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Bifunctional Rhodium Intercalator Conjugates as Mismatch-Directing DNA Alkylating Agents

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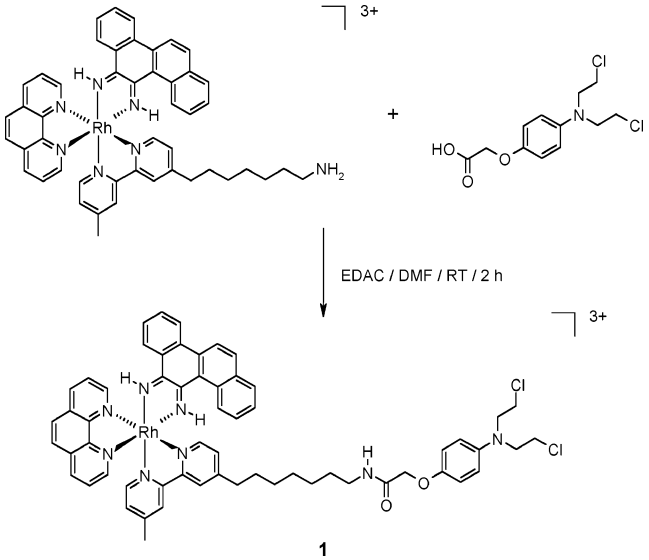
To maintain the integrity of the genome, a complex cellular repair machinery has evolved.[1−3] Defects in this machinery, notably deficiencies in mismatch repair, are associated with an increased cancer susceptibility.[4,5] In an effort to probe these mispairs, we have designed metallointercalators that target base pair mismatches with high selectivity.[6−10] Metal complexes containing a bulky, intercalating ligand that is too expansive to insert within well-matched B-DNA bind preferentially to mismatched sites. Thus, while rhodium complexes containing the phenanthrenequinone diimine (phi) ligand bind by intercalation without major perturbation to the B-form duplex,[11] complexes containing the more expansive chrysenequinone diimine (chrysi) ligand bind poorly to well-matched DNA and instead selectively target mismatches. $[Rh(bpy)_2(chrysi)]^{3+}$ binds and, upon photoactivation, cleaves the DNA backbone neighboring the destabilized mismatch site;[6,7] the site selectivity correlates with the thermodynamic instability of the mismatch.[8] Specific DNA cleavage is observed with over 80% of mismatched sites in all sequence contexts. Moreover, the Rh complex has been shown to target a single base mismatch in a 2725 base pair-linearized plasmid heteroduplex.[7] Recently, $[Rh(bpy)_2(phzi)]^{3+}$ (phzi = benzo[*a*]phenazinequinone diimine), a mismatch-specific intercalator of higher affinity, was applied in the differential cleavage of DNA obtained from cell lines deficient versus proficient in mismatch repair.[9]

To explore alkylation of mismatch-containing DNA, we have synthesized a conjugate **1** of $[Rh(phen)(chrysi)(bpy)]^{3+}$ tethered to an aniline mustard known to form covalent adducts at 5′-GNC-3′ sites.[12−14] Bifunctional conjugates containing DNA alkylating agents tethered to nonselective[15,16] or site-specific[17−20] DNA binding moieties have been reported, but conjugates of metallointercalators containing pendant alkylators have not been prepared. The Rh conjugate **1** (Scheme 1) was constructed by sequential introduction of the phenanthroline, chrysi, and aminoalkyl-substituted bipyridine to the Rh center as described for the parent compound (Supporting Information).[21] The key step involved a mild coupling reaction of the amino-functionalized Rh complex with the carboxylate-bearing aniline mustard.

Scheme 1



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Reaction of **1** with 17mer oligonucleotides with two 5′-GNC-3′ binding sites[12−14] either containing (**AB**) or lacking (**AC**) a central CC mismatch was examined by an electrophoretic mobility shift assay (Figure 1). Duplexes **AB** and **AC** (5 μM), 5′-^{32}P-end-labeled

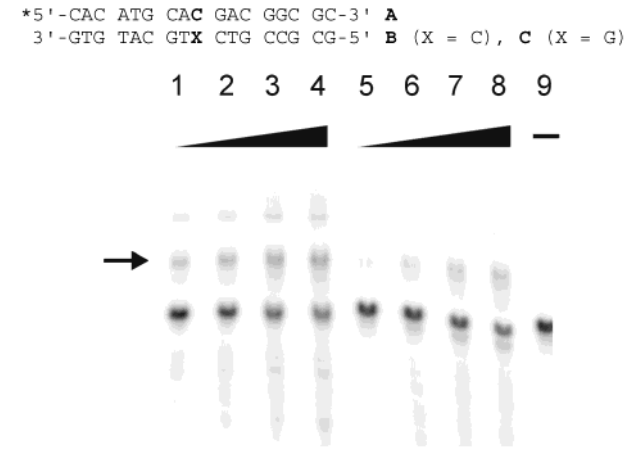

Figure 1. Autoradiogram of a denaturing gel showing the concentration dependence of alkylation of **AB** (lanes 1−4) and **AC** (lanes 5−8) by conjugate **1** (2, 5, 12.5, and 25 μM). * indicates 5′-^{32}P-end-label of **A**. Conditions: incubation for 1 h at 37 °C in the dark, 5 μM duplex DNA in buffer (0.7 mM phosphate, 20 mM NaCl) at pH = 7. Lane 9: DNA in buffer alone. The arrow indicates the primary alkylation product relative to the higher intensity parent band.

on the **A** strand, were incubated with increasing concentrations of conjugate **1** (2−25 μM) at 37 °C for 1 h in the dark, and the reaction was then quenched by freezing. Autoradiography after denaturing PAGE shows a band of retarded mobility that we assign to a covalent DNA adduct containing conjugate **1**; the retarded mobility is expected with covalent attachment of the Rh complex. A weaker band of still lower intensity is visible above. Importantly, the amount formed is up to seven times higher with mismatched **AB** compared to the fully matched duplex **AC**, with the difference most pronounced at lower concentrations. Incubations as a function of time with 2 μM conjugate **1** also show a more rapid increase in formation of the covalent adduct with mismatched **AB** versus the matched duplex **AC**, as well as a corresponding decrease in the intensity of the parent band. The presence of the Rh-chrysi unit thus facilitates alkylation of the DNA by the tethered aniline mustard preferentially on the mismatch-containing duplex.

To determine the binding site of the Rh intercalator as well as the position of covalent modification by the aniline mustard, both DNA photocleavage[22] by the Rh complex and the enhanced

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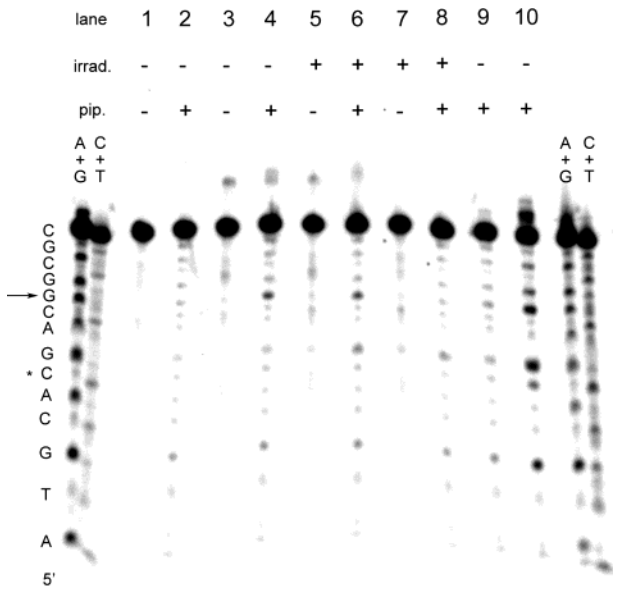

Figure 2. Autoradiogram of a denaturing gel to determine the site specificity of DNA alkylation. Conditions: 5 μM duplex DNA **AB** and 2 μM metal complex in buffer (0.7 mM phosphate, 20 mM NaCl) at pH = 7, incubated for 1 h at 37 °C in the dark prior to subsequent irradiation (HeCd laser, 442 nm, 1 h, 12.5 mW) and/or piperidine treatment (30 min at 90 °C). A+G and C+T, Maxam−Gilbert sequencing reactions. Lanes 1 and 2, buffer alone. Lanes 3−6, conjugate **1**. Lanes 7 and 8, $[Rh(bpy)_2(chrysi)]^{3+}$. Lanes 9 and 10: melphalan (5 and 50 μM). Arrow: preferential site of alkylation. *: position of the mismatch.

depurination associated with *N*-alkylation[12] were exploited. Figure 2 shows autoradiography after denaturing PAGE of 5′-^{32}P-labeled **AB** (5 μM) following incubation with 2 μM **1** for 1 h with or without subsequent piperidine treatment and/or irradiation to promote direct strand cleavage. Control samples contained either buffer alone, untethered $[Rh(bpy)_2(chrysi)]^{3+}$, or melphalan, an aniline mustard.

The formation of a slow-moving covalent adduct above the parent band is clearly visible after incubation with conjugate **1** (lanes 3−6). Interestingly, incubation with melphalan alone at about the same concentration (5 μM, lane 9) does not yield a resolved adduct, and higher concentrations (50 μM, lane 10) lead to nonspecific alkylation of the guanines. Subsequent treatment with piperidine then reveals the site of alkylation.[12] The primary alkylation site on the labeled strand is at the G four bases away from the central CC mismatch (lanes 4 and 6). Note that some damage, although of lower intensity, is visible also at the G directly adjacent to the mismatch. The preferential alkylation at the distal G is not surprising given the length of the tether and the likely shielding of the proximal site by the ancillary ligands of **1**.[23] Direct photocleavage, marking the site of Rh-chrysi binding, occurs with still lower intensity at the 5′-G neighboring the CC mismatch. It is noteworthy that quantitation of these bands shows that the combined effects of photocleavage and alkylation by **1** are similar to the sum of reactions of the component parts.[24] Thus, the tethered alkylator does not inhibit binding of the intercalator at the mismatched site. 3′-end-^{32}P-labeling of the complementary **B** strand gives consistent results. Direct photocleavage neighboring the mismatch is evident with higher intensity than on the **A** strand. Piperidine treatment to reveal alkylation also shows significant reaction at the G 3 bases to the 5′ side of the mismatch.

These results demonstrate that the bifunctional rhodium complex **1** yields site-selective alkylation of mismatch-containing DNA. This preferential targeting of mismatched DNA by **1** at low concentrations, where untethered organic mustards show little reaction, renders these compounds useful tools for the covalent tagging of mismatched DNA and, potentially, for new chemotherapeutic design.

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Supporting Information Available: Schemes outlining the synthesis of conjugate **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) Given the lack of complete reaction with piperidine, the relative intensities at the two sites may not reflect the relative amounts of alkylation.
(24) The amount of cleavage due to alkylation at the 5′-G of the distal 5′-GNC-3′ site is 1.0%, and at the 5′-G of the proximal site it is 0.4% (lane 4). The damage due to direct photocleavage alone is 0.3% (lane 5). The sum of these two values is similar to the combined effects of alkylation and photocleavage, 0.7% in lane 6. Also, the amount of photocleavage at the 5′-G of the proximal site in the presence of conjugate **1** is similar to that observed with untethered intercalator **2** (0.3% in lane 7).

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