C500 Project: Revision of Structure, Spectroscopy and DNA Binding of Bichromophoric Mixed Ligand Ruthenium Metalloenediyne Complexes

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ABSTRACT

An analysis of the bichromophoric behavior and binding to the DNA base stack of the novel complex ruthenium 2,3-bis(phenylethynnyl)-1,4,8,-tetraazatriphenylene (Ru-bptt) and its *tert*-butyl substituted analogue (Ru-tbptt). This project attempts to revise the previously submitted manuscript of Structure, Spectroscopy and DNA Binding of Bichromophoric Mixed Ligand Ruthenium Metalloenediyne Complexes in the form of quantitating a binding constant for the insertion into the bases stack of DNA. As well as, to further draw a distinction between the bindings of the Ru-bptt compared with the binding of Ru-tbptt.

BACKGROUND

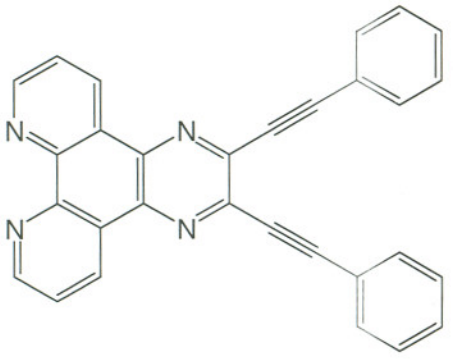
Metalloenediyne complexes that show affinity to bind strongly to DNA are of considerable interest to the scientific community due to their potential use as chemotherapeutic and gene-targeting applications.[1-9] By specializing molecules to interact with specific regions of the genome or a particular base series the compounds are able to zero in on a specific target area rather than attacking large areas of cells thereby many of the ill effects of currant gene therapy and chemotherapeutic treatments may be eliminated. Many papers have been published looking at the prospective significance of transition metal complexes in modifying this area of research, due to their large coordination numbers. These open coordination positions leave room for interaction with DNA base pairs.[10-11] The paper being revised Structure, Spectroscopy and DNA Binding of Bichromophoric Mixed Ligand Ruthenium Metalloenediyne Complexes does not deal directly with molecules designed for chemotherapeutic purposes but rather looks at some transition metal complexes and their effectiveness in binding to DNA, using multiple spectroscopic techniques to characterize this union. The compounds of interest are $[Ru(bptt)(phen)_2]^{2+}$ (Ru-bptt) and $[Ru(tbptt)(phen)_2]^{2+}$(Ru-tbptt).

The way in which the transition metal interacts with the DNA is many times of interest. All transition metal complexes bind in one of three ways. The first is electrostatic interactions between the anionic backbone of DNA and the cationic metal complex.[10] The second is direct coordination of heterocyclic bases to the metal ion as seen in cis-platin.[11-13] The final and most relevant to this manuscript is the insertion of an aromatic ligand into the DNA base stack.[12]

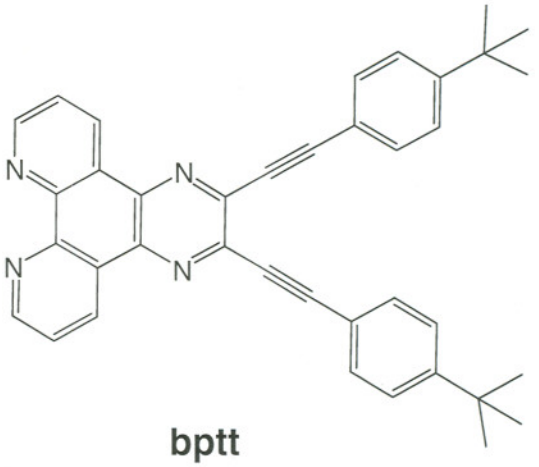
A notable DNA binding transition metal complex is $[Ru(phen)_2(dppz)]^{2+}$, which displays what is commonly referred to as "light switch" behavior.[1,15,] This is characterized by increased phosphorescence quantum yield only when binding occurs, which effectively is caused by changes in the environmental interaction with the ligand.[1] This is helpful in the development of gene-targeting diagnostics due to the phosphorescence is monitored easily via spectroscopic techniques.[10,16,17] Presently it is accepted that the "light switch" behavior is a product of the insertion of the phenazine portion of the molecule into the major grove of the DNA double helix.[14,18,9] This creates a solvent

shielding effect eliminating hydrogen interaction with the naked nitrogen. This in turn is able to eliminate a pathway for non-radiative decay, thereby increasing the quantum yield.[9] There is evidence that the "light switch" mechanism is bichromophoric, having two MLCT bands populated in the excited state, originating from two separate areas if nitrogen population yet localized on a single chromospheres.[19] This interesting phenomenon is what sparked the interest in $[Ru(diimine)_2(diimine\text{-}enediyne)]^{2+}$ compounds, such as compounds Ru-bptt and Ru-tbptt, metallochromophores whose electronic structure and spectroscopic properties will be probed and compared to those of $[Ru(phen)_2(dppz)]^{2+}$. These probes will allow for the detection for the presence of bichromaphoric behavior by not only comparisons to $[Ru(phen)_2(dppz)]^{2+}$, but also looking at bptt versus *tert*-butyl substituted bptt behaviors when spectroscopically probed.

The authors first achievement on their way to proving their theories was to prove that the ligands (shown below) themselves possess the bichromophoric nature as well as bind to DNA. They found that the fluorescence, absorbance spectra, and DFT calculations showed characteristics of



bptt



bptt

having frontier orbital with character localized at the terminal phenyl group and weaker n-π* transitions localized on the pyrazine ring in both ligands.[1] They then synthesized the ruthenium compounds and found that much of the spectroscopic results were similar to the non-metallated ligands. Except for the absorbance peak at ~450 nm, which was expected to red shift as seen in the

ligand spectra, has almost no red shift in the t-butyl substituted compound.[1] The absence of the MLCT shift in transitions in Ru-bptt and Ru-tbptt implies that there is in fact a bichromophoric character similar to that seen in $[Ru(phen)_2(dppz)]^{2+}$.[1, 20]

In further support of the electronic spectrum data, resonance Raman spectra of the Ru-bptt compound was analyzed relying on general locations of common shifts in the spectra. It is expected that upon excitation at 406.7nm vibrations associated with the phenyl portion of the ligand will be enhanced, where as at 457.9 nm the alkyne will be enhanced.[1] When excited at 457.9 nm, 1457, 1572 and 1599 cm^{-1} are enhanced and the alkyne at 2204 cm^{-1} is not.[1] (Fig. 1a) Yet, when excited at 406.7 nm the vibrations associated with the bptt ligand (ν_{89}, ν_{189}, ν_{246}) including modes associated with the phenanthroline (ν_{96}, ν_{199}, ν_{222} and ν_{238}), the pyrazine-enediyne (ν_{80}, ν_{166}, ν_{206} and ν_{246}), and the terminal phenyl (ν_{89} and ν_{242}) fragments of the bptt ligand take over the spectrum (Fig. 1b) Raman also insinuates things about the earlier electronic spectra including that at least one transition is detecting both delocalized fragments of the bptt ligand, phenanthroline and pyrazine-enediyne.[1] As far as the second hypothesis that these compounds not only possess similar spectroscopic character as

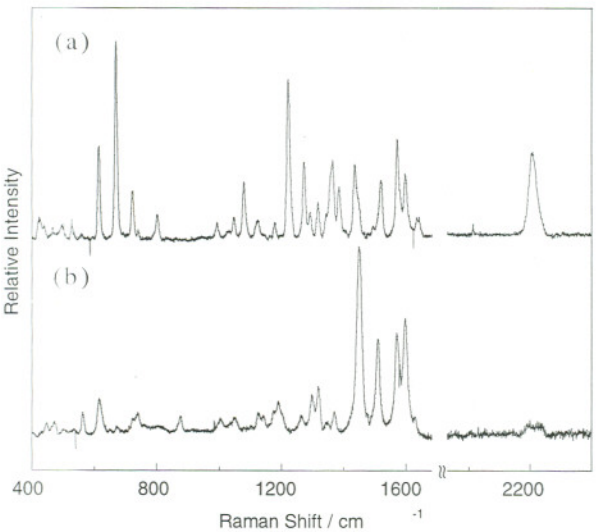


Figure 1[1]: Resonance Raman Spectra of **3** collected at 298 K in DMSO (20 mM) with λ_{ex} = 406.7 nm (a) and λ_{ex} = 457.9 nm (b).[1]

$[Ru(phen)_2(dppz)]^{2+}$, they also bind to DNA in a similar fashion. The addition of DNA affect the intensity and red shift of the 395 nm transition as well cause a 9-fold increase in luminescence quantum yield. (Fig. 2a) This result is similar to the spectral signatures of many other transition metal complexes known to bind in the base stack of DNA. [15,16,18,21] In comparison Ru-tbptt shows only minor changes in both the electronic absorption and emission spectra upon addition of DNA (Fig. 2b), suggesting that the *tert*-butyl group hinders binding to DNA. [1] The increase in luminescence coupled by the lower intensity and shift of the 395 nm transition suggest that the binding causes the molecule to be quenched less by the solvent and therefore is able to sustain the lifetime to luminescence. This is a clear signal that the ruthenium ligand is in fact binding into the base stack of the DNA, which is shielding the lone pair of electrons on the nitrogen from solvent quenching in the excited state. The

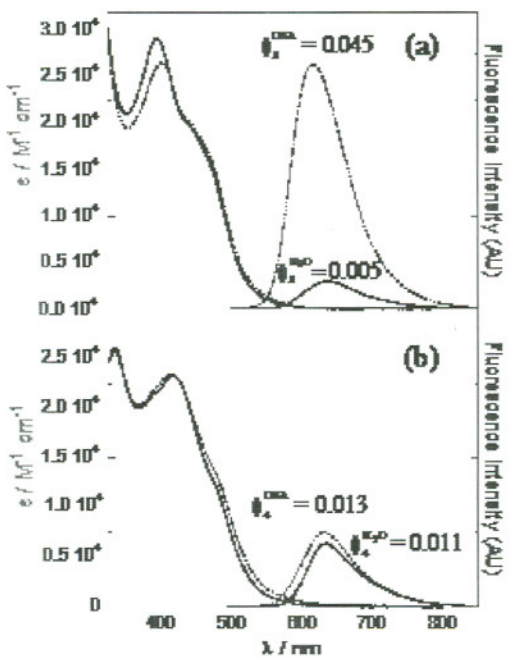


Figure 2[1]: Electronic absorption and emission spectra of Ru-bptt (a) and Ru-tbptt (b) in the absence (—) and presence (·····) of 10 molar equivalents (nucleobase) CT-DNA in 10 mM TrisHCl at pH 7.2 and 298 K. Luminescence quantum yields for Ru-bptt and Ru-tbptt in the absence (ϕ^{H2O}) and presence (ϕ^{DNA}) of DNA are shown above the emission profiles.[1]

authors go on to explain the kinetics of the ruthenium complex and binding to DNA. They conclude that two enantiomers are present in the ruthenium DNA complex, Δ and Λ, which possess unique biphasic decay kinetics in the presence of DNA. They however are unable to quantitative the kinetics of the binding and release of the ruthenium compound to DNA.

Largely, the authors did a good job of proving their conclusions, that the ligands bptt and tbptt do in fact show bichromophoric behavior, which is maintained when metallated with the ruthenium and when binding to DNA; as well as proving that there is definite binding to DNA demonstrated by the changes observable by the shift in quantum yield. However, Knowing that I am revising this paper's reviewers must have had some issue with its contents. One of the more notable and tricky issues was that though binding was proven within reason there was no quantitative data for the kinetics of this binding or release. By quantizing the on and off kinetics of the ruthenium molecule it would dispose of any doubt of the proposed binding.

There are many different methods to probe the kinetics behind the binding of Ru-bptt to DNA. Quite a few methods were examined for suitability for our purposes yet, surface plasmon resonance (SPR) using the Biacore 3000 instrument seemed like the most effective, due to its simple mode of detection coupled with its flexible procedure. Signals are able to be obtained from even small quantities of material or even impure samples.[22]

SPR is a phenomenon where light is reflected off thin metal films and the angle and intensity that the light emerges is analyzed.[22] The angle change is caused by the delocalized atoms in the gold film, plasmon, on the chip.[22] The angle of incidence is determined by many factors, yet Biacore instruments are designed so that the principle factor becomes the refractive index at the surface of the gold film-glass interface.[22] When using the Biacore system one of the molecules is immobilized on the sensor surface, the ligand, and the other is passed over the surface in the mobile phase, analyte.[23] This mobile phase contains various other molecules which are able to interact with the molecule bound to the chip. If binding occurs, the local refractive index changes, therefore the SPR angle is also changed.[22] This angular change can be monitored by detecting changes in the intensity of the

reflected light producing a sensorgram. A sensorgram is a plot of response over time which is seen in real time on the computer monitor.[23] The SPR change is measured in response units (RU) and is proportional to the mass on the surface, hence when binding occurs, response increases due to the increased weight on the surface.[23]

The rates of change of the sensogram can be analysed to yield appropriate rate constants for the association and dissociation phases of the reaction and this ratio gives the apparent equilibrium constant. The change in the SPR signal is directly proportional to the mass being immobilized and can be roughly estimated using the equation: [23]

$$R_{max} = \frac{\text{analyte MW}}{\text{ligand MW}} \text{ x immobilized amount x stochiometric ratio}$$

Where R_{max} is the maximum response, and describes the binding capacity of the surface in terms of a saturated response. Most of the time this theoretical R_{max} is larger than the experimental one; this could be due to having a ligand that is not fully active, or that has a sterical hindrance. [23]

There are three major steps in performing the Biacore experiment. The first is immobilization of the ligand onto the chip surface. The ruthenium DNA experiment required a special streptavitan coated gold surface is used, and biotin labelled DNA is attached to the surface. The affinity of this interaction, biotin and streptavitan has been measured in solution to be $K= 10^{15} M^{-1}$.[24] Therefore to attach the DNA two injections were used to regulate the amount of the DNA solution was deposited two 200 µL /s (flow rate 10µL/min) injections of 500 nM biotin labelled DNA (5`-[bioTEG]TTTGGCTTCACTCATTGCTC-3`)(Operon, diluted with 10 µM Tris-HCl buffer). Using the equation mentioned earlier the target for response was approximately 450-650 RU. This target was important to hit in order to reduce diffusion controlled factors in the kinetics, eliminating a common problem found in kinetic experiments on a Biacore.

The second step is injection of the analyte over the modified sensor surface. The analyte used is a $2.5x10^{-6}$ M Ru-bptt solution in 10 µM Tris-HCl buffer 5% (v/v) DMSO. The binding is proposed

to be strong therefore one injection at 100 μL/min (flow rate 10μL/min) should attach Ru-bptt to all available DNA groves. This will generally be hindered by steric and/or conformational obstructions.

The final step is regeneration of the surface removing all traces of the analyte so that the experiment can be repeated using the same channel repetitively for good results. Here in lies the first of many problems encountered with this experiment. When washed with 1000 μL of 3M KCl at a flow rate of 200 μL/min the Ru-bptt does not release from the DNA. This is a major problem due to the need for an off rate to get an on rate via the computer, and this would cause all experiments to be systematically irreproducible. Yet there were a few things this identified. First that K_{off} is less than 10^{-12}, implying that K_{on} is very large and that the on rate must therefore be quite large.[24] This is the currant state the inquiry at time of submission, further experiments will quantitate the actual rate.

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