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MOLECULAR DYNAMICS SIMULATIONS OF DEOXYRIBONUCLEIC ACIDS AND REPAIR ENZYME T4 ENDONUCLEASE V.

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Molecular Dynamics Simulations of Deoxyribonucleic Acids and Repair Enzyme T4 Endonuclease V.

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This report describes the results of molecular dynamics (MD) simulation of deoxyribonucleic acids (DNA) and specific repair enzyme T4 endonuclease V. Namely research described here is focused on the examination of specific recognition process, in which this repair enzyme recognizes the damaged site on the DNA molecule-thymine dimer (TD). TD is frequent DNA damage induced by UV radiation in sun light and unless properly repaired it may be mutagenic or lethal for cell, and is also considered among the major causes of skin cancer. T4 endonuclease V is a DNA specific repair enzyme from bacteriophage T4 that catalyzes the first reaction step of TD repair pathway. MD simulations of three molecules - native DNA dodecamer (12 base pairs), DNA of the same sequence of nucleotides as native one but with TD, and repair enzyme T4 endonuclease V - were performed for 1 ns individually for each molecule. Simulations were analyzed to determine the role of electrostatic interaction in the recognition process. It is found that electrostatic energies calculated for amino acids of the enzyme have positive values of around +15 kcal/mol. The electrostatic energy of TD site has negative value of approximately-9 kcal/mol, different from the nearly neutral value of the respective thymines site of the native DNA. The electrostatic interaction of TD site with surrounding water environment differs from the electrostatic interaction of other nucleotides. Differences found between TD site and respective thymines site of native DNA indicate that the electrostatic energy is an important factor contributing to proper recognition of TD site during scanning process in which enzyme scans the DNA. In addition to the electrostatic energy, the important factor in recognition process might be structural complementarity of enzyme and bent DNA with TD. There is significant kink formed around TD site, that is not observed in native DNA.

Keywords: Molecular Dynamics, T4 Endonuclease V, Recognition Process, Electrostatic Energy

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デオキシリボ核酸と修復酵素Ｔ４エンドヌクレアーゼＶの分子動力学シミュレーション

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本レポートは、デオキシリボ核酸（ＤＮＡ）と修復酵素Ｔ４エンドヌクレアーゼＶの分子動力学シミュレーションの結果を報告する。ここで述べるのは、ＤＮＡ損傷のひとつであるチミンダイマーをＤＮＡ修復酵素が認識する過程に着目した研究である。チミンダイマーは、太陽光にも含まれる紫外線によって頻繁に生じるＤＮＡ損傷である。これが修復されなければ、細胞の突然変異や致死を招き、皮膚癌の原因にもなると考えられている。Ｔ４エンドヌクレアーゼＶは、バクテリオファージＴ４由来のＤＮＡ修復酵素であり、チミンダイマーの修復過程における最初の反応を担う。分子動力学シミュレーションは、損傷のない12塩基対のＤＮＡ、損傷のない場合と塩基配列は同じだがチミンダイマーのあるＤＮＡ、修復酵素Ｔ４エンドヌクレアーゼＶ、の３つの分子についてそれぞれ１ナノ秒（ns）ずつ行った。これらのシミュレーション結果は、認識過程における静電作用の役割を調べるために解析された。この結果、酵素のアミノ酸の静電エネルギーは、約＋15 kcal/molの正の値であることがわかった。チミンダイマーの部分の静電エネルギーは約－９kcal/molであり、損傷のないＤＮＡのチミンの部分での静電エネルギーがほぼ中性を示したのとは異なった。この違いは、静電エネルギーが、修復酵素がＤＮＡをスキャンする過程において正確にチミンダイマーを認識するための重要な因子であることを示唆している。さらに、酵素とチミンダイマーのあるＤＮＡの構造的な相補性も、認識過程の重要な因子に加えられるだろう。チミンダイマーのあるＤＮＡには、チミンダイマーの部分ではっきりとした歪みが見られたが、これは損傷のないＤＮＡには見られなかったものである。

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1. Introduction

The continuity of life depends upon the successful repair of damages caused by environmental radiation to deoxyribonucleic acid (DNA) /1/. This radiation, ranging from cosmic rays to ultraviolet light (UV), could lead to variety of damages in DNA. Several types of damages may prevent accurate replication or transcription during cell proliferation and can potentially be mutagenic or lethal for the cell. The major damage produced by UV in DNA are cyclobuthane pyrimidine photodimers /2,3/. Many studies indicate, that the photolesion - thymine dimer (TD) - induced in DNA by UV in sunlight are among major cases of skin cancer /4/. Thymine dimer is formed as a covalently bonded complex of two adjacent thymines on the single strand of DNA. This damage is very frequent but almost 90% of TDs are repaired within short time, order of minutes, and only few are experimentally observable and originate future changes on cell level.

In the study of biochemical defense against UV induced DNA damages the resistance of bacteriophage T4 suggested that a specific molecular mechanism might be responsible for this property. The observed resistance was attributed to the specific repair enzyme - T4 endonuclease V. T4 endonuclease V is a DNA repair enzyme from bacteriophage T4 that catalyzes the first reaction step of TD excision repair pathway. This repair pathway consists of the following steps:

1. binding to DNA molecule non-specifically;
2. scanning the DNA surface;

3. recognizing of TD damaged site on DNA strand;
4. and finally scission of glycosyl bond at C5' side and incision of phosphodiester bond at C3' position of TD (Fig.1).

This way the damaged part of DNA is removed from the strand and resulting gap is filled by repair synthesis.

Recently, a high resolution X-ray crystal structure of the repair enzyme T4 endonuclease V has been reported /5/. The surface of the enzyme is concave which may be an essential structural feature in order to form a close contact with the bent structure of DNA near TD. The concave area is populated by monthly positively charged amino acids which indicate that the electrostatic interaction between enzyme surface and DNA may play a significant role in either non-specific or specific binding. Among these amino acids there is Glutamin 23 (Glu23), which has been shown in mutagenic studies as playing an important role in the repair enzymatic activity /6/. The speed of the recognition and entire repair processes, and small size of molecules make it difficult to study them by experimental techniques. On the other hand the computational simulations are useful tools to get detail insight into this important process at the level of cell nucleus. Reported X-ray crystal structure of the enzyme enables molecular dynamics study of the recognition process by which enzyme recognizes the TD damaged site on DNA. The significance of structural features of enzyme as well DNA molecule, in addition to the role of electrostatic energy is discussed in this paper.

2. Materials and Methods

2.1 Molecular Dynamics Simulation

Molecular Dynamics (MD) simulation is a simulation technique that yields static and dynamic properties of a molecular system. The behavior of a system is simulated by solving Newton's equation of motion for each atom in the system. Solving of these equations produces new atomic coordinates, which can be used to calculate a new set of forces. This procedure is repeated many times and a trajectory of each atom is obtained that describes in detail the behavior of the system as a function of time. In solving Newton's equations the knowledge of acting forces is required. These forces are obtained as the derivatives of potential energy, which describes also interactions among atoms. It consists of several terms as hydrogen bonding, electrostatic interactions, energetic cost of structural changes, etc.

In order to perform MD simulations, a simulated system is created as consisting of a solute molecule (e.g. DNA, enzyme, etc.) immersed in a few thousands water molecules ensuring proper solvation of the solute molecule. All atoms are placed in a simulation cell and periodic boundary conditions are applied in most cases to eliminate undesirable surface effects. In this approach a three-dimensional array of cell is formed replicating the original simulation cell, forming an infinite structure whose single unit is a simulated system.

2.2 Computational Details

MD simulations of the three systems were performed for 1 ns. The following molecules were the solute molecules of the respective system:

1. native DNA dodecamer of the sequence $d(TCGCGTTGCGCT)_2$;
2. DNA dodecamer with TD; the central TT (T6 and T7) part of native dodecamer was replaced by the TD; DNA sequence $d(TCGCG'TD'GCGCT)_2$;
3. repair enzyme T4 endonuclease V.

The MD software AMBER 5.0 /7/ was used for simulations and for calculation of electrostatic force fields. Each system was treated individually and independently from each other, and the same simulation protocol was used in each simulation. The initial structure of the enzyme was that of the crystal structure and the crystallographic coordinates were taken from the Nucleic Acid Database Project /8/. No crystal water molecules were included in further MD simulation and an extensive equilibration period was performed to ensure the stability of the enzyme molecule. The initial structure of native DNA dodecamer $d(TCGCGTTGCGCT)_2$ were used as an idealized B-DNA molecule generated by program NUCGEN (part of AMBER 5.0 program package). For the simulation of lesioned DNA, the two thymines at the positions 6 and 7 were replaced by cis.sin cyclobuthane thymine dimer.

Formation of thymine dimer

TD was formed in the process of preliminary minimization of the hydrated dodecamer. In the first step of minimization the positions of surrounding water molecules were optimized with atoms of DNA fixed. This first step was followed by the 2^{nd} and 3^{rd} minimization. During 2^{nd} minimization

the atom-atom distance restraints between C(5)-C(5) and C(6)-C(6) atoms of thymines at positions 6 and 7 (T6 and T7) were applied as 3 and 2 Å respectively. These restraint were removed at the final 3rd stage of minimization and the covalent bonds C(5)-C(5) and C(6)-C(6) were formed creating TD molecule.

Molecular Dynamics Protocol

The preparation of systems for MD simulations consisted of several steps:

1. Solvating the solute molecules, i.e. immersing the respective solute molecules into the water box consisting of several thousands water molecules. The final systems have around 20,000 atoms 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 the P-O-P angle at the distance of 5 Å from the phosphorus atom. In the following 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 not at 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 (bending, repulsion, formation of hydrogen bonds, etc.) during the following MD process. The potential energy of each system was minimized in two parts. In the first part the geometry of the solute was kept frozen and only surrounding water molecules were

allowed to move. When water molecules arranged itself around the solute, the second minimization was performed, that included all atoms.

4. Heating up to 300 K; the temperature upon the formation of the system is 0 K, i.e. the atoms have no velocities. In the beginning of the heating the velocities were assigned from the Maxwell-Boltzman distribution at 30 K. The heating was performed in 10 subsequent 30 K steps, avoiding large temperature gradient that might result in breakage of some bonds. Each 30 K step represented 1 ps of MD simulation.
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 system box. In this step the box sizes were released and the density of system stabilized at the saturated level during 10 ps of MD simulation. The final box sizes were then fixed and the density was kept at the constant value during the entire MD simulation.

After these preparatory steps the MD simulations of each system were performed for 1 ns. All simulations were performed on AP 3000 parallel supercomputer and some preparatory steps were performed on SGI Silicon Graphics computer. One ps of MD required around 3 hours of CPU time on AP 3000.

Force Field and Calculation of Electrostatic Energy

Atomic charges, van der Waals, hydrogen bonds, angles and other atomic parameters were taken from AMBER 5.0 all-atom force field /7/. The atomic charges of TD were taken as those calculated by Miaskiewicz at al. /9/.

Potential energy function in AMBER 5.0 is calculated as contribution from bond, single angle, torsional, improper dihedral, van der Waals, hydrogen bonding, electrostatic and constraint functions:

$$E_{potential} = \sum_{bonds} K_r(r - r_{eq})^2 + \sum_{angles} K_\theta(\theta - \theta_{eq})^2 + \sum_{dihedrals} \sum_{\eta} \frac{V_\eta}{2}[1 + \cos(\eta\phi - \gamma)] + \frac{1}{VDW_{scale}} \sum_{j=1}^{atoms} \sum_{i>j}^{atoms} \varepsilon_{ij}^{*}[(\frac{R_{ij}^{*}}{r_{ij}})^{12} - (\frac{R_{ij}^{*}}{r_{ij}})^{6}] + \sum_{j=1}^{Hbonds} \sum_{i>j}^{Hbonds} (\frac{C_{ij}}{r_{ij}^{12}} - \frac{D_{ij}}{r_{ij}^{10}}) + \frac{1}{EEL_{scale}} \sum_{j=1}^{atoms} \sum_{i>j}^{atoms} \frac{q_i q_j}{\varepsilon r_{ij}} + \sum_{constr.} K_{constr}(x - x_o)^2 + \sum_{cap\ atoms} K_{cap}(y - y_o)^2 \quad \text{Eq. 1}$$

Here r, θ and ϕ and are bond lengths, planar angles, and dihedral angles, respectively; r_{eq}, and θ_{eq} are their equilibrium values; η is periodicity of dihedral potential function and γ is the phase; r_{ij} is the distance between atoms i and j; q_i is the partial charge on atom i ; ε_{ij} is dielectric constant; K_{constr}, K_{cap}, K_θ, V_η, R_{ij}, C_{ij} and D_{ij} are empirical parameters depending on atom types. The point charges q_i and q_j in calculation of electrostatic function (sixth term in Eq. 1) are centered an each atom and are derived by fitting to a quantum mechanical electrostatic potential /10/. In our MD simulation the constant dielectric function was used, 1-4 electrostatic interactions were scaled by factor 1.2 (recommended value for Amber 5.0 force field) and Particle Mesh Ewald Sum

technique was used as implemented in Amber 5.0 /7, 11/.

Using Ewald Sum method the electrostatic energy was calculated for infinite volume of repeating units through periodic boundary conditions. Ewald sum method is computational technique for calculating long range electrostatic interactions which are often neglected while using a finite non-bonded cutoff in MD simulations. In the Ewald sum method, a Gaussian charge distribution of opposite sign is superimposed upon the original point charges, producing a screened charge distribution. The electrostatic interaction between the screened charges are short ranged. The original distribution is recovered by adding a second Gaussian charge distribution identical to the first, but of opposite sign. The interaction between these canceling distributions is calculated in reciprocal space. The total electrostatic interaction is then calculated as:

$$E_{total} = E_{real} + E_{reciprocal} - E_{self} - E_{excl} \qquad \text{Eq. 2}$$

$E_{(real)}$ is the screened interaction and $E_{(reciprocal)}$ is due to the canceling Gaussian charge distribution. $E_{(self)}$ corrects for the interactions of the canceling charge distribution with itself. $E_{(excl)}$ corrects for the interactions of the canceling charge distribution between an atom and all excluded atoms (i.e. atoms that are linked through a bond, angle or torsion to an atom).

3. Results and Discussion

3.1. Structural Analysis

It is expected that each solute molecule undergoes structural changes in the process of MD. The dynamic properties of the molecule, such a differential flexibility of different parts (nucleotides, amino acids, phosphodiester bonds, etc.) can be determined and analyzed from the trajectory of MD simulation. Calculation of the respective root mean square deviations (r.m.s.d.) of all solute atoms (except hydrogen ones) from the original positions in the beginning of MD may suggest which conformational changes of solute molecules were undergoing.

R.m.s.d. of the T4 endonuclease V doesn't show stabile period up to 280 ps and is steadily increasing. After 280 ps it stabilizes and remains stabile throughout the following MD up to performed 1 ns (Fig.2a)). Similar situation is in the case of native DNA, its r.m.s.d. increases during the first 200 ps, and then largely oscillates up to 400 ps. After 400 ps oscillations become smaller (±0.5 Å) showing that DNA structure keeps stabile (Fig.2b)). R.m.s.d. of DNA with TD shows smaller oscillations than those observed in native DNA, except the period between 400 and 600 ps (Fig.2c)). In this period there is sharp increase in r.m.s.d. reflecting the movement of edge residues. After 600 ps these residues return to the original positions and resume corresponding hydrogen bonding. There was no restraint applied on nucleotides during simulation and similar motion of edge nucleotides was observed in our previous MD simulations /e.g. 12/. With aim to investigate the changes in backbone structure and clarify in which extend each nucleotide contributes to

the total r.m.s.d., the calculation of r.m.s.d. for each nucleotide was performed. It is found, that the r.m.s.d. of all nucleotides, except TD site, have comparable r.m.s.d. and contribute equally to the total r.m.s.d. The only significant difference is observed between r.m.s.d. of TD site and r.m.s.d. of corresponding thymine region (T6- T7) on the native DNA (Fig.3). TD site is more stabile and its r.m.s.d. doesn't have large oscillations comparing with those of T6-T7 site on native DNA. The potential energy of all systems stabilized quickly and remained stabile throughout the entire simulation (Fig.4). Temperature of each system was oscillating around the average value of 300 K (Fig.5).

Analyzing the structure of enzyme throughout the entire MD simulation, it is found that the molecule itself didn't undergo significant changes. Fig.6 shows how each solute molecule was developing during the process of MD simulation. Comparing the structure after "constant pressure" MD (referred as "cp") and the final structure at 1 ns it is seen, that enzyme slightly changes its position and rotates inside the entire system box. The structure of the enzyme itself remains stabile. Observed stability of enzyme molecule may be an important factor for its alignment with DNA during scanning process, recognition of TD lesion and consequent binding into DNA. The crystallographic study combined with mutational analyses revealed that the carboxyl side chain of Glu-23 plays a crucial role in the cleavage of N-glycosyl bond in DNA /13, 14/. Glu-23, as part of estimated catalytic site (Glu-23, Thr-2, Arg-22 and Arg-26) is located at the center of the concave surface of enzyme that may be exposed to TD site of the DNA during scanning process (Fig.7).

Examining the structures of both DNA molecules in the same moments of MD simulation as enzyme ones it is found that the double helical structure and hydrogen bonding are kept for both DNA molecules except the edge nucleotides (Fig.6). DNA molecule with TD has sharp bending around the TD site formed approximately after 200 ps of MD, comparing with bending around T6-T7 site of native DNA. This bending is originated by two covalent bonds C(5)-C(5) and C(6)-C(6) between the adjacent thymine basis forming TD (see Fig.1). These two bonds also contribute to the decreased mobility of TD site that is seen on Fig.3. Observed bending also originates the formation of the DNA shape which is complementary to the shape of enzyme. This result is in agreement with crystallographic study of DNA-enzyme complex, in which the crystal structure of DNA in complex is strongly kinked at the TD site and the catalytic site of the enzyme creates possible contacts with TD /15/.

3.2. Analysis of Electrostatic Energy

The electrostatic energy of individual parts of enzyme and DNA molecules was calculated as a contribution to the total potential energy of the system, in order to examine its possible role in recognition process.

The electrostatic energy of the accessible surface of the enzyme indicates the obviously biased positive energy distribution and it is assumed to participate in recognition of TD site and consequent binding to DNA. The electrostatic energy is calculated individually for each amino acid of the enzyme and it has positive value oscillating around +10 kcal/mol with 12 arginine amino acids having significantly negative value (around -50 kcal/mol)

(Figs.8a) and 9). Focusing on the enzyme site participating on the catalytic process - Glu-23, Thr-2, Arg-22 and Arg-26, it is found that amino acid Glu-23 has the electrostatic energy of positive value of +12.19±0.56 kcal/mol calculated as an average value over the r.m.s.d. stabile period of MD. Looking at the value of electrostatic energy of amino acids located at the surface surrounding catalytic site, there are several other amino acids having the electrostatic energy of the positive values similar to that one of Glu-23 (Fig.8b)).

The electrostatic energy was also calculated for nucleotides of both DNA molecules. Fig.10a) shows the distribution of the electrostatic energy calculated for each nucleotide of native DNA. It can be seen that cytosine nucleotides have negative values around -45 kcal/mol, guanine nucleotides around -37 kcal/mol, adenine nucleotides around -14 kcal/mol and thymine nucleotides nearly neutral value of approximately +0.6 kcal/mol. Thymine nucleotides at the positions 6 and 7 have values of +0.64±0.72 and +0.61±0.67 kcal/mol, respectively (average values calculated over the r.m.s.d. stabile intervals). Performing the same calculation of electrostatic energy for DNA with TD, it is found, that all nucleotides have the same values of electrostatic energy within their respective r.m.s.d. as those of native DNA, except TD site. This has the negative value of -9.23±0.64 kcal/mol calculated as an average value over the stabile period 240-400 ps of MD (Fig.10b)). Figs.11a) and 11b) display in color the electrostatic energy of nucleotides of both DNA molecules.

In scanning process, the electrostatic interaction between the TD site and surrounding environment in cell nucleus (including enzyme) may become important. With aim to clarify the differences among these interactions, the electrostatic interaction between each nucleotide of DNA and surrounding water was calculated. Electrostatic interaction was calculated as an interaction between two groups of atoms (respective nucleotide as 1^{st} group and water molecules as 2^{nd} group). Such a calculation is a simplification in certain extent, as it doesn't include real interaction between DNA and enzyme. In MD simulation the Particle Mesh Ewald Sum technique was used, which doesn't introduce any cut-off distance for electrostatic interactions, therefore the calculated electrostatic energy represents the interaction between individual nucleotide and all surrounding water molecules in the box. Absolute value of difference in electrostatic interaction between respective nucleotides of native DNA and DNA with TD is shown on Fig.12. It can be seen, that there is significant difference in electrostatic interaction with surrounding water molecules between TD site and respective thymines site (T6 and T7) in the native DNA.

These results lead to a suggestion that the electrostatic energy of TD site on the DNA may be an important factor in recognition of this site by repair enzyme. It is not clear in which extend the electrostatic energy contributes to the proper orientation of enzyme molecule with respect to DNA molecule ensuring that catalytic site of enzyme is exposed to TD site on DNA. Also the occurrence of amino acids having similar positive value of electrostatic energy in the vicinity of catalytic site, (Fig.8b)), suggests the idea, that the electrostatic

energy is not the only factor in recognition process and other factors are needed to properly recognize lesion site and consequently bind to DNA. Among other important factors are structural complementarity of DNA and enzyme, and specific structural aberrations at the TD site /16,17/. The structure of the enzyme itself and its alignment with DNA in such a way as to allow electrostatic contact with active amino acids is also of great importance. This could be clarified in MD simulation of DNA molecule containing TD in complex with T4 endonuclease V which, up to our knowledge, has not been reported yet.

4. Conclusion

In this study we try to identify the structural changes and energy properties that are observed after formation of thymine dimer in DNA molecule and to compare them with those observed in native DNA. In addition to DNA molecules there is studied the thymine dimer repairing specific enzyme T4 endonuclease V. These three molecules are explored by using molecular dynamics simulation - technique that allows to derive the mechanical and energetically properties in the time scale. It has been found that the DNA molecule with TD forms a sharp kink around the TD site that is different from the bending of native DNA. This kink originates shape of DNA molecule that is complementary to the concave shape of repair enzyme. This structural complementarity is important in binding of enzyme to DNA. The calculation of electrostatic energy of individual structural parts of each molecule shows that the TD site has negative value of around -9 kcal/mol and that most amino

acids of surface of the enzyme have positive value between +5 and +15 kcal/mol. The negative electrostatic energy of TD is specific for this site and differs from the electrostatic energy of respective native thymine site that shows nearly neutral value. In addition, there is also difference among electrostatic interactions of individual DNA nucleotides with surrounding water, namely between thymine and thymine dimer site. These two factors - structural complementarity and specific electrostatic energy of TD site - play an important role in proper recognition of damaged site on DNA molecule by repair enzyme.

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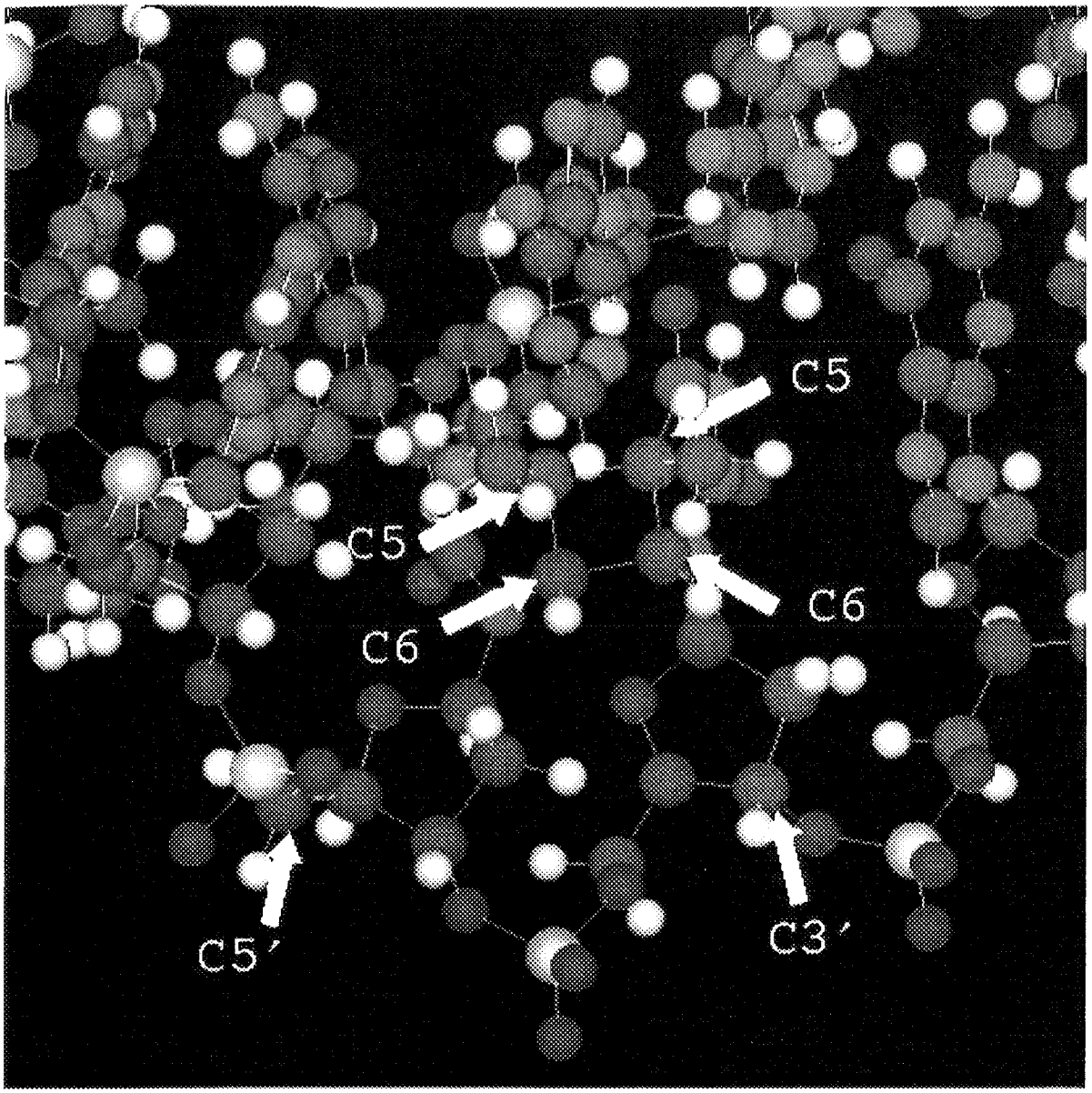

Fig.1

Thymine dimer formed by covalent bonds C(5)-C(5) and C(6)-C(6) between respective carbon atoms of thymines. C5' and C3' are atoms of DNA backbone of which the bonds are cleaved during the enzymatic repair process.

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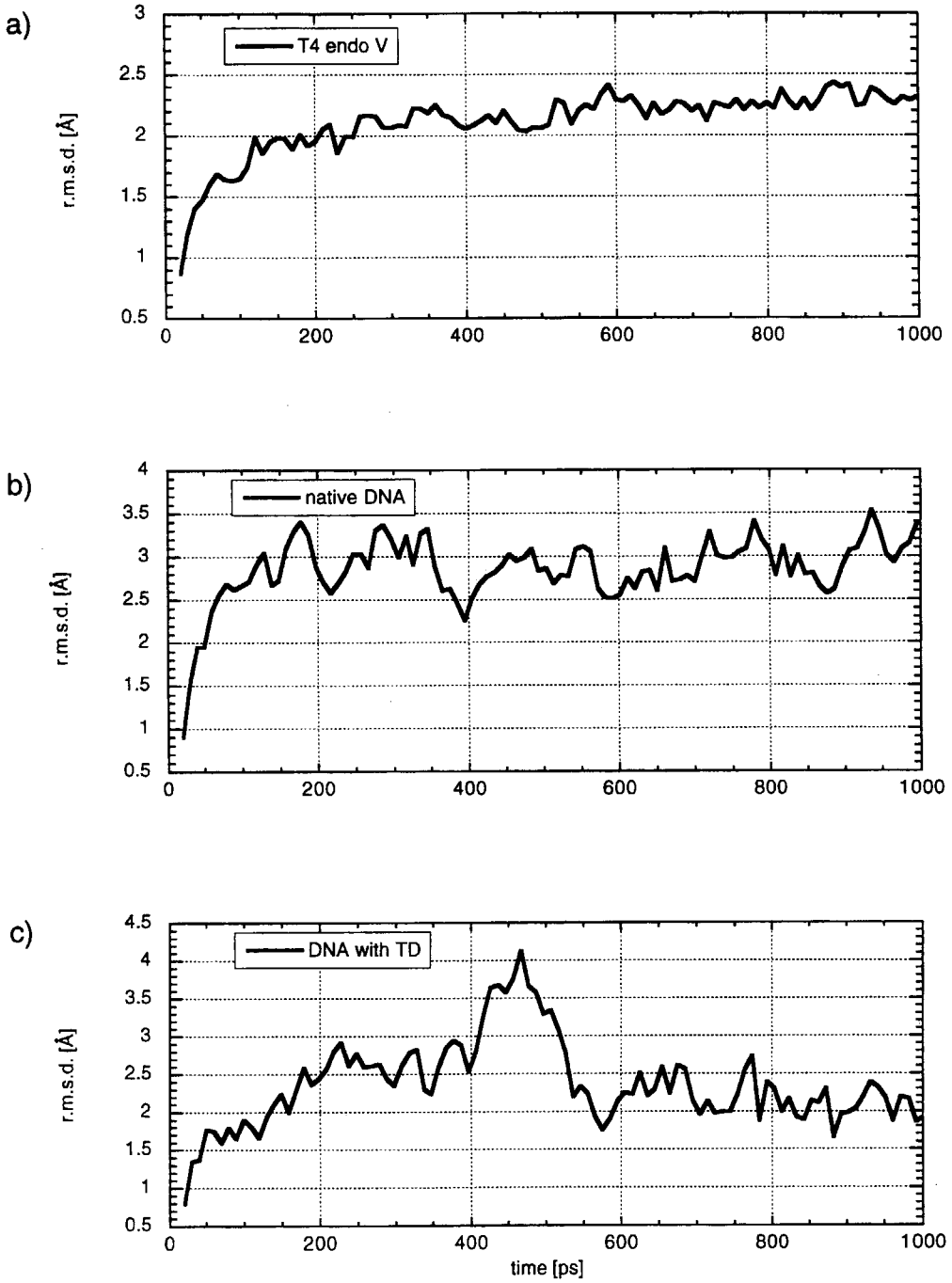

Fig.2

R.m.s.d. of T4 endonuclease V (a)), native DNA (b)) and DNA with TD (c)). T4 endonuclease V stabilizes after 280 ps and remains stabile up to 1 ns. R.m.s.d. of native DNA rises quickly during first 100 ps, oscillates largely between 200 and 400 ps and then oscillations become smaller. R.m.s.d. of DNA with TD shows smaller oscillations than those of native DNA, except the period between 400 and 600 ps. Increase of r.m.s.d. in this period is attributed to the movement of edge residue.

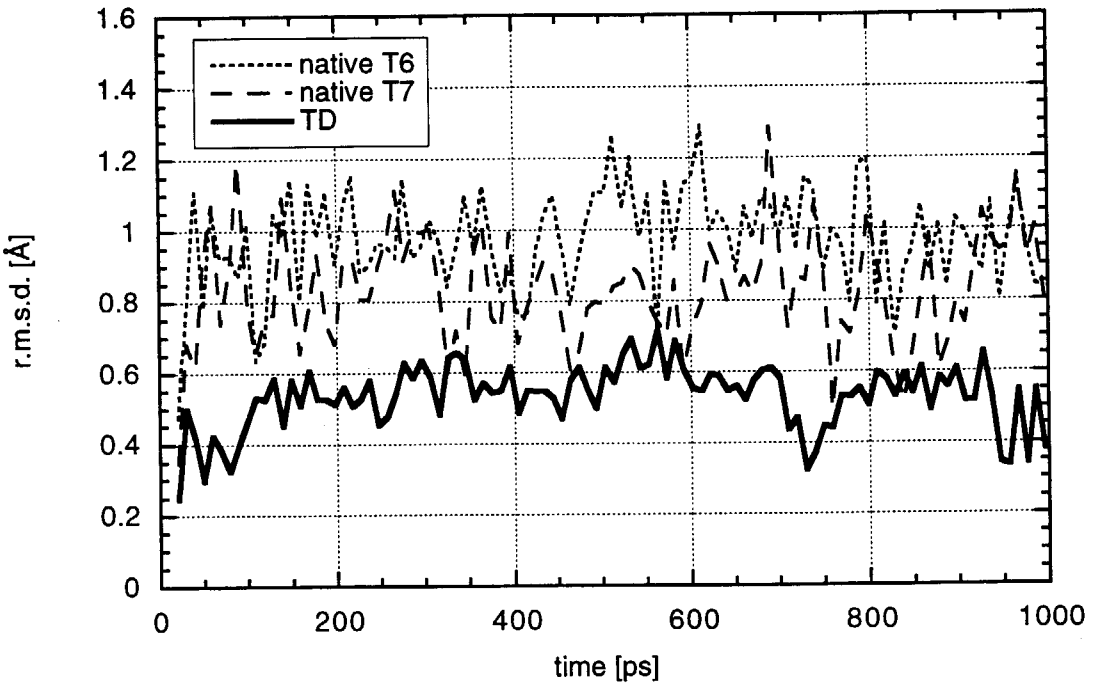

Fig.3

R.m.s.d. of TD site and two thymine nucleotides T6 and T7 of the native DNA. TD site is more stabile and its r.m.s.d. doesn't have large oscillations as those of T6 and T7.

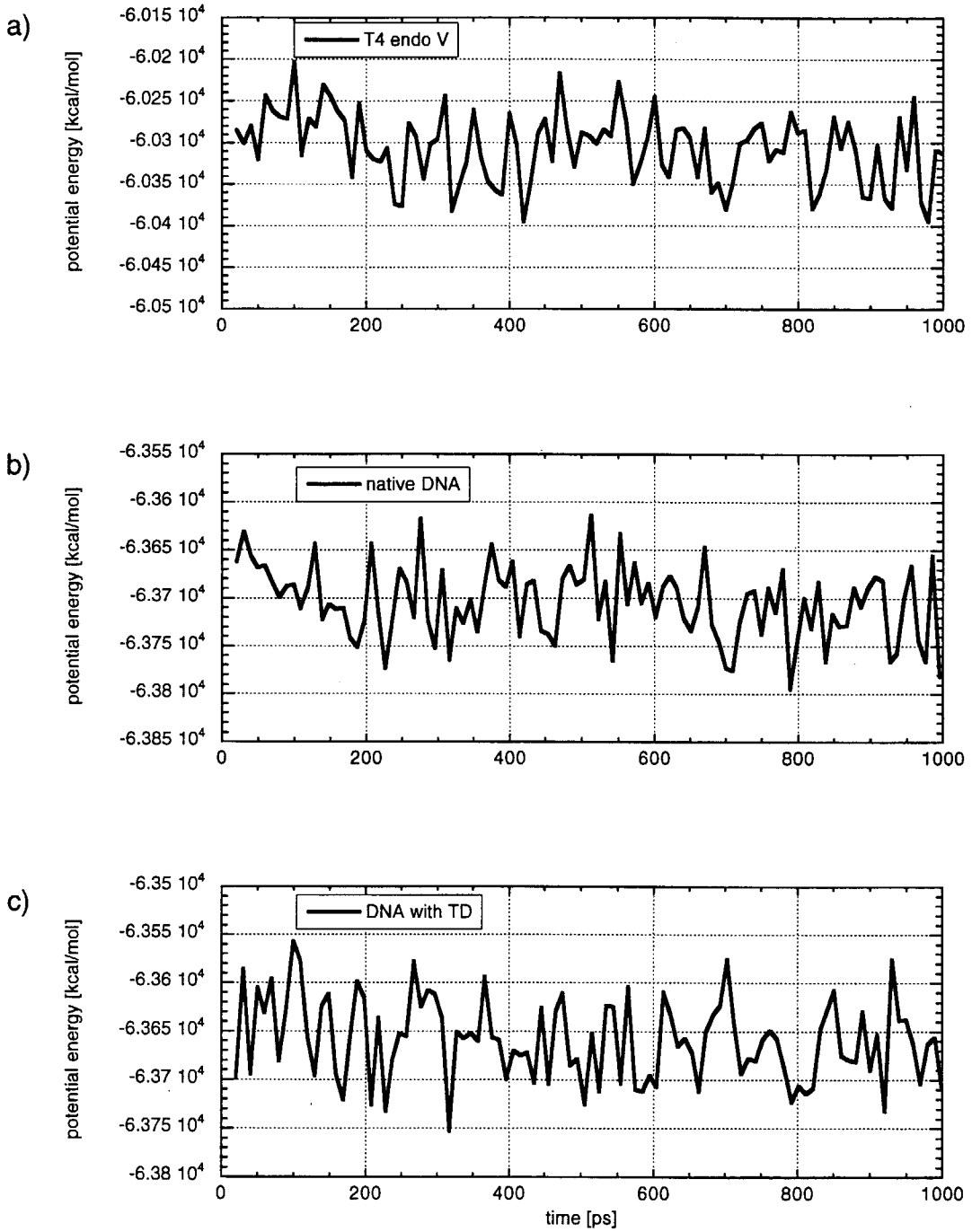

Fig.4

Trajectory of potential energy of each solute molecule during 1 ns of MD. Potential energy was minimized in the preparatory minimization prior the entire MD (graphs not shown). After the energy reached minimal value during minimization, it remained stabile throughout the following MD.

a) - T4 endonuclease V

b) - native DNA

c) - DNA with TD

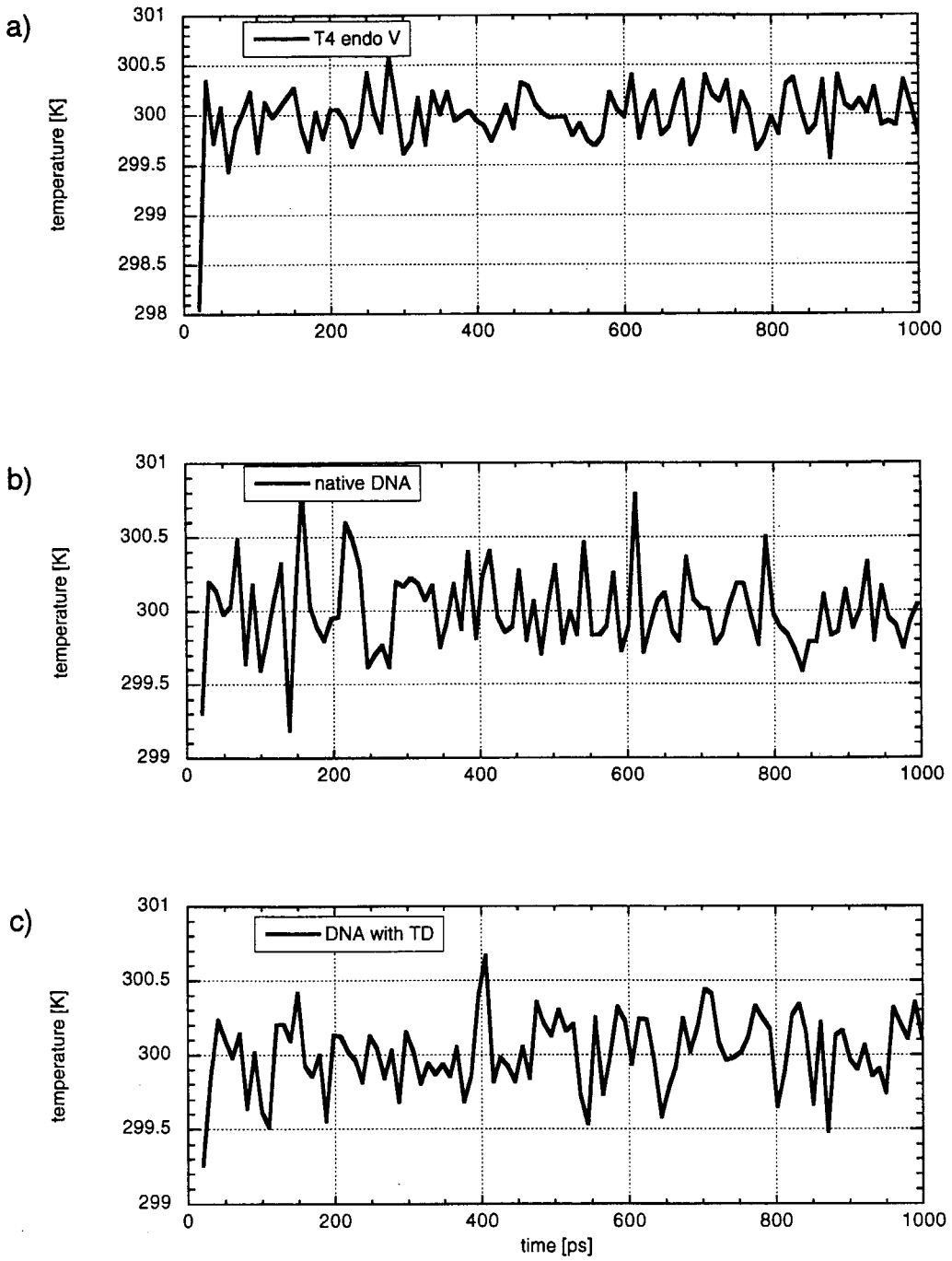

Fig.5

Trajectory of temperature of each solute molecule during 1 ns of MD. System were heated up to 300 K from original 0 K during preparatory heating MD (10 ps, graphs not shown). After each system reached 300 K, the temperature was kept stabile and oscillates around the average value of 300 K (±0.5 K).

a) - T4 endonuclease V

b) - native DNA

c) - DNA with TD

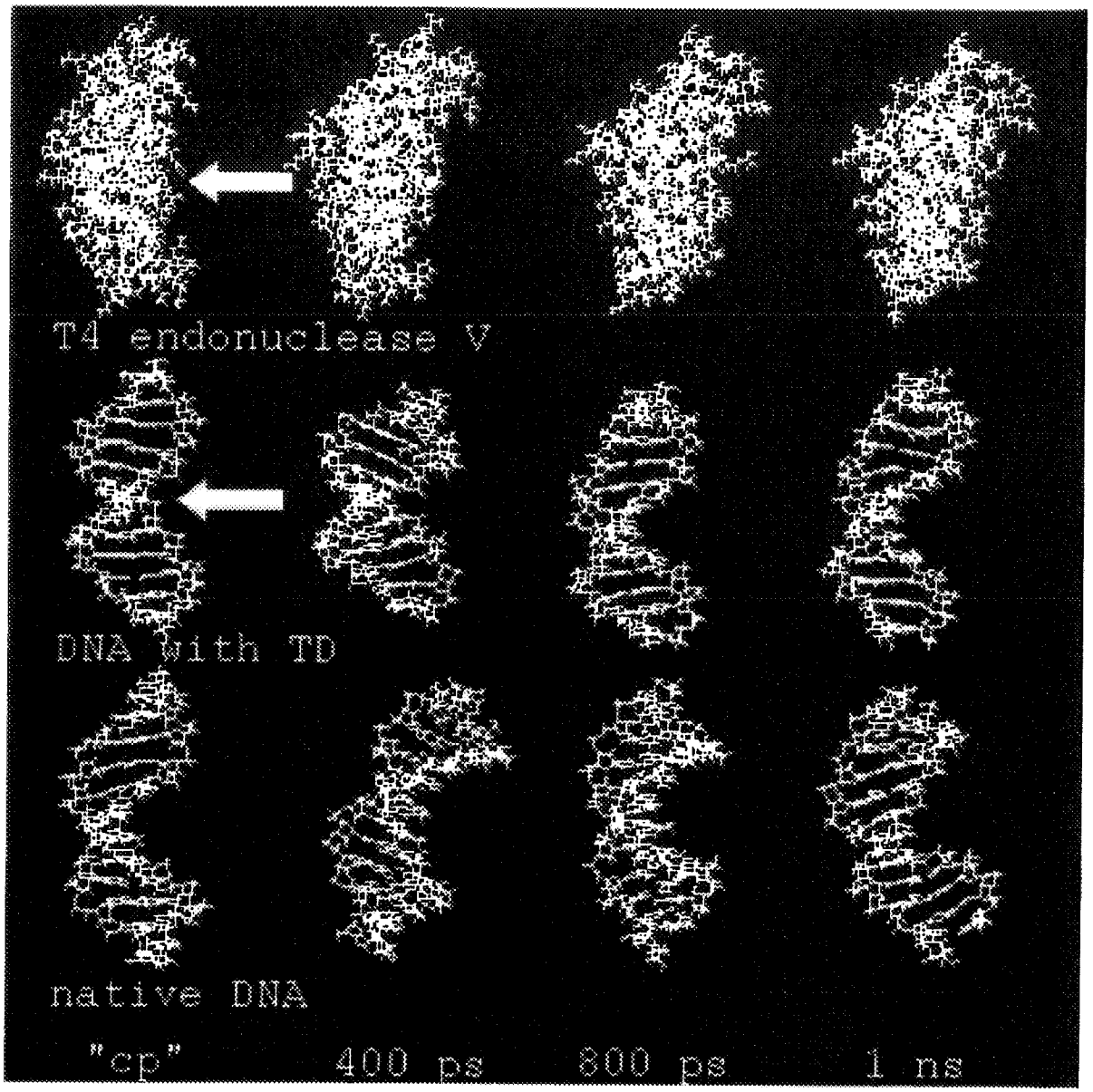

Fig.6

Snapshots of solute molecules during 1 ns of MD. A "cp" marks the structure after constant pressure run, i.e. after heating and after stabilization of saturated density. Arrows mark the position of catalytic site on enzyme surface and the position of thymine dimer, respectively.

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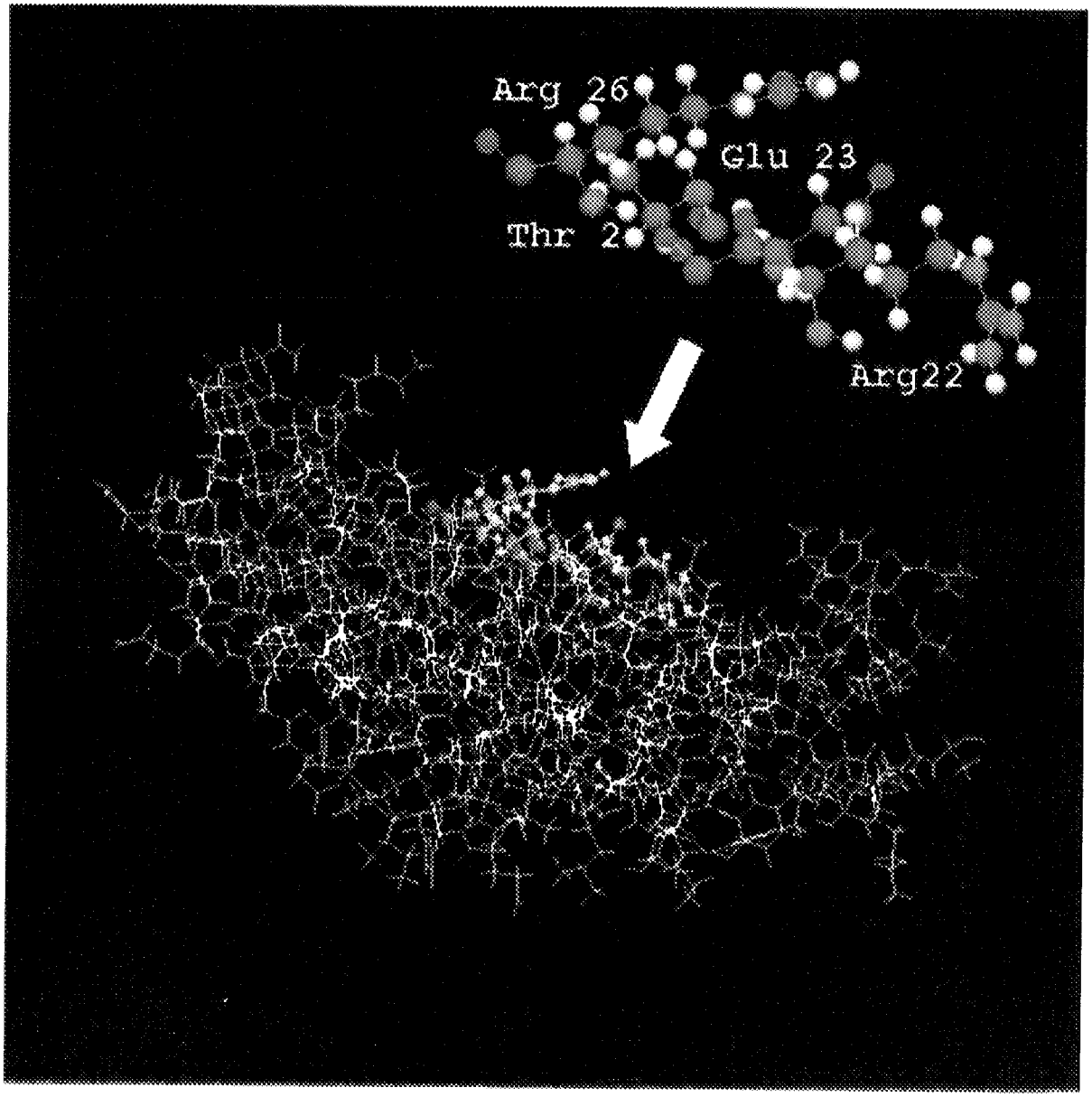

Fig.7

Catalytic site of T4 endonuclease V - Glu-23, Thr-2, Arg-22 and Arg-26. These amino acids are located at the central part of concave surface of enzyme that may be exposed to TD site during scanning process.

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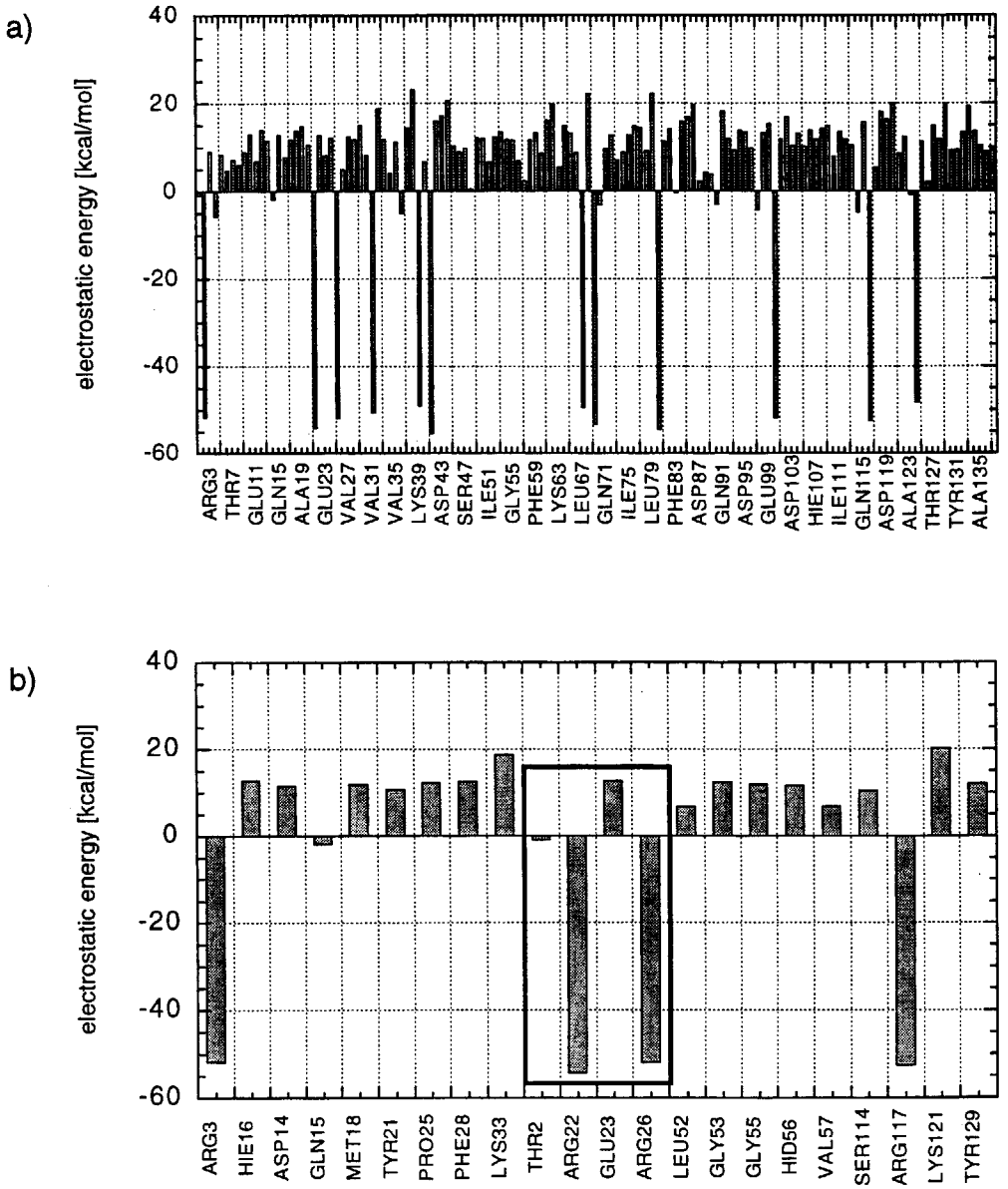

Fig.8

a) Electrostatic energy of amino acids of enzyme. The energy of most amino acids has positive value between +5 and +15 kcal/mol. The negative values around -50 kcal/mol are those of arginines. (average values calculated over stabile interval 280 - 1000 ps of r.m.s.d., error bars not shown)

b) Electrostatic energy of amino acids located at the surface of the enzyme close to the catalytic site. Amino acids of catalytic site (Glu-23, Thr-2, Arg-22 and Arg-26) are within open rectangular.

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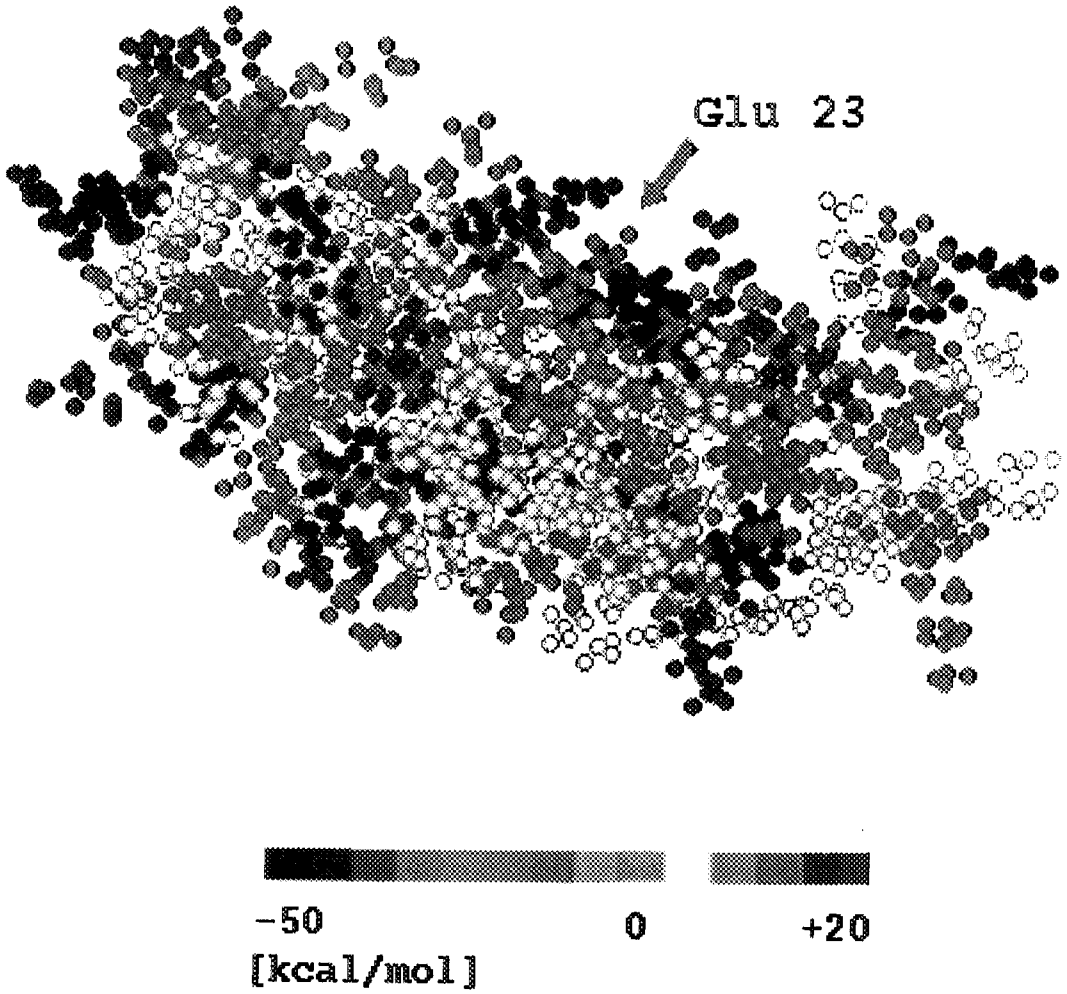

Fig.9

Electrostatic energy of aminoacids on the enzyme surface. All amino acids at the surface have positive electrostatic energy, except arginines (black). The structure of molecule is the one at 400 ps. The value of electrostatic energy is colored equally for each atom of single amino acid and represents its average value as shown on Fig.8a).

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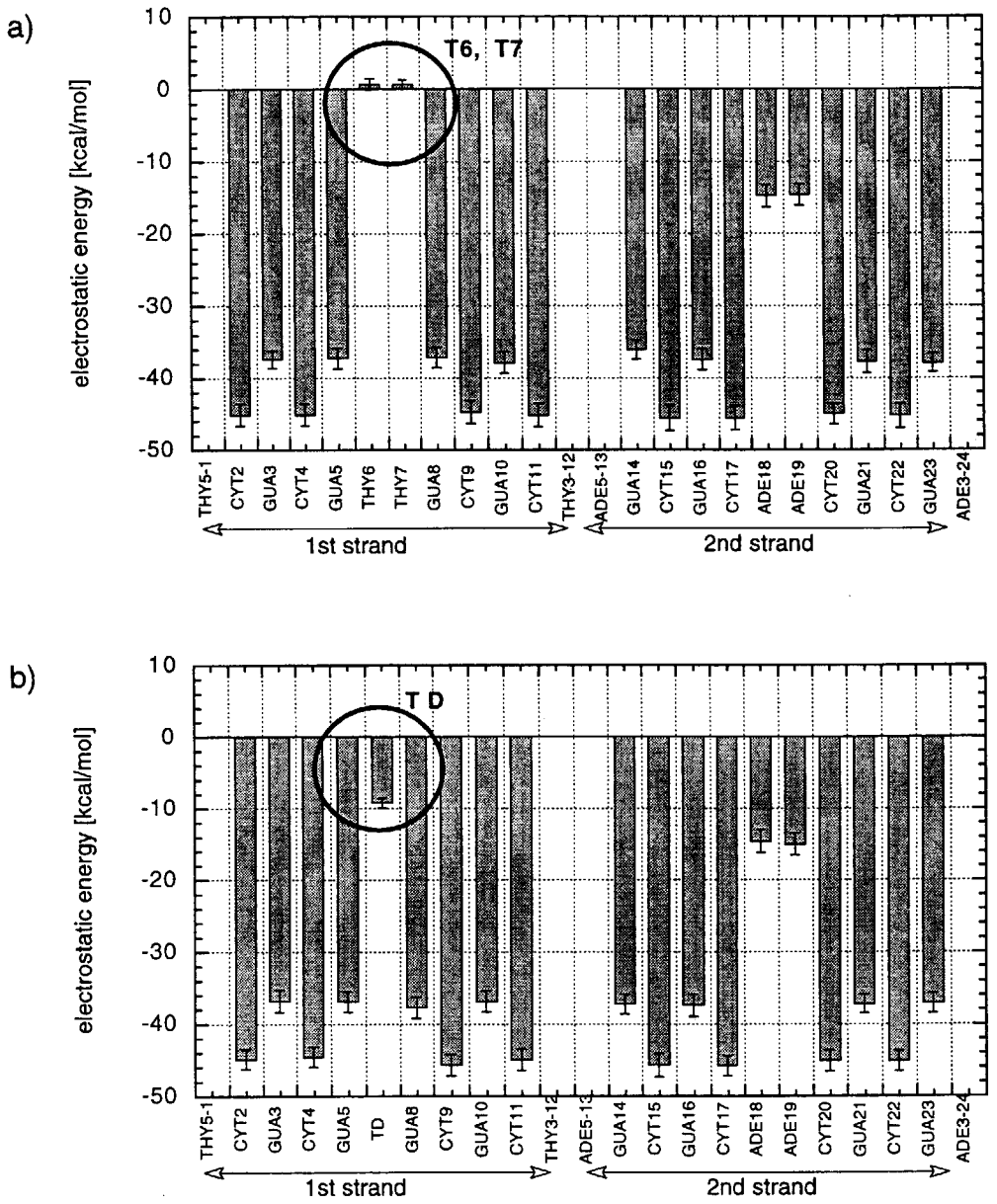

Fig.10

Electrostatic energy of nucleotides of DNA molecules. The values of edge nucleotides are not shown as they are affected by their specific positions. (average values calculated over respective stabile intervals of r.m.s.d.)

a) - native DNA; thymine nucleotides T6 and T7 at the central part of DNA have nearly neutral values around +0.6 kcal/mol (marked by open circle).

b) - DNA with TD; TD site has negative value of -9.23±0.64 kcal/mol (marked by open circle).

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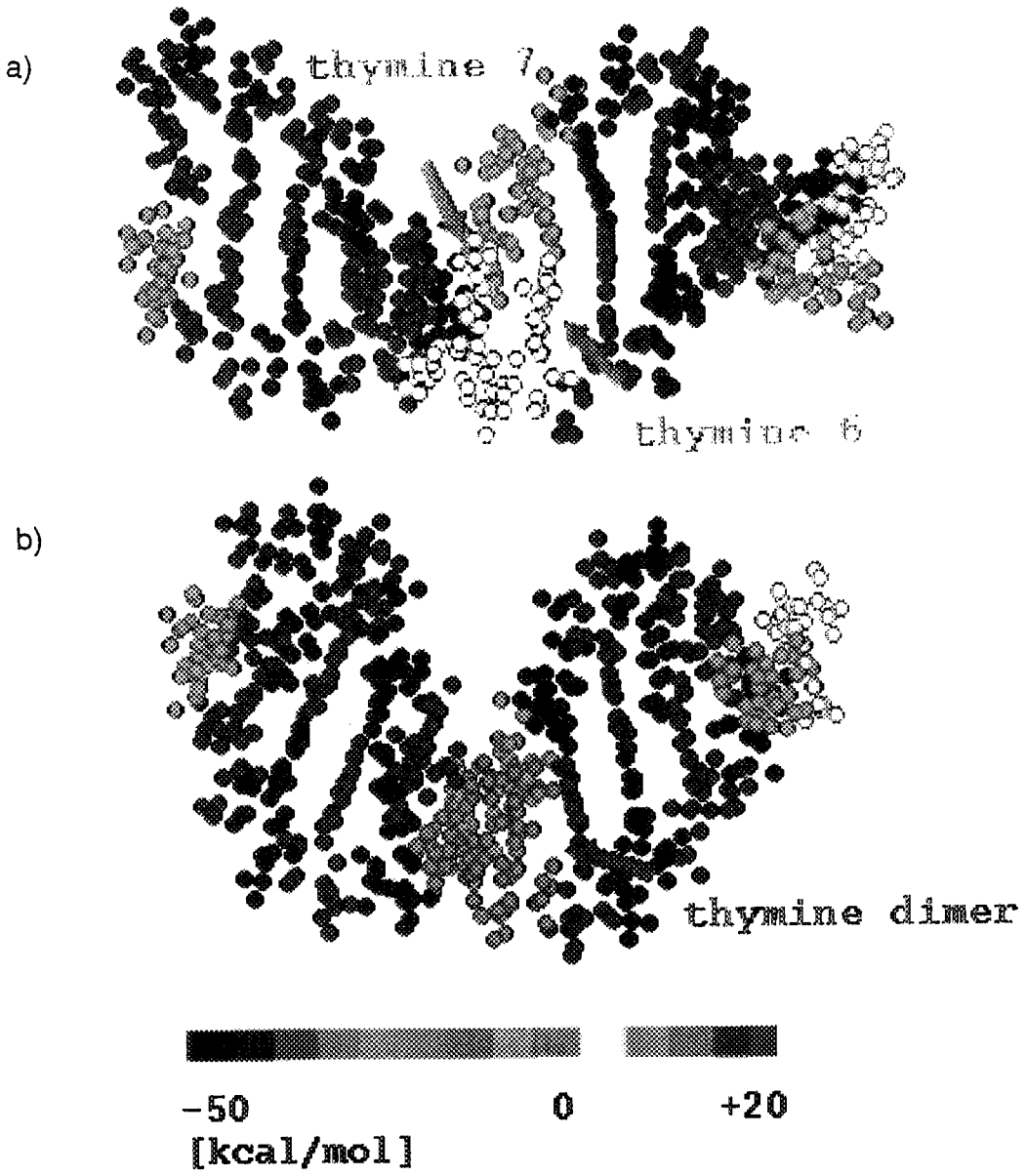

Fig.11

Electrostatic energy of nucleotides of DNA molecules. The structures of molecules are those at 400 ps. The value of electrostatic energy is colored equally for each atom of single nucleotide and represents its average value as shown on Fig.10.

a) - native DNA; nearly neutral value of T6 and T7 nucleotides (white)

b) - DNA with TD; negative value of TD site ≈ -9 kcal/mol (green)

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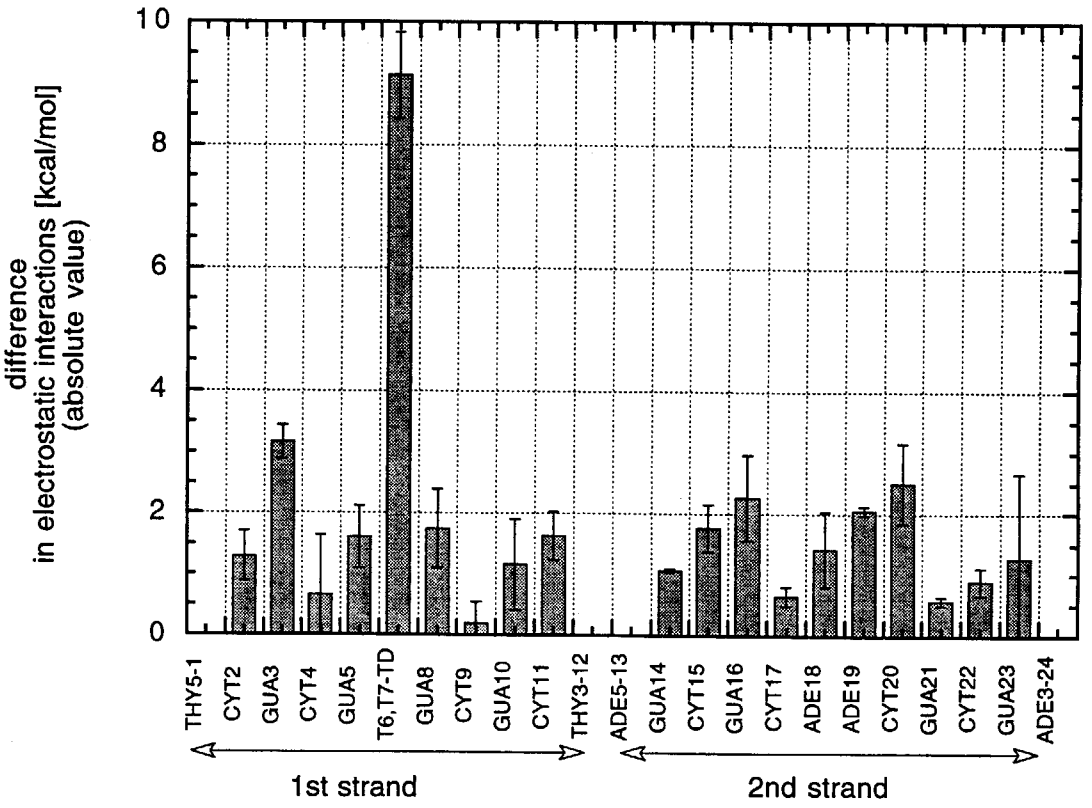

Fig.12

Absolute value of difference between electrostatic interactions of respective nucleotides (native DNA and DNA with TD) with surrounding water. The significant difference is found between TD site and T6 and T7 nucleotides of the native DNA.

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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
線量等量	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=0.1nm=10^{-10}m

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

1 bar=0.1MPa=10^{5}Pa

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

1 Ci=3.7×10^{10}Bq

1 R=2.58×10^{-4}C/kg

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

1 rem=1cSv=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　1m^2/s=10^{4}St(ストークス)(cm^2/s)

圧力	MPa(=10bar)	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=4.18605J（計量法）

=4.184J（熱化学）

=4.1855J（15℃）

=4.1868J（国際蒸気表）

仕事率　1 PS(仏馬力)

=75 kgf·m/s

=735.499W

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 SIMULATIONS OF DEOXYRIBONUCLEIC ACIDS AND REPAIR ENZYME T4 ENDONUCLEASE V.