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J. Am. Chem. Soc., **2008**, 130 (30), 9646-9647 • DOI: 10.1021/ja8030642 • Publication Date (Web): 01 July 2008

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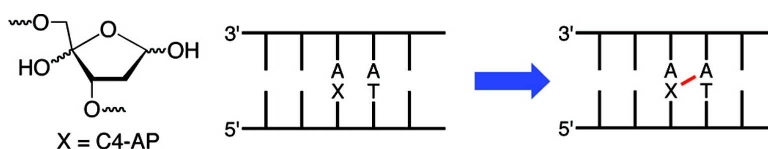

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Published on Web 07/01/2008

Self-Promoted DNA Interstrand Cross-Link Formation by an Abasic Site

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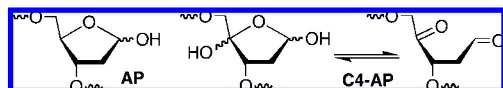
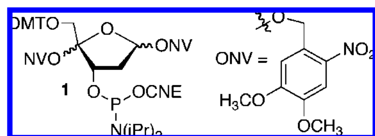
DNA interstrand cross-links (ICLs) are deleterious to cells because they are potent blocks to replication and transcription.[1] Although ICLs have long been associated with drugs such as mitomycin C, the scope of chemical processes that produce them has expanded to include DNA lesions and a radical.[2,3] Gates recently characterized an ICL between an AP site and a dG opposite a 5′-dC.[4] In addition, cyclic adducts formed between dC and the C4′-oxidized abasic site (C4-AP) in cellular DNA were detected by mass spectrometry, but it was uncertain whether the adducts were derived from intra- and/or interstrand cross-links.[5] Although C4-AP is a commonly observed lesion that is formed in high yields by the antitumor agent, bleomycin,[6,7] ICLs had not previously been attributed to this lesion.



A suitable sequence from which to investigate ICL formation was identified by detecting a hotspot for C4-AP formation in a 50 nt fragment of pBR322 irradiated in the presence of Co•pepleomycin.[8] Subsequently, a 31 bp duplex (**2a**) containing C4-AP embedded in the local sequence identified from the PCR fragment was synthesized using **1** and modifed solid-phase synthesis cycles in which the length of the acidic detritylation step was shortened.[9,10] The advantages of **1** compared to the previous phosphoramidite reported by our group include its compatibility with standard oligonucleotide synthesis reagents and higher yielding syntheses.[10,11] C4-AP was generated on an as needed basis in duplexes via brief photolysis (30 min).



Incubation of freshly prepared **2a** at 37 °C produced two higher molecular weight products detected by denaturing gel electrophoresis. Size markers indicated that the slower moving product (**3a**) corresponded to the complement bonded to full-length oligonucleotide containing C4-AP, whereas the lower molecular weight ICL (**4a**) was consistent with the cleaved C4-AP oligonucleotide bonded to the full-length complement. These ICLs were formed in significantly higher yield than the respective product from AP in far shorter time.[4] The growth of **3a** could be fit to first order kinetics over the first 13 h ($k = 5.5 \pm 0.1 \times 10^{-5}\ s^{-1}$), but that of **4a** could not (Figure 1A). The higher molecular weight cross-link proved to be unstable and was not stabilized by treatment with $NaCNBH_3$. However, interrogation of rapidly isolated material with hydroxyl radical (OH•) revealed that cross-linking occurred exclusively at A_{15}.[9,12] The lower molecular weight cross-link product (**4a**) was stable and hydroxyl radical (OH•) cleavage showed that it too involved exclusive reaction with A_{15}. Cross-

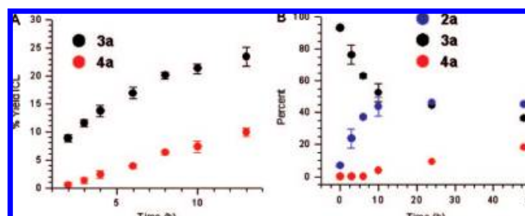
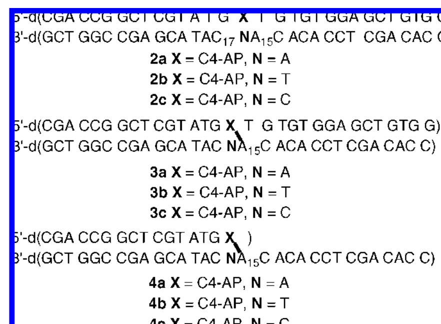


Figure 1. Growth and decay of interstrand cross-links from C4-AP: (A) yield of **3a** and **4b** as a function of time; (B) decomposition of **3a** as a function of time.

linking to A_{15} is very different from the reaction of AP, which produced cross-links exclusively with the dG opposite the 5′-adjacent nucleotide.[4] Isolated **3a** slowly reverted to duplex DNA (**2a**) under the incubation conditions, and over time **4a** appeared (Figure 1B). The lag time for the appearance of **4a** indicated it arises from **2a** but not directly from the higher molecular weight cross-link (**3a**).



Although dA_{16} in **2a** does not form cross-links with C4-AP, replacing it with dT (**2b**) had a dramatic effect on the ICL reaction. A higher molecular weight ICL product consisting of two intact strands (**3b**) was still formed in a first order process ($k = 8.7 \pm 1.5 \times 10^{-5}\ s^{-1}$) exclusively at dA_{15}. The yield of the corresponding lower molecular weight products (**4b**, Note: reaction occurs at A_{15} and C_{17}[9]) were reduced by almost 3-fold (2.6 ± 0.3%) even though nucleotide substitution occurred at the adjacent, nonreactive site. Hypothesizing that dA_{16} was involved at least indirectly in the generation of the lower molecular weight ICL, we attempted to rescue its formation by incubating **2b** with adenine.[13] Indeed, at 0.1 mM adenine a lower molecular weight ICL at dA_{15} (**4b**) was produced in even greater yield (12.6 ± 0.4%) over 10 h than the corresponding product (**4a**) from **2a** (7.2 ± 1.3%). Although **4b** formed at the expense of **3b**, it had little if any effect on the rate constant for formation ($k = 1.0 \pm 0.1 \times 10^{-4}\ s^{-1}$) of the latter. Of several other small molecules tested (Figure 2A) 2-aminopurine and purine were also effective additives for rescuing ICL formation, albeit less so than adenine. The magnitude of adenine's effect on the yield of **4b** was dependent upon its concentration, but was saturated at at 1−2 mM. The lack of an effect by cytosine is consistent with the lower yield of **4c** (3.1 ± 0.3%, 10 h) from a

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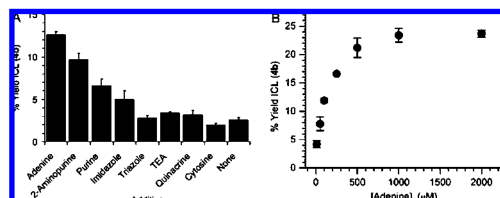


Figure 2. Rescue of ICL formation: (A) additive (0.1 mM) effects on the yield of **4b** from **2b**; (B) adenine concentration effect on the yield of **4b**.

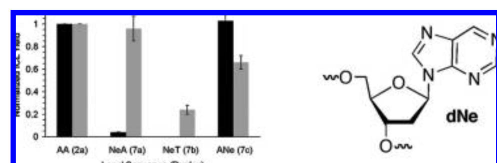
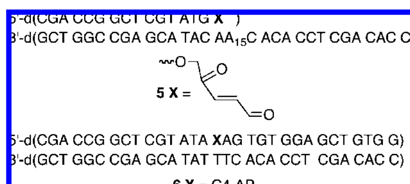


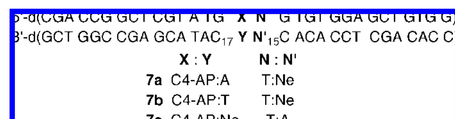
Figure 3. Effect of nebularine (dNe) on ICL formation: higher molecular weight ICL (black); lower molecular weight ICL (gray).

duplex (**2c**) containing dC opposite C4-AP, while the yield of **3c** (15.3 ± 0.5%) was only slightly lower.

Since these data suggested that adenine catalyzes the formation of cross-link **4a**,**b** from C4-AP, we sought to elucidate its role in this unusual reaction. Since the formation of **4a**,**b** involved cleavage of the strand containing C4-AP, the possibility that adenine-catalyzed elimination precedes reaction with the opposing strand was investigated. Treatment of **2a** with endonuclease III, which acts as a lyase on abasic sites producing the trans α,β-unsaturated aldehyde (**5**) rapidly gave rise to the lower molecular weight ICL (**4a**) between the remnants of C4-AP and A_{15}.[9,14] The rate constant for the growth of **4a** ($k \approx 4.9 \pm 0.4 \times 10^{-4}$ s^{-1}) under these conditions showed that **5** yields the ICL considerably faster than it is produced from duplex DNA containing C4-AP. Having shown the kinetic competence of **5** for producing **4a**, the possibility that adenine catalyzes production of the α,β-unsaturated aldehyde was explored using a duplex (**6**) that does not readily offer a position for ICL formation. The total yield of ICLs produced from **6** was <3%, and although the respective cleavage product builds up over 10 h (4.6 ± 0.9%), adding adenine (0.1 mM) has an insignificant effect on its yield (5.6 ± 1.1%). This increase is small compared to adenine's effect on ICL yield from **2b** (Figure 2), indicating that formation of **5** is not the major outcome of the interaction between adenine and C4-AP.



Insight into the structure of the cross-links, as well as adenine's role in promoting ICL formation was sought by subsituting nebularine (dNe) for dA in duplexes containing C4-AP (Figure 3). Substitution of dA_{15} in **2a** by dNe (**7a**) drastically reduced ICL product containing uncleaved oligonucleotides. In addition, OH• cleavage showed that the majority of lower molecular weight cross-links occur approximately equally at dA_{16} and dC_{17}. The change in reaction pattern from **2a** suggests that cross-link formation involves reaction with the exocyclic amine of dA_{15}, which is consistent with the reaction of C4-AP with dC and other bis-electrophiles with dA.[5,15] Replacing the opposing dA with dNe (**7c**) had no effect on the yield of the higher molecular weight cross-link compared to in **2a** and resulted in a small decrease in the yield of the corresponding lower molecular weight ICL. The latter is consistent with the relative abilities of adenine and purine at rescuing the formation of **2b** (Figure 2A). Finally, replacement of dA_{15} and dA_{16} by dNe and dT, respectively (**7b**), eliminated the higher molecular weight cross-link and reduced the yield of the lower molecular weight ICL relative to the level observed from **2a**.



The effect of the nucleotide opposing C4-AP on the lower molecular ICL (dN, pK_a; dA, 3.5 > dNe, 2.1 > dC, 4.2) does not correlate with their respective pK_a values and suggests that the heterocycles do not act as acid/base catalysts.[16,17] Nucleophilic catalysis could explain adenine's effect on ICL formation. In this regard there is precedent to suggest that the hypothetical adenine–aldehyde adduct could act as a storage site for the aldehyde or as a more reactive aldehyde equivalent.[18,19] Regardless, aside from DNA enzymes selected by in vitro methods, this is a rare example in which DNA promotes a process that leads to its own modification.[20–22] The mechanism warrants further investigation, especially given the relatively high yields in which this biologically significant family of lesions is produced.

Acknowledgment. We are grateful for support from the National Institute of General Medical Sciences (Grant GM-063028). We thank Professors Kent Gates (Missouri) for helpful discussions and Hiroshi Sugiyama (Kyoto) for Co•pepleomycin.

Supporting Information Available: Experimental procedures, ESI-MS of oligonucleotides, and sample autoradiograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA8030642