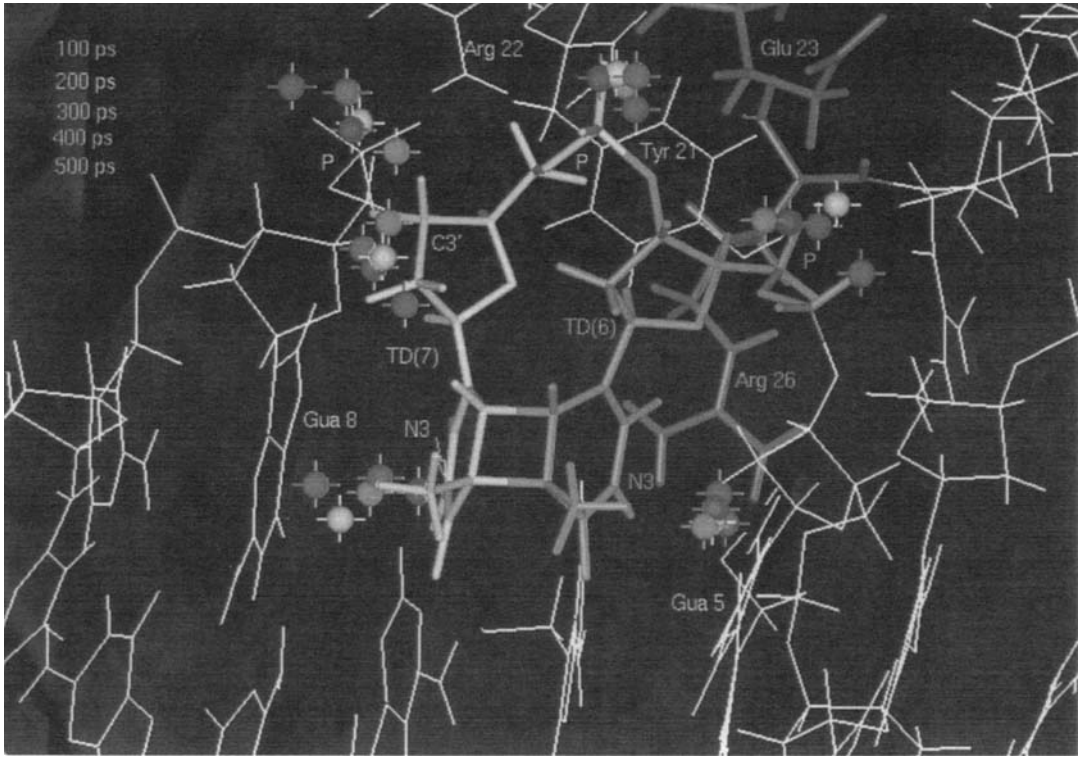

FIGURE 7 Detail view at the contact area of DNA and catalytic center of enzyme. There are 6 stabile positions close to atoms P, C3′ and N3 occupied by water molecules. These waters contribute to the stability of complex by forming the hydrogen bonds between enzyme and DNA. The stability is necessary condition for the onset of enzymatic repair. Positions of waters are colored according to time of MD (see legend). (See Color Plate IV).

close to atoms P, C3′ of phosphodiester bond and to N3 atoms of TD. These 6 positions are occupied by water molecules throughout the performed 500 ps of MD (Fig. 7). Considering the close proximity of these water molecules of DNA atoms as well to atoms of enzyme it can be concluded that they participate at the direct enzyme-DNA interaction through water mediated hydrogen bonds [13, 25]. Amino acid Glu-23 lies in close vicinity to phosphodiester bond (~ 3 Å) and Arg-26 is docked between TD and guanine (Gua-5).

In this simulation only the relatively small part of enzyme is included and if complex of whole enzyme and DNA molecule would be simulated, other water mediated binding sites would be probably detected ensuring the stability of complex of enzyme and DNA. This finding confirms a suggestion that water plays an important role in keeping enzyme-DNA complex stabile and thus is important factor for the successful repair process takes place.

4. CONCLUSION

In this paper the results of MD study of hydration and residence time of water molecules around DNA and TD lesioned DNA in complex with catalytic center of repair enzyme T4 Endonuclease V has been presented. It represents an attempt to better understanding of the recognition and repair process at the molecular level and the role of water in this process. DNA with TD has more water oxygens located closer than 3 Å to the DNA atoms than those observed around native DNA. Most water molecules are located closely to DNA oxygens O1 and O2 bound to phosphates and in the TD part to atoms P, C1′, C2′, C3′, N1 and C2. This results suggests that the TD part is densely populated by water molecules which might be detected by enzyme during scanning process.

The water plays an important role also in the formation of complex molecule and in ensuring its stability which is an important factor for the onset of catalytic repair process. There are found 6 stabile positions of water molecules between the catalytic center and TD part (close to atoms P, C3′ and N3) which are involved in the hydrogen bonds between enzyme and DNA.

The specific water environment found for the TD lesioned DNA suggests that water participates in the proper recognition of TD site by the repair enzyme T4 Endonuclease V. The water is also showing as playing role in the formation and in the stability of complex molecule through hydrogen bonds and thus is one of the important factors in recognition and repair process.

Acknowledgments

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APPENDIX

MD Protocol

MD simulations were performed using the same MD protocol for the each system – native DNA, DNA with TD and complex DNA-catalytic center of enzyme. No crystal water molecules were included in MD simulations and an extensive equilibration period was performed to ensure the stability of each molecule. Simulations consisted of several steps:

1. Solvating of solute molecules, *i.e.*, immersing the solute molecule into the water box consisting of several thousands water molecules. The final boxes have around 20,000 atoms in total each.
2. Neutralizing the negative charges of DNA phosphates by sodium counterions to assure the neutral total charge of the system. The sodium ions were placed at the initial positions bisecting P—O—P angle at the distance of 5 Å from the phosphorus atom. During the MD simulation no restraint on the position of sodium atoms was applied.
3. Minimizing the potential energy of each system. The potential energy of system upon its formation is in general not at the minimal value as water is placed around solute molecule without considering optimal energetic criteria. Some water molecules may be very close to solute atoms, that may cause unfavorable artificial changes in its structure during MD (bending, repulsion, formation of hydrogen bonds, *etc.*). The potential energy was minimized in two steps. In first step the geometry of solute molecule was kept frozen and only surrounding water molecules were allowed to move. When water molecules arranged themselves around the solute, the second step was performed that included all atoms.
4. Heating up to 300 K. The temperature upon the formation of the system was 0 K, *i.e.*, the atoms had no velocities. In the beginning the velocities were assigned from the Maxwell-Boltzman distribution at 30 K. Then the heating was performed in 10 subsequent steps of 30 K to avoid large temperature gradient that might result in the breakage of some bonds. Each 30 K step represented 1 ps of MD, *i.e.*, heating up to 300 K lasted 10 ps of MD.

5. Adjusting of saturated density. Upon the formation of the system there may be holes and spots with high concentration of atoms in the surrounding water that are caused by the fixed size of the box and fixed number of atoms. In this step the box sizes were released and the density of system stabilized at the saturated level (for the given box size of around 20,000 atoms the density saturation took up to 10 ps of MD).
6. Entire MD simulation. After density stabilized at a saturated level, the box sizes were fixed and the MD simulation was performed, *i.e.*, the density, temperature and volume of the box were kept at the constant levels.

To be able to handle the larger system around 20,000 atoms, the original code AMBER 5.0 was changed and partly vectorized. Its sequential and parallel flags were also changed to compile program on the VPP500/42 computer. After these changes, the program was capable to deal with system up to 30,000 atoms within reasonable CPU time. Simulations were performed on FUJITSU VPP500/42 vector/parallel type supercomputer and on the AP3000 parallel supercomputer of the Japan Atomic Energy Research Institute. One ps of MD simulation of constructed systems required approximately 1.7 hours and 3 hours of CPU time respectively.

Construction of Complex DNA-catalytic Center

This step consisted of docking of the isolated catalytic center of enzyme (Thr-2, Arg-22, Glu-33 and Arg-26) with 6 surrounding amino acids (Arg-3, Glu-20, Tyr-21, Leu-24, Pro-25 and Val-27) onto the DNA with TD (the selected part of enzyme is described in Methods). The major consideration in construction of complex was to minimize initial mutual van der Waals interactions between the respective molecules. The initial position of part of enzyme with respect to DNA was determined using the molecular graphic software InsightII [26] in such a manner that position of TD was near the catalytic center of enzyme (distance between the closest atoms of enzyme and DNA was ~ 7 Å) and to ensure minimal overlap of VDW surfaces of the DNA molecule and enzyme.

The constructed structure was partially optimized by energy minimization, with the complex DNA-part of enzyme kept rigid in order to relieve bad contacts between the two parts. After minimization the complex was fully relaxed with a simulated annealing (SA) protocol in duration of 15 ps:

- 1 ps, heating up to 1,200 K from initial 0 K;
- 2 ps, keeping at 1,200 K;
- 12 ps, cooling to 0 K in 3 stages with different coupling constants.

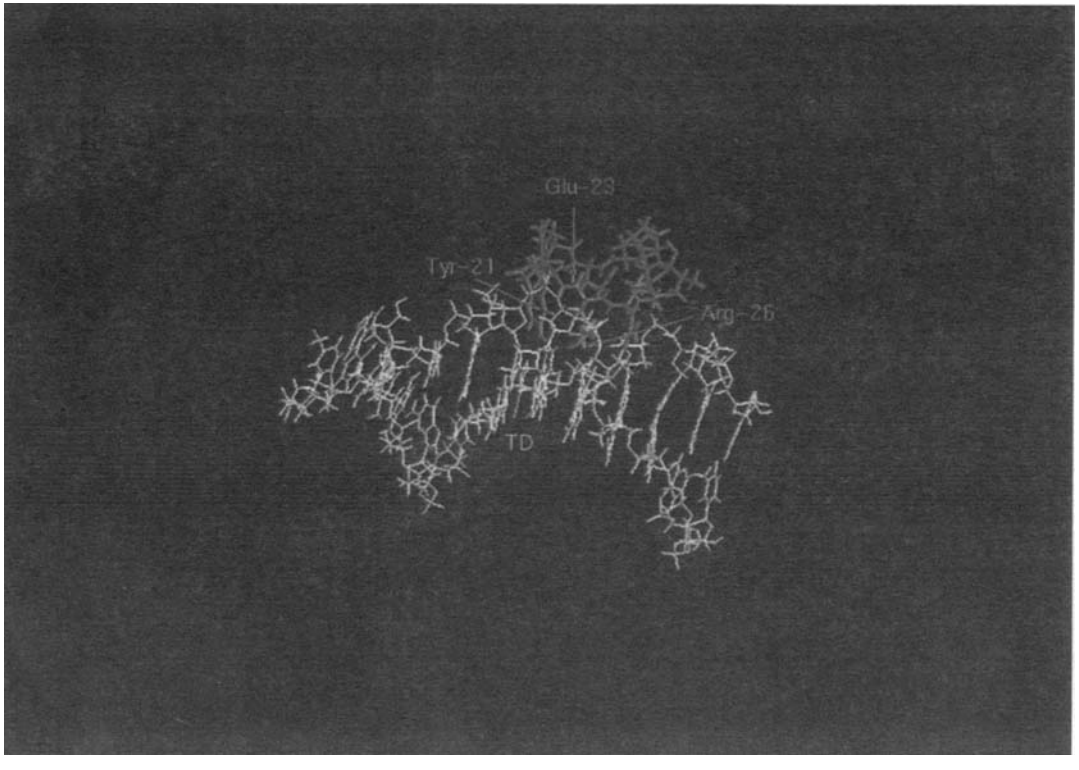

FIGURE 8 The structure of complex DNA (yellow) and part of enzyme inclusive catalytic center (red) after simulated annealing. This is initial structure subjected to 500 ps of MD. The Glu-23 cleaves the phosphodiester bond at C3′ and C5′ atoms of DNA. Tyr-21 and Arg-26 enable docking of part of enzyme into the DNA. (See Color Plate V).

During simulated annealing the all nucleotides of DNA dodecamer were restrained in Cartesian space using a harmonic potential with harmonic force constant of 1 kcal/mol. The reason for constraining DNA atoms was high annealing temperature of 1,200 K that might result in breaking of hydrogen bonds between corresponding nucleotides of DNA strands. No restraint was applied on the amino acids of the enzyme. The resulting annealed structure was used as the input for the MD simulation as has been described above (Fig. 8).

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