DNA cleavage by intercalatable cobalt–bispicolylamine complexes activated by visible light

Santanu Bhattacharya*† and Subhrangsu S. Mandal

Department of Organic Chemistry, Indian Institute of Science, Bangalore, 560012, India

Two intercalatable Co^{II}-complexes of anthryl or anthraquinone attached bispicolylamine derivatives cleave plasmid pTZ19R DNA spontaneously upon exposure to visible light under ambient conditions.

Redox-active coordination complexes of either synthetic or natural origin that induce DNA cleavages are useful tools in modern molecular biology and in medicine.[1] For example naturally occurring glycopeptide antibiotics such as bleomycin (BLM)[2] which is in use in combination therapy against several types of cancer also utilizes a metal complex to cause DNA cleavage either through ambient O_2 activation or *via* a photonucleolytic pathway.[3] In contrast examples of synthetic systems that effect DNA scission either through activaton of O_2[4] or by light[5] are few. The photonucleases are in particular quite useful as they have greater potential than that of the thermal reagents either in biological chemistry or in photo-therapy.[6] However, many of the reagents either require high concentration or intense UV irradiation to effect appreciable DNA scission. Since the UV light itself is known to induce DNA damage and cross-linking,[7] the use of many such reagents are often compromised. Herein, we introduce two new, water-soluble, bis(2-picolyl)amine based metal complexes that induce pronounced DNA scission at micromolar concentration upon brief exposure to visible light under physiological conditions of pH and ambient temperature.

From the start we considered ligands **1–3**.‡ *N,N*-Bis(2-picolyl)amine (BPA), is a ligand (of complex **1**) that can effectively coordinate with a wide variety of metal ions.[8] Thus it offers scope for structural manipulation and flexibility in the redox potencies of the transition metal complexes. In order to provide stronger binding of such complexes towards DNA, we considered the fact that a number of cytostatic drugs which are presently in clinical use bind DNA by outside binding or by intercalation.[9] This led us to attach an anthryl moiety[10] to BPA to give stronger intercalative capacities towards DNA duplexes. We also tethered BPA with a 2-methyl anthraquinone moiety. This group was chosen because it is present in several antitumour drugs including the well-known radical producing anticancer drug adriamycin.[11]

We first examined the DNA binding properties of the metal complexes **1–3**. When examined by UV–VIS absorption spectroscopy using calf-thymus DNA (CT-DNA) with dark brown **1**, no red-shift could be seen although a small degree of hypochromism was detected. In contrast, complexes **2** (dark brown) or **3** (light brown) which contained anthryl or anthraquinone moieties respectively were found to bind the above DNA avidly. Thus binding of **2** to duplex CT-DNA led to a decrease [>41% at 371 nm, *ca.* 40% at 396 nm. Fig. 1(*a*)] of molar extinction coefficients and *ca.* 7 nm red-shifts (both at 371 and 396 nm). Upon addition of saturating concentration of CT-DNA to **3**, *ca.* 44% hypochromism at *ca.* 335 nm [Fig. 1(*b*)] was also seen. The binding constants were determined as $8.4 \pm 0.5 \times 10^4$ and $9.8 \pm 0.3 \times 10^4$ dm^3 mol^{-1} respectively for **2** and **3** using the method described (inset, Fig. 1).[12] Taken together, these observations indicate a strong electrostatic binding–intercalation[10] by **2** or **3** towards CT-DNA (Fig. 1).

The photoinduced DNA cleavage§ abilities of **1–3** at pH 7.4 at 25 °C were examined by following the conversion of the supercoiled (F-I) plasmid pTZ19R to nicked circle (F-II)

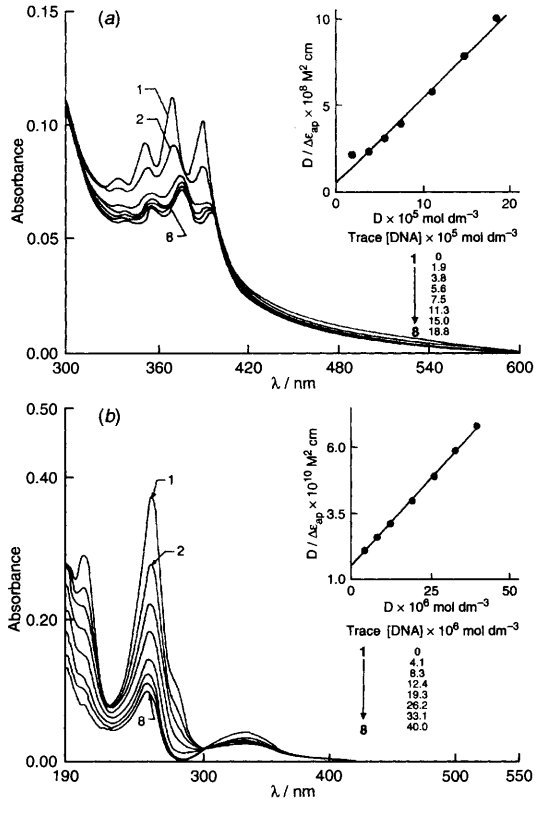

Fig. 1 Absorption titration of the metal complexes with DNA. Performed by adding an increasing amount of CT-DNA to a solution of fixed concentration of the metal complex until saturation in hypochromism was seen. (*a*) Absorption titration spectra of **2** *vs.* CT-DNA: [**2**] = 2.45×10^{-5} mol dm^{-3}, Final concentration of CT-DNA = 18.8×10^{-5} mol dm^{-3}; Inset: Corresponding half-reciprocal plot; Binding constant = $8.4 \pm 0.5 \times 10^4$ dm^3 mol^{-1}. (*b*) Absorption titration of **3** *vs.* CT-DNA: [**3**] = 4.7×10^{-6} mol dm^{-3}, Final concentration of CT-DNA = 4.0×10^{-5} mol dm^{-3}; Inset: corresponding half-reciprocal plot; Binding constant = $9.8 \pm 0.3 \times 10^4$ dm^3 mol^{-1}.

followed by linear (FIII) DNA in 1% agarose gel using supercoiled plasmid pTZ19R. Fig. 2(*a*) shows the effect of the reaction of the complex **2** (at various concentrations) on DNA for 20 min either in visible light or in the dark. Lanes 1 and 2 show supercoiled DNA alone that were kept in the dark and in the light respectively. Lanes 3 and 8 show supercoiled DNA that were treated with 100 and 50 μmol dm^{-3} of the complex **2** respectively but not exposed to light. Lanes 4–7 contained DNA to which 1, 10, 50 and 100 μmol dm^{-3} of the complex **2** were added respectively and were then exposed to light. No significant DNA scission was observed when it was simply exposed to light in the absence of **2** (lane 2) or when it was kept in the dark even though **2** was present (lanes 3 and 8). DNA cleavages were seen in the presence of various concentrations of **2** only when the mixture was exposed to either visible light or sunlight, even on a cloudy day, as evidenced by the conversion of the supercoiled (F-I) DNA to nicked circle (F-II) and then to linear form (F-III) (lanes 4–7). In the absence of light, **1** could not nick DNA at all, while upon exposure to visible light only a small (*ca.* 1% relative to **2**) DNA scission was seen.

Fig. 2(*b*) shows the effects of 20 min incubation of various concentrations of **3** on plasmid pTZ19R in the dark and in the light. Lanes 1 and 2 contained supercoiled plasmid DNA alone in the light and in the dark. Lanes 3–5 show a mixture of DNA and 1, 10 and 50 μmol dm^{-3} of the reagent **3** respectively that were exposed to visible light. Lane 6 shows DNA that was kept in the dark with 50 μmol dm^{-3} of **3**. As observed earlier with **2**, the compound **3** also effected DNA scission efficiently only upon exposure to visible light.

It is noteworthy that the DNA scission activities were detectable even at 1 μmol dm^{-3} concentration of either **2** and **3** [lane 4 in Fig. 2(*a*) and lane 3 in Fig. 2(*b*)]. At 10 μmol dm^{-3} of either of **2** or **3** almost complete conversion of the supercoiled DNA to nicked circle forms (F-II) were seen [lane 5 in Fig. 2(*a*) and lane 4 in Fig. 2(*b*) respectively). At 50 μmol dm^{-3} of either of **2** or **3**, the F-II form started producing linear DNA (F-III) [lane 6 in Fig. 2(*a*) and lane 5 in Fig. 2(*b*) respectively]. At still higher concentration, further degradation of both F-II and F-III took place to produce smaller fragments as evidenced by the smearing in the agarose gel [lane 8 in Fig. 2(*a*)]. The metal complexes of **2** and **3** with a Co^{III} central metal-ion also produced comparable cleavages on plasmid DNA. The analysis of the DNA cleavage products in high resolution sequencing gel using primer extension assay[4] showed almost sequence-neutral fashion DNA cleavage pattern.

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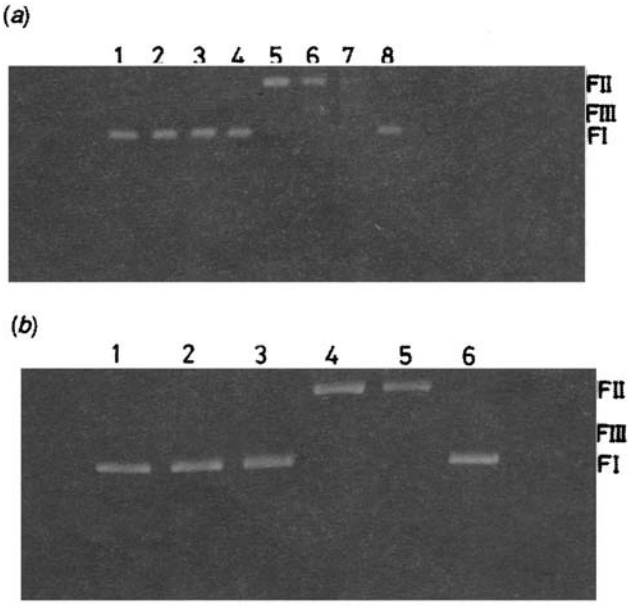

Fig. 2 Cleavage of supercoiled plasmid pTZ19R with **2** or **3**. (*a*) Cleavages of supercoiled plasmid DNA pTZ19R by **2** in the presence and absence of visible light in 20 mmol dm^{-3} tris. HCl for 20 min: Lanes 1 and 2: DNA alone which were kept in dark and light respectively for 20 min; lanes 3 and 8 contain plasmid DNA treated with 100 and 50 μmol dm^{-3} of **2** but kept in the dark; lanes 4–7 contain DNA that was treated with 1, 10, 50 and 100 μmol dm^{-3} of **2** respectively and then were exposed to visible light for 20 min. (*b*) Cleavages of supercoiled plasmid pTZ19R in the presence and absence of light: Lanes 1 and 2 contain supercoiled plasmid pTZ19R in the absence and presence of light; lanes 3–5 contain DNA which were kept in light for 20 min with 1, 10 and 50 μmol dm^{-3} of **3** respectively; lane 6 contained the same supercoiled DNA with 50 μmol dm^{-3} of the **3** but was kept in the dark.

Footnotes

† E-mail: sb@orgchem.iisc.ernet.in

‡ Ligands **2** and **3** were synthesized by the treatment of *N,N*-bis(2-picolyl)amine (BPA) with 9-chloromethylanthracene (reflux, C_6H_6) or 2-bromomethylanthraquinone (reflux, $CHCl_3$) respectively. Column chromatography over silica gel gave pure ligands. The complexes were prepared by addition of a slight stoichiometric excess [Co^{II}:BPA = 1:2.1] of methanolic solutions of the respective ligands **1–3** to a solution of $Co(OAc)_2{\cdot}4H_2O$. The solidification of the complexes were induced by the addition of a few drops of Et_2O and the resulting solids were recrystallized several times from MeOH. All new compunds were thoroughly characterized and the spectroscopic data (^1H NMR, IR and UV–VIS) and elemental analyses were consistent with their given structures.

§ Supercoiled plasmid DNA pTZ19R [plasmid DNA pTZ19R (2.9 kbp, Pharmacia), (250 ng/reaction) was incubated in a reaction mixture (10 μl) containing various concentrations of **1–3** in 20 mmol dm^{-3} Tris.HCl buffer, pH 7.4, at 25 °C (20 min either in light or in dark). Reactions were initiated by exposure to light. After 20 min gel loading dye containing, 20% glycerol, 25 mmol dm^{-3} EDTA and 0.05% (1:1 bromophenol blue and xylene cyanol) was added in the dark. The samples were loaded on 1% neutral agarose gel and were subjected to electrophoresis in a horizontal slab gel apparatus (approximately 100 V for *ca.* 1.5 h). The gel was stained with a solution of 0.5 μg ml^{-1} ethidium bromide for *ca.* 60 min.

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