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Dramatic 5′-Residue Effect on Conformer Distribution of Short Oligonucleotide Retro Models of the Cisplatin−DNA Cross-Link: Implications for the Lippard and Cross-Link Distorted Base Pair Steps Present in Cisplatin−DNA Duplex Adducts

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An unusually distorted base pair (bp) step, which we refer to as the Lippard bp step, was discovered recently[1] next to the distorted G*G* step containing the adjacent intrastrand cross-link, the main DNA lesion formed by the anticancer drug cisplatin (G* = N7-platinated dG). The distortions arise mainly from a large movement of the 5′-G*•C bp relative to the X•X′ and 3′-G*•C bps (Chart 1). The Lippard bp step was identified by solid-state X-ray methods in a DNA 16-mer duplex bound to an HMG protein.[1] By assessing in-depth NMR data, we concluded that the distorted step exists in all G*G* DNA duplexes characterized well by NMR methods.[2] The Lippard bp step is thus intrinsic to intrastrand lesions formed by this very widely used therapeutic drug.[2] Within the accuracy of the methods,[2] the distortions appear to involve the DNA, not the Pt geometry,[3] and to be similar in solution and solid protein-bound states. Such good correlations between solution and solid-state results have been elusive because the dynamic nature and conformational diversity of DNA increase on cross-link formation, making structural assessments even more difficult.[2,4]

Now that the distorted structure is well defined, we can compare features of the duplex with those of single-strand (ss) G*G* adducts to define the forces stabilizing the distortions. We chose to examine ss G*G* adducts with T residues because GGT is part of the repetitive sequence in ss telomeres, a potential cisplatin ss target.[5,6] In solution, G*G* favors an HH1 form [head-to-head (HH) bases, *anti* G* residues, and the B-DNA phosphodiester backbone propagation direction]; this form is highly left-handed (L) canted in XG*G* ss[7−9] and right-handed (R) canted in duplex adducts (Chart 2).[4] This long-recognized difference in canting[10] is poorly understood. Recent duplex studies[1,2] provide the new information that the degree of R canting is low, and ammine H-bonds are either absent or very weak. The X residue has an N pucker,[1,2,4] which may either cause the distortion exemplified by the Lippard bp step or be a consequence of the essentially normal X•X′ WC H-bonding.[1,2] In contrast, in L ss X-ray structures the ammine cis to the canted 5′-G* models always has an H-bond to the oligo,[11,12] and the X residue has an S sugar.[11]

The S pucker and the L canting in ss models[7−9,11] may be favored by H-bonding with the carrier ligand; alternatively, the N pucker and R canting in the duplex may arise because such H-bonding is absent. Thus, it is of some interest to determine if the X-residue pucker and L canting are related to flanking residue H-bonding by examining **Me$_2$ppz**Pt retro models because the **Me$_2$ppz** (*N*,*N*′-dimethylpiperazine) ligand cannot form NH H-bonds and does not influence conformer structure and distribution.[13] Retro models, which have bulky carrier ligands, exhibit an ∼10^9 reduction of the rotation rate of the guanine base relative to cisplatin,[13] allowing us both to define structure and to identify new conformers of dinucleotide d(G*pG*) adducts in solution by NMR methods.[4,14] (Phosphodiester linkage denoted by "p" only for dinucleotides.) The NMR data (Figure 1, Table 1, Supporting Information "**SI**") establish that **Me$_2$ppz**Pt(d(TG*G*T)) exists at equilibrium as ∼100% HH1 form. This is the first reported case in which an ss G*G* adduct has been shown to favor one conformer. Usually the HH1 form is in equilibrium with a second appreciable form, a ΔHT1 or an HH2 form (Chart 2).[14] For **Me$_2$ppz**Pt adducts at equilibrium, d(G*pG*)[13] and d(G*G*T) uniquely have *three* significant forms, but, in contrast, d(TG*G*) has only HH1 (Table 1). Thus, a 3′-T has virtually no effect, but the 5′-T has a dramatic effect. *Carrier-ligand H-bonding cannot cause the high preference of the HH1 form because **Me$_2$ppz** cannot form H-bonds.* For all new adducts,

Chart 1. Distorted X•X′, 5′-G*•C, and 3′-G*•C bp Region

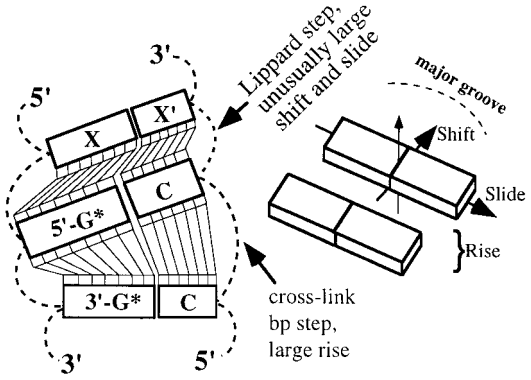
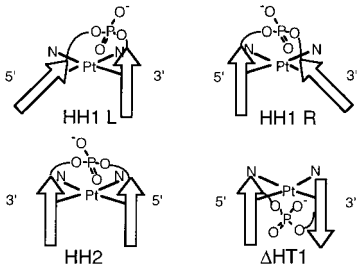


Chart 2. Known Forms of d(G*pG*) Retro Models Showing Both L-Canted (HH1 L) and R-canted (HH1 R) Forms (G base shown as an arrow with the H8 atom at tip)



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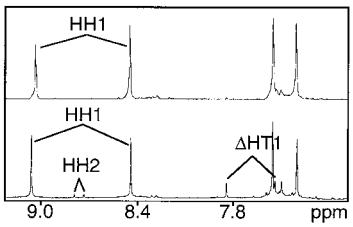

Figure 1. G H8/T H6 NMR signals for **Me$_2$ppz**Pt(d(TG*G*T)), pH ∼4 at ∼14 days (bottom). Only the HH1 signals were present after ∼60 days (top). Lines point to G H8 signals for each form.

Table 1. Distribution and NMR Data for **Me$_2$ppz**Pt Adducts[a]

Me$_2$ppz Pt adduct	HH1 (%)	G*	H8 shifts (ppm)[b]	$J_{H1'-H2'}$ (Hz)[b]	$J_{H1'-H2''}$ (Hz)[b]
d(G*pG*)[c]	50	5′	8.51	0	7.6
		3′	8.93	10.0	4.9
d(G*G*T)[c]	50	5′	8.48	0	8.3
		3′	8.93	9.2	4.8
d(TG*G*T)[d]	>97	5′	8.45	0	7.3
		3′	9.14	10.4	5.3
d(TG*G*)[d]	>97	5′	8.47	0	7.5
		3′	9.10	9.8	5.0
d(pG*pG*)[d]	>95	5′	8.59	0	7.2
		3′	9.02	9.1	4.7

[a] D_2O at 5 °C, pH ∼4. [b] HH1. [c] ∼20% HH2 and 30% ΔHT1 found after ∼14 and ∼60 days. [d] After ∼60 days (or more); distributions at ∼14 days were ∼80% HH1, ∼4−8% HH2, and ∼9−16% ΔHT1.

unambiguous NMR signals indicated formation of HH1, HH2, and ΔHT1 conformers as kinetic and sometimes equilibrium products (**SI**). This is the first reported characterization of multiple conformers in ss G*G* adducts longer than d(G*pG*).

From downfield 3′-G* H8 shifts of *cis*-Pt(NH$_3$)$_2$ ss adducts[4,7−10] and X-ray structures [d(CG*G*), three independent, very L-canted molecules with ammine 5′-G* O6 H-bonding;[11] d(pG*pG*), two molecules L with the 5′-p H-bonded[12]], L canting decreases in the order: 5′-residue > 5′-p > no substituent. This order is reflected in **Me$_2$ppz**Pt adducts by very downfield 3′-G* H8 NMR shifts (Table 1), clearly indicating that HH1 L canting decreases in the series: d(TG*G*T) ≈ d(TG*G*) > d(pG*pG*) > d(G*pG*) ≈ d(G*G*T). *The degree of L canting clearly depends on 5′-X steric effects, not on ammine H-bonding.*

From coupling data (**SI**), 5′-T of **Me$_2$ppz**Pt(d(TG*G*T)) has an S pucker. Thus, the S pucker of the 5′-X residue is not related to ammine H-bonding. Indeed, by assessing models of hybrid structures constructed from pieces of known structures (**SI**), we find that L models with known C3′−O3′ torsion angles (ϵ) have severe 5′-X 5′-G* clashes unless the 5′-X has S sugar pucker and $\epsilon = -146°$, the X-ray value for the highly L-canted *cis*-Pt(NH$_3$)$_2$(d(CG*G*)).[11] The latter has close 5′-X-5′-G* contacts, consistent with the very upfield 5′-T H2′ signals of **Me$_2$ppz**Pt adducts (**SI**). A striking feature for all adducts is that the shifts and couplings of the G*G* sugar signals of the HH1 conformer are nearly identical when G* lacks a flanking T (**SI**). Thus, the 5′-T residue, although positioned close to the G*G* lesion, causes no detectable changes in backbone geometry, consistent with the X-ray findings.[11,12]

Hybrid models (**SI**) provide additional insight. In ss models, the severe clashes involving an L-canted 5′-G* and an N-puckered 5′-X residue disappear for R canting. The 5′-X N pucker and the backbone torsion angles in the Lippard and G*G* bp steps allow the X·X′ bases to occupy positions favorable for stacking with the 5′ flanking region. In this position, the 5′-X base does not clash with the 5′-G* only when G*G* has R canting. (When the 5′-X in duplex hybrid models has the ∼180° ϵ value of the distorted duplex,[2] R-canted 5′-G* steric clashes with X are minimal even if the 5′-X has an S pucker, but the X and X′ amino groups clash and no X·X′ WC H-bonding is possible, **SI**.) The equal numbers of L- and R-canted molecules in the solid state for *cis*-Pt(NH$_3$)$_2$(d(pG*pG*))[12] and NMR data for d(G*pG*) retro models[14] provide evidence that L-to-R canting changes require little energy. The R molecules[12] simulate several aspects of the Lippard and G*G* bp steps[1,2] (5′-p of 5′-G* position and lack of NH$_3$ H-bonding, G*G* backbone structure, normal Pt geometry).

In conclusion, the highly L nature of ss adducts with a 5′-X residue is chiefly a consequence of the bulk of the 5′ residue; H-bonding interactions are inconsequential. Any 5′ substituent, including the 5′-p group, favors the HH1 form. However, the 5′-p group induces less L canting than a 5′-X residue. Most importantly, the 5′ residue maintains an S pucker in ss adducts even in the absence of H-bonding, suggesting strongly that the N pucker of this residue in the Lippard bp step of duplexes is related to stacking and WC H-bonding, not to the insignificance of ammine H-bonding. Hybrid models built with the *cis*-Pt(NH$_3$)$_2$(d(pG*pG*)) X-ray structure[12] reveal that R canting effectively eliminates clashes with the 5′-X of an X·X′ bp. Our analysis rationalizes key solid-state and solution data in a satisfying fashion.

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Supporting Information Available: Tables and figures of NMR data and of superimposed structures derived from X-ray and NMR data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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