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Interstrand Cross-Links Generated by Abasic Sites in Duplex DNA

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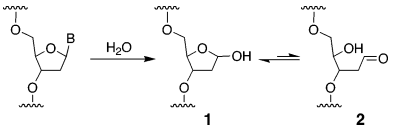
Abasic sites (**1**, Scheme 1), which are generated by hydrolysis of the glycosidic bonds connecting the heterocyclic nucleobases to the deoxyribose backbone, represent the most common type of damage suffered by genomic DNA.[1] Spontaneous hydrolysis yields approximately 10,000 abasic sites per cell per day. In addition, exposure of DNA to radiation, mutagens, and anticancer drugs can lead to the generation of abasic sites.[2] Unrepaired abasic sites are mutagenic or cytotoxic.[1]

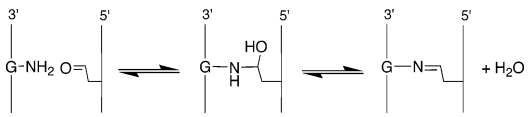
Scheme 1



Interestingly, an early report suggested that abasic sites can generate interstrand cross-links in duplex DNA.[3] Subsequent studies confirmed this finding, but the structure of these lesions has remained unclear.[4] This is a striking observation because interstrand DNA cross-links have profound biological consequences.[5] For example, a single unrepaired interstrand cross-link can be sufficient to kill a eukaryotic cell.[6] The widespread occurrence of abasic sites in cellular DNA along with the potent biological activities associated with cross-links make it important to characterize interstrand cross-links derived from abasic sites in duplex DNA.

Abasic sites in duplex DNA consist of an equilibrating mixture of the ring-closed acetal (99%) and the ring-opened aldehyde (1%, Scheme 1).[7] When considering the possible chemical structure(s) of abasic-site-derived cross-links, it is important to note that aldehydes can covalently modify DNA, with a preference for reactions involving the exocyclic N^2-amino group of guanine residues.[8] Furthermore, recent studies have shown that an aldehyde residue tethered in the minor groove of duplex DNA can generate an interstrand cross-link via carbinolamine/imine formation with the exocyclic N^2-amino group of a guanine residue on the opposite strand of the double helix (Scheme 2).[8c,9] In some cases, reduction of the initially formed imine with $NaCNBH_3$ can stabilize these cross-links. This type of reductive amination is favored at pH $\approx$ 5.[10]

Scheme 2



On the basis of these literature precedents, we used molecular modeling to predict B-DNA sequences that might be preferred sites for interstrand cross-link formation involving reaction of the abasic aldehyde **2** with the exocyclic N^2-amino group of an opposing guanine residue.[11] The modeling results summarized in Figure 1 inspired us to investigate formation of interstrand DNA cross-links in the context of duplexes **A** and **B** (Scheme 3) which were expected to place the reacting groups in close proximity.

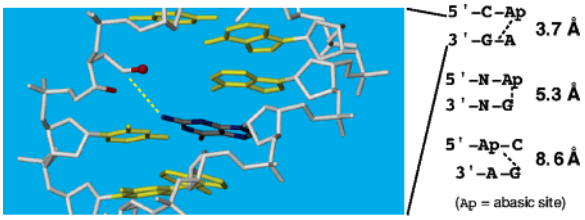


Figure 1.

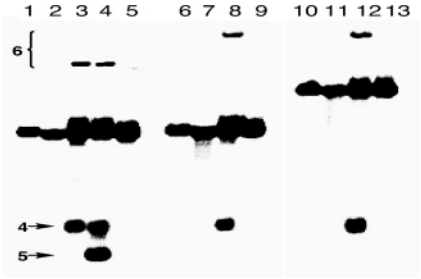


Figure 2. Interstrand cross-linking of duplexes **A**, **C**, and **D**. In a typical cross-linking reaction, the abasic-site-containing DNA duplex was incubated in MES buffer (50 mM, pH 5.5) for 7 d in the presence of $NaCNBH_3$ (270 mM) at 30 °C. The sample was lyophilized and dissolved in formamide loading buffer; the DNA fragments were resolved on a 20% denaturing polyacrylamide gel and visualized by phosphorimaging. Lanes 1, 6, and 10: uracil-containing precursors to duplexes **A**, **C**, and **D**, respectively (as size markers); lanes 2−5: duplex **A**; 7−9: duplex **C**; 11−13: duplex **D**. Lanes 2, 7, 11: freshly prepared abasic duplex (without subsequent incubation); 3, 8, 12: standard cross-linking reaction; 4: standard cross-linking reaction + piperidine workup; 5, 9, 13: standard cross-linking reaction + methoxyamine (CH_3ONH_2). In lane 4, the non-piperidine-labile band co-migrating with **4** likely consists of the $NaCNBH_3$-reduced, alcohol analogue of **4**.

DNA duplexes containing abasic sites were prepared by treatment of 2′-deoxyuridine-containing duplexes with uracil deglycosylase.[12] This enzyme hydrolytically removes uracil from the DNA backbone to generate the desired abasic site. To facilitate detection of interstrand DNA cross-links, the abasic strand in these duplexes was ^{32}P-labeled on the 5′-end. In the event, incubation of duplex **A** in MES buffer (pH 5.5) in the presence of $NaCNBH_3$, followed by 20% denaturing polyacrylamide gel electrophoresis, reveals a substantial yield (3 ± 0.3%) of a slow-moving band in the region of the gel where interstrand cross-links are expected[5] to migrate (Figure 2, lane 3). Consistent with the anticipated involvement of the abasic aldehyde residue in formation of the slow-moving band, addition of methoxyamine (20 mM) inhibits formation of this species (lane 5). Methoxyamine "caps" abasic sites through conversion of the aldehyde group to the oxime derivative (**3**, Scheme 3).[13] Importantly, when the reaction is carried out at pH 7 rather than 5.5, the slow-moving cross-link band is still observed in significant yield (~1%).

Extension of the strand opposing the abasic oligonucleotide (duplex **C**) retards migration of the slow-moving band (lane 8, Figure 2), indicating that this band contains *both* strands of the

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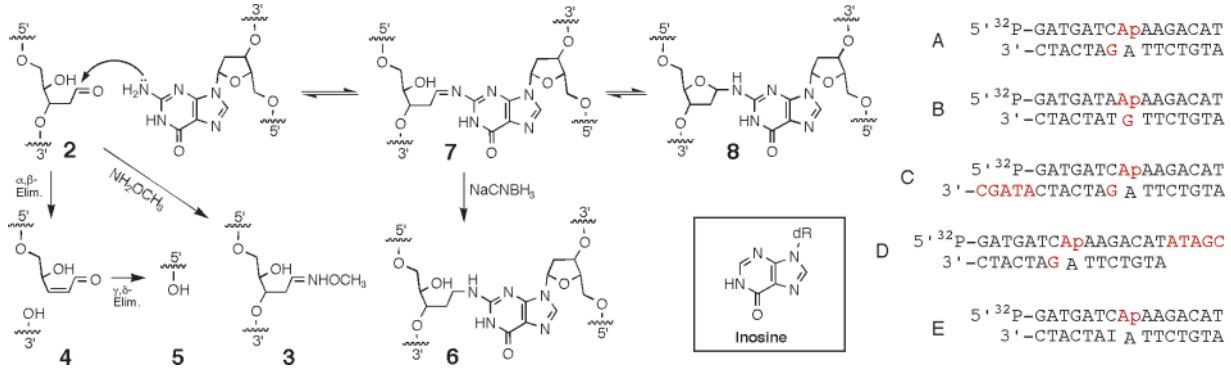
Scheme 3



starting duplex. Extension of the 3′-end of the abasic oligonucleotide (duplex **D**) similarly retards migration of the slow-moving band (lane 12, Figure 2), indicating that the slow-moving band contains the *full length* abasic oligo and not a truncated strand (**4** or **5**) stemming from elimination of the oligonucleotide fragment to the 3′-side of the abasic site (Scheme 3). Overall, these results indicate that the slow-moving bands observed in Figure 2 are abasic-aldehyde-derived interstrand cross-links.

Consistent with our expectation that interstrand cross-link formation would involve reaction of the abasic site with an opposing guanine residue, cross-linking is strongly dependent upon the location of this base. For example, a slow-moving cross-link band is not generated by duplex **B**, in which the guanine lies directly across from the abasic site (Figure 3, lane 7). Evidence implicating the involvement of the guanine N^2-amino group in the cross-linking reaction is provided by the observation that replacement of the key guanosine in duplex **A** with inosine (to yield duplex **E**) completely abrogates cross-link formation (Figure 3, lane 11). This base substitution amounts to the targeted deletion of a single guanine N^2-amino group from duplex **A** (for the structure of inosine, see inset Scheme 3). Finally, negative ion nanospray mass spectrometric analysis of the slow-moving band derived from the incubation of duplex **A** in the presence of $NaCNBH_3$ reveals an m/z of 9018.2 ± 0.4 consistent with that expected (m/z 9018.0) for the cross-link structure **6**, stemming from hydride reduction of the parent cross-link **7**/**8** (Scheme 3).

In summary, the results provide evidence that the most common type of cellular DNA damage, the abasic site, has the potential to generate one of the most biologically deleterious lesions, an interstrand DNA cross-link, via reaction of the abasic aldehyde group with the exocyclic N^2-amino group of an opposing guanine residue in 5′-d(CAp) sequences. Further work is required to determine whether these cross-links contribute to the chemical and biological properties of abasic sites in duplex DNA.

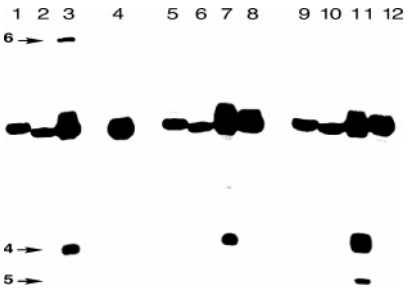


Figure 3. Evidence for the role of the opposing guanine residue in the cross-linking reaction. Cross-linking reactions were conducted as described in the legend of Figure 2. Lanes 1, 5, 9: uracil-containing precursors to duplexes **A**, **B**, and **E**, respectively (as size markers). Lanes 2−4: duplex **A**; 6−8: duplex **B**; 10−12: duplex **E**. Lanes 2, 6, 10: freshly prepared abasic duplex (without subsequent incubation); 3, 7, 11: standard crossl-inking conditions; 4, 8, 12: standard cross-linking conditions + CH_3ONH_2. The bands marked "**4**" likely consist of a mixture of **4** and the $NaCNBH_3$-reduced alcohol analogue of **4**.

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Supporting Information Available: Experimental procedures for all reactions and analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

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