DNA Binding

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Temperature-Switched Binding of a Ru^{II}(dppz)/DNA Light-Switch Complex**

*Michael G. Walker, Veronica Gonzalez, Elena Chekmeneva, and Jim A Thomas**

Dipyridylphenazine (dppz)-based ruthenium complexes are extremely well studied DNA binding substrates. The prototypical system in such studies is the "DNA light-switch complex", $[Ru(N\text{-}N)_2(dppz)]^{2+}$ (N-N = 2,2′-bipyridyl, 1,10-phenanthroline; Figure 1). In aqueous solutions, the

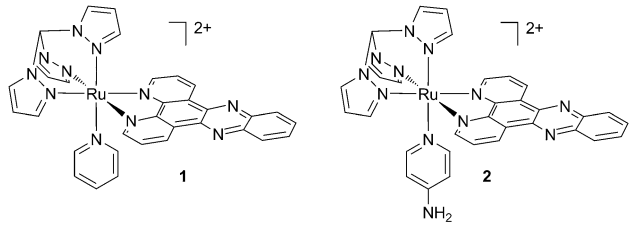

Figure 1. Ru^{II}(dppz)-based complexes.

^{3}MLCT-based emission of this chiral complex is only "switched on" when it intercalates into the duplex.[1] Numerous studies designed to identify the exact nature of the binding site of the complex, to outline the binding differences between its Λ and Δ enantiomers, and to delineate the photophysical details of its binding-induced optical response have been carried out.[1–3]

In order to facilitate the construction of mixed-motif and oligonuclear DNA binding substrates based on the Ru^{II}(dppz) moiety, we investigated the properties of achiral $[Ru(tpy)(L)(dppz)]^{2+}$ complexes (tpy = tris(pyrazolyl)methane, L = monodentate N-donor ligand).[4–8]

Recently, we demonstrated that the DNA binding properties of such systems are highly dependent on small changes in the structure of L; for example, while complex **1** is a typical light-switch DNA intercalator with high binding affinities for duplexes ($K_b > 10^6\,M^{-1}$), complex **2** shows reduced interaction with DNA.[7] Through experimental and computational studies, we ascribed this effect to unfavorable steric interactions involving the amino group of the pyridyl-based $pyNH_2$ ancillary ligand preventing intercalation of the dppz moiety. To explore this unusual behavior more fully, the thermodynamic interaction of **2** with calf thymus (CT)-DNA at 25 °C as well as 10 °C and 35 °C were initially investigated through isothermal calorimetry (ITC). To aid comparisons, the interaction of **1** with CT-DNA under the same conditions was also studied. Although we have previously reported an ITC study on the interaction of **1** with synthetic homopolymers,[4] this is the first study on genomic B-DNA.

At all three temperatures, complex **1** binds to CT-DNA through an endothermic, entropically driven interaction (see Table 1 and the Supporting Information). This kind of thermodynamic signature was seen for several other Ru^{II}-(dppz)-based metallointercalators; indeed the parameters at 25 °C are very similar to those reported for Λ-$[Ru(phen)_2(dppz)]^{2+}$ at this temperature ($\Delta H = 2.9$ kcal M^{-1}, $-T\Delta S = -11.4$ kcal M^{-1}).[9] In contrast, the temperature dependence for the interaction of **2** with CT-DNA is much more complex.

Table 1: ITC-derived CT-DNA binding parameters for complex **1** at selected temperatures.

	10 °C	25 °C	35 °C
ΔH [kcal M^{-1}]	3.67	2.82	1.50
$-T\Delta S$ [kcal M^{-1}]	−10.6	−9.36	−9.09
ΔG [kcal M^{-1}]	−6.93	−6.54	−7.60
S [bp]	2.3	4.9	3.8
K [M^{-1}]	1.2×10^5	7.4×10^4	2.3×10^5

Despite carrying out experiments at different concentrations of complex and/or DNA, the titration of **2** with CT-DNA at 25 °C only showed heat changes because of the dilution of the complex, thus indicating that the free energy of binding at this temperature is entirely due to favorable entropic changes. However, identical studies at other temperatures give contrasting results.

At 10 °C, **2** clearly interacts with CT-DNA through two binding modes (see the Supporting Information for ITC traces). The first few injections of **2** lead to a relatively high affinity interaction ($\Delta G = -7.18$ kcal M^{-1}; $K_b = 3.6\times10^5\,M^{-1}$), which is entirely enthalpically driven ($\Delta H = -9.96$ kcal M^{-1}) and actually displays an unfavorable entropic contribution ($-T\Delta S = 2.38$ kcal M^{-1}). The thermodynamic signature for this interaction is reminiscent of that observed for the initial binding mode of **1** with A-tract duplexes;[4] a second similarity is that the site size for the initial interaction with CT-DNA is also very large (18.7 bp). It has been previously suggested that such interactions are due to binding at the fraying ends and/or gaps of duplexes[4,10] and, given its thermodynamic signature, it seems likely that this mode involves the amino group of **2**

[*] M. G. Walker, Dr. V. Gonzalez, Dr. E. Chekmeneva, Dr. J. A. Thomas
Department of Chemistry, University of Sheffield
Sheffield, S3 7HF (UK)
E-mail: james.thomas@sheffield.ac.uk
Dr. V. Gonzalez
School of Chemistry, University of Cardiff
Cardiff (UK)

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dppz = Dipyridylphenazine.

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making specific hydrogen-bonding contacts with base residues. The main DNA binding phase involving **2** possesses a very similar site size and a virtually identical thermodynamic signature to that observed for the intercalative interaction of **1** with CT-DNA at 10 °C, with the free energy being dominated by a favorable entropic contribution.

The large heat changes observed at 35 °C clearly confirm that **2** binds CT-DNA at this temperature. However, the fact that there are three phases of binding (see the Supporting Information prevented quantitative fits to all the data using the calorimeter software. Attempts to obtain more usable data by employing different concentrations and mixing ratios were also unsuccessful. Nevertheless, in some respects, the data obtained at 35 °C is consistent with those obtained at 10 °C: following some heat changes initiated by the initial injections (presumably these are again due to binding to a number of atypical sites) a main phase of lower affinity binding to duplex is observed. To obtain insight into the thermodynamics of this interaction, fits of this isotherm to a single site model were carried out; although this method does not provide the accuracy of a full fit, it allows a qualitative analysis of the thermodynamics of the interaction (Table 2). While the binding affinity for the interaction

Table 2: ITC-derived CT-DNA binding parameters for complex **2** at selected temperatures.

	10 °C	25 °C[a]	35 °C
ΔH [kcal м$^{-1}$]	3.17	–[a]	−2.33
$-T\Delta S$ [kcal м$^{-1}$]	−10.4	−6.86[b]	−4.03
ΔG [kcal м$^{-1}$]	−7.18	−6.86[b]	−6.36
S [bp]	2.7	1.3[c]	1.7
K [м$^{-1}$]	1.8×10^5	6.5×10^5[c]	3.2×10^4

[a] No detectable heat change observed. [b] Because the interaction at 25 °C produced no detectable heat change, this thermodynamic parameter was calculated from standard equations using K_b values obtained from previously reported optical titrations.[20] [c] Previously reported data obtained through optical titrations.

appears to be slightly lower than that obtained at 10 °C, a closer analysis reveals a very different thermodynamic signature, which is unique for such systems. The interaction now exhibits a favorable enthalpy ($\Delta H = -2.33$ kcal mol^{-1} ± 15 %) and a much reduced, but nonetheless still favorable entropy term ($T\Delta S \approx 4.03$ kcal mol^{-1}). This profile suggests the presence of favorable hydrogen bonding/van der Waals contacts between the amino group of **2** and suitable residues within the duplex groove, but in this case, the smaller site size shows that this interaction does not involve atypical sites within the duplex.

The differing effect of temperature on the binding characteristics of **1** and **2** is clearly seen in Figure 2; these plots also allow the changes in heat capacity (ΔC_p) to be calculated ($\Delta C_p = d\Delta H/dT$). For complex **1**, a ΔC_p of -84 cal mol^{-1} K^{-1}, a value which is comparable to those reported for other small molecule DNA intercalators; for example methylene blue, which has a strong structural resemblance to dppz, displays a ΔC_p of -78 cal mol^{-1} K^{-1}.[11] Negative ΔC_p values are associated with the release of water

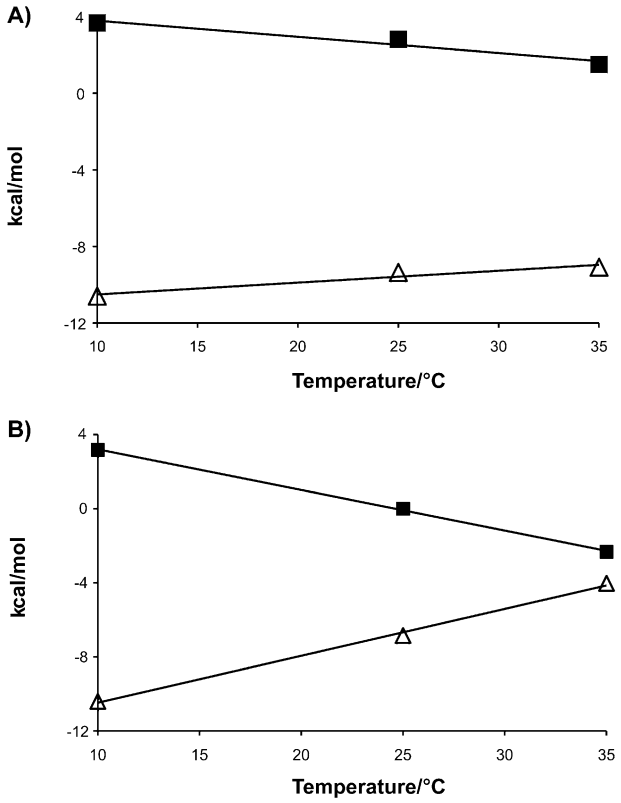

Figure 2. The variance of ΔH (■) and $-T\Delta S$ (△) with temperature for the interaction with CT-DNA of: A) [**1**]Cl_2 and B) [**2**]Cl_2.

during hydrophobic interactions and the free energy of hydration (ΔG_{hyd}) can be estimated by the relationship $\Delta G_{hyd} = 80\Delta C_p \pm 25\%$.[12] For **1**, this relationship gives $\Delta G_{hyd} = -6.7$ kcal mol^{-1}; again this value is typical for a hydrophobically driven intercalative interaction and is comparable with previously reported values for structurally related systems.[13]

In contrast, although the thermodynamic signature of **2** is very similar to that of **1** at 10 °C, and as for **1**, apparent enthalpy–entropy compensation is observed, the equivalent temperature-variance plots for the interaction of **2** with CT-DNA gives a steeper gradient (-219 cal mol^{-1} K^{-1}), indicating a much larger contribution from solvent release and hydrophobic effects.

Given the large temperature-induced changes in the DNA binding properties of **2** revealed by this analysis, and in particular similarities in the thermodynamic signatures of **1** and **2** at 10 °C, variable temperature–viscosity experiments were carried out.

DNA binding substrates have distinctive effects on the relative viscosity of aqueous DNA solutions, as they change the hydrodynamic length of duplexes: intercalators induce an increase in relative viscosity, whereas classical groove binders (such as netropsin), which do not lengthen the DNA helix, have no effect on relative viscosity.[14] In previous studies carried out at room temperature, we have established that although **1** is clearly an intercalator, **2** has no effect on the viscosity.[7] Surprisingly, when such studies were extended to

10 °C and 35 °C, it was found that viscosity changes induced by **2** are actually temperature dependent (Figure 3).

As expected, at 27 °C, while progressive addition of the intercalator ethidium bromide clearly induces progressive increases in relative viscosity, the orthodox groove binder H33258 produces very little change in the relative viscosity of

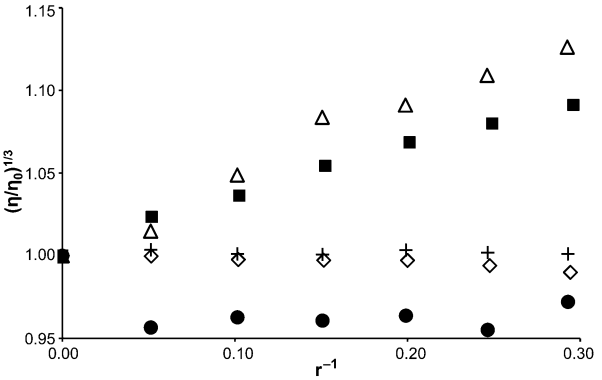

Figure 3. Plot of relative viscosity $(\eta/\eta_0)^{1/3}$ of CT-DNA versus r (r = [complex]/[DNA]) upon addition of [**2**]Cl_2 at 10 °C (△), 27 °C (+), and 35 °C (●), as well as the intercalator ethidium bromide (■) and the groove binder H33258 (◇), both at 27 °C. Conditions in all cases: tris buffer (5 mM tris(hydroxymethyl)aminomethane, 25 mM NaCl), pH 7.

CT-DNA, and, as observed previously under these conditions, **2** also produces no change in viscosity.

However, at 35 °C, addition of **2** led to a distinctive reduction in viscosity at low **2**:DNA ratios, followed by an slightly smaller increase at higher mixing ratios that brings the viscosity close to its initial value. This response is virtually identical to the changes observed when Δ-$[Ru(phen)_3]^{2+}$ is added to CT-DNA.[14,15] In this case, the effect is assigned to kinking of the duplex structure caused by partial insertion of a phen ligand of the complex bound to the minor groove. There is evidence that conventional metallointercalators can also bind DNA using this mode. For example, thermodynamic and kinetic studies on the DNA binding of *rac*-$[Ru(phen)_2(dppz)]^{2+}$ suggest that it binds by partial insertion at low complex loading.[16] Indeed, two recently reported X-ray structures of Λ-$[Ru(TAP)_2(dppz)]^{2+}$ and Λ-$[Ru(phen)_2(dppz)]^{2+}$ bound to duplex sequences reveal that although both complexes intercalate through the minor groove, they also bind through partial insertion.[17,18] Given these observations, and the thermodynamics and viscosity experiments at 35 °C, it seems highly likely that **2** binds to DNA in this manner. Because the dppz ligand contains the largest extended aromatic surface within the complex, it seems likely that this is the partially inserted moiety; certainly, NMR studies on an organic cation, which is structurally related to the dppz ligand, have also confirmed that it can bind to short duplex sequences through partial insertion.[19] Alternatively, partial insertion of the $pyNH_2$ ligand would allow it to make hydrogen bond contacts with base residues, and this would explain the distinctive thermodynamic profile of binding at 35 °C revealed by the ITC data.

A very different viscosity response is observed at 10 °C. At this temperature, progressive addition of **2** now causes large increases in relative viscosity; indeed, the increases are larger than those induced by ethidium bromide at 27 °C. This is a priori evidence that **3** is an intercalator at 10 °C. However, to confirm this hypothesis, the effect of temperature on the optical response of **3** to DNA binding was also investigated.

Again as reported previously,[7] aqueous solutions of **2** remain entirely non-emissive at 25 °C, even after the addition of large excesses of CT-DNA; the same lack of response was also observed at 35 °C. However, at 10 °C the complex displays an obvious light-switch effect (Figure 4), thus demonstrating that the dppz unit of the complex is intercalated into an environment that is less accessible to solvents.

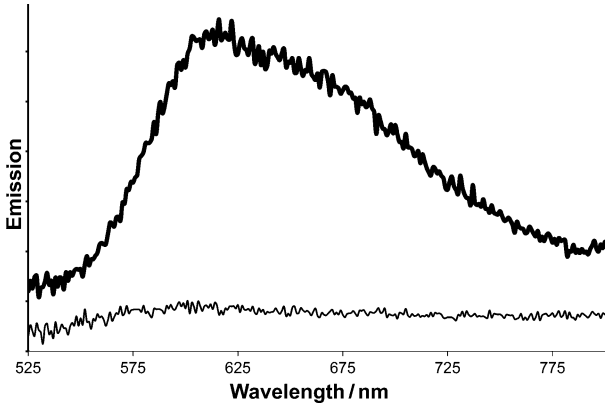

Figure 4. Luminescence of [**2**]Cl_2 at 10 °C in the absence (fine line) and presence of CT-DNA (bold line).

Uniquely, complex **2** displays path-dependent hysteresis in its DNA binding properties: if it is intercalated into duplex at 10 °C and then warmed to 25 °C, the intensity of its luminescence is lowered, but it does not "switch off"; indeed, even after heating to 35 °C for over two hours, luminescence is clearly detectable (see the Supporting Information). These observations demonstrate that, once bound at 10 °C, the complex is capable of staying intercalated even at elevated temperatures. To the best of our knowledge, this is the first time such a phenomenon has been observed.

Taken together, the ITC, viscosity, and luminescence data all point to the same conclusion: unprecedentedly, the DNA binding properties of complex **2** are temperature dependent. At room temperature, the complex interacts with DNA duplexes through groove binding, leading to partial insertion at biologically relevant temperatures; at lower temperature its main binding mode is through intercalation, which induces the expected light-switch effect. The basis of this phenomenon may be due to kinetic or thermodynamic factors, or a combination of both. For example, favorable hydrogen bonding/van der Waals contacts possess an activation barrier, as participating residues must first be desolvated;[20] these effects would disfavor the groove binding mode at lower temperatures. Furthermore, recent studies on DNA persistence lengths and base pair openings have confirmed that, at premelting temperatures of 25 °C and above, duplex defor-

mation and flexibility is enhanced.[21] Again, such effects would destabilize stacking interactions and favor groove binding. Future studies aimed at delineating the precise molecular details of this fascinating phenomenon are under way.

This present work underlines how the binding properties of light-switch systems can be fundamentally changed by small structural changes, and shows the possibility that sequence-selective light-switch systems may be designed through a similar approach.

Experimental Section

Viscosity experiments were performed on a 1 mL Cannon-Manning semi-micro viscometer (size 50) immersed in a thermostated water bath. The temperature was maintained at the desired temperature (± 0.1 °C). The concentration of CT-DNA in the viscometer was kept at ≈ 50 μM bp^{-1}. Additions of the ligand were made so that the values of $1/R$ (R = [DNA]/[ligand]) were between 0 and 0.3. An equilibration time of 20 min was allowed before the flow times (time taken for the solution to pass through the capillary tube) were recorded. Times were recorded in triplicate to within 0.1 second of each other, and averaged.

Details of the synthesis of **1** and **2**, and descriptions of all other instrumentation and methods used in this study have been published previously.[5,7,8]

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