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Diepoxybutane Interstrand Cross-Links Induce DNA Bending

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Abstract

The bifunctional alkylating agent 1,2,3,4-diepoxybutane (DEB) is thought to be a major contributor to the carcinogenicity of 1,3-butadiene, from which it is derived *in vivo*. DEB forms DNA interstrand cross-links primarily between distal deoxyguanosine residues at the duplex sequence 5'-GNC. In order for the short butanediol tether to span this distance, distortion of the DNA target has been postulated. We determined that the electrophoretic mobility of ligated DNA oligomers containing DEB cross-links was retarded in comparison with control, uncross-linked DNA. Our data are consistent with DNA bending of ~34° per lesion towards the major groove.

Keywords

DNA cross-linking; diepoxybutane; DNA bending; DNA damage; alkylating agent

1. Introduction

The carcinogen 1,3-butadiene (BD) undergoes conversion by mammalian detoxification systems to the water-soluble, DNA-reactive epoxides 1,2-epoxy-3-butene, 1,2-epoxy-3,4-butanediol, and 1,2,3,4-diepoxybutane (DEB) [1]. Although all of these metabolites have the potential to alkylate DNA, DEB has the highest genotoxicity by 100-fold, a fact attributed in part to its ability to induce DNA cross-links [2]. DEB cross-links disrupt DNA replication and transcription, leading to cytotoxic and mutagenic events such as point mutations, rearrangements, and deletions [3]. Formation of these lesions is believed to contribute to the high rates of leukemia in workers who are exposed occupationally to BD, such as in the synthetic polymer industry [2, 4]. Other common human exposure routes to BD include cigarette smoke and automobile exhaust [2].

Genotoxic DEB lesions include DNA-DNA cross-links at deoxyguanosine and deoxyadenosine residues [5, 6], with 1,4-bis-(guan-7-yl)-2,3-butanediol (bis-N7G-BD) the most abundant lesion DNA in the tissues of laboratory animals exposed to BD by inhalation [7; Chart 1]. This product results primarily from interstrand cross-linking between distal deoxyguanosine residues at duplex 5'-GNC sequences [8, 9, 10]. The structure of this adduct was confirmed through mass spectrometry [5] decades after it was originally proposed [11]. Because of the relatively long distance spanned by the butanediol tether,

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Disclosure Statement

The authors declare that there are no conflicts of interest.

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considerable DNA distortion has been postulated to occur upon N7-to-N7 cross-linking at 5'-GNC sites [8].

The 5'-GNC consensus sequence for DEB interstrand cross-linking is shared by the nitrogen mustard anti-cancer drug mechlorethamine (HN2) [12, 13], suggesting that cross-linking at N7 of deoxyguanosine residues may play a role in the biological outcomes of both compounds. The fact that these agents span a distance of about 8.9 Å in canonical B-DNA despite their relatively short chain lengths (~4 Å for DEB [14] and ~7.5 Å for HN2 [15]) is evidence for the conformational flexibility of DNA. Mechlorethamine cross-linking at the sequence 5'-GGC induces a bend of about 17° per lesion, as demonstrated through analysis of anomalously low electrophoretic mobility of cross-linked oligomers in native polyacrylamide gels [16]. Curved DNA's possess an anomalously low electrophoretic mobility relative to straight DNAs of the same length [17]. We used a similar method to characterize bending induced by DEB cross-linking. Our data suggest that a DEB cross-link at 5'-GGC induces a bend of approximately 34° towards the major groove. This bend may trigger recruitment of cellular proteins that mediate the biological effects of DNA cross-linking.

2. Materials and methods

2.1 Materials

Enzymes were purchased from New England Biolabs (Ipswich, MA), [γ -³²P]ATP was from Perkin Elmer (Waltham, MA), oligonucleotides were from Integrated DNA Technologies (Coralville, IA), and DEB (racemic) was from Sigma-Aldrich Chemical Company (Milwaukee, WI). All other chemicals were of reagent grade.

2.2 Preparation of Cross-Links

Oligonucleotides were purified via 20% denaturing polyacrylamide gel electrophoresis (PAGE; 19:1 acrylamide: bisacrylamide; 40% urea) followed by the crush-and-soak procedure [18]. Duplexes for cross-linking contained a single central 5'-GGC site for cross-linking and a 5'-base overhang for ligation. DEB cross-links were prepared with 5 OD duplex DNA and purified as described previously [9].

2.3 Ligation and Electrophoresis

Triplicate samples of cross-linked and control native duplexes were phosphorylated and ligated as described by Rink and Hopkins [16]. Ligated samples were electrophoresed on native 8% polyacrylamide gels (acrylamide: bisacrylamide ratio of 29:1) run at 1000 V and 4°C. Gels were dried and analyzed via autoradiography. PCR marker (New England Biolabs) was used as a size reference (765 bp, 500 bp, 300 bp, 150 bp, and 50 bp, with the 300-bp marker resolving as two bands). Mobilities were measured as the distances to the centers of the major bands and compared to those of the ligated products of a 21-bp unbent control duplex [19]. The relative length, R_L , was then calculated for each ligation product as the ratio of apparent length (L_a , as determined through a standard curve of log bp versus mobility for the reference 21mer) to the real length (L_r) of the multimer in base pairs [17]: $R_L = L_a/L_r$. We then determined the angle of absolute curvature per helix turn, C , through the empirical relationship derived by Koo and Crothers [17, 16]:

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

We corrected bend angles per cross-link to a helical repeat of 10.5 bp per turn.

3. Results

3.1 Electrophoretic retardation of DEB-cross-linked DNA

We used the method of Koo and Crothers [17] to examine possible bending induced upon the formation of DEB cross-links. Drug-induced bending leads to DNA with an anomalously low electrophoretic mobility on native polyacrylamide gels relative to straight DNA of similar length [20]. Ligation of a bent oligomer can either amplify or reduce this mobility difference depending on the phasing of the bend, which can be constructive or destructive. In-phase bends that are spaced at integral numbers of helical repeats are constructive, resulting in a minimal mobility, whereas out-of-phase bends spaced at half-helical repeats are destructive, resulting in a maximal mobility. Thus, ligated duplexes with cross-links in phase would be more retarded relative to those with cross-links out of phase if cross-linking induces bending.

A family of DNA duplexes, containing a single central 5'-GGC site and an overhang for ligation (Figure 1A), was cross-linked with DEB under standard conditions. The 30mer in this family has been shown previously to be efficiently cross-linked by DEB and related compounds [21]. Interstrand cross-links were purified via denaturing PAGE by excising the lowest mobility band, which was verified to contain dG-to-dG linkages at central distal deoxyguanosines via piperidine treatment and subsequent analysis of the fragments through denaturing PAGE (data not shown; for similar studies, see [9]). Cross-linked DNAs and appropriate controls, including a 21 bp unbent duplex [19], were exhaustively phosphorylated with [γ - 32 P]ATP on their 5'-ends, ligated, and then analyzed via native PAGE. Products appeared as a ladder of bands, with cross-linked duplexes retarded relative to the corresponding controls of the same length (Figure 1B), consistent with cross-link-induced bending. Cross-linked duplexes were somewhat smeared relative to control duplexes, suggesting a subtle heterogeneity in products that could have arisen from some depurination of the heat-labile adducts during the experiment or copurification of some duplexes containing both a central cross-link and a monoalkylated residue.

Relative length (R_L , the ratio of the apparent length to the actual length) was calculated for each ligation product through reference to the 21 bp unbent control (Figure 1C). The relative lengths of all multimers of the control, uncross-linked duplexes were close to 1.0, consistent with little or no bending. On the other hand, multimers of the cross-linked DNAs generally displayed length and phase dependence consistent with bending, with the R_L values of 30mer and 31mer higher than those of the 32mer, the 33mer, and the 35mer. For example, for the multimers corresponding to ligation of five units of each duplex (~150 bp), average R_L values were as follows: 30mer, 1.13; 31mer, 1.14; 32mer, 1.05; 33mer, 1.08; 35mer, 1.06. Therefore, cross-link-induced bends that are separated by close to an integral number of turns (with approximately 10.5 bp/turn) have decreased mobility relative to control, uncross-linked DNAs of the same length. In contrast, DNAs with cross-links separated by about half-helical repeats migrate similarly to controls because of interference. The reduced R_L value for the 32mer relative to the 30mer and 31mer suggests that cross-linking could induce overwinding so that the helix repeat is slightly less than 10.5 bp/turn, as has been observed previously for mechlorethamine [16]. However, even for the cross-linked 31mer with its maximal retardation, the R_L value was not in the range of $R_L \geq 1.2$, where the equation of Koo and Crothers is valid for multimers 120–170 bp in length [17], so the angle of absolute curvature could not be calculated from these data.

3.2 Quantification of the degree of bending in a DEB-cross-linked 42mer

Assuming a helical repeat of about 10.5 bp per turn, neither a 30mer nor a 31mer has an optimal spacing of cross-links following ligation for maximal retardation. Shorter duplexes

are inefficiently cross-linked by DEB, a phenomenon we attribute to end effects. Therefore, we constructed a 42-bp duplex similar to the family of duplexes already tested (Figure 2A). This 42mer was DEB cross-linked, phosphorylated, ligated, and then analyzed via native PAGE. Relative lengths of the cross-linked multimers were in an appropriate range ($R_L \geq 1.2$) for determination of the angle of curvature (Figure 2B), which corresponded to $33.8 \pm 4.7^\circ$ per cross-link for the 168 bp multimer. The control 42mer also had reduced mobility, but it was too small to quantitate ($R_L < 1.2$ for multimers 120–170 bp in length) [17]. The natural curvature of the unmodified 42mer could reinforce the bending induced by the cross-link, yielding slightly less bending than calculated for the cross-link, or oppose it, yielding more bending than calculated. Furthermore, if the helical repeat is less than 10.5 bp per turn, as suggested by the data for the 32mer, then the degree of bending per cross-link would be higher than the calculated value.

3.3 Determination of the direction of bending

Runs of adenine (A-tracts) induce bending towards the minor groove [22]. We constructed duplexes containing an A-tract either in phase or out of phase with a cross-linking site to determine the direction of bending induced by DEB cross-linking. These 30-bp duplexes had a tract of five deoxyadenosine residues spaced either 5 bp (out-of-phase) or 10 bp (in phase) from the center of a 5'-GGC site (Figure 2C). Electrophoretic retardation and relative length were higher for the cross-linked duplex containing the out-of-phase A-tract than for the duplex containing the in-phase A tract (Figure 2D). This finding suggests that the bends induced by the A-tract and the DEB cross-links are constructive when they are out of phase and destructive when they are in phase. Therefore, we conclude that DEB cross-links induce bending towards the major groove.

4. Discussion

DEB interstrand cross-links were originally postulated to form exclusively between deoxyguanosine residues on opposite strands of DNA at the duplex sequence 5'-GC, which contains the minimal N7-to-N7 distance in B DNA [23]. However, experimental evidence revealed that DEB forms interstrand cross-links preferentially at 5'-GGC sites in duplex DNA [8, 9, 10], the same consensus sequence for nitrogen mustard cross-linking [12, 13]. Because both agents bridge a distance that exceeds that predicted to be possible from their chain lengths, they have been proposed to induce distortion in the normal B-DNA structure upon cross-linking [8, 13]. Indeed, Rink and Hopkins experimentally confirmed mustard-induced bending of $12.4 - 16.8^\circ$ per lesion, although the direction of bending was not determined [16]. Even larger distortions would be expected to accommodate DEB cross-linking because its four-carbon atom chain is shorter than that of mechlorethamine by one nitrogen atom. Evidence for structural changes upon DEB cross-linking includes blockage of the 3'-exonuclease activity of *E. coli* Polymerase I one nucleotide ahead of interstrand *bis*-N7-BD lesions [10].

In this study, our data is consistent with a bend of approximately 34° per DEB cross-link at the 5'-GGC sequence, with some overwinding possible. This confirms the prediction that DEB induces more distortion than mechlorethamine because of its shorter chain length [8]. The magnitude of the DEB-induced bend is comparable to that induced by the major adduct of *cis*-diamminedichloroplatinum(II) (cisplatin), which bends DNA about 40° towards the major groove [24]. This bending is believed to contribute to the antitumor activity of cisplatin through recruitment of HMG domain proteins, shielding the lesion from repair proteins and ultimately triggering cell death [25, 26, 27, 28]. Indeed, the sensitivity of human cancer cells to cisplatin can be increased through overexpression of HMGB1 and other HMG domain proteins [29].

DNA bending plays a key role in such cellular events as transcription, replication, and repair. Bending can arise from protein binding, specific DNA sequences (such as A-tracts), or the binding of small molecules [20]. Compounds that bend DNA can enhance regulatory protein binding, an activity that could contribute to the ultimate biological effects of these agents. These effects can include anticancer activity, such as for cisplatin [25, 26, 27], or carcinogenicity, such as for benzo[a]pyrene diol epoxide [30].

In conclusion, our results show that a DEB cross-link at the sequence 5'-GGC induces a bend of approximately 34° in the direction of the major groove. Overwinding could also be a consequence of cross-linking. The significance of this structural deviation from B-form DNA could arise through recruitment of regulatory proteins. Although DEB has recently been shown to induce cross-links between DNA and many cellular proteins [31], specific proteins that recognize DEB-DNA interstrand cross-links remain largely uncharacterized [32].

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Highlights

- We examined potential DNA bending of interstrand cross-links induced by diepoxybutane.
- Cross-linked DNA had anomalously low electrophoretic mobility in native polyacrylamide gels.
- Our findings are consistent with DNA bending of $\sim 34^\circ$ per lesion towards the major groove.

Figure 1.

Panel A shows the DNA duplexes used. *Panel B* is a representative autoradiogram of the ligation products generated from these duplexes. Lane 1: size markers (150 bp and 300 bp); Lanes 2–6: control duplexes, 30, 31, 32, 33, and 35, respectively; Lanes 7–11: cross-linked (XL) duplexes, 30, 31, 32, 33, and 35, respectively; Lane 12: standard ligated DNA (21 bp). *Panel C* is the average (N=3) relative length of these duplexes as a function of actual length.

Figure 2.

Panel A shows the 42mer used. *Panel B* is the average relative length of 42mer, both cross-linked (XL) and uncross-linked (control), as a function of actual length. *Panel C* shows the A-tract-containing DNA duplexes. *Panel D* is the average relative length of these duplexes, both cross-linked (XL) and uncross-linked (control), as a function of actual length.

Chart 1.
Reaction of DEB with DNA to give a monoadduct (**1**) and cross-link (**2**) at deoxyguanosine residues.