Assembly of DNA Recognition Elements on an Octahedral Rhodium Intercalator: Predictive Recognition of 5′-TGCA-3′ by Δ-[Rh[(*R*,*R*)-Me$_2$trien]phi]$^{3+}$

Achim H. Krotz, Brian P. Hudson, and Jacqueline K. Barton*

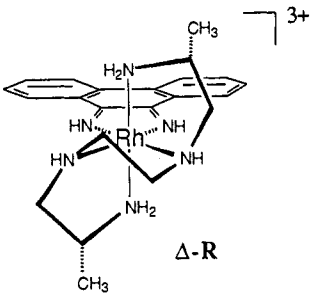
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California 91125

Received August 9, 1993

Here we explore the *de novo* design of rhodium intercalators for predictive four base pair recognition of duplex DNA. Complexes have been constructed which, like DNA-binding proteins,[1] pose several functionalities for site-specific noncovalent interaction with DNA. Octahedral rhodium intercalators[2-4] are particularly useful in this design since (i) the octahedral coordination specifies the stereochemistry of ligand functionalities and (ii) the intercalating ligand[5] orients these functionalities with respect to the DNA major groove.

Recently we showed that a novel class of metallointercalators containing the phenanthrenequinone diimine ligand (phi) and ancillary aliphatic amines, [Rh(L4)phi]$^{3+}$ (L4 = 4NH$_3$, 2en, [12]aneN$_4$ or [12]aneS$_4$), bind in the major groove of B-form DNA (K_b > 10^6 M^{-1}) and promote strand cleavage upon photoactivation with ultraviolet light.[4,5] Preferential recognition of a 5′-GC-3′ base step is afforded by these small complexes through specific hydrogen-bonding interactions by the axial amine ligands.[6] Site recognition may now be extended to 5′-*TGCA*-3′ by placement of methyl groups adjacent to the axially coordinated amines on the parent [Rh(en)$_2$phi]$^{3+}$ for specific van der Waals contacts in the major groove with thymine methyls.

Four C_2-symmetrical isomers of [(2,9-diamino-4,7-diazadecane)(phenanthrenequinone diimine)rhodium(III)]$^{3+}$, [Rh(Me$_2$trien)phi]$^{3+}$, namely, Λ,α-(2*S*,9*S*) (Λ-**S**), Δ,α-(2*S*,9*S*) (Δ-**S**), Δ,α-(2*R*,9*R*) (Δ-**R**), and Λ,α-(2*R*,9*R*) (Λ-**R**), have been prepared and crystallographically characterized.[7] On the basis of molecular modeling,[8] we expected, in addition to the hydrogen-bonding interactions between the axial amines and the O6 of guanines, attractive methyl–methyl interactions between thymine and Δ-**S** or Δ-**R** for 5′-TGCA-3′ sites (Figure 1). As a consequence, modeling predicts an increased affinity of Δ-**S** and Δ-**R**[10] for a 5′-TGCA-3′ site as compared to other 5′-XGCX-3′ sites and also higher specificity of Δ-**S** and Δ-**R** compared to Δ-[Rh(en)$_2$phi]$^{3+}$ for a 5′-TGCA-3′ site. In 5′-AXXT-3′ sites, because of the right-handed helicity of DNA, the distances between thymine methyls and the methyl groups of the complexes are too large (≥7 Å) for direct interaction. For Λ-**S** and Λ-**R**, molecular modeling suggested repulsive interactions between the methyl groups for sites 5′-TXXA-3′.



Δ-**R**

In order to test these predictions, site-selective DNA recognition by Λ-enantiomers Λ-[Rh(en)$_2$phi]$^{3+}$, Λ-**S**, and Λ-**R** and Δ-enantiomers Δ-[Rh(en)$_2$phi]$^{3+}$, Δ-**S**, and Δ-**R** was experimentally compared through photoinduced DNA cleavage. Figure 2 shows the DNA photocleavage by these metal complexes on a 140 base pair 5′-^{32}P-end-labeled restriction fragment[11] and well represents the results obtained on a series of DNA fragments containing the 5′-TGCA-3′ site.

In comparing Δ-enantiomers, the addition of the adjacent methyl group onto the Δ-[Rh(en)$_2$phi]$^{3+}$ skeleton is found to increase affinity and specificity. Δ-**S** and Δ-**R**, like Δ-[Rh(en)$_2$phi]$^{3+}$, both show a preference for 5′-GC-3′ sites owing to hydrogen-bonding interactions by the axial amines of the ligand; Δ-[Rh(en)$_2$phi]$^{3+}$ and Δ-**S** show comparable site-selectivity.[12] However, Δ-**R** shows increased cleavage intensity at 5′-TGC-3′ sites compared to several 5′-XGCX-3′ steps and significantly enhanced specificity for a 5′-TGCA-3′ site, as predicted from molecular modeling. Quantitation shows almost a doubling of cleavage at this site compared to the neighboring 5′-GGCA-3′ site and a factor of approximately 3 increase in intensity compared to cleavage at an isolated 5′-GC-3′ site.[13] This stabilization can reasonably be attributed to the designed methyl–methyl van der Waals contact.[4,14] Together the axial amines and the added

* To whom correspondence should be addressed.

(1) (a) Pabo, C. O.; Sauer, R. T. *Annu. Rev. Biochem.* **1992**, *61*, 1053. (b) Steitz, T. Q. *Q. Rev. Biophys.* **1990**, *23*, 205.

(2) (a) Pyle, A. M.; Barton, J. K. *Prog. Inorg. Chem.* **1990**, *38*, 413. (b) Sitlani, A.; Barton, J. K. *Handbook of Metal Ion Ligand Interactions in Biological Fluids*; Marcel Dekker: New York, in press. (c) Chow, C. S.; Barton, J. K. *Methods Enzymol.* **1992**, *212*, 219.

(3) Sitlani, A.; Dupureur, C. M.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 12589.

(4) Krotz, A. H.; Kuo, L. Y.; Shields, T. P.; Barton, J. K. *J Am. Chem. Soc.* **1993**, *115*, 3877.

(5) (a) David, S. S.; Barton, J. K. *J. Am. Chem. Soc.* **1993**, *115*, 2984. (b) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303.

(6) Λ-[Rh(en)$_2$phi]$^{3+}$ was also shown to be stabilized at 5′-TX-3′ sites through methyl–methyl interactions with the 5′-thymine.[4]

(7) Krotz, A. H.; Barton, J. K. Submitted for publication.

(8) For molecular modeling, the coordinates for the metal complexes were taken from their crystal structures, and the coordinates for the intercalation site from the crystal structure of (terpyridyl)(ethanethiolate)platinum(II) with d(CpG)$_2$.[9] Additional base pairs were added to the 5′- and 3′-sides using MacroModel.

(9) Wang, A. H. J.; Nathan, J.; van der Marel, G.; van Boom, J. H.; Rich, A. *Nature* **1978**, *276*, 471.

(10) Both complexes Δ-**S** and Δ-**R** may be intercalated in the 5′-TGCA-3′ site with distances of 3 Å for hydrogen bonding (N_{axial}–$O6_{guanine}$ = 3 Å) and 4 Å between the methyl group of the thymine and the methyl group of the complex for attractive van der Waals interactions.

(11) At all sites cleaved, 3′-^{32}P-end-labeling reveals cleavage on the opposing strand with a single base 5′-asymmetry, an observation which is consistent with major groove reaction. See ref 5b. Also evident at some sites on the 3′-^{32}P-end-labeled fragment is cleavage directly across from the base cleaved in the 5′-end-labeled fragments.

(12) Although a small enhancement of Δ-**S** for sites containing 5′-TGC-3′ sites is apparent, the effect is not remarkable compared to Δ-**R**. The crystal structures reveal that the actual position in space of the methyl group depends sensitively upon the puckering of the metal chelate, which differs for the two isomers in the crystals.

(13) It is noteworthy that in close proximity to this strong 5′-TGCA-3′ site there is another 5′-TGCA-3′ site which displays lower reactivity. Weaker cleavage, however, is also evident at this second site with [Rh(en)$_2$phi]$^{3+}$. Results on the several fragments examined indicate that flanking sequences clearly affect the local topology of a 5′-TGCA-3′ step for recognition by both Δ-[Rh(en)$_2$phi]$^{3+}$ and Δ-**R**. Strongest cleavage by Δ-**R** is apparent at 5′-TGCA-3′ sites which are flanked by T's and A's.

(14) (a) Goeddel, D. V.; Yansura, D. G.; Caruthers, M. H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3578. (b) Williams, D. H.; Cox, J. P. L.; Diog, A. J.; Gardner, M.; Gerhard, U.; Kaye, P. T.; Lal, A. R.; Nicholls, I. A.; Salter, C. J.; Mitchell, R. C. *J. Am. Chem. Soc.* **1991**, *113*, 7020.

0002-7863/93/1515-12577$04.00/0

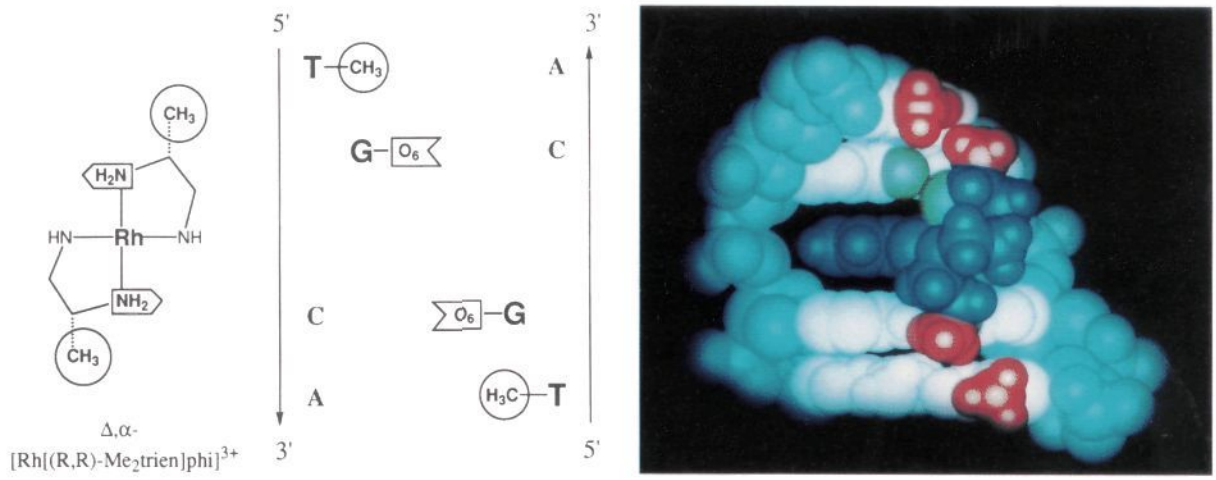


Figure 1. Site-selective design for 5′-TGCA-3′ recognition. Left: Schematic representations of the ancillary ligand of Δ-**R** and the recognition elements of 5′-TGCA-3′, showing the complementarity of the hydrogen-bond-donating axial amines of Δ-**R** and the hydrogen-bond-accepting O6 of the guanine residues as well as the approximate positions of the methyl groups of Δ-**R** and their interactions with thymine residues. Right: Molecular modeling of Δ-**R** intercalated into the 5′-GC-3′ step of double-stranded 5′-TGCA-3′. Groups well-positioned to participate in hydrogen-bonding interactions (axial amines of Δ-**R**, O6 of guanine) are shown in green. The methyl groups of Δ-**R** and the thymine residues are shown in red. Note that they are proximate but nonoverlapping. The remainder of Δ-**R** is shown in dark blue, the DNA base pairs in white, and the deoxyribose-phosphate backbone in light blue.

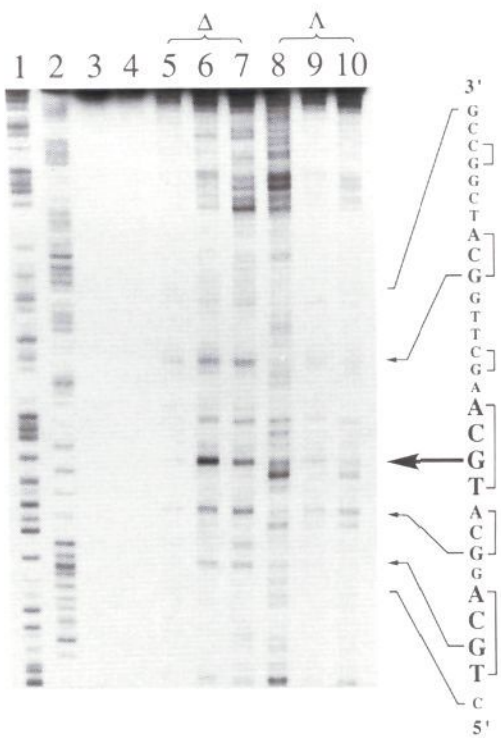


Figure 2. Photocleavage by rhodium isomers showing their site-selectivities. Autoradiogram of an 8% denaturing polyacrylamide gel after photocleavage of the 5′-^{32}P-labeled *Eco*RI/PvuII fragment of pUC18: lanes 1 and 2, Maxam–Gilbert A + G and C + T reactions, respectively; lane 3, untreated fragment; lane 4, fragment irradiated in the absence of rhodium complex; lanes 5–10, fragment irradiated in the presence of Δ-[Rh(en)$_2$phi]$^{3+}$, Δ-**R**, Δ-**S**, Λ-[Rh(en)$_2$phi]$^{3+}$, Λ-**S**, and Λ-**R**, respectively. The sequence containing several preferred cleavage sites is shown to the right, with brackets marking sites containing 5′-GC-3′ steps and arrows denoting sites of strong cleavage. Irradiations were conducted using a HeCd laser at 325 nm for 20 min. Samples contained 100 nM rhodium and 1 μM base pairs in 5 mM Tris, 50 mM NaCl, pH 7.0. Note the strong cleavage by Δ-**R** at one of the 5′-TGCA-3′ sites. Also note the decrease in cleavage by the methyl-containing Λ isomers (Λ-**S** and Λ-**R**) versus Λ-[Rh(en)$_2$phi]$^{3+}$.

methyl groups with defined stereochemistry on the ancillary ligand yield *predictive* four-base sequence-specific recognition.

The addition of the adjacent methyl groups onto the parent Λ-[Rh(en)$_2$phi]$^{3+}$ leads also to an increase in selectivity as predicted from molecular modeling, but in contrast to Δ-isomers, here steric clashes provide the selectivity. Λ-[Rh(en)$_2$phi]$^{3+}$ shows cleavage at 5′-TX-3′ as well as 5′-GC-3′ steps.[15] The methyl derivatives Λ-**S** and Λ-**R** show cleavage only at 5′-GG-3′ or 5′-GC-3′ sites which do not contain thymines. This preference is apparent in particular at the strongest 5′-TG-3′ site for Λ-[Rh(en)$_2$phi]$^{3+}$, which is weaker with Λ-**S**, and which shows no detectable cleavage by Λ-**R**. This increased selectivity of Λ-**S** and Λ-**R** for 5′-GC-3′ steps compared to Λ-[Rh(en)$_2$phi]$^{3+}$, which lacks the adjacent methyl group, may be attributed to steric repulsion between these methyl groups and the thymine methyl groups in the major groove.

These results exemplify the rational design of a metallointercalator for sequence-specific recognition of double-helical DNA. Such design illustrates the application of both attractive and repulsive interactions in achieving site-selectivity. The construction of octahedral metallointercalators containing an array of functional groups assembled on a rigid, stereochemically well defined ligand framework now offers a strategy to prepare sequence-specific DNA-binding complexes with predictability.

Acknowledgment. We are grateful to the National Institutes of Health (GM33309) for their financial support. In addition we thank the Deutsche Forschungsgemeinschaft (A.H.K.) for postdoctoral and the National Science Foundation (B.P.H.) for predoctoral fellowships.

Supplementary Material Available: Autoradiogram showing cleavage by the rhodium complex on a different DNA fragment containing 5′-TGCA-3′ (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(15) Overall cleavage and binding is largest for Λ-[Rh(en)$_2$phi]$^{3+}$.