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DISTRIBUTION OF IONS AROUND THYMINE DIMER CONTAINING DNA: A POSSIBLE RECOGNITION ELEMENT FOR ENDONUCLEASE V

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Introduction

The molecular link between sunlight exposure and skin cancer can be traced to the formation of cyclobutane pyrimidine dimers together with (6-4) photoadducts of pyrimidines in DNA upon exposure to UV radiation (Hutchinson 1987; Cadet and Vigny 1990). The mutagenicity of these lesions is frequently explained by miscoding during DNA replication due to perturbations of base-pairing interactions. However, the mutagenicity of UV photoproducts depends of their sequence context (Brash et al. 1987), suggesting that more global structural changes in DNA contribute to mutation induction.

One of the most effective protections against the deleterious effects of cyclobutane pyrimidine dimers is the wide range of repair of this lesion by different enzymatic pathways. DNA photolyases (Sancar 1990) catalyze photocleavage of the CC bonds linking the adjacent thymines of the dimer, thereby restoring the pyrimidines to their native state. Cyclobutane pyrimidine dimers are also removed by base-excision. The most extensively studied enzyme of this type is the bacteriophage T4 endonuclease V. This enzyme hydrolyses the glycosyl bond of the 5' pyrimidine in the dimer and also exhibits an apurinic/apyrimidinic endonuclease activity proceeding through the β-elimination of the 3'-phosphate of an abasic site (Dodson and Lloyd 1989). The enzyme has also been shown to bind to DNA duplex non-specifically and to scan it in a one-dimensional diffusion along the DNA until

it encounters a thymine dimer. Upon encounter with the thymine dimer the enzyme binds to DNA with higher affinity and engages in the enzymatic excision of the damage. The nonspecific binding is sensitive to ionic strength: below 50mM NaCl the enzyme performs a one-dimensional scanning, whereas above that concentration the enzyme activity is controlled by a three dimensional diffusion (Gruskin and Lloyd 1986; Dowd and Lloyd 1990). This fundamental property of rapid one-dimensional scanning along DNA (Von Hippel and Berg 1989; Dowd and Lloyd 1990; von Hippel 1994) is shared by many DNA binding proteins. Thus, the understanding of the underlying mechanism of such a process is of major importance.

In spite of the high resolution X-ray crystal structure of endonuclease V (Morikawa et al. 1992) and numerous site directed mutagenesis studies (Dowd and Lloyd 1989; Lloyd and Augustine 1989; Augustine et al. 1991; Dodson et al. 1991; Doi et al. 1992; Dodson et al. 1993), the mechanism by which it recognizes the pyrimidine dimer lesion in DNA is still obscure. NMR studies performed on DNA oligonucleotides containing *cis,syn*-pyrimidine dimers showed that the distortions caused by the lesion are surprisingly small (Kemmink et al. 1987; Kemmink et al. 1987; Kemmink et al. 1987; Taylor and ODay 1990; Lee et al. 1994). Nevertheless, endonuclease V is able to recognize this change with very high fidelity. Since distortions of DNA structure are usually associated with changes in the electrostatic shielding by counterions and in solvation of DNA it is possible that the enzyme is acutely sensitive to this property, as is demonstrated by the dependence of the scanning ability on ionic strength.

This paper presents the results of a 200 ps molecular dynamics simulation on the dodecamer d(CGCGAATTCGCG)$_2$ containing a *cis,syn*-cyclobutane thymine dimer, explicit water and counterions. The averaged structure calculated from the simulation shows good agreement with the available NMR data. The distribution of counterions around the damaged DNA is different from that around a non damaged DNA and suggests a possible mechanism of damage recognition by the

enzyme.

Methods

A *cis-syn*-thymine cyclobutane dimer was incorporated into the double helical dodecamer d(CGCGAATTCGCG)$_2$ with a canonical B-DNA initial geometry. To neutralize the phosphates, 22 Na^+ ions were placed around the DNA and the system was surrounded by a box of water with periodic boundary conditions. The complete system of DNA, water and counterions contained 6435 atoms.

The thymine dimer was built inside DNA during the initial energy optimization of the system. First, the positions of counterions and water were optimized with the DNA fixed in the initial conformation. This was followed by a stepwise minimization of the whole system with a restraining force constant of 100 kcal/mole on the distances between the thymines. The restraints were removed in the final stage of minimization and the covalent bonds between the thymines were defined by changing the atom types and the atomic charges.

Minimizations were followed by ten 0.2ps molecular dynamics 'heating' steps in which the temperature was gradually raised from 0K to 300K. This was followed by a 1.0ps run at 300K assigning initial velocities from a Maxwell-Boltzmann distribution. A 200ps molecular dynamics simulation was performed with constant temperature and volume. The SHAKE constraint was applied to all bonds and a 0.001ps time step was used. A non bonded cutoff distance of 10Å was used and the matrix of non bonded interactions was updated every 20 steps.

The resulting trajectories were used for structure analysis and for calculations of counterion density distribution.

Results and Discussion

The simulation can be divided into three distinct regions. The initial 20ps can be considered as an equilibration period followed by additional 80ps during which the structure of the DNA evolved with time. The RMS deviation of backbone

dihedral angles and helical parameters from their starting values shows a gradual increase between 20 and 80ps. The third period, 100 - 200ps, is characterized by fluctuations about an apparently stable structure judged by the values of structural and energetic parameters.

Several structural parameters, e.g., the *base pair axis* displacement and inclination, are very good indicators of the global character of DNA structure, which appears to be close to a canonical B-DNA. On the other hand, values of the tip angle for the T7-A18 base pair indicate that the position of the 5′ thymine of the dimer is slightly distorted within the helix. The *axis* parameters defined by relative arrangements of base pairs also show the distortions of the helical axis induced by the thymine dimer. Axis inclination at the A6-T19/T7-A18 step as well as axis tip and axis displacement at the T7-A18/T8-A17 step are significantly increased, causing a slight bending of the helical axis at the lesion.

Backbone torsional angles and fluctuations of the sugar puckering phase angle ϕ cover the range of conformations characteristic for A-DNA and B-DNA without extending into the high energy structures. The thymine dimer does not change the pattern of backbone angles in a significant way. The only change is in χ, the glycosyl bond dihedral at the 5' thymine of the dimer, which shows a *syn* instead of an anti conformation. Also, the coupling of the thymines produces a more rigid deoxyribose ring. The puckering of the 5' thymine is mostly a B-DNA type whereas the 3' thymine is more A-DNA type. This dynamic behavior of the DNA backbone is consistent with that observed in simulations performed on the native sequence (Swaminathan et al. 1991; Miaskiewicz et al. 1993).

Effects of the thymine dimer on *intra base pair* parameters, that describe the geometry of base pairing, show an increase in buckle and in propeller twist at T7-A18. The *inter base pair* parameters, that describe base stacking, show an increase in tilt angle for the A6-T19/T7-A18 step (more positive) and a decrease for the T7-A18/T8-A17 step (more negative). Most Watson-Crick hydrogen bonds are preserved in the dodecamer during the simulation except for the T7-A18 base pair

that contains the 5′ thymine of the dimer. It is substantially stretched and bent suggesting a weakening of this bond. The other hydrogen bond in the T7-A18 base pair does not show significant distortion. The major DNA groove width does not show changes attributable to the presence of lesion, but the minor groove seems to be widened by 2-3Å in the vicinity of the dimer.

NMR studies give strong evidence that distortions to DNA structure due to cyclobutane pyrimidine dimer formation are small. The results of our molecular dynamics simulation on a DNA dodecamer containing a cyclobutane thymine dimer are in line with these NMR studies in that they predict only small structural perturbations close to the site of the lesion. Our results are also consistent with NMR spectra of thymine dimers in the dinucleotide d(TpT) (Kim and Alderfer 1992; Kim et al. 1993), which suggest significant deviations of the glycosyl torsional angle from an *anti* conformation and varying preferences in sugar puckering. The dynamic simulations also suggest an increased rigidity of the deoxyribose moieties in the dimer relative to native DNA nucleotides in the dodecamer.

The major structural changes predicted by our calculations are not in the backbone but rather in the position, base-pairing and base stacking geometries of the damaged bases within the helix. This finding is consistent with 2D-NOE spectra of an octamer duplex containing a thymine dimer (Kemmink et al. 1987) from which it was deduced that the 5′ thymine was more reoriented with respect to helical axis than the 3′ thymine. This effect is clearly seen from our results. As a consequence of this reorientation, our calculations predict that one of the hydrogen bonds of the 5′ thymine with complementary adenine is significantly weakened. This result is in full agreement with observations based on NMR spectra of imino protons that the hydrogen bonds of base pairs in a thymine dimer are weakened but not broken (Kemmink et al. 1987; Lee et al. 1994).

In a recent study using synthetic oligonucleotides, the minor groove of DNA containing a thymine dimer was implicated as the binding site for the repair enzyme T4 endonuclease V (Iwai et al. 1994). Our calculations show that the minor

groove is widened by 2-3Å in the vicinity of the thymine dimer. This raises the question whether the subtle structural changes in the DNA are a sufficient recognition element identified by the enzyme. In view of the dependence of the enzyme-DNA interaction on the ionic strength of the environment it would be reasonable to expect that the distribution of counterions around the damaged DNA may be sufficiently different from the distribution around normal DNA. We have analyzed the distribution of counterions from the present simulation to assess the effect of DNA distortion of counterion distribution and compared it to the counterion distribution in a similar simulation of a normal DNA that does not contain a thymine dimer.

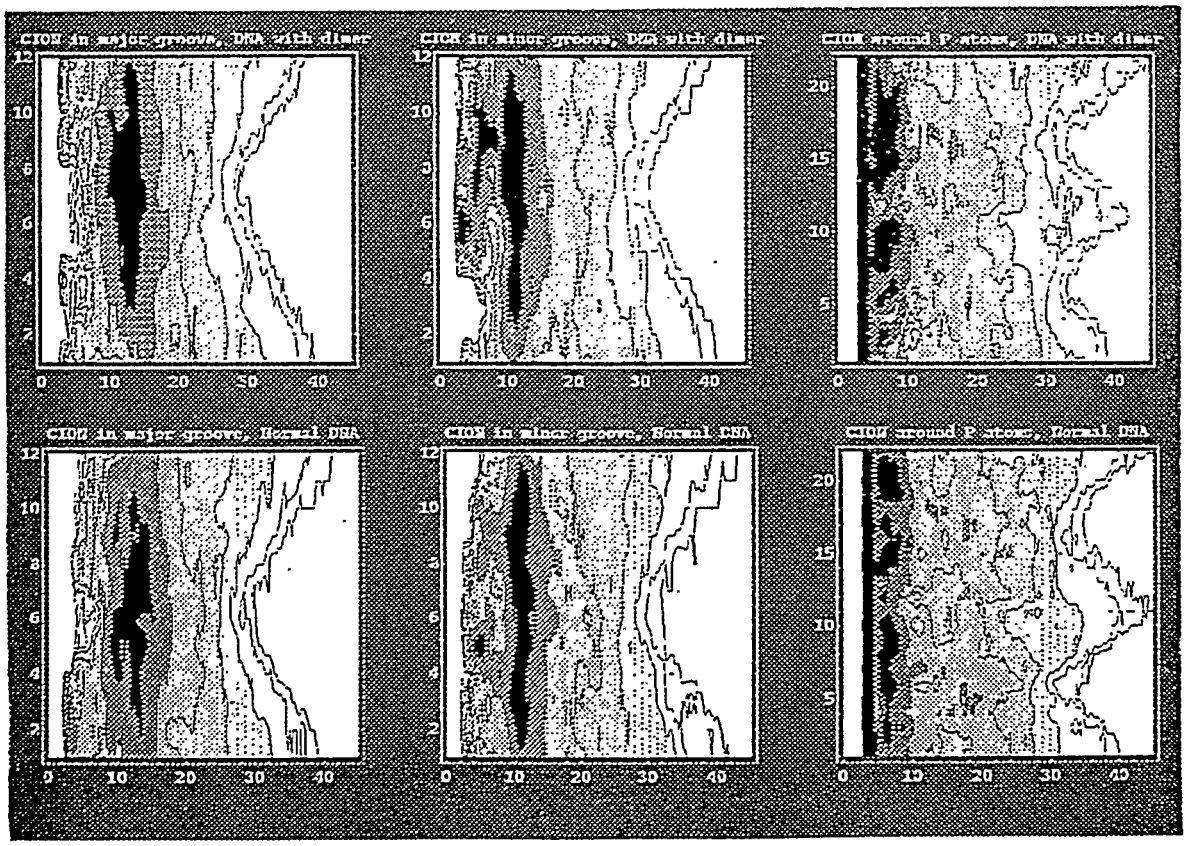

Figure 1. Contour density plots of the distribution of ions in major groove (left), minor groove (center), and along the phosphates (right).

The contour density plots shown in Figure 1 represent three types of distributions that were calculated from the trajectories of the simulations. The increasing dark colors in the plots depict increasing densities of counterions as a function of a radial distance from the DNA (horizontal displacement) and as a function of the position of the base pairs along the sequence (vertical displacement). The pair of plots on the left show the distribution of counterions in the major groove of the damaged DNA (top) and normal DNA (bottom). The density of counterions increases as the distance from the bottom of the minor groove increases and reaches a maximum near 14 Å. Moving further away from the DNA the density is reduced reflecting the fact that a considerable number of counterions is condensed on the DNA. It is clear that the differences between the distributions in the damaged and normal DNAs is small. The only region in which the densities of counterions differ is in the vicinity of T7 of the DNA with thymine dimer. Thus, the area near the thymine dimer in the major groove of the damaged DNA has a lower density than the corresponding place in normal DNA.

The center pair of plots shows the distribution of counterions in the minor groove of damaged DNA (top) and normal DNA (bottom). The radial distribution of counterions in the minor groove peaks around 10 Å from the bottom showing a closer packing of ions than in the major groove. The distribution in the damaged DNA shows an interesting polarization of counterions which is not observed in the normal DNA. There is a clear depletion of counterions near the C3 and a concomitant increase near C9. In the normal DNA the distribution is more uniform. Thus, the depletion of counterions on the 5′ side of the thymine dimer may act as an electrostatic trigger for the enzyme to change its affinity to the particular sequence that contains the lesion. A reduction in the density of counterions will reduce the required energy to displace the counterions and consequently will increase the affinity of the protein to DNA.

A comparison of the densities around the phosphates, shown in the pair of plots on the right, demonstrates that the difference between the distributions is very

small. This would suggest that the phosphates and the counterions condensed around these negatively charged groups may not be important for the recognition of the damage. It is noteworthy that the changes in counterion distribution should also have an effect on the structure of water around the damaged site. These studies are now in progress.

Conclusions

Structural analysis of the results of molecular dynamics simulations on the dodecamer d(CGCGAATTCGCG)$_2$ with a *cis,syn* cyclobutane dimer at T7-T8 reveals several distortions that are easily attributable to lesion. These perturbations of DNA structure are largest for the 5′ thymine of the dimer which exhibits a *syn* conformation about the glycosyl bond, extensive reorientation of the base relative to the helical axis, and weakening, but not breaking, of the hydrogen bond to complementary adenine. Significant distortions of the 3′ thymine in the dimer were not detected in our simulations. Base stacking is perturbed within the dimer and between the linked thymines and their nearest neighbors. The dimer increases the minor groove width by 2-3Å but significant bending or kinking was not detected at sight of the lesion. Our calculations agree with NMR results in that they predict only small distortions of DNA structure localized at the site of the lesion. This level of agreement suggests that molecular dynamics simulations can provide reliable data on the structure of damaged DNA sequences for studies of pyrimidine dimer recognition by repair enzymes.

The subtle changes in DNA structure produce a significant redistribution of counterions. The area near the T7-T8 in the major groove is slightly depleted from counterions but large change in the density of counterions is observed in the minor groove. The area 5′ to the damage is strongly depleted from counterions suggesting an electrostatic mechanism for the recognition of the damage by the enzyme.

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