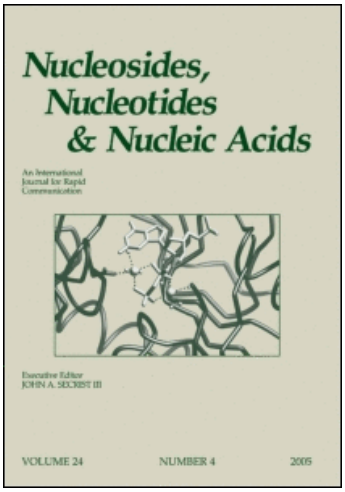

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Site-Specific Formation of A basic Lesions in DNA

Robert S. Coleman[a]; Richard M. Pires[a]
[a] Department of Chemistry, The Ohio State University, Columbus, Ohio, USA

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Site-Specific Formation of Abasic Lesions in DNA

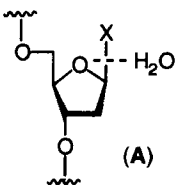
Robert S. Coleman* and Richard M. Pires

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210-1185, USA

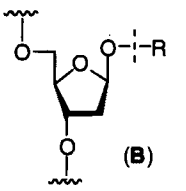
Abstract: A method for the introduction of depurinated lesions in DNA is described, and is based on the formation of a covalent cross-link between an antisense oligonucleotide probe and the target DNA sequence followed by an unexpectedly mild thermal depurination.

Apyrimidinic and apurinic (abasic) sites in DNA oligomers result from the inherent susceptibility of the glycosidic bond of nucleosides towards hydrolysis.[1] The lesions can be produced by the action of DNA damaging agents,[2] spontaneously,[3] and are intermediates in the enzymatic repair of DNA damage.[4] Abasic sites are thought to be carcinogenic and mutagenic,[5] and they have a significant impact on the stability and conformation of DNA duplexes.[6]

As a result of their biological importance, there is a need for methods to introduce abasic sites into prespecified positions within oligonucleotides. There have been reports of the synthetic incorporation of abasic sites into oligonucleotides using a non-natural base that imparts increased hydrolytic lability to the glycosidic bond (process A),[7] or by incorporation of a protected 2-deoxyribofuranoside followed by post-synthetic deprotection of the glycosidic oxygen (process B).[8] The selective enzymatic hydrolysis of dU residues from synthetic oligonucleotides using uracil-DNA glycoslase is routinely used for introduction of abasic sites,[9] but this technique necessitates the synthesis of dU-containing DNA oligomers. Deoxyribose analogs have also served as abasic site models.[10] Without exception, the methods reported to date rely on the synthetic incorporation of a non-natural base into DNA.

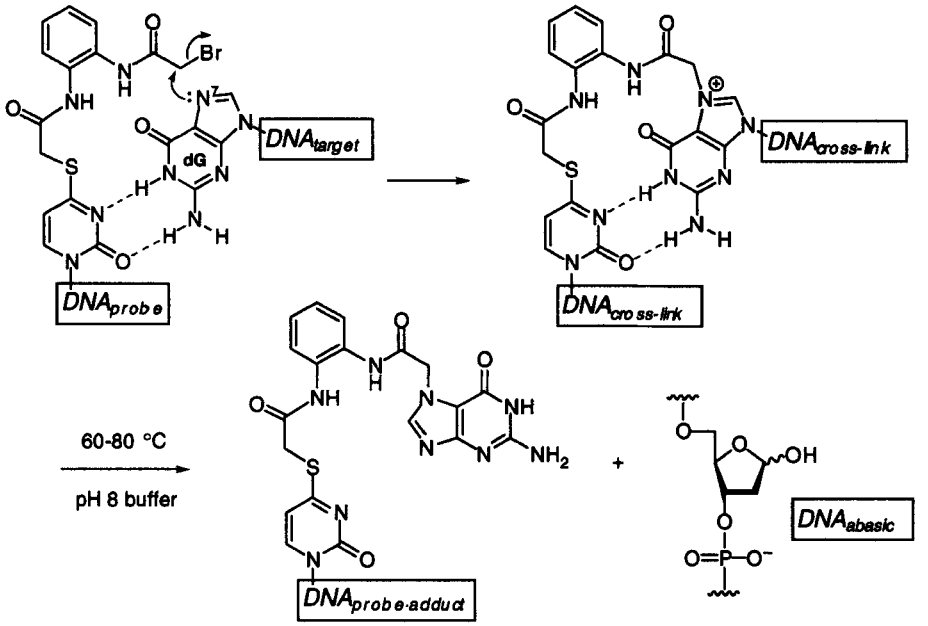


(A)



(B)

We report our preliminary observation of the formation of apurinic sites in DNA oligomers using an approach that is based on the formation of a covalent interstrand cross-link between an electrophilic antisense oligonucleotide probe and a deoxyguanosine (dG) residue in a native sequence of DNA.[11,12,13] This affords a unique dG glycosidic bond with enhanced hydrolytic lability that can be cleaved by neutral and mild thermal hydrolysis to afford an oligonucleotide with a site-specifically incorporated abasic lesion. Although the increased lability of alkylated nucleic acids is a well studied phenomenon,[14] being the basis for the Maxam-Gilbert G-specific sequencing reaction,[15] this aspect of nucleoside chemistry has not been used for introduction of abasic sites. In addition, we report a preliminary observation on the sequence dependence of this thermal depurination.



Cross-linking probe oligonucleotides bearing an α-bromoacetamide tethered to 4-thio-2′-deoxyuridine ($d^{S4}U$) undergo site-selective cross-linking to the deoxyguanosine residues at the N7 position of target oligomers.[11,12] As the cross-linking reaction between the DNA_{probe} and ^{32}P end-labeled DNA_{target} went to completion to afford $DNA_{cross\text{-}link}$ as a lower mobility band on denaturing polyacrylamide gel electrophoresis, we observed the concomitant formation of a new band that was of very similar mobility to the starting DNA_{target}. We supposed that this new product was oligonucleotide DNA_{abasic} containing an abasic site formed as a result of spontaneous hydrolytic depurination of the $DNA_{cross\text{-}link}$. This hypothesis was confirmed by treatment of the reaction mixture with piperidine at 95 °C, whence the product DNA_{abasic} was converted to strand cleaved products, as expected for an oligonucleotide containing an abasic lesion.

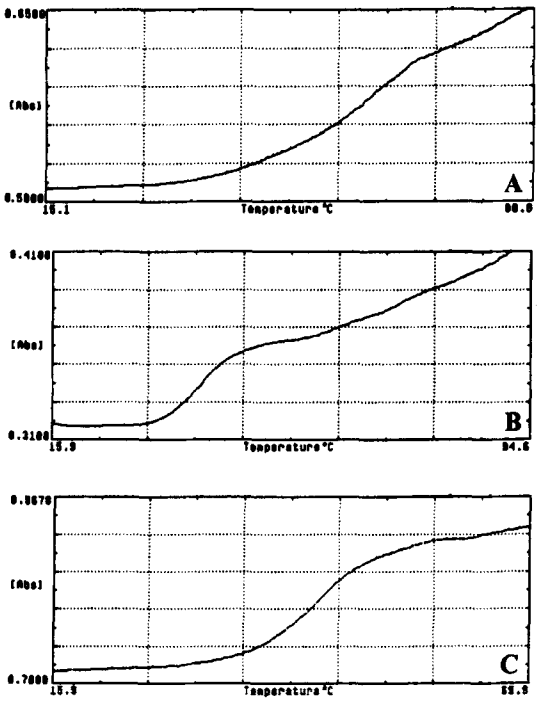


Figure 1. Melting curves: A = $DNA_{cross\text{-}link}$; B = re-melt, now of depurinated strand DNA_{abasic} and $DNA_{probe\text{-}adduct}$ strand; C = control duplex.

5′- T A A T A C G A C X C A C T A T T -3′
3′- A T T A T G C T G G G T G A T A A -5′

Evidence for this mechanism came from melting studies of duplex $DNA_{cross\text{-}link}$ (Figure 1) in 100 mM phosphate buffer (pH 8) containing 100 mM NaCl, which showed a poorly defined T_m = 75 °C with an anomalous, irreversible transition near 60 °C (Curve A). When this sample was re-annealed and the melting repeated, it showed a clear, well defined T_m = 40 °C (Curve B) indicating that the cross-link was not present: the species was the duplex of $DNA_{probe\text{-}adduct}$ and the apurinic DNA_{abasic}. The non-cross-linked duplex containing an *N*-acetyl phenylenediamine tether as a control[12] gave a clearly defined T_m = 45 °C (Curve C).

The mild conditions under which this depurination took place were remarkable. Similar treatment with $(CH_3O)_2SO_2$ gave no thermally induced abasic site formation (data not shown). This means that depurination is a result specifically of the cross-linked lesion, and not just due to N-alkylation of the complementary base. The *o*-phenylenediamine tether was the only system that showed this effect, contrasting the related *m*-phenylenediamine and 1,2-ethylenediamine systems.[12] With the sequence **1**, a single cross-link is formed to the complementary dG residue in **2** with the *o*-phenylenediamine linker (Figure 2, Lane 2).[12] Subsequent warming of the PAGE-isolated cross-linked duplex at 65 °C for 30-60 min in pH 8 buffer caused near quantitative depurination at the site of the lesion (Figure 2, Lane 3), as demonstrated by Maxam-Gilbert sequencing of the depurinated oligomer **3** (data not shown).

5′- T A A T A C G A C X C A C T A T T -3′ **1**
3′- A T T A T G C T G G G T G A T A A C C G G A A G G C A T A T -5′ * **2**
3′- A T T A T G C T G – G T G A T A A C C G G A A G G C A T A T -5′ * **3**

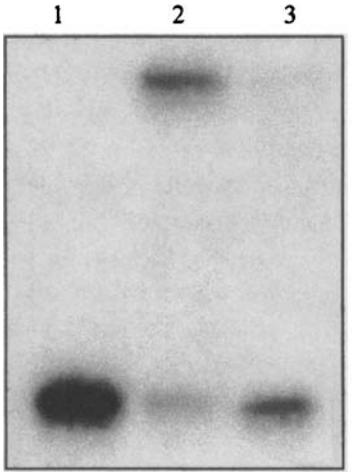


Figure 2. Thermal depurination of probe **1** to target **2** oligomers. Lane 1: ^{32}P labeled target oligomer **2**; Lane 2: cross-link formation; Lane 3: thermal (65 °C, 1 h) formation of depurinated oligomer **3**.

Figure 3. Cross-linking of probe **4** to target **5** oligomers. Lane 1: ^{32}P labeled target oligonucleotide **5**; Lane 2: cross-link formation (upper band) and spontaneous depurination to form **6** (lower band).

The rate of thermal depurination was surprisingly dependent upon the specific base that was N-alkylated. In the DNA **4** containing the 5′ -XCC-3′ sequence with the *o*-phenylenediamine linker, cross-linking to **5** occurs in high yield at both the complementary dG and the dG two bases to the 5′ side.[12] The *complementary* cross-link underwent spontaneous depurination to form **6** at 25 °C (Figure 3, Lane 2), as demonstrated by piperidine induced cleavage (95 °C) of this material, isolated by PAGE (Figure 4, Lane 3). The more stable 5′ -disposed cross-link could be isolated intact from the same gel, and underwent a thermally induced (65 °C) depurination to form **7**. Piperidine induced cleavage (95 °C) of this material demonstrated it resulted from cross-linking to the dG residue two bases to the 5′ side. (Figure 4, Lane 4) Thus, it seems, the rate of depurination is dependent either on the sequence context of the cross-link, or more likely, on the localized conformational distortion induced by cross-link formation.

5′-T A A T A C G A X C C A C T A T A-3′	**4**
3′-A T T A T G C T G G G T G A T A T-5′ *	**5**
3′-A T T A T G C T – G G T G A T A T-5′ *	**6**
3′-A T T A T G C T G G – T G A T A T-5′ *	**7**

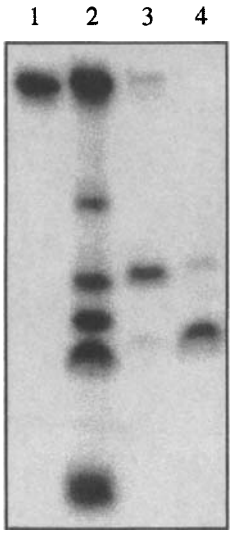


Figure 4. Sequence-dependent depurination of cross-linked oligomers **4** and **5**. Lane 1: ^{32}P-end labeled target oligonucleotide **5**; Lane 2: Maxam-Gilbert G-reaction of **5**; Lane 3: piperidine cleavage of spontaneously depurinated oligomer **6**; Lane 4: piperidine cleavage of thermally depurinated oligomer **7**.

This thermal depurination may prove effective as a method for the synthesis of apurinic site-containing DNA oligomers with which to study repair mechanisms. The present protocol has the possibility of generating a range of sequences containing abasic sites from a single target sequence. Our work complements existing methods for the introduction of abasic sites into DNA, and these preliminary studies will provide the basis for a more thorough examination of their generality.

These results do not change the conclusions of our earlier work[11,12] on *o*-phenylenediamine-based duplex DNA cross-linking, although we now realize that the occasionally modest yields we observed in the cross-linking reactions were due to spontaneous depurination, rather than less-than-quantitative yields as originally believed.

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