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Cross-Linking of a DNA Conjugate Tethering a *cis*-Bifunctional Platinated Complex to a Target DNA Duplex

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Cis-diamino dichloro platinum and numerous related derivatives have been shown to be effective anticancer agents that bind DNA.[1,2] The primary targets of these agents are the sites GpG, ApG, and GpNpG, and in all three cases complexation occurs with the two purine N7-nitrogens.[3−5] The binding/cross-linking ability of a large number of platinum complexes to oligonucleotides has been studied in detail.[6] Complexation at target sites appears to occur randomly within the context of the genomic sequence, although in studies of DNA polymerase inhibition some sequence specificity for telomeres[7] or for the GC box in SV40[8] DNA has been reported. The ability to deliver a platinated complex to a specific target could result in more effective agents.[9] Lippert et al.[10] demonstrated via a triple helix model that oligonucleotide specificity could feasibly be applied to deliver a platinated complex to a specific DNA site. One report of an interstrand platinum cross-linking reaction in triplexes employed a monofunctional transplatin dG adduct.[11] Interstrand cross-linking in DNA duplexes using mono/binuclear platinum complexes has been well established.[12]

There are only a few reports in the literature describing the synthesis of platinated oligonucleotides, and they often involve a *trans*-a_2Pt^{II} species (a = NH_3 or amine),[13−16] although the preparation of *cis* derivatives is also known.[17] Incorporation of a preplatinated nucleotide building block into an oligonucleotide by solid-phase DNA synthesis results in the formation of an inactivated species.[18]

We report a simple approach toward the synthesis of an oligodeoxynucleotide-tethered bifunctional *cis*-Pt^{II} complex that retains cross-linking ability. The platinated oligodeoxynucleotide was synthesized in two steps: (i) preparation of a DNA fragment tethering a 2-(2-aminoethylamino)ethanol chelator and (ii) metal complexation to the chelator. With the low reactivity of dT toward a platinum source such as K_2PtCl_4, we chose dT_8 and dT_{15} as our initial synthetic targets for platination. The chelator was coupled to the 5′ terminus of either strand by solid-phase phosphoramidite coupling of the corresponding Fmoc (9-fluorenyl-methoxycarbonyl) protected derivative (see Supporting Information). After deprotection and purification of the conjugate, platination was observed to occur with highest yields simply in DMF/water (9/1). Platination of either sequence with K_2PtCl_4 (10 equiv, 0.05 M) in DMF/water (9/1) at 37 °C for 48 h occurred in 80% yield (HPLC). The reaction was nearly quantitative after a reaction time of ∼96 h, but the product was usually purified after 48 h using reversed phase HPLC. MALDI-TOF mass spectrometry indicated that the oligonucleotide−platinum conjugates were obtained as the dichloro species. This postsynthetic procedure may prove difficult with sequences containing the other common nucleosides, but suitable analogues will prove useful for expanding sequence diversity.

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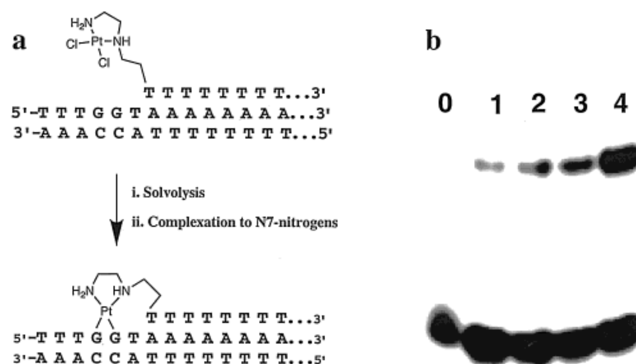

Figure 1. (a) Cross-linking to a DNA duplex facilitated by oligonucleotide-mediated triplex formation. (b) PAGE analysis of the cross-linking between the dT_{15}−Pt third strand and the target of entry 1 Table 1 monitored at reaction times of 0, 20, 40, 60, 120 min (Lanes 0, 1, 2, 3, 4).

Since either oligonucleotide conjugate has two *cis* chlorine atoms attached to the platinum center, they have the capability of reacting very much like *cis*-$Pt(en)Cl_2$ or related *cis*-dichoro platinum compounds and binding to a G-rich target. Previous work has indicated that the reaction course for cisplatin or *cis*-$Pt(en)Cl_2$ requires solvolysis of the chlorine atoms ($t_{1/2} \approx 2$ h, pH 7.0) followed by reaction of the bis-aquo species with the purine N7-nitrogens of the DNA.[5,19−20] Our initial studies indicated that the cross-linking reaction (as monitored by PAGE) occurred between the homopyrimidine third strand tethering the bifunctional platinum adduct and the complementary duplex with a $d(Gp)_4$ target site when using the dT_{15}-conjugate, but no corresponding high-molecular weight cross-linked product was observed with the dT_8-conjugatge. Thermal melting analyses confirmed the presence of a triplex-to-duplex transition for the dT_{15} sequence tethering the ethylenediamine (∼35 °C); a similar transition was not observed for the dT_8 sequence. These results suggest that reaction of the platinated conjugate with the target duplex in the absence of sequence-specific binding was inconsequential.

When the purine-rich strand of the duplex was radiolabeled, the higher-molecular weight band was visible on the gel, and it increased in intensity over a 6-h time period. When the pyrimidine-rich strand was radiolabeled, no new product band was detected, suggesting that only the purine-rich strand represented the target sequence for platinum cross-linking.With a stoichiometric amount of platinated 15-mer, 20% cross-linking was observed after a 6 h incubation at 30 °C with the 25-mer duplex containing a $d(Gp)_4$ target site. This value increased to 48% cross-linking with 2 equiv of the 15-mer (entry 1, Table 1). No significant enhancement in cross-linking was observed by further increases in either the incubation period or the concentration of the 15-mer (up to 10 equiv). However, with higher concentrations of platinated 15-mer, additional more slowly migrating bands began to appear at the

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Table 1. Extent of Platinum-Mediated Cross-Linking between a Third Strand and a Target Duplex

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        Pt
         5' T T T T T T T T T T T T T T T
5' X X X X X X A A A A A A A A A A A A A A A A C G C G
3' Y Y Y Y Y Y T T T T T T T T T T T T T T T T G C G C C C C C
```

Entry	Sequence	% X-linking	Entry	Sequence	% X-linking
	5' X X X X X X 3' Y Y Y Y Y Y		7	T T T T C C A A A A G G	19
1	T T G G G G A A C C C C	48	8	T T T T C G A A A A G C	14
2	T T T T G G A A A A C C	37	9	T T T T G C A A A A C G	27
3	T T T G G T A A A C C A	58	10	T T T T T T A A A A A A	0
4	T T G G T T A A C C A A	43	11	T T T T A A A A A A T T	19
5	T G G T T T A C C A A A	20	12	T T T T T G A A A A A C	10
6	T T T 7 7 T A A A C C A	0	13	T T T T G T A A A A C A	12

expense of the described cross-linked product, suggesting the presence of additional, less specific complexation reactions.

On establishing the cross-linking ability of the tethered platinum complex to a GpGpGpG site of DNA, we wished to identify the specific guanines targeted most effectively by the conjugate. Since the predominant reaction site for agents such as *cis*-platin is the GpG site,[6] we synthesized pairs of complementary 25-mers and 29-mers (entries 2−5, Table) each differing in the position of a GpG target. Cross-linking to the platinum strand was observed upon radiolabeling of the 25-mer but not upon radiolabeling of the 29-mer. The maximum extent of cross-linking to a GpG site occurred with entry 3 (Table 1) and decreased if the GpG target was moved closer (entry 2) or farther (entries 4 and 5) from the triplex binding site. To confirm the nature of the cross-linking reaction, we replaced the GpG target of entry 3 with two 7-deazaguanine residues (7p7, entry 6). No cross-linking occurred with this sequence, confirming the participation of the purine N7-nitrogens in platinum complexation. Although the third strand is bound to the purine-rich strand of the duplex, it remains located in the major groove. We prepared the sequence in which the GpG site was located in the 29-mer rather than the 25-mer (entry 7). However, cross-linking was only observed when the 25-mer was radiolabeled, indicating that sequences adjacent to the triplex binding site, even CpC targets, were preferred over GpG targets positioned across the major groove in the complementary 29-mer. Similarly, the positioning of the two target guanines in complementary strands (which could potentially result in cross-linking between all three strands) only resulted in complexation to the 25-mer (presumably to a GpC or CpG target, entries 8 and 9). No cross-linking was observed when the 25-mer contained all dT residues adjacent to the triplex binding site (entry 10), but did occur with the ApA target (entry 11). While bifunctional reactions with the tethered platinum complex are expected, monofunctional reactions are also possible. To examine such effects, we prepared two sequences each containing a single dG residue either adjacent to the triplex binding site (entry 12) or removed by one residue (entry 13). In both cases roughly 10% cross-linking to the 25-mer was observed. While this latter experiment suggests that some of the observed cross-linked products might be the result of reaction with a single nucleotide, it is also likely that proximity effects will result in the vast majority of products being bifunctional in nature—when two adjacent (nonthymine) bases are present. The lower yield of monofunctional versus bifunctional products may reflect overall lower stability for the former relative to the latter. Proximity effects as the result of triplex binding are likely responsible for the ability to target a variety of dinucleotides.

These experiments suggest that oligonucleotide-directed DNA triplex formation can be used to deliver a *cis*-dichoro platinum complex to a non-thymine-containing preselected site in duplex DNA in a sequence-specific fashion. Sequence-specific delivery of such complexes should enhance the effectiveness of such agents while lowering the associated toxicity effects.

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Supporting Information Available: Synthetic procedures for the chelator and its oligonucleotide conjugate, the HPLC profile, and MALDI-TOF spectral data for the platinum complexes of oligonucleotides (dT_8 and dT_{15}), and autoradiograms of platinum cross-linking with varying DNA sequences (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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