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A Mismatch-Selective Bifunctional Rhodium−Oregon Green Conjugate: A Fluorescent Probe for Mismatched DNA

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Accurate DNA replication is the basis of cellular survival. Therefore, an intricate cellular machinery has evolved to maintain the fidelity of the genome.[1] Nonetheless, DNA base pair mismatches can arise from polymerase errors, genotoxic chemicals, or UV or ionizing radiation.[2] To probe DNA mismatches, we have developed bulky rhodium intercalators that target these mismatches with high selectivity.[3−6] These complexes employ sterically demanding intercalating ligands that are too wide to insert into matched B-form DNA and bind instead preferentially to mispaired sites. Complexes bearing these ligands, such as 5,6-chrysenequinone diimine (chrysi), not only bind to mismatched DNA but also, upon photoactivation, cleave the DNA backbone neighboring the mismatched site. Indeed, a single base mismatch can be detected and cleaved in a 2725 base pair DNA fragment.[5] In all, more than 80% of mismatches are targeted by these metallointercalators, with only the most thermodynamically stable mispairs avoiding detection. To exploit further this unique selectivity, we have also recently developed bifunctional metallointercalators that are mismatch-selective, including conjugates bearing alkylating and platinating functionalities.[7,8]

A fluorescent probe for mismatched DNA would be particularly useful as a diagnostic for mismatch repair deficiencies. Earlier, a bulky luminescent ruthenium intercalator was applied to this effort, but the hydrophobicity of the complex was cumbersome.[9] Here we describe the application of the parent rhodium complex conjugated to an organic fluorophore to detect mismatched DNA. We hypothesized that a mismatch-selective probe could be designed by conjugating a negatively charged fluorophore to the cationic rhodium intercalator (Figure 1). In free solution or with matched DNA, the fluorescence of the conjugate would be quenched as a result of ion pairing of the fluorophore with the intercalator. In the presence of mismatched DNA, however, the metal complex would intercalate, and the negatively charged DNA backbone would repel the fluorophore away from the rhodium, thus reducing quenching and increasing fluorescence.[10]

To test this idea, we have synthesized a conjugate of [Rh(phen)(bpy)(chrysi)]$^{3+}$ tethered to Oregon Green 514, **1**. The trisheteroleptic intercalator moiety of conjugate **1** was synthesized through the sequential addition of phenanthroline, chrysi, and ethylene glycol-modified bipyridine (peg-bpy) to the Rh center (Supporting Information).[11] The completed rhodium complex, **2**, was then coupled to the succinimidyl ester of Oregon Green to yield the final conjugate (Scheme 1).

Fluorescence studies of compound **1** reveal excitation and emission maxima at 519 and 530 nm, respectively, slightly shifted relative to the parent Oregon Green.[12] As expected, in the presence of DNA, the fluorescence of **1** is dramatically quenched versus an equimolar solution of unconjugated Oregon Green and **2** with a relative intensity of about 0.02.[13,14] Fluorescence titrations of untethered Oregon Green with **2** eliminate energy transfer as a quenching mechanism and instead support electron transfer as the source of quenching.

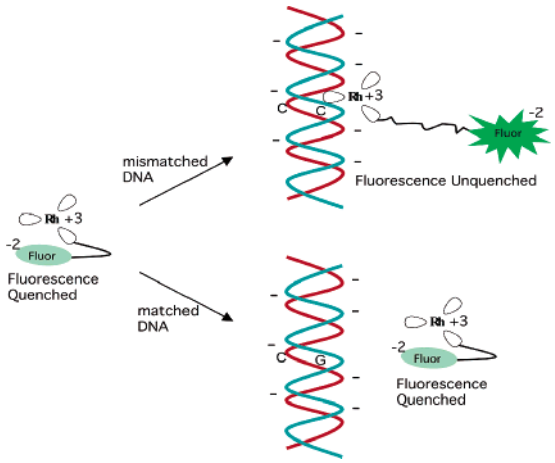
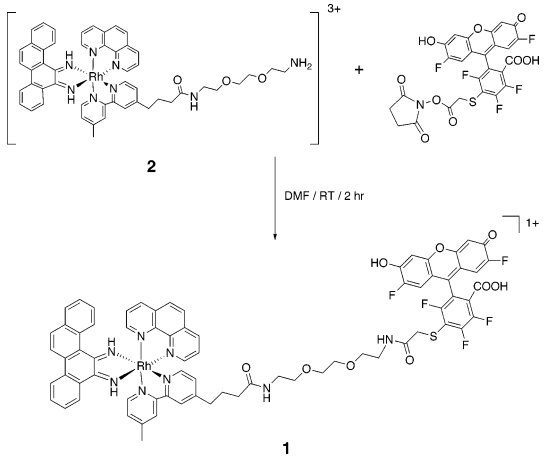


Figure 1. Illustration of the design of a mismatch-specific fluorophore.

Scheme 1. Assembly of Rh/Fluorophore Conjugate, **1**



Fluorescence measurements of 1 μM **1** in the presence of variable salt concentrations (10 mM Na_2HPO_4, 0−500 mM NaCl, pH 7.1) reveal a strong dependence in fluorescence on ionic strength. The fluorescence of **1**, while still significantly quenched relative to free Oregon Green, increases almost 15-fold over the range of NaCl concentrations tested. Oregon Green, not conjugated to the Rh complex, shows no variation in fluorescence with ionic strength. These observations support an intramolecular ion-pair mechanism of quenching; as the salt concentration increases, the ion-pair can separate more easily, resulting in less quenching and greater fluorescence.

In fluorescence titrations with DNA, 1 μM **1** was added to variable amounts of two 17-mer oligonucleotide solutions that either contained or lacked a central CC mismatch (Figure 2). Over the concentration range examined, conjugate **1** shows significantly greater fluorescence with mismatched DNA than with matched

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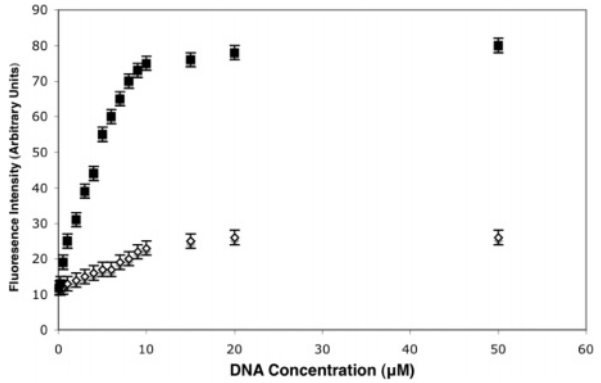

Figure 2. Plot of fluorescence emission (530 nm) of **1** versus increasing concentrations of matched (○) and mismatched (■) oligonucleotides. Excitation wavelength = 475 nm. Fluorescence measurements are in 20 mM NaCl, 10 mM NaPi, pH 7.1. Prior to measurement, DNA was incubated with 1 μM **1** for 15 min. The DNA sequence used was 5′-CACATGCA**C**-GACGGCGC-3′ with complements either containing or lacking a mismatched C at the bold site. For comparison, 1 μM untethered Oregon Green shows a fluorescence intensity of 1500 on the same scale.

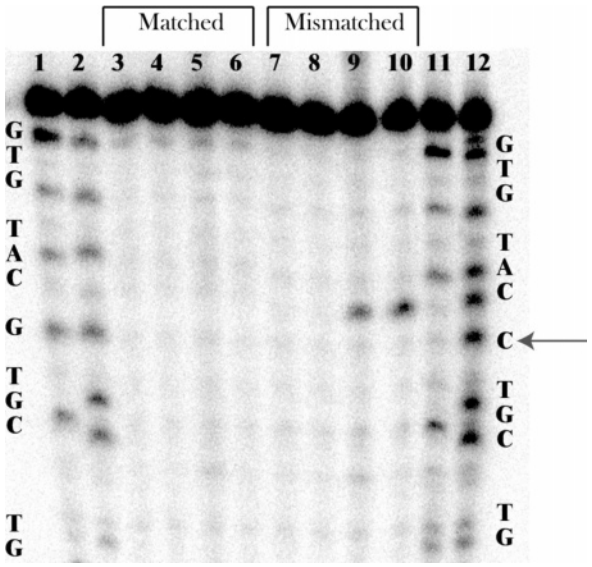

Figure 3. Autoradiogram of a denaturing 20% polyacrylamide gel revealing photocleavage for **1** and **2** with fully matched and mismatched oligonucleotides. Conditions are duplex (1 μM), Rh (1 μM) in 20 mM NaCl, 10 mM NaPi, pH 7.1 for 30 min at ambient temperature followed by irradiation for 5 min with a solar simulator (325−450 nm). Lanes 1, 2, 11, and 12 show Maxam−Gilbert sequencing reactions for matched (1, 2) and mismatched (11, 12) DNA. For matched and mismatched DNA, respectively: lanes 3 and 7 show fragments irradiated with no metal complex; lanes 4 and 8 show fragments with **1** but no irradiation; lanes 5, 6, 9, and 10 show fragments after irradiation in the presence of **1** (5, 9) and **2** (6, 10). The DNA sequence is 5′-^{32}P-end-labeled-GCGCCGTCGT**X**CATGTG-3′ where X = C, G with a complement containing a matched or mismatched C at the bold site. The arrow marks the mismatched site.

DNA; at saturating DNA concentrations, the relative intensity with mismatched versus matched DNA is 3.2 ± 0.2.[15] Significantly, even in the presence of mismatched DNA, the fluorescent conjugate remains very quenched, with a maximum absolute fluorescence equal to 6% of that of an equimolar solution of unconjugated Oregon Green and **2** in the presence of DNA.

In control experiments, no mismatch-dependent differences in fluorescence are found for Oregon Green alone or for noncovalently linked **2** and Oregon Green. In addition, the conjugate **1** shows no increased fluorescence with single-stranded DNA. Interestingly, no DNA-dependent (matched or mismatched) changes in fluorescence anisotropy are observed, suggesting that the fluorophore is exceedingly mobile in the DNA-bound form.

Mismatch targeting by the bifunctional conjugate can also be examined in photocleavage experiments.[16] Denaturing PAGE experiments with 5′-^{32}P-end-labeled oligonucleotides containing or lacking a central CC mismatch were employed to test specific site targeting (Figure 3). Duplex DNA (1 μM) was incubated with variable concentrations of **1** (100 nm to 5 μM) for 30 min and then irradiated for 5 min. Autoradiography reveals specific photocleavage neighboring the mismatched site. DNA photocleavage titrations yield 6×10^5 M^{-1} for the binding affinity to the mismatched site.[17] Significantly, no cleavage is evident with matched DNA. These results also agree well with measurements for the parent Rh complex and conjugates.[3,4]

This work establishes a strategy for the design of a bifunctional rhodium/fluorophore conjugate that serves as a fluorescent probe for mismatches. The complex selectively binds, cleaves, and, importantly, shows enhanced fluorescence with mismatched DNA.

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Supporting Information Available: Synthesis and characterization of conjugate **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) Several linker-modified bipyridine linkers have been examined. Alkane linkers in particular lead to significant quenching under all conditions.
(12) For Oregon Green excitation and emission maxima are 514 and 525 nm.
(13) A 475-nm excitation wavelength was employed in all experiments.
(14) For comparison, without DNA, the relative fluorescent intensity for 1 μM **1**: Oregon Green + **2**: Oregon Green are 1/73/100.
(15) These titrations do not allow quantitation of affinities. Qualitatively, results for **1** with mismatched DNA agree with those for the parent complex. See refs 3 and 4 as well as photocleavage results in Figure 3.
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(17) This value is ∼10% lower, which may reflect the decreased charge for **1**.

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