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# Detection of an Interchain Carbinolamine Cross-Link Formed in a CpG Sequence by the Acrolein DNA Adduct γ-OH-1,$N^2$-Propano-2′-deoxyguanosine

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Acrolein **1**, a potential mutagen and carcinogen,[1,2] reacts with dG to form hydroxylated 1,$N^2$-propanodeoxyguanosine (OH-PdG) adducts (Scheme 1).[2−4] Most abundant are the stereoisomeric 3-(2-deoxy-β-D-*erythro*-pentofuranosyl)-5,6,7,8-tetrahydro-8-hydroxypyrimido[1,2*a*] purin-10(3*H*)-ones, γ-OH-PdG adduct **2**.[2,5] Adduct **2** was detected in animal and human tissue,[2] suggesting its involvement in mutagenesis and carcinogenesis.[6] Methods for site-specific synthesis of **2** in oligonucleotides were developed.[7,8] When placed into duplex DNA opposite dC at neutral pH, **2** opened spontaneously to aldehyde **3**.[9] Kozekov et al.[10] trapped a trimethylene cross-link upon insertion of **2** into an oligonucleotide duplex at a 5′-CpX-3′ sequence, followed by $NaCNBH_3$ treatment. This implied the presence of cross-linked imine **5**, in equilibrium with cross-linked carbinolamine **4**. We now report $^{15}N$ HSQC NMR detection of **4** in situ, in the same 5′-CpX-3′ sequence.

The γ-OH-PdG modified oligonucleotide 5′-d(GCTAGCX-AGTCC)-3′, X = **2**, was annealed with 5′-d(GGACTCYCTAGC)-3′, Y = $^{15}N^2$-dG. The $^{15}N$ HSQC spectrum[11] revealed four signals exhibiting a 90 Hz $^1H$ coupling (Figure 1A). A $^{15}N$ HSQC-filtered TOCSY experiment established that $^1H$ signals at δ 8.5 and 8.6 ppm arose from isomeric adducts **4** (Figure 1B). Scalar coupling was observed between $Y^{19}$ $N^2H$ and protons of the cross-link. The signal observed at δ 5.7 ppm indicated coupling to Hγ. Cross-peaks at δ 1.53 and δ 2.02 ppm were observed to the β protons. Diastereomeric cross-links **4** were not formed equally. Because of its low abundance, only a single cross-peak was observed for the minor isomer, attributed to vicinal coupling with the γ proton.

A $^{15}N$ HSQC-filtered NOESY experiment[12] (Figure 1C) suggested the two cross-links **4** were accommodated without disruption of base pair $C^6$·$Y^{19}$. For the major isomer, the $Y^{19}$ $N^2H$ → $Y^{19}$ N1H NOE was observed at δ 12.8 ppm, in the expected chemical shift range for the N1H proton, which participates in Watson−Crick hydrogen bonding. NOEs were observed from $Y^{19}$ $N^2H$ to α, β, and γ protons of the cross-link. The minor stereoisomer exhibited an NOE from $Y^{19}$ $N^2H$ to $Y^{19}$ N1H, and to a resonance at δ 4.5 ppm, probably Hγ. That formation of **4** in the CpG sequence was apparently accommodated with minimal perturbation corroborated studies of a duplex containing a trimethylene cross-link.[13]

Formation of **4** was not quantitative at pH 7 (Figure 2). A peak corresponding to aldehyde **3** was observed at δ 9.5 ppm, confirming previous work.[9] The presence of aldehyde **3** was consistent with $^1H$ cross-peaks at δ 8.1 ppm and δ 6.7 ppm in the HSQC spectrum (Figure 1A). These were assigned to hydrogen-bonded and non-hydrogen-bonded protons at base pair $C^6$·$Y^{19}$ in the noncross-linked species. The CpG cross-link was stable in duplex DNA, but reverted within 1 h in $H_2O$.[10]

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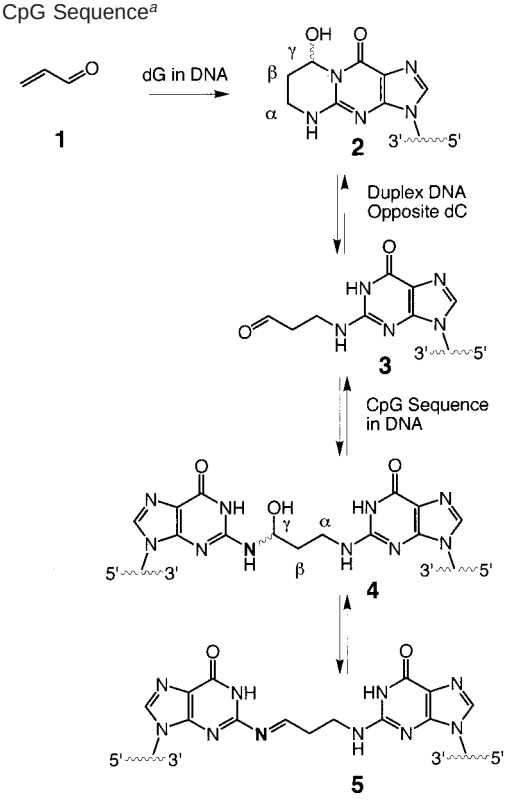
***Scheme 1.*** Formation of the Acrolein Carbinolamine Cross-Link **4** in the CpG Sequence[a]




[a] Adduct **2** spontaneously opened to the $N^2$-(3-oxopropyl)-deoxyguanosine derivative **3**. Reaction of **3** with the guanine exocyclic amino group yielded carbinolamine **4**. Imine **5** existed in equilibrium with **4** evidenced by $NaCNBH_3$ reduction of the cross-link.[10]

At pH 5.5, signals located at δ 8.9 and δ 10.2 ppm appeared (Figure 2). They disappeared upon raising pH, as reported,[9] and were assigned to hydrogen-bonded and non-hydrogen-bonded $C^{18}$ $N^4H$ protons in a Hoogsteen pair at $X^7$·$C^{18}$, in which **2** was intact. Hoogsteen pairing is characteristic of PdG adducts at acidic pH.[14−17] The downfield shift of the $N^4H$ resonances was attributed to the positive charge in the Hoogsteen pair. Ring-opening of **2** was pH dependent and likely facilitated by cytosine in the complementary strand,[9] as is observed for the malondialdehyde $M_1G$ lesion.[18,19]

We conclude that when placed into a CpG sequence in duplex DNA, adduct **2** exists not only in equilibrium with **3**[9] and imine cross-link **5**,[10] but also with carbinolamine cross-link **4**. The biological processing of acrolein damage in DNA may be modulated by the positions of these equilibria. Acrolein is mutagenic in bacterial,[20] mammalian,[21] and human[22,23] cells, and carcinogenic in rats.[24] Its stable analogue 1,$N^2$-propanodeoxyguanosine (PdG) induced G → T transversions and G → A transitions.[25,26] In contrast,



10.1021/ja020333r CCC: $22.00 

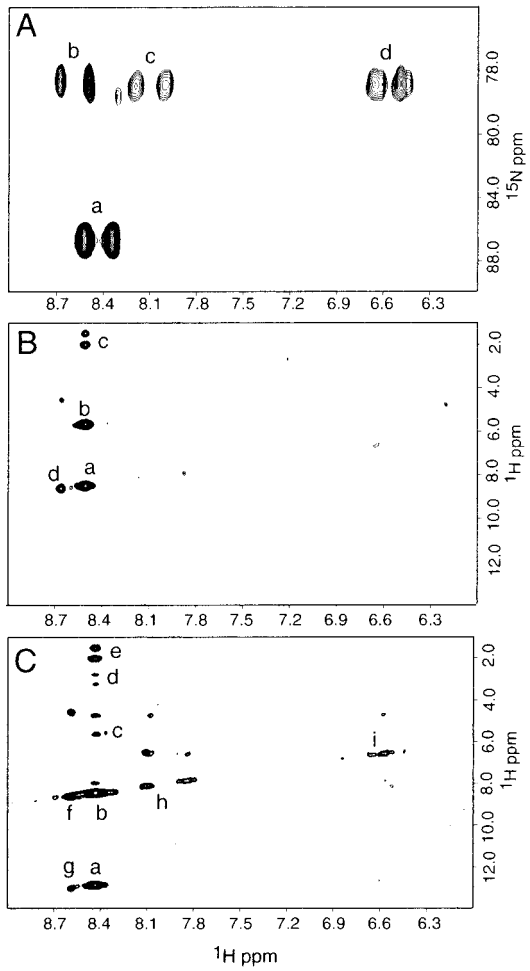



***Figure 1.*** (A) $^{15}$N HSQC spectrum at pH = 7. Peaks a, major; b, minor isomeric cross-links **4**; c, d, hydrogen- and non-hydrogen-bonded $\mathbf{N}^2$ protons, noncross-linked pair $C^6{\cdot}Y^{19}$. (B) $^{15}$N TOCSY HSQC spectrum. Peaks a, autocorrelation for major isomer **4**; b, coupling to H$\gamma$; c, couplings to H$\beta_{1,2}$; d, autocorrelation peak for minor isomer **4**. (C) $^{15}$N NOESY HSQC spectrum. NOEs a, $Y^{19}$ $N^2H \rightarrow Y^{19}$ N1H; b, $Y^{19}$ $N^2H$ autocorrelation; c, $Y^{19}$ $N^2H \rightarrow$ H$\gamma$; d, $Y^{19}$ $N^2H \rightarrow$ H$\alpha_{1,2}$; e, $Y^{19}$ $N^2H \rightarrow$ H$\beta_{1,2}$; f, $Y^{19}$ $N^2H$ autocorrelation (minor isomer); g, $Y^{19}$ $N^2H \rightarrow Y^{19}$ N1H; h, i, hydrogen- and non-hydrogen-bonded $N^2$ protons of noncross-linked pair $C^6{\cdot}Y^{19}$. 5′-d(GCTAGCX-AGTCC)-3′, X = 2, was purified by C8 HPLC in 0.1 M ammonium formate (pH 6.5). Negative ion MALDI-TOF mass spectrometry yielded *m*/*z* 3700.9 (calcd $[M - H]^-$ 3700.7). 5′-GGACTCYCTAGC-3′, Y = $^{15}\mathbf{N}^2$-dG, was prepared by deprotection of 5′-GGACTCZCTAGC-3′, Z = $O^6$-TMSE-2-fluoro-inosine,[34] using 6 M $^{15}\mathbf{N}H_4OH$, desilyated with 5% acetic acid, and purified by C8 HPLC in 0.1 M ammonium formate (pH 6.5). Negative ion MALDI-TOF mass spectrometry yielded *m*/*z* 3645.9 (calcd for $[M - H]^-$ 3645.6).

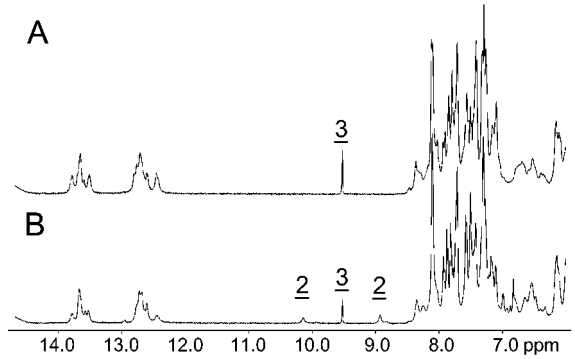



***Figure 2.*** (A) At pH 7.0, adduct **2** converts to aldehyde **3** as evidenced by the resonance at $\delta$ 9.5 ppm. (B) At pH 5.5, adduct **2** exists in equilibrium with **3**. Signals observed at $\delta$ 8.9 and $\delta$ 10.2 ppm arise from a duplex containing adduct **2**, and in which the base pair $X^7{\cdot}C^{18}$ exists in the Hoogsteen conformation.

adduct **2** was weakly mutagenic in *Escherichia coli*,[27] and in HeLa, XP-A, and XP−V cells.[28,29] This was attributed[27−29] to its rearrangement to **3** in duplex DNA.[9] The cross-links **4** and **5** formed when **2** is located in CpG sequences may contribute to the mutagenic spectrum of acrolein and may interfere with DNA repair.[30] Similar spectroscopic experiments will facilitate in situ characterization of additional acrolein-induced reversible DNA cross-links, and others,for example, those arising from crotonaldehyde,[31] malondialdehyde,[32] or the epoxide of vinyl chloride.[33]

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