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# Symmetrical dinuclear complexes with high DNA affinity based on $[Ru(dpq)_2(phen)]^{2+}$†

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*Received (in Cambridge, UK) 5th January 2003, Accepted 11th March 2004*
*First published as an Advance Article on the web 23rd March 2004*

**Symmetrical homometallic dinuclear complexes of the type $[\{Ru(dpq)_2\}_2(phen–SOS–phen)]^{4+}$, with a flexible 2-mercaptoethyl ether linker joining the two $[Ru(dpq)_2(phen)]^{2+}$-based subunits, have DNA dissociation constants ($K_d$) in the nM range.**

Mononuclear ruthenium(II) complexes have been extensively investigated as probes for DNA.[1–9] They are, however, relatively small and can only span 1–2 DNA base pairs as a monomer, but can span 4–6 base pairs if an informal dimer is formed.[10] Monomeric interactions are characterised by weak binding ($K_b \approx 10^4$–$10^6$ $M^{-1}$, depending on the intercalator)[11] and are easily displaced from DNA at ionic strengths far below *in vivo* conditions. These are significant drawbacks, as recognition of at least 8–10 base pairs is essential if metal complexes are to approach the binding footprint of a DNA-binding protein. Binding affinities need to emulate the nanomolar level at concentrations of 150 mM NaCl.[12] Dinuclear metal complexes have been recognised as probes for DNA as they have increased size, charge and variety of molecular shapes for greater DNA-binding affinity. To date, a limited number of dinuclear ruthenium(II) complexes have been synthesised and their binding to DNA investigated.[2–24] These can be categorised as either bridged or linked dimers.

Dinuclear complexes that share a bridging ligand, such as $[\{Ru(phen)_2\}_2(\mu\text{-HAT})]^{4+}$ [17,19] (phen = 1,10-phenanthroline; HAT = 1,4,5,8,9,12-hexaazatriphenylene) and $[\{Ru(Me_2bpy)_2\}_2(\mu\text{-bpm})]^{4+}$ [21,22] ($Me_2$bpy = 4,4′-dimethyl-2,2′-bipyridine; bpm = 2,2′-bipyrimidine), have been shown to groove bind weakly to DNA, with some preference for denatured or deformed segments along the DNA helix.[17,19,21,22] Linked dinuclear complexes consist of two discreet metal complexes connected *via* linkers of various lengths, types and compositions, such as $[\{Ru(L)_2\}_2\{Mebpy\text{-}4\text{–}(CH_2)_x\text{–}4\text{-}Mebpy\}]$[12,15,18] (L = phen or bpy = 2,2′-bipyridine; $x$ = 5 or 7) and $[\{Ru(phen)_2\}_2\{\mu\text{-dppz(11–11′)dppz}\}]^{4+}$ (dppz = dipyrido[3,2-*a*:2′,3′-*c*]phenazine).[14,23] Symmetrical dimers of this type have been shown to associate with DNA either by groove binding[12,15,17–22] or intercalation.[14,23]

Symmetrical dinuclear complexes of the type $[\{Ru(dpq)_2\}_2\text{-}(phen\text{-}x\text{–}SOS\text{–}x\text{-}phen)]^{4+}$ (dpq = dipyrido[3,2-*d*:2′3′-*f*]quinoxaline; SOS = 2-mercaptoethyl ether; $x$ = 3, 4 or 5) have been synthesised (Fig. 1)‡ to realise DNA binding affinity and some measure of selectivity. These linked dimers bind through the dpq ligand, which has been shown to have a greater affinity for purine–purine sequences than mixed-base sequences in the minor groove. This is in contrast to the dppz ligand, which displays no such preference.[7] The systematic variation of the phen attachment position (3, 4 or 5) of the linker produces complexes with slightly different distances between the metal centres. An effective aliphatic linker length has been reported to be a critical factor in determining binding efficiency[12,18] for a series of $[\{(phen)_2Ru\}_2\{Mebpy\text{-}4\text{–}(CH_2)x\text{–}4\text{-}Mebpy\}]$ compounds. 2-Mercaptoethyl ether was chosen as the linker because it affords a similar chain length to $\text{–}(CH_2)_7\text{–}$ and the oxygen and sulfur atoms may contribute to hydrogen bonding with the groove.

The fluorescent emission intensities of these dimers at concentrations of $10^{-8}$ M are small, but measurable (see ESI†), whereas no emission was detected for the monomer at a concentration of $10^{-6}$ M. The luminescence of the dimers is enhanced upon addition of CT-DNA.§ Binding affinity was measured in 10 mM phosphate buffer solutions with 100 mM NaCl to approximate physiological conditions. It should be noted that the characteristic emission curve of each complex is conserved after each successive titration (see ESI†). The emissions are slightly shifted (see Table 1) upon addition of CT-DNA, although no consistent trend was observed. The data for each complex were analysed using the intrinsic approach and the Scatchard model.[25] The binding results for the mononuclear compound $[Ru(dpq)_2(phen)]Cl_2$ ($K_b = 5.4 \times 10^4$ $M^{-1}$, $n$ = 3.4) are comparable to those of $[Ru(bpy)_2dpq]^{2+}$ ($K_b = 5.9 \times 10^4$ $M^{-1}$).[11] The binding affinity is increased upon addition of 2-mercaptoethyl ether to the mononuclear compound to form $[Ru(dpq)_2(phen\text{-}4\text{–}SOS)]^{2+}$ ($K_b = 2.3 \times 10^6$ $M^{-1}$, $n$ = 8.9).

The observed affinity is a good indication that the dpq ligands of the linked metal complexes are intercalating. The particular attachment position (3, 4 or 5) of the linker produces complexes with different binding affinities and binding site sizes. The **4**–**4** and **5**–**5** dimers have similar binding affinities, $K_b = 6.2 \times 10^7$ and $5.9 \times 10^7$ $M^{-1}$, respectively, and both are higher than that of the **3**–**3** dimer ($K_b = 3.2 \times 10^7$ $M^{-1}$). The observed binding affinities for the symmetrical dinuclear dimers are a factor of 1000 greater than that of the monomer $[Ru(dpq)_2(phen)]Cl_2$, a factor of 10 greater

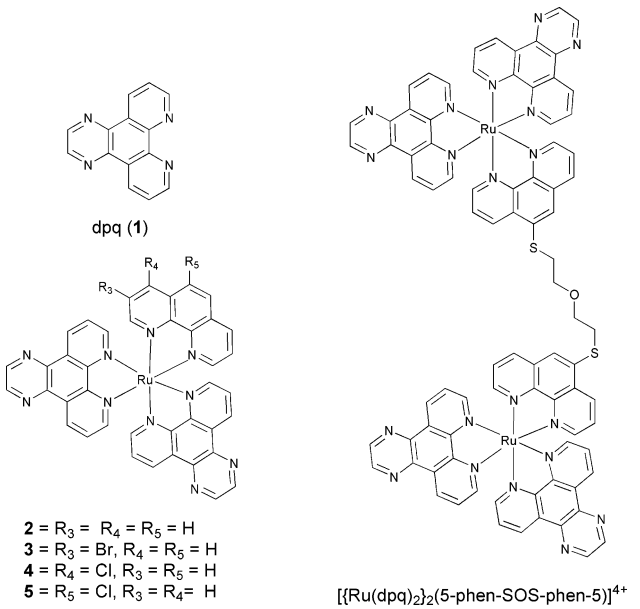


**Fig. 1** Structures of the ligand dpq (**1**), the mononuclear complexes $[Ru(dpq)_2(phen\text{-}X)]^{2+}$ (X = H, Br or Cl; **2**–**5**) and the dinuclear complex $[\{Ru(dpq)_2\}_2(phen\text{-}5\text{–}SOS\text{–}5\text{-}phen)]^{4+}$ (**5**–**5**; where phen-3 and phen-4 produce **3**–**3** and **4**–**4**, respectively).

† Electronic supplementary information (ESI) available: method for determining equilibrium binding constants and representative spectroscopic data for each experiment. See http://www.rsc.org/suppdata/cc/b3/b316917k/

DOI: 10.1039/b316917k

**Table 1** Luminescence-derived binding affinity ($K_b$), the apparent site size ($n$) in terms of base pairs and the changes in duplex melting temperature ($\Delta T_m$)[a] for the complexes

| Complex | $I_{DNA}/I_{free}$[bc] | Quantum yield | $\lambda_{max}$[c] / nm | $K_b$[c]/M$^{-1}$ | $K_d$[c]/nM | $n$[c]/base pair$^{-1}$ | $\Delta T_m$[a]/°C |
|---|---|---|---|---|---|---|---|
| $[Ru(dpq)_2(phen)]^{2+}$ | 2.31 | 0.061 | 606 | $(5.1 \pm 1.7) \times 10^4$ [de] | 19 488 | 3.4 ± 1.5 | −1 ± 0.2 |
| $[Ru(dpq)_2(phen\text{-}4\text{–}SOS)]^{2+}$ | — | — | 602 | $(2.3 \pm 0.3) \times 10^6$ [fg] | 447 | 8.9 ± 1.5 | — |
| $[\{Ru(dpq)_2\}_2(phen\text{-}3\text{–}SOS\text{–}3\text{-}phen)]^{4+}$ | 1.76 | 0.061 | 603–608 | $(3.9 \pm 1.1) \times 10^7$ [ef] | 27 | 6.5 ± 1.2 | 9 ± 2 |
| $[\{Ru(dpq)_2\}_2(phen\text{-}4\text{–}SOS\text{–}4\text{-}phen)]^{4+}$ | 2.25 | 0.053 | 607–610 | $(4.7 \pm 2.2) \times 10^7$ [fg] | 24 | 7.3 ± 0.1 | 11 ± 2 |
| $[\{Ru(dpq)_2\}_2(phen\text{-}5\text{–}SOS\text{–}5\text{-}phen)]^{4+}$ | 1.84 | 0.048 | 602–603 | $(8.9 \pm 4.3) \times 10^7$ [ef] | 11 | 19.0 ± 0.2 | 20 ± 2 |
| $[\{Ru(phen)_2\}_2\{Mebpy\text{-}4\text{–}(CH_2)_7\text{–}4\text{-}Mebpy\}]^{4+}$ [12] | 2.36 | — | 610 | $0.36 \times 10^7$ | 278 | 8.8 | — |

[a] The concentration of the duplex 5′-TCGGGATCCCGA-3′was 1 μM, while the Ru complexes were at concentrations of 2 μM. Melting was performed at a rate of 2 C° min$^{-1}$ from 25 to 90 °C. Average $T_m$ for 12 mer = 54 ± 3°. [b] $I_{DNA}$ is the fluorescence of the metal complex and DNA, $I_{free}$ is the fluorescence of the free metal complex; molar ratio [DNA]/[metal complex] = 50. [c] Phosphate buffer solution: 5 mM $NaH_2PO_4$, 5 mM $Na_2HPO_4$, 100 mM NaCl, 1 mM EDTA; adjusted to pH 7.0. [d] Average values determined from the emission data using the McGee–von Hippel method.[25] [e] $n$ = 3. [f] Average values determined from the emission data using the intrinsic approach and Scatchard model.[25] [g] $n$ = 2.

than for the dimeric groove binder $[\{Ru(phen)_2\}_2(Mebpy\text{-}4\text{–}(CH_2)_7\text{–}4\text{-}Mebpy)]^{4+}$ and approach values reported for the treading bis-intercalator Δ,Δ-$[\{Ru(phen)_2\}_2\{\mu\text{-}dppz(11\text{–}11')\text{-}dppz\}]^{4+}$ ($K_b = 2.6 \times 10^8$ M$^{-1}$).[14,23] The position of attachment seems to profoundly affect the apparent binding site size, which varies from $n$ = 5.9 base pairs for the **3**–**3** dimer to $n$ = 8.3 for the **4**–**4** dimer and $n$ = 17.1 for the **5**–**5** dimer. Linker attachment through either the 3 or the 4 position seems to provide ample separation for the orientation of the dpq ligands on each of the metal centres to allow both ends of the complex to bind to the same strand of DNA. The apparent binding site size of 17.1 for the **5**–**5** dimer seems to fall outside this explanation, unless there is some sequence preference or this dimer interacts with two strands of DNA through interstrand binding.

UV melting experiments¶ were conducted in both the absence and presence of complex to assess the impact, if any, of these metal dimers on the thermal stability of the duplex 5′-TC**GGG**AT**CCC**GA-3′ studied here. This duplex was chosen since it has two potential binding sites, two trimeric purine–purine base pairs (highlighted in bold), separated by the central AT. The resulting $\Delta T_m$ values are tabulated in Table 1. The extent of this thermal enhancement follows the trend **5**–**5** > **4**–**4** > **3**–**3** > $[Ru(dpq)_2(phen)]Cl_2$.

In conclusion, we have shown that these symmetrical dinuclear complexes bind strongly to DNA at 100 mM NaCl concentrations and that dimerisation can have an effect on observed binding affinity and binding site size. All three dimers exhibit relatively intense luminescence at room temperature in the presence of DNA. The choice of ligands (in this case, dpq and phen) and the linker (2-mercaptoethyl ether) influences the degree of hydrogen bonding within the groove of DNA and, hence, the specificity. Binding studies of these symmetrical dinuclear complexes using short duplexes (~20 mers), designed to further probe the specificity and geometry of their interaction, as well as synthesis of derivative and homochiral forms to fully explore binding interactions, are currently under way.

We gratefully acknowledge the support of the Australian Research Council (Grant no. DP0343480), The University of Western Sydney and Dr S. Ralph (University of Wollongong, Australia) for carrying out the ESI-MS analyses. We also thank Dr D. Haines from Varian for constructive discussions.

## Notes and references

‡ All compounds were isolated as $PF_6$ salts and characterised by ESI mass, UV and $^1$H NMR spectroscopies. Details of the syntheses will be reported elsewhere. Chloride salts of the complexes were used for the DNA melting experiments and equilibrium binding studies.

§ Equilibrium binding studies were performed with a Perkin Elmer LS 50B luminescence spectrometer that utilises WinLab. Titrations were conducted in 10 mM phosphate buffer (10 mM sodium phosphate, 100 mM sodium chloride, pH 7.0). Multiple experiments were conducted for each metal complex. Representative titrations and the resulting intrinsic and Scatchard plots are included in the ESI†. An excitation wavelength of 450 nm was used and the total luminescence intensity was recorded from 500 to 800 nm. The change in the emission spectra of a fixed concentration of each metal complex was measured after aliquots of CT-DNA (Gilbco BRL, used without futher purification) were titrated into the solution.

¶ DNA melting experiments were conducted using a Beckman Coulter Du 640 spectrophotometer with temperature control, a cell path length of 1 cm and samples in 10 mM phosphate buffer (10 mM sodium phosphate, 100 mM sodium chloride and 1 mM EDTA) adjusted to pH 7.0. The concentration of 5′-TCGGGATCCCGA-3′ duplex was 1 μM and the ruthenium complexes were at 2 μM. Continuous heating from 25 to 90 °C was applied at a rate of 2 C° min$^{-1}$.

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