Influence of cisplatin intrastrand crosslinking on the
conformation, thermal stability, and energetics of
a 20-mer DNA duplex

(differential scanning calorimetry/thermodynamics/crosslink-induced conformational/structural changes/DNA recognition)

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ABSTRACT cis-Diamminedichloroplatinum(II) (cisplatin) is a widely used anticancer drug that binds to and crosslinks DNA. The major DNA adduct of the drug results from coordination of two adjacent guanine bases to platinum to form the intrastrand crosslink cis-[Pt(NH3)2(d(GpG)-N7(1), -N7(2))] (cis-Pt-GG). In the present study, spectroscopic and calorimetric techniques were employed to characterize the influence of this crosslink on the conformation, thermal stability, and energetics of a site-specifically platinated 20-mer DNA duplex. CD spectroscopic and thermal denaturation data revealed that the crosslink alters the structure of the host duplex, consistent with a shift from a B-like to an A-like conformation; lowers its thermal stability by ~9°C; and reduces its thermodynamic stability by 6.3 kcal/mol at 25°C, most of which is enthalpic in origin; but it does not alter the two-state melting behavior exhibited by the parent, unmodified duplex, despite the significant crosslink-induced changes noted above. The energetic consequences of the cis-Pt-GG crosslink are discussed in relation to the structural perturbations it induces in DNA and to how these crosslink-induced perturbations might modulate protein binding.

The chemotherapeutic efficacy of the anticancer drug cisplatin is derived from its ability to bind and crosslink DNA, the major adduct being the cis-Pt-GG intrastrand crosslink (1). Previous crystallographic, NMR, and gel electrophoretic investigations have evaluated the impact of this crosslink on the structure and conformation of the host duplex (1–9). These studies indicate that formation of the cis-Pt-GG crosslink unwinds DNA by 13° and bends it by 34°–55° in the direction of the major groove. A very recent crystallographic study revealed that the crosslink can induce formation of an A-form structural subdomain, thereby creating a hybrid DNA duplex with an A-B junction (8). Such cis-Pt-GG-induced alterations in duplex structure have been implicated in the promotion of specific interactions with cellular proteins that contain one or more high mobility group domains—for example, HMG1, Inr1, and human upstream-binding factor (hUBF) (8, 10–15). When bound by such cellular proteins, the cis-Pt-GG sites are shielded from excision repair (11, 13, 16, 17), thereby enhancing the cytotoxic properties of the drug.

Unlike the detailed structural information about the major cisplatin-DNA adduct, comparatively little is known about its corresponding energetic consequences. Such thermodynamic data would reveal how the crosslink influences duplex stability, a property that has been implicated in the modulation of protein recognition and binding (18–20). To address this deficiency, we have used a combination of calorimetric and spectroscopic techniques to characterize the effect of a single cis-Pt-GG intrastrand crosslink on the conformation, thermal stability, and energetics of a 20-mer DNA duplex. The sequences of unmodified (GG20) and modified (cis-Pt-GG20) duplexes examined are depicted in Fig.1. Investigation of these two duplexes has revealed that formation of the cis-Pt-GG crosslink reduces both the thermal and thermodynamic stabilities of the host DNA. We discuss how such adduct-induced energetic consequences may correlate with the structural perturbations believed to mediate recognition by high mobility group domain proteins.

MATERIALS AND METHODS

Oligonucleotide Synthesis, Purification, and Characterization. Oligonucleotides were prepared on a Perkin-Elmer PS250 DNA Synthesizer by using standard cyanomethylphosphoramidite chemistry. Deprotected oligonucleotides were purified initially by size exclusion chromatography on a 25-250 Sephadex (Pharmacia) column, with distilled water as the eluent. Before platination, d(TCTCTTTGCGTCTCCTTCT) was converted to the sodium salt on a Dowex (Bio-Rad) cation-exchange column. The activated complex, cis-[Pt(NH3)2(H2O)2]2+, was formed from the reaction of cisplatin with 1.97 mol equiv of AgNO3. This activated complex was incubated with ≥100 μM strand of d(TCTCTTTGCGTCTCCTTCT) for 3 h at 23°C in 10 mM sodium phosphate (pH 6.0). Oligonucleotides were purified further by preparative ion exchange HPLC through a Dionex Nucleopac PA-100, 9 × 250 mm column, with linear gradients of 0.1 M NH4OAc (pH 6.0) and 0.1 M NH4OAc, pH 6.0/1 M NaCl. Oligomer purity was verified by analytical ion exchange HPLC through a Dionex Nucleopac PA-100, 4 × 250 mm column. Platinum atomic absorption spectroscopy confirmed the presence of only a single platinum atom per strand of d(TCTCTTTGCGTCTCCTTCT).

Determination of Oligonucleotide Extinction Coefficients. Molar extinction coefficients (ε) for the single-stranded oligonucleotides were determined by phosphate analysis as de-

Abbreviations: cis-Pt-GG, cis-[Pt(NH3)2(d(GpG)-N7(1), -N7(2))]; DSC, differential scanning calorimetry.

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Fig. 1. Base sequences of the unmodified 20-mer DNA duplex (GG20) and the corresponding platinated 20-mer DNA duplex (cis-Pt-GG20). The strand numbering system is indicated in parentheses.
scribed (21). The following extinction coefficients at 260 nm and 25°C in units of (mole strand/liter)^(-1)cm^(-1) were obtained: 164,000 for d(TCTCCCTTTGCTCTTCCT) [unmodified strand 1 in the GG20 duplex]; 188,000 for d(TCTCCTTGTGCTTCTTCCT) [modified strand 1 in the cis-Pt-GG20 duplex, hereafter designated as strand 1']; and 181,000 for d(AGAGGAAAGCCAGAGAAGAG) [strand 2 in both the GG20 and cis-Pt-GG20 duplexes]. Isothermal mixing experiments (22, 23) using strand 2 and either strand 1 or strand 1' revealed 1:1 stoichiometries for both complexes, a ratio consistent with duplex formation.

Circular Dichroism (CD) Spectropolarimetry. CD experiments were performed on an Aviv model 62A DS spectropolarimeter (Aviv Associates, Lakewood, NJ) equipped with a thermoelectrically controlled cell holder. The cell pathlength was 0.5 cm. Isothermal CD spectra were recorded from 220 to 320 nm in 1-nm increments with an averaging time of 5 s. The DNA concentration was 5 µM in duplex, and the buffer conditions were 10 mM Mops (sodium salt of 3-(N-morpholino)propanesulfonate) (pH 7.4), 100 mM NaCl, 10 mM MgCl2, and 0.1 mM EDTA.

UV Absorption Spectrophotometry. UV absorbance measurements were conducted on an Aviv model 14DS Spectrophotometer (Aviv Associates, Provo, UT). In these experiments, the heating rate was 60°C/h. Transition enthalpies (ΔH_cal) and entropies (ΔS_cal) were calculated from the areas under the experimental ΔCp versus T and the derived ΔCp/T versus T curves, respectively, by using the ORIGIN version 1.16 software (Microcal, Northampton, MA). Lyophilized DNA duplex samples were dissolved in 1.2 ml of buffer containing 10 mM sodium cacodylate (pH 7.2), 100 mM NaCl, 10 mM MgCl2, and 0.1 mM EDTA. Isothermal mixing experiments were performed on an Aviv model 62A DS spectropolarimeter (Aviv Associates, Lakewood, NJ) equipped with a thermoelectrically controlled cell holder. The cell pathlength was 0.5 cm. Isothermal CD spectra were recorded from 220 to 320 nm in 1-nm increments with an averaging time of 5 s. The DNA concentration was 5 µM in duplex, and the buffer conditions were 10 mM Mops (sodium salt of 3-(N-morpholino)propanesulfonate) (pH 7.4), 100 mM NaCl, 10 mM MgCl2, and 0.1 mM EDTA.

Differential Scanning Calorimetry (DSC). Excess heat capacity (ΔCp) versus temperature profiles for the thermally induced transitions of both the GG20 and cis-Pt-GG20 duplexes were measured by using a prototype model 5100 Nano calorimeter (Aviv Associates, Lakewood, NJ). Lyophilized DNA duplex samples were dissolved in 1.2 ml of buffer containing 10 mM sodium cacodylate (pH 7.2), 100 mM NaCl, 10 mM MgCl2, and 0.1 mM EDTA. Isothermal mixing experiments were performed on an Aviv model 62A DS spectropolarimeter (Aviv Associates, Lakewood, NJ) equipped with a thermoelectrically controlled cell holder. The cell pathlength was 0.5 cm. Isothermal CD spectra were recorded from 220 to 320 nm in 1-nm increments with an averaging time of 5 s. The DNA concentration was 5 µM in duplex, and the buffer conditions were 10 mM Mops (sodium salt of 3-(N-morpholino)propanesulfonate) (pH 7.4), 100 mM NaCl, 10 mM MgCl2, and 0.1 mM EDTA.

RESULTS AND DISCUSSION

The Unmodified and Crosslinked Single Strands Each Associate With Their Complement Strand to Form Duplex Structures. Fig. 2A shows the isothermal mixing curve (22, 23) for complex formation between strands 1 and 2 (see Fig. 1). Fig. 2B shows the corresponding curve for complex formation between strands 1' and 2. The break points in both mixing curves occur near 0.5 and indicate that the strands associate to form 1:1 complexes. This stoichiometry is consistent with duplex formation and demonstrates that the crosslink does not prevent a single strand from hybridizing with its complement, as previously assumed (1).

Crosslink Formation Induces CD Changes Consistent with a Partial Shift from a B-Like Toward an A-Like Duplex Conformation. Fig. 3 shows the CD spectra of the unmodified, parent duplex (GG20) and the modified, platinated duplex (cis-Pt-GG20). The GG20 duplex exhibits a nearly conservative CD spectrum (solid curve) characteristic of B-form DNA (25, 26). In the CD spectrum of the cis-Pt-GG20 duplex (dashed curve), which is qualitatively similar, there is an increase in the intensity of the positive band at 278 nm and a decrease in the intensity of the negative band at 241 nm. To a first approximation, the qualitative similarity of the two spectra suggests that the crosslink does not significantly alter the global B-conformation of the host duplex. Such an interpretation would be consistent with previously reported NMR studies on oligomeric DNA duplexes containing a single cis-Pt-GG crosslink (2, 3, 6, 9, 27, 28). These studies revealed that crosslink-induced changes were restricted to base pairs surrounding the platinated nucleosides and that the presence of the adduct neither disrupted Watson–Crick hydrogen bonding nor altered the global B-like helical parameters of the host duplex. The platinum-induced quantitative CD differences we observe (Fig. 3), however, are significant and similar to those reported previously for two decameric DNA duplexes (3, 28). We suggest that these CD spectral changes may reflect a shift from a B-like DNA structure toward one with some contributions from an A-like conformation (25, 26). This CD-based conclusion is consistent with recent crystallographic results (8), in which a single cis-Pt-GG crosslink induced an A-type DNA segment in a dodecamer having both A- and B-like conformational domains. Thus, although the crosslink does not prevent hybridization to form a duplex, its presence does alter the global duplex conformation. In the sections that follow, we evaluate the thermal and thermodynamic consequences of such crosslink-induced alterations by analyzing calorimetric and spectroscopic melting profiles. For such analyses to be valid, however, we first must establish that the duplex melting transitions are reversible. This assessment is described in the next section.

The Melting Transitions of Both the Unmodified GG20 Duplex and the Platinated cis-Pt-GG20 Duplex Are Fully
Reversible. Fig. 4 shows the UV denaturation (heating) and renaturation (cooling) curves for the unmodified GG20 duplex (A) and the platinated cis-Pt-GG20 duplex (B). These data were acquired with heating and cooling rates of 0.5°C/min and a maximum temperature of 95°C. Inspection of Fig. 4 reveals the denaturation and renaturation curves for the GG20 duplex to be virtually superimposable, an observation consistent with the reversibility of this melting equilibrium. By contrast, inspection of Fig. 4B reveals that the heating and cooling curves for the cis-Pt-GG20 duplex are not fully superimposable, with the cooling curve exhibiting an altered shape, a lower \( T_m \) (\( \Delta T_m = 0.8^\circ \text{C} \)) and a failure to reach the 5°C starting absorbance of the heating curve. These observations indicate that this melting transition is not completely reversible, but, as shown in the Fig. 4B, inset, superimposable heating and cooling curves can be obtained for the cis-Pt-GG20 duplex by heating to a final temperature of only 75°C rather than 95°C. This differential renaturation behavior suggests a heat-induced change in the properties of the platinated single strand between 75°C and 95°C, with this alteration kinetically limiting rehybridization to the complementary strand. Upon cooling at 0.5°C/min, this altered form of the platinated single strand, which may involve heat-induced changes in platinum ligation, could become kinetically trapped in a metastable state. Consistent with this possibility is our observation that, when a cis-Pt-GG20 sample that had been heated to 95°C was allowed to equilibrate for 1 to 2 h at a temperature near the \( T_m \) and then further cooled down to the initial starting temperature of 5°C, the repeat heating curve was superimposable on the initial 95°C heating curve (data not shown). Such a staged cooling process probably acts to reequilibrate any altered platinated single strands that are kinetically trapped during the faster 0.5°C/min cooling process, perhaps including states that involve changes in platinum bonding to the nucleobases. Caution must therefore be exercised in studies of platinated nucleic acids to assess whether one is characterizing an equilibrium or kinetically trapped state. Based on these results, we conclude that, under appropriate conditions, the melting transitions of both the platinated and unmodified duplexes are fully reversible. Consequently, we can obtain meaningful thermodynamic data from our calorimetric and spectroscopic measurements, as described below.

Formation of the cis-Pt-GG Intrastrand Crosslink Lowers the Thermal Stability of the Host Duplex. Fig. 5 shows the UV thermal denaturation profiles of the unmodified GG20 duplex and the platinated cis-Pt-GG20 duplex at concentrations (\( C_{\text{dup}} \)) of 1 \( \mu \text{M} \) (A) and 50 \( \mu \text{M} \) (B). The following two features are worthy of emphasis. First, as expected for oligomeric bimolecular complexes (29), the thermal stabilities of both the GG20 and cis-Pt-GG20 duplexes depend on the DNA concentration. Specifically, increasing the duplex concentration from 1 to 50 \( \mu \text{M} \) increases the \( T_m \) of the GG20 duplex from 64.3 to 70.2°C, while increasing the \( T_m \) of the cis-Pt-GG20 duplex from 55.4 to 61.8°C. These observations are consistent with both the parent and platinated duplexes melting with apparent molecularities greater than one, whereas the isothermal mixing experiments described above and shown in Fig. 2 reveal a 1:1 stoichiometry for each complex. Second, at both concentrations studied, the presence of the crosslink reduces the thermal stability of the host duplex by a similar amount (\( \Delta T_m = -9.1^\circ \text{C} \) at 1 \( \mu \text{M} \) duplex; \( \Delta T_m = -8.4^\circ \text{C} \) at 50 \( \mu \text{M} \) duplex), while also slightly reducing the hyperchromic change that accompanies denaturation. These results are consistent with previous studies probing the effects of cis-Pt-GG formation on the thermal stabilities of double-helical DNA and RNA targets (2, 28, 30, 31). Our observation of a cis-Pt-GG-induced reduction in the extent of the melting hyperchromism may reflect crosslink-induced perturbations in the initial duplex state (e.g., unstacking). In the section that follows, we evaluate the thermodynamic origins of the crosslink-induced decrease in duplex thermal stability.

Formation of the cis-Pt-GG Intrastrand Crosslink Results in an Enthalpically Driven Destabilization of the Host Duplex. Calorimetry. Fig. 6 shows DSC melting profiles (\( \Delta C_p \) versus \( T \)) for the parent, unmodified GG20 duplex (solid curve) and the platinated cis-Pt-GG20 duplex (dashed curve). These curves were analyzed as described in Materials and Methods to obtain the results listed in Table 1. Inspection of these thermodynamic parameters reveals a number of interesting features. First, crosslink formation reduces the duplex thermal stability by \( \sim 9^\circ \text{C} \), a result consistent with our optical melting data. Second, crosslink formation results in a large decrease in the
duplex transition enthalpy of 17 kcal/mol. In other words, the intrastrand crosslink enthalpically destabilizes the duplex. Third, crosslink formation results in a substantial increase in duplex transition entropy of 36 cal/K·mol ($T_A = 10.7$ kcal/mol at 25°C). In other words, the intrastrand crosslink entropically stabilizes the duplex. Thus, the crosslink-induced 17 kcal/mol enthalpic destabilization of the duplex is partially, but not fully, compensated by a crosslink-induced entropic stabilization of the duplex at 25°C of $\sim$11 kcal/mol. The net result of these enthalpic and entropic effects is that crosslink formation at 25°C induces a decrease in duplex stability (Δ$G_{25}$) of 6.3 kcal/mol, with this destabilization being enthalpic in origin.

**Spectroscopy.** The melting transitions of the unmodified and platinated duplexes also can be characterized thermodynamically from optical measurements by using a van’t Hoff analysis (32–34). This indirect approach is more commonly employed, although it yields model-dependent parameters, in contrast to the model-independent data more directly obtained via the calorimetric measurements (24, 29).

For transitions with molecularities greater than one, the concentration dependence of $T_m$ can be determined from families of optical melting curves (24, 29, 34). The resulting data usually are cast as, and analyzed from, plots of the reciprocal of the melting temperature (1/$T_m$) versus the logarithm of the total strand concentration (ln$C_{Tot}$). Such plots for the two duplexes studied here are shown in Fig. 7. For bimolecular associations of two non-self-complementary strands, such as are encountered in this work, van’t Hoff transition enthalpies and entropies can be derived from the slopes and y-intercepts of these lines, respectively, with the use of the Eq. 1 (29), where $C_{Tot}$ is the total strand concentration (i.e. twice the duplex concentration) and $R$ is the gas constant.

$$\frac{1}{T_m} = \frac{R}{\Delta H_{vH}} \ln C_{Tot} + \frac{\Delta S_{vH} - 1.39R}{\Delta H_{vH}}.$$  \[1\]

This equation predicts that a plot of 1/$T_m$ versus ln$C_{Tot}$ should be linear, as we observed for both duplexes, with a slope of $R/\Delta H_{vH}$ and a y-intercept of $(\Delta S_{vH} - 1.39R)/\Delta H_{vH}$. From these $\Delta H_{vH}$ and $\Delta S_{vH}$ values, we calculated the corresponding free energy change for duplex formation at 25°C ($\Delta G_{25}^{vH}$) by using the standard thermodynamic relationship given in Eq. 2.

$$\Delta G_{25}^{vH} = \Delta H_{vH} - (298.15)\Delta S_{vH}.$$  \[2\]

The van’t Hoff thermodynamic parameters derived in this manner are listed in Table 1. Inspection of these results reveals that the cis-Pt-GG intrastrand crosslink lowers the enthalpy of duplex formation ($\Delta H_{vH}$) by 15 kcal/mol. This enthalpic cost is partially compensated by an increase in entropy ($\Delta S_{vH}$) of 35 cal/K·mol thereby resulting in a net crosslink-induced duplex destabilization at 25°C ($\Delta G_{25}^{vH}$) of 4.8 kcal/mol. These optically derived van’t Hoff values are in good agreement with those we independently and more directly determined by using DSC.

The van’t Hoff analysis described above assumes that duplex formation is an all-or-none, two-state process, with no significant thermodynamic contributions from intermediate states. In the next section, we evaluate the validity of this assumption.

**Crosslink Formation Does Not Alter the Nature of the Melting Transition.** Inspection of the data in Table 1 reveals that the calorimetrically-derived enthalpies of duplex formation (~152 kcal/mol for GG20 and ~135 kcal/mol for cis-Pt-GG20) are virtually identical to the corresponding optically derived van’t Hoff transition enthalpies (~151 kcal/mol for GG20 and ~136 kcal/mol for cis-Pt-GG20). Thus, for the two duplexes studied here, $\Delta H_{vH} \approx \Delta H_{vH}$, an equality consistent with both duplexes exhibiting two-state melting behavior. In other words, despite dramatically reducing the stability of the duplex, crosslink formation does not alter the “stateness” or cooperativity of the transition by causing a significant population of intermediate species. This conclusion suggests that the presence of the crosslink does not serve as a barrier to the coupling and/or propagation of forces required to cause the duplex to behave as a single thermodynamic entity. We have observed similar behavior for other DNA lesions, such as 8-oxo-guanosine and 1,N2-propano-guanosine, which in principle have the potential to act as insulators and/or spacers of cooperative effects but which in practice do not create thermodynamically separate domains (18–20, 35).

**Correlation Between the Structural and Thermodynamic Impacts of the cis-Pt-GG Crosslink on Duplex Properties.** As discussed above, our thermodynamic data (Table 1) reveal an enthalpically driven destabilization of the crosslinked duplex...
relative to its unmodified counterpart. It is of interest to assess whether this destabilizing influence can be rationalized in terms of crosslink-induced structural perturbations in the host duplex. As noted earlier, NMR, crystallographic, and gel electrophoresis studies indicate that formation of a cis-Pt-GG crosslink bends the helix axis of the host duplex by 34°-55° in the direction of the major groove (2, 7, 8). Concomitant with this bend is a change in the propeller twists of the 4 bp at the platination site (8). The NMR and crystallographic studies suggest that, although these changes in propeller twist do not significantly disrupt the Watson–Crick hydrogen bonds, they do lead to at least partial disruption of the stacking interactions between the four neighboring base pairs at the platination site (3, 8, 36). Such a disruption should be enthalpically unfavorable, as we observe here. In an attempt to quantify this effect, we can use published nearest-neighbor data (37) to estimate an upper limit for this crosslink-induced perturbation by calculating the enthalpic cost that results from complete disruption of the three relevant nearest-neighbor stacking interactions, namely, TG/AC, GG/CC, and GT/CA. This calculation yields a ∆ΔH upper limit value of 23.3 kcal/mol. Our experimental calorimetric data (see Table 1) reflect a crosslink-induced ∆ΔHcal value of 17 ± 8 kcal/mol. If one were to take seriously the difference between these two numbers, which is within the error limit, one could perhaps naively suggest that the three interbase stacking interactions at the site of adduct formation are significantly, but not completely, enthalpically disrupted. This interpretation of our data, as well as the others discussed below, ascribes all the ∆ΔG, ∆ΔH, and ∆ΔS values to differences in the initial duplex states, thereby implying that the final single-stranded states are thermodynamically equivalent at the high temperatures at which they are formed. This assumption is bolstered here by the coalescence of the high temperature, single-stranded CD spectra (data not shown) and is commonly employed when interpreting thermodynamic data on nucleic acids (20, 38). Nevertheless, when evaluating any of our proposed interpretations, it should be kept in mind that the observed differential thermodynamic properties could, in part, reflect differences in the final, single-stranded states.

With this qualification in mind, an alternative explanation for the small and perhaps insignificant difference between the predicted upper limit ∆ΔH value of 23.3 kcal/mol and the measured value of 17 kcal/mol invokes the energetic consequences of DNA bending. Enthalpy lost owing to crosslink-induced disruption of stacking interactions, as suggested by crystallographic studies (8, 36), is partially compensated by an enthalpy gained by a crosslink-induced helical bending. Previously we estimated a lower-limit value of 1.6 kcal/mol per bp for thermally induced unbending of an “A tract” in an unmodified decameric duplex (39). Although the sequence as well as the cause of bending are different, we can use this value to estimate crudely that the four “bent” base pairs in our cis-Pt-GG20 duplex contribute ~6.4 kcal toward stabilization of the global duplex structure. Adding this value to our experimentally determined ∆ΔH of 17 kcal/mol yields a final predicted ∆ΔH of 23.4 kcal/mol, in perhaps unjustifiably good agreement with the ∆ΔH value of 23.3 kcal/mol predicted by the nearest-neighbor analysis.

Another possible explanation for the large crosslink-induced enthalpic effects we measure is suggested by the crystal structure of a cisplatin-modified duplex dodecamer (8). cis-Pt-GG crosslink formation induced a hybrid duplex with an A–B junction and deformation of the platinum atom from the planes of the coordinated guanine rings. Such crosslink-induced perturbations in duplex structure undoubtedly also contribute to the observed energetic impact of the adduct. The limited nature of the current thermodynamic database on A-form duplexes, A–B junctions, and distortion of Pt-nucleobase geometries from planarity, however, does not allow us to predict the energetic consequences of such structural alterations.

Irrespective of these possible microscopic interpretations of our macroscopic data, the calorimetric and spectroscopic results reported here reveal that cisplatin crosslink formation induces a substantial thermal and thermodynamic destabilization of the host duplex that is enthalpic in origin but that does not alter the cooperativity of the duplex-to-single strand melting transition.

**Comparison Between the Energetic Impact of the cis-Pt-GG Intrastrand Crosslink and Other Guanine Adducts.** Table 2 summarizes the thermodynamic consequences on duplex properties of the cis-Pt-GG crosslink and other exocyclic guanosine adducts. Only the cis-Pt-GG crosslink investigated is a bifunctional adduct, the others being monoadducts. Inspection of the data in Table 2 reveals that none of monoadducts, in which only a single guanine residue is modified, is as energetically

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**Table 1. Thermodynamic parameters for formation of the GG20 and cis-Pt-GG20 duplexes**

<table>
<thead>
<tr>
<th>Duplex</th>
<th>T_m, °C</th>
<th>∆H^C, kcal/mol duplex</th>
<th>∆S^C, cal/K·mol duplex</th>
<th>∆G^C25, kcal/mol duplex</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG20</td>
<td>70.2 ± 0.2</td>
<td>-152 ± 8</td>
<td>-151 ± 8</td>
<td>-400 ± 22</td>
</tr>
<tr>
<td>cis-Pt-GG20</td>
<td>61.8 ± 0.2</td>
<td>-135 ± 7</td>
<td>-136 ± 12</td>
<td>-404 ± 20</td>
</tr>
</tbody>
</table>

^Tm values were derived from UV melting profiles at 50 μM duplex as described (24). Each Tm value is an average derived from three different profiles acquired at 255, 260, and 267 nm, with the indicated errors corresponding to a single standard deviation.

Calorimetric (Cal) experiments were conducted as described in the text. The calorimetric ∆H^C and ∆S^C values are averages derived from two independent experiments, with the indicated errors corresponding to the average deviation from the mean.

^Van’t Hoff (vH) parameters were derived as described in the text. The van’t Hoff ∆H^C and ∆S^C values are averages derived from three families of UV melting profiles acquired at 255, 260, and 267 nm, with the indicated errors corresponding to a single standard deviation.

^ΔG^C25 is the free energy of duplex formation at 25°C, as determined using Eq. 2 in the text and the corresponding values of ∆H^C and ∆S^C. The indicated uncertainties reflect the maximum possible errors in ∆G^C25 that result from the corresponding uncertainties noted above in ∆H^C and ∆S^C, as propagated through Eq. 2.
### Table 2. The impact of various exocyclic deoxyguanosine adducts on the thermodynamic properties of different oligomeric host duplexes

<table>
<thead>
<tr>
<th>Adduct</th>
<th>Host sequence*</th>
<th>$\Delta H^\ddagger$ kcal/mol</th>
<th>$\Delta S^\ddagger$ cal/Kmol</th>
<th>$\Delta G_{298}^\ddagger$ kcal/mol</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-Pt-GG‡</td>
<td>5'-TCTCTCTTCTGTTCTCTC-3'</td>
<td>17</td>
<td>36</td>
<td>6.3</td>
<td>This study</td>
</tr>
<tr>
<td>1,N2-propano-2'-deoxyguanosine‡</td>
<td>5'-GCGAGGTTACCCG-3'</td>
<td>13</td>
<td>32</td>
<td>3.8</td>
<td>35</td>
</tr>
<tr>
<td>2'-deoxy-7-hydro-8-oxo-guanosine‡</td>
<td>5'-GGCTACGCACTCGG-3'</td>
<td>7.3</td>
<td>17.8</td>
<td>2.0</td>
<td>40</td>
</tr>
<tr>
<td>2'-deoxy-7,8-dihydro-8-oxo-guanosine‡</td>
<td>5'-GCGCCCAGCCTCGG-3'</td>
<td>6.5</td>
<td>16</td>
<td>1.7</td>
<td>20</td>
</tr>
<tr>
<td>N-acetyl-5-(2-N7-guanylyethyl)Cys methyl ester‡</td>
<td>5'-TGCTCAGCAAC-3'</td>
<td>-11.2</td>
<td>-42.4</td>
<td>1.4</td>
<td>41</td>
</tr>
<tr>
<td>2'-deoxy-N7-methyl-guanosine‡</td>
<td>5'-CCGGAATTCGCC-3'</td>
<td>-10.3</td>
<td>-37.4</td>
<td>0.9</td>
<td>41</td>
</tr>
<tr>
<td>*‡G denotes a single added guanosine residue, while GG denotes a bidentate crosslink of two guanosine residues. In each case, cytosis is the base opposing the added guanosine(s).</td>
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<tr>
<td>$\Delta G = \Delta H - T \Delta S$</td>
<td></td>
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<tr>
<td>*‡Thermodynamic parameters were obtained calorimetrically.</td>
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<tr>
<td>†Thermodynamic parameters were derived from a combination of calorimetric and concentration-dependent UV melting data.</td>
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<tr>
<td>‡Thermodynamic parameters were derived from van't Hoff UV melting profiles.</td>
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**CONCLUDING REMARKS**

We have used a combination of spectroscopic and calorimetric techniques to demonstrate that formation of a cis-Pt-GG intrastrand crosslink reduces the thermal and thermodynamic stabilities of a host duplex without altering its melting cooperativity. The decrease in thermodynamic stability is enthalpic in origin. We propose correlations between crosslink-induced reduction in duplex stability and crosslink-induced perturbation in the structure (base unstacking, DNA bending, and A-DNA formation), previously revealed by NMR, crystallography, and gel electrophoresis (1–9). Such cross-correlations between macroscopic and microscopic observables should provide insight into the physicochemical consequences of the crosslink on duplex properties, including protein recognition and binding specificity, and help to delineate the biological basis for the cytotoxic properties of the drug. Ultimately, it may be possible to establish empirical, semiquantitative correlations between the biophysical and biological consequences of cisplatin binding to DNA, including factors that contribute to its cytotoxic activity, thereby contributing to a rational basis for designing and screening of new candidates for chemotherapy.

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