Modeling and Molecular Mechanical Studies of the *cis*-Thymine Glycol Radiation Damage Lesion in DNA†

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ABSTRACT: Computer graphics and energy minimization techniques were used to construct a model of DNA containing *cis*-thymine glycol, an oxidation product of thymine formed in DNA by ionizing radiation. The model simulated an experimental DNA substrate used to study the effects of this lesion on DNA synthesis in vitro. The results derived from the model indicate that *cis*-thymine glycol lesions introduce localized perturbations of DNA structure. Specifically the model shows that interactions with the neighboring base pair on the 5′ side are significantly destabilized by thymine glycol whereas interactions with the 3′ base pair are stabilized by the lesion. The magnitude of these effects is modulated by the nucleotide sequence around the lesion, particularly by the nature of the base on the 3′ side. The base pair formed between adenine and thymine glycol is energetically stable and shows minimal distortion, suggesting that this lesion retains the ability to direct the insertion of the correct nucleotide during DNA synthesis.

Structural alteration of DNA bases caused by radiation or chemical carcinogens constitutes a significant hazard to living organisms. Such lesions, if unrepaired, can lead to lethality, mutagenesis, or carcinogenesis (Trosko & Chang, 1981). The biochemical pathways that lead from the initial DNA lesion to the observed biological end points are not well understood; however, the ability of cells to replicate DNA that contains structural abnormalities clearly plays an important role in these processes (Strauss, 1985). Damaged DNA bases produced by numerous agents have been shown to impair the ability of DNA to serve as a template for replication by DNA polymerases (Moore & Strauss, 1979; Moore et al., 1982; Piette & Hearst, 1983; Pinto & Lippard, 1985; Clark & Beardsley, 1986). At present, however, little is known about the precise molecular basis for the inhibitory effects that such lesions have on DNA synthesis. To achieve such an understanding requires a detailed knowledge of both the structural perturbations introduced by the lesion and the effects that these perturbations have on the interaction between the polymerase and its DNA substrate.

Most of the detailed structural data about lesions in DNA has come from the application of physical methods such as X-ray crystallography and NMR spectroscopy to simple model compounds. However, the direct application of such methods to DNA containing structural abnormalities has been limited by the difficulties involved in preparing sufficient quantities of a homogeneous DNA fragment with the lesion at a specific site. As an alternative approach, X-ray crystallographic data from model compounds have been used in conjunction with molecular model building to deduce the effects on DNA structure of lesions such as the thymine photodimer or psoralen cross-link (Broyde et al., 1980; Rao et al., 1984; Pearlman et al., 1985). In this paper we present a molecular model and energy calculations for a DNA structure in which a single *cis*-thymine glycol lesion (t′)[1] has been introduced near the 5′ end of the strand corresponding to the template for DNA synthesis. In the accompanying paper (Clark & Beardsley, 1987), we present the results of DNA synthesis assays carried out with synthetic oligodeoxynucleotide primer/template substrates that correspond to several of the sequences used in the modeling studies. The results of the modeling studies help to explain the structural and physicochemical basis for the biochemical effects of the lesion.

The cis and trans isomers of thymine glycol represent a significant component of oxidative damage produced in DNA by ionizing radiation (Teoule & Cadet, 1978). We (Clark & Beardsley, 1986, 1987) and others (Rouet & Essigmann, 1985; Ide et al., 1985; Hayes & LeClerc, 1986) have shown that the cis isomer of thymine glycol arrests synthesis by procaryotic DNA polymerases in vitro. Moreover, the structure of the cis isomer of thymine glycol has been determined by X-ray crystallography (Flippen, 1973); thus coordinate data were available to which energy minimization and model building techniques could be readily applied.

Model Building and Molecular Mechanical Studies

Construction of Models. All molecular models were displayed on an Evans & Sutherland PS2 system at the Computer Graphics Laboratory at the University of California (San Francisco); the modeling program MIDAS was used (Jarvis et al., 1984). Results obtained from a corresponding experimental system indicate that DNA polymerases can insert nucleotides before and opposite the site of the thymine glycol but are unable to add the next nucleotide beyond the lesion (Clark & Beardsley, 1987). The model was designed specifically to evaluate the structural basis for the observed effects

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[1] Abbreviation: t′, thymine glycol.

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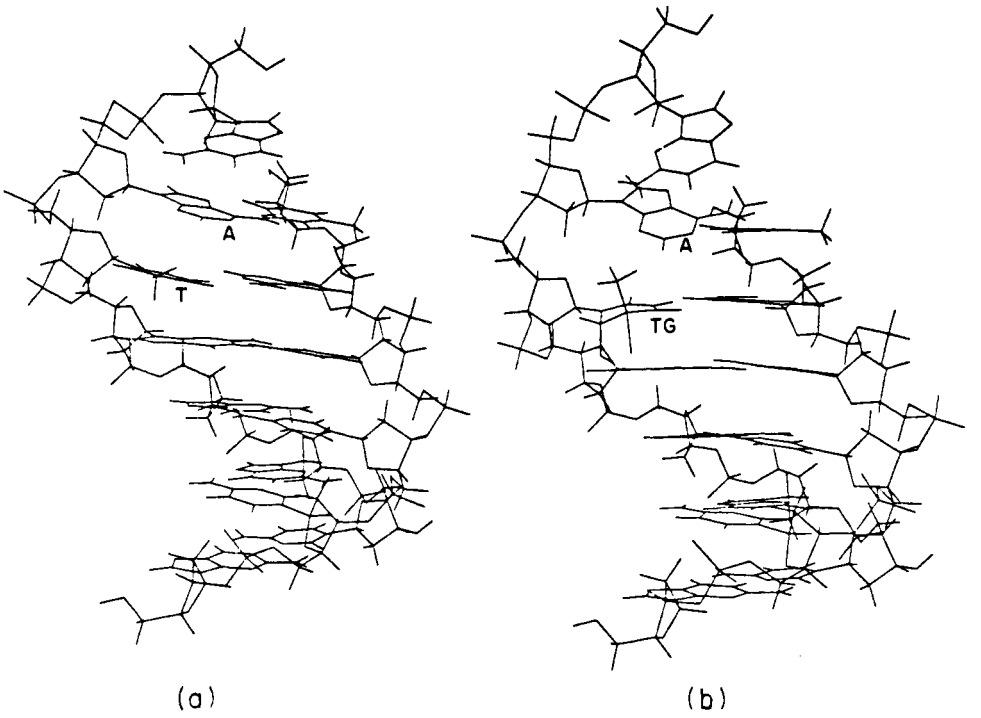

FIGURE 1: A portion of the energy-minimized DNA structures containing (a) thymine or (b) thymine glycol. The sequence shown is

5′ GCCCAT 3′
3′ CGGGTAG 5′

of this lesion on DNA synthesis. The models were constructed as follows. Segments of DNA (not energy minimized), 18 base pairs in length, were generated by using standard coordinates for the four normal bases in a B DNA conformation (Arnott & Hukins, 1972). The 3′-terminal nucleotide of the "primer" strand was then deleted to form a partial duplex:

5′ CCTTTTCGTCGGCCCAT 3′
3′ GGAAAAGCAGCCGGGTAG 5′

The structure served as the basic model from which all subsequent models were derived. The model was constructed in this particular fashion to study the stability of base pairs on either side of thymine glycol, in a structure that would necessarily be formed as an intermediate during replication past this lesion. The thymine glycol lesion was introduced by replacing the coordinates of the base moiety of the single thymidine residue in the "template" (bottom) strand with the corresponding coordinates for thymine glycol obtained from X-ray crystallography (Flippen, 1973). Partial charges were calculated for thymine glycol by use of the program Gaussian 80—UCSF (Singh & Kollman, 1984). The replacement was done by superimposing thymine glycol on thymine and matching the coordinates of the four least perturbed atoms of the thymine glycol ring (N_1, C_2, N_3, C_4) to the corresponding atoms of thymine.

The potential energy function for the molecule was then minimized by using the AMBER molecular mechanics program (Weiner et al., 1986) in the absence of solvent molecules and without constraining the thymine glycol structure to the crystallographic coordinates. For the thymine glycol moiety the constants were taken from the X-ray crystal structure (Flippen, 1973). The various constants and functions required were all taken from Weiner et al. (1984), including values for the sugar ring parameters and a distance-dependent function for the dielectric constant used in calculating the electrostatic interaction energy. No other input parameters were adjusted. Only the terminal duplex portion of the model (underlined above) was included in the calculations. The minimization was carried out iteratively until the root-mean-square value was less than 0.1 kcal/(mol A). Following the minimization, the revised structure was displayed on the graphics terminal.

The above model has a purine base on both sides of the thymine glycol. Substitution of a pyrimidine for a purine represents the greatest possible change in local DNA structure both sterically and in terms of the stacking interactions between neighboring base pairs. Therefore, additional models were also generated in which the sequence context around the lesion was altered by systematically changing one or the other of the purine bases (G or A) on either side of the lesion to a pyrimidine (C) prior to the energy minimization.

RESULTS

Structural Features of the Model. A portion of the energy-minimized DNA structures obtained with thymine glycol or the natural base thymine is shown in Figure 1. This particular sequence has an A·T base pair on the 5′ side of the lesion (the polarity is defined with respect to the lesion-containing strand). Although the molecular mechanical calculations were carried out without constraining the thymine glycol structure to the values specified by the crystallographic data, the structural parameters derived for the altered base in DNA differ only slightly from the values determined for free thymine glycol. The methyl group of the glycol remains in an axial configuration with respect to the (nonplanar) pyrimidine ring while the *cis*-hydroxyl groups project out into the major groove of the helix. The DNA molecules into which the lesion is introduced undergo several significant structural changes. These changes occur primarily in close proximity to thymine glycol, and their magnitude depends upon the particular sequence context surrounding the lesion. The most striking effect is the large increase in the tilt angle of the template base immediately 5′ to the lesion site when thymine glycol replaces thymine. This effect is more readily apparent in Figure 2, which shows the van der Waals surfaces for

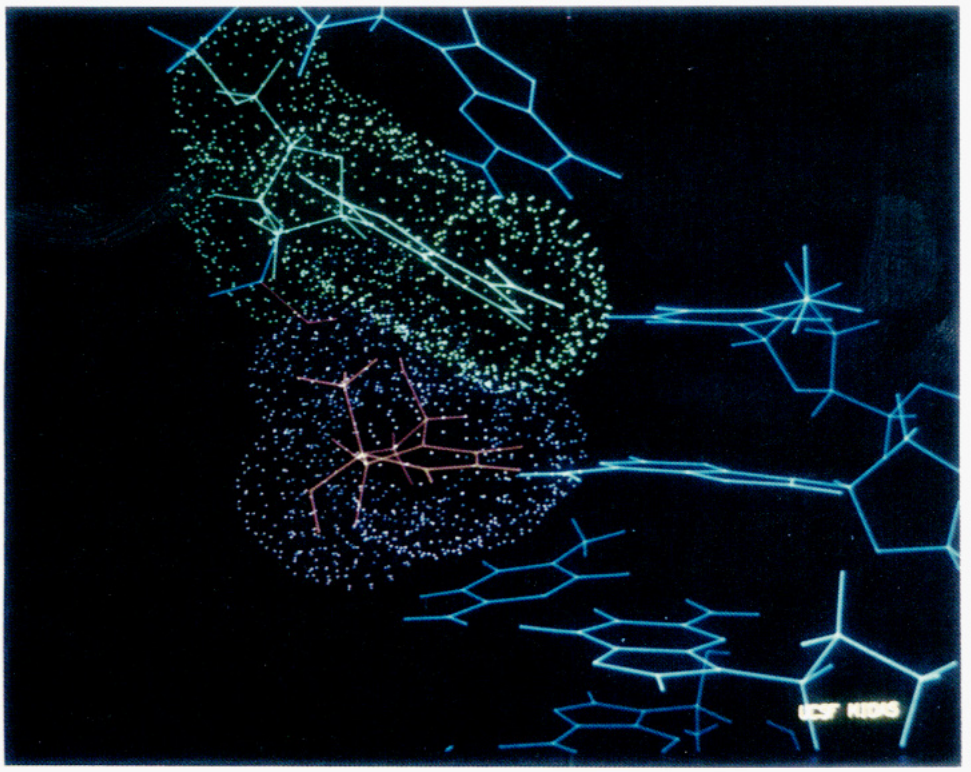

FIGURE 2: Detailed view showing the van der Waals surfaces of thymine glycol and its 5′ neighbor, adenine. The sequence shown is identical with that shown in Figure 1 except that the G·C base pair immediately 3′ to the lesion has been replaced by a C·G base pair, i.e., C replaces G 3′ to thymine glycol.

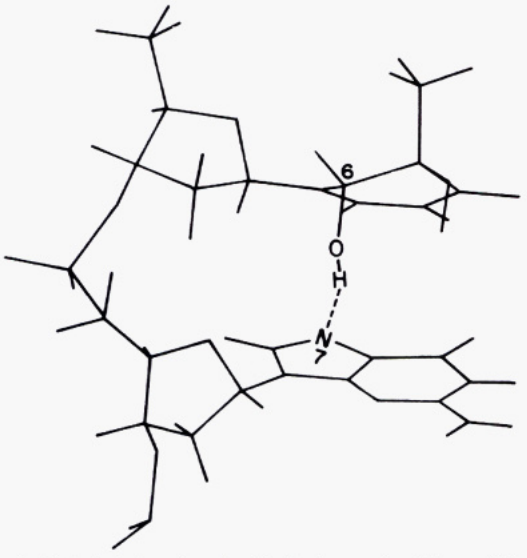

FIGURE 3: Detailed view showing the hydrogen bond formed between the hydroxyl attached to C6 of thymine glycol and N7 of the guanine residue on the 3′ side.

thymine glycol and its 5′ neighbor (adenine). A corresponding increase in the propeller twist of the base pair 5′ to the A·t′ base pair is also evident. Both of these effects are primarily due to an unfavorable steric overlap between the 5′ adenine base and the methyl group of thymine glycol, which lies on the 5′ side of, and is nearly orthogonal to, the nonplanar pyrimidine ring.

An additional feature of thymine glycol containing structures is the possibility of forming a hydrogen bond between the hydroxyl group attached to C6 of the glycol and N7 of a guanine residue on the 3′ side of the lesion (Figure 3). As a consequence of this hydrogen bond, the glycosyl torsion angle, χ (O1′–C1′–N1–C6), for thymine glycol is increased by more than 50°, and thus the base moiety shifts from an anti to a syn conformation (Table I). However, when cytosine

Table I: Selected Values of Torsion Angles for DNA Structures Containing Thymine or Thymine Glycol

Sequence	Residue(s)	Torsion Angle (Degrees)[a] χ T	χ t′	δ T	δ t′	ζ T	ζ t′
5′ G_1 C_2 C_3 C_4 A_5T_6 3′							
3′ $C_{13}G_{12}G_{11}G_{10}T_9A_8G_7$ 5′	T6	53	58	104	114	–	–
	T9	50	101	118	139	–	–
	G10	61	60	129	143	–	–
	A5–C4	–	–	–	–	225	235
	T6–A5	–	–	–	–	276	274
	A8–T9	–	–	–	–	244	233
	T9–G10	–	–	–	–	265	224
	G10–G11	–	–	–	–	250	262
	G11–G12	–	–	–	–	240	242
5′ G_1 C_2 C_3 C_4 A_5G_6 3′							
3′ $C_{13}G_{12}G_{11}G_{10}T_9C_8G_7$ 5′	G6	58	57	135	138	–	–
	T9	47	103	115	133	–	–
	G10	58	59	127	144	–	–
	A5–C4	–	–	–	–	272	273
	G6–A5	–	–	–	–	256	250
	C8–T9	–	–	–	–	252	258
	T9–G10	–	–	–	–	268	218
	G10–G11	–	–	–	–	248	259
	G11–G12	–	–	–	–	237	244
5′ G_1 C_2 C_3 G_4 A_5T_6 3′							
3′ $C_{13}G_{12}G_{11}C_{10}T_9A_8G_7$ 5′	T6	52	56	117	132	–	–
	T9	51	68	124	112	–	–
	C10	41	50	97	107	–	–
	A5–G4	–	–	–	–	229	240
	T6–A5	–	–	–	–	270	257
	A8–T9	–	–	–	–	242	260
	T9–C10	–	–	–	–	264	247
	C10–G11	–	–	–	–	273	275
	G11–G12	–	–	–	–	235	237
5′ G_1 C_2 C_3 G_4 A_5G_6 3′							
3′ $C_{13}G_{12}G_{11}C_{10}T_9C_8G_7$ 5′	G6	54	49	131	132	–	–
	T9	53	65	132	121	–	–
	C10	41	47	95	104	–	–
	A5–G4	–	–	–	–	229	236
	G6–A5	–	–	–	–	262	261
	C8–T9	–	–	–	–	248	256
	T9–C10	–	–	–	–	263	250
	C10–G11	–	–	–	–	273	273
	G11–G12	–	–	–	–	234	238

[a] T and t′ represent structures containing thymine or thymine glycol, respectively, at position T9. χ is the glycosyl torsion angle, O1′–C1′–N1–C6 for pyrimidines and O1′–C1′–N9–C8 for purines, δ is C5′–C4′–C3′–O3′, and ζ is C3′–O3′–P–O5′.

Table II: Interaction Energies for DNA Molecules Containing Thymine or Thymine Glycol in Various Sequence Contexts

Sequence[a]	Total	Energy (kcal)					
		Base Pairing[b]				Stacking[c]	
	kcal/mole	A:T	A:t'	3'bp	5'bp	3'bp	5'bp
3'5'							
5'GCCCAT 3'							
3'CGGGTAG 5'	-504	-13.1	-	-22.7	-13.0	-18.7	-17.9
5'GCCCAT 3'							
3'CGGGTAG 5'	-512	-	-13.8	-22.5	-12.9	-29.2	-15.9
5'GCCCAG 3'							
3'CGGGTCG 5'	-525	-13.1	-	-22.7	-22.6	-19.0	-18.7
5'GCCCAG 3'							
3'CGGGTCG 5'	-530	-	-13.8	-22.7	-22.2	-28.8	-14.7
5'GCCGAT 3'							
3'CGGCTAG 5'	-502	-13.2	-	-22.8	-12.8	-18.2	-17.4
5'GCCGAT 3'							
3'CGGCTAG 5'	-499	-	-13.9	-22.7	-12.9	-19.3	-13.6
5'GCCGAG 3'							
3'CGGCTCG 5'	-527	-13.2	-	-22.8	-22.3	-18.5	-17.9
5'GCCGAG 3'							
3'CGGCTCG 5'	-523	-	-13.5	-22.7	-21.7	-19.3	-13.5

[a] T designates thymine glycol in the sequence; 3' and 5' flanking base pairs are shown by numerals above the top sequence. [b] Interaction energies are given for the adenine·thymine glycol (A·t') base pair, the corresponding natural base pair (A·T), and the 3' and 5' flanking base pairs. [c] Stacking energies are computed as the sum of all pairwise interactions between the four bases in each dinucleotide pair, excluding the contribution due to base pairing.

replaces guanine as the 3' neighbor, the change in the glycosyl torsion angle introduced by the glycol is less than 20°, and the base remains in the anti conformation.

In addition to the structural features noted above, numerous changes in sugar puckering occur when thymine glycol is introduced into DNA. These changes are generally small and are localized to within one or two base pairs on either side of the glycol. The largest changes occur within the deoxyribose moiety attached to thymine glycol itself and are influenced by sequence context as indicated by changes in the torsion angle δ (C5'–C4'–C3'–O3') [following the convention of Seeman et al. (1976); Table I]. The hydrogen bonding that occurs when guanine is present 3' to the lesion leads to a change in sugar puckering of greater magnitude and of opposite sense to that seen when cytosine occurs at this position. The only significant changes along the sugar phosphate backbone introduced by the glycol are rotations about the P–O3' bond as indicated by changes in the torsion angle ζ (C3'–O3'–P–O5'; Table I). The largest of these changes is a counterclockwise rotation of 40–50° about the P–O3' bond between the thymine glycol residue and the 3' guanine residue in the template strand.

Energetics. The total minimized energies for the various models showed relatively small overall differences between structures that contain thymine glycol and those that contain thymine (Table II). The effect of thymine glycol on the stability of the structure varies, depending upon the particular sequence context surrounding the lesion. Structures that contain guanine as the template base immediately 3' to thymine glycol are more stable than their natural counterparts whereas those that contain cytosine on the 3' side of the lesion are less stable than the corresponding natural DNA. Interestingly, the base pairing between adenine and thymine glycol does not appear to be significantly impaired since the interaction energies for the A·T and A·t' base pairs are very similar (Table II). Furthermore, the 3'- and 5'-flanking base pairs also form adequate hydrogen bonds since the base-pairing interaction energies for these base pairs are comparable in the presence or absence of thymine glycol. That this is true for the 5'-flanking base pair despite the angular distortions described earlier reflects the nondirectional nature of hydrogen bonds.

The energetics of the stacking interactions with the 3'- and 5'-flanking base pairs are significantly altered by the presence of thymine glycol. The base pair on the 3' side of the lesion is stabilized by the presence of the glycol. Conversely, the 5' base pair is significantly destabilized (Table II). This may underly the failure of most polymerases to extend primers beyond a thymine glycol lesion site in the template (see Discussion). The magnitude of these effects depends upon the sequence context. Stabilization of the 3' base pair is more dramatic when the base 3' to the lesion is guanine and much less when cytosine occurs at this position. The greater stability of the 3' base pair that occurs when guanine is adjacent to the thymine glycol is largely due to the formation of a hydrogen bond between the hydroxyl group attached to C_6 of the glycol and N_7 of guanine as described above (Figure 3). The substitution of cytosine for guanine at this position results in the elimination of this hydrogen bond between thymine glycol and the adjacent 3' base.

Discussion

Molecular modeling is a useful technique for studying the perturbations introduced by structural abnormalities in DNA, particularly in cases where direct information from physical methods is lacking. A major advantage of the method is the ease with which the effects of altering the sequence context around the lesion can be studied. A structure generated in this way should be the most probable one since it represents a minimum in the potential energy function. However, an important caveat should be noted with regard to the use of this technique. The minimized structure generated by molecular mechanics calculations represents a local, and not a global, minimum energy conformation. Therefore, it is possible that other acceptable conformations exist that are inaccessible to the minimization program because they are separated by potential energy barriers. The spectrum of accessible conformations also depends upon the assumptions employed in constructing the model. For example, two rather different models of a thymine dimer in DNA have been generated when energy minimization techniques have been used. Rao et al. (1984) concluded that thymine dimers produce little overall distortion of the DNA helix whereas the model of Pearlman et al. (1985) predicts that the dimer introduces a kink of about 30° in the helix. The lack of helix distortion reported by Rao et al. (1984) presumably arises because the energy minimization was unable to reach any minimum energy conformation that deviated significantly from that of the starting dodecanucleotide employed by these workers. Conversely, Pearlman et al. (1985) started with the crystallographically determined thymine dimer structure and initiated their analysis on a six base pair fragment that was small enough to allow energy minimization to propagate the structural changes introduced by the dimer. Our analysis was also carried out on a fragment small enough to allow structural changes introduced by thymine glycol to be adequately reflected in the energy minimization calculations. Furthermore, the structural perturbations introduced by thymine glycol are considerably less than those imposed by thymine dimers, and the modeling of this lesion should consequently be somewhat less dependent upon the initial assumptions. The basic structural features of our model for *cis*-thymine glycol in DNA should therefore be a reasonable approximation to the true structure. We point out, however, that our model was specifically designed to evaluate the stability of the first base pair that would be added by a DNA polymerase beyond the lesion site. Thus it does

not address the question of how far the perturbations introduced by thymine glycol extend in the 5′ direction.

The model that we generated using this approach simulates an experimental system in which an oligonucleotide template/primer substrate was used to study the effects of *cis*-thymine glycol lesions on DNA synthesis in vitro. Our experimental results indicate that synthesis carried out by several DNA polymerases terminates at the site of a *cis*-thymine glycol lesion in the template strand and that the correct nucleotide, dAMP, is preferentially inserted opposite the lesion (Clark & Beardsley, 1987). The results obtained with our model are generally consistent with the experimental findings and suggest several factors that may contribute to the observed effects of thymine glycol lesions on DNA synthesis in vitro. Specifically, the model indicates that the insertion of nucleotides prior to, and at the site of, the lesion can occur without major deleterious effects upon either base pairing opposite thymine glycol or stacking interactions with the 3′ base pair. In fact, the interaction between the A·t′ base pair and its 3′ neighbor appears to be energetically more stable than the corresponding interaction between the A·T base pair and its 3′ neighbor. This is particularly evident when guanine occurs immediately 3′ to thymine glycol and a hydrogen bond is made between the hydroxyl group on C6 of the glycol and N7 of guanine. It is also of interest that hydrogen bond formation for the 5′ base pair, as judged by the base-pairing interaction energies, is unaffected by the presence of thymine glycol. This result, taken together with the experimental data, indicates that hydrogen bond formation per se is not a sufficient condition for the addition of the next nucleotide beyond the site of the lesion. Thus it appears that additional structural features of the DNA, e.g., an appropriate stacking geometry between adjacent base pairs at the primer terminus, are important determinants of the suitability of the substrate for DNA polymerase catalyzed nucleotide insertion. In this regard it is noteworthy that the stacking interaction with the 5′ base pair is considerably destabilized by the introduction of thymine glycol in place of thymine. The destabilization is due, at least in part, to an unfavorable steric overlap between the 5′ base and the (axial) methyl group of the glycol. As a consequence of this steric overlap, the tilt angle of the template base immediately 5′ to the glycol is significantly increased. This increase in tilt angle of the 5′ template base presumably distorts the overall geometry of the primer/template junction in such a way as to inhibit stable addition of the next nucleotide beyond the lesion.

Since the base-pairing interaction energy for the A·t′ base pair does not differ significantly from that calculated for the A·T base pair, it is not surprising that DNA polymerases preferentially insert dAMP opposite thymine glycol in vitro (Clark & Beardsley, 1986, 1987). However, our recent data indicate that a 3′-terminal dAMP residue is removed somewhat more rapidly by the 3′–5′-exonuclease activity of the Klenow fragment from an A·t′ base pair than from an A·T base pair (Clark & Beardsley, 1987). This suggests that an A·t′ base pair can be distinguished from an A·T base pair, at least by the editing function of DNA polymerase I. The physicochemical basis for this discrimination is not evident from the model. However, we note that the electrostatic surface potential in the major groove near thymine glycol will be altered significantly by the presence of the two additional hydroxyl groups. It is possible that this difference contributes to the observed discrimination between A·t′ and A·T base pairs exercised by the editing function of polymerase I.

We note that *cis*-thymine glycol, as the free base, has only one isomer whereas two cis diastereomers are possible in the nucleoside (and in DNA). Our models contain only one of the two diastereomers: *cis*-5(*R*),6(*S*)-dihydroxy-5,6-dihydrothymidine. Both cis diastereomers as well as two trans diastereomers of thymidine glycol are formed in DNA by ionizing radiation (Teoule & Cadet, 1978). A final point should also be made about the significance of the hydrogen bond predicted by the model when guanine (and presumably adenine as well) occurs immediately 3′ to thymine glycol. Since the models were generated in the absence of solvent molecules, it is not clear that such hydrogen bonds would actually form in DNA in solution. However, it is intriguing that DNA polymerase I (Klenow fragment) can bypass some thymine glycol lesions when DNA synthesis is carried out on single-stranded M13 templates if the sequence around the lesion is either 5′-CTA-3′ or 5′-CTG-3′ (Hayes & LeClerc, 1986). These results at least raise the possibility that the structural alterations imposed upon the DNA substrate by hydrogen bond formation between thymine glycol and a 3′ purine facilitate bypass of these lesions in this particular sequence context.

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Assessment of Preferential Cleavage of an Actively Transcribed Retroviral Hybrid Gene in Murine Cells by Deoxyribonuclease I, Bleomycin, Neocarzinostatin, or Ionizing Radiation†

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ABSTRACT: Preferential cleavage induced by bleomycin, neocarzinostatin, or ionizing radiation in a transcribed cellular gene was evaluated through comparisons with deoxyribonuclease I. The glucocorticoid-inducible LTL gene (a hybrid viral gene derived from mouse mammary tumor virus DNA) previously described [Zaret, K. S., & Yamamoto, K. R. (1984) *Cell (Cambridge, Mass.) 38*, 29–38] served as the specific DNA target. A Southern blot analysis was used to specifically assess cleavage of the LTL gene in nuclei isolated from cells either treated or untreated with the synthetic glucocorticoid dexamethasone. Hypersensitivity of the gene to bleomycin or neocarzinostatin, which paralleled deoxyribonuclease I hypersensitivity, was evident only in nuclei isolated from dexamethasone-treated cells. Like deoxyribonuclease I, sites of dexamethasone-inducible drug hypersensitivity were coincident with the binding region for the glucocorticoid receptor found within the regulatory sequences of the LTL gene. In contrast, no hypersensitivity to ionizing radiation was evident. Although bleomycin and neocarzinostatin showed qualitatively similar preferences for the transcribed LTL gene, quantitative evaluations of damage to total cellular DNA by filter elution showed that the relative specificity of bleomycin for the hypersensitive region was much less than that of either deoxyribonuclease I or neocarzinostatin.

Active or potentially active chromatin regions have been shown to be preferential targets for deoxyribonuclease I (DNase I)[1] catalyzed cleavage (Weintrab & Groudine, 1970; Garel & Axel, 1976). In particular, hypersensitivity to this endonuclease has been associated with regions involved in the control of transcription and replication [for a review, see Eissenberg et al. (1985)]. The precise mechanisms which render the DNA within these regions more vulnerable to the action of endonucleases are not completely elucidated. However, the accepted view is that the increased susceptibility of active chromatin to enzymatic cleavage is a consequence of a more "relaxed" chromatin conformation (Eissenberg et al., 1985).

The cytotoxic antibiotics bleomycin (BLM) and neocarzinostatin (NCS) are pharmacological equivalents of endonucleases since they also have the intrinsic ability to cleave DNA (Suzuki et al., 1969; Beerman & Goldberg, 1974). Like many endonucleases, these drugs are sequence selective (Sugiura & Suzuki, 1982; Kross et al., 1982; Takeshita et al., 1981) and can cleave chromatin nonrandomly. Apparently, conformational factors contribute to this nonrandom activity on chromatin since drug-induced cleavage was shown to be predominantly confined to the nucleosomal linker (Kuo & Hsu, 1978; Kuo & Samy, 1978; Beerman et al., 1982). In addition, specific chromatin domains may also represent sites of preferential cleavage. Notably for BLM, differential activity on transcribing chromatin has been observed in at least one system (Kuo, 1981), while indirect evidence suggests that NCS shows no such preference (Hatayama & Yukioka, 1982).

Since preferential cleavage of specific chromatin domains, as exemplified by DNase I, may be an important determinant for drug action in vivo, we sought to further assess this possibility by evaluating scission activity on a well-characterized gene target. Accordingly, we chose to study drug activity in

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[1] Abbreviations: DNase I, deoxyribonuclease I; BLM, bleomycin; NCS, neocarzinostatin; kbp, kilobase pair; MMTV-LTR, mouse mammary tumor virus long terminal repeat; HSVtK, herpes simplex virus thymidine kinase; krad, kilorad; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; DMEM, Dulbecco's modified Eagle's medium; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; PBS, phosphate-buffered saline; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

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