A Mechlorethamine-Induced DNA Interstrand Cross-Link Bends Duplex DNA†

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Received August 25, 1994; Revised Manuscript Received November 10, 1994®

ABSTRACT: The dG-to-dG, DNA−DNA interstrand cross-link at the duplex sequence 5′-d(GNC) formed by the antitumor drug mechlorethamine (bis(2-chloroethyl)methylamine) was studied both theoretically and experimentally. Computer models of cross-linked DNA were energy minimized using molecular mechanics. The energy minimized structures possessed local distortion of the DNA helix, especially propeller twisting and buckling, caused by the tether length being too small to bridge the spacing of N7 atoms of dG at the sequence 5′-d(GNC) in B DNA. Overwinding of 2−6° was present at each of the two dinucleotide steps spanned by the cross-link. The predicted structural changes were compatible with the possibility that this cross-link would introduce a static bend into the DNA double helix axis. An experimental study provided evidence for this induced bending of the helix axis in interstrand cross-linked samples. DNAs containing multiple mechlorethamine-induced interstrand cross-links exhibited anomalously low electrophoretic mobility in polyacrylamide gels when the lesions were separated by one or two turns. From the degree of gel retardation, the cross-linked DNAs were estimated to be bent by 12.4−16.8° per lesion; estimation of the extent to which this bend was induced by the lesion was complicated by a preexisting bend in the non-cross-linked DNAs used. The data did not allow distinction of a static from an anisotropic dynamic bend; "universal" and "hinge" joints were excluded. Anomalous mobility was maximal when the lesion spacing was 21 bp, suggesting a helical repeat of 10.5 bp per turn.

The discovery in the 1940s that mechlorethamine [bis(2-chloroethyl)methylamine, HN2, **1**] is useful in the treatment of human cancer marked the beginning of cancer chemotherapy (Gilman & Philips, 1946; Pratt & Ruddon, 1979). The ability of mechlorethamine to inhibit protein, RNA, and DNA synthesis provided early evidence for the hypothesis that DNA is the biologically relevant target of mechlorethamine (Johnson & Ruddon, 1967; Ruddon & Johnson, 1968); numerous other observations support this conclusion (Gray & Phillips, 1993, and references therein). As is the case with several antitumor agents (e.g., BCNU, cisplatin, mitomycin C), mechlorethamine is a bifunctional electrophile and can create both interstrand (Geiduschek, 1961) and intrastrand (Chun et al., 1969) covalent cross-links in DNA. It has long been recognized that these lesions may be important in the cytotoxic action of these agents, as they would be expected to block or inhibit the passage of polymerases. More recently, it has become appreciated that covalent lesions, especially cross-links, can alter the global structure of DNA and, in turn, modulate its affinity for DNA-binding proteins, providing an alternative avenue for exerting biological effects (Toney et al., 1989; Donahue et al., 1990, 1991). We report herein theoretical and experimental evidence that a mechlorethamine-induced DNA−DNA interstrand cross-link induces a bend in the helix axis of duplex DNA. Mechlorethamine thus joins the growing list of agents whose lesions in DNA cause global structural reorganization of the biopolymer.

In vitro treatment of duplex DNA with mechlorethamine produces a variety of interstrand cross-linked products. In the DNAs thus far studied, the most abundant and best structurally characterized interstrand cross-link bridges two deoxyguanosine residues at the duplex sequence 5′-d(GNC) (Ojwang et al., 1989a,b; Millard et al., 1990; Grueneberg et al., 1991; Rink et al., 1993). In this lesion, the N7 atoms of the two deoxyguanosine residues on opposite strands and separated by an intervening base pair are linked to one another by an *N*-methyldiethyleneamine linkage (i.e., **2**). This connectivity poses a structural puzzle, as the tether depicted in **2** is insufficient in length to bridge the separation between two N7 sites at this trinucleotide sequence in B DNA. The constraints of bond lengths and angles in the diethyleneamine tether mandate that the bridged sites be separated by 7.5 Å or less, while the N7-to-N7 distance in B DNA at the canonical 5′-d(GNC) sequence is 8.9 Å. As such, it is assured that the structure of DNA at the lesion site must depart from that of canonical B DNA, in which adjacent base pairs reside in approximately parallel planes separated by some 3.4 Å. Because examination of models of duplex DNA suggested to us that bending of the helix axis might provide one solution to this mechanical problem, we tested this hypothesis both theoretically and experimentally.

We report here molecular mechanics energy minimization computations which illustrate the appreciable structural distortions potentially imparted to B DNA by the mechlorethamine-induced interstrand cross-link. Experimental evidence indicative of bending of the helix axis by the cross-link is also reported: DNAs containing multiple mechlorethamine-induced interstrand cross-links exhibited anomalously low electrophoretic mobility in polyacrylamide gels

† This work was supported in part by the National Institutes of Health (GM 32681). P.B.H. acknowledges a Cope Scholarship from the American Chemical Society. S.M.R. was a National Institutes of Health Predoctoral Fellow (GM 08437) and recipient of a fellowship from the Division of Medicinal Chemistry of the American Chemical Society.

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® Abstract published in *Advance ACS Abstracts,* January 1, 1995.

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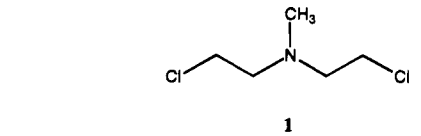

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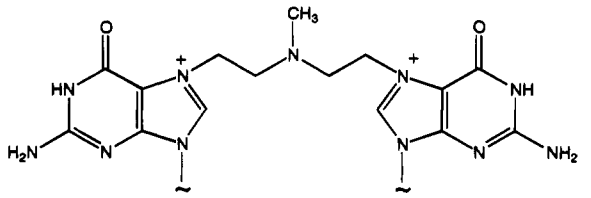

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when the lesions were separated by one or two turns. Other spacings reduced or eliminated the effect. This behavior is characteristic of bent or curved DNA.

EXPERIMENTAL PROCEDURES

Materials and Methods. The materials and methods used are described in Rink et al. (1993) with the following exceptions. T4 ligase was obtained from Amersham and ATP from Aldrich. The nondenaturing loading buffer consisted of 30% (aqueous) glycerol, 0.25% bromophenol blue, and 0.25% xylene cyanol dyes. OD refers to the calculated absorbance at 260 nm of the sample in 1 mL of solvent in a 1 cm path length cell. The extinction coefficients at 260 nm for DNAs were estimated to be 10 000 M^{-1} cm^{-1}residue^{-1} (Maniatis et al., 1989).

Computational Studies. Computation was performed on a Silicon Graphics 4D-25 workstation utilizing Insight II (version 2.0.0) (Biosym Technologies, San Diego, CA) for energy minimizations. All calculations with Discover used the AMBER force field and a distance-dependent dielectric of $4r$ in the absence of solvent and counterions. Starting structures were generated from B DNA as described in the text. Structures were refined using the method of steepest descents for ca. 300 iterations using diagonal terms only and a simple harmonic potential followed by 6000 iterations using the method of conjugate gradients. The maximum derivative for displacement of any single atom was at this point <0.1 kcal/(mol·Å); the root mean square derivative for the ensemble was <0.01 kcal/(mol·Å). The resulting structures were analyzed using the program Newhelix91.

Oligonucleotide Synthesis and Purification. The DNA was synthesized and purified as described by Rink et al. (1993). Complementary strands were admixed in a 1:1 ratio as determined by UV absorbance, heated to 90 °C for 1 min, and cooled to 25 °C over ca. 1 h.

Cross-Linking. Nonradiolabeled oligonucleotides possessing 3′- and 5′-hydroxyl termini were cross-linked as described by Rink et al. (1993) (0.1 mM in duplex DNA, pH 8.0, and 0.4 mM mechlorethamine, 37 °C, 3 h). The interstrand cross-linked products were separated by denaturing PAGE[1] (DPAGE), visualized by UV shadowing, and recovered as described by Rink et al. (1993).

Phosphorylation. The non-cross-linked and cross-linked duplexes (0.2 OD) and a *Bam*H1 -linker duplex (0.4 OD) were independently phosphorylated at their 5′-ends with [γ-^{32}P]ATP (20 μCi for cross-linked samples, 10 μCi for non-cross-linked and *Bam*H1 DNAs) using T4 kinase (12 units for cross-linked DNA, 6 units for non-cross-linked and *Bam*H1 DNAs) in kinase buffer (10 μL final volume). The samples were incubated at 25 °C for 1 h, followed by the addition of cold ATP (0.5 μL of 5 μM stock solution), T4 kinase (6 units), and kinase buffer for a final volume of 20 μL. After gently mixing, the samples were incubated for another 1.5 h at 25 °C to ensure exhaustive phosphorylation of the 5′-ends.

Ligation. T4 ligase (32 units for non-cross-linked duplexes, 48 units for cross-linked and *Bam*H1) and ATP (2.5 mM final concentration) were added directly to the phosphorylated duplex samples. Following mixing, the samples were incubated at 4 °C for 16 h. Cold absolute ethanol, 1 mL (−20 °C), was added to each of the samples which was then cooled to −20 °C for 15 min. DNA was collected by centrifugation, the supernatants were discarded, and the pellets were washed once with cold (−20 °C) 85% (aqueous) ethanol solution. The samples were chilled as previously described, and the recovered pellets dried in vacuo at 25 °C. After dissolution of the pellet in 20 μL of water, an aliquot (2 μL) of each sample was taken up in nondenaturing buffer (4 μL) for electrophoretic anaylsis.

Electrophoresis. Electrophoretic analysis was performed using a nondenaturing 8% polyacrylamide gel (mono-:bis-(acrylamide) ratio 29:1). The gels were polymerized for 1 h and then pre-run in TBE buffer (0.1 M TBE, pH 8.3) for 1 h at 2 °C with a constant voltage of 1000 V. After loading the samples, the gels were run at 1000 V until the bromophenol blue dye had migrated ca. 22 cm. The gels were dried for 15 min at 80 °C and subsequently analyzed by autoradiographic and phosphorimage analyses.

Calculation of Band Mobilities. The electrophoretic molibility of a given band was assigned as the maximum in a densitometric plot derived from the phosphorimage of ligation ladder gels. Only bands in which the peak in the densitometric trace had a height/width at half-height ratio of 1 or greater were included in the analysis. Mobilities were converted to relative length, R_L, as described by Koo and Crothers (1988):

$$R_L = L_a/L_r$$

where L_a is the apparent length of the multimer, as described by the size (in bp) of the *Bam*H1 multimer whose mobility corresponds to the multimer of interest, and L_r is the real length of the multimer of interest (in bp). The angle of absolute curvature per turn of DNA, C, for selected multimers was estimated following the empirical relation of Koo and Crothers (1988):

$$C = [(R_L - 1)/((9.6 \times 10^{-5})L_r^2 - (0.47\ \text{bp})^2)]^{1/2} \times (19.75 \pm 2.75\ \text{deg bp turn}^{-1})$$

Bend angles reported as per lesion were corrected to a helical repeat of 10.5 bp per turn. As a precaution, the assignment of actual length of several non-cross-linked multimers was verified by their excission and isolation from a ligation ladder gel. The isolated multimers were then sized on DPAGE by comparison to synthetic DNA samples of known length.

RESULTS

Computation. The impact of a mechlorethamine-derived DNA interstrand cross-link was modeled using molecular

[1] Abbreviations: bp, base pairs; PAGE, polyacrylamide gel electrophoresis; TBE, Tris−borate−EDTA.

mechanics energy minimization calculations with the AMBER force field. Structures calculated by the molecular mechanics approximation are most reliable where atomic positions are governed by high force constant interactions such as bond lengths and bond angles. Bending or curvature of the helix axis of DNA is, on the contrary, the result of deformations constrained by relatively low force constants and is thus probably not reliably studied by the relatively simple approximation used here (von Kitzing, 1992). For this reason, we generally constrained ourselves to inspection of the calculated structures for deformations induced locally by the cross-link, particularly to the impact of the cross-link on the relative orientation of the two cross-linked deoxyguanosyl residues, their Watson–Crick partners, and the intervening base pair.

A B-like conformation (10.0 bp/turn, untilted base pairs, propeller twist of ca. 4°) of the undecanucleotide duplex **I** was selected as a starting structure. An *n*-pentylene chain was substituted for the diethylenemethylamino linker actually present in the mechlorethamine cross-link; charges on the heavy atoms of the two cross-linked guaninyl residues were not altered to reflect the net unit charge formally added to each by the alkylation reactions. These choices reflected the desire to explore exclusively the consequences of the end-to-end length of the linking chain in the absence of other steric and electronic effects. Although the fully extended conformation of the pentylene tether (all torsional relationships anti, or aaaa) allowed for maximal separation of the N7 atoms which were bridged by the cross-link, this conformer did not appear to be an optimal match to the bonding sites presented by the lone pairs on the two N7 atoms of the 5′-d(GNC) sequence. Plausible structures could also be generated by incorporation of a single gauche minus (g^-) torsional angle at any one of the four nonequivalent carbon–carbon bonds of the pentylene tether. Thus, to enhance the chances that relevant conformations of the tether would be sampled, five structures for energy minimization were constructed. These consisted of the fully extended (aaaa) conformer along with the four conformational isomers possessing a single gauche minus torsional angle, g^-aaa, ag^-aa, aag^-a, and $aaag^-$. Because model building did not reveal any simple way to incorporate a single gauche positive interaction without even greater distortion of B DNA, no such tether geometries were employed as starting structures. Furthermore, because each gauche interaction itself brings an energetic cost of ca. 0.7 kcal/mol, combinations of two or more gauche interactions were not exhaustively studied.

	1	2	3	4	5	6	7	8	9	10	11
5′-	T	T	A	T	G	A	C	C	T	T	A
3′-	A	A	T	A	C	T	G	G	A	A	T
	22	21	20	19	18	17	16	15	14	13	12

I

The five cross-linked starting structures and the DNA without the cross-link were energy minimized in vacuo. No attempt was made to free the resulting structures from local minima. The two lowest energy structures are shown in Figure 1.

The energies of the cross-linked structures varied from one to another by some 7 kcal/mol, with the two lowest energy structures, ag^-aa and aag^-a, differing insignificantly in energy. All of the calculated cross-linked structures possessed structural distortions in the vicinity of the cross-link clearly visible on visual inspection. These presumably result from the diminished interatomic spacing of the two linked N7 atoms from the ca. 9 Å found in the non-cross-linked structure to the 6.5–7.1 Å spacing seen in the cross-linked structures.

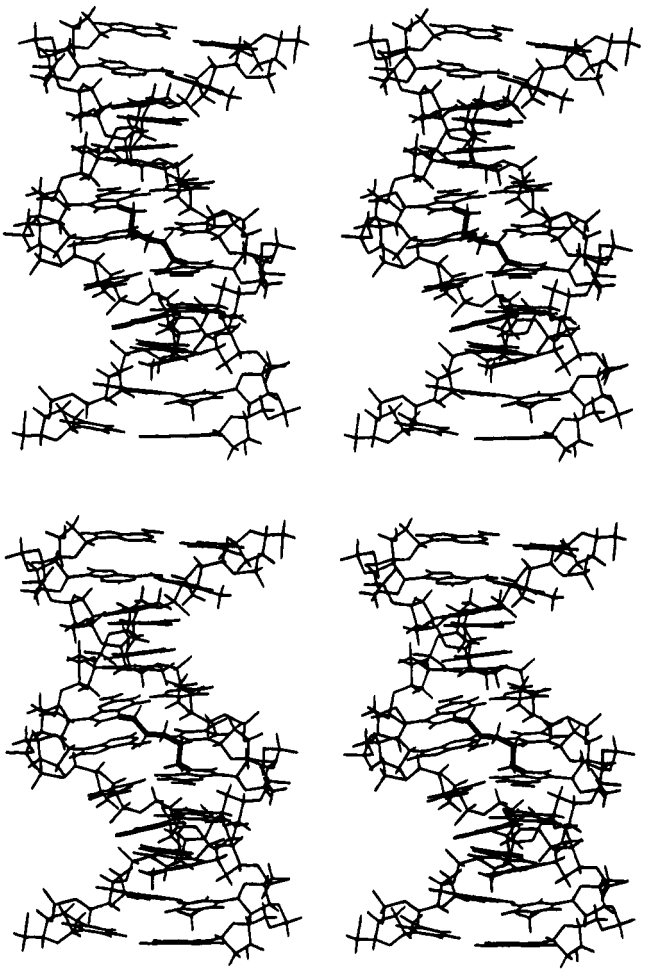

FIGURE 1: Stereoviews of the interstrand cross-linked structures found by molecular mechanics energy minimization to be lowest in energy. ag^-aa (upper) and aag^-a (lower). Cross-link indicated by bold bonds.

The structures were analyed using the computer program Newhelix91, which provided, among other things, expressions for best helix axes (local or global) and vectors for normals to the best planes of bases and base pairs. Some information provided by the program or conveniently calculated from it is summarized in Table 1. For reasons noted above, we focused attention on the distortions caused locally by the cross-link. [The nomenclature of nucleic acid structure follows the Cambridge conventions as described by Dickerson et al. (1989).] Obvious on visual inspection was that the best planes through the two bridged guaninyl moieties deviated substantially from being parallel. The angle between the normals to these planes, which was ca. 10° in the unminimized DNA and ca. 16° after minimizations without a cross-link, varied from 16° to 31° in the cross-linked structures, with the two lowest energy structures having angles of 22° and 29°. If the base pairs external to and flanking the cross-linked bases were to stack on these purines, then a net curvature would be predicted for the overall duplex.

Table 1: Computational Results

tether geometry[a]	minimized energy (kcal/mol)	G5(N7)−G16(N7) distance (Å)	G5/G16 angle[b] (deg)
none[e]		9.0	10.4
none[f]	−202.0	8.7	16.2
aaaa	−187.5	7.1	18.9
g⁻aaa	−189.6	6.6	31.1
ag⁻aa	−193.7	6.5	22.3
aag⁻a	−193.7	6.6	28.7
aaag⁻	−186.8	6.6	15.9

tether	twist[c] (deg)		roll (deg)		tilt[d] (deg)	
geometry[a]	G5-A6	A6-C7	G5-A6	A6-C7	G5-A6	A6-C7
none[e]	36.00	36.00	−0.84	0.20	−0.09	0.09
none[f]	33.36	33.45	−1.40	−4.89	0.22	−2.08
aaaa	36.81	35.90	2.06	−7.61	−2.51	1.51
g⁻aaa	38.60	37.70	1.11	−8.83	−5.58	−0.87
ag⁻aa	38.95	36.15	3.70	−7.29	−2.65	1.65
aag⁻a	38.14	36.41	3.71	−6.34	−5.52	1.53
aaag⁻	39.67	38.76	2.61	−10.86	−0.46	2.17

tether	propeller twist (deg)		buckle (deg)	
geometry[a]	G5•C18	C7•G16	G5•C18	C7•G16
none[e]	4.37	4.37	−0.01	0.01
none[f]	−14.46	−17.66	−4.46	1.53
aaaa	−17.17	−15.58	−11.26	8.75
g⁻aaa	−9.83	−21.98	−34.32	−8.52
ag⁻aa	−16.08	−18.17	−17.72	1.95
aag⁻a	−14.55	−22.23	−24.72	−2.85
aaag⁻	−15.16	−15.26	−15.34	4.45

[a] a = anti, g⁻ = gauche minus. [b] Angle between best planes for guanines only. [c] Residue 5 to 6 and 6 to 7. [d] For base pairs. [e] B DNA, no energy minimization. [f] B DNA, after energy minimization.

Table 2: DNA Duplexes Used in this Study

Duplex Sequence	Descriptor
5'TTATGACCTTA ATACTGGAATA	11-mer
5'TATTATGACCTTATT TAATACTGGAATAAA	15-mer
5'TTATTATGACCTTATTA ATAATACTGGAATAATA	17-mer
5'ATTATTATGACCTTATTAT AATAATACTGGAATAATAT	19-mer
5'AATTATTATGACCTTATTAT TAATAATACTGGAATAATAT	20-mer
5'AATTATTATGACCTTATTATT TAATAATACTGGAATAATAAT	21-mer A
5'AATATTAAGGGCTATATTATT TATAATTCCCGATATAATAAT	21-mer B
5'ATTATTATGACCTTATTATTAT AATAATACTGGAATAATAATAT	22-mer
5'TAATTATTATGACCTTATTATTA TTAATAATACTGGAATAATAATA	23-mer
5'CGGGATCCCG GCCCTAGGGC	Bam H1 Linker

Despite the reservations noted above concerning the reliability of aspects of these structures governed by low force constants, the computed structures were inspected for evidence of bending outside of the cross-linked trinucleotide duplex. For the non-cross-linked and cross-linked structures, best double helix axes were calculated separately above and below the central three residues. The angle between these axes (ca. 4−8°) and the direction of the bend (e.g., toward major or minor groove) were found to vary with the identity and number of residues included in the calculation. We judged these values not to be meaningful and did not pursue this further.

The twist angles in the two dinucleotide steps involved in the cross-link reflected 3−6° of overwinding relative to the minimized structure (33° per step). Assuming that this twist is not propagated outside the cross-link site (for which there was no evidence in the cross-linked structures), no large change in helical repeat was predicted.

The propeller twisting of the cross-linked base pairs varied typically by a few degrees from the −14° and −18° found in the non-cross-linked, minimized structure. Extrema of −10° and −22° were observed, the most negative being found in the lowest energy structure. This deformation is one which allows the bridged N7 atoms to more closely approach one another.

Another mechanism by which these atoms approach one another is by buckling of the base pairs. The most significant deviations between non-cross-linked and cross-linked structures were in fact found in the value of buckle, the angle between the best planes to the two partners in a base pair. Whereas the non-cross-linked, energy-minimized DNA showed buckle values of only −4° and 2° for the cross-linked base pairs, these ranged from −34° to 4° in the cross-linked structures. In every case, the *difference* between the buckle values for the two cross-linked base pairs within a duplex (the "cupping" of the two cross-linked base pairs) was 20° or greater, compared to a difference of only 6° in the non-cross-linked structure. Like propeller twisting, these differences in buckle offer a mechanism for the bridged N7 atoms to approach one another.

The computed structures thus predicted local distortion caused by the cross-link, with significant relocation of the planes of the cross-linked guaninyl residues and with only slight overwinding of the helix. No conclusion was drawn as to whether this distortion would or would not translate into net bending of the helix axis. This question was addressed experimentally.

Aberrant Electrophoretic Mobility of Mechlorethamine-Cross-Linked DNA. A panel of self-complementary DNAs which ranged in length from 11 to 23 residues (Table 2) was designed and synthesized. Each contained a single, centrally located 5′-d(GNC) sequence and had a single 5′-base overhanging to ensure proper polarity of ligation. The central sequence was identical to that employed in the computational study described above. The DNAs were separately exposed to mechlorethamine under previously established conditions, and the interstrand cross-linked

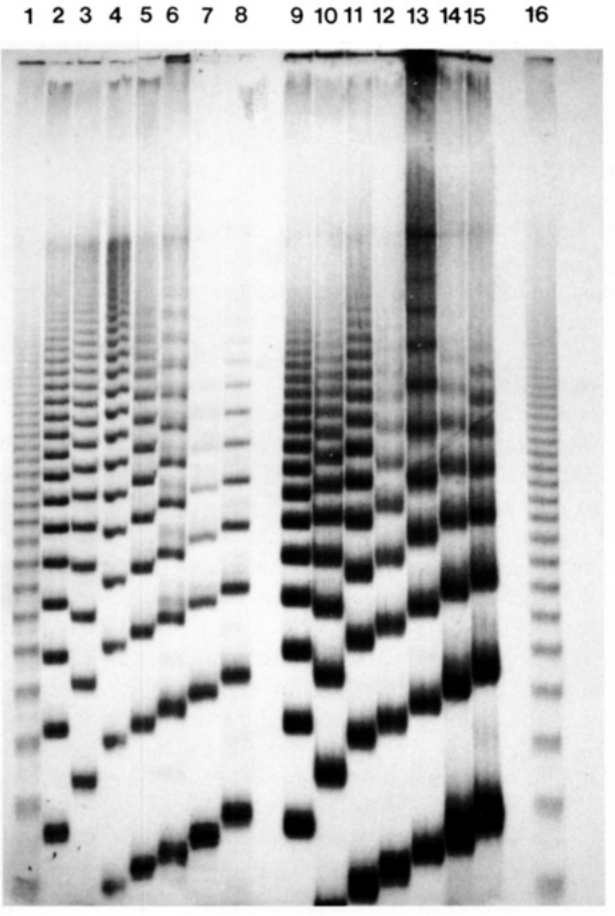

FIGURE 2: Autoradiogram of ligation ladder. Lanes 1 and 16: *Bam*H1 linker; lanes 2−8: non-cross-linked DNA 15, 17, 19, 20, 21A, 22, and 23, respectively; lanes 9−15: cross-linked DNAs 15, 17, 19, 20, 21A, 22, and 23, respectively.

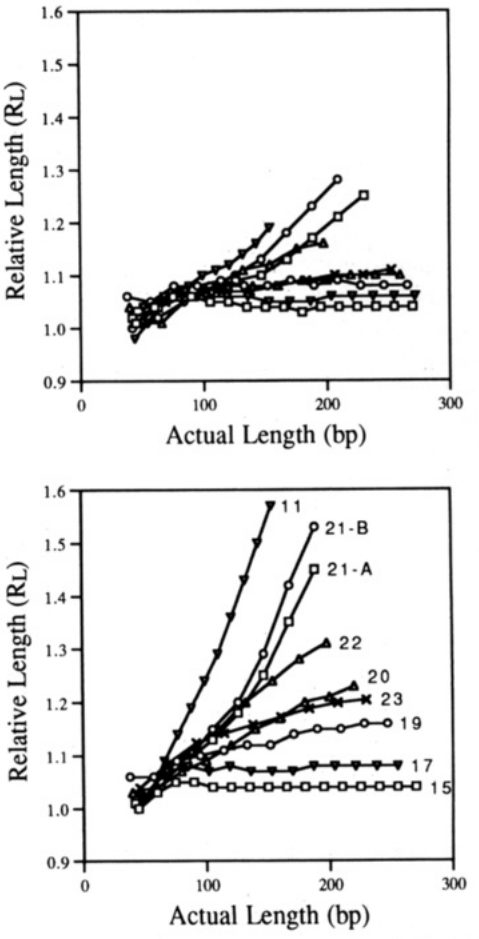

FIGURE 3: Relative length of non-cross-linked (upper) and cross-linked (lower) DNAs as a function of actual length. Identity of data points in upper panel is the same as is indicated in the lower panel. The nucleotide sequence of the monomers is shown in Table 2.

product from each was isolated by denaturing PAGE. [That the cross-links were in fact dG-to-dG at 5′-d(GNC) was demonstrated for one of these by piperidine treatment and analysis of the fragment lengths by denaturing PAGE, the method used in the original assignment of the sequence specificity of cross-linking by mechlorethamine (Millard et al., 1990).] These duplex DNAs in both the non-cross-linked and cross-linked forms and a *Bam*H1 linker were exhaustively phosphorylated using T4 kinase and [γ-^{32}P]ATP. The resulting products were ligated using T4 ligase at 4 °C and analyzed by nondenaturing PAGE (acrylamide:bis(acrylamide) ratio 29:1, 1000 V, 4 °C, Figure 2). The mobilities observed for the non-cross-linked and cross-linked multimers were converted to relative mobilities (R_L) by reference to the multimers derived from the *Bam*H1 linker and then plotted versus multimer length (Figure 3).

The relative mobilities (R_L) of the multimers derived from the non-cross-linked duplexes in Table 2 showed the length and phase dependence expected of a weakly bent structure (Figure 3, upper). Of the eight DNAs tested, the ligated forms of the approximately one- (11-mer) and two-turn (21-mer) duplexes showed the greatest retardation of electrophoretic mobility relative to the *Bam*H1 linker. This retardation increased with the length of the multimer. The ligated non-cross-linked 15-mer (one and one-half turns) deviated only slightly from an R_L of 1.0, and this value did not vary with length. This weakly bent structure, when phased with the helical repeat of ca. 10.5 bp/turn, thus produced miminal electrophoretic mobility.

The multimers of cross-linked DNAs displayed electrophoretic mobility properties qualitatively similar to the non-cross-linked DNAs, but the magnitude of the R_L values was significantly increased, consistent with the cross-linked DNAs being more bent (Figure 3, lower). The ligated cross-linked 11-mer and 21-mers displayed the largest R_L values, which increased with length. The ligated cross-linked 15-mer showed an R_L value which differed only slighly from 1.0, and this value did not vary with length. The impact of helical phasing of the cross-links was quite evident in this case, with the order of R_L values for multimers over 150 bp in length being $11 \gg 15 < 17 < 19 < 20 < 21 > 22 > 23$. This suggests not only that the mechlorethamine cross-link bends the helix axis of DNA, but also that the helical repeat is not grossly altered from that of B DNA, roughly 10.5 residues per turn, or 21 bp per two turns. The direction of the lesion-induced bend in duplex DNA was not directly determined.

To ensure that the aberrant electrophoretic mobility of mechlorethamine interstrand cross-linked DNA was not unique to the nucleotide sequences we had arbitrarily selected for study we prepared a second 21-mer (21-mer B) distinct in sequence from the 21-mer (21-mer A) originally selected for study, and measured the electrophoretic mobility of its multimers in non-cross-linked and cross-linked forms (Figure 3). Multimers of 21-mer B in these two forms displayed electrophoretic behavior analogous to that of the correspond-

ing multimers of 21-mer A. The R_L values for 21-mer B were somewhat larger than those for 21-mer A for a given length, but whether this difference exceeded the error of the experiment was not determined. Two DNAs of different sequence thus behave as expected for increased bending in the cross-linked form, suggesting this result may be general.

A consideration in the present study was the impact on electrophoretic mobility of charge density differences in the non-cross-linked and cross-linked samples arising from introduction of the cross-links. Assuming that alkylation at N7 does not alter the protonation state of the alkylated deoxyguanosine residues (e.g., N1) and that the amino group in the diethylenemethylamino chain is protonated, then each cross-link formally reduces the net negative charge on the biopolymer by 3 units. It might thus be predicted that as the cross-link to base pair ratio increased, the effective charge on the biopolymer would be less negative, and the electrophoretic mobility would decrease. While this is the direction of the effect in progressing from 23-mer to 21-mer, it is opposite that observed from 21-mer to 15-mer. Charge density changes alone, therefore, cannot account for the observed mobilities. Evidence that introduction of positive charge associated with the cross-link had not significantly altered the electrophoretic mobility can also be found in the essentially identical mobilities of the non-cross-linked and cross-linked 15-mers, R_L 1.0 for all multimers. That these putative differences in charge density do not dominate is fully consistent with theoretical and experimental results on DNA, which indicate that at low levels of charge modification, compensating changes in counterion association provide an effective counterbalance (Heath & Schurr, 1992).

The extents of the curvature of several of these multimers were calculated using the empirical relationship of Koo and Crothers (1988). The cross-linked 21-mers A and B and the 11-mer were bent by 15.6 ± 2.2° (147 bp), 16.8 ± 2.3° (147 bp), and 12.4 ± 1.7° (132 bp) per cross-link, respectively. This formula is not directly applicable to the non-cross-linked multimers, because the observed R_L values were below the cutoff (R_L 1.2) for multimers 120−170 bp in length. An upper limit on the extent of bending of the non-cross-linked multimers can be calculated using an R_L of 1.2 and 170 bp, affording 11.6 ± 1.6° per 21 bp for the 21-mers and 6.1 ± 0.8° per 11 bp for the 11-mer. Because this study did not define the direction of these bends (e.g., toward the major vs minor groove), it remains unknown whether these bends are reinforcing, in which case the lesion induces a *minimum* of a modest 4.0 ± 3.8° of bending, or opposing, in which case the lesion introduces a *maximum* of a 28.4 ± 3.9° of bend.

DISCUSSION

Mechlorethamine creates interstrand cross-links in synthetic oligodeoxynucleotides by joining the N7 atoms of deoxyguanosine residues on opposite strands at the sequence 5′-d(GNC) (Ojwang et al., 1989a,b; Millard et al., 1990; Grueneberg et al., 1991; Rink et al., 1993). This assures some deformation of B DNA, because in this structure the distance between these atoms exceeds that which can be spanned given the bond angles and length of the resulting diethylenemethylamine bridge. Reported herein are several computer models of mechlorethamine-cross-linked DNA derived from molecular mechanics energy minimizations. The calculated structures showed local deformations which resulted from shortening of the N7−N7 distance to accommodate the cross-link. These deformations resulted in reorganization of the linked guaninyl residues, particularly by buckling and propeller twisting, which might be expected to propagate with the result of a bent helix axis. A minor net overwinding of up to 12° per cross-link was observed for the trinucleotide step which encompassed the cross-link.

A general approach to the investigation of helix axis geometry of DNA and its conjugates uses electrophoretic mobility as an experimental observable (Wu & Crothers, 1984; Koo et al., 1986; Zinkel & Crothers, 1987; Koo & Crothers, 1988; reviewed by Harrington, 1993). Substantial evidence indicates that curved DNAs possess an anomalously low electrophoretic mobility relative to the linear counterpart of identical number of base pairs. This effect can be observed directly in DNAs when the angle of curvature is substantial (ca. 90°). The anomalous mobility can be used to study curvature smaller in magnitude by incorporation of several such curves *separated by an integral number of turns of double helix* into a single DNA molecule (i.e., a planar curve). Separation by other than an integral number of turns diminishes the aberrant electrophoretic mobility. We have applied this technique to the mechlorethamine cross-link.

By the criterion of anomalous electrophoretic mobility, we find mechlorethamine interstrand cross-linked DNA, on average, to have a bent helix axis. Anomalous mobility was maximal at inter-cross-link spacings of 11 and 21 bp, suggesting a B-like helical repeat of ca. 10.5 bp per turn for the cross-linked DNA. Using the empirical relation derived by Koo and Crothers (1988), each lesion was estimated to be bent by 12.4−16.8°. Estimation of the extent to which this bend was *induced* by the cross-link was complicated by bending in the DNAs prior to cross-linking which was not only too small to quantitate, but was also of undetermined orientation. Induced bends as small as 6° and as large as 31° were compatible with the data presented herein. Similar values have been estimated for a variety of other bent structures, including 7° (Wang et al., 1991) and 30° (Husain et al., 1988) for the T-T photodimer, 14−19° for the CC-1065 adduct (Lee et al., 1991), 17−22.5° for the A-tract (Koo & Crothers, 1988), 26° for the transplatin interstrand cross-link (Brabek et al., 1993), 32° for the cisplatin intrastrand cross-link (Bellon & Lippard, 1990), and 55° for the cisplatin interstrand cross-link (Sip et al., 1992).

From the available data, we cannot distinguish whether the mechlorethamine cross-link introduces a static bend or a dynamic flexibility leading to a time-average bent structure. The phase dependence of the electrophoretic retardation assures that any such flexibility must be anisotropic. The data thus argue against the presence of a "universal joint", which would shorten the end-to-end length of a DNA and thus retard its mobility regardless of the spacing of the joints. Likewise, a "hinge joint", as has been observed with the trans-GTG intrastrand cross-links derived from transplatin (Bellon et al., 1991), is ruled out, by the normal electrophoretic mobility of the cross-linked isomer (1.5 turn spacing of cross-links).

Bends in the helix axis of DNA, induced by complexation with proteins or present by virtue of DNA sequence alone, are widely believed to be functionally significant (Travers, 1990; Harrington & Winicov, 1994). Present models hold that bent DNA is necessary for the formation of important

DNA−protein complexes. Bending of DNA is observed in the three-dimensional structure of several DNA−protein complexes (Steitz, 1990). Bending is necessitated by the looping mechanism which orients regulatory sequences which act in *cis* at a distance (Ptashne, 1986). Bent DNA in multiprotein-DNA complexes precisely orients DNA sequence for events such as transcription, recombination, and replication (Echols, 1986). It is reasonable to extrapolate that lesions in DNA which alter the static or dynamic structure of DNA might thus detrimentally alter recombination, DNA repair, replication, and transcription, all of which depend upon protein−DNA complexation.

The best characterized examples of DNA−protein interactions being altered by lesion formation are in the platinum anticancer drug family. A human high mobility group protein which binds specifically to cisplatin-modified DNA (Bruhn et al., 1992) has been discovered. It has been suggested that it is the unwound and bent structure of the intrastrand cross-link which is recognized by the protein. The functional significance of the complex is unknown. The unwinding caused by cisplatin has also been proposed to be the major determinant in its recognition by the *Escherichia coli* (A)BC excinuclease (Bellon, et al., 1991).

Mechlorethamine thus joins the list of alkylating agents whose lesions alter the structural or dynamical properties of double-helical DNA. While the biological significance of these changes remains unknown, the growing library of bent and under- or overwound, lesion-containing DNAs provides a potentially valuable tool for the further study of the role of DNA structure and dynamics in its recognition by proteins.

ACKNOWLEDGMENT

We thank Professor B. Reid for use of the Iris workstations and Drs. O. Federov and L. Zhu for helpful discussions. We thank Mr. S. Alley and Mr. K. A. Brameld for technical contributions. Phosphorimaging was performed by the PhosphorImager Facility, Markey Molecular Center, University of Washington. We thank Ms. K. Bennett for assistance in the preparation of the manuscript.

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BI941987I