Conf-9307160--1
PNL-SA-22964

STRUCTURE-FUNCTION STUDIES OF DNA DAMAGE
USING AB INITIO QUANTUM MECHANICS AND
MOLECULAR DYNAMICS SIMULATION

J. H. Miller
K. Miaszkiewicz
R. Osman^(a)

July - August 1993

Presented at the
New York Academy of Sciences Conference
on DNA Damage: Effects on DNA Structure
and Protein Recognition
July 31 - August 4, 1993
Burlington, Vermont

Work supported by
the U.S. Department of Energy
under Contract DE-AC06-76RL0 1830

Pacific Northwest Laboratory
Richland, Washington 99352

(a) Mount Sinai School of Medicine
City University of New York
New York, New York 10029

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

MASTER

VED

DEC 20 1993

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

OSTI

STRUCTURE-FUNCTION STUDIES OF DNA DAMAGE USING *AB INITIO* QUANTUM MECHANICS AND MOLECULAR DYNAMICS SIMULATION

**John Miller and Karol Miaskiewicz
Biology and Chemistry Department
Pacific Northwest Laboratory
Richland, Washington**

and

**Roman Osman
Department of Physiology and Biophysics
Mount Sinai School of Medicine of the City University of New York
New York, New York**

ABSTRACT

Studies of ring-saturated pyrimidine base lesions are used to illustrate an integrated modeling approach that combines quantum-chemical calculations with molecular dynamics simulation. Electronic-structure calculations on the lesions in isolation reveal strong conformational preferences due to interactions between equatorial substituents to the pyrimidine ring. Large distortions of DNA should result when these interactions force the methyl group of thymine to assume an axial orientation, as is the case for thymine glycol but not for dihydrothymine. Molecular dynamics simulations of the dodecamer d(CGCGAATTCGCG)₂ with and without a ring-saturated thymine lesion at position T7 support this conclusion. Implications of these studies for recognition of thymine lesions by endonuclease III are also discussed.

INTRODUCTION

DNA in living cells is exposed continuously to a number of environmental agents which may include UV and ionizing radiation as well as various chemical species. In many cases, the mechanism of action of these agents involves the generation of free radicals that attack DNA and produce a variety of lesions including sugar and base modifications, strand

breaks, and cross-links. Some active oxygen radicals (e.g. $O_2^{\cdot-}$, $\cdot OH$) are also generated endogenously during cellular aerobic metabolism and the damage they cause may be an important factor in aging and age-dependent diseases including cancer ^{1, 2, 3, 4}.

Pyrimidine bases are a major target of free-radical induced DNA damage. Free radicals add easily to the C(5)C(6) unsaturated bond of pyrimidines. The base centered radicals formed in such addition reactions can be further transformed to yield different stable products. One group of stable products, which includes 5,6-dihydroxy-5,6-dihydropyrimidines (pyrimidine glycols), 5,6-dihydropyrimidines, 5-hydroxy-5,6-dihydropyrimidines and 6-hydroxy-5,6-dihydropyrimidines (often referred as pyrimidine hydrates), is characterized by pyrimidine derivatives with a saturated ring. These pyrimidine lesions have been observed in DNA after radiolysis, UV irradiation or oxidation by chemicals. Pyrimidine glycols, 5,6-dihydropyrimidines, and 5-hydroxy-5,6-dihydropyrimidines have been detected upon radiolysis of polynucleotides and DNA both *in vitro* (reviewed by ^{5, 6}) and *in vivo* ^{7, 8}. On the other hand pyrimidine hydrates are a common product of UV damage to DNA ^{9, 10}. Similar products to those seen in DNA radiolysis are also observed in the damage caused by oxidative chemicals including some antibiotics ¹¹. Substantial amounts of endogenously generated thymine glycol are also detected in the human and animals urine ².

The pyrimidine DNA lesions may cause significant biological consequences including mutagenesis and inhibition of replication. Surprisingly, the biological consequences of ring-saturated pyrimidine lesions can be very different. Strong inhibition of DNA polymerase I activity has been observed for thymine glycol ^{12, 13, 14, 15}, which is in sharp contrast to the chemically similar lesion 5,6-dihydrothymine that is at most a very weak block for polymerase I action ¹⁶. Similarly, only slight inhibition of DNA replication by UV-induced thymine hydrate (6-hydroxy-5,6-dihydrothymine) was detected ¹⁷. The 5'-triphosphate of dihydrothymine can serve as an efficient substrate for DNA polymerase I but the 5'-triphosphate of thymidine glycol is not incorporated into DNA by this polymerase ¹⁸. Addition evidence that DNA structural perturbations by chemically related pyrimidine

lesions are very different comes from the activity of the UvrABC nuclease complex toward these lesions. This repair enzyme, which is generally considered to be the enzyme responsible for removing bulky adducts that cause extensive distortions to DNA structure^{19, 20}, recognizes and incises DNA that contains thymine glycol^{20, 21} but it is not active toward 5,6-dihydrothymine²¹.

Although the biological consequences of damaged pyrimidine DNA bases have been studied quite extensively, a mechanistic understanding of the differences in the biological activity of chemically related lesions such as the ring-saturated pyrimidines has not been achieved. Theoretical studies of the effects of damaged bases on the conformation and dynamics of DNA, tested whenever possible by experimental data, can make a significant contribution to this endeavor. This paper describes quantum-chemical calculations that show the energetic basis for the conformational preferences of ring-saturated pyrimidines in isolation. The most stable stereoisomers of the isolated thymine lesions have been incorporated into the dodecamer d(CGCGAATTCGCG)₂ at the T7 position. Molecular dynamics simulations suggest that the major source of DNA distortion by ring-saturated thymine lesions is their nonplanarity, which varies greatly depending on which of the substituents at C(5) and C(6) are axially oriented.

The principal repair pathway for pyrimidine lesions is the base-excision mechanism that involves the release of a modified base by a DNA N-glycosylases and then hydrolysis of the phosphodiester bond by apurinic/aprimidinic (AP) endonucleases. Endonuclease III of *Escherichia coli*, which requires double stranded DNA for its activity but no cofactor or metal ions, has both of these activities. It catalyses the hydrolysis of base-sugar bonds to liberate damaged pyrimidines and cleaves the phosphodiester bond 3' to the lesion by β -elimination in a concerted way²². Interestingly, the enzyme repairs a broad spectrum of pyrimidine lesions formed by ring saturation and fragmentation even though these lesions behave so differently in many other aspects of their biological activity as discussed above. Activities of endonuclease III towards thymine glycols, 5,6-dihydrothymine, urea, cytosine and thymine hydrates have been observed^{22, 23, 24, 25}. Thymine glycol strongly inhibits

endonuclease III activity towards DNA containing thymine glycol and urea ²² indicating that thymine glycol binds to the same site of endonuclease III as lesioned DNA. This observation together with atomic coordinates for the enzyme obtained from recent crystallographic data ²⁶ are the basis of our model of the complex between endonuclease III and DNA containing thymine glycol.

QUANTUM-CHEMICAL CALCULATIONS OF STRUCTURAL PROPERTIES OF PYRIMIDINE LESIONS

Free-radical addition to the C(5)C(6) double bond of pyrimidines leads to a class of nonplanar ring-saturated lesions which includes 5,6-dihydroxy-5,6-dihydropyrimidines (pyrimidine glycols), 6-hydroxy-5,6-dihydropyrimidines (pyrimidine hydrates), 5-hydroxy-5,6-dihydropyrimidines, and 5,6-dihydropyrimidines. Each of these lesions can exist in several stereoisomeric forms which may be energetically distinct depending on the environment. The stereochemistry of thymine glycol (Tg) and thymine hydrate are particularly complex because they have two asymmetric carbon centers while rest of the lesions have only one. The stereochemistry of Tg is illustrated in Fig.(1). Each asymmetric carbon gives rise to two stereoisomers; however, since the ring is nonplanar, each isomer can exist in two conformations with different substituents in the equatorial and axial orientations. In the absence of an external perturbation, such as deoxyribose or other components of DNA, the eight stereoisomers of Tg can be grouped into four pairs of degenerate enantiomers. Hence, for quantum-chemical studies of the relative stability of Tg stereoisomers in isolation, only the four conformations on the left-hand side of Fig.(1) need to be considered. These conformations can be denoted as 5-OH_{eq}-6-OH_{eq}, 5-OH_{ax}-6-OH_{ax}, 5-OH_{eq}-6-OH_{ax}, and 5-OH_{ax}-6-OH_{eq} according to the pseudo equatorial and pseudo axial orientation of the hydroxyl groups at C(5) and C(6). The stereochemistry of thymine hydrates is analogous to Tg with 5-OH_{eq} and 5-OH_{ax} replaced by 5-Met_{eq} and 5-Met_{ax}, respectively, to denote the orientation of the methyl group at C(5).

Only two conformations must be considered to calculate the relative stability of isolated stereoisomers of ring-saturated pyrimidine lesions with only one asymmetric carbon center. For uracil and cytosine hydrates, we use 6-OH_{eq} and 6-OH_{ax} to denote these energetically distinct conformers. Similarly, 5-OH_{eq} and 5-OH_{ax} can be used to label the energies that we calculate for stereoisomers of 5-hydroxy-5,6-dihydropyrimidines. The energetically distinct conformations of 5,6-dihydrothymine in isolation will be denoted by 5-Met_{eq} and 5-Met_{ax}. All of our quantum-chemical calculations were performed using the GAUSSIAN90 ²⁷ package for *ab initio* calculations. Geometries were optimized without constraints at the HF/6-31G level and single-point calculations at MP2/6-31G* level were done on the optimized structures. Solvation energies were calculated using a representation of the solvent as a dielectric continuum and the solute as a cavity in the solvent ²⁸. The charge distribution of the solute is described as a collection of point charges that induce a reaction field in the continuum. The point charges were obtained from a Mulliken population analysis of a 6-31G wave function. Our results are summarized in Table 1 where the energies calculated at various levels of approximation are given in kcal/mole relative to the most stable conformation.

Table 1 shows that 5,6-dihydrothymine prefers a conformation with the methyl group at C(5) in an equatorial orientation. The energy difference for this structural preference is about an order of magnitude smaller than that found for the other ring-saturated pyrimidine lesions which involve addition of hydroxyl groups rather than hydrogen atoms. The OH group of the hydroxy-dihydro-pyrimidines shows different stereochemical preference at C(5) and C(6). A pseudoaxial orientation of OH is preferred at C(6), whereas a pseudoequatorial OH is energetically favored at C(5). Interactions between polar groups in the equatorial plane are primarily responsible for these preferences. The interaction between the equatorial OH group at C(5) and the adjacent C(4)O(4) carbonyl bond in uracil can be clearly seen from the structure of 5-OH_{eq}-5,6-dihydrouracil presented in Fig.(2a). The dipoles of the OH and C(4)O(4) bonds are almost in an ideal antiparallel orientation, producing a favorable electrostatic interaction. A similar interaction occurs in

thymine derivatives. An equatorial orientation of OH groups at C(5) is also preferred in cytosine derivatives because the amino group at C(4) gives rise to a similar favorable electrostatic interaction between the C(5)O(5) and the N(4)H(4) bond dipoles. The OH group in an equatorial orientation at C(6) experiences an unfavorable electrostatic interaction with the N(1)H(1) dipole since the direction of the N(1)H(1) bond dipole is opposite to that of the C(4)O(4) bond. To minimize this unfavorable interaction the OH bond dipole is rotated away from the N(1)H(1) bond as is illustrated by the structure of 6-OH_{eq}-5,6-dihydrouracil in Fig.(2b).

The calculated conformational preferences described above agree very well with x-ray crystallographic data on *cis*- thymidine glycol ²⁹ which show both the 5-CH₃ and 6-OH groups in the pseudo axial orientation. In addition, an axial orientation of OH at C(6) is suggested by NMR spectra on a variety of 5,6-saturated derivatives of uracil ³⁰ and thymine ³¹. The structural preferences calculated for 5,6-dihydrothymine are also in good agreement with x-ray ³² and NMR data ³³ which indicate that the methyl group at C(5) prefers the pseudo equatorial orientation.

Our calculations predict the changes in pyrimidine structure that are expected from saturation of the C(5)C(6) bond. The bond length increases by about 0.2 Å and bond angles at C(5) and C(6) decrease to approximately the tetrahedral values. The pyrimidine ring adopts a half-chair conformation, with C(5) and C(6) on opposite sides of the mean plane passing through the N(1)C(2)N(3)C(4) atoms. The rms deviation of these atoms from the calculated mean plane was between 0.02 and 0.06 Å for all of the calculated structures. Calculated displacements of the C(5) atom from this plane were between 0.22 and 0.27 Å. The C(6) atom is displaced between 0.43 and 0.48 Å in the opposite direction.

In spite of the changes in the C(5)C(6) region, the structure of the base-pairing regions of the pyrimidine bases is very well preserved in the ring-saturated pyrimidine lesions that we have studied. Cytosine derivatives may be an exception to this general finding. Small distortions in the geometry of the amino group may contribute to a slight weakening of the hydrogen bond to guanine. Changes of the electronic charge distribution in the base-pairing

regions are negligible in all cases. Based on these results, one may expect a low mutagenic potential for ring-saturated pyrimidine lesions; nevertheless, it should be noted that mutagenicity can be caused by steric distortions of DNA as well as by changes in base-pairing ability of the pyrimidine base itself.

Variations in the both magnitude and the direction of pyrimidine-base dipole moments result from saturation of the C(5)C(6) bond; however, we do not believe that these changes in base dipole moments are the source of the biological effects observed from these ring-saturated pyrimidine lesions. The molecular dynamics simulations of a DNA dodecamer with different thymine lesions described in the next section strongly support the idea that nonplanarity is the primary source of these effects. Our results particularly emphasize the importance of hydroxylated thymine lesions. In contrast to hydroxylated derivatives of cytosine and uracil where the axial position at C(5) is always occupied by a hydrogen atom, this position is occupied by a much bulkier methyl group when an OH group is present at C(5) of thymine. This means that larger DNA distortions are anticipated for thymine glycol and 5-hydroxy-5,6-dihydrothymine than for cytosine lesions and other thymine lesions. These conclusions from our quantum calculations, which are consistent with the experimental data on biological activity discussed above, have been tested by molecular dynamics simulations that are described in the next section.

MOLECULAR DYNAMICS SIMULATION OF DNA CONTAINING RING-SATURATED THYMINE LESIONS

The quantum-chemical calculations discussed above provide useful information about the structure and properties ring-saturated pyrimidine lesions that is not easily available experimentally. They also provide the input data needed for molecular dynamics simulations (MDS) of DNA sequences containing these lesions. The dodecamer d(CGCGAATTCGCG)₂ was chosen for these studies because this sequence, which contains the EcoRI recognition site, has been extensively studied experimentally in both solid state and solution as well as being the subject of numerous MDS, including our own work ³⁴

Starting from the ideal right-handed Arnott B-DNA geometry, MDS have been carried out on the dodecamer d(CGCGAATTCGCG)₂ with T7 replaced by one of the following lesioned thymine bases: 5-hydroxy-5,6-dihydrothymine, 6-hydroxy-5,6-dihydrothymine, 5,6-dihydroxy-5,6-dihydrothymine and 5,6-dihydrothymine. The lesions were taken in their most stable conformations as predicted by the *ab initio* quantum-chemical calculations described in the previous section. Atomic charges were obtained by fitting to the electrostatic potential evaluated from the quantum-chemical wavefunction. Energy minimization and MDS were performed with the AMBER4.0 program ³⁵ using the standard unmodified all-atom force-field parameters for nucleic acids included in the program. The ring geometry of lesions was constrained to the half-chair geometry predicted by our quantum-chemical calculations and observed in crystal structures of thymine glycol and 5,6-dihydrothymine. When this constraint is removed, MDS of DNA containing these lesions predict significant distortions of the ring if the standard AMBER force field parameters are used with sp³-type carbon atoms at C(5) and C(6) instead of the native sp² type. Existing experimental data are not adequate to determine if these distortions are reasonable; therefore, while we continue to carry out simulations with constraints on the ring geometry of lesions, we are also developing new force field parameters for the ring-saturated pyrimidines that are based on quantum-chemical calculations of the barriers for the ring inversion in saturated pyrimidines.

22 Na⁺ ions were placed around the DNA dodecamer containing the modified thymine base and the system of DNA and ions was placed in a rectangular cube of water molecules. Periodic boundary conditions were applied within a constant volume algorithm. After minimization, the system was heated from 0 to 300K in ten 0.2 ps runs followed by a 1.0 ps run at 300K. This initial 3.0 ps of simulation insures equilibration of the system and removes steric strain that could remain after minimization. After 'heating', MDS were carried out for 120 ps using the constant temperature algorithm with a bath temperature of 300K. The final 60 ps of the simulations were used for structural analysis.

As illustrated in Fig.(3), ring-saturated pyrimidine lesions can be incorporated into DNA with the axial substituent at C(5) pointing in either the 5' or the 3' direction of the damaged strand. In isolation, as modeled by the quantum-chemical calculations, these two conformations of the modified base are enantiomers; however, inside DNA the two configurations will have different energies. Interactions of the modified base with other components of DNA are very different for these two configurations. An axial substituent at C(5) pointing in the 3' direction will be located in the major groove of DNA whereas this substituent pointing in the 5' direction will result in significant steric clashes with the adjacent base.

Most of the MDS that we have carried out thus far have been for conformation **A** shown in Fig.(3). In this case, C(5) is displaced from the base plane in the 5' direction and C(6) is displaced in the 3' direction of damaged strand. The accessibility of DNA bases to free-radical attack should favor conformation **A** for the following reasons. Since $\cdot\text{OH}$ and $\cdot\text{H}$ addition to pyrimidine bases is an attack on the π -electron system of a double bond, transition-state calculations (Miaskiewicz & Osman, prepared for publication) show that the radicals must approach from a direction that is perpendicular to the base plane. Molecular modeling of the accessibility of bases in B-DNA readily shows that radicals must approach the base from the 3' direction to satisfy the requirements on transition states for addition reactions. Hence, stable products in the **A**-type configuration with hydroxyl groups in the 3' direction should be more abundant. Details of the stereospecific mechanism leading to the formation of *cis*-thymine glycol in the **A** conformation are illustrated in Fig(4).

Production of pyrimidine lesions in the **B** configuration cannot be totally excluded because experimental data indicate the presence of two stereoisomers of *cis*-thymine glycol inside DNA ^{36, 37}, presumably structures **A** and **B** in Fig(3). Structure **B** could result from $\cdot\text{OH}$ -radical attack on DNA in a distorted geometry that allows accessibility to thymine from the 5' direction. Alternatively, an epimerization process like that suggested by Lustig et al. ³⁸ might explain the production of both stereoisomers by $\cdot\text{OH}$ radicals approaching from the 3' direction. Hence, while the remainder of this section is primarily devoted to

MDS of DNA containing ring-saturated thymine lesions in type **A** configurations, the final paragraph will give a comparison of results for thymine glycol in both of the configurations shown in Fig.(3).

Since detailed analysis of a simulation is rather voluminous, only the most important results will be presented here. Surprisingly, all four of the ring-saturated thymine lesions that we have studied can be incorporated into DNA without significant perturbation of the pattern of backbone torsional angles. As illustrated in Fig.(5) for thymine glycol, the torsional angles around the lesion do not differ from those of other nucleotides in the dodecamer and are in the range expected for right-handed DNA structures. Hence, the DNA structure must distort in some other way to accommodate these modified bases. Fig.(6) shows that the **Rise** distance between the stacked bases of the damaged strand is dramatically affected by the bulky non-planar lesions. For thymine glycol and 5-hydroxy-5,6-dihydrothymine, which have a pseudo axially oriented methyl group, the increase in **Rise** between A6 and T7* is accompanied by a decrease in the **Rise** between T7* and T8. This effect is smaller for thymine hydrate and 5,6-dihydrothymine where the axially oriented substituent at C(5) is a less bulky hydrogen atom.

The time course of the length of Watson-Crick hydrogen bonds in base pairs at or near the lesion is shown in Fig.(7). All of the hydrogen bonds in a given base pair show essentially the same time dependence. Surprisingly, we find greater perturbation of the hydrogen bonds in the A6-T19 base pair than at the site of the lesion in all four cases. Since the largest perturbations of base pairing occur on the 5' side of the lesion and are most pronounced when the dodecamer contains thymine glycol or 5-hydroxy-5,6-dihydrothymine, it is reasonable to associate these effects on base pairing with the pseudo axially oriented substituents at C(5). Clearly, base-stacking will also be compromised in the vicinity of the lesion due to the increase in rise for the A6/T7* step and the simultaneous decrease for the T7*/T8 step that were discussed above in connection with the results shown in Fig.(6).

The pattern of DNA perturbation observed in our MDS is qualitatively similar for the four lesions; however the magnitude of the distortions clearly distinguishes thymine glycol

and 5-hydroxy-5,6-dihydrothymine from thymine hydrate and 5,6-dihydrothymine. The major structural difference between these lesions is the orientation of the methyl group at the C(5) position of the thymine ring, which is axial for the former pair and equatorial for the latter. Axial substituents at C(5) are perpendicular to the base plane and cause significant steric clashes with the adjacent base in the 5' direction, as is illustrated in Fig.(8). To accommodate these axial substituents the distance between the bases must increase significantly, as is apparent for the time dependence of the rise parameters shown in Fig.(6). This in turn perturbs the hydrogen bonding for the base pair located 5' to the lesion. Since a methyl group is significantly more bulky than a hydrogen atom, these distortions of DNA structure are significantly larger for thymine glycol and 5-hydroxy-5,6-dihydrothymine than they are for thymine hydrate and 5,6-dihydrothymine. These results correlate very well with the biological consequences of the lesions described in the INTRODUCTION.

Variations in the **Rise** coordinate and the length of Watson-Crick hydrogen bonds observed in MDS of d(CGCGAATgTCGCG)₂ when the axial methyl group of thymine glycol is oriented in the 3' direction of the damaged strand are shown in Fig.(9). In this case, perturbations in **Rise** occur primarily between T8 and Tg7 but they are significantly smaller than that shown in Fig.(6) for the **Rise** between Tg7 and A6 when methyl group of thymine glycol is orientated in the 5' direction. Hydrogen bond distances in T8-A17 are not affected by these smaller changes in **Rise** so that, unlike the stereoisomer of thymine glycol with methyl pointing in the 5' direction, the major effect on base pairing is at the lesion site. These results clearly show the importance of the conformation adopted by ring-saturated pyrimidine lesions when formed inside DNA. An axial OH at C(6) pointing in the 5' direction produces significantly less distortion on the 5' side of the lesion than an axial methyl group at C(5) with a similar orientation. Hence, for both stereoisomers of thymine glycol, the hydroxyl groups are mainly accommodated in the major groove of the double helix where they are easily accessible to water of hydration. On average, about 3 water molecules are found in close proximity to the hydroxyl substituents of thymine glycol at C(5) and C(6). The

effect of these substituents on the electrostatic potential in the groove may play a role in recognition of the lesion by repair enzymes.

RECOGNITION OF RING-SATURATED PYRIMIDINE LESIONS BY ENDONUCLEASE III.

Several features of endonuclease III facilitate molecular modeling of complexes between this *Escherichia coli* repair enzyme and DNA substrates that contain ring-saturated and ring-fragmented pyrimidine lesions. First, the enzyme does not require any cofactors and is active only on double-stranded DNA. Second, thymine glycol is a very effective inhibitor of the binding of endonuclease III to DNA containing lesions that are normally recognized by the enzyme ²². This suggests that thymine glycol and lesioned DNA have the same binding site. Third, the binding site of thymine glycol has been identified crystallographically ²⁶ to be a β -hairpin loop composed of amino-acid residues 113 through 119. This binding loop lies on a surface of the enzyme with a positive electrostatic potential that will engage long-range electrostatic interactions with negatively charged DNA. Finally, a high-resolution x-ray crystallographic structure of endo III has been determined ²⁶ and atomic coordinates derived from this structure were kindly provided to us by Dr. John A. Tainer of the Scripps Research Institute.

The first step in our modeling strategy to take advantage of these features is to investigate the interaction of thymine glycol (Tg) and the enzyme. The electrostatic potential at the surface of the protein calculated by the simplified DelPhi algorithm ³⁹ included in the program GRASP by Anthony Nicholls, shows that while the vicinity of the loop has a highly positive potential, a region of negative potential in the center of the loop can be observed, that could enable efficient interaction with the positive charge on the hydroxyl groups of Tg. Hence, we performed docking calculations between Tg and endonuclease III followed by energy minimizations performed with the AMBER force field. In all of the binding configuration that were identified, at least one of the hydrogen bonds involved the C=O groups of Glu 112, Leu 114, or Val 117. The location of these and other carbonyl groups on the exposed side of the binding loop is shown in Fig.(10).

Three binding configurations for thymine glycol found by our procedure are shown in Fig.(11) along with their energies of interaction with the loop and with the entire protein. The most stable, configuration **A**, has hydrogen bonds between the hydroxyl groups of Tg and C=O groups of Leu 114 and Val 117 with lengths of 2.5 and 2.7 Å, respectively. In configuration **B**, the hydrogen bond with the C=O group of Val 117 is replaced by a hydrogen bond with an N-H group of Gly 116. In configuration **C**, which is stabilized primarily by a hydrogen bonds between 6-OH and a C=O group of Glu 112, the N(1) position of the pyrimidine ring lies close to Glu 112 and the pseudo axial methyl at C(5) points into the loop. This contrast with configurations **A** and **B** for which N(1) is near Gly 118 and the 5-Me_{ax} of Tg points out of the loop. Configuration **C** might be more favorable to the catalytic activity of endonuclease III than **A** or **B**, since Glu 112 and Lys 120 are currently the most likely candidates for the N-glycosylase and AP lyase activities, respectively. The advantage of **C** for AP lyase activity centered on Lys 120 is a consequence of the fact that the results illustrated in Fig.(11) are for (-)cis-5(R),6(S)-5,6-dihydroxy-5,6-dihydrothymine. Inside DNA, this stereoisomer of Tg will have the methyl group pointing in the 5' direction of the damaged strand; hence, the 3' side of the lesion, where the backbone will be cleaved by β -elimination, will be oriented in the direction of Lys 120. Docking calculations with (+)cis-5(S),6(R)-thymine glycol also revealed a binding configuration with these same advantages for the catalytic activity of the enzyme.

Fig.(12) shows the spatial relationship between a typical binding configuration of Tg and features of endonuclease III that are likely to be involved in interactions with DNA sequences. The second step in our modeling strategy is to superimpose Tg in the binding loop with the lesion in the dodecamer d(CGCGAATgTCGCG)₂ from our MDS. Superposition of this DNA dodecamer on the Tg in its most stable configuration (**A** in Fig.(11)) gives a very reasonable starting structures for modeling the DNA-endonuclease III complex. DNA faces the part of the enzyme that shows positive electrostatic potential. The interaction between α -helix 82-99 and the major groove of DNA can be also seen; however, steric clashes in the neighborhood of Lys 120 tend to cause intertwinement of the backbones of enzyme and

substrate. To avoid this problem, the damaged DNA sequence must be shifted away from the protein. Refinement of the starting structures formed this way, lead to structures with the Tg of lesioned DNA much farther from the binding loop of the enzyme than is the case for binding of Tg in the absence of DNA. Hence, successful application of our modeling strategy will probably require molecular dynamics simulations to investigate the flexibility of the protein structure, especially in the neighborhood of the binding loop, and the dynamics of the structures of both enzyme and DNA during the complex formation.

Tg also inhibits the binding of urea-containing DNA by endonuclease III ²². Thus, it is reasonable to expect that the binding of urea-containing DNA to endonuclease III is similar to that for Tg-containing DNA. If the nonspecific global interaction of endonuclease III with d(CGCGAATTCGCG)₂ is independent of which of the two lesions (urea or Tg) replaces T7 in the dodecamer, then the optimum placement of the dodecamer in the complex with DNA containing Tg should provide a starting point for modifications in the neighborhood of the urea residue to achieve significant binding with the flexible β -hairpin loop. Modeling strategies of this type will be used to address the relative affinity of endonuclease III for different pyrimidine lesions.

CONCLUSIONS:

Our approach to modeling structure-function relationships for damaged DNA sequences, that is based on quantum-chemical analysis of lesions in isolation followed by investigations of their impact on DNA structure through the use of semiempirical force fields, has several advantages over approaches that use NMR data as a starting point. The most obvious of these is that it can be immediately applied to lesions of interests for which NMR data are not currently available. An approach that does not require an experimental starting structure has the additional advantage that it can provide information on a given lesion that is not readily available from experiments and insights that come from comparison with closely related lesions. This advantage is illustrated in the current work by the *ab initio* quantum results that reveal a relatively simple energetic basis for structural

preferences among the many possible stereoisomers of ring-saturated pyrimidine lesions. General principles that emerge from investigation of related lesions can suggest new experiments to test the theory. The strong energetic preference for equatorially orientated OH at C(5) and the implications of this preference for the difference in perturbations of DNA structure by thymine and cytosine glycol is an example of this process. Finally, an approach that does not require an experimental starting structure can more easily investigate the effects of a lesion on different DNA sequences.

The main disadvantages of our approach are the uncertainty of existing force fields for nucleic acids, the limited ability of MDS to sample the conformation space of solvated macromolecules with existing computer resources, and the limited opportunity to check intermediate results by comparison with experimental data. Generally, the available experimental structures for modified pyrimidine nucleosides show that the results of our quantum calculations are very reasonable but direct comparison of theory and experiment is not possible without consideration of the effects of the ribose moiety. As sample preparation techniques improve and the availability of NMR spectrometers increases, we expect to make more exacting tests of our modeling approach.

ACKNOWLEDGEMENTS

This work was supported by the Office of Health and Environmental Research (OHER) of the United States Department of Energy under contract DE-AC06-76RLO 1830 and DOE grant DE-FG02-88ER60675. We also gratefully acknowledge receiving atomic coordinates for endonuclease III from Dr. John Tainer of The Scripps Research Institute in La Jolla California.

REFERENCES

1. LUTZ, W. K. 1990. Mutation Res. **238**: 287-295.
2. AMES, B. N. 1989. Mutation Res. **214**: 41-46.
3. AMES, B. N. 1989. Environ. Mol. Mutagen. **14 (Suppl.16)**: 66-77.

4. AMES, B. N. & L. S. GOLD. 1991. Mutation Res. **250**: 3-16.
5. TÉOULE, R. 1987. Int. J. Radiat. Biol. **51**: 573-589.
6. DIZDAROGLU, M. 1991. Free-Radical Biol. Med. **10**: 225-242.
7. LEADON, S. A. & P. C. HANAWALT. 1983. Mutation Res. **112**: 191.
8. BREIMER, L. H. & T. LINDAHL. 1985. Biochemistry **24**: 4018.
9. BOORSTEIN, R. J., T. P. HILBERT, R. P. CUNNINGHAM & G. W. TEEBOR. 1990. Biochemistry **29**: 10455-10460.
10. GANGULY, T. & N. J. DUKER. 1991. Nucleic Acids Res. **19**: 3319-3323.
11. QUINLAN, G. J. & J. M. C. GUTTERIDGE. 1991. Biochem. Pharmacol. **42**: 1595-1599.
12. IDE, H., Y. W. KOW & S. S. WALLACE. 1985. Nucleic Acids Res. **13**: 8035-8052.
13. CLARK, J. M. & G. P. BEARDSLEY. 1986. Nucleic Acids Res. **14**: 737-749.
14. CLARK, J. M. & G. P. BEARDSLEY. 1987. Biochemistry **26**: 5398-5403.
15. HAYES, R. C. & J. E. LECLERC. 1986. Nucleic Acids Res. **14**: 1045-1061.
16. IDE, H., L. A. PETRULLO, Z. HATAHET & S. S. WALLACE. 1991. J. Biol. Chem. **266**: 1469-1477.
17. GANGULY, T. & N. J. DUKER. 1992. Mutation Res. **293**: 71-77.
18. IDE, H., R. J. MELAMEDE & S. S. WALLACE. 1987. Biochemistry **26**: 964-969.
19. SANCAR, A. & G. B. SANCAR. 1988. Annu. Rev. Biochem. **57**: 29-67.
20. LIN, J. & A. SANCAR. 1989. Biochemistry **28**: 7979-7984.
21. KOW, Y. W., S. S. WALLACE & B. VAN HOUTEN. 1990. Mutation Res. **235**: 147-156.
22. KOW, Y. K. & S. S. WALLACE. 1987. Biochemistry **26**: 8200-8206.
23. DEMPSE, B. & S. LINN. 1980. Nature **287**: 203-208.
24. BOORSTEIN, R. J., T. P. HILBERT, J. CADET, R. P. CUNNINGHAM & TEEBOR, G. W. 1989. Biochemistry **28**: 6164-6170.
25. GANGULY, T., K. M. WEEMS & N. J. DUKER. 1990. Biochemistry **29**: 7222-7228.
26. KUO, C.-F., D. E. MCREE, C. L. FISHER, S. F. O'HANDLEY, R. P. CUNNINGHAM & J. A. TAINER. 1992. Science **258**: 434-440.

27. FRISCH, M. J., M. HEAD-GORDON, G. W. TRUCKS, J. B. FORESMAN, H. B. SCHLEGEL, K. RAGHAVACHARI, M. ROBB, J. S. BINKLEY, C. GONZALEZ, D. J. DEFREES, D. J. FOX, R. A. WHITESIDE, R. A. R. SEEGER, C. F. MELIUS, J. BAKER, R. L. MARTIN, L. R. KAHN, J. J. P. STEWART, S. TOPIOL & J. A. POPLE. 1990. GAUSSIAN 90. Gaussian, Inc., Pittsburgh, PA.
28. RASHIN, A. & B. HONIG. 1985. J. Phys. Chem. **89**: 5588-5593.
29. HRUSKA, F. E., R. SEBASTIAN, A. GRAND, L. VOITURIEZ & J. CADET. 1987. Can. J. Chem. **65**: 2618-2623.
30. DUCOLOMB, R., J. CADET, C. TAIEB & R. TEOULE. 1976. Biochim. Biophys. Acta **432**: 18-27.
31. CADET, J., R. DUCOLOMB & F. E. HRUSKA. 1979. Biochim. Biophys. Acta **563**: 206-215.
32. KONNERT, J., I. L. KARLE & J. KARLE. 1970. Acta Cryst. **B26**: 770-778.
33. CADET, J., L. VOITURIEZ, F. E. HRUSKA, S.-L. KAN, F. A. A. M. DE LEEUW & C. ALTONA. 1985. Can. J. Chem. **63**: 2861-2868.
34. MIASKIEWICZ, K., R. OSMAN & H. WEINSTEIN. 1993. J. Am. Chem. Soc. **115**: 1526-1537.
35. PEARLMAN, D. A., D. A. CASE, J. C. CALDWELL, G. L. SEIBEL, U. CHANDRA SINGH, P. WEINER & P. A. KOLLMAN. 1991. AMBER4.0. Univ. of California, San Francisco, CA.
36. SHARMA, M., H. C. BOX & D. J. KELMAN. 1990. Chem.-Biol. Interactions **74**: 107-117.
37. TEEBOR, G. W., A. CUMMINGS, K. FRENKEL, A. SHAW, L. VOITURIEZ & J. CADET. 1987. Free Rad. Res. Commun. **2**: 303-309.
38. LUSTIG, M. J., J. CADET, R. J. BOORSTEIN & G. W. TEEBOR. 1992. Nucleic Acids Res. **20**: 4839-4845.
39. NICHOLLS, A. & B. HONIG. 1991. J. Comp. Chem. **12**: 435-445.
40. SAENGER, W. 1984. Principles of Nucleic Acid Structure. Springer-Verlag, New York.
41. RAVISHANKER, G., S. SWAMINATHAM, D. L. BEVERIDGE, R. LAVERY & H. SKLENAR. 1989. J. Biomol. Str. Dyn. **6**: 669.

FIGURE CAPTIONS

1. Relationship between stereoisomers of 5,6-dihydroxy-5,6-dihydrothymine (thymine glycol). Results of electronic structure calculations carried out on the four isomers on the left side of the figure are given in Table 1 under the notation 5-OH_{eq}-6-OH_{eq}, 5-OH_{ax}-6-OH_{ax}, 5-OH_{eq}-6-OH_{ax}, and 5-OH_{ax}-6-OH_{eq}. The degeneracy of enantiomeric pairs in isolation will be removed in the asymmetric environment of a DNA macromolecule.

2. Intramolecular interactions between equatorial substituents in 5-OH_{eq}-5,6-dihydrouracil (a) and in 6-OH_{eq}-5,6-dihydrouracil (b).

3. Schematic of **cis**-5R-6S (A) and **cis**-5S-6R (B) stereoisomers of thymine glycol in DNA. Although they are degenerate in isolation, the isomers perturb DNA structure in different ways due to the difference in the interaction of axial substituents with neighboring bases.

4. Schematic of the reaction pathway leading to **cis**-5R-6S thymine glycol in DNA. (I) OH addition from the accessible 3' side gives an intermediate radical with spin density mainly on C(6). (II) Energetically preferred ring inversion allows oxygen to approach the radical intermediate from the accessible 3' side. (III) O₂ addition at C(6) gives the more stable peroxy radical. (IV) Further transformations of peroxy radical leads to the stable product.

5. Time sequence of backbone torsional angles in a molecular dynamics simulation of d(CGCGAATTCGCG)₂ with T7 replaced by thymine glycol. The standard Greek-letter designation for DNA backbone torsional angles⁴⁰ is used to identify the column of dials⁴¹ that show the time course of the indicated torsion angle for the nucleotides in the lesioned strand of the duplex. The columns labeled by χ and ϕ show results for the glycosyl torsional angle and the pseudorotation of the sugar pucker, respectively. The radial coordinate is the time variable and the initial value of the torsional angle is indicated by a line from the center to the edge of the dial. Similar lines in the dials at the bottom of each column show the standard values for A and B-DNA.

6. Time sequence of rise coordinates in $d(\text{CGCGAATTCGCG})_2$ when T7 is replaced by a ring-saturated thymine lesion. Time increases from 60 to 120 ps between the bottom and the top of the windows. Horizontal axis is the rise in Angstroms for the indicated dinucleotide step.

7. Time sequence of Watson-Crick hydrogen bonds in $d(\text{CGCGAATTCGCG})_2$ when T7 is replaced by a ring-saturated thymine lesion. Time increases from 60 to 120 ps between the bottom and the top of the windows. Horizontal axis is the separation in Angstroms between the hydrogen atom and the heavy atom involved in hydrogen bonding. A solid and a dotted line are used to illustrate the two hydrogen bonds in A-T base pairs. A heavy line is added for the third hydrogen bond in the C-G base pair.

8. Snapshots showing the structure of DNA around the lesion site after 100 ps of molecular dynamics simulation on $d(\text{CGCGAAT}^*\text{TCGCG})_2$, where T^* denotes a ring-saturated thymine base.

9. Fluctuations in rise and the length of Watson-Crick hydrogen bonds near the lesion in $d(\text{CGCGAAT}^*\text{TCGCG})_2$, where T^* is *cis*-5S-6R thymine glycol with the pseudo axial methyl group pointing in the 3' direction of the damaged strand. The methods used to display of the time sequences are the same as those described in Figs.(6) and (7).

10. Orientation of C=O groups in the β -hairpin binding loop of endonuclease III with the greatest potential for hydrogen bonding with hydroxyl groups of thymine glycol. Dark filled circles indicate oxygen atoms in these groups that lie over the interior of the loop. Lighter filled circles denote oxygen atoms on the periphery of the binding loop but also on the surface that is accessible to DNA.

11. Three orientations of *cis*-5R-6S thymine glycol bound to the β -hairpin loop of endonuclease III found in energy minimizations. Thymine glycol-protein and thymine glycol-loop interaction energies are in kcal/mole.

12. Spatial relationship for the most stable binding configuration found for *cis*-5R,6S thymine glycol and structural elements of endonuclease III believed to be important in recognition of DNA damage.

Fig. 1

a)

b)

Fig. 3

Fig.4

Fig. 5

Fig 6

Fig. 17

5,6-dihydroxy-
5,6-dihydrothymine

5-hydroxy-
5,6-dihydrothymine

6-hydroxy-
5,6-dihydrothymine

5,6-dihydrothymine

RIS

Hbonds

Fig. 10

Fig. 11

**DATE
FILMED**

5/23/94

END