A Molecular Staple for DNA: Threading Bis-intercalating $[Ru(phen)_2dppz]^{2+}$ Dimer

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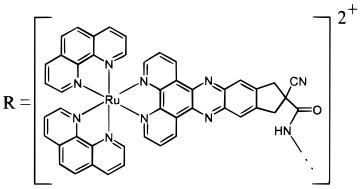
Among the attempts to develop drugs that form strong DNA complexes with slow dissociation rates, properties that are believed important for antitumor potency, one approach is to link several DNA-intercalating subunits together to form multi-intercalating ligands.[1−3] For example, Iverson et al. recently reported on a series of polyintercalating molecules, constructed by connecting several naphthalenetetracarboxylic diimide chromophores in a head-to-tail arrangement with flexible peptide linkers, which show extremely slow dissociation rates from $[poly(dG\text{-}dC)]_2$.[4] We here report a novel diruthenium complex with unique binding geometric and dissociation mechanistic properties relative to DNA. The complex **1**, $[\mu\text{-c4(cpdppz)}_2-(phen)_4Ru_2]^{4+}$,[5] consists of two units of the extensively studied chiral mono-intercalator $[Ru(phen)_2dppz]^{2+}$,[6] tethered to each other via the dppz moieties and an aliphatic diamide linker. We have synthesized the three stereoisomers of **1** and characterized their binding to DNA. Earlier we reported on a semirigid dimer of $Ru[(phen)_2dppz]^{2+}-[\mu\text{-(dppz-(11−11′)dppz)}-(phen)_4Ru_2]^{4+}$, in which the dppz moieties are connected by a single bond—whose enantiomers were found to bind nonintercalatively to DNA.[7] By contrast, as will be concluded, all three stereoisomers of **1** bind to B-DNA by intercalating both of their dppz moieties between nucleobases with a two base-pairs separation.

We have used linear dichroism (LD) spectroscopy to characterize the angular binding geometry of **1** bound to flow-oriented calf thymus DNA. A detailed spectroscopical analysis of $[Ru(phen)_2dppz]^{2+}$ bound to DNA has revealed that, although the visible spectral range comprises a multitude of overlapping electronic transitions, two transition moment directions dominate:[6e] One (*A*) polarized along the dppz ligand long axis, and one ($B_{(E)}$) polarized perpendicular to the latter and along the line connecting the middle rings of the phen-ligands. The *A* polarized transitions dominate at 480 and 380 nm, the $B_{(E)}$ transitions around 420 nm. Since the $B_{(E)}$ polarization makes an oblique angle to the molecular plane of the dppz ligand, it constitutes a sensitive indicator of intercalation geometry due to the $\cos^2$ dependence of the LD on the angle between the transition moment and the DNA helix axis. Thus, the dramatically different LD spectra between enantiomeric forms of $[Ru(phen)_2dppz]^{2+}$ and congeners intercalated into DNA can be quantitatively explained by a small deviation (common to both enantiomers) from the idealized intercalation geometry: a clock-wise rotation by about 10° around the dppz long axis.

1 = R-CH_2- CH_2- CH_2- CH_2-R

2 = R-CH_2- CH_2- CH_2- CH_2-NH_3^+



LD spectra[8] are shown in Figure 1 for the three stereoisomers, as well as for the enantiomers of the corresponding monomeric chromophore **2**, ($[Ru(phen)_2cpdppzC4NH_2]^{2+}$). The shapes of the spectra immediately indicate that the chromophoric subunits of the Δ,Δ and Λ,Λ dimers bind with orientations very similar to those of the respective isolated Δ and Λ monomers. The LD spectra further show good agreement with those previously obtained for Δ and Λ $[Ru(phen)_2dppz]^{2+}$ intercalated into calf thymus DNA,[6e] indicating a bis-intercalative binding mode for the three stereoisomers of **1**. A striking observation is that the experimentally obtained LD spectrum of the meso (Δ,Λ) stereoisomer bound to DNA could be reproduced as the arithmetic mean of the LD spectra of the two opposite enantiomers (Δ,Δ and Λ,Λ). This result suggests that the binding geometry of each subunit depend solely upon its absolute configuration and not upon any diastereomeric intramolecular interactions between the $Ru(phen)_2$ moieties.

For each stereoisomer, the shape of the LD spectrum was practically identical, whether obtained at different P/Ru-values[9] (20 and 40) or at different salt concentrations (10 and 200 mM NaCl) (not shown), indicating each of the binding geometries to be independent of both salt concentration and binding density.

The two homochiral stereoisomers of **1** show luminescence properties very similar to those of the appropriate parent $[Ru(phen)_2dppz]^{2+}$ enantiomer when bound to DNA[10] (unlike the nonintercalated semirigid 11,11′-bidppz binuclear complex, which

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(5) Abbreviations: $c4(cpdppz)_2$ = *N*,*N*′-bis(12-cyano-12,13-dihydro-11H-cyclopenta[*b*]dipyrido[3,2-*h*:2′,3′-*j*]phenazine-12-carbonyl)-1,4-diaminobutane; phen = 1,10-phenanthroline; dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine; $cpdppzC4NH_2$ = *N*-(12-cyano-12,13-dihydro-11H-cyclopenta[*b*]dipyrido[3,2-*h*:2′,3′-*j*]phenazine-12-carbonyl)-1,4-diaminobutane; SDS = sodium dodecylsulphate.

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(8) Linear dichroism (LD) is defined as the difference in absorbance of light linearly polarized parallel and perpendicular to a macroscopic axis of orientation (the flow direction); $LD(\lambda) = A_{\parallel}(\lambda) - A_{\perp}(\lambda)$. Samples of ruthenium complex and calf thymus DNA were oriented in flow gradient (700 rpm) in a Couette flow cell with an outer rotating cylinder and LD spectra measured on a Jasco J-500 spectrodichrometer. The orientation factor *S* (which can take values between 0 (isotropic sample) and 1 (perfectly oriented sample)) was calculated by comparing LD-spectra of DNA in the presence of Ru-complex at two different P/Ru values, and free DNA. (Lincoln, P., Ph.D. Thesis, Chalmers University of Technology, Gothenburg, 1998).

(9) The DNA to ligand ratio in the experiments is given by the ratio P/Ru, where P is the concentration of DNA phosphate (=[DNA bases]) and Ru the concentration of $Ru(phen)_2dppz$ *subunits*. Note that for a nearest neighbor-exclusion model, saturation of the DNA occurs at P/Ru = 4 for both **1** and **2**.

(10) Lincoln, P.; Önfelt, B.; Nordén, B., manuscript in preparation.

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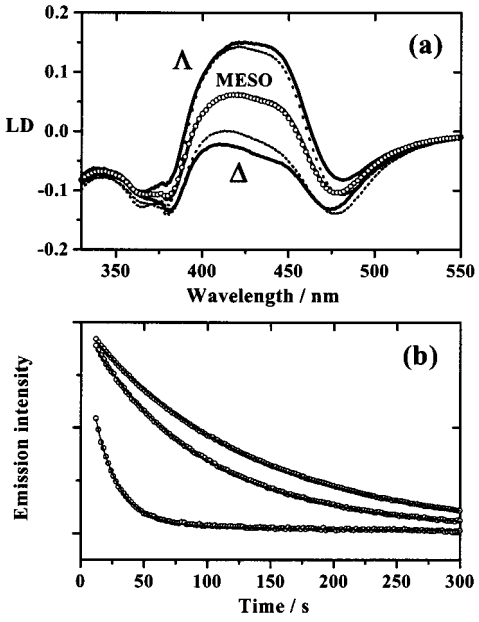

Figure 1. (a) Linear dichroism (LD) spectra of the three stereoisomers of **1** (solid curves) and the enantiomers of the corresponding monomer **2** (dotted curves), bound to flow-oriented calf thymus DNA in 10 mM NaCl. The experimental spectrum for the *meso* complex shows excellent agreement with the arithmetic mean (as [(Δ,Δ)+(Λ,Λ)]/2, opened circles) of the (Δ,Δ)- and (Λ,Λ) spectra. Ru concentration was 10 μM and the DNA concentration 400 μM nucleotides. The spectra were normalized with respect to the LD orientation factor, S, of DNA.[8] (b) Normalized emission intensity as a function of time upon addition of SDS to the three stereoisomers of **1** bound to [poly(dA-dT)]$_2$.[11] From top to bottom: (Δ,Δ), (Δ,Λ), (Λ,Λ) complexes. Solid curves are least-squares best fits to monoexponential decays, see text. Temperature was 20 °C and salt concentration 100 mM NaCl. The concentration of **1** was 2 μM, and the P/Ru-ratio was 38.5.

shows no luminescence upon binding to DNA),[7] affording further support for identical intercalative binding modes, i.e., bis-intercalation for **1**. Binding titrations (Supporting Information) indicate that the size of the binding site is about four base pairs, consistent with a nearest-neighbor-exclusion binding model, in which the two intercalated dppz subunits of **1** are separated by two base pairs.

Molecular modeling confirms that the linker is long enough to allow such a bis-intercalated binding conformation; however, restricting the two Ru(phen)$_2$-moieties to be bound in the same groove (see Supporting Information for a molecular model). This type of binding can be envisaged to give a very rigid structure in which **1** acts as a staple, holding the bases firmly stacked together.

A most interesting topological conclusion regarding the binding mode of **1** is that formation of the complex must involve threading: either the Ru(phen)$_2$ moieties must pass through the DNA strands to reach their final positions or the linker has to sling itself around opening base-pairs. Also the binding of **2** must involve threading through DNA of either the Ru(phen)$_2$ moiety or (more probably) the butylamine chain. The parent monomer [Ru(phen)$_2$dppz]$^{2+}$ can be expected to more easily reach its intercalated binding geometry by just sliding its dppz-ligand between the base pairs without further perturbing the DNA structure. By contrast, for **1** to enter or exit its bis-intercalative binding site, the DNA is likely to undergo a much larger (transient) conformational reorganization. If such a conformational change is rate-limiting, we would expect slow association and, in particular, extremely slow dissociation rates.

Table 1. Dissociation Rate Constants of **1** from Calf Thymus DNA at Varying Ionic Strengths

compd	[NaCl]/mM	$10^3 \times k_1/s^{-1}$	amplitude (%)	$10^3 \times k_2/s^{-1}$	amplitude (%)
1 (meso)	10	0.5	16	0.08	84
1 (meso)	50	2.9	51	0.8	49
1 (meso)	100	6.4	76	1.5	24
1 (meso)	200	12.1	48	3.3	52

The P/Ru value was 40 in all cases. Typical error levels are 15% for the amplitudes and 15% for the rate constants.

The dissociation of the three stereoisomers of **1** bound to [poly(dA-dT)]$_2$ at 20 °C (100 mM NaCl), upon addition of SDS,[11] was followed by luminescence spectroscopy and indeed found to be remarkably slow (Figure 1b). The decay curves from [poly(dA-dT)]$_2$ were monoexponential, with dissociation rate constants being 7.3×10^{-3} s^{-1} for (Δ,Δ), 42×10^{-3} s^{-1} for (Λ,Λ) and 10×10^{-3} s^{-1} for the meso form of **1**. Also, the dissociation of the Δ enantiomers of **2** and [Ru(phen)$_2$dppz]$^{2+}$ (measured under similar conditions using stopped flow[12]) were monoexponential, giving rate constants 7.0×10^{-2} s^{-1} for **2** and 2.0 s^{-1} for [Ru(phen)$_2$dppz]$^{2+}$. The monoexponential decay indicates that, at equilibrium, for each compound there is only one effective form of binding. Comparison of the dissociation rates clearly shows that the threading of **2** and in particular that of **1**, significantly slows down the dissociation process.

The dissociation of **1** from calf thymus DNA, over an extended ionic strength range, was found to always demand a two-exponential function to fit the decay curves (exemplified in Table 1 with the *meso* form). Interestingly, not only the rate constants but also the amplitudes depend strongly on the ionic strength. This complex kinetics observed with mixed sequence DNA indicates pronounced sequence selectivity for the dissociation of **1** from DNA.

These bis-intercalating ruthenium complexes represent a novel type of DNA-threading compounds that can provide not only new possibilities for the design of gene-targeting drugs but also mechanistic insight about the structure and dynamics of the DNA itself in solution. For example, the fact that these large molecules do bis-intercalate demonstrates that large amplitude conformational changes occur in DNA.

Supporting Information Available: Emission titration of **1** (Δ,Δ) with [poly(dG-dC)]$_2$. Molecular model of the binding of **1** to DNA. Scheme of synthesis of **1** and **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) The dissociation of **1** from DNA was studied with the detergent-sequestration technique. Sodium dodecyl sulphate (SDS), 3% in buffer, was added to an equilibrium mixture of Ru-complex and polynucleotide, to a final concentration of 0.6% SDS. The decay of emission intensity at 620 nm was monitored with a SPEX fluorolog τ2 fluorimeter in a thermostated cell at 20 °C. The time delay between mixing and collection of datapoints was typically 8 s.

(12) SDS, 1.2% in buffer, was mixed in equal proportions with an equilibrium mixture of **2** or [Ru(phen)$_2$dppz]$^{2+}$ ([Ru] = 2.5 μM) and poly(dA-dT)$_2$ ([P] = 100 μM) in a computer controlled stopped-flow instrument from Bio Logic. The sample was excited at 436 nm, and emission collected trough a 540 nm cutoff filter. Typically five spectra were averaged for each output file. The time delay between mixing and data collection (dead time) was 5.2 ms. Syringes, mixing chamber, and cell were held at constant temperature (20 ± 0.5 °C) by a water thermostat.