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STRUCTURE-PROPERTY CORRELATIONS IN DNA BINDING AND PHOTOCLEAVAGE CHARACTERISTICS OF METALLOINTERCALLATORS

C V SASTRI#, M MARIAPPAN, TAMAL GHOSH AND BHASKAR G MAIYA*

School of Chemistry, University of Hyderabad, Hyderabad-500 046 (India)

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Studies probing the interactions of metal complexes with DNA are actively being pursued in relation with the development of synthetic restriction enzymes, foot-printing agents, metal-based drugs, fluorescent probes etc. We have recently initiated studies in this exciting research area. Our overall strategy is to employ photoactive metal complexes to bind and then on to cleave the DNA in the presence of light. In our efforts, we have been successful in 'linking' either electroactive or photoactive subunits to 1,10-phenanthroline/dipyridophenazine ligands. Mixed-ligand complexes of these new systems with ruthenium(II), cobalt(III) and nickel(II) are found to be avid binders of DNA via, generally, the intercalative mode as suggested by the results of various spectroscopic and biochemical investigations. These complexes are also capable of photocleaving the DNA; in a few cases the reactive species that are responsible for the cleaving of DNA have been identified with the help of 'inhibitor' studies/gel electrophoresis experiments. The DNA binding and photocleavage proclivities of our complexes are observed to be dependent on not only the structural intricacies of the new intercalating ligands but also on the metal ion present in the complexes. These aspects of the new metallointercalators recently developed by us are discussed in detail.

Key Words: Metal Complexes; DNA Binding; DNA Photocleavage; Intercalation; Structure-Property Correlations

1 Introduction

The interaction of metal complexes with DNA is a recent focus of research in bioinorganic chemistry. A major interest in this field concerns binding and cleavage of DNA by metal complexes, and it is related to the utility of such metal complexes in the design and development of synthetic restriction enzymes, new drugs, DNA foot printing agents etc.[1-5]. Metal complexes have been found to be particularly useful for the above-mentioned purposes because of their potential to bind DNA *via* multitude of interactions and to cleave the duplex by virtue of their intrinsic chemical, electrochemical and photochemical reactivities[6-8].

First major break through in the use transition metal complexes as DNA targets came from discovery of the potent anticancer agent cis-platin in 1969 by Rosenberg *et al.*[9]. The copper complex of 1,10-phenanthroline (phen), $[Cu(phen)_2]^+$, was the first synthetic coordination compound demonstrated to have an efficient nucleolytic activity[10]. In recent years, there is an increasing emphasis on photoactivated DNA cleavage agents, because this methodology possesses significant practical advantages. In particular, photonucleases can be triggered by exposure to light; light is an attractive 'co-factor' since it is easy to manipulate. This realization has led us to the research in this area and our work deals with the design, DNA - binding and cleavage of new, rationally-designed photonucleases based, mainly, on DNA intercalating metallo-polypyridyl complexes.

Keck and Lippard first established that square planar platinum(II) complexes containing an aromatic heterocyclic ligand could bind to DNA by intercalation[11]. This metallointercalation was later extended to three dimension using octahedral complexes. Octahedral complexes of the type $[M(LL)_3]^{n+}$, where LL is either phen or a modified phen ligand, are particularly attractive species for studies with DNA and are of relevance to the present article. The ligands or the metal in these complexes can be varied in an easily controlled manner to facilitate an individual application thus providing an easy access for the understanding of details involved in DNA-binding and cleavage. Amongst such complexes, a great deal of attention is being paid to DNA interactions of mixed-ligand ruthenium(II) complexes that contain both phen (or bpy = 2, 2'-bipyridyl) and modified phen (or modified

#*e-mail*: sastricv@yahoo.com
*since deceased

bpy) ligands, the later of which so designed to augment the intercalative interaction by the complexes. Notable examples are $[Ru(phen)_2(dppz)]^{2+}$ and $[Ru(bpy)_2(dppz)]^{2+}$ (dppz = dipyrido[3,2-a:2',3'-c]-phenazine, a modified phenanthroline ligand) both which have been reported to be avid binders of DNA and more importantly, to be remarkable luminescent reporters of DNA structure[12 - 17]. In addition, it has been shown, by Barton and co-workers, that the application of mixed-ligand ruthenium(II) complexes of the type $[Ru(phen)_2(LL')]^{2+}$ (LL' = a modified phenanthroline ligand belonging to the dppz family) permits the variation in geometry, size, hydrophobicity and hydrogen-bonding ability of the complexes and allows a variation in the strength of their DNA-binding and 'light-switching' ability[16,17].This avid DNA-binding ability of the dppz-based complexes has been rationalized in terms of their expansive aromatic surface area.

We reasoned that further strategic derivatization of phen/dppz might serve to explore and also to modulate not only the DNA binding and photocleaving abilities but also the other interesting functions associated with the ensuing complexes. In addition, a survey of the literature suggested that notwithstanding the well documented importance of dppz in DNA interactions of the complexes containing it, binding studies using such complexes having the metal ion other than ruthenium have attracted much less attention[18–20]. Bearing the above points in mind, we initiated studies on the design, synthesis and DNA interactions of a series of complexes containing modified phen/dppz ligands. Such new ligands have been so designed that besides containing the expansively aromatic 'dppz' type of structure, they are also endowed with either electro- or photoactive subunits that impart special properties for the complexes in the presence of DNA. Molecular structures of the ligands employed by us are given in Fig. 1 and the expansions of the abbreviations used to denote the ligands are presented in Table I. The metal ions that we employed for complexation with these new ligands include the well-known ruthenium(II) and also the less well known cobalt(III) and nickel(II).

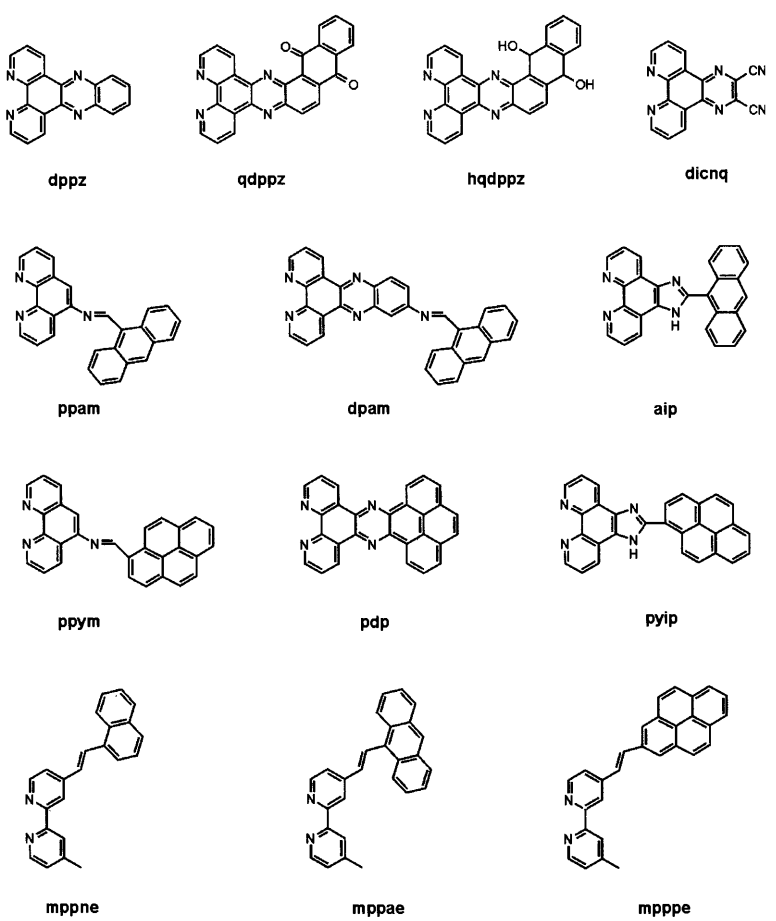


Fig. 1 Ligands employed during this study (See Table I for expansion of abbreviations)

Table I

Ligands Employed in this Study[a]

Abbreviation	Chemical Nomenclature
dppz[b]	dipyrido[3,2-a:2',3'-c]-phenazine
qdppz	naptho[2,3-*a*]dipyrido[3,2-*h*:2',3'-*f*]phenazine-5,18-dione
dicnq	6,7-dicyanodipyridoquinoxaline
ppam	N-[1,10]phenanthrolin-5-yl-9-anthrylmethanimine
dpam	N-dipyrido[3,2-a:2,3-c]phenazin-11-yl-9-anthrylmethanimine
aip	2-(9-anthryl)-1H-imidazo[4,5-f][1,10]phenanthroline
ppym	N-[1,10]phenanthrolin-5-yl-1-pyrenylmethanimine
pdp	phenanthro[4,5-abc]dipyrido[3,2-h:2,3-j]phenazine
pyip	2-(1-pyrenyl)-1H-imidazo[4,5-f][1,10]phenanthroline
mppne[c]	(E)-1-[2-(4-methyl-2-pyridyl)-4-pyridyl]-2-(1-napthyl)-1-ethene
mppae[c]	(E)-1-(9-anthryl)-2-[2-(4-methyl-2-pyridyl)-4-pyridyl]-1-ethene
mpppe[c]	(E)-1-[2-(4-methyl-2-pyridyl)-4-pyridyl]-2-(1-pyrenyl)-1-ethene

a: See Fig. 1 for the structures of these ligands.
b: dppz has been reported earlier (see refs. 29 and 39)
c: These ligands have been synthesized in Dr. Amitava Das' group (CSMCRI, Bhavanagar)

Binding of these new complexes with calf thymus (CT) DNA has been explored by a range of physical and biochemical techniques that include (i) absorption titration, (ii) luminescence titration, (iii) thermal denaturation, (iv) viscometry, (v) differential pulse voltammetry, (vi) topoisomerase assay, (vii) agarose gel electrophoresis etc. The DNA photocleavage experiments were carried out using the supercoiled pBR 322 DNA and were monitored by the gel electrophoresis method. Presented below in this article are some key results that primarily focus on the roles played by the ligand architecture and type of the metal ion on the DNA interactions of the complexes containing modified phen/dppz ligands.

2 Complexes of Modified 'dppz' Ligands Containing Electroactive Groups

In our efforts 'link' electroactive groups onto the dppz architecture, two new ligands, viz. qdppz and dicnq (see Fig. 1 and Table I), have been synthesized[21-23]. These ligands were easily synthesized by the condensation of 1,10 – phenanthroline 5,6-dione (phen-dione) with either 2,3-diaminoanthraquinone or diaminomaleonitrile. Reaction of $[Ru(phen)_2Cl_2]$ with qdppz or dicnq afforded mixed ligand complexes of the type $[Ru(phen)_2(LL)]^{2+}$ where LL = qdppz/dicnq. Bis- and tris- complexes of dicnq, i. e. $[Ru(phen)(dicnq)_2]^{2+}$ and $[Ru(dicnq)_3]^{2+}$ and also $[Ru(phen)_2(hqdppz)]^{2+}$ - the complex containing the reduced qdppz (*i.e.* hydroquinone containing ligand hqdppz; see Fig. 1 and Table I) - were also synthesized. Corresponding Co(III) and Ni(II) complexes have also been investigated and the results will be summarized in a later Section. Structures of the Ru(II) complexes of qdppz/hqdppz and dicnq are given in Fig. 2. Both the ligands and their complexes have been fully characterized by various spectroscopic methods[21-23].

DNA Binding and Photocleavage Studies Using the Ru(II) Complexes

Initially, interaction of $[Ru(phen)_2(qdppz)]^{2+}$ and $[Ru(phen)_2(dicnq)]^{2+}$ (chloride salts) with DNA was monitored by absorption titration (MLCT band) and thermal denaturation (monitoring A_{260} of DNA) methods. In the presence of increasing amounts of CT DNA the complexes showed bathochromic shift and hypochromism in their UV/VIS spectra and increased values (at [DNA nucleotide phosphate]/[Ru] = 25) of both the DNA melting temperature (DT_m) and the curve width (Ds_T) in the thermal denaturation experiments. Typical results of absorption titration and thermal denaturation experiments are illustrated in Figs. 3 and 4, respectively. While $[Ru(phen)_2(qdppz)]^{2+}$ gave a very high binding constant ($K_b > 10^6\ M^{-1}$), the dicnq analogue binds DNA only moderately strongly ($K_b = 3.3 \times 10^4\ M^{-1}$).

It is of interest to know which ligand between the available two on $[Ru(phen)_2(qdppz)]^{2+}$/ $[Ru(phen)_2(dicnq)]^{2+}$, i. e. phen or qdppz / phen or dicnq intercalates with DNA. As far as $[Ru(phen)_2(qdppz)]^{2+}$ is concerned, it may be noted that the strength of DNA binding by $[Ru(phen)_2(qdppz)]^{2+}$ is much higher than that by $[Ru(phen)_3]^{2+}$ ($K_b \sim 6.2 \times 10^3\ M^{-1}$)[24] but, it is in the same range as that for the various complexes containing dppz (or modified dppz) [24-30]. In addition, the reduced complex,

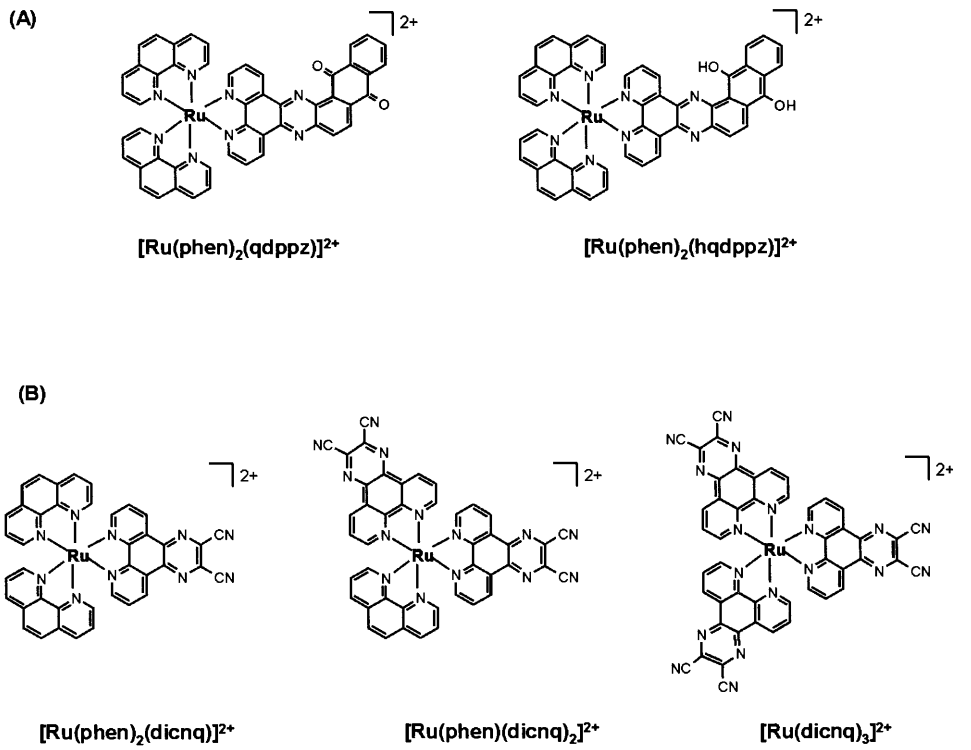


Fig. 2 (A) Ru(II) complexes bearing electroactive qdppz/hqdppz ligand, (B) Mono-, bis- and tris- Ru(II) complexes of dicnq.

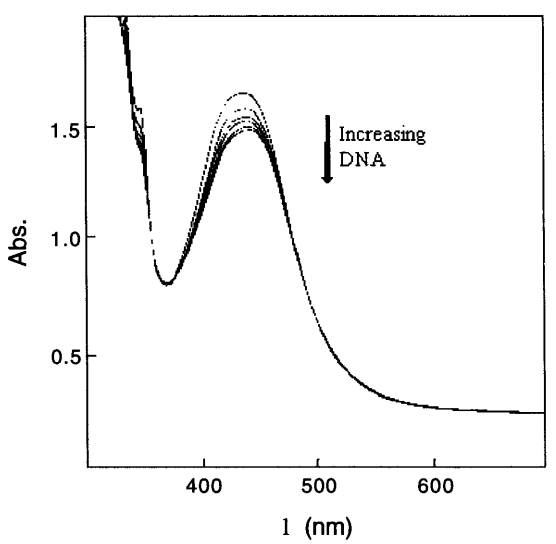


Fig. 3 UV/VIS spectra of $[Ru(dicnq)_3]^{2+}$ (25 mM) in the absence (top curve) and presence (subsequent curves) of increasing concentrations of CT DNA (0 – 150 mM).

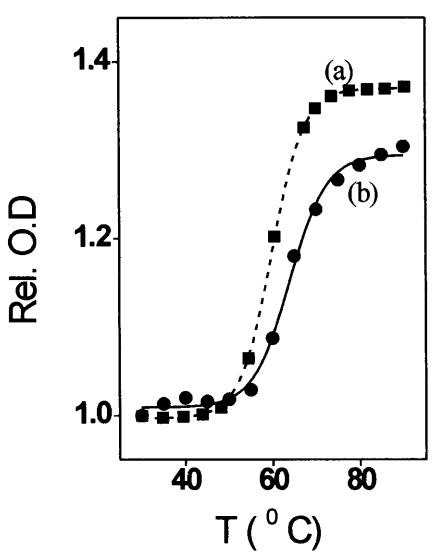


Fig. 4 Melting curves of CT DNA ([C] = 170 mM in nucleotide phosphate) (a) in the absence and (b) in the presence of $[Ru(phen)_2(qdppz)]^{2+}$ ([DNA]/[drug] = 25).

$[Ru(phen)_2(hqdppz)]^{2+}$, binds to DNA ~ 12.6 times less strongly than the qdppz complex as determined by the differential pulse voltammetric studies carried out in the presence of CT DNA[22]. These observations together with the fact that quinones are strong intercalators of DNA[31] argue in favour of an interaction of the bound modified dppz ligands with DNA in these complexes. With regard to $[Ru(phen)_2(dicnq)]^{2+}$, the binding constant value of this complex was compared with the two other dicnq ruthenium(II) complexes, viz, $[Ru(phen)(dicnq)_2]^{2+}$ and $[Ru(dicnq)_3]^{2+}$. It was observed that the strength of DNA binding varies as

$[Ru(phen)_2(dicnq)]^{2+}$ ~ $[Ru(phen)(dicnq)_2]^{2+}$ [3] $[Ru(dicnq)_3]^{2+}$. It is thus possible that it is dicnq, which is responsible for the intercalative mode of binding by these complexes although, a weak non-intercalative mode of binding by the tris- complex cannot be ruled out altogether[23].

Intercalation of a ligand to DNA is known to cause a significant increase in the viscosity of a DNA solution due to an increase in the separation of the base pairs at the intercalation site and, hence, an increase in the overall DNA molecular length[32]. In contrast, a ligand that binds in the DNA-grooves causes a less pronounced change (positive or negative) or no change in the viscosity of a DNA solution. While $[Ru(phen)_3]^{2+}$ does not affect the DNA viscosity as previously reported[33], there is a positive change of viscosity with increasing addition of the other complexes being discussed here suggesting intercalation. Consistent with this analysis, unwinding of pBR 322 DNA by $[Ru(phen)_2(qdppz)]^{2+}$ was noticed following incubation with topoisomerase I in the presence of increasing amounts of the ruthenium complex; an unwinding angle of 34 ± 10^0 per bound ruthenium is obtained for $[Ru(phen)_2(qdppz)]^{2+}$. On the other hand, topoisomerase assay carried out in presence of the reduced complex $[Ru(phen)_2(hqdppz)]^{2+}$ gave a lower value for the unwinding angle (16 ± 7^0) per bound ruthenium, as expected.

During the DNA photocleavage experiments, no DNA nicking was perceptible for the plasmid pBR 322 in the presence of each of these complexes in the dark experiments. Control experiments also suggested that photolysis of untreated plasmid does not produce Form II (relaxed circular) from the native Form I (supercoiled) upon irradiation of the sample at 440 nm. In addition, phen, dppz, qdppz and dicnq (dissolved in 10% DMF) are not detectably active either in dark or upon irradiation. In the light experiments, each complex caused single strand nicking of DNA with the conversion of Form I to Form II, as described below.

Irradiation (30 min. inside the sample chamber of a JASCO Model FP - 777 spectrofluorimeter; l_{exc} = 440 ± 5 nm, slit width = 5 nm) of DNA in the presence of the $[Ru(phen)_2(qdppz)]^{2+}$ caused complete conversion of the supercoiled form generating the relaxed circular DNA. The reduced complex, obtained from dithionite reduction of $[Ru(phen)_2(qdppz)]^{2+}$, was also seen to cleave DNA albeit, with less efficiency under the similar set of experimental conditions. Indeed, under comparable experimental conditions, DNA nicking efficiencies were seen to roughly follow the trend: '$[Ru(phen)_3]Cl_2$ << $[Ru(phen)_2(hqdppz)]Cl_2$ £ $[Ru(phen)_2(dppz)]Cl_2$ < $[Ru(phen)_2(qdppz)]Cl_2$ '. While DNA photocleavage by $[Ru(phen)_3]^{2+}$ has been reported to involve $^1O_2$ - based mechanism[34,35] and, to a large extent, that by $[Ru(phen)_2(dppz)]^{2+}$ does also involve oxygen-centered reactive species including $^1O_2$,[36] nature of the reactive intermediates as well as the mechanism of their action involved in the efficient DNA photocleavage by the two new complexes have not been explored in detail so far. However, it is interesting to note that whereas excitation of $[Ru(phen)_2(hqdppz)]^{2+}$ at 440 nm can activate only the MLCT state, that of $[Ru(phen)_2(qdppz)]^{2+}$ can, in principle, activate both its MLCT and localized quinone (p-p*) states owing to a partial overlap of the corresponding absorption bands. Thus, irradiation into the MLCT band of $[Ru(phen)_2(qdppz)]^{2+}$ can generate a species containing oxidized ruthenium and reduced qdppz (1 e^- transfer) and direct excitation of the bound-qdppz is expected to provide the triplet quinone. Both these quinone-based, transient species are known to be potent DNA cleaving agents capable of reacting with the duplex via various mechanisms[31, 37] thus explaining the superior DNA nicking ability of $[Ru(phen)_2(qdppz)]^{2+}$.

In the light experiments, both $[Ru(phen)_2(dicnq)]^{2+}$ and $[Ru(phen)(dicnq)_2]^{2+}$ caused single strand nicking with the conversion of Form I to Form II with the former complex being more active; however, $[Ru(dicnq)_3]^{2+}$ showed no appreciable photocleavage[23]. This result may not reflect the binding strengths of these complexes if one considers the fact that their K_b values are close to each other (vide supra). It is, rather, a consequence of the influence of the number of 'unprotected' dicnq ligands present on each complex in its DNA bound state. Presence of more such non-intercalating ligands in a given DNA bound complex causes a more efficient deactivation of its photochemically active MLCT excited state, resulting in diminished DNA photocleavage efficiency. The precise rationale behind this supposition is discussed in detail in the next section.

Electro-photo Switch and Molecular Light Switch Properties

During the course of investigations on the DNA interactions of the qdppz and dicnq complexes, we discovered that the redox couple $[Ru(phen)_2(qdppz)]^{2+}$/ $[Ru(phen)_2(hqdppz)]^{2+}$ acts as a 'electro-photo' switch, whereas the Ru(II) complexes of dicnq, especially

$[Ru(phen)_2(dicnq)]^{2+}$, act as 'molecular light switches' for DNA[21]. These interesting aspects of the new complexes are described below.

Exhaustive Coulometric reduction of $[Ru(phen)_2(qdppz)]^{2+}$, conducted in deaerated, aqueous CH_3CN at -0.5 V, generated $[Ru(phen)_2(hqdppz)](PF_6)_2$ as identified by its UV/VIS and NMR spectra. The solution containing this reduced complex showed an oxidation wave at +0.92 V and the bulk exhaustive Coulometry conducted at +1.1 V was seen to regenerate $[Ru(phen)_2(qdppz)]^{2+}$ The redox-cycle was repeated many times with < 5% loss of the material. In addition, while the quinone form was found to be almost non-luminescent, the electrochemically generated hydroquinone form of the complex showed the MLCT luminescence at 601 nm (f = 0.02), Fig. 5. Thus, the $2e^-/2H^+$ couple $[Ru(phen)_2(qdppz)]^{2+}/[Ru(phen)_2(hqdppz)]^{2+}$, which combines an electroactive component with a light-emitting centre, represents a redox-activated luminescence on/off switching device. The lack of luminescence observed for $[Ru(phen)_2(qdppz)]^{2+}$ can be rationalized in terms of an intramolecular photoinduced electron transfer (PET) quenching of its MLCT state by the appended quinone fragment. The reduced complex, on the other hand, does not show this PET process and hence is luminescent. However, an additional process, which has been previously reported for $[Ru(phen)_2(dppz)]^{2+}$, involving the sensitivity of the excited state to quenching by water and the subsequent increase in the non-radiative decay rate seems to be responsible for the weak luminescence observed for this complex in the aqueous environments[13-18, 29, 30, 38].

The Ru(II) complexes containing dicnq were found to be moderately good luminescent probes for DNA. Steady state emission spectra of $[Ru(phen)_2(dicnq)]^{2+}$ and $[Ru(phen)(dicnq)_2]^{2+}$ were measured in the absence and presence of increasing concentrations of DNA. It was seen that luminescence due to $[Ru(phen)_2(dicnq)]^{2+}$ increases steadily with increasing addition of CT DNA and reaches a maximum (~16 times) at [DNA nucleotide phosphate]/[Ru] ratio of 36 (see Fig. 6). In the case of $[Ru(phen)(dicnq)_2]^{2+}$, luminescence increases initially at low [DNA nucleotide phosphate]/[Ru] ratio but, reaches a plateau with the apparent enhancement factor of ~ 8 at higher [DNA nucleotide phosphate]/[Ru] ratio. $[Ru(phen)_3]^{2+}$ also shows an intensity enhancement in the presence of DNA but, only moderately; the enhancement factor is only 2 for this complex even at [DNA nucleotide phosphate]/[Ru] ratio of ~ 90 (see Fig. 6). On the other hand, $[Ru(phen)_2(dppz)]^{2+}$ has been reported to show a $>10^4$ times enhancement of emission in the presence of DNA[16, 17, 29, 30]. In this latter case, emission

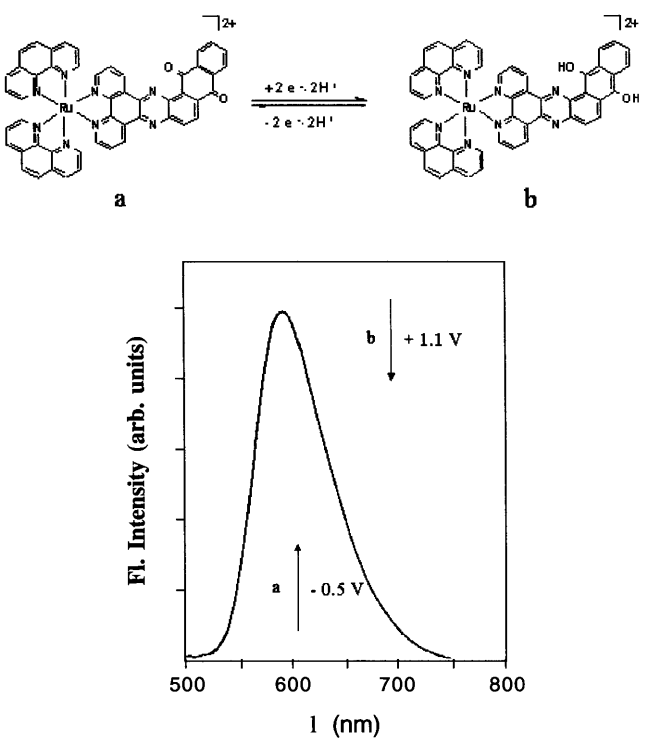


Fig. 5 Luminescence spectra (CH_3CN/5% H_2O, 0.1 M $TBAPF_6$, l_{exc} = 440 nm) of $[Ru(phen)_2(qdppz)]^{2+}$ (a) and $[Ru(phen)_2(hqdppz)]^{2+}$ (b) as obtained by exhaustive electrolyses at the indicated potentials in each case. The arrows refer to the reversible changes observed upon electrochemical interconversion of these complexes.

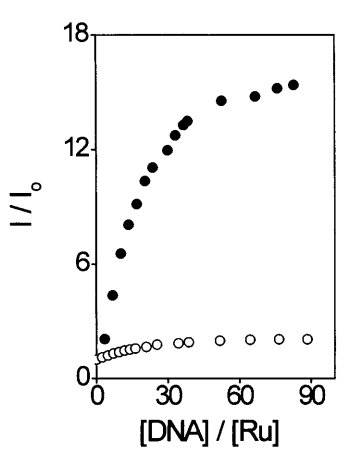


Fig. 6 Plots of I/I_0 (I_0 and I refer to luminescence intensities in the absence and presence of DNA) for 10 mM solutions of $[Ru(phen)_2(dicnq)]^{2+}$ (·) and $[Ru(phen)_3]^{2+}$ (o) *vs.* increasing ratios of [DNA nucleotide phosphate]/[Ru].

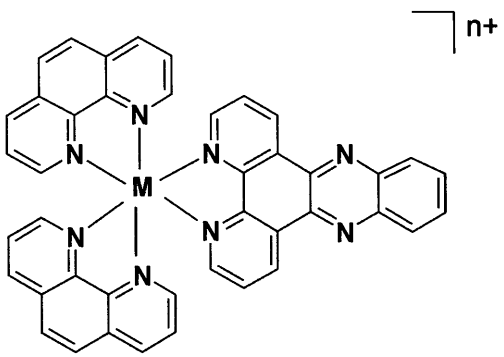
enhancement has been ascribed to the protection of the imine nitrogens from attack by water and a consequent decrease in the non-radiative processes upon intercalation[13-18, 29, 30, 38]. It is reasonable to expect that, with dicnq being a quinoxaline ligand bearing imine nitrogens, the increase in emission intensity observed for $[Ru(phen)_2(dicnq)]^{2+}$ and $[Ru(phen)(dicnq)_2]^{2+}$ in the presence of DNA is also a consequence of a decrease in the non-radiative deactivation process of each excited complex due to the protection of this ligand consequent to intercalation.

Interestingly, although the DNA binding constants of $[Ru(phen)_2(dicnq)]^{2+}$ and $[Ru(phen)(dicnq)_2]^{2+}$ are close to each other (vide supra), the former complex shows relatively higher emission enhancement compared to the latter upon addition of DNA. It should be noted here that in the event of one dicnq ligand in $[Ru(phen)(dicnq)_2]^{2+}$ being intercalated with DNA, the second, non-intercalating, spectator dicnq on this complex is essentially exposed to water. Being 'unprotected', this ligand is amenable for attack by the surrounding water molecules resulting in the non-radiative luminescence quenching mentioned above. On the other hand, intercalation by the quinoxaline ligand in $[Ru(phen)_2(dicnq)]^{2+}$ leaves only the two 'innocuous', ancillary phenanthrolines exposed to the surrounding aqueous medium. This analysis strongly suggests that dicnq is involved in the DNA intercalation by both $[Ru(phen)_2(dicnq)]^{2+}$ and $[Ru(phen)(dicnq)_2]^{2+}$, as indicated in the previous Section.

Co(III) and Ni(II) Complexes of dppz, qdppz and dicnq

Early during our studies on metal complex – DNA interactions, we recognized that further studies using various $[M(phen)_2(LL)]^{n+}$ (LL is a modified polypyridyl ligand) type species are needed to evaluate the influence of metal-ion-induced geometry, charge, spin-state, redox potential etc. changes on the DNA binding and cleavage mechanisms in this important class of complexes. Thus, we sought to find out the effect of varying the metal ion in dppz/qdppz/dicnq based complexes on their DNA interactions.

DNA-binding and photochemical DNA cleavage characteristics of $[M(phen)_2(dppz)]^{n+}$ (M = Co(III) or Ni(II) and n =3 or 2, respectively) were taken up first, Fig. 7[39]. Based on the similarities in structures, absorption titration characteristics and also apparent binding constants between the previously studied dppz complexes[7,12-19,29,30] and $[Co(phen)_2(dppz)]^{3+}$ and $[Ni(phen)_2(dppz)]^{2+}$, it was suggested that DNA-binding by the latter complexes also involves an intercalation of dppz[39]. This suggestion is further supported by thermal denaturation and differential-pulse voltammetric experiments (Fig. 8). $[Co(phen)_2(dppz)]^{3+}$ was found to effect photocleavage of the supercoiled pBR 322 DNA when irradiated by 350 nm light. On the other hand, no perceptible DNA cleavage was observed when samples of pBR 322 containing $[Co(phen)_3]^{3+}$ or $[Co(phen)_2(phen\text{-}dione)]^{3+}$ were irradiated at 350 nm



M = Co , n = 3 : $[Co(phen)_2(dppz)]^{3+}$

M = Ni , n = 2 : $[Ni(phen)_2(dppz)]^{2+}$

Fig. 7 Molecular structures of $[M(phen)_2(dppz))]^{n+}$ (M = Co(III) or Ni(II) and n =3 or 2, respectively)

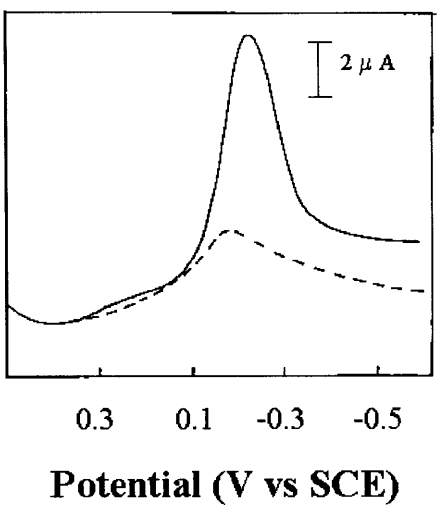
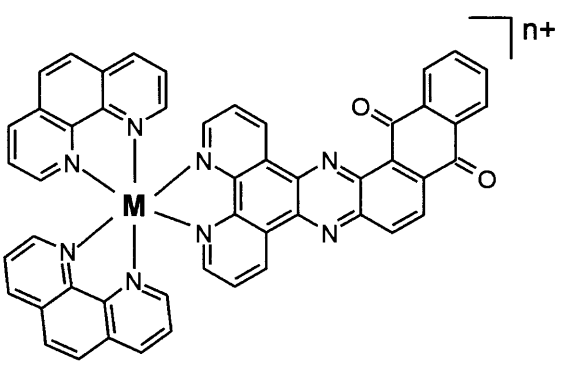


Fig. 8 Differential-pulse voltammograms (scan rate = 10 mV/s; modulation amplitude = 10 mV PP) of $[Co(phen)_2(dppz)]^{3+}$ (0.1 mM, buffer B) in the absence (¾ ¾ ¾) and in the presence (----------) of CT DNA (3 mM nucleotide phosphate)

as these complexes do not absorb light at this wavelength. The d^8 $[Ni(phen)_2(dppz)]^{2+}$ system did not show any light-induced nuclease activity probably because of the paramagnetic nature of the complex that, in principle, would render the excited state of the molecule ineffective. Thus these results while underscoring the importance of dppz in the DNA-binding, also demonstrate that substitution by different metal ions can bring about subtle modulation in the properties and, consequently, in the DNA interaction of this class of mixed-ligand complexes containing the versatile dipyridophenazine ligand[39].

Next, four new mixed-ligand complexes, namely $[Co(phen)_2(qdppz)]^{3+}$, $[Ni(phen)_2(qdppz)]^{2+}$, $[Co(phen)_2(dicnq)]^{3+}$ and $[Ni(phen)_2(dicnq)]^{2+}$, have been synthesized and characterized by FAB-MS, UV/VIS, IR, ^1H NMR, cyclic voltammetry and magnetic susceptibility methods, Fig. 9[40]. Overall, analysis of the spectroscopic and electrochemical properties of the four new complexes reveals that, in general, these properties are rather sensitive to the type of the ligand, i. e. dicnq or qdppz, rather than to the central metal ion, i. e. Co(III) or Ni (II). Therefore, overall charge on the complex does not seem to influence the properties of these polypyridyl complexes. However, both the Co(III) and Ni(II) complexes differ from their corresponding Ru(II) analogues in that the MLCT transition typical of the Ru(II) complexes is absent in the UV/VIS spectra of the former complexes. In addition, $[Ru(phen)_2(qdppz)]^{2+}$ and $[Ru(phen)_2(dicnq)]^{2+}$ are weakly/moderately luminescent in dry organic solvents but, the corresponding Co(III) and Ni(II) complexes are non-luminescent under the similar set of experimental conditions.

Absorption and viscometric titration as well as thermal denaturation studies reveal that each of these octahedral complexes is an avid binder of the CT DNA. The apparent binding constants for the dicnq and qdppz bearing complexes are in the order of 10^4 and $> 10^6\ M^{-1}$, respectively. Based on the data obtained, an intercalative mode of DNA binding has been suggested for these complexes. Irradiation of $[Co(phen)_2(qdppz)]^{3+}$ and $[Ni(phen)_2(qdppz)]^{2+}$ results in the conversion of nearly 50% of pBR 322 from Form I to Form II under the set of experimental conditions in which the corresponding Ru(II) complex completely converts Form I DNA to Form II, Fig. 10. While $[Ru(phen)_2(dicnq)]^{2+}$ has been reported[23] to photocleave DNA, both $[Co(phen)_2(dicnq)]^{3+}$ and $[Ni(phen)_2(dicnq)]^{2+}$ were found to be ineffective as is the case with $[Ni(phen)_2(dppz)]^{2+}$, the DNA cleavage properties of which have been mentioned earlier[39]. On the other hand, the observed ability of $[Ni(phen)_2(qdppz)]^{2+}$ and $[Co(phen)_2(qdppz)]^{3+}$ to photocleave DNA underscores the importance of the quinone functionality present in qdppz towards the photochemical cleavage process. Excited states of quinones are known to photocleave DNA both by electron transfer and H-abstraction mechanisms[31, 37]. More studies are clearly needed to ascertain the exact mechanism of the DNA photocleavage by this class of complexes.



M = Co , n = 3 : $[Co(phen)_2(qdppz)]^{3+}$

M = Ni , n = 2 : $[Ni(phen)_2(qdppz)]^{2+}$

Fig. 9 Molecular structures of $[M(phen)_2(qdppz)]^{n+}$ (M = Co(III) or Ni(II) and n =3 or 2, respectively)

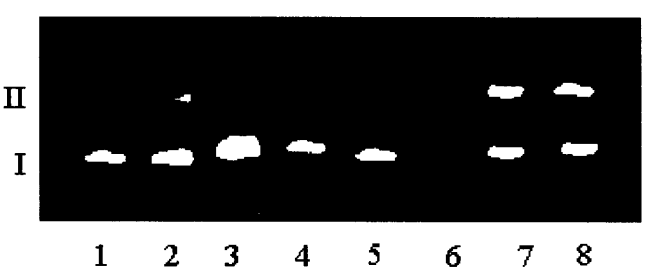


Fig. 10 Light-induced nuclease activity of $[Ru(phen)_2(qdppz)]^{2+}$, $[Co(phen)_2(qdppz)]^{3+}$ and $[Ni(phen)_2(qdppz)]^{2+}$. Dark and Light Experiments: Lanes 1 and 5: Untreated pBR 322 (100 mM) in the dark and upon irradiation ($l_{irrd.}$ = 360 nm, 30 min.). Lanes 2, 3 and 4: pBR 322 + $[Ru(phen)_2(qdppz)]^{2+}$, $[Co(phen)_2(qdppz)]^{3+}$ and $[Ni(phen)_2(qdppz)]^{2+}$ respectively (10 mM). Lanes 6 – 8: pBR 322 + $[Ru(phen)_2(qdppz)]^{2+}$, $[Co(phen)_2(qdppz)]^{3+}$ and $[Ni(phen)_2(qdppz)]^{2+}$, respectively upon irradiation ($l_{irrd.}$ = 360 nm, 30 min.)

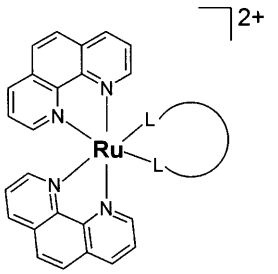
3 Complexes of Modified 'dppz/phen/bpy' Ligands Containing Photoactive Groups

After having successfully tested the DNA binding and photocleavage properties of the Co(III), Ni(II) and Ru(II) complexes containing electroactive dppz based ligands, we sought to probe the effects due to the presence of photoactive hydrocarbon groups on the phen/dppz architecture. phen or bpy ligands linked with aromatic hydrocarbon groups, such as napthalene, anthracene or pyrene, have been reported to exhibit unusual photochemical reactivities[41 - 45]. In particular, Ru(II) complexes of such ligands have long lived excited states that are potentially useful in applications such as, for example, solar energy conversion. We envisaged that it should be possible to harness this particular property for photocleaving the DNA as well. In addition, the flat, aromatic surfaces of the aromatic hydrocarbons present in such ligands are well suited for the interaction into the DNA base pairs. With these ideas in mind, naphthalene, anthracene or pyrene moiety was appended to the phen / dppz/ bpy systems to develop a new generation of photoactive ligands.

Ru(II) Complexes of phen/dppz Based Ligands Endowed with Anthracene/Pyrene Subunits

Ligands phen and dppz were connected to anthracene via an imine spacer to yield ppam and dpam, respectively. phen-dione was condensed with 9-anthraldehyde (9-AA) giving aip. Similarly, three different pyrene appended ligands ppym, pdp and pyip were synthesized (see Fig. 1 and Table I for identification of these ligands). The Ru(II) complexes of the type $[Ru(phen)_2(LL)]^{2+}$ (LL = ppam, dpam, aip, ppym, pdp or pyip) were also synthesized and spectrally characterized[46]. Structures of these six complexes are given in Fig. 11.

The DNA binding affinity of these complexes is found to vary in accordance with the architectural intricacies of the new ligands. For example, the DNA binding strengths of $[Ru(phen)_2(ppam)]^{2+}$, $[Ru(phen)_2(dpam)]^{2+}$ and $[Ru(phen)_2(aip)]^{2+}$ were ~ 4 $\times 10^4\ M^{-1}$, ~ 5 $\times 10^5\ M^{-1}$ and ~1 $\times 10^6\ M^{-1}$, respectively. The luminescent titration experiments showed that all the complexes act as weak 'molecular light switches' in the presence of DNA; the order of their emission enhancement was found to be: $[Ru(phen)_2(dpam)]^{2+}$ > $[Ru(phen)_2(aip)]^{2+}$ > $[Ru(phen)_2(ppam)]^{2+}$. DNA interactions of the pyrene appended complexes also showed characteristics similar to the complexes having



= **ppam, dpam, aip, pyip, pdp or ppym**

Fig. 11 Molecular structures of the mixed-ligand Ru(II) complexes containing photoactive ligands ppam, dpam, aip, ppym, pdp or pyip (see: Table 1 for expansion of abbreviations and Fig. 1 for the structures of these ligands).

anthracene-based ligands. Large hypochromism was observed during the absorption titration experiments with $[Ru(phen)_2(pyip)]^{2+}$ (~ 61%) while the other two complexes showed only marginal hypochromism of about 11 %. The binding constant values for $[Ru(phen)_2(pyip)]^{2+}$, $[Ru(phen)_2(ppym)]^{2+}$ and $[Ru(phen)_2(pdp)]^{2+}$ were evaluated as 1.57 x 10^6, 9.19 x 10^4 and 8.50 x $10^5\ M^{-1}$, respectively. In the luminescence experiments, a two fold increase of emission intensity and a red shift of 41 nm was noticed for $[Ru(phen)_2(pdp)]^{2+}$. The other two pyrene appended Ru(II) complexes, $[Ru(phen)_2(pyip)]^{2+}$ and $[Ru(phen)_2(ppym)]^{2+}$, showed small blue shifts (6 and 1 nm respectively) of their MLCT luminescence bands, in the presence of DNA.

DNA photocleavage experiments with these complexes also showed variations of the nicking ability depending on the ligand structure. Ru(II) complexes of ppam and dpam were found to be nearly as active as $[Ru(phen)_2(dppz)]^{2+}$ in the conversion of Form I to Form II upon irradiation at their MLCT band. However, the corresponding aip bearing complex was not so active under similar set of experimental conditions. In attempts to unravel the probable DNA photocleavage mechanism of these complexes, a few control experiments in the presence of various 'inhibitors' were conducted. The results seem to indicate that photocleavage by $[Ru(phen)_2(ppam)]^{2+}$ and $[Ru(phen)_2(dpam)]^{2+}$ is mediated by reactive oxygen species such as $OH^{\cdot}$ and O_2^{-} .

DNA photocleavage experiments conducted with complexes of pyrene appended ligands revealed that the imine and phenazine linked systems (viz. $[Ru(phen)_2(ppym)]^{2+}$ and $[Ru(phen)_2(pdp)]^{2+}$ respectively) are better candidates compared to the

imidazole linked system, as is the case with the anthracene linked systems discussed above. 'Inhibition' studies reveal that DNA photocleavage by $[Ru(phen)_2(pdp)]^{2+}$ is inhibited by DMSO and mannitol ($OH^{\cdot}$ and $O_2^{-}\cdot$ quenchers, respectively) to a large extent, and DABCO ($^1O_2$ quencher) to a minor extent.

Ru(II) Complexes of 'bpy' Based Ligands Endowed with Naphthalene/Anthracene/Pyrene Subunits

$[Ru(bpy)_2(mppne)]^{2+}$, $[Ru(bpy)_2(mppae)]^{2+}$ and $[Ru(bpy)_2(mpppe)]^{2+}$ (see Fig. 1 and Table I for identification of mppne, mppae and mpppe) have been synthesized by Das and co-workers[47] and are being examined by us for their DNA binding and photocleavage abilities[47]. Ligands mppne, mppae and mpppe are structurally different from the anthracene/pyrene linked phen/dppz ligands described above in that the naphthalene, anthracene or pyrene chromophores have been connected to bpy via a C=C bond in the former ligands, whereas the aromatic hydrocarbons are either directly fused or linked via a C=N bond to phen/dppz in the latter cases. It is thus of interest to examine the effect due to these structural variations on the DNA interactions of the mixed ligand complexes of mppne, mppae and mpppe. However, aqueous solutions of $[Ru(bpy)_2(mpppe)]^{2+}$ were seen to precipitate out in the presence of DNA and studies with this complex could not be carried out satisfactorily. In what follows now, preliminary results carried out with $[Ru(bpy)_2(mppne)]^{2+}$ and $[Ru(bpy)_2(mppae)]^{2+}$ are described.

Despite the fact that mppne and mppae possess pendant naphthalene and anthracene moieties respectively, the DNA binding strengths of their mixed ligand complexes are found to be comparable. On the other hand, $[Ru(bpy)_2(mppne)]^{2+}$ showed ~ 2 times enhancement in the emission intensity in the presence of DNA but, $[Ru(bpy)_2(mppae)]^{2+}$ showed no change in the emission intensity even in the presence of excess DNA. The results of DNA photocleavage experiments with these complexes have shown that both $[Ru(bpy)_2(mppne)]^{2+}$ and $[Ru(bpy)_2(mppae)]^{2+}$ are far more efficient in single strand nicking of supercoiled pBR 322 compared with the nicking efficiency of $[Ru(bpy)_3]^{2+}$. However, the photocleavage activity of $[Ru(bpy)_2(mppae)]^{2+}$ was found to be inferior to that exhibited by the analogous Ru(II) complexes of anthracene linked phen/dppz ligands (i.e. ppam, dpam and aip) described above.

4 Conclusions

In summary, modified phen/dppz/bpy ligands that are endowed with electro- or photo-active subunits have been designed and their complexes with Ru(II), Co(III) and Ni(II) ions have been synthesized. Most of the complexes seem to bind DNA via intercalative mode by virtue of the intentionally designed flat, aromatic extensions present in these ligand architectures. Variations in their binding abilities could be traced back to the subtleties of these ligand architectures rather than to the presence of different metal ions in the complexes. However, the metal ions play a crucial role in the DNA photocleavage by these complexes. In general, the DNA photocleavage abilities seem to follow the order Ru(II) > Co(III) >> Ni(II). During these studies, several novel features related/unrelated to the DNA interactions of these complexes have also been noticed. For example, while the redox couple $[Ru(phen)_2(qdppz)]^{2+}/[Ru(phen)_2(hqdppz)]^{2+}$ acts as an 'electro-photoswitch', the Ru(II) complexes of dicnq and the pyrene/anthracene appended ligands are moderately efficient "molecular light switches" for DNA. Overall, our studies underscore the importance of the both the structural features of the ligands and presence of different metal ions in bringing about subtle modulation in the properties and, consequently, in the DNA binding and cleaving proclivities of the ensuing complexes.

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