Tuning the DNA Reactivity of *cis*-Platinum: Conjugation to a Mismatch-Specific Metallointercalator

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Non-Watson−Crick base pairs can be generated in genomic DNA as a result of errors in replication or base lesions.[1] If not corrected by the mismatch repair machinery,[2] these mispairs result in mutations and increased cancer susceptibility.[3] A molecular probe capable of limiting replication and/or transcription of mismatched DNA would thus be a valuable tool. Therefore, we have designed a bifunctional conjugate that combines a metallointercalator directed to DNA mismatches[4] and a reactive platinum moiety of the *cis*-platin family, since the latter has been shown to form adducts with DNA that inhibit transcription and replication.[5] Conjugates of *cis*-platinum with organic intercalators have been reported previously.[6−8] Our strategy involves targeting the nonmismatch-selective but reactive Pt species toward mismatched sites in DNA, directing the formation of coordinated adducts using a bulky metallointercalator.[9] Here we describe the preparation of such a bifunctional conjugate (1) as well as an examination of its reactivity with matched and mismatched oligonucleotides.

Conjugate **1** is derived from its parent $[Rh(chrysi)(bpy)_2]^{3+}$ (chrysi = chrysenequinonediimine), an expansive metallointercalator that targets thermodynamically destabilized mismatched sites in DNA.[4] The conjugate is prepared by functionalization of the ancillary bpy-NH$_2$ ligand of $[Rh(chrysi)(phen)(bpy-NH_2)]^{3+}$ **2** through amide coupling with a carboxyl-functionalized analogue of dichloroethylenediamine−platinum(II) (see Figure 1 and Supporting Information).[10] Binding of **1** was examined with several duplexes lacking or containing a mispair, where the distance between the mismatch site and the preferred *cis*-platinum coordination site (GG) was varied (Figure 1). The permanent link between the Rh and Pt subunits constrains the mismatch recognition unit and the reactive Pt center to be closer than 20 Å and thus imposes an upper limit of ∼6 bases between the mispair and the Pt reaction site if both units are simultaneously bound. In duplex **CC3**, the CC mismatch is positioned three base pairs (bp) away from GG, which, on the basis of model building, should allow both the Rh and Pt units to be satisfied. In **CC0** and **CC9**, however, the CC mismatch and the GG reactive sites are either too close or too far apart for both units to be accommodated.

Recognition of the mismatch in **CC3** by conjugate **1** was first investigated by photoinduced DNA strand cleavage[11] after incubation at 37 °C for 12 h[12] (Figure 2). The precursor complex **2**, in which the bpy bears a free amine group, was used as a control (lanes 2 and 3). Like its parent complex $[Rh(chrysi)(bpy)_2]^{3+}$, **1** promotes photocleavage only in the mismatched oligonucleotide at the 3′ side of the mismatch.[13] Similarly, **2** targets the mismatched duplex; neither **1** nor **2** promote cleavage on the matched duplex with irradiation. Hence, the fully assembled conjugate is able to recognize the mismatched DNA duplex with selectivity.

Upon incubation of **1** with duplexes of different sequences (Figure 3), two bands (denoted **ad1** and **ad2**) of slower mobility than the intense parent band consistently appear, indicating irreversible binding.[14] Mass spectrometry (MALDI) on the isolated bands extracted from the gel indicates that the slowest moving adduct contains both DNA strands,[15] while the one of intermediate mobility
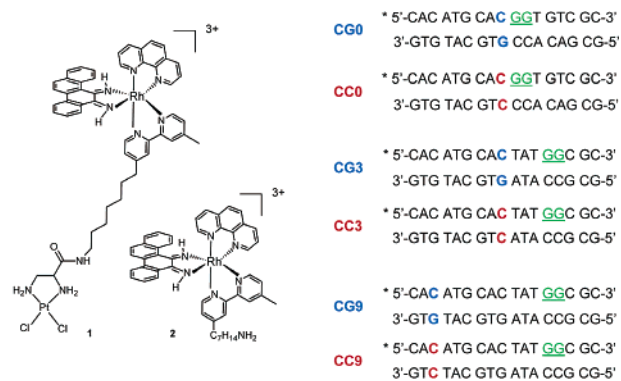

Figure 1. Schematic structures of conjugate **1** and its precursor **2** (left) as well as the three families of DNA duplexes (right). The asterisk indicates sites of ^{32}P-5′-end-labeling.
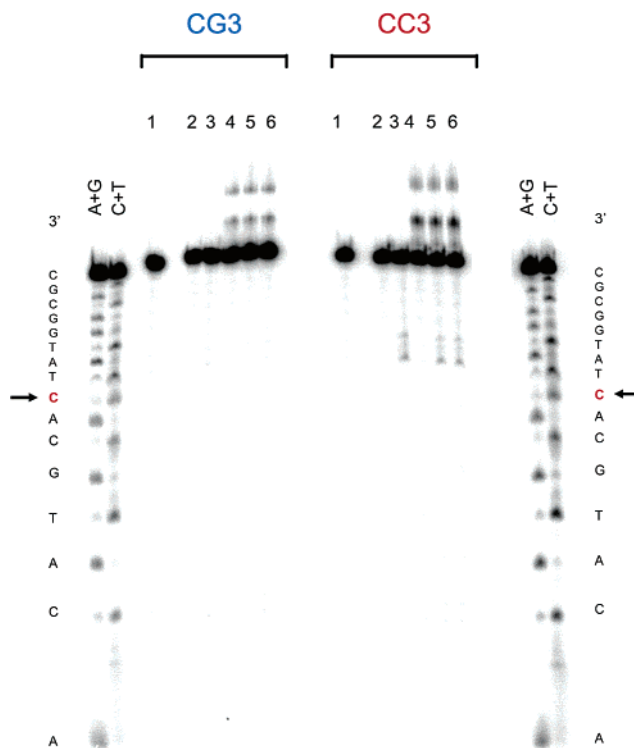
Figure 2. Autoradiogram of the denaturing gel revealing the photocleavage pattern after incubation of **1** and **2** with duplexes **CG3** (left) and **CC3** (right). Incubation conditions: duplex (5 μM) with complexes **1** or **2** (5 μM) in 10 mM NaCl, 10 mM Na phosphate, pH 7.0, at 37 °C for 12 h, protected from light. Irradiation conditions: 15 min at 442 nm wavelength (HeCd laser; 12.5 mW power). Lanes 1: duplex alone irradiated in the absence of complex. Lanes 2 and 4: incubation with **2** and **1**, respectively, in the absence of light. Lanes 3 and 5: irradiation after incubation with **2** and **1**, respectively. Lanes 6: irradiation after incubation with **1** and removal of the unbound conjugate. The arrows mark the mismatched site.
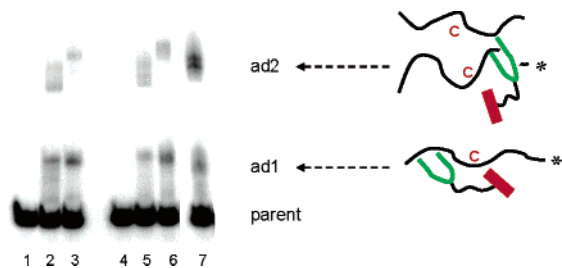
Figure 3. Autoradiogram of the denaturing gel after incubation of **CG0** in the absence (lane 1) and presence (lane 2) of **1**, **CC0** with **1** (lane 3), **CG3** in the absence (lane 4) and presence (lane 5) of **1**, **CC3** with **1** (lane 6), and **CC9** with **1** (lane 7), protected from light. Adduct assignments are on the right.
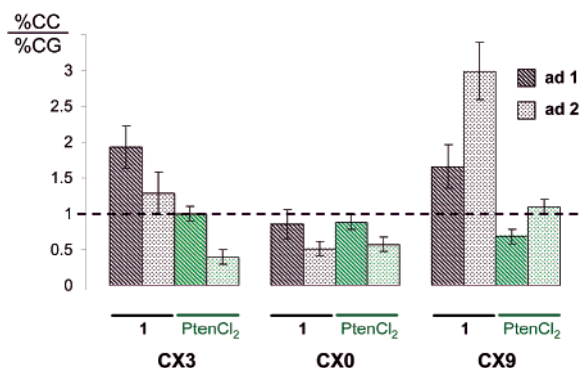
Figure 4. Selectivity ratios (%CC/%CG) upon quantitation of the gel autoradiograms after incubation of **1** with duplexes **CX3**, **CX0**, and **CX9** (**X** = **G** or **C**) for 12 h at 37 °C in the absence of light.

includes only the GG-containing strand. Thus, we assign **ad1** and **ad2** as intra- and interstrand cross-links, respectively.[16] Note also that control reactions with single-stranded DNA show only **ad1**.

The selectivity ratios with each sequence quantitated by phosphorimagery (ImageQuant) of the adducts[17] are given in Figure 4. For duplexes **CG3** versus **CC3**, adduct formation is higher with mismatched than with matched DNA.[18] This selectivity is, moreover, greater for intra- versus interstrand cross-linking. The selectivity may be attributed to the compatibility of the **CC3** duplex with the simultaneous recognition of the mismatch and reaction of the Pt unit with its preferential GG coordination site. Significantly, PtenCl$_2$, as a control, shows no selectivity for the mismatched over the matched duplex.

Interestingly, reactions with duplexes **CX0** and **CX9** (**X** = **G** or **C**) show very different behaviors, indicating the importance of how the mismatch is positioned relative to the preferred Pt binding site. With duplex **CC0**, where the CC mismatch is adjacent to the GG, conjugate **1** shows no selectivity. This lack of selectivity likely results from the close proximity of the two potential binding sites; once the intercalator is inserted, the bulk of the ancillary ligands on the Rh complex limits the accessibility of the adjacent bases, thus preventing reaction with the appended Pt unit within the conjugate.

Most importantly, in duplex **CC9**, where the CC mismatch is 10 bp away from the preferential GG, the total mismatch selectivity is highest. Despite the large separation between the target sites for the two components of **1**, the presence of the mismatch still influences the Pt coordination. Remarkably, it is the interstrand cross-link that is formed in higher yield[19] with **CC9** and with the highest selectivity.[20] Thus, it appears that because the mismatch is 10 bp away from the preferred GG coordination site, interstrand cross-links are formed instead as primary adducts.

The studies reported here therefore show that the presence of a permanent link to a site-specific intercalator is able to affect the reactivity of a *cis*-platin analogue. When sterics allow, the recogni-

tion of a mismatch by the bulky Rh intercalator directs the Pt unit to react preferentially with mismatched DNA at a site that may (duplex **CC3**) or may not (duplex **CC9**) be its preferred site of coordination. In the latter case, the Rh targeting dominates over the Pt reactant, since the conjugation leads to the formation of otherwise minor interstrand products, showing that the Rh unit is able to tune the *cis*-platinum reactivity. Targeting DNA with such bifunctional conjugates could have distinctive biological consequences.

Supporting Information Available: Preparation of conjugate **1** and an autoradiogram of the denaturing gel after incubation, photocleavage, and cyanide reversal (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Adduct formation levels off after 10 h under these conditions.
(13) Since the observed photocleavage could have been due to residual unreacted conjugate, we also separated the incubated duplex from free conjugate via molecular sieves prior to irradiation. The irradiation patterns of the purified samples (Figure 2, lanes 6) are very similar to unfiltered mixtures (lanes 5), suggesting that the intercalator is still able to interact with the mismatched site within DNA adducts. In addition, since the coordination adducts with the oligonucleotide targets may have a different migration than the related Maxam Gilbert fragments, the irradiated incubated samples were treated with cyanide in order to reverse the adducts to yield unmetalated oligonucleotide fragments. Product analysis does not reveal new species, confirming that the intercalator mainly resides close to the mismatch, even in the coordination adducts (see Supporting Information).
(14) Coordination of **1** but not **2** to the DNA duplex is also evident in Figure 2.
(15) For **CC9**, M (interstrand) calcd 11 438 g/mol, M (**ad2**) found 11 440 g/mol.
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(17) With **CC3**, there is ∼6 and 2%, respectively, obtained for **ad1** and **ad2** after incubation for 15 h at 37 °C with **1**, and ∼30 and ∼4% after incubation with PtenCl$_2$ using 5 μM DNA, 5 μM Pt.
(18) Varying the reaction conditions (nature and concentration of the buffer, temperature) affects the yield of adduct formed but does not affect the selectivity significantly.
(19) Absolute yield of **ad2** is noteworthy. Whereas reaction with **CC3** results in ∼5.2% **ad1** and ∼2% **ad2**, with **CC9**, adducts **ad2** are more abundant (∼7%) than adducts **ad1** (∼3.6%).
(20) 5′ labeling of the complementary strand (not containing the GG) confirms that the interstrand adducts are more abundant than their faster moving intrastrand counterparts (by a factor of ∼1.4 in this G-rich complement).

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