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Photoredox behavior and chiral discrimination of DNA bound M(diimine)$_3^{n+}$ complexes (M = Ru^{2+}, Cr^{3+})

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Dedicated to Professor Arthur W. Adamson on the occasion of his 80th birthday

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Abstract

An overview is given of the rapidly developing field of bioinorganic chemistry involving the interaction of $Ru(diimine)_3^{2+}$ complexes with double-stranded DNA. This contribution focusses on the photoredox behavior of these DNA bound systems and the presence of chiral discrimination in the binding process. $Cr(diimine)_3^{3+}$ complexes are attractive new candidates for such studies in view of their long-lived room temperature luminescence and strong excited state oxidizing power. For these Cr(III) systems we observe strong quenching of the emission signal in the presence of B-DNA, and provide evidence that this quenching is associated with a photoredox process involving guanine base oxidation. We also demonstrate the value of capillary electrophoresis (CE) as a technique for assessing the relative binding constants and the stereoselectivities of transition metal interactions with DNA. In addition, we explore the use of CE as an alternative approach to help resolve literature disagreements between different $Ru(phen)_3^{2+}$/DNA binding models.

Keywords: Photochemistry; Luminescence; Capillary electrophoresis; Diimine; DNA

1. Introduction

Since the seminal reports by Adamson and Demas [1] and Adamson and Gafney [2] on the involvement of the ^{3}MLCT excited state of $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) in energy transfer and electron transfer processes, $Ru(diimine)_3^{2+}$ complexes have played a central role in the explosive growth of transition metal (TM) photochemistry and photophysics [3–5]. Among their attractive attributes are their thermal robustness and the presence of a strong, relatively long-lived ^{3}MLCT phosphorescence signal in room temperature solution. The last 15 years have witnessed the emergence of a rich sub-field of bioinorganic chemistry associated with the interaction of these $Ru(diimine)_3^{2+}$ species with duplex DNA, with the ultimate goal of developing new diagnostic and therapeutic agents [6–13]. From the outset there has been considerable interest in the extent of enantiomeric discrimination in the binding of these chiral molecules to double helical DNA, and this topic is the primary focus of this review (Section 3). We have recently proposed [14] that capillary electrophoresis may provide a rapid screening method for determining both relative binding constants and stereoselectivities of TM interactions with DNA, and our results for $Ru(diimine)_3^{2+}$ and $Cr(diimine)_3^{3+}$ systems are incorporated in Sections 3.2 and 3.4.

Of greater long-term interest is the potential utility of $Ru(diimine)_3^{2+}$ systems as DNA photocleavage agents via excited state redox processes, and a brief literature review of this subject is provided in Sections 2.1 and 2.2. Our research group has had a long-standing interest in the photobehavior of Cr(III) complexes, motivated in no small part by the challenge presented by 'Adamson's Rules' [15–18]. This interest had extended to $Cr(diimine)_3^{3+}$ systems [19], and we have recently initiated

a detailed investigation of their interactions with nucleotides [20]. We were drawn to these $Cr(diimine)_3^{3+}$ complexes because of their strong room temperature luminescence signature ($^2E_g \rightarrow {}^4A_{2g}$ phosphorescence) [19a,21,22], and the well-documented oxidizing power of the ^2E_g excited state [21–24]. The results of our emission studies in the presence and absence of added polynucleotides and mononucleotides are presented in Section 2.3, and are consistent with selective guanine base oxidation by the Cr(III) ^2E_g excited state. The structures of the diimine ligands of concern in this presentation are depicted in Fig. 1.

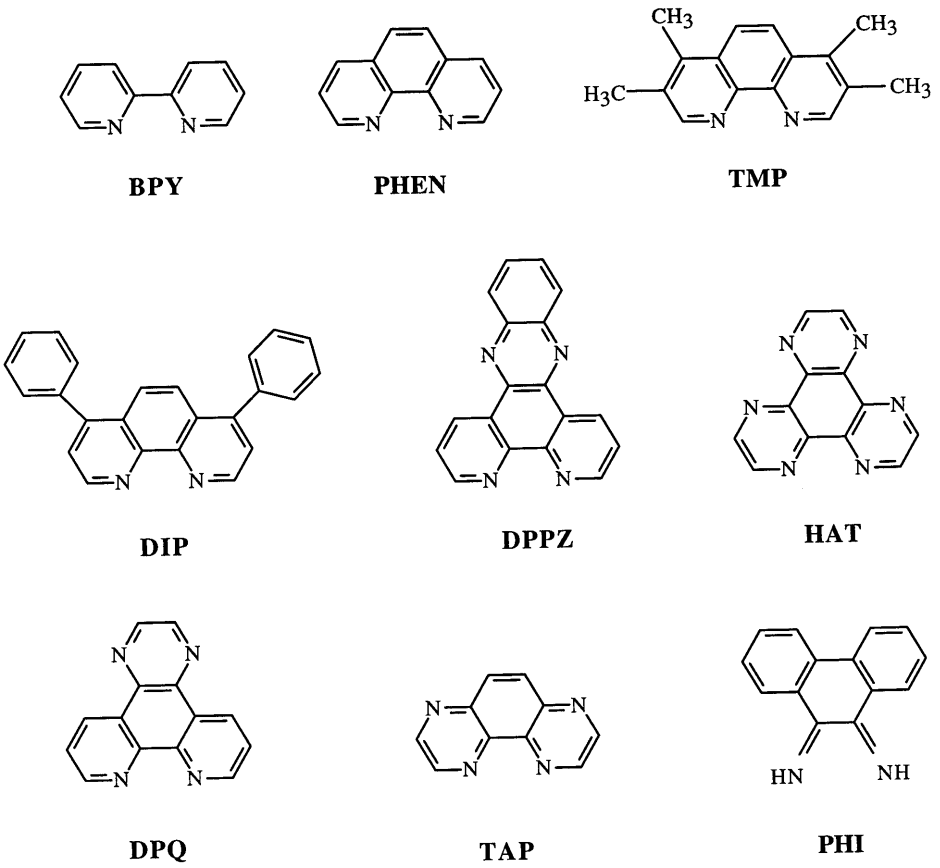

Fig. 1. Structures of diimine ligands. BPY = 2,2′-bipyridine; PHEN = 1,10-phenanthroline; TMP = 3,4,7,8-tetramethyl-1,10-phenanthroline; DIP = 4,7-diphenyl-1,10-phenanthroline; DPPZ = dipyrido[3,2-a:2′,3′-c]phenazine; DPQ = dipyrido[2,2-d:2′,3′-f]quinoxaline; TAP = 1,4,5,8-tetraazaphenanthrene; HAT = 1,4,5,8,9,12-hexaazatriphenylene; PHI = 9,10-phenanthrenequinonediimine.

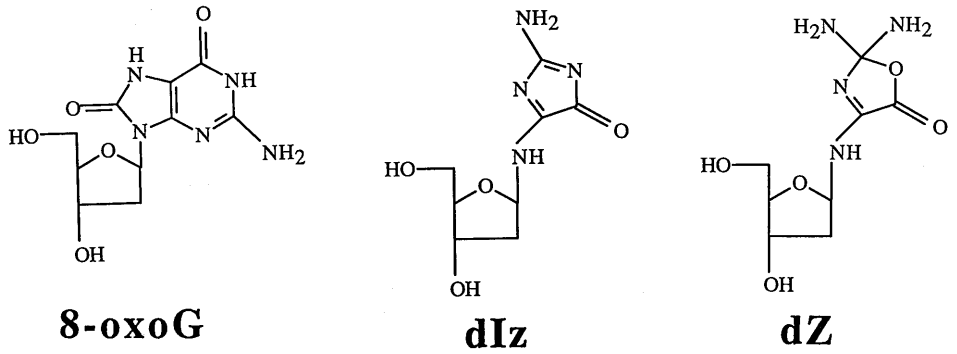

Fig. 2. Structures of guanine base oxidation products: 8-oxoG = 8-oxo-2′-deoxyguanosine; dIz = imidazolone; dZ = oxazolone.

2. Photoredox behavior of DNA bound complexes

2.1. General considerations

The subject of oxidative strand scission of nucleic acids has been comprehensively reviewed in the recent literature [25–27]. Two important pathways for photoinitiated DNA strand cleavage resulting from DNA oxidation are discussed in Sections 2.1.1 and 2.1.2.

2.1.1. Path A: deoxyribose oxidation via hydrogen atom abstraction

This process leads to a carbon-based sugar radical, and often culminates in direct (frank) strand scission [25]. The majority of known chemical nucleases initiate cleavage via sugar H abstraction, and Barton and co-workers [28] have demonstrated that photocleavage of DNA by $Rh(diimine)_3^{3+}$ complexes containing the strongly intercalating phi ligand (phi = 9,10-phenanthrenequinonediimine) also proceeds via this route.

2.1.2. Path B: nucleobase oxidation via electron abstraction

Direct strand scission is not observed in this case, but alkaline-labile sites are formed which undergo strand cleavage on subsequent treatment with a base such as hot piperidine [26]. A variety of photochemical oxidants (including anthraquinones, riboflavin, napthalimides, and benzophenone) are known to cause photocleavage, at least in part, via this mechanism [26,27]. Of the DNA nucleobases (guanine, adenine, cytosine, and thymine) guanine is thermodynamically the easiest to oxidize [26]. Two alternative fates (hydration followed by one-electron oxidation or deprotonation followed by reaction with O_2) have been explored for the guanine radical cation, $G^{\bullet+}$, initially generated [26]. The hydration pathway leads to formation of 8-oxo-2′-deoxyguanosine (8-oxoG), while the deprotonation route results in the generation of imidazolone (dIz) and its hydrolysis product oxazolone (dZ). The structures of 8-oxoG, dIz, and dZ are depicted in Fig. 2. Studies by Cadet and

co-workers [29] have established that dZ and dIz formation is favored for mononuleotides as targets because of rapid deprotonation of $G^{\bullet+}$, while 8-oxoG [26,27], and dZ and dIz [30] all appear to be important nucleobase oxidation products for studies involving duplex DNA. The recent findings of Cullis and co-workers suggest that dZ and dIz are the nucleobase oxidation products actually responsible for alkali activated strand scission in double helical DNA [31]. In related studies, Burrows and co-workers have established that an oxidized form of 8-oxoG (possibly guanidinohydantoin) may initiate misreading and misinsertion of dNTPs during in vitro DNA synthesis [32].

2.2. *Ru(diimine)$_3^{2+}$ systems*

Depending on the specific diimine ligands involved, Ru(diimine)$_3^{2+}$ complexes have binding constants, K_{DNA}, to duplex DNA in the range 10^3–10^7 M^{-1} [33]. The photoredox behavior of these DNA bound systems has been recently reviewed by Kirsch-De Mesmaeker [13]. With several notable exceptions (see later), the ^{3}MLCT excited state of Ru(diimine)$_3^{2+}$ complexes is incapable of causing a direct one-electron oxidation of guanine (the most easily oxidized nucleobase). For example, the ^{3}MLCT excited states of Ru(bpy)$_3^{2+}$ and Ru(phen)$_3^{2+}$ are estimated [4] to have an oxidizing power of ≈ 0.8 V versus NHE, whereas guanine oxidation in DNA is disfavored by ≈ 1.2 V versus NHE [26]. In such cases, DNA binding is usually accompanied by increases (sometimes spectacular) in the Ru(diimine)$_3^{2+}$ emission intensity [34,35], and photoinduced DNA strand scission occurs with low efficiency. The photocleavage observed in these instances is believed to involve singlet oxygen ($^1O_2$) mediated guanine base oxidation [36,37].

However, for several Ru(diimine)$_3^{2+}$ complexes containing strongly oxidizing ligands such as TAP and HAT (Fig. 1), more efficient photocleavage is observed, and convincing evidence for direct guanine base oxidation has been obtained [38,39]. For such systems, the ^{3}MLCT excited state has an oxidation potential exceeding that required for one-electron oxidation of guanine. For example, Ru(hat)$_3^{2+}$ and Ru(tap)$_3^{2+}$ have oxidizing powers of ≈ 1.7 and 1.6 V versus NHE, respectively. In the presence of B-DNA (and other guanine containing polynucleotides) quenching of the Ru(diimine)$_3^{2+}$ emission signal is observed, and transient absorption spectral evidence is obtained for formation of $G^{\bullet+}$ and Ru(diimine)$_3^{+}$ [13,38,39]. Emission quenching was therefore attributed to an electron transfer process involving guanine oxidation (i.e. Path B, Section 2.1). Further compelling evidence for this mechanism comes from continuous irradiation experiments on Ru(tap)$_2$(bpy)$^{2+}$ in the presence of double-stranded DNA, which induces the formation of covalent adducts with guanine [40].

In related studies, Thorp and co-workers have demonstrated that electrochemically generated Ru(bpy)$_3^{3+}$ may be used as an effective chemical oxidant with duplex and single strand DNA [41], leading to piperidine-labile lesions specifically at guanine [42]. More recently, Barton and co-workers have achieved similar results by photochemically generating the oxidant Ru(phen)$_2$(dppz)$^{3+}$ in the presence of guanine containing polynucleotides via a flash–quench technique [43].

2.3. $Cr(diimine)_3^{3+}$ systems

It can be readily shown from a combination of cyclic voltammetry and emission data that the ^2E_g excited states of $Cr(diimine)_3^{3+}$ species are potentially strong oxidizing agents. For example, $E°$ ($^*Cr(diimine)_3^{3+}/Cr(diimine)_3^{2+}$) $\approx +1.4$ V versus NHE for $Cr(phen)_3^{3+}$ and $Cr(bpy)_3^{3+}$ [22]. This oxidizing ability has been experimentally demonstrated for a wide range of non-biological substrates [22]. We have recently observed [20] that these $Cr(diimine)_3^{3+}$ complexes also function as photooxidants for guanine containing duplex DNA. In these studies we have employed the Cr^{3+} phosphorescence signal (both steady-state intensity and lifetime) as a key reporter of the DNA/TM complex interaction. The results of these findings are presented in Sections 2.3.1 and 2.3.2.

2.3.1. $Cr(phen)_3^{3+}$ and $Cr(bpy)_3^{3+}$ emission quenching by B-DNA

In the presence of calf thymus B-DNA, strong quenching of the $^2E_g \rightarrow {}^4A_{2g}$ (O_h) phosphorescence signal of $Cr(phen)_3^{3+}$ and $Cr(bpy)_3^{3+}$ is observed in air-saturated 50 mM Tris–HCl buffer, pH 7.1 [20]. Such behavior is reminiscent of that noted earlier for the highly oxidizing $Ru(hat)_3^{2+}$ and $Ru(tap)_3^{2+}$ systems, and is an indication that an electron transfer quenching process may be operative. The associated Stern–Volmer intensity and lifetime plots for $Cr(phen)_3^{3+}$ emission quenching are provided in Fig. 3. The SV lifetime plot shows a good straight line fit, and dividing the slope by the $Cr(phen)_3^{3+}$ lifetime in the absence of DNA yields a bimolecular quenching rate constant, k_q, of 1.1×10^8 M^{-1} s^{-1}. In contrast, the corresponding intensity plot exhibits strong upward curvature at higher DNA concentrations. This latter behavior is indicative of the additional presence of a static quenching pathway [44,45], which we attribute to formation of a non-luminescent DNA/$Cr(phen)_3^{3+}$ ion-pair (i.e. to DNA binding of the lumophore). The absence of detectable emission from DNA bound $Cr(phen)_3^{3+}$ is consistent with our observation of strict single exponential decay for pulsed emission decay traces in the presence and absence of DNA.

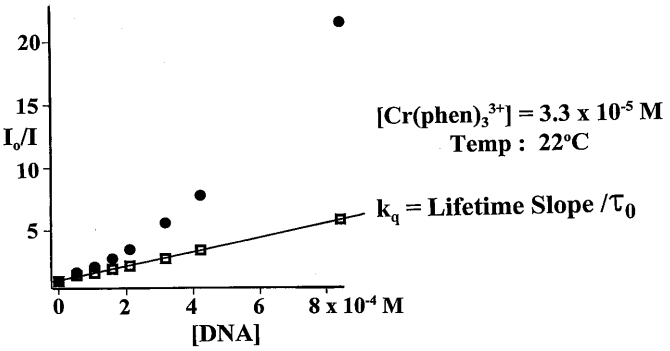

Fig. 3. Stern–Volmer plots for emission quenching of an air saturated $Cr(phen)_3^{3+}$ solution in 50 mM Tris–HCl buffer (pH 7.1) by calf thymus B-DNA: ● steady-state emission intensity data, $I_o/I = \Phi_o/\Phi$ ($\lambda_{exc} = 320$ nm); □ emission lifetime data, $I_o/I = \tau_o/\tau$.

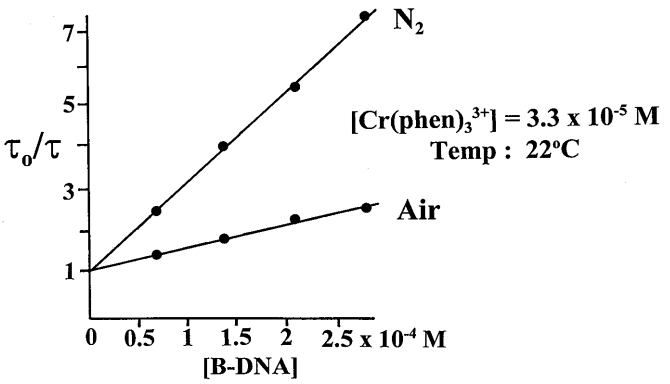

Fig. 4. Stern–Volmer plots for emission lifetime quenching of air and N_2 saturated solutions of $Cr(phen)_3^{3+}$ in 50 mM Tris–HCl buffer (pH 7.1) by calf thymus B-DNA.

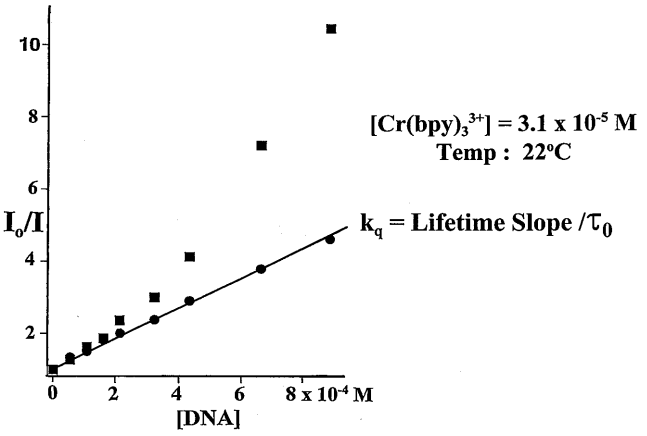

Fig. 5. Stern–Volmer plots for emission quenching of an air saturated $Cr(bpy)_3^{3+}$ solution in 50 mM Tris–HCl buffer (pH 7.1) by calf thymus B-DNA: ■ steady-state emission intensity data, $I_o/I = \Phi_o/\Phi$ ($\lambda_{exc} = 320$ nm); ● emission lifetime data, $I_o/I = \tau_o/\tau$.

A binding constant value of $K_{DNA} = 3500\ M^{-1}$ for $Cr(phen)_3^{3+}$ may be estimated from the differences in the intensity and lifetime SV plots at low DNA concentrations [20,44]. Literature K_{DNA} values for $Ru(phen)_3^{2+}$ are comparable under reasonably similar conditions [33], in agreement with our capillary electrophoresis (CE) data (see Section 3.4). Analogous emission quenching studies have been carried out in N_2 purged solution, and the lifetime SV plots for $Cr(phen)_3^{3+}$ under air and N_2 conditions are compared in Fig. 4. The difference in slopes is due to the differences in lifetimes in the absence of DNA in the two cases, with the two plots yielding essentially identical values for the DNA bimolecular quenching rate constant. This observation eliminates a significant role for O_2 in the DNA quenching process.

Fig. 5 shows the corresponding intensity and lifetime SV plots for $Cr(bpy)_3^{3+}$ emission quenching by B-DNA under air saturated conditions. Once again, upward curvature is present in the steady-state intensity plot indicative of $Cr(bpy)_3^{3+}$/DNA binding. As expected, the lifetime SV plot is linear, from which a bimolecular quenching rate constant of 1.0×10^8 M^{-1} s^{-1} is determined. Within experimental error this value is indistinguishable from that found for the phenanthroline analog, and is close to the diffusion controlled value estimated using diffusion coefficient information available for DNA [46,47].

2.3.2. Emission quenching by mononucleotides and synthetic polynucleotides

In an effort to determine whether emission quenching by B-DNA is associated with an electron transfer process involving selective guanine base oxidation, comparable studies have been carried out using mononucleotides and several polynucleotides as potential quenching agents [20]. Since the ^2E_g excited states of $Cr(phen)_3^{3+}$ and $Cr(bpy)_3^{3+}$ have an oxidizing power of 1.4 V versus NHE, they are thermodynamically capable of oxidizing guanine, while oxidation of the other nucleobases is improbable [26].

Consistent with these expectations, only deoxyguanosine-5′-monophosphate (dGMP) exhibits bimolecular quenching of the $Cr(phen)_3^{3+}$ phosphorescence, while for the non-guanine containing mononucleotides only a mild decrease in lifetime is detected (Fig. 6(A)). Importantly, for these latter cases a limiting lifetime is reached at high nucleotide concentration, which we associate with the intrinsic lifetime of the $Cr(phen)_3^{3+}$/mononucleotide ion-pair species. For the $Cr(phen)_3^{3+}$/dGMP system, the SV lifetime plot yields a bimolecular quenching rate constant of 2.2×10^9 M^{-1} s^{-1} (Fig. 6(B)). This latter value is very close to the diffusion controlled rate constant reported for GMP quenching of the emission of the strongly oxidizing Ru(II) complexes $Ru(hat)_3^{2+}$ and $Ru(tap)_3^{2+}$ [39]. For these Ru(II) systems, conclusive evidence has been presented for an electron transfer mechanism involving direct guanine oxidation.

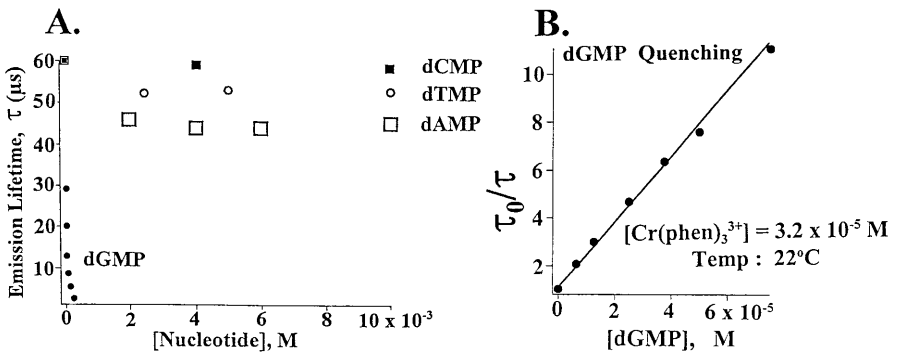

Fig. 6. (A) Emission lifetime quenching of an air saturated solution of $Cr(phen)_3^{3+}$ by mononucleotides in 50 mM Tris–HCl buffer (pH 7.1). (B) Stern–Volmer plot for emission lifetime quenching of an air saturated solution of $Cr(phen)_3^{3+}$ by dGMP in 50 mM Tris–HCl buffer (pH 7.1).

In related studies on $Cr(phen)_3^{3+}$ with three synthetic polynucleotides (data not shown), we find that only the guanine containing systems poly(dG-dC)·poly(dG-dC) and poly(dG-dG)·poly(dC-dC) exhibit bimolecular quenching of the Cr(III) emission signal. In contrast, for poly(dA-dT)·poly(dA-dT) only a minor decrease occurs in the intensity and lifetime of $Cr(phen)_3^{3+}$ phosphorescence, with the limiting values observed at high nucleotide concentration being taken as characteristic of the $Cr(phen)_3^{3+}$/poly(dA-dT)·poly(dA-dT) ion-pair.

We conclude from these studies that the quenching of $Cr(diimine)_3^{3+}$ emission by nucleotides most probably proceeds via an electron transfer mechanism involving direct oxidation of a guanine nucleobase (i.e. Path B, Section 2.1). The absence of bimolecular quenching by the mononucleotides dAMP, dCMP, and dTMP and the polynucleotide poly(dA-dT)·poly(dA-dT) provides compelling evidence against an alternative redox mechanism involving the deoxyribosyl units (Path A, Section 2.1). Many important questions still remain to be answered, one of which is whether permanent oxidative damage to DNA may be realized using $Cr(diimine)_3^{3+}$ systems as photoxidants, i.e. whether they may function as efficient photocleavage agents. Our results in this area are still at a preliminary stage, and are restricted to continuous irradiation studies with guanosine as a target nucleoside. These experiments have confirmed the generation of the lesion products dZ and dIZ expected in a Path B mechanism, via a fluorescence assay method developed by Cadet and co-workers [29,48] for released guanidine (which is an alkali breakdown product of dZ and dIZ).

3. Chiral discrimination in DNA binding

3.1. General considerations

The interaction of TM complexes with double helical DNA may be divided into either:

1. direct covalent binding of the metal to N atoms of one or more of the DNA bases, an example of which is the case of the *cis*-$Pt(NH_3)_2Cl_2$ species [49];
2. non-covalent binding, where no direct metal–nucleotide coordination takes place.

The $Ru(diimine)_3^{2+}$ and $Cr(diimine)_3^{3+}$ complexes of interest exist in two enantiomeric forms, designated as the Λ and Δ optical isomers (see Fig. 7). In room temperature aqueous solution these compounds are kinetically inert to ligand substitution [4,22] and racemization [4,50], and are coordinatively saturated. As a result their contacts with polynucleotides are non-covalent, and may be categorized as either:

1. Electrostatic binding, involving coulombic attraction between the positively charged metal complex ion and the negatively charged DNA phosphate backbone. This interaction is not expected to exhibit sensitivity to the chirality of the TM cation [6].

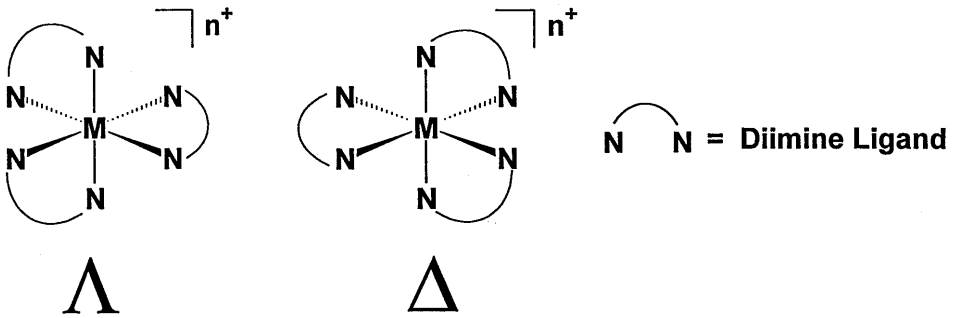

Fig. 7. Structures of the Λ and Δ enantiomers of $M(diimine)_3^{n+}$ complexes.

2. Surface binding in the major or minor groove of the DNA, where hydrophobic and H bonding interactions are possible.
3. Intercalative binding in the major or minor groove, where a planar ligand component inserts between the stacked base pairs.

Chiral molecular recognition is anticipated for both surface and intercalative binding, and Barton and co-workers have argued that the extent of chiral discrimination is likely to be larger for intercalative binding due to the more extensive substrate-receptor contacts [6].

3.2. $Ru(bpy)_3^{2+}$ and $Ru(phen)_3^{2+}$ systems

3.2.1. Background

The interaction of $Ru(diimine)_3^{2+}$ complexes with double helical DNA has been most extensively investigated by the Barton group. They were the first to report (on the basis of equilibrium dialysis data) a net binding discrimination in favor of the Δ isomer for the interaction of $Ru(phen)_3^{2+}$ with B-DNA [51]. In contrast, no net binding stereopreference was observed for $Ru(bpy)_3^{2+}$ [52]. The net enantiomeric selectivity reported for $Ru(phen)_3^{2+}$ has since been confirmed by Chaires and co-workers using both equilibrium dialysis and emission titration data [53]. Although $Ru(phen)_3^{2+}$ binding with DNA is known to be dominated by electrostatic forces [53], the observation of enantiomeric discrimination, coupled with recent thermodynamic data from Thorp and co-workers, demonstrates that non-electrostatic forces are of significance [54].

Several NMR studies on $Ru(phen)_3^{2+}$ employing oligonucleotides [55–58] indicate that these non-electrostatic interactions occur primarily via surface binding in the minor groove near AT base pair sites. Barton and co-workers [6,7,55] and others [56–58] have proposed that this surface binding mode favors the Λ optical isomer. The Barton model assigns an additional binding mode with an unusually strong Δ preference to partial intercalation of a phenanthroline ligand in the major groove. Thus, although this latter mode may make only a small contribution to the overall binding constant, a net discrimination for the Δ isomer is observed. However, the more recent NMR studies suggest that the binding mode character-

ized by a strong Δ preference also occurs in the minor groove [57,58]. Furthermore, in one of these latter NMR studies [57] it is argued that the Δ selective binding mode does not involve partial intercalation. Instead, it is proposed that this mode is associated with insertion of two of the phenanthroline ligands into the minor groove, while for the preferential Λ binding mode only one phenanthroline ligand inserts into the minor groove (Eriksson model).

3.2.2. Capillary electrophoresis studies

Despite the widespread recognition of capillary electrophoresis (CE) as a highly efficient, versatile analytical separations tool [59], its use by the inorganic community is still in its infancy. Our results below (and in Section 3.4) demonstrate that the CE technique provides an attractive method for the rapid assessment of relative binding constants and stereoselectivities of $M(diimine)_3^{n+}$ interactions with DNA [14,20]. Furthermore, we show that CE offers an alternative approach to help resolve the disagreements in the $Ru(phen)_3^{2+}$/DNA binding models noted above with regard to minor versus major groove binding.

3.2.2.1. Relative binding constants and stereoselectivities. A representative electropherogram is shown in Fig. 8 for the injection of a mixture of *rac*-$Ru(phen)_3^{2+}$ and *rac*-$Ru(bpy)_3^{2+}$ into a capillary containing 2.5 mM B-DNA as a chiral additive in ammonium acetate buffer at pH 5. Near-baseline separation of the Λ and Δ isomers of both complexes is achieved, and similar results are obtained when a B-DNA/phosphate buffer at pH 7 is utilized. It is clear that $Ru(phen)_3^{2+}$ has the longer capillary residence time, and thus a stronger interaction with the capillary DNA. Furthermore, coinjection of the racemic analyte mixture with first Δ-$Ru(phen)_3^{2+}$ and then Δ-$Ru(bpy)_3^{2+}$ (data not shown) establishes the enantiomer component with the longer migration time as the Δ isomer for both complexes. We have also performed experiments on each complex separately, and the migration velocities

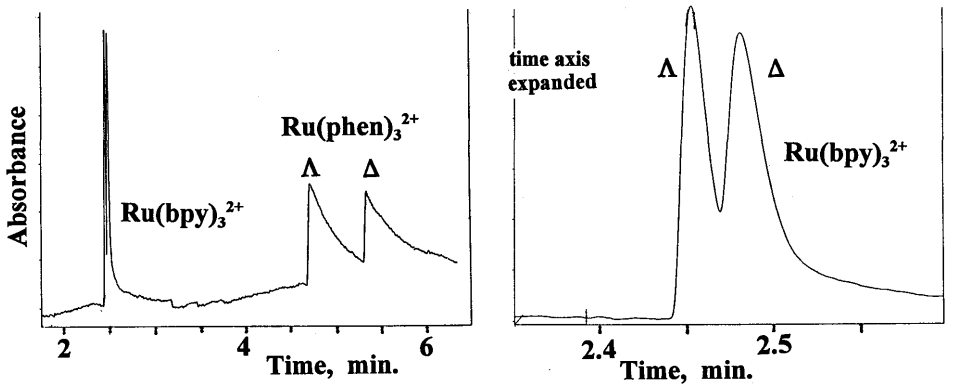

Fig. 8. Electropherogram of a mixture of *rac*-$Ru(bpy)_3^{2+}$ and *rac*-$Ru(phen)_3^{2+}$ in 50 mM ammonium acetate buffer, pH 5.0, containing 100 mM NaCl and 2.5 mM calf thymus B-DNA. Electrophoretic field strength 452 V cm^{-1} (run temperature 30°C). Detection: $\lambda = 450$ nm.

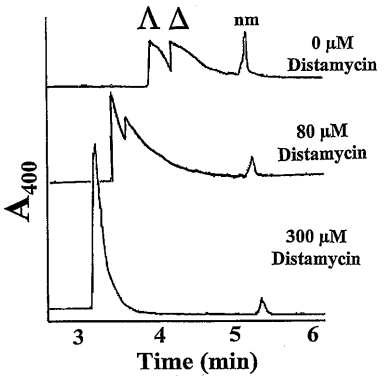

Fig. 9. Effect of added distamycin on the electropherogram of *rac*-$Ru(phen)_3^{2+}$ (1 mM) in 50 mM Tris–HCl buffer, pH 7.4, containing 100 mM NaCl and 0.92 mM B-DNA. Electrophoretic field strength 319 V cm^{-1} (run temperature 35°C). Detection: $\lambda = 400$ nm. (nm = neutral marker)

and stereoselectivity results are identical to those noted above when an analyte mixture was employed [60]. These results provide dramatic confirmation of earlier literature studies involving equilibrium dialysis [51,53] and emission titration [53] data (i.e. a greater DNA binding equilibrium constant for $Ru(phen)_3^{2+}$ and net preferential binding by the Δ isomer). It is particularly significant that the small degree of enantiomeric discrimination present in the case of the $Ru(bpy)_3^{2+}$ is difficult to detect in equilibrium dialysis experiments [52], yet readily detectable by CE (Fig. 8). The observation of a much larger breadth for the $Ru(phen)_3^{2+}$ isomer peaks also provides support for the greater importance of a non-electrostatic binding component for this system [54].

3.2.2.2. Minor versus major groove binding. In order to assess the relative importance of minor versus major groove binding in the $Ru(phen)_3^{2+}$/DNA system, we have carried out a CE investigation with both B-DNA and distamycin A in the capillary buffer medium. Distamycin A is a strongly AT selective minor groove binder with a large binding constant for B-DNA. It has been widely used in competitive binding experiments to distinguish between minor and major groove binding by various DNA binding agents [61]. Its presence in the capillary medium would be expected to result in the release of both $Ru(phen)_3^{2+}$ enantiomers if the Λ and Δ isomers were both bound in the minor groove. In contrast, if the binding mode favoring the Δ isomer were associated with the major groove, then the addition of distamycin would lead to the observation of an even greater net preference for Δ binding accompanying reduced overall binding. Our CE data for the $Ru(phen)_3^{2+}$/B-DNA/distamycin system are shown in Fig. 9. It is apparent that the presence of distamycin results in reduced migration times, accompanied by the complete loss of enantiomeric discrimination. These observations suggest that the binding mode characterized by a strong Δ preference occurs in the minor groove.

3.2.2.3. A modified DNA binding model. The ability of CE to detect even very modest net binding stereoselectivities, such as that for $Ru(bpy)_3^{2+}$ (see Fig. 8), allows for a more careful assessment of current DNA binding models with regard to surface versus intercalative binding modes. Based on a large body of experimental evidence, it is generally agreed that intercalative binding is not operative for the $Ru(bpy)_3^{2+}$ system. Consequently, our observation of a net enantiomeric discrimination for the Δ isomer for both $Ru(phen)_3^{2+}$ and $Ru(bpy)_3^{2+}$ is difficult to reconcile with the Barton model (Section 3.2.1), since it would predict a small net Λ preference for the $Ru(bpy)_3^{2+}$ system. In contrast, in the Ericksson model two groove binding modes are postulated, with a Λ preference predicted if just one bipyridine ligand or phenanthroline is inserted in the groove (mode A) and a Δ preference for two ligands inserted (mode B). The degree of isomer discrimination is expected to be considerably greater when two bipyridines or phenanthrolines are inserted, due to the opportunity for more substrate-receptor contacts. Thus, even if mode A makes a greater contribution to the overall binding constant, a net observed discrimination for the Δ isomer is still possible (as observed).

However, the Ericksson model is unable to explain satisfactorily our CE results when additional salt is added to the capillary buffer medium. Surface groove binding modes should be seriously affected by added salt, and one might expect the loss of chiral discrimination. Our CE results confirm this expectation for $Ru(bpy)_3^{2+}$. In marked contrast, in CE studies on $Ru(phen)_3^{2+}$ the net observed preference for the Δ enantiomer increases as additional NaCl is added to the run buffer (data not shown). This latter result is in accord with literature equilibrium dialysis data for the $Ru(phen)_3^{2+}$/B-DNA system [62], and is consonant with at least some intercalative binding favoring the Δ isomer being present. This binding mode is anticipated to be the least affected by added salt, a contention that receives strong support from the recent ESR study by Turro et al. on Ru(II) polypyridyl/B-DNA systems [63].

In order to accommodate these mutual inconsistencies, we propose a modified binding model, which integrates aspects of both the Barton and Ericksson schemes. This integrated model requires consideration of three binding modes for the case of $Ru(phen)_3^{2+}$ — namely two minor groove binding modes (modes A and B of the Ericksson model, favoring the Λ and Δ isomers, respectively) and a partial intercalation mode favoring the Δ isomer (also in the minor groove). In Section 3.4 we find that this integrated model also provides a satisfactory explanation for our CE data on the corresponding $Cr(phen)_3^{3+}$/B-DNA system.

3.3. $Ru(TMP)_3^{2+}$, $Ru(DIP)_3^{2+}$, $Ru(phen)_2(dppz)^{2+}$, and $Rh(diimine)_3^{3+}$ systems

Barton and co-workers have pioneered the development of a library of Ru^{2+} and Rh^{3+} diimine complexes which recognize specific nucleic acid binding sites based on shape selection [64,65]. These systems are briefly reviewed below.

The $Ru(TMP)_3^{2+}$ species (where TMP is 3,4,7,8-tetramethyl-1,10-phenanthroline) exhibits only very weak binding to B-DNA, an observation that has been attributed to the steric bulk of the methyl groups [37,66]. This bulkiness not only precludes

intercalative binding, but also places constraints on surface groove binding. Much stronger binding is found with A-DNA, which has a wider and shallower minor groove than normal B-DNA. Preferential binding of the Λ isomer is observed, with a level of discrimination of 92% being reported at low loading levels [66].

Chiral discrimination has also been reported for $Ru(DIP)_3^{2+}$ (where DIP is 4,7-diphenyl-1,10-phenanthroline), with a preference for the Δ isomer for B-DNA and the Λ isomer for Z-DNA [67]. This reversal in enantioselectivities is associated with the reverse helicities of the two polynucleotide targets (B-DNA: right-handed; Z-DNA: left-handed), while the isomer discrimination observed is consistent with a significant intercalative binding component (assisted by the presence of the phenyl groups). However, the limited solubility of this complex in aqueous buffer necessitated the addition of 10% DMSO to the test solutions. The acetate salt exhibits an enhanced water solubility, and a recent study with this salt reveals no binding stereoselectivity for B-DNA in this medium [68].

Among the most remarkable of these DNA reporters are the species $Ru(bpy)_2(dppz)^{2+}$ and $Ru(phen)_2(dppz)^{2+}$, where dppz is the diimine ligand dipyrido [3,2-a:2′,3′-c] phenazine. While non-luminescent in aqueous solution, these complexes are strongly emissive in the presence of double-stranded nucleic acids, i.e. they function as molecular 'light switches' for DNA [35,69]. The unusual absence of aqueous emission is attributed to excited state quenching by solvent hydrogen bonding with the phenazine nitrogens [70]. It has been established that the DNA binding involves intercalation [69–73], where the planar dppz ligand inserts between the DNA base pairs (thus providing solvent protection for the phenazine nitrogens). Binding constants in excess of 10^6 M^{-1} per DNA base pair are observed for solutions 50 mM in NaCl [33,69,72].

On the basis of ^1H and ^{31}P NMR evidence, Dupureur and Barton have argued that $Ru(phen)_2(dppz)^{2+}$ intercalates from the major groove [74].

However, in recent studies by Nordén et al. [71,75] it is proposed that intercalation occurs in the minor groove. In a very recent response, Barton and co-workers reconfirm their original major groove assignment for intercalation [61]. Interestingly, Collins and co-workers [58] have reported a two-dimensional ^1H NMR investigation of the interaction of the closely related $Ru(phen)_2(dpq)^{2+}$ complex (where dpq is dipyrido[2,2-d:2′,3′-f]quinoxaline) with the same hexanucleotide used in the Dupureur and Barton study [74]. Their observation of intercalation by dpq from the minor groove illustrates the exquisite sensitivity of these systems to changes in the intercalating ligand's structure.

Although outside the primary focus of this review, the family of $Rh(diimine)_3^{3+}$ complexes developed by Barton and co-workers containing the phi ligand and derivatives (where phi = 9,10-phenanthrenequinonediimine) provides another interesting illustration of the critical influence of ligand structure on the nature of polynucleotide interactions. This behavior has been discussed in detail elsewhere [25,27,28,65], and in an exciting recent extension of these studies, the Barton group has demonstrated the ability of a Rh(III) phi complex to recognize DNA base mismatches [65]. Germane to the present discussion, these Rh(III) phi species have large binding affinities for duplex DNA via intercalation of the phi ligand into the major groove with a stereopreference for the Δ isomer [76].

3.4. $Cr(bpy)_3^{3+}$ and $Cr(phen)_3^{3+}$ systems

We discuss below the results of our capillary electrophoresis studies on *rac*-$Cr(bpy)_3^{3+}$ and *rac*-$Cr(phen)_3^{3+}$ in the presence of B-DNA in the capillary buffer medium. In the case of $Cr(bpy)_3^{3+}$ no CE evidence was obtained for enantiomeric selectivity in DNA binding, an observation compatible with a greater contribution by purely electrostatic forces to the overall binding constant for tripositively charged bipyridine species [77]. Hydrophobic interactions are anticipated to be stronger for analogous tripositive phenanthroline systems [77]. In accord with this expectation, chiral discrimination is clearly apparent in our CE data for the $Cr(phen)_3^{3+}$ system. Fig. 10 shows the resultant electropherogram when a 1:1 mixture of *rac*-$Cr(phen)_3^{3+}$ and *rac*-$Ru(phen)_3^{2+}$ is injected into a capillary buffer medium (pH 7.4) containing 3 mM calf thymus B-DNA. After correcting for the electroosmotic flow component using a neutral marker, added DNA causes a slightly greater reduction in electrophoretic mobilities for $Ru(phen)_3^{2+}$, indicative of a slightly larger K_{DNA} value. Significantly, the enantiomeric order of migration and thus net binding stereoselectivities (established by co-injections with samples of the Λ isomer) are reversed for the Cr^{3+} and Ru^{2+} complexes. In a pertinent earlier NMR investigation on the oligonucleotide $d(GTGCAC)_2$ in the presence of Cr(-phen)$_3^{3+}$ [78], the results were interpreted in terms of minor groove surface binding in which Λ isomer binding was favored. Only very weak evidence was found for an additional intercalative binding mode favoring Δ isomer binding. In a related NMR study involving $Ru(phen)_3^{2+}$ and $Rh(phen)_3^{3+}$, Rehmann and Barton noted that increasing complex charge is an additional factor favoring surface binding over partial intercalation [55]. A lower probability of intercalation with increase in complex charge is also in accord with the reported lower DNA binding constant of $Co(phen)_3^{3+}$ compared with $Co(phen)_3^{2+}$ (i.e. the relative binding constants are counter to the charge preference) [46b,77].

Our observation (Fig. 10) of net preferential binding by the Λ isomer of $Cr(phen)_3^{3+}$ may therefore be interpreted solely in terms of the two competing groove binding modes described earlier in the integrated model for DNA binding

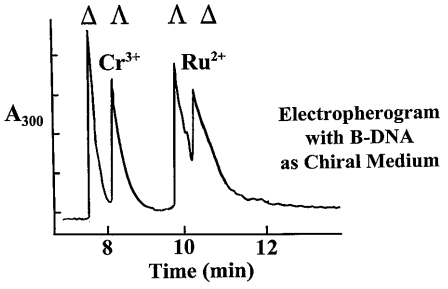

Fig. 10. Electropherogram of a 1:1 mixture of *rac*-$Cr(phen)_3^{3+}$ and *rac*-$Ru(phen)_3^{2+}$ (0.8 mM) in 50 mM Tris–HCl buffer, pH 7.4, containing 100 mM NaCl and 3 mM B-DNA. Electrophoretic field strength 260 V cm^{-1} (run temperature 35°C). Detection $\lambda = 300$ nm.

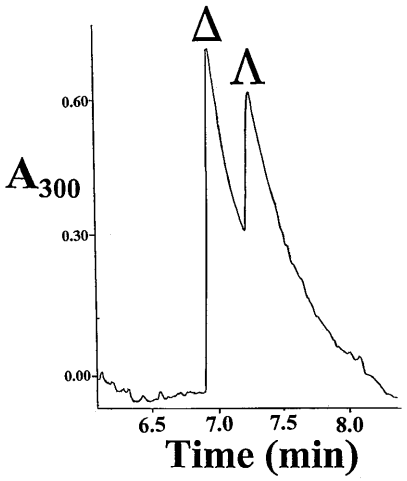

Fig. 11. Electropherogram of *rac*-Co(phen)$_3^{3+}$ (1.5 mM) in 50 mM Tris–HCl buffer, pH 7.4, containing 100 mM NaCl and 6 mM B-DNA. Electrophoretic field strength 260 V cm^{-1} (run temperature 35°C). Detection: $\lambda = 300$ nm.

(Section 3.2.2.3). For tripositive systems one might expect a larger contribution from mode A binding (i.e. for just one phenanthroline ligand inserted in the groove), thus leading to a net Λ preference. Consistent with this interpretation, we note that our preliminary CE studies on the analogous Co(phen)$_3^{3+}$ complex in the presence of B-DNA also reveal a similar binding constant to that for Cr(phen)$_3^{3+}$ and an observed net enantioselectivity in favor of the Λ isomer (Fig. 11). We have also conducted distamycin CE studies for the Cr(phen)$_3^{3+}$/B-DNA system. In the presence of increasing concentrations of distamycin in the buffer medium, a progressive loss of enantiomeric stereoselectivity is observed. This result suggests that both binding modes involve the minor groove.

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