DNA Binding of Semi-rigid Binuclear Ruthenium Complex Δ,Δ-[μ-(11,11'-bidppz)(phen)$_4$Ru$_2$]$^{4+}$: Extremely Slow Intercalation Kinetics

L. Marcus Wilhelmsson, Fredrik Westerlund, Per Lincoln, and Bengt Nordén*

Department of Physical Chemistry, Chalmers University of Technology, S-41296 Gothenburg, Sweden

RECEIVED DATE (will be automatically inserted after manuscript is accepted)

DNA-binding small molecules have since long attracted interest because of their interference with important mechanisms in the cell, some inducing mutations and cancer while others have found use as cancer therapeutics. To learn more about the molecular mechanisms of DNA binding in general and the prerequisites for specificity in particular, various bulky and sterically constrained molecules have been studied, including chiral substitution-inert transition metal compounds,[1] such as the complex [Ru(phen)$_2$dppz]$^{2+}$ (phen=1,10-phenanthroline; dppz=dipyrido- [3,2-a:2',3'-c]phenazine)[2] which binds to DNA by intercalating the dppz moiety between the base pairs of the DNA.[2a,3] In order to increase specificity, including chiral discrimination, and to decrease the dissociation rate, which is considered important for antitumor activity,[4] one approach has been to make dimeric compounds. For a bis-intercalating dimer with a flexible linker ([μ-c4(cpdppz)$_2$(phen)$_4$Ru$_2$]$^{4+}$), it was indeed shown that the dissociation rate was reduced by several orders of magnitude,[5] as an effect of the complex being threaded through DNA in a way that requires a huge, transient conformational change to occur in the DNA structure.[1c] One class of [Ru(phen)$_2$dppz]$^{2+}$ dimers reported on earlier, and to which the present [μ-(11,11'-bidppz)(phen)$_4$Ru$_2$]$^{4+}$ (Figure 1) belongs, has been found to display very high binding affinity for DNA and to show marked variations in their binding geometry to DNA depending on enantiomeric form (Δ,Δ or Λ,Λ).[6]

We here report the observation of an extremely slow rearrangement of the initial DNA binding mode of the complex Δ,Δ-[μ-(11,11'-bidppz)(phen)$_4$Ru$_2$]$^{4+}$ (11,11'-bidppz=11,11'-bi(dipyrido[3,2-a:2',3'-c]phenazinyl)) (**1**), earlier assigned as a groove-bound mode on the evidence of the positive linear dichroism, lack of fluorescence and modest hypochromism upon DNA-binding.[6] A serendipitous observation that a sample of calf thymus (ct) DNA and **1** that had been left for two weeks at room temperature, surprisingly showed a change of LD from positive to negative indicating a change of binding geometry. Further studies by CD indicated that the integrity of the complex was intact. In order to be able to examine the binding process more efficiently it was accelerated by adding salt and raising the temperature.[5]

Figure 2 showing the flow linear dichroism[7] (LD) spectra of **1** in presence of ct-DNA, recorded at different times after mixing,[8] unambiguously reveals that a major change in binding geometry occurs. Despite elevated temperature (45°C) and high salt concentration (100 mM Na^+), equilibrium takes almost one day to reach. For comparison the DNA-binding of the bis-intercalating Δ,Δ-[Ru(phen)$_2$dppz]$^{2+}$-dimer ([μ-c4(cpdppz)$_2$(phen)$_4$Ru$_2$]$^{4+}$), is complete after half an hour at room temperature and in 100 mM Na^+.[5] We propose the complex is rearranged from a groove-binding to an intercalative geometry which final binding mode is reached by threading one of the Ru(phen)$_2$ moieties through the DNA duplex, thereby intercalating one of the bridging dppz

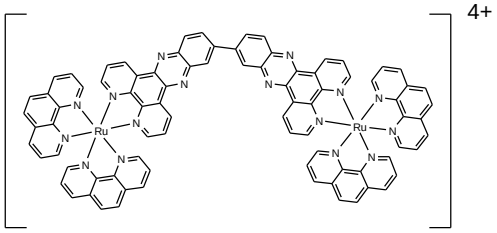


Figure 1. (**1**) Δ,Δ-[μ-(11,11'-bidppz)(phen)$_4$Ru$_2$]$^{4+}$; phen=1,10-phenanthroline, 11,11'-bidppz=11,11'-bi(dipyrido[3,2-a:2',3'-c]phenazinyl).

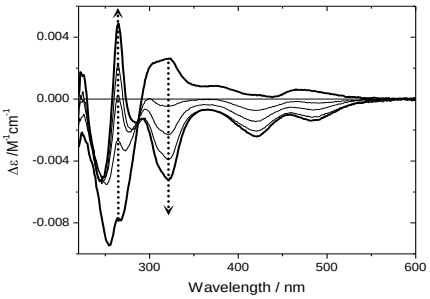


Figure 2. Flow linear dichroism spectra of 10 μM **1** and 160 μM calf thymus DNA (P/Ru-ratio[9] of 8) at different times after mixing (100 mM Na^+, 1 mM sodium cacodylate, pH 7). The two distinct spectra are the start and final ones and the arrows specify spectral change with time (0, 1, 2, 4 and 16 h of storage at 45°C). The arrow at 320 nm indicates the change at the wavelength where the transition moment originating from the bidppz-ligand is dominating and the arrow at approximately 270 nm illustrate the change where the transition of the phenanthroline-ligands makes the largest contribution. Experiments performed at room temperature.

ligands between the DNA base pairs and thus placing one metal center in each groove.

An absorption peak at 320 nm which originates from a $\pi \rightarrow \pi^*$ transition polarized parallel to the long-axis of the bidppz ligand has been used to estimate the angle between this direction in **1** and the DNA helix axis to be about 70°,[10] an angle though deviating from 90° still consistent with intercalation when regarding the fact that not both dppz moieties have to be aligned parallel with the bases. The isosbestic point at 287 nm (Figure 2) and the result of a singular-value-decomposition analysis of the LD-spectra (not shown) indicate that two binding geometries are enough to accurately describe the spectral variations: an initial groove-bound and a final intercalated geometry. This hypothesis is consistent with the observation that the complex dissociates almost instantaneously from DNA from the initial binding mode upon addition of SDS, while it needs several days at 45°C to dissociate from its final binding mode (see Supporting Information S1).

Both the rearrangement between the two DNA binding modes of **1** and the subsequent SDS-induced dissociation[11] from its final DNA binding mode were followed by observing the change in fluorescence of the complex. The processes are extremely slow even at elevated temperatures (Figure 3). The kinetic data for the

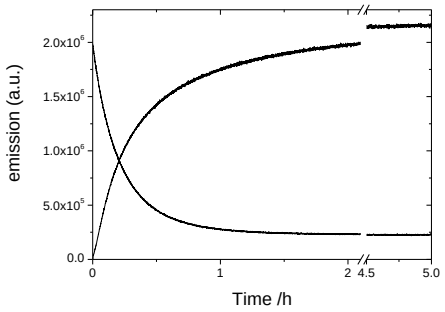


Figure 3. Rearrangement (emission build up curve) of **1** bound to calf thymus DNA and also dissociation (emission decay curve) of the complex from its final DNA binding mode. A solution of 10 μM **1** and 160 μM ([bases]) calf thymus DNA was used. For the dissociation, SDS was added to a final concentration of 0.6 %. The experiments performed at 50°C in a 1 mM sodium cacodylate buffer (100 mM Na^+, pH 7).

Table 1. Arrhenius parameters for rearrangement of DNA binding geometry and DNA dissociation from the final binding mode of **1**.

	$\ln A_1$	E_{a1} (kJ/mol)	$\ln A_2$	E_{a2} (kJ/mol)
Rearrangement	28.3	94.0	22.3	83.0
Dissociation	17.9	65.1	17.5	66.4

Table 2. Luminescence properties of **1** in the presence of various types of DNA (P/Ru-ratio of 8). Included are also the luminescence properties of Δ-$[Ru(phen)_2dppz]^{2+}$ in poly(dA-dT)$_2$.

	calf thymus	poly(dA-dT)$_2$		Δ-$[Ru(phen)_2dppz]^{2+}$
ϕ^a	0.02	0.09		0.07
τ_1/ns[b]	406 (0.19)	707 (0.79)		756 (0.56) [2e]
τ_2/ns	156 (0.44)	131 (0.21)		129 (0.44) [2e]
τ_3/ns	36 (0.37)			

[a] Luminescence quantum yield. [b] Excited state lifetimes (τ) and in parenthesis normalized pre-exponential factors reflecting mole fractions of the different luminescing species at t=0 (directly after illumination).

two processes obtained at four different temperatures had to be fitted by a double exponential function ($C_1\exp(-k_1t)+C_2\exp(-k_2t)$) in order to get a good fit. It is not yet clear whether this biphasic intercalation kinetics is due to sequence or ligand-distribution heterogeneity. The rate constants (see Supporting Information S2) were used to estimate the Arrhenius parameters for the processes (Table 1). The high activation energies agree well with the proposed intercalative final binding mode. In order to rearrange or dissociate, **1** obviously has to thread one of the $Ru(phen)_2$ moieties through the DNA duplex, which requires the unstacking and base pair opening of at least one base pair. Indeed, our activation energies (Table 1) are in reasonable agreement with those experimentally determined for base pair openings (AT: 71-80 kJ/mol[12], GC: 69-88 kJ/mol[12]). However, it should be noted that according to these data, the SDS-induced dissociation rate is faster than the rearrangement rate, which is inconsistent with the final species being thermodynamically more stable. A plausible explanation is that, contrary to what has been found previously for small DNA binding dyes,[13] but supported by preliminary experiments in our laboratory, SDS monomers can catalyze the DNA dissociation process of hydrophobic cations.

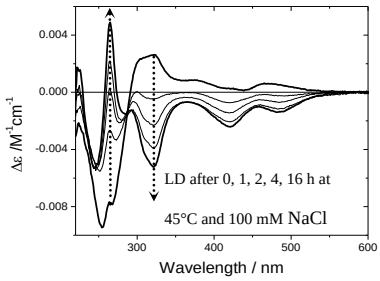
That the final binding mode in DNA is of intercalative nature is further supported by luminescence measurements (Table 2).[14] The luminescence quantum yield of **1** in its final binding mode in ct-DNA is approximately 1500 times higher than that found for the complex free in water solution. This quantum yield increase is of the same order of magnitude as that established for the intercalated ruthenium monomer under the same conditions. A higher quantum yield when the complex is bound to poly(dA-dT)$_2$ suggests that the aza-nitrogens of the bidppz ligand are more protected from water quenching in this case than in the mixed sequence DNA. The similar lifetimes observed for **1** in this case and those found for the ruthenium monomer (Table 2) under the same conditions[2e] are striking, indeed supporting our conclusions that **1** is intercalated in its final binding state.

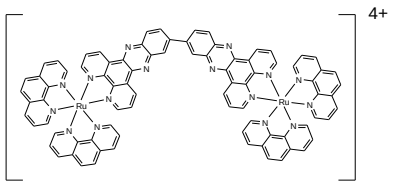
Remarkable properties of **1** are its extremely slow reorganization from an initial to a final binding mode upon interaction with DNA and the very slow dissociation of the complex whether with ct-DNA or poly(dA-dT)$_2$. That intercalation is being reached much faster for the closely related $[\mu\text{-c4(cpdppz)}_2(phen)_4Ru_2]^{4+}$ complex compared to **1** is most likely due to the more flexible linker between the ruthenium centra. The slowness of both processes is probably due to that the final binding mode is intercalative and that the threading of the complex through DNA, which is required to reach intercalation, is sterically hindered. The unique slow dissociation of **1** in its final DNA-binding mode suggests that this class of threading, partially intercalated, binuclear complexes may be interesting in the context of cancer therapy. Also, their unique optical and photophysical properties could make such complexes, either alone or scaffolded by DNA structures, of interest for the development of nanometer-sized molecular opto-electronic devices.[15]

Supporting Information Available: Dissociation from the two different DNA binding modes of **1** studied by linear dichroism; temperature dependence of rates of DNA binding mode rearrangement and DNA dissociation for **1**.

* Corresponding author. Telephone: +46-(0)31-7723041. Fax: +46-(0)31-7723858. E-mail: norden@phc.chalmers.se

(1) (a) Nordén, B.; Lincoln, P.; Åkerman, B.; Tuite, E. In *Metal Ions In Biological Systems*; Siegel, A., Siegel, H., Eds.; Marcel Dekker, Inc: New York, Basel, Hong Kong, 1996; Vol. 33, pp 177-252. (b) Lincoln, P.; Nordén, B. *J. Phys. Chem. B* 1998, *102*, 9583-9594. (c) Önfelt, B.; Lincoln, P.; Nordén, B. *J. Am. Chem. Soc.* 1999, *121*, 10846-10847. (d) Chow, C. S.; Barton, J. K. *Methods Enzymol* 1992, *212*, 219-242. (e) Pyle, A. M.; Barton, J. K. *Prog. Inorganic Chem.* 1990, *38*, 413-475. (f) Balzani, V.; Ballardini, R. *Photochem. Photobiol.* 1990, *52*, 409-416.
(2) (a) Friedman, A. E.; Chambron, J.-C.; Sauvage, J.-P.; Turro, N. J.; Barton, J. K. *J. Am. Chem. Soc.* 1990, *112*, 4960-4962. (b) Friedman, A. E.; Kumar, C. V.; Turro, N. J.; Barton, J. K. *Nucleic Acids Res.* 1991, *19*, 2595-2602. (c) Jenkins, Y.; Friedman, A. E.; Turro, N. J.; Barton, J. K. *Biochemistry* 1992, *31*, 10809-10816. (d) Lincoln, P.; Broo, A.; Nordén, B. *J. Am. Chem. Soc.* 1996, *118*, 2644-2653. (e) Tuite, E.; Lincoln, P.; Nordén, B. *J. Am. Chem. Soc.* 1997, *119*, 239-240.
(3) Hiort, C; Lincoln, P.; Nordén, B. *J. Am. Chem. Soc.* 1993, *115*, 3448-3454.
(4) Muller, W.; Crothers, D. M. *J. Mol. Biol.* 1968, *35*, 251-290.
(5) Önfelt, B.; Lincoln, P.; Nordén, B. *J. Am. Chem. Soc.* 2001, *123*, 3630-3637.
(6) Lincoln, P; Nordén, B. *Chem. Commun.* 1996, 2145-2146.
(7) Nordén, B.; Kubista, M.; Kurucsev, T. *Q. Rev. Biophys.* 1992, *25,* 51-170.
(8) A buffered solution (1 mM cacodylate, pH 7, 100 mM Na^+) of 10 μM **1** and 160 μM ([bases]) CT DNA was used in the experiments performed on a Jasco J-700 spectrodichrometer at room temperature.
(9) The P/Ru-value is a DNA to ligand ratio, where P is the concentration of phosphates (DNA-bases) and Ru is the concentration of ruthenium-centra.
(10) The value was arrived at by comparing the LD^r-values ($LD^r(\lambda)=LD(\lambda)/A_{iso}(\lambda)$=reduced linear dichroism) of free unperturbed ct-DNA and the same DNA with added **1** (P/Ru=8). In the calculation it has been assumed that the bases are directed perpendicularly (90°) to the DNA helix axis, i.e. the direction of orientation, and not changed during the experiment.
(11) Dissociation of **1** from ct-DNA was studied using the detergent-sequestration technique. SDS in a 1 mM sodium cacodylate buffer (100 mM Na^+, pH 7) was added to a pre-equilibrated (24 h at 50°C) solution of ct-DNA and **1** to an SDS concentration of 0.6 %.
(12) (a) Leijon, M.; Sehlstedt, U.; Nielsen, P. E.; Graslund, A. *J. Mol. Biol.* 1997, *271*, 438-455. (b) Moe, J. G.; Russu, I. M. *Biochemistry*, 1992, *31*, 8421-8428.
(13) Wilson, W. D.; Krishnamoorthy, C. R.; Wang, Y.-H.; Smith, J. C. *Biopolymers* 1985, *24*, 1941-1961.
(14) Both the luminescence quantum yield measurements and the time-resolved measurements were performed on a SPEX fluorolog τ2 spectrofluorimeter at room temperature. The time-resolved measurements were carried out using the phase shift and modulation technique. The various DNAs and **1** were mixed at a P/Ru-ratio of 8 in a 1 mM cacodylate buffer (pH 7, 100 mM Na^+). To reach the final binding mode, all solutions were pre-equilibrated at 50°C over night.
(15) Staffilani, M.; Belser, P.; De Cola, L.; Hartl, F. *Eur. J. Inorg. Chem.* 2002, 335-339.





Abstract: We here report a remarkably slow rearrangement of binding modes for a binuclear ruthenium(II) complex upon interaction with DNA. It has been previously shown that Δ,Δ-[μ-(11,11'-bidppz)$(phen)_4Ru_2]^{4+}$ binds to DNA in one of the grooves. However, we find that this is only an initial, meta-stable, binding mode, which is extremely slowly reorganized into an intercalative binding geometry. The slow rearrangement and dissociation, revealed by flow linear dichroism and fluorescence spectroscopy, are concluded to be a result from the complex being threaded through the DNA, with one of the bridging aromatic dppz ligands intercalated between the base pairs of the DNA placing one metal center in the minor groove and one in the major groove. A negative LD, a high luminescence quantum yield and long luminescence lifetimes, similar to the intercalating complex Δ–$[Ru(phen)_2dppz]^{2+}$, indicate intercalation of the bidppz moiety. The unique slow dissociation of the complex in its final DNA-binding mode suggests that this class of threading, partially intercalated binuclear complexes may be interesting in the context of cancer therapy. Also, their unique optical and photophysical properties could make such complexes, either alone or scaffolded by DNA structures, of interest for the development of nanometer-sized molecular opto-electronic devices.