A dinuclear ruthenium(II) complex that functions as a label-free colorimetric sensor for DNA

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A dinuclear ruthenium(II) complex groove binds to DNA and this interaction results in distinctive color changes that are dependent on both DNA sequence and structure.

Systems capable of sensing small molecules and biomolecules are the target of much research.[1–3] In particular, due to possible applications in fields as diverse as forensics, basic medical research, and the identification of genetically based diseases including cancer, there is a burgeoning interest in methods of sensing nucleic acids.[4,5] While systems that sense specific sequences have received much attention, the possible role of four-stranded quadruplex structure in a number of biological processes and specific disease states[6] has meant that structurally specific nucleic acid probes are also beginning to be targeted.[7]

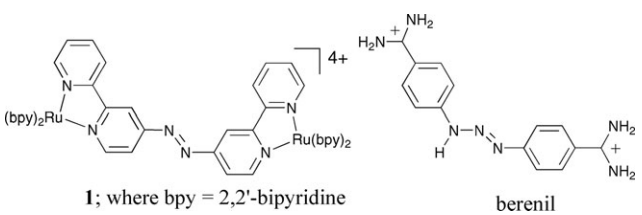
In many cases, colorimetric-based sensing is particularly attractive, as it allows naked eye detection of nucleic acids. By exploiting the optical properties of nanoparticles the Mirkin group,[8] and others,[9] have created hybrid oligonucleotide–gold nanoparticle conjugate systems that hybridize with a target sequence, showing distinctive colour changes as target sequence templated aggregation occurs. These systems—and conventional technologies[4,5]—all require chemically labeled oligonucleotides for sensing to occur. Recently Li, *et al.* reported that a hemin-G-quartet DNAzyme complex can act indirectly as a label free colorimetric sensor for single stranded oligonucleotides analytes by catalyzing a H_2O_2-mediated oxidation resulting in the generation of a colored radical cation, but this requires an overnight incubation.[10] Herein, we report on a dinuclear ruthenium-based complex that functions as a prototypical label-free colorimetric sensor capable of differentiating between specific duplex sequences; and also G-rich duplex and quadruplex DNA structures.

Due to their rich optical and electronic properties oligonuclear polypyridyl d^6-metal complexes have found use in a variety of applications including light energy conversion and molecular devices.[11–14] Specific moieties such as azo groups have also been used to confer photochromic, proton-response and redox active properties to systems that include metal complexes.[15,16]

An example of this latter approach comes from the Otsuki group, where Ru^{II} complexes containing azo based ligands such as 4,4″-azobis(2,2′-bipyridine), (4-azo), have been synthesized. The previously reported dinuclear complex $[\{(bpy)_2Ru\}_2(4\text{-azo})]^{4+}$ (bpy = 2,2′-bypyridine), **1** (Fig. 1), functions as an electrochemically switched system: in its neutral form the 4-azo ligand quenches the ^{3}MLCT-based emission usually observed in $[Ru(bpy)_3]^{2+}$ analogues.[17] On reduction of the ligand, luminescence from **1** is activated.

Concurrent with such molecular device studies, interest in dinuclear Ru^{II} complexes as DNA probes[18] has also burgeoned. While much of this latter work has focused on metallo-intercalators, several recent studies have explored the properties of oligonuclear groove binding complexes. Research by the De Mesmaeker[19] and Keene[20] groups has revealed that non-intercalating complexes based on rigid oligotopic bridging ligands can display distinctive DNA binding properties. For example, enantiopure ΔΔ-$[\{(Ru(Me_2bpy)_2)\}_2bpym]^{4+}$ (Me_2bpy = 4,4′-dimethyl-2,2′-bipyridine, bpym = 2,2′-bipyrimidine) binds preferentially to bulge regions of DNA.[20] In another approach, the Kelly group have investigated the DNA binding properties of more extended dinuclear complexes containing $[Ru(bpy)_3]^{2+}$ units tethered by flexible alkyl linkers.[21] They found that, in contrast to their monomeric analogue, these systems display appreciable duplex binding affinities, even at higher ionic strengths. More recently, the Keene and Collins groups have separated the stereoisomers of these flexibly linked systems and investigated their interaction with bulged DNA.[22]

In the light of these previous studies, and especially as we were struck by its structural similarities to minor groove binding molecules—particularly berenil (Fig. 1)—we decided to investigate the DNA binding properties of complex **1**. The aim of this work was to explore the effect of azo-based tethers on the binding properties of linked systems. In this preliminary study, a racemic mixture of $[\mathbf{1}]Cl_4$ (synthesized using



1; where bpy = 2,2'-bipyridine berenil

Fig. 1 The structure of complex **1** and the minor groove binder berenil.

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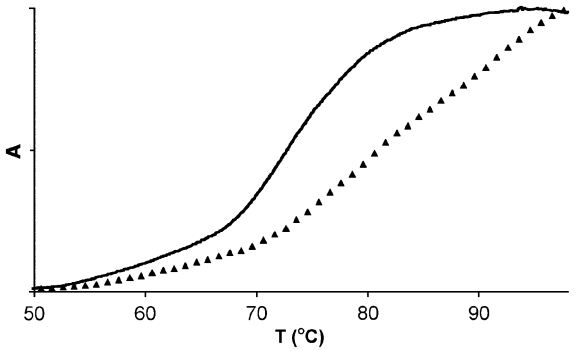

Fig. 2 T_m data for CT-DNA (—) and complex **1** (▲). Conditions: 25 mM NaCl and 5 mM tris (pH 7.0) buffer, using typical concentrations of 50 mM of DNA and 8.3 mM of metal complex. T_m measurements were initiated near 35 °C and temperature was increased at a rate of 0.25 °C per min until 98 °C. The wavelength used to follow the changes in absorbance (melting point) was 260 nm.

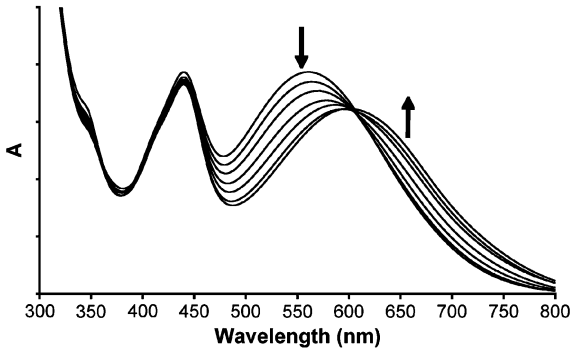

Fig. 3 Absorption titration for **1** on progressive addition of CT-DNA. Conditions: [**1**] = 50 mM; buffer = 25 mM NaCl and 5 mM tris (pH 7.0) made with doubly distilled water (Millipore).

previously reported methods[23]) was employed. The interaction of **1** with calf thymus DNA, CT-DNA, was first investigated through melting point studies—Fig. 2. In the experimental conditions employed, the transition melting temperature, T_m, of CT-DNA is 72.7 °C, however on addition of **1** a large stabilization effect is observed; indeed the exact T_m value for **1** cannot be determined because the end of the melting curve is not reached, indicating that $\Delta T_m > 20$ °C.

This interaction was further investigated using UV-visible spectroscopic titrations. Previous absorption studies have determined that **1** displays two distinctive MLCT transitions a higher energy bpy based MLCT (centred at 438 nm in water) and a longer wavelength 4-azo based MLCT (558 nm in water). Although both bands show considerable hypochromicity (<10% and 17%, respectively), the most striking change on addition of CT-DNA is the large bathochromic shift of over 40 nm for the 4-azo based MLCT; at binding saturation the band is centred at 603 nm, where an isosbestic point is also observed—Fig. 3. Presumably, this is due to the change of environment for the azo group as it moves from the bulk aqueous solvent into the DNA groove.

The hypochromism of the azo-based MLCT on addition of CT-DNA was used to construct a Scatchard plot, which was then fitted to the McGhee–von Hippel model[24] for non-cooperative binding to an isotropic lattice. This analysis revealed that **1** binds to CT-DNA with micromolar affinity giving binding parameters ($K_b = 1.61 \pm 0.17 \times 10^6$ M^{-1}; N(bp) = 3.00 ± 0.04) that are almost identical to those obtained for the dinuclear complex $[(bpy)_2Ru(Mebpy)\text{-}(CH_2)_5\text{-}(bpyMe)Ru(bpy)_2]^{4+}$ ($K_b = 1.5 \times 10^6$ M^{-1}; N(bp) = 3.00 in 10 mM phosphate buffer) reported by the Kelly group.[21] A similar analysis using the bathochromic shift of this band also produced, within experimental error, identical binding parameters.

The nature of the interaction of **1** with duplex DNA was further probed using viscosity studies. While moieties that intercalate into DNA produce a lengthening of the duplex, resulting in increases in the relative viscosity of aqueous DNA solutions, classical groove binders such as netropsin do not affect duplex length and so viscosity is minimally affected. However, non-classical groove binders, such as $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline) can often produce a distinctive lowering of viscosity. This is due to the, non-intercalative, partial insertion of a moiety such as a phen ligand between base pairs resulting in a decrease in DNA length due to bending.[25,26]

Such studies on **1** reveal that the complex induces a large negative viscosity change compared to the classical groove binder H33258—Fig. 4. These data confirm that **1** is a groove binder and, given the changes in the absorption bands associated with the 4-azo unit (Fig. 3) and the similarity of the overall structure to known minor groove binders, particularly berenil, it seems likely that the bridging azo ligand is located in the duplex minor groove.

Since it is well established that minor groove binders favour the narrow deep minor groove of A/T sequences,[27,28] the colorimetric response of complex **1** to selected oligonucleotides in similar conditions and mixing ratios was explored, leading to striking results—Fig. 5. When poly(dG)-poly(dC) (Fig. 5B) was added no colour change visible to the naked eye was observed. However, addition of the same quantity of CT-DNA (Fig. 5C) or poly(dA)-poly(dT) (Fig. 5D) produced a distinctive purple → green color change.

The structure and function of telomeric DNA and other G-rich putative quadruplex sequences are currently attracting much interest,[6,29,30] particularly as it has been calculated that genomic DNA contains a large number of putative quadruplex

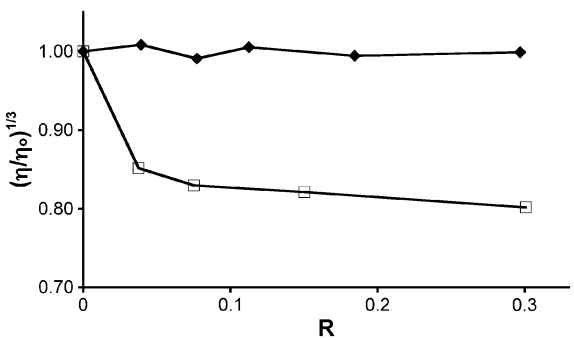

Fig. 4 Relative viscosity change in aqueous buffered solution of CT-DNA on addition of H33258 (♦) and complex **1** (□). Conditions: measurements carried out at 27 °C. [CT-DNA] kept constant at 0.5 mM (bp).

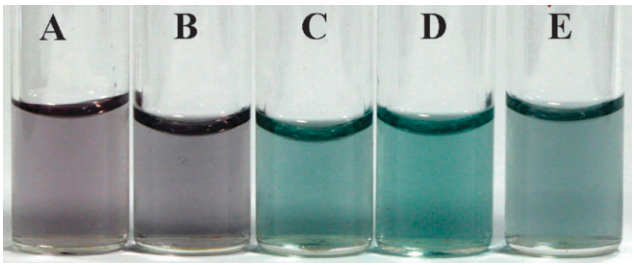

Fig. 5 Colorimetric response of buffered solutions of complex **1** to DNA. A = Pure **1**; B = **1** + poly(dG)-poly(dC); C = **1** + CT-DNA; D = poly(dA)-poly(dT); E = **1** + $AG_3(T_2AG_3)_3$ quadruplex. Conditions: buffer = 10 mM KH_2PO_4/K_2HPO_4, 1 mM K_2EDTA in 50 mM KCl (pH 7.0). [DNA] = 0.067 mM (per bp for duplex/per tetrad for quadruplex), [**1**] = 0.60 mM.

sequences.[31,32] Detection of quadruplex structures can be carried out using techniques such as fluorescence *in situ* hybridization,[33] but again chemically labeled oligonucleotides are required. A colorimetric means to detect quadruplex structures has yet to be developed. Given this context, and the fact that minor groove duplex binders such as netropsin and distamycin A[34,35] also bind to G-rich quadruplexes through groove binding and/or stacking interactions, we also investigated the effect that addition of the human telomere sequence, HTS, $AG_3(T_2AG_3)_3$ has on solutions of complex **1**.

Gratifyingly, in contrast to the G-rich *duplex* (Fig. 5B) the G quadruplex structure induced a very clear, but visually different, purple → blue colour change (Fig. 5E). We believe these divergent colorimetric responses reflect how deeply the 4-azo group of **1** is embedded into the very different structures of the respective oligonucleotides and we are currently further investigating this issue.

In summary the dinuclear DNA groove binding complex **1** produces distinctive colorimetric responses on binding to DNA. This response can be used to distinguish between different DNA sequences and structures. Studies to delineate further details of the interaction of this molecule with nucleic acids are under way and will form the basis of future reports, while the synthesis of derivatives with modulated binding preference will also be pursued.

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