JAERI-Research
2002-016

MOLECULAR DYNAMICS SIMULATION OF 8-OXOGUANINE LESIONED DNA COMPLEXED WITH REPAIR ENZYME hOGG1

September 2002

Miroslav PINAK

日本原子力研究所
Japan Atomic Energy Research Institute

本レポートは，日本原子力研究所が不定期に公刊している研究報告書です。
入手の問合わせは，日本原子力研究所研究情報部研究情報課（〒319-1195 茨城県那珂郡東海村）あて，お申し越しください。なお，このほかに財団法人原子力弘済会資料センター（〒319-1195 茨城県那珂郡東海村日本原子力研究所内）で複写による実費頒布をおこなっております。

This report is issued irregularly.
Inquiries about availability of the reports should be addressed to Research Information Division, Department of Intellectual Resources, Japan Atomic Energy Research Institute, Tokai-mura, Naka-gun, Ibarakiken 319-1195, Japan.

編集兼発行　日本原子力研究所

Molecular Dynamics Simulation of 8-oxoguanine Lesioned DNA Complexed with Repair Enzyme hOGG1

Miroslav PINAK

Department of Health Physics
Tokai Research Establishment
Japan Atomic Energy Research Institute
Tokai-mura, Naka-gun, Ibaraki-ken

(Received June 4, 2002)

The molecular dynamics (MD) simulations of DNA mutagenic oxidative lesion - 7,8-dihydro-8-oxoguanine (8-oxoG), single and complexed with the repair enzyme - human oxoguanine glycosylase 1 (hOGG1), were performed for 1 nanosecond (ns) in order to determine structural changes at the DNA molecule and to describe a dynamical process of DNA-enzyme complex formation. The molecule of 8-oxoG was inserted into central part of B-DNA 15-mer d(GCGTCCA'8-oxoG'GTCTACC)$_2$ replacing the native guanine 8. In the case of simulation of single DNA molecule the broken hydrogen bonds resulting in locally collapsed B-DNA structure were observed at the lesion site. In addition the adenine on the complementary strand (separated from 8-oxoG by 1 base pair) was flipped-out of the DNA double helix. In the case of simulation of DNA and repair enzyme hOGG1, the DNA-enzyme complex was formed after 500 picoseconds of MD that lasted stable until the simulation was terminated at 1 ns. Proximity of both molecules in complex satisfied the Van der Waals interactions between the closest atoms. The N-terminus of arginine 313 was located close to the phosphodiester bond of nucleotide with 8-oxoG enabling chemical reactions between amino acid and lesion. Phosphodiester bond at C5'of 8-oxoG was at the position close to the N-terminus of arginine 313. The water mediated hydrogen bonds network was formed in each contact area between DNA and enzyme further enhancing the stability of complex. In the background simulation of the identical molecular system with the native DNA, neither the complex nor the water mediated hydrogen bond network were observed.

Keywords: Molecular Dynamics, B-DNA, 8-oxoguanine, Flipped-out Adenine, hOGG1

8 オキソグアニン損傷 DNA と修復酵素 hOGG1 複合体の分子動力学的シミュレーション

日本原子力研究所東海研究所保健物理部
Miroslav PINAK

（２００２年６月４日受理）

突然変異を誘発する DNA 酸化損傷 である 7,8-ジヒドロ-8-オキソグアニン (8-oxoG)について、損傷 DNA の単独存在下、およびヒト修復酵素オキソグアニングリコシレース 1 (hOGG1)との共存下で 1 ナノ秒(ns)の分子動力学的シミュレーションを行い、DNA 分子の構造変化と DNA-酵素複合体の形成に関わる動力学的過程の検討を行った。シミュレーションには、d(GCGTCCA'8-oxoG'GTCTACC)2 の中心位の正常グアニンの替わりに 8-oxoG 分子を挿入した B 型 DNA を用いた。DNA のみのシミュレーションでは、水素結合の切断によって部分的に構造が破壊された B 型 DNA が観察された。また、8-oxoG 挿入位から 1 塩基対分離れた相補鎖側のアデニンが DNA 二重鎖からフリップアウトしていた。DNA と修復酵素 hOGG1 共存下のシミュレーションでは、分子動力学的シミュレーション開始後 500ps で DNA-酵素複合体が形成され、シミュレーションが終了する 1ns 後まで安定していた。複合体は主に DNA と酵素のファンデルワール
ス面の重なり合いで定義されている。アルギニン 313 の N 末端は、8-oxoG を持つヌクレオチドのリン酸ジエステル結合に近接し、酵素のアミノ酸と DNA 損傷との化学反応を可能にしている。8-oxoG の 5'位のリン酸ジエステル結合は、アルギニン 313 の N 末端に近接した位置に移動していた。さらに、DNA と酵素の近接箇所では水分子を介した水素結合が形成され、複合体の安定性を高めていた。正常な DNA を用いた同様の分子系で行ったシミュレーションでは、複合体や水分子を介した水素結合は観察されなかった。

東海研究所：〒３１９－１１９５　茨城県那珂郡東海村白方白根２－４

Contents

目　次

This is a blank page.

1. Introduction

Living organisms are constantly exposed to oxidative stress from environmental agents and from endogenous metabolic processes. Among many environmental agents the ionizing radiation represents a significant factor producing the active water radicals [1]. These oxidants with free-radical character are among the known causes of DNA damage, which may either block replication and transcription or generate mutation by miscoding during replication. The most significant consequence of oxidative stress are DNA modifications, which can result in mutations and other types of genomic instability. The group of other damage-producing agents includes certain activated antibiotics, metal complexes, redox-active metalloenzymes and oxygen metabolites, for example anticancer agents such as bleomycin [2]. To counter the threat posed by the genotoxic lesion on DNA, cells express enzymes that function solely to recognize and repair structural aberrations in their genomes [3, 4, 5, 6]. Considerable information regarding enzyme/DNA interaction has been gained from many biological experiments [e.g. 7, 8]. It is known that sequence specific DNA binding by repair and regulatory enzymes occurs as a result of multistage hydrogen bonding and van der Waals interactions between the DNA recognition amino acid chains of enzyme and nucleotide base sites of DNA. However, the underlying mechanisms by which repair enzymes recognize aberrant sites on DNA are still subject of a debate [e.g. 9, 10].

The 7,8-dihydro-8-oxoguanine (8-oxoG) is formed by oxidation of a guanine base in DNA. It is considered to be one of the major endogenous mutagens contributing broadly to spontaneous cell transformation. Its frequent mispairing with adenine during replication increases the number of G-C → T-A transversion mutations. This mutation is among the most common somatic mutations in human cancers [11, 12].

The 8-oxoG is recognized and subsequently repaired by the DNA glycosylase (hOGG1 in humans). DNA glycosylases acting on single-base lesions use an extrahelical repair mechanism during which enzyme recognizes oxidative damaged guanines and excludes normal DNA bases [13]. The docking of enzyme into DNA is a necessary intermediate ensuring the onset of repair process and in many cases is facilitated by existence of a hole in DNA double helix caused by extrahelical position of bases.

The current computational study focuses on the 8-oxoG lesioned DNA molecule. Its main aim is to describe profound structural and energetic changes on the 8-oxoG lesioned DNA molecule. In addition it is focusing on the description of a dynamical mechanism by which repair enzyme hOGG1 recognizes oxidatively damaged guanine on DNA. The proper recognition of damaged part of DNA is an onset of repair process that prevents its malignant transformation.

2. Materials and Methods

The method for study the time evolution of the 8-oxoG lesioned DNA was the classical molecular dynamics (MD) simulation using the program AMBER 5.0 [14].

The module NUCGEN of AMBER 5.0 software package was used to prepare the native sequence of the 15 base pairs B-DNA duplex (DNA 15-mer). The force field used in simulation was parm96.dat [15], which is modified version of parm94.dat force field [16]. The difference between the two force fields is that in parm96.dat there have been introduced new torsional parameters according to the results of *ab initio* calculations performed by Beachy MD et al. [17]. The potential energy function is calculated as contributions from bonds, single angles, torsional, and electrostatic functions (Eq. 1):

$$E_{pot} = 1/2 \sum_{bonds} K_r (r - r_{eq})^2 + 1/2 \sum_{angles} K_\theta (\theta - \theta_{eq})^2 + 1/2 \sum_{dihedrals} K_\varphi [1 + \cos(n\varphi - \chi)] + \sum_{j=1}^{atoms} \sum_{i>j}^{atoms} \left[\left(\frac{R_{ij}^*}{r_{ij}} \right)^{12} - \left(\frac{R_{ij}^*}{r_{ij}} \right)^{6} + \frac{q_i q_j}{\varepsilon r_{ij}} \right] \quad \text{Eq. (1)}$$

Here r, θ and φ are bond lengths, planar angles and dihedral angles, respectively; r_{eq} and θ_{eq} are their equilibrium values; r_{ij} is the distance between atoms i and j; q_i is the partial charge on atom i; ε is dielectric constant; K_r, K_θ, K_ϕ and R_{ij} are empirical parameters depending on atom types. The point charges q_i and q_j in a calculation of electrostatic function are centered on each atom and are derived by fitting to quantum mechanical electrostatic potential. In MD simulation a constant dielectric function was used and 1-4 electrostatic interactions were scaled by factor 1.2 that is recommended value for parm96.dat force field. The Particle Mesh Ewald method (PME), [18] was used for calculation of electrostatic interactions with no cut-off distance. Throughout the simulation the periodic boundary conditions were employed to eliminate undesirable edge effects.

2.1 Formation of 8-oxoG molecule

The 8-oxoG was built as a 7,8-dihydro-8-oxoguanine by addition of oxygen atom on the C8 atom of guanine. After addition of oxygen (O8) at the C8, hybridization of the N7 and transforming the double bond C8-N7 into single one (using graphical molecular software INSIGHT II [19]), the new molecule was subjected to a two-stage energy minimization process at 30 K by the SANDER module of the AMBER 5.0. In the first stage, 5,000 steps of minimization were performed while keeping bond lengths and

angles of the guanine atoms constrained except for the heavy atoms of the C8-O8 group. In this process the parameters of the double bond C8-N7 were transformed to a single bond and stabilized at length of a 1.52 Å. After the first stage a second stage minimization of 10,000 steps was performed which relaxed all atoms. At this stage only bonds involving hydrogens were constrained for the first 2,000 steps. The molecule of the 8-oxoG is shown on Fig. 1. Partial atomic charges of 8-oxoG were taken as those calculated by Poltev VI et al. [20]. If compared with native guanine, the most significant change is the charge of oxygen O8 bound to C8 atom of which the partial charge is negative of ~ -0.54. (the partial charge of H8 of native guanine is ~ +0.19). The molecule of 8-oxoG was used to replace the native guanine at position 8 of the DNA 15-mer.

2.2 MD protocol

The following molecules were subjects of 1-nanosecond (ns) of MD simulations:

- 8-oxoG lesioned DNA 15-mer, d(GCGTCCA'8-oxoG'GTCTACC)$_2$;
- hOGG1 repair enzyme [21];
- native DNA 15-mer, d(GCGTCCAGGTCTACC)$_2$, - background simulation.

The DNA molecules were simulated as a single water solvated molecule and in complex with the hOGG1, i.e. four simulation configurations were prepared:

- 8-oxoG lesioned DNA, single;
- 8-oxoG lesioned DNA + hOGG1;
- native DNA, single, - background simulation;
- native DNA + hOGG1, - background simulation.

The MD protocol consisted of several preparatory steps - minimization, stepwise heating up to 310 K, density stabilization and production dynamics. The MD protocol is described bellow and details have been described elsewhere [e.g. 22, 23].

To neutralize the negative charge of DNA molecules sodium counterions (Na^+) were placed at the initial positions bisecting the O-P-O angle at an initial distance of 5 Å from each phosphorus atom. During the MD simulation no restrictions were applied on the position of counterions.

Solvating of solute molecules - native DNA, 8-oxoG lesioned DNA and enzyme, i.e. immersing solute molecules into the water box consisting of several thousands water molecules, resulted in the system consisting of approximately 25,000 (single DNA) and 53,000 (DNA + hOGG1) atoms in total.

The MD simulations were performed at constant temperature of 310 K (~ 36.5°C, the temperature of human body). Heating from the initial temperature of 0 K was performed in 10 sequential MD runs each of 1 picosecond (ps). The temperature coupling constants

for solute (DNA, enzyme, counterions) and solvent (water) molecules were 0.1 and 0.2 ps, respectively. The saturated density of the simulated system was achieved during 10 ps of constant pressure run with the pressure relaxation time of 0.2 ps.

During preparatory steps bond length constraints were applied as follows: heating - bonds involving hydrogen; stabilization of density – all bonds (this was intended to remove bond stretching freedom and protect bonds from disrupting). These constraints were then removed to ensure that the DNA molecules were fully flexible during the entire 1 ns production MD simulations. The time step (interval between each force evaluation) was 1 femtosecond and coordinates and energies of the each atom were stored every ps for further analysis. The production simulations were performed with constant cubic volume sizes (92.0 x 62.9 x 43.7 $Å^3$ – DNA with 8-oxoG, single; 92.1 x 63.6 x 43.2 $Å^3$ – native DNA, single; 87.2 x 87.3 x 71.4 $Å^3$ – DNA with 8-oxoG + hOGG1; 88.4 x 86.6 x 71.6 $Å^3$ – native DNA + hOGG1).

The simulations were performed on the Hitachi SR8000 parallel supercomputer at the Center for Promotion of Computational Science and Engineering of the Japan Atomic Energy Research Institute (JAERI). One picosecond of MD simulation (i.e. 1,000 iterations) required around 16 min. of CPU time – systems with single DNA, and around 36 min. of CPU time – systems with hOGG1; (8 CPU parallel run). Analysis of the structural properties and energy characteristics were evaluated using ANAL module of AMBER 5.0 and electrostatic potential was calculated with DELPHI software, [24].

2.3 Initial conditions of DNA and enzyme hOGG1

Prior the MD simulation the DNA (native or with 8-oxoG) and the enzyme were inserted into the center of simulated systems. The major consideration in their entire position was to minimize initial mutual van der Waals interactions between the DNA and enzyme. The initial position of the enzyme with respect to the DNA was determined using the molecular graphic software INSIGHT II, [19] in such a manner that the enzyme faced the 8-oxoG lesion (native guanine 8 in background simulation) and that there was a minimal overlap of VDW surfaces of the DNA molecule and the enzyme (distance between the closest atoms of enzyme and DNA was ~5 Å). The constructed structure was partially optimized by energy minimization, with bonds of both solute molecules kept constrained in order to relieve bad contacts between them (Fig. 2)

3. Results

3.1 Structural analysis of B-DNA structure around lesion site

In order to investigate the dynamics of DNA molecules and enzyme in the simulated systems, the root mean square deviations (r.m.s.d.) for each solute molecule were

calculated. R.m.s.d. value represents an average deviation calculated for all heavy atoms of the solute molecule with respect to its structure that was reached after preparatory steps - minimization, stepwise heating and density stabilization. The analysis of the r.m.s.d. was performed in order to reveal the significant structural changes that DNA and enzyme developed during the MD simulation. The Fig. 3a) shows r.m.s.d. of systems with single native and lesioned DNA molecules. In the 8-oxoG lesioned DNA molecule the disruptions of weak hydrogen bonds between complementary bases close to lesion (adenine 7 – thymine 24, guanine 9-cytosine 22) cause local instability that is reflected in higher r.m.s.d. values after 550 ps compared with those of native DNA. In the case of the native DNA the B-DNA structure around native guanine 8 is well kept. The r.m.s.d. of both DNA molecules increased during the first 550 ps of MD indicating a movement and structural changes of the molecules. The r.m.s.d. of both DNA molecules stabilized after 700 ps and remained stable oscillating around their respective average values up to the 1 ns of the MD simulation. The systems with DNA and hOGG1 showed similar r.m.s.d. trajectories of DNA (graphs are not shown). The molecular structures of hOGG1 in both systems (with native and lesioned DNA) were well preserved with small fluctuations of r.m.s.d. values (Fig. 3b)).

To determine the local impact of the lesion on the DNA structures the r.m.s.d. of atoms in the vicinity to the 8-oxoG were calculated. The atoms included in the calculation were those belonging to the following nucleotide pairs: 'cytosine 6 – guanine 25', 'adenine 7 – thymine 24', '8-oxoG (guanine 8) – cytosine 23', 'guanine 9 – cytosine 22' and 'thymine 10 – adenine 21' (each number refers to the sequentional position of nucleotide on the DNA; e.g. cytosine 6 refers to heavy atoms of deoxycytosine at position 6, adenine 7 to deoxyadenosine at position 7, etc.). The large deviations of lesioned DNA part suggest that this part is less stable than the identical part of native DNA due to the presence of 8-oxoG (Fig. 4).

The r.m.s.d. trajectory of the atoms close to the lesion is associated with structural changes that may be represented as disruptions of weak hydrogen bonds between respective bases and locally collapsed B-DNA structure. Analysis of hydrogen bonds shows that in the case of the native DNA the hydrogen bonds close to native guanine 8 are well preserved, (Fig. 5a)). In the case of the lesioned DNA, the hydrogen bonds between 8-oxoG and opposite cytosine 23 are kept while between neighboring base pairs (adenine 7 – thymine 24, and guanine 9 – cytosine 22) are broken. The hydrogen bonding of base pair thymine 10 – adenine 21 cease to exist very early (after 50 ps of MD simulation), (Fig. 5b)).

Fig. 6 shows snapshots of single DNA molecules with 8-oxoG during the entire MD

simulation. It can be seen that at 500 ps there are already missing several hydrogen bonds between complementary base pairs around the lesion. In the system with single DNA, the adenine 21 on the complementary strand (separated from 8-oxoG by 1 base pair) was flipped-out of DNA double helix after around 900 ps of MD simulation. The cytosine 22 is also severely dislocated form its intrahelical position and its hydrogen bonding to guanine 9 is not existing (Fig. 7). The extrahelical position of adenine 21 forms a hole in the double helix that may favor docking of repair enzyme into DNA during repair process.

In the case of the single native DNA molecule the B-DNA structure was well preserved and no distortions around the native guanine 8 were observed (snapshots not shown).

The flipped-out adenine 21 was not observed for the system with hOGG1 indicating that the presence of enzyme stabilizes DNA structure and flipping out occurs only if there is a favorable structural configuration enabling latching of flipped-out base into enzyme.

3.2 Analysis of the movement of DNA and enzyme

In the case of systems with hOGG1, the relative positions of DNA and enzyme were analyzed. Fig. 8a) shows the distances between closest heavy atoms of DNA and enzyme in system with native DNA. It is seen that both molecules were keeping their closest distances oscillating around 5 Å that was the distance at the beginning of the simulation. Different situation was observed for system with lesioned DNA molecule (Fig. 8b)). From the original distance of nearly 5 Å both molecules distanced from each other, then approached around 400 ps, distanced again and finally formed a very close contact after 900 ps of MD (closest heavy atoms around 2 Å). The distance of around 2 Å would satisfy the formation of hydrogen and even covalent bonds between atoms. To confirm the formation of bonded situation between DNA and enzyme, the quantum chemical studies on the orbital electron transfers between DNA and enzyme atoms shall be performed.

3.3 Analysis of electrostatic energy

In calculation of electrostatic energy the PME method was used. In this method a Gaussian charge distribution of opposite sign was superimposed upon the original point charges, producing a screened charge distribution. The electrostatic interaction between the screened charges was then short ranged. The original distribution was recovered by adding a second Gaussian charge distribution identical to the first, but of opposite sign. In the calculation of electrostatic interactions no cut-off distance was applied and thus all molecules in the system were included. Since PME method assumes the electronegativity of the simulated system, this was satisfied by adding of sodium

counterions (Na^+) as is described in Materials and Methods.

The electrostatic energy is the only long-range nonbonded interaction in the potential energy function, (Eq. 1) and its value is calculated for pairs of atoms that are separated by three or more bonds. Thus it is assumed that the well-separated atoms are interacting mainly electro-statically since no cut-off distance was used in the calculation of the electrostatic interactions. To evaluate the impact of the 8-oxoG lesion on the DNA molecule the intermolecular electrostatic interactions were calculated between the nucleotide with 8-oxoG and the neighboring nucleotides with respective bases (cytosine 6, adenine 7, guanine 9, thymine 10, adenine 21, cytosine 22, cytosine 23, thymine 24 and guanine 25). Interaction energy was calculated between two groups of atoms. Atoms belonging to the nucleotide with 8-oxoG formed one group, second group contained atoms of the respective neighboring nucleotide. During the 1 ns of MD simulation the electrostatic interaction energy in lesioned DNA largely oscillated around an average value of around 21 kcal/mol (Fig. 9). The positive value of the interaction electrostatic energy between the lesion and the rest of DNA represents a repulsion that may cause the disruption of hydrogen bonds in the vicinity of the lesion and also may contribute to the lesser stability of the surrounding atoms as it is seen in the respective r.m.s.d. (compare with Fig. 4). The electrostatic repulsion between the atoms in the region and disruption of hydrogen bonds caused extrahelical position of adenine 21 as discussed above. The electrostatic interaction energy calculated for the nucleotide with the native guanine 8 in the non-lesioned DNA is less repulsive and has a stable average value of 7.5 ± 1.4 kcal/mol.

According to the electrostatic term in the potential energy function (4^{th} term in Eq. 1), the value of electrostatic energy depends on the partial charges of the atoms and on the distance between them. Since the charges are preserved during the classical MD simulation, the distances between each two atoms are crucial for the final values of their electrostatic interaction. The higher and largely fluctuating values of this interaction between atoms of the lesioned DNA (if compared with the native DNA) mean that some atoms are at a certain moment very close to each other, causing a repulsive (or attractive) force and thus further contribute to the instability in the lesioned region. This instability may lead to the observed disruption of hydrogen bonds, flipping-out of a base and generally to the locally collapsed B-DNA structure.

The electrostatic energies are calculated for a large number of atoms (calculated values are per 1 mol) and the real difference of the interaction may be smaller and its significance would need further confirmation by experimental studies.

The effect of the ionic charge on the ionic distribution around a simple B-DNA model at

the continuum solvent level was not specifically investigated since no restrictions on the positions of Na^+ were applied throughout the entire MD simulation. Nevertheless, no overscreening of the DNA charge has been observed in this system. The screening effect, the "end –up" position of Na^+ including residence time, and role in the enzymatic recognition shall be subject of further study.

3.4 Van der Waals contacts between DNA and enzyme

During 1 ns of MD simulation of the system with hOGG1 and lesioned DNA, the enzyme approached DNA and formed a close contacts between several atoms (Fig. 10). The contacts between enzyme and DNA are important since molecular recognition is mediated by direct contacts between amino acids and DNA bases. There were three regions forming Van der Waals contacts:

- arginine 266 - guanine G5,
- arginine 313 - guanine, 8-oxoG , adenine
- serine 43, glutamine 42 - adenine, guanine.

Interaction between Van der Waals surfaces (encircled parts on Fig. 10) affects directly the binding affinity and also deters the exclusion of water and counterions from the interface. This way it may serve as a major driving force for the enzyme-DNA binding. The Van der Waals contacts between the DNA and the enzyme were established after 500 ps of MD simulation and lasted stable until simulation was terminated at 1 ns. Fig. 11 shows the detail view on the contact region including Van der Waals surfaces of nucleotides at the lesion site and arginine 313. N terminus of arginine 313 is located close to C5' atom of 8-oxoG, forming 2 hydrogen bonds between O1P (8-oxoG) and N atoms (arginine 313). N terminus of arginine is very basic due to its positive charge ^+H_2N. This positive charge establishes an attractive force to the negative charge of DNA phosphorus. In addition to the observed Van der Waals interaction, the electrostatic attractive interaction between N terminus and negative charge of DNA phosphorus contributes to the stability of the DNA-enzyme complex. N terminus of arginine 313 is also located close to the phosphodiester bond of nucleotide with 8-oxoG that is supposed to be sequentially cleaved during the repair process.

3.5 Hydrogen bonds network between DNA and enzyme

In addition to the Van der Waals and electrostatic interactions between solute molecules in the system, the water mediated hydrogen bonds between DNA and enzyme may contribute to its stability. To determine the existence of hydrogen bonds network between DNA and enzyme, each atom of DNA and enzyme was examined for the presence of water molecules located closely to both of them. In calculation, only

positions of water oxygens were considered. Fig. 12 shows the average number of water oxygens within distance of 3 Å from the atoms of both solute molecules - DNA and enzyme. In the case of system with lesioned DNA (Fig. 12b)), there are more water molecules located close to both solute molecules if compared with system with native DNA (Fig. 12a)). Most hydrogen bonds are formed with the atoms that belong to parts of molecules with the closest Van der Waals surfaces that are discussed above. Water mediated hydrogen bonds network further enhances stability of the complex that is required for the onset of the entire repair process.

4. Conclusions

The MD simulations of the 8-oxoguanine lesioned DNA segments (15 base pairs) single and in complex with specific repair enzyme human OGG1 are being discussed in this paper. It represents an attempt to theoretically describe the impact the oxidative DNA lesion 8-oxoG on the structural and energetic properties of B-DNA molecule, and aims to determine the changes originated by the lesion that may be important for recognition of this lesion by the repair enzyme. Simulations of single DNA molecules show that there are broken the hydrogen bonds and locally collapsed B-DNA structure at the lesion site. In addition the adenine 21 on the complementary strand (separated from 8-oxoG by 1 base pair) is flipped-out of the DNA double helix. Since simulations of native DNA molecule doesn't show any significant changes, the changes at 8-oxoG are caused by the presence of the lesion. The strong repulsive electrostatic interaction energy between the nucleotide with 8-oxoG and neighboring nucleotides suggests that the local electrostatic repulsion contributes to the instability of lesioned region and to the disruption of hydrogen bonds. In the case of simulation of 8-oxoG lesioned DNA complexed with hOGG1 it has been found that the lesioned DNA and the enzyme made Van der Waals molecular contacts at the three regions after 500 ps of MD simulation and maintained them until the end of simulation at 1 ns. Proximity of both molecules in the complex satisfying the Van der Waals interactions between the closest atoms established the conditions for the onset of chemical reactions of the entire repair process. In addition to the Van der Waals interactions there are several water molecules located between enzyme and DNA forming hydrogen bonds network that contributes to the stability of complex. In the system with native DNA, the DNA did not form any Van der Waals contacts with enzyme and number of water molecules located close to both solute molecules was significantly lower. The lack of Van der Waals contacts and non-existence of water mediated hydrogen bonds network between the native DNA and the enzyme suggest that the DNA-enzyme stabile complex is formed only for the

lesioned DNA molecule. Contrary to the simulation of single DNA, the flipped out adenine 21 was not observed for the complex. The possible explanation of this observation is that the presence of enzyme is a stabilizing factor for DNA structure. The achieved results shall serve as a template for further theoretical and experimental studies of other factors contributing to proper recognition of the oxidative base lesion and for the formation of complex. Among factors requiring further studies are for example electrostatic attractive interaction and dynamical process of formation of structural complementarity that enables insertion of damaged base into an enzyme pocket.

Acknowledgments

The author wishes thank to Mr. Toshiyuki Nemoto of The Research Organization for Information Science and Technology for the installation, maintenance and adjustment of the AMBER 5.0 code on supercomputer SR8000. The valuable support from the all members of The Radiation Risk Analysis Laboratory, JAERI Tokai Research Establishment is also highly acknowledged.

References

[1] J.F. Ward, Radiat. Res., 104 (1985) S103.

[2] Marie-Helen David-Cordonier, S. Boitex and P. O'Neill, Nucl. Acid Res. Vol. 29, No.5 (2001) 1107.

[3] S. Harrison and A. Aggarwal, Annu. Rev. Biochem. 59 (1990) 933.

[4] B. Gicquel-Sanzey and P. Cossart, EMBO J. 1 (1982) 591.

[5] J. Ham, A. Thompson, M. Nedham, P. Webb and M. Parker, Nucl. Acid Res. 16:12 (1988) 5263.

[6] M. Beato, Cell 56 (1989) 335.

[7] L. Harris, M. Sullivan and D. Hickok, Computers and Mathematics with Applications 20 (1990) 25.

[8] J. Marx, Science 229 (1985) 846.

[9] B. Matthews, Nature 335 (1988) 294.

[10] L. Harris, M. Sulliwan and D. Hickok, Proc. Natl. Acad. Sci. USA 90 (1993) 5534.

[11] M. Hollestein, B. Shomer, M. Greenblatt, T. Soussi, E. Hovig, R. Montesano, C.C. Harris, Nucl. Acid Res. 24 (1996) 141.

[12] T. K. Hazra, J. G. Muller, R. C. Manuel, C. J. Burrows, R. S. Lloyd and S. Mitra, Nucl. Acid Res. Vol. 29, No. 9 (2001) 1967.

[13] H. M. Nash, R. Lu, W. S. Lane and G. L. Verdine, Chem Biol. 4 (1997) 693.

[14] D. A. Case, D. A. Pearlman, J. W. Caldwell, T. E. Cheathman III, W. S. Ross, C. L. Simmerling, T. A. Darden, K. M. Merz, R. V. Stanton, A. L. Cheng, J. J. Vincent, M. Crowley, D. M. Ferguson, R. J. Radmer, G. L. Seibel, P. K. Weiner and P. A. Kollman, AMBER 5.0 (1997) University of California San Francisco.

[15] A. Wilkinson, P. Weiner, W. van Gunsteren, in F. Wilfred, Van Gunsteren, P. K. Weiner (Eds), Computer Simulation of Biomolecular Systems: Theoretical and Experimental Applications, Vol. 1, 1997.

[16] W. D. Cornell, P. Ciepelak, C. I. Bayly, I. R. Gould, K. M. Merz Jr, D. M. Ferguson, D. C. Spellmeyer, T. Fox, J. W. Caldwell and P. A. Kollman, J. Am. Chem. Soc. 117 (1995) 5179.

[17] M. D. Beachy, D. Chasman, R. B. Murphy, T. A. Halgren and R. A. Friesner, J. Am. Chem. Soc. 119 (1997) 5908.
[18] T. Darden, D. York and L. Pedersen, J. Chem. Phys., 98 (1993) 10089.
[19] Insight II 97.0, Molecular Simulations, Inc. 1977, San Diego, CA, USA.
[20] V. I. Poltev, S. L. Smirnov, O. V. Issarafutdinova and R. Lavery, J. Biomol. Struct. Dyn. 11(2) (1993) 293.
[21] S. D. Bruner, P. G. Derek, N. L. Verdine, and G. L. Verdine, Nature 403 (2000) 859.
[22] M. Pinak, J. Mol. Struct.: (Theochem) 466 (1999) 219.
[23] M. Pinak, Molecular Simulations 23 (2000) 307.
[24] DELPHI, Molecular Simulations, Inc., 2000.

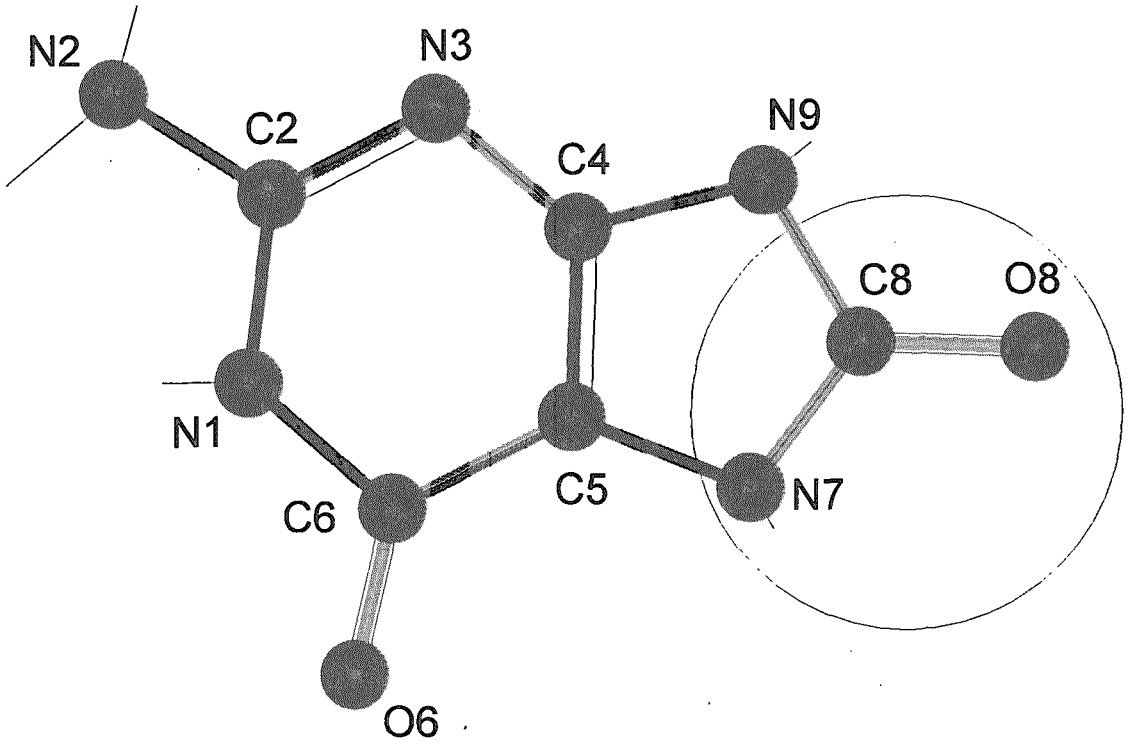

Fig. 1
The 8-oxoG molecule. The oxygen O8 is added at the C8 transforming the double bond C8-N7 into single one. The N7 atom is hybridized. The molecular parameters of the encircled atoms were changed and molecule was optimized as described in Materials and Methods.

This is a blank page.

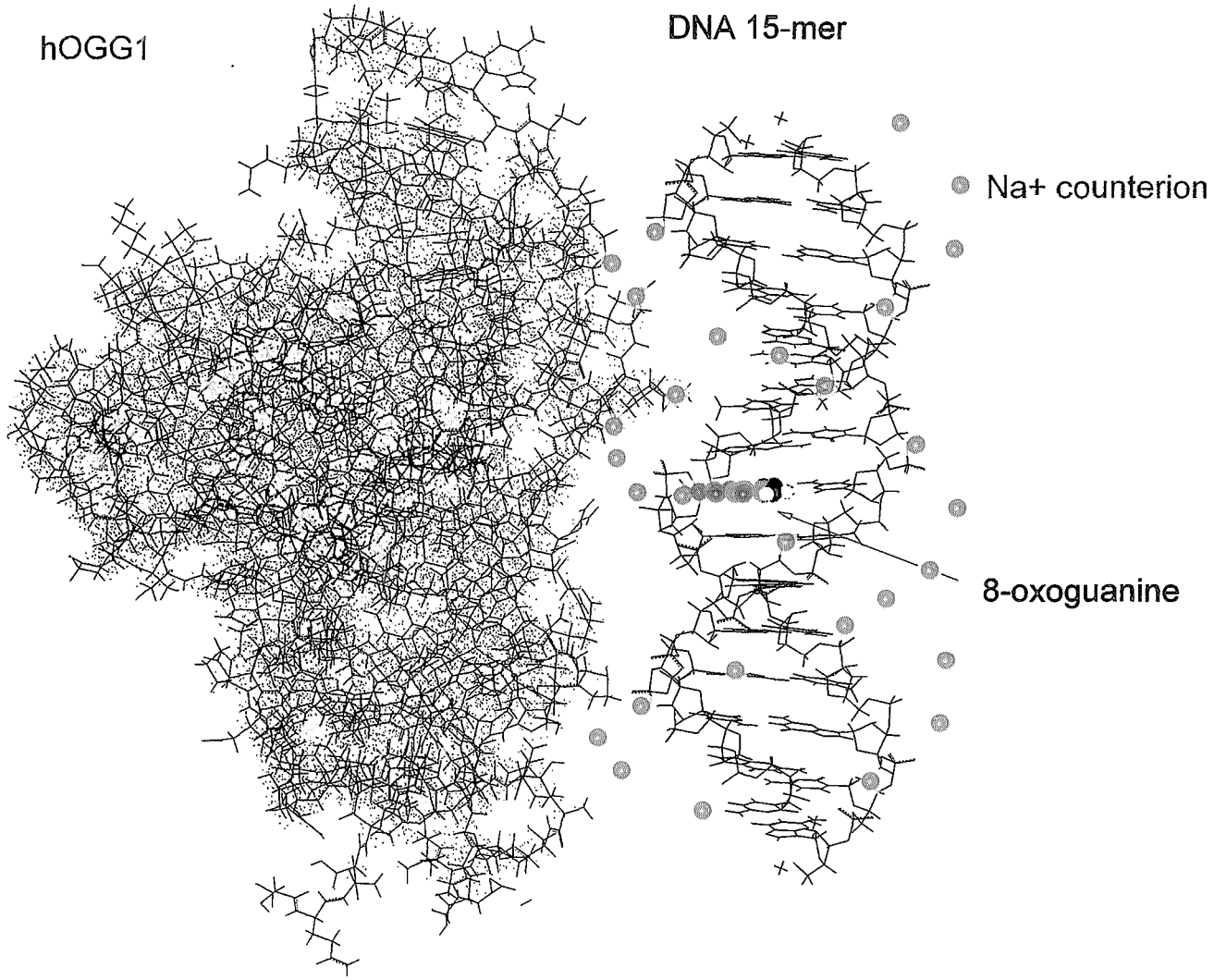

Fig. 2
The initial positions of enzyme and DNA. There is a minimal overlap of VDW surfaces of the DNA molecule and enzyme (distance between the closest atoms of enzyme and DNA is ~5 Å). Enzyme is represented in red including Connolly surface, DNA is shown in black except 8-oxoG atoms that are colored by atom type.

This is a blank page.

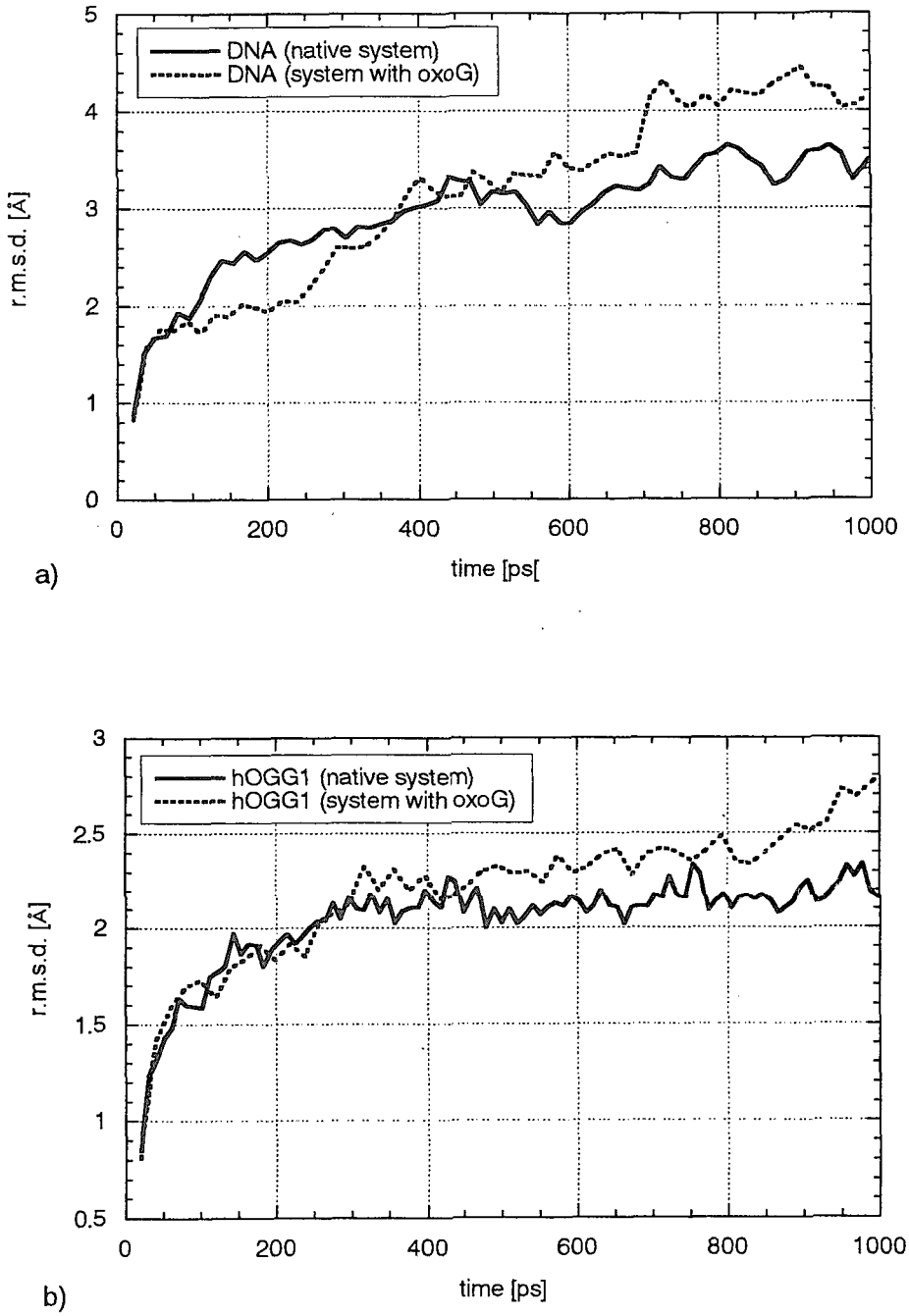

Fig. 3

R.m.s.d. of the solute molecules during the 1 ns of MD simulation. In the 8-oxoG lesioned DNA molecule the disruptions of weak hydrogen bonds between complementary bases close to lesion cause local instability that is reflected in higher r.m.s.d. values observed after 550 ps if compared with native DNA, a). In the case of the native DNA the B-DNA structure around native guanine 8 is well kept. The molecular structures of hOGG1 in both systems are well preserved in both systems, b).

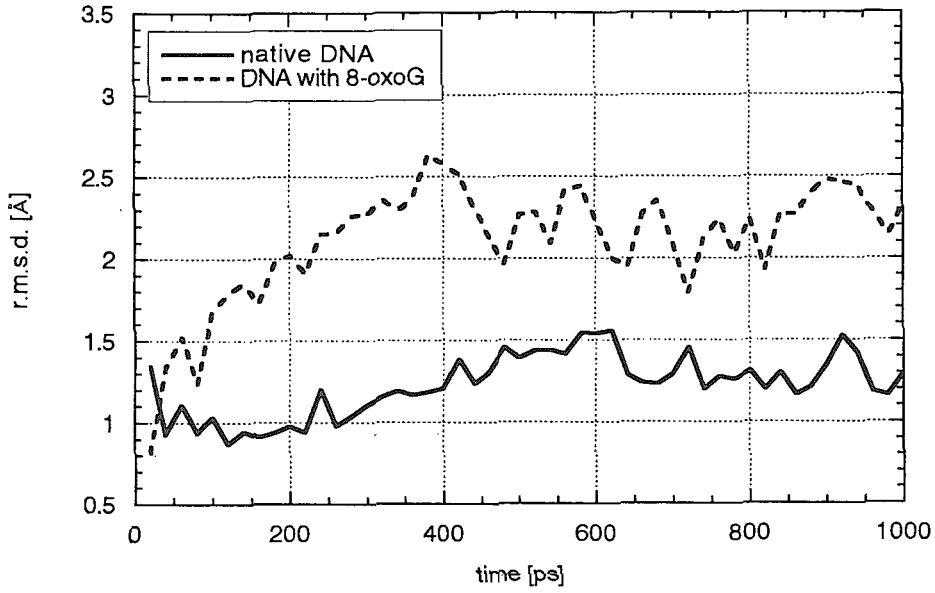

Fig. 4

R.m.s.d. of the atoms belonging to the nucleotide pairs located in the close vicinity of the lesion: cytosine 6, adenine 7, 8-oxoG (guanine 8), guanine 9, thymine 10, adenine 21, cytosine 22, cytosine 23, thymine 24 and guanine 25. The atoms around the lesion are less stable if compared with non-lesioned ones that is reflected in the higher values of r.m.s.d.

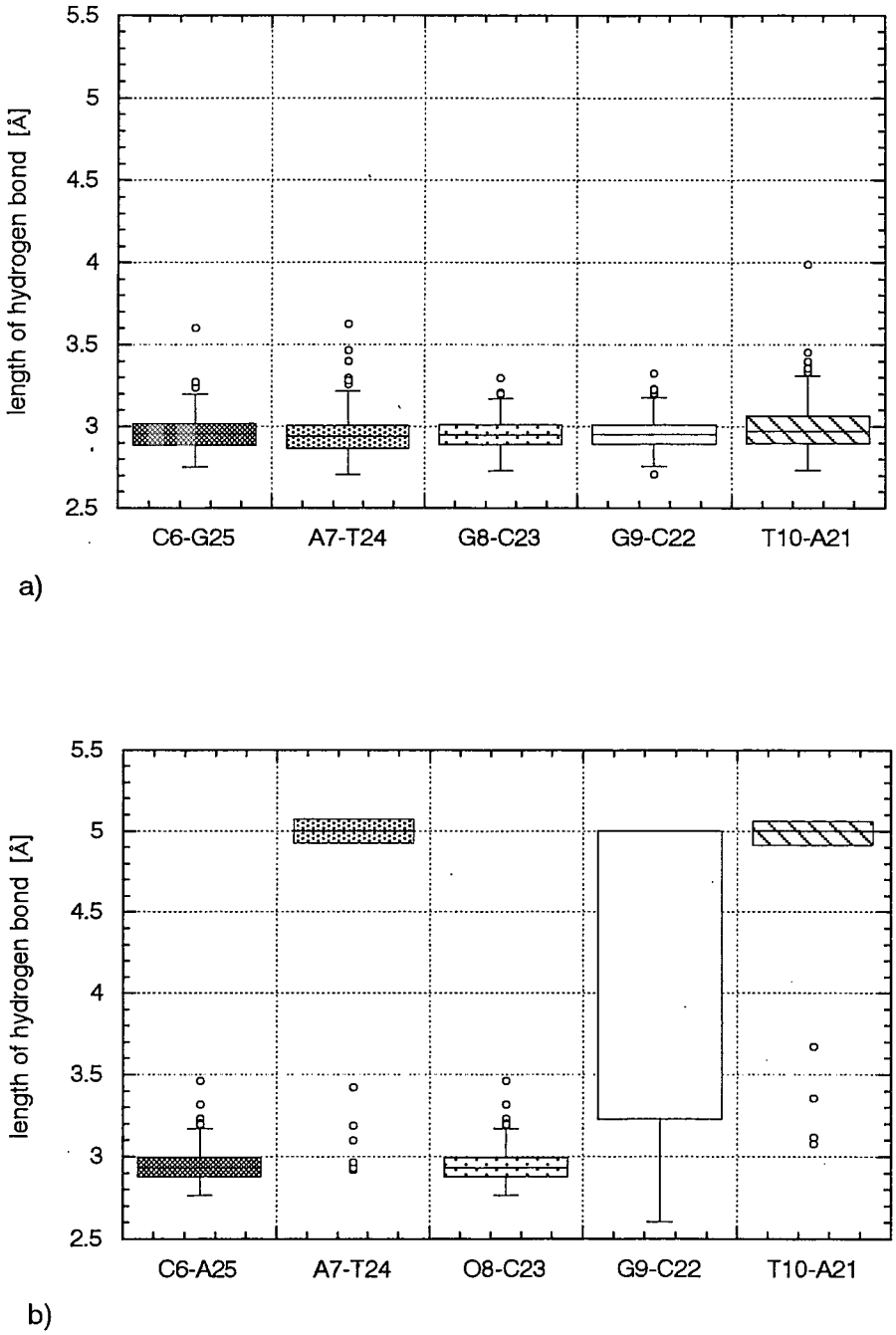

Fig. 5

The length of hydrogen bonds between complementary bases in the vicinity of native guanine, a) and in the vicinity of the lesion, b). Hydrogen bonds between 8-oxoG and opposite cytosine 23 are well kept, the neighboring base pairs (adenine 7 – thymine 24, and guanine 9 – cytosine 22) are broken, b). The length longer than 3.5 Å indicates non-existence of the hydrogen bond (lengths of hydrogen bonds in B-DNA are: -N-H...O ~ 2.84 Å; -N-H...N ~ 2.92 Å). The hydrogen bonds of the native DNA are well preserved, a). The bars show the average values calculated over the 2 ns of MD simulation. (A - adenine, G - guanine, C - cytosine, T - thymine, O - 8-oxoG; numbers refer to the sequentional position of nucleotide on the DNA).

This is a blank page.

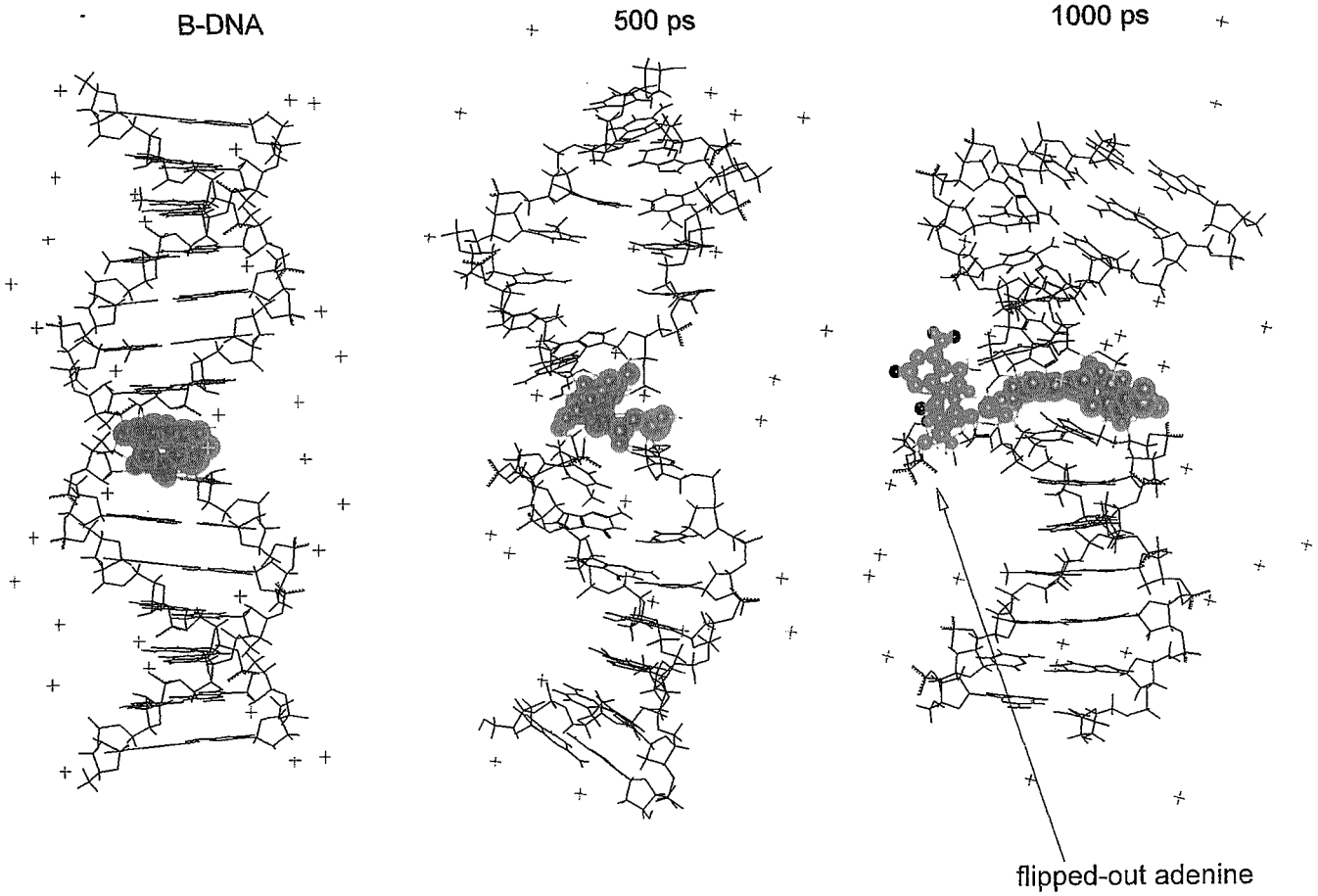

Fig. 6

The snapshots of DNA with 8-oxoG during the MD simulation. The flipped-out adenine 21 is seen at 1 ns. The large distortion and disruption of hydrogen bonds between complementary bases at the lesion site is already seen at the 500 ps of MD simulation. The nucleotide with 8-oxoG is shown in blue and the atoms of the flipped-out adenine 21 are red. The scattered green atoms around DNA are Na^+ counterions.

This is a blank page.

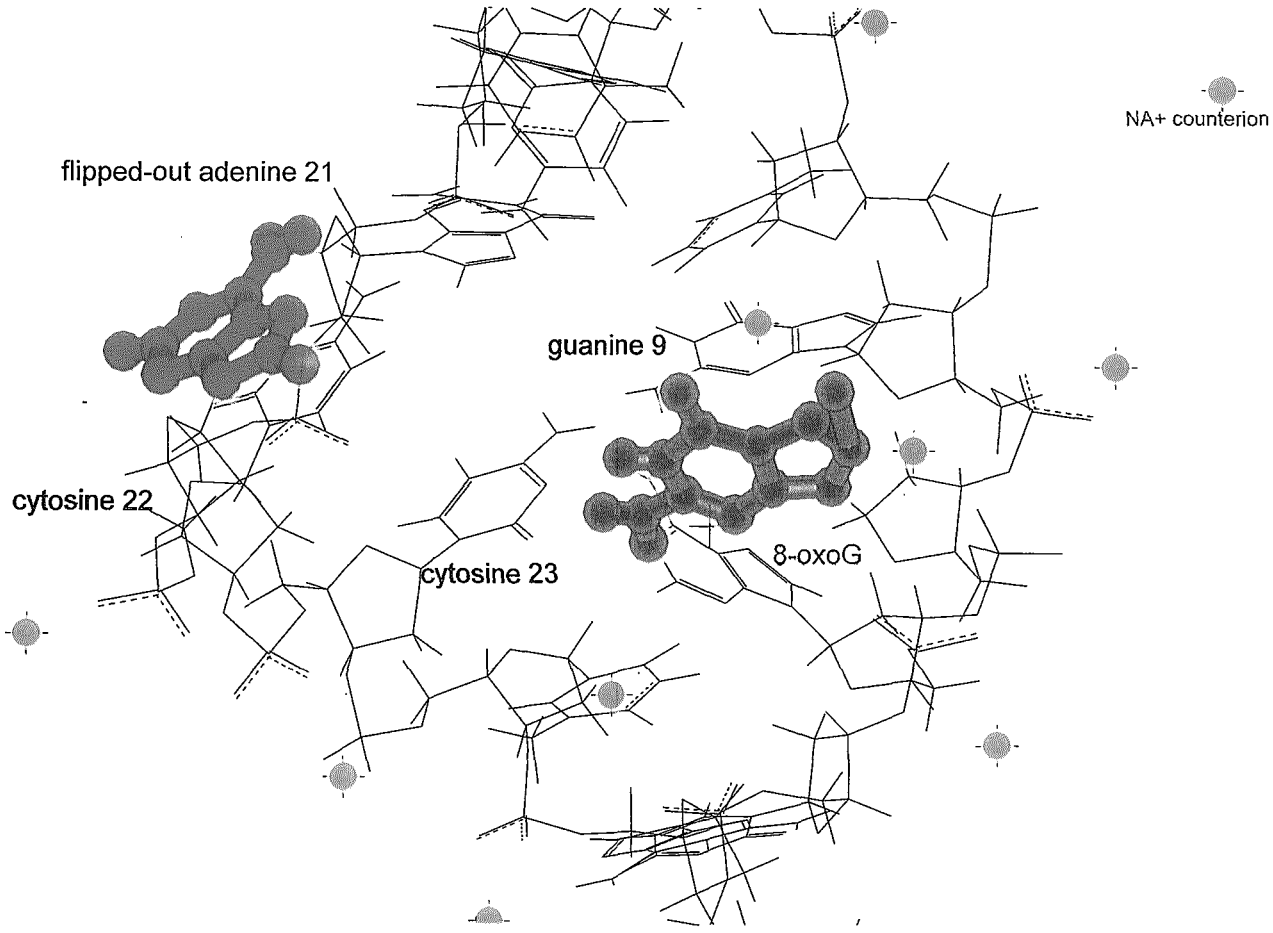

Fig. 7

Flipped-out adenine 21 (red) on the complementary strand to strand with 8-oxoG (blue). The figure also indicates non-existence of hydrogen bonds between guanine 9 and cytosine 22 since the cytosine 22 is also severely dislocated form its intrahelical position. The figure shows situation at 1 ns of MD simulation. The green atoms are Na^+ counterions.

This is a blank page.

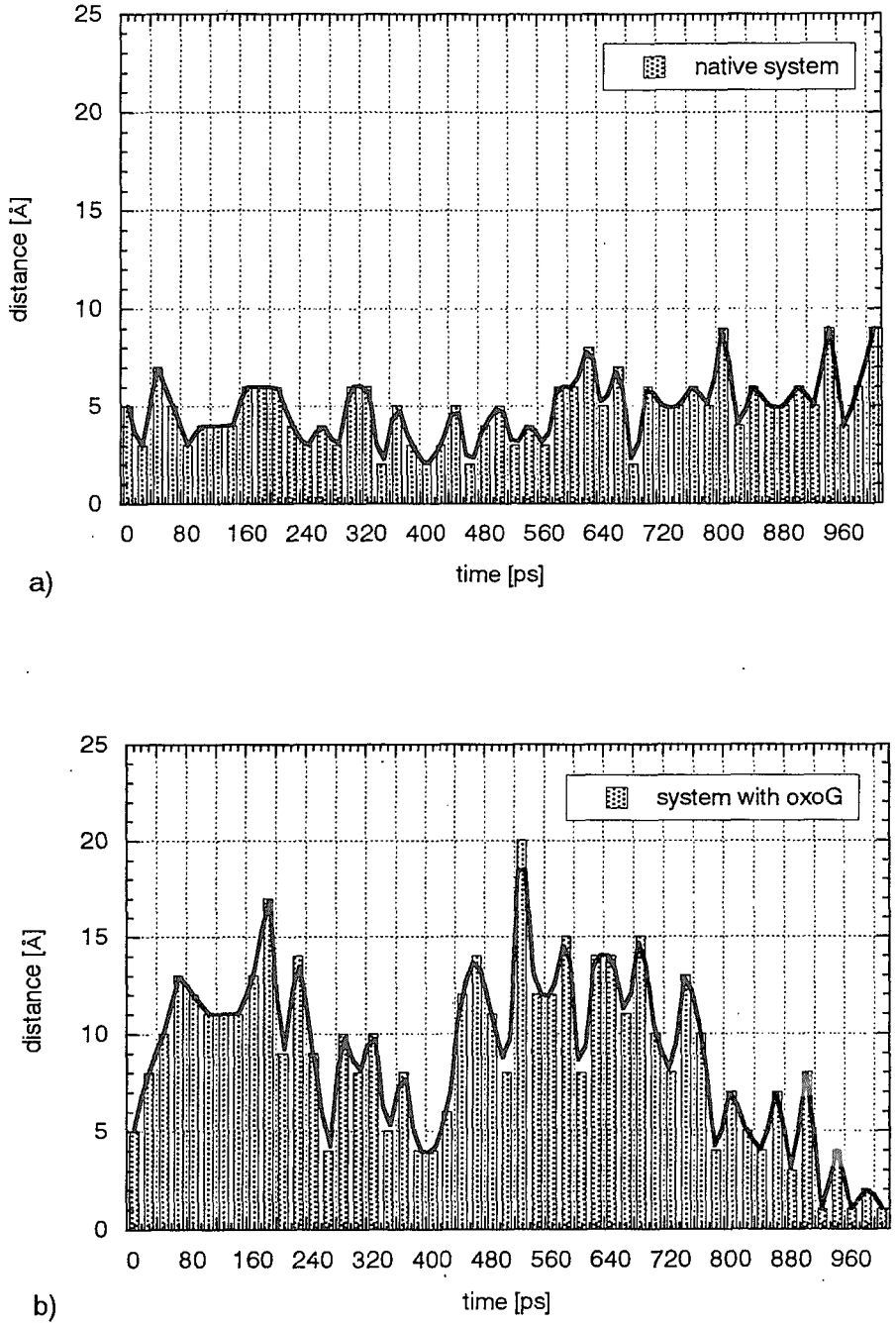

Fig. 8

Minimal distances between heavy atoms of DNA and enzyme. It is seen that native DNA and enzyme were keeping its minimal distance oscillating around 5 Å that was the distance at the beginning of the simulation, a). In the case of simulation system with lesioned DNA molecule, b) both molecules distanced from each other (~15 Å), approached around 400 ps, and finally formed a close contact after nearly 900 ps of MD (~2 Å).

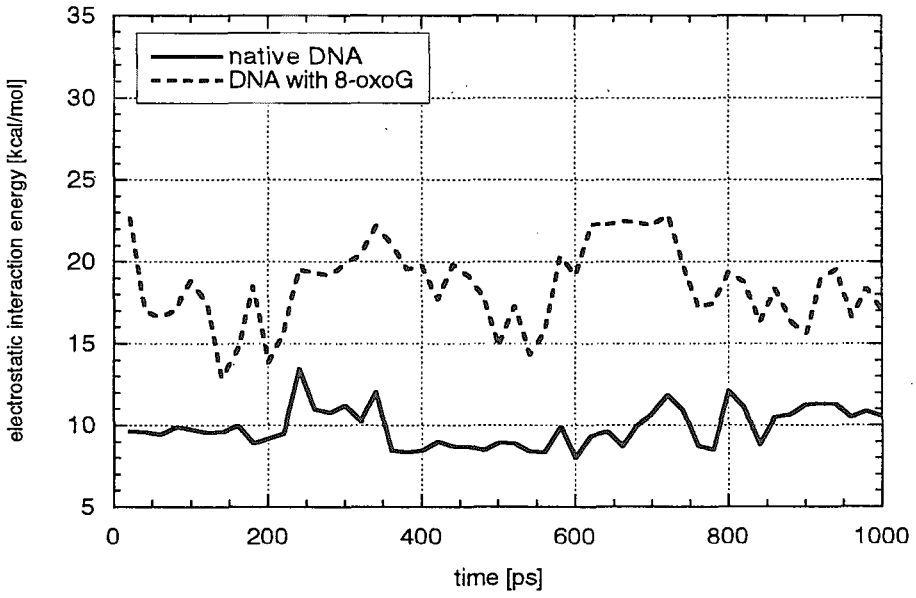

Fig. 9

The intermolecular electrostatic interaction between the nucleotide with 8-oxoG and the neighboring nucleotides with respective bases (cytosine 6, adenine 7, guanine 9, thymine 10, adenine 21, cytosine 22, cytosine 23, thymine 24 and guanine 25). The electrostatic interaction energy in lesioned DNA largely oscillated around an average value of around 21 kcal/mol. The electrostatic interaction energy calculated for the nucleotide with the native guanine 8 has a stable average value of 7.5 ± 1.4 kcal/mol.

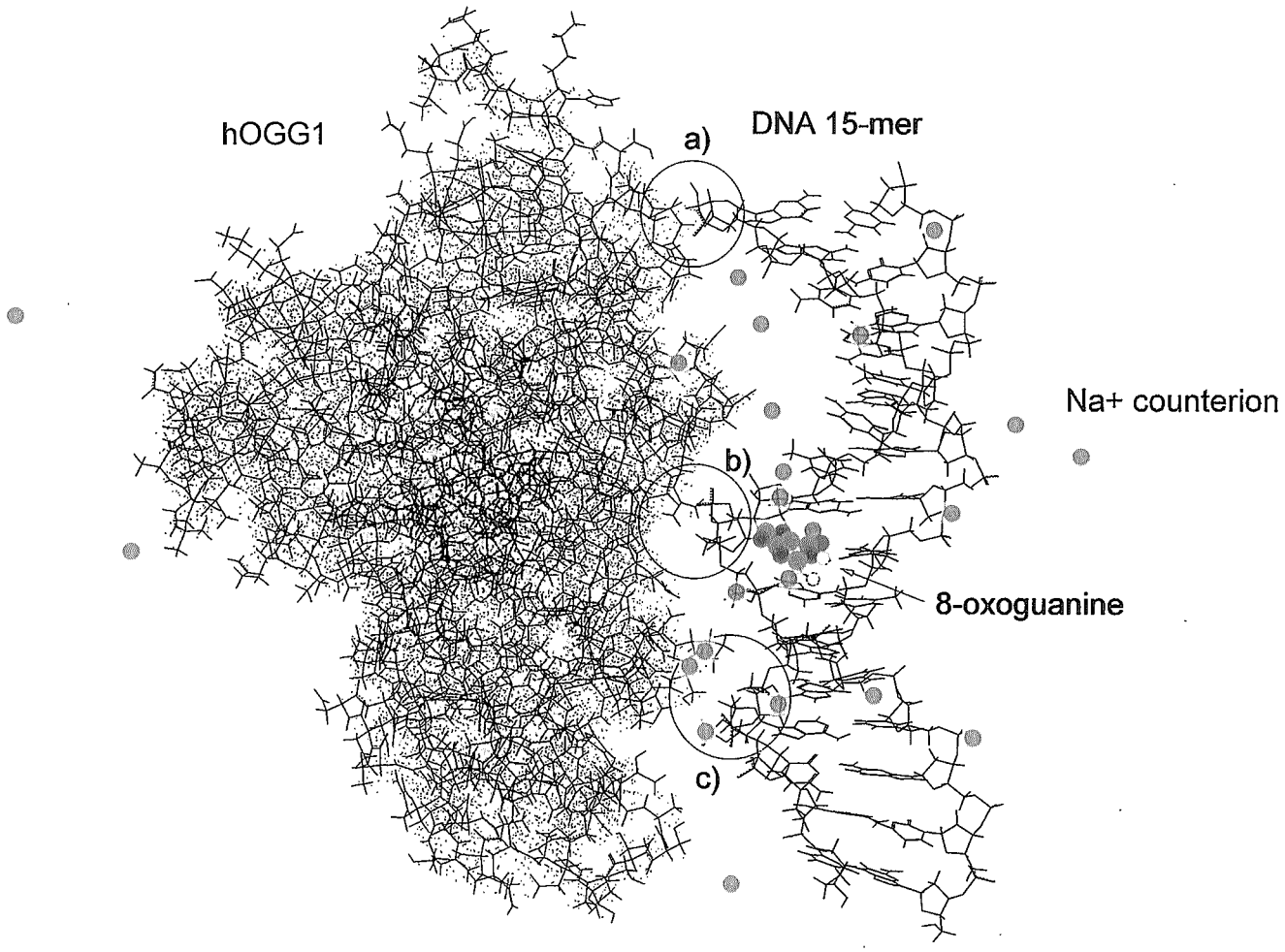

Fig. 10

The molecular configuration of the lesioned DNA and the enzyme at 1 ns of MD simulation. The enzyme and the DNA form a close Van der Waals contacts between several atoms in three regions (encircled):

- a) arginine 266 - guanine G5;
- b) arginine 313 - guanine, 8-oxoG; adenine;
- c) serine 43, glutamine 42 - adenine, guanine.

This is a blank page.

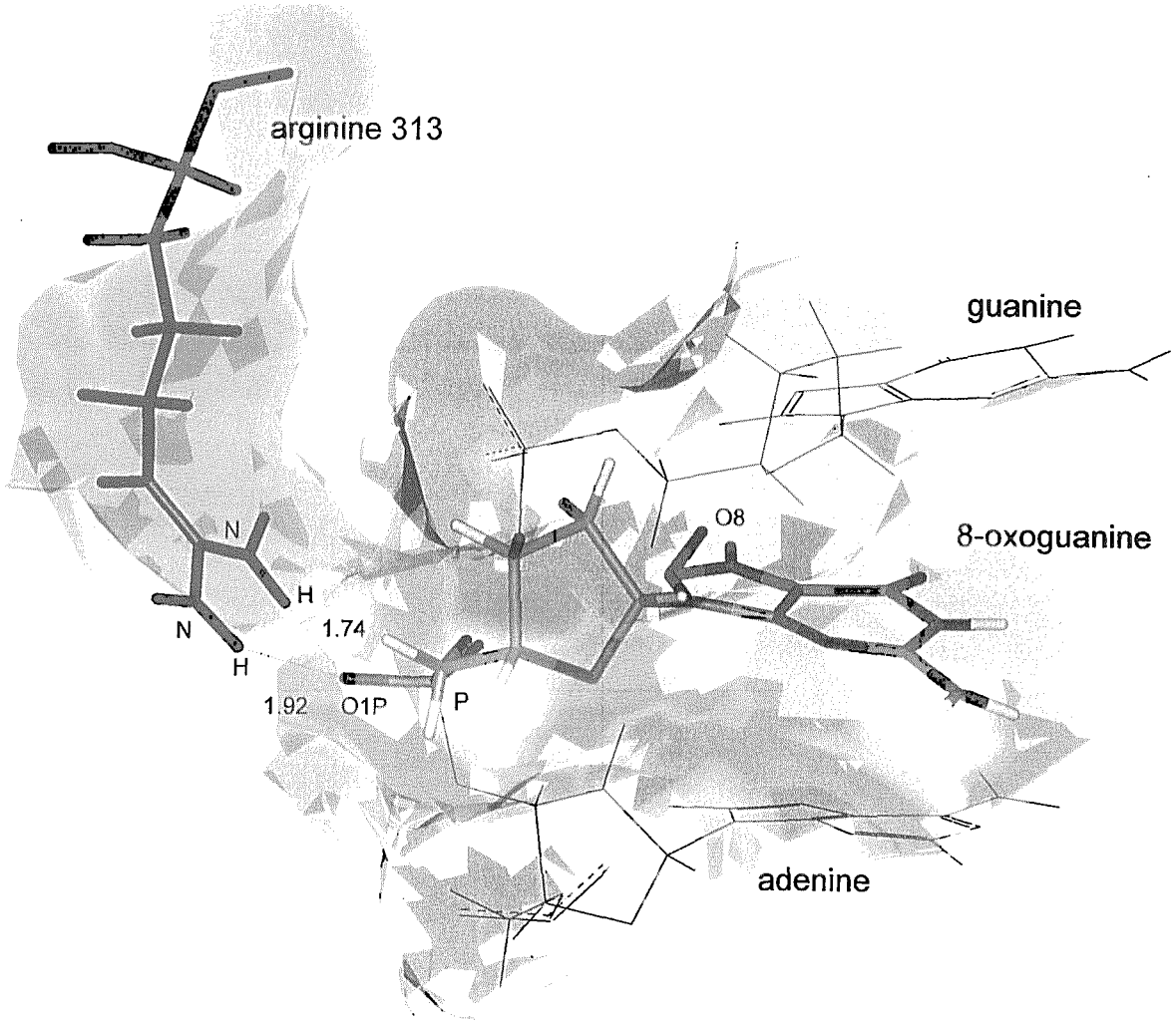

Fig. 11

Detail view in the contact site between arginine 313 and nucleotide with 8-oxoG displaying Van der Waals surfaces. N terminus of arginine 313 is located close to C5' atom of 8-oxoG, forming 2 hydrogen bonds between O1P (8-oxoG) and N atoms (arginine 313). The attractive electrostatic interaction between $^{+}H_2N$ and P contributes to the stability of the complex.

This is a blank page.

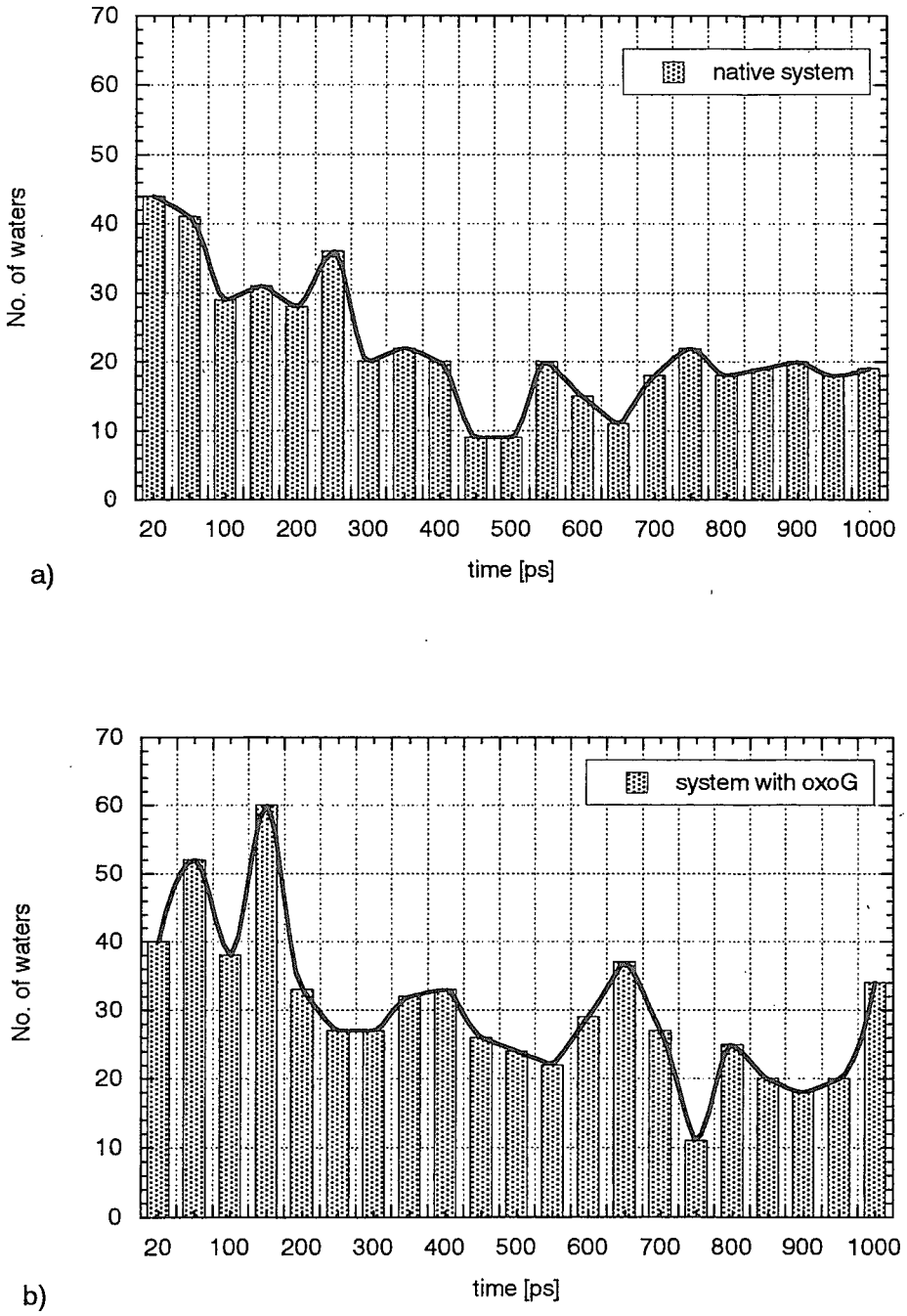

Fig. 12
The average number of water oxygens located within distance of 3 Å from the atoms of both solute molecules - DNA and enzyme. In the case of system with lesioned DNA, b) there are more water molecules that are close to both solute molecules if compared with system with native DNA, a). The presence of water molecules indicates the existence of water mediated hydrogen bonds between the lesioned DNA and the enzyme.

This is a blank page.

国際単位系 (SI) と換算表

表1　SI基本単位および補助単位

量	名称	記号
長さ	メートル	m
質量	キログラム	kg
時間	秒	s
電流	アンペア	A
熱力学温度	ケルビン	K
物質量	モル	mol
光度	カンデラ	cd
平面角	ラジアン	rad
立体角	ステラジアン	sr

表2　SIと併用される単位

名称	記号
分，時，日	min，h，d
度，分，秒	°，′，″
リットル	l，L
トン	t
電子ボルト	eV
原子質量単位	u

1 eV=1.60218×10^{-19} J

1 u=1.66054×10^{-27} kg

表3　固有の名称をもつSI組立単位

量	名称	記号	他のSI単位による表現
周波数	ヘルツ	Hz	s^{-1}
力	ニュートン	N	m·kg/s^2
圧力，応力	パスカル	Pa	N/m^2
エネルギー，仕事，熱量	ジュール	J	N·m
工率，放射束	ワット	W	J/s
電気量，電荷	クーロン	C	A·s
電位，電圧，起電力	ボルト	V	W/A
静電容量	ファラド	F	C/V
電気抵抗	オーム	Ω	V/A
コンダクタンス	ジーメンス	S	A/V
磁束	ウェーバ	Wb	V·s
磁束密度	テスラ	T	Wb/m^2
インダクタンス	ヘンリー	H	Wb/A
セルシウス温度	セルシウス度	℃	
光束	ルーメン	lm	cd·sr
照度	ルクス	lx	lm/m^2
放射能	ベクレル	Bq	s^{-1}
吸収線量	グレイ	Gy	J/kg
線量当量	シーベルト	Sv	J/kg

表4　SIと共に暫定的に維持される単位

名称	記号
オングストローム	Å
バーン	b
バール	bar
ガル	Gal
キュリー	Ci
レントゲン	R
ラド	rad
レム	rem

1 Å=0.1 nm=10^{-10} m

1 b=100 fm^2=10^{-28} m^2

1 bar=0.1 MPa=10^5 Pa

1 Gal=1 cm/s^2=10^{-2} m/s^2

1 Ci=3.7×10^{10} Bq

1 R=2.58×10^{-4} C/kg

1 rad=1 cGy=10^{-2} Gy

1 rem=1 cSv=10^{-2} Sv

表5　SI接頭語

倍数	接頭語	記号
10^{18}	エクサ	E
10^{15}	ペタ	P
10^{12}	テラ	T
10^{9}	ギガ	G
10^{6}	メガ	M
10^{3}	キロ	k
10^{2}	ヘクト	h
10^{1}	デカ	da
10^{-1}	デシ	d
10^{-2}	センチ	c
10^{-3}	ミリ	m
10^{-6}	マイクロ	μ
10^{-9}	ナノ	n
10^{-12}	ピコ	p
10^{-15}	フェムト	f
10^{-18}	アト	a

（注）

1. 表1－5は「国際単位系」第5版，国際度量衡局 1985年刊行による。ただし，1 eVおよび1 uの値はCODATAの1986年推奨値によった。
2. 表4には海里，ノット，アール，ヘクタールも含まれているが日常の単位なのでここでは省略した。
3. barは，JISでは流体の圧力を表わす場合に限り表2のカテゴリーに分類されている。
4. EC閣僚理事会指令ではbar，barnおよび「血圧の単位」mmHgを表2のカテゴリーに入れている。

換算表

力	N(=10^5 dyn)	kgf	lbf
	1	0.101972	0.224809
	9.80665	1	2.20462
	4.44822	0.453592	1

粘度　1 Pa·s(N·s/m^2)=10 P(ポアズ)(g/(cm·s))

動粘度　1 m^2/s=10^4 St(ストークス)(cm^2/s)

圧力	MPa(=10 bar)	kgf/cm^2	atm	mmHg(Torr)	lbf/in^2(psi)
	1	10.1972	9.86923	7.50062×10^3	145.038
	0.0980665	1	0.967841	735.559	14.2233
	0.101325	1.03323	1	760	14.6959
	1.33322×10^{-4}	1.35951×10^{-3}	1.31579×10^{-3}	1	1.93368×10^{-2}
	6.89476×10^{-3}	7.03070×10^{-2}	6.80460×10^{-2}	51.7149	1

エネルギー・仕事・熱量	J(=10^7 erg)	kgf·m	kW·h	cal(計量法)	Btu	ft·lbf	eV
	1	0.101972	2.77778×10^{-7}	0.238889	9.47813×10^{-4}	0.737562	6.24150×10^{18}
	9.80665	1	2.72407×10^{-6}	2.34270	9.29487×10^{-3}	7.23301	6.12082×10^{19}
	3.6×10^6	3.67098×10^5	1	8.59999×10^5	3412.13	2.65522×10^6	2.24694×10^{25}
	4.18605	0.426858	1.16279×10^{-6}	1	3.96759×10^{-3}	3.08747	2.61272×10^{19}
	1055.06	107.586	2.93072×10^{-4}	252.042	1	778.172	6.58515×10^{21}
	1.35582	0.138255	3.76616×10^{-7}	0.323890	1.28506×10^{-3}	1	8.46233×10^{18}
	1.60218×10^{-19}	1.63377×10^{-20}	4.45050×10^{-26}	3.82743×10^{-20}	1.51857×10^{-22}	1.18171×10^{-19}	1

1 cal = 4.18605 J（計量法）
= 4.184 J （熱化学）
= 4.1855 J （15 °C）
= 4.1868 J（国際蒸気表）

仕事率　1 PS（仏馬力）
= 75 kgf·m/s
= 735.499 W

放射能	Bq	Ci
	1	2.70270×10^{-11}
	3.7×10^{10}	1

吸収線量	Gy	rad
	1	100
	0.01	1

照射線量	C/kg	R
	1	3876
	2.58×10^{-4}	1

線量当量	Sv	rem
	1	100
	0.01	1

（86年12月26日現在）

Molecular Dynamics Simulation of 8-oxoguanine Lesioned DNA Complexed with Repair Enzyme hOGG1