A New Class of DNA Metallobinders Showing Spectator Ligand Size Selectivity: Binding of Ligand-Bridged Bimetallic Complexes of Ru(II) to Calf Thymus DNA†

David L. Carlson, Daniel H. Huchital, Edgardo J. Mantilla, Richard D. Sheardy, and W. Rorer Murphy, Jr.*

Department of Chemistry, Seton Hall University
400 South Orange Avenue
South Orange, New Jersey 07079-2694

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The development of new molecules that bind strongly and selectively to nucleic acids expands the range of potential pharmaceutical agents whose mode of bioactivity is through interaction with DNA or RNA.[1] Metal complexes containing planar aromatic ligands have been shown to interact with both native and synthetic nucleic acids.[2-10] Barton[3] has shown that racemic $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline; all ligand structures are shown in Figure 1) binds to calf thymus DNA with a binding constant K of 6.2×10^3 M^{-1} in solutions containing 50 mM Na^+. The binding site size s, expressed in numbers of base pairs, is typically 3–4 for these complexes. The dominant modes of binding for the enantiomers of this complex have been a subject of vigorous debate in the literature.[5,6] Phenanthrenequinone diimine (phi) Rh(III) complexes have been shown to bind strongly to DNA, with a K greater than 10^7 M^{-1}.[5] NMR data support the partial intercalation of the phi of Δ-$[Rh(phen)_2(phi)]^{3+}$ at the 5′-CG-3′ step of $[d(GTCGAC)_2]$.[5] Extension of the planarity at the 5,6 sites of phen increases the strength of interaction of these complexes with DNA. Complexes containing dipyrido[3,2-*a*:2′,3′-*c*]phenazine (dppz) such as $[Ru(bpy)_2(dppz)]^{2+}$ also have large binding constants ($K > 10^6$ M^{-1}).[7] Partial intercalation and major groove binding has been reported by Strekas et al. for $[Ru(bpy)_2(ppz)]^{2+}$ (ppz = 4,7-phenanthrolino[6,5-*b*]pyrazine), although the binding constants are only on the order of 10^3 M^{-1}.[8-10] Baker et al. have also observed the enantiospecific cleavage of DNA using the bimetallic complex Λ-$[Ru(bpy)_2(ppz)CuL_2]^{4+}$ prepared *in situ* and activated with hydrogen peroxide and 3-mercaptopropionic acid.[10] The planar aromatic region extending away from the metal center is the common structural motif in all of the complexes.

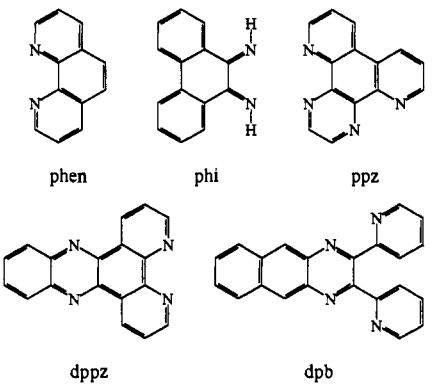

Figure 1. Ligand structures.

Our research has focused on the chemistry of ligand-bridged bimetallic complexes of Ru(II) and Re(I), where the bridging ligand is based on 2,3-bis(2-pyridyl)benzo[*g*]quinoxaline (dpb).[11-13] Bridging ligands such as these hold the two metal centers in relatively close proximity (ca. 700 pm) while leaving the metal-centered properties relatively unaltered. The dpb ligand also provides a planar aromatic region similar to the one in dppz, phi, and ppz, except that in dpb this region extends away from the molecule at a 90° angle to the intermetallic axis. We have used this ligand to prepare $[Ru(NH_3)_4]_2(dpb)^{4+}$ and $[Ru(bpy)_2]_2(dpb)^{4+}$. The synthesis of the latter complex is reported in ref 12.

The $[Ru(NH_3)_4]_2(dpb)^{4+}$ complex was prepared from $[Ru(NH_3)_5(OH_2)]^{2+}$ and dpb using a modification of a previously reported method.[14] The complex identity and purity were verified by electronic spectroscopy, thin-layer chromatography, electrochemistry, and ^1H NMR. The absorption spectrum of the complex in buffer I[15] shows bands at 640 and 318 nm, with extinction coefficients of 1.7×10^4 and 4.12×10^4 M^{-1} cm^{-1}, respectively. These absorbances are assigned to the $d\pi(Ru) \rightarrow p\pi^*(dpb)$ and $p\pi(dpb) \rightarrow p\pi^*(dpb)$ transitions. Reduction potentials of 1.31 and 1.48 V (vs 3 M NaCl Ag/AgCl reference electrode) were obtained by Osteryoung square-wave voltammetry in acetonitrile containing 0.1 M tetrabutylammonium hexafluorophosphate. These potentials are comparable to those measured by Ruminski[14] for $[Ru(NH_3)_4]_2(dpp)^{4+}$ and are characteristic of bimetallic complexes of this type.

The ^1H NMR for the complex shows an upfield shifts consistent with the complexation of the metal to the ligand.[16] The specific assignments for the resonances in the complex have not been made, but the lack of peaks associated with uncomplexed 2,2′-pyridine is consistent with the $[Ru(NH_3)_4]_2(dpp)^{4+}$ formulation. The electrochemical results also support a structure with the two metal centers in close proximity.[12]

* To whom correspondence should be addressed.

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(15) Buffer I is composed of 10 mM phosphate ion, 0.1 mM EDTA, and 115.4 mM total Na^+ ion. The pH was adjusted to 7.00 with HCl.

(16) The ^1H NMR showed resonances for free dpb of δ 8.24 (m), 8.80 (s), 7.66 (dd), 7.94 (ddd), 7.30 (ddd), 8.06 (d). The ^1H NMR showed resonances for $[Ru(NH_3)_4]_2(dpb)^{4+}$ of δ 9.18 (d), 9.10 (s), 8.82 (d), 8.82 (d), 8.58 (d), 8.38 (d), 8.33 (dd), 8.07 (t), 7.93 (d), 7.78 (m), 7.69 (m), 7.62 (d), 7.47 (m). (s = singlet; d = doublet; dd = doublet of doublets; ddd = doublet of doublet of doublets; t = triplet; m = multiplet.) The complete NMR data will be available in a subsequent publication.

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Addition of a 30-fold excess of sonicated and dialyzed calf thymus DNA to $[Ru(bpy)_2]_2(dpb)^{4+}$ and $[Ru(NH_3)_4]_2(dpb)^{4+}$ in buffer I causes dramatically different changes in the absorption spectra of the two complexes. The absorption of DNA was removed by adding equal amounts of the DNA solution to both the sample and reference cells. The $p\pi(dpb) \rightarrow p\pi^*(dpb)$ band at 317 nm of $[Ru(NH_3)_4]_2(dpb)^{4+}$ shows a percent hypochromicity (defined as $(A_{free} - A_{bound})/A_{free}$) of 29.0% and a bathochromic shift of 4 nm, which are consistent with the binding of this complex to DNA. Since the maximum hypochromism is observed for the dpb-localized band, the binding probably involves insertion of the benzo[*g*]quinoxaline moiety either into one of the grooves or between two base pairs. The sizes of these bathochromic shifts are typical for these types of transitions.[3b,8-10] The $[Ru(bpy)_2]_2(dpb)^{4+}$ complex shows no significant spectral changes, indicating that this complex binds weakly if at all.

The discrimination of DNA between $[Ru(NH_3)_4]_2(dpb)^{4+}$ and $[Ru(bpy)_2]_2(dpb)^{4+}$ is striking and is probably due to the difference in the sizes of the ligand extending parallel to the benzo[*g*]-quinoxaline moiety. Examination of molecular models suggests that the difference in size between ammonia and pyridine is sufficient to prevent significant interaction of the benzo[*g*]-quinoxaline moiety with DNA in the latter case. The ammonia groups may also increase the strength of the interaction due to hydrogen bonding, as in the case of $[Co(NH_3)_6]^{3+}$ binding to $(dC\text{-}dG)_3$.[17] The steric bulk of the spectator ligands has been observed to have an important effect in $[Ru(phen)_3]^{2+}$-type complexes, where the fit into the major groove was apparently an important feature of the interaction.[3b] It is likely that $[Ru(NH_3)_4]_2(dpb)^{4+}$ also has to fit in the major groove.

Initial results for the direct titration of $[Ru(NH_3)_4]_2(dpb)^{4+}$ with DNA yielded the data shown in Figure 2. These data were fit to the following function, which was first developed by Bard:[4,18]

$$C_b = \frac{1}{2K}(b - (b^2 - 2K^2C_t[DNA]/s)^{1/2})$$

$$b = 1 + KC_t + \frac{K[DNA]}{2s}$$

where C_b is the concentration of the bound complex, K is the microscopic binding constant for each site, C_t is the total metal complex concentration, and s is the site size in base pairs. This treatment is valid for cases of noncooperative, nonspecific binding to DNA.[4] The values of K and s obtained from Marquart–Levichberg nonlinear least-squares analysis are 8.3×10^5 M^{-1} and 6 base pairs, respectively. These values are probably accurate to within 20%[18] and have been reproduced with two separate preparations of the complex and DNA. Fitting of the titration data to the McGhee–Von Hippel equation[19] yielded values for K of ca. 10^5 M^{-1}, but due to the error of the determination of the free complex concentration resulting from direct titration, the Bard approach gave more unique fits. The value of K is ca. 10-fold smaller than observed for dppz and phi containing complexes, although it should be emphasized that these latter values were determined in buffer solutions containing 50 mM Na^+ ion, while our measurements were done in solutions containing 115 mM Na^+ ion. We are in the process of determining the Na^+ ion dependence of K for $[Ru(NH_3)_4]_2(dpb)^{4+}$, but given the high charge of our complex, lowering the ionic strength of the buffer should increase K for this complex.

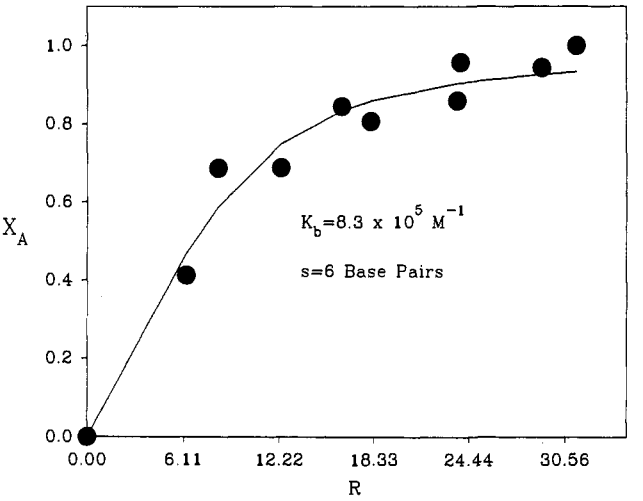

Figure 2. Plot of X_a ($=C_b/C_t$) vs R (= [DNA] in base pairs/C_t) for the titration of $[Ru(NH_3)_4]_2(dpb)^{4+}$ with DNA. Data points are shown as ●, and the nonlinear least-square fit of the data to the equation described in the text is shown as –.

The significant features of the interaction of $[Ru(NH_3)_4]_2(dpb)^{4+}$ with calf thymus DNA are the combination of the large binding site size and large binding constant and the rich potential for tailoring the molecular shape. By incorporating different spectator ligands, the architecture of the binding moiety can be refined to enhance specific interactions, particularly by unsymmetrical substitution of the spectator ligands, as in $[Ru(NH_3)_4(dpb)Ru(ethylenediamine)_2]^{4+}$. The binding site size of 6 for $[Ru(NH_3)_4]_2(dpb)^{4+}$ is consistent with the larger size of this complex and corresponds to more than 50% of one turn of the DNA helix. Further studies are underway to determine the isotherms, mode, and specificity of its interaction with DNA.

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