Recognition of DNA Base Mismatches by a Rhodium Intercalator

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DNA base mismatches arise during the course of genetic recombination and replication as a consequence of enzymatic errors or DNA damage.[1,2] Although studies have been directed toward unraveling the roles mismatch structure,[3−6] dynamics,[7−9] and biochemistry[10,11] play in their recognition and repair, a detailed chemical understanding of the process is still elusive. As part of the effort to gain insight into natural recognition systems and to produce useful DNA probes, the design of molecules which site-specifically recognize mismatches is an attractive experimental goal.[12,13] Strategies have exploited isolated mismatch recognition proteins,[14,15] hybridization of fluorescent conjugates,[16,17] DNA chip methodologies,[18,19] and differential chemical cleavage with reagents assaying for base accessibility.[20−22]

Here, we describe DNA mismatch recognition by a novel rhodium intercalator, $[Rh(bpy)_2(chrysi)]^{3+}$ (chrysi = 5,6-chrysenequinone diimine). The phenanthrenequinone diimine (phi) complexes of rhodium,[23−26] which bind DNA via intercalation in the major groove[27,28] and promote strand scission upon photoactivation,[29] have proven to be versatile frameworks for the design of novel DNA recognition agents. Base mismatch recognition poses a new challenge in the design of specific DNA binding molecules. Unlike the recognition of a base sequence where interacting functionalities can be placed to "read" the order of the bases,[30] mismatch recognition must identify mismatches independent of the bases involved. We have chosen to exploit the thermodynamic destabilization[9,31,39] near a mismatch site as a basis for site discrimination. $[Rh(bpy)_2(chrysi)]^{3+}$ was designed as a derivative of the phi family of intercalators but with the broader four-ring chrysene for DNA intercalation. Molecular modeling suggested that this ligand, unlike phi, is too large to intercalate easily into standard DNA base steps but that the locally perturbed site of a base mismatch might accommodate the large chrysene ring system.

$[Rh(bpy)_2(chrysi)]^{3+}$ was synthesized by condensation of $[Rh(bpy)_2(NH_3)_2](PF_6)_3$[32] with 5,6-chrysenequinone[33] by a method analogous to that developed by Sargeson and co-workers (see the Supporting Information).[34,35] The mismatch binding properties of the separated Δ- and Λ-enantiomers were then examined in DNA photocleavage experiments on a set of 17-mer oligonucleotides, each containing one DNA base mismatch (Figure 1). At 10 μM DNA duplex concentration, photoinduced cleavage by the rhodium complex is not apparent at B-form sites. Instead, the strongest cleavage intensity is observed with the Δ-enantiomer on the duplex containing the CC mismatch. Here, cleavage occurs to the 3′ side of the mismatch. Similar but less intense cleavage is observed at the other pyrimidine−pyrimidine mismatches TT and TC. CA, the single purine−pyrimidine mismatch that is recognized, shows a very different cleavage pattern. Strong cutting is evident at the mismatched C and neighboring the base 3′ to the mismatch site. Unlike the other recognized mismatches, the cleavage 3′ to CA results in two products of differing gel mobility.[36] Photocleavage on the duplex containing AA, the purine−purine mismatch recognized by the complex, also shows distinctive characteristics. Δ-$[Rh(bpy)_2(chrysi)]^{3+}$ promotes strong cleavage at the base 3′ to AA and a small amount of cleavage at the mismatched A itself. It is only at the AA site, however, that the Λ-enantiomer shows detectable recognition with a cleavage pattern similar to, but weaker than, its Δ counterpart. When the end label is placed on the other strand of the duplex, significant cleavage is observed only with the CC and TC mispairs (data not shown); here cleavage is at the guanine 3′ of the mismatch site.

We also examined recognition of the CA mispair as a function of orientation and sequence context. In a series of DNA hairpins containing central CA mismatches (bold) we see cleavage as follows: C**C***C* > C***C***T* > T**C***AG* = G****C**G > A*****C***G (italics indicates cleavage at indicated base, and an asterisk indicates cleavage opposite; see the Supporting Information).[38] Irrespective of the flanking sequences considered in this experiment, the CA mismatch is specifically targeted by $Rh(bpy)_2(chrysi)^{3+}$. Differences in cleavage pattern arise with changes in sequence context just as with different mismatches; these variations

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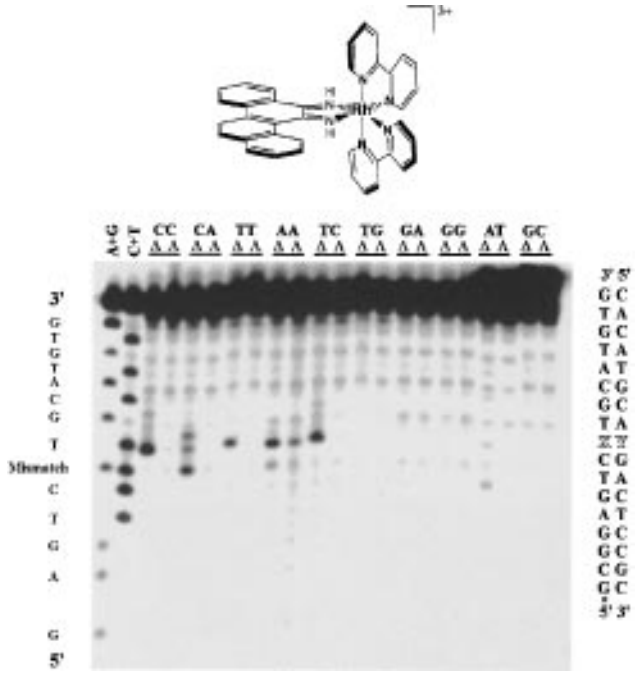

Figure 1. (Top) Δ-[Rh(bpy)$_2$(chrysi)]$^{3+}$. (Bottom) DNA photocleavage by Δ- and Λ-[Rh(bpy)$_2$(chrysi)]$^{3+}$ on the 5′-^{32}P-end-labeled oligonucleotide duplex (the star indicates the position of the label). Molecular Dynamics Phosphorimager scanned image of 20% denaturing polyacrylamide gel showing fragments after irradiation of duplexes (10 μM oligonucleotide) with 1 μM Δ- or Λ-[Rh(bpy)$_2$(chrysi)]$^{3+}$ in 50 mM Tris, 20 mM NaOAc, 18 mM NaCl, pH 7.0. Each sample was preequilibrated for 11 min before irradiation for 13 min at 365 nm using an Oriel Hg Xe arc lamp. Gel lanes labeled A + G and C + T are standard Maxam−Gilbert sequencing reactions.[43] Mismatch sequences identified above each set of lanes correspond to XY base positions, and Δ or Λ indicates the enantiomer used to promote photocleavage. All oligonucleotides were made using standard phosphoramidite chemistry and purified by reversed phase HPLC. They were 5′-end-labeled with γ-[^{32}P]ATP (Dupont-NEN) and T4 polynucleotide kinase (New England Biolabs).

underscore the different geometries likely to exist at the various sites. Since the individual structures may lead to different efficiencies of photocleavage, relative cleavage intensities do not directly reflect relative binding affinities.

In a quantitative photocleavage titration on a 35-mer DNA hairpin, the thermodynamic binding constant for Δ-[Rh(bpy)$_2$(chrysi)]$^{3+}$ at the CC mismatch was found to be 8.4(1.0) × 10^5 M^{-1}. (Figure 2). The binding affinity of the complex for its target site is an order of magnitude smaller than those of analogous phi complexes of rhodium at their DNA binding sites.[37] This difference is understandable given that steric clash between the intercalator and DNA is designed into the complex as its source of specificity. To examine site selectivity, the average binding constant of Δ-[Rh(bpy)$_2$(chrysi)]$^{3+}$ to B-form DNA was determined. Photocleavage titration on a 33-mer hairpin and competitive titration with unlabeled 25-mer B-DNA yielded a value of 4(2) × 10^4 M^{-1} for the average nonspecific binding affinity (see the Supporting Information.)

Destabilization at a mismatch appears to accommodate the expansive intercalator. This notion is supported by the correlation of helix destabilization caused by a mismatch with strong targeting by [Rh(bpy)$_2$(chrysi)]$^{3+}$. In Figure 1, the mismatches are ordered (left to right) by decreasing thermodynamic destabilization; in this set, the CC mismatch is the most destabilizing and GG is the least disruptive.[7,39,40] Although possible variations in DNA photocleavage efficiency need to be considered, it is interesting that significant cutting is observed only at the more helix-destabilizing mismatches.

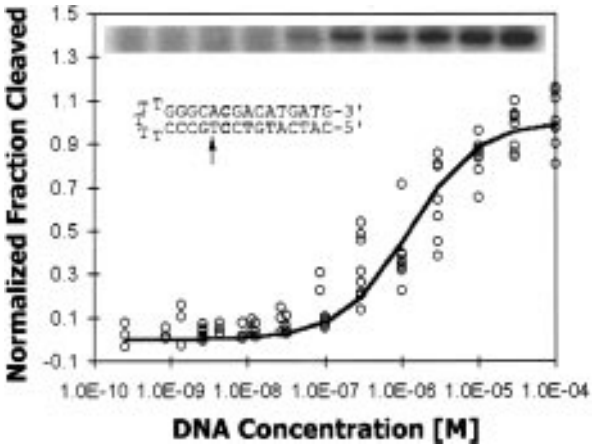

Figure 2. Binding isotherm for Δ-[Rh(bpy)$_2$(chrysi)]$^{3+}$ targeted to a CC mismatch- containing oligonucleotide. Top inset: Excised gel bands from a representative photocleavage titration experiment. The binding constant was determined on a hairpin oligonucleotide, 5′-CATCAT-GTC**C**TGCCCTTTTTGGGCA**C**GACATGATG-3′ (bottom inset) containing a single CC base mismatch (involved bases boldface). Photocleavage reactions were performed at either 313 or 365 nm for 7.5−15 min. The concentration of hairpin DNA varied from 3 × 10^{-10} to 1 × 10^{-4} M with the rhodium complex at 10-fold lower concentration. Samples were eluted through 20% denaturing polyacrylamide gels and the data analyzed using a Molecular Dynamics Phosphorimager and ImageQuant software. Cleavage is observed 3′ to the CC mismatch on both sides of the hairpin; only the cleavage band closest to the end label (indicated by the arrow) was quantitated. Data from multiple trials were normalized (open circles) and fit to a standard single binding site binding model (solid line).[37]

These results show that the destabilized structure distinctive to a mispaired site can be exploited for the specific recognition of DNA mismatches. This strategy may be applied in the recognition of other sites of DNA damage or modification involving thermodynamic disruption and could be useful in the development of new molecular diagnostics or chemotherapeutic agents. It is noteworthy that similar mechanisms of DNA damage recognition are observed in cellular nucleotide excision repair systems.[41,42]

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Supporting Information Available: Synthesis and characterization of [Rh(bpy)$_2$(chrysi)]$^{3+}$, gel data showing CA mismatch recognition in various sequence contexts, and binding isotherms to nonspecific sites (6 pages). See any current masthead for ordering and Internet access information.

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