

Efficient DNA Cleavage Induced by $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in the Presence of Ascorbic Acid

Nanda Gunawardhana¹, Shingo Homi¹ & Masaaki Tabata¹

¹ Department of Chemistry, Faculty of Science and Engineering, Saga University, Japan

Correspondence: Masaki Tabata, Department of Chemistry, Faculty of Science and Engineering, Saga University, 1 Honjo-machi, Saga, 840-8502, Japan. Tel: 81-952-288-560. E-mail: tabatam@cc.saga-u-ac.jp

Received: January 17, 2012 Accepted: February 6, 2012 Online Published: May 27, 2012

doi:10.5539/ijc.v4n3p42

URL: <http://dx.doi.org/10.5539/ijc.v4n3p42>

This research is funded by the JSPS post-doctoral Fellowship for Foreign Researchers; Ministry of Education, Science, Technology, Sports and Culture of Japan, Fellowship, PE-007

Abstract

The ability of Rh(III) tetrakis-*N*-methylpyridyl porphyrin, $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$, to interact with and cleave DNA was investigated by UV-visible, luminescence, circular dichroism (CD), electron spin resonance (ESR) and gel electrophoresis methods in the presence or absence of ascorbic acid. UV-absorption data showed that $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ is capable of interacting with DNA, as indicated by the appearance of a red shift and hypochromicity of the Soret band. The CD data revealed that $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ was capable of binding to DNA via an external binding mode. The $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ showed fluorescence and phosphorescence at room temperature. The phosphorescence increased in the presence of DNA and this could be attributed to the shielding of the metal-porphyrin by DNA. Gel electrophoresis studies revealed that $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ was only able to cleave DNA in the presence of the reducing agent ascorbic acid. ESR data indicated the formation of $\text{Rh}^{\text{II}}(\text{TMPyP})^{5+}$ by reduction of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ with ascorbic acid. The involvement of Rh(III)/Rh(II) species in catalytic DNA cleavage and a possible DNA cleavage mechanism is discussed.

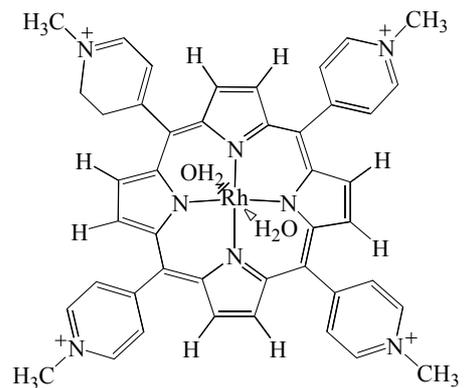
Keywords: $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$, DNA cleavage, ESR, CD spectra, gel electrophoresis

1. Introduction

Porphyrins and metalloporphyrins have been studied extensively by various research groups because of their uses in photodynamic therapy, cancer detection and virus inhibition (McMillin et al., 2005; Romera et al., 2011; Mező et al., 2011). These complexes readily bind to DNA through three binding modes: electrostatic interactions with the negatively charged sugar-phosphate back bone; intercalation between the base pairs of negatively charged DNA; and interactions within the two grooves of the DNA double helix. ESR, CD, Raman, fluorescence and absorption spectroscopic methods have been widely used to understand these binding modes (Pasternack, 2003). A number of physiochemical methods, e.g., gel electrophoresis, melting temperature measurements and hydrodynamic methods such as sedimentation and viscosity measurements, have also been used to determine the binding modes of porphyrins to DNA (Drexler et al., 1998; Pratviel et al., 1989).

The binding modes of metalloporphyrins to DNA depend on many factors, including experimental conditions such as ionic strength, pH, molar ratio of porphyrin to DNA base pair, the properties of the metal center and the porphyrin core and the ligand structure and/or metal coordination geometry. The pioneering work of Pasternack et al. showed that metalloporphyrins lacking axial ligands will intercalate into DNA and exhibit a negative CD in the Soret band (Pasternack, 2003). In contrast, metalloporphyrins that have axial ligands, such as Zn(II)-, Mn(III)-, Fe(III)-, V(IV)- and Co(III)-TMPyP do not intercalate into DNA (Gibbs et al., 1998; Yellappa et al., 2006; Nyarko et al., 2004). Intercalation is believed to be prevented by axial ligands that sterically impede the insertion of the porphyrin ring between the DNA base pairs. In general, these types of metalloporphyrins bind to DNA via electronic interactions between the negatively charged phosphate groups of DNA and the positively charged porphyrin ring. Previous studies have been conducted with plasmid DNA to understand the relative spectral changes in fluorescence and phosphorescence emission of Pt(II), Pd(II) and Au(III) porphyrins (Nyarko et al., 2004; Tabata et al., 2003; Nyarko et al., 2001; Habib et al., 2004; Tabata et al., 1998).

In an attempt to understand more about the interactions of DNA with metals, especially with noble metals such as Pt(II), Pd(II) and Au(III), we have previously studied the relative luminescence changes these types of metalloporphyrins with DNA (Nyarko et al., 2004). The spectral changes of Rh(III)-porphyrins (d6 systems) are of particular interest due to their apparent similarities to Pd(II), Pt(II) and Au(III)-porphyrins (d8 systems). We have also demonstrated the DNA cleavage mechanisms in the presence of Hg(II), Cd(II) and Pb(II)-porphyrins (Tabata et al., 2003; Nyarko et al., 2001; Habib et al., 2004; Tabata et al., 1998). Based on the knowledge that changing the redox properties of metal center changes the ability to generate reactive oxygen species such as O_2 and H_2O_2 , we were able to demonstrate that DNA could be oxidatively damaged by Au(III)-porphyrins. This type of redox activation of metalloporphyrins can be achieved by reducing agents such as ascorbic acid or superoxide anion.



Scheme 1. Structures of the $Rh^{III}(TMPyP)^{5+}$

Although numerous investigations have been carried out to understand DNA interactions with metalloporphyrins that have axial ligands, only one report has indicated the attraction of DNA to a noble-metalloporphyrin (Nyarko et al., 2004). The present paper reports a DNA cleavage by $Rh^{III}(TMPyP)^{5+}$ that occurs only in the presence of ascorbic acid. A more efficient DNA cleavage was observed for $Rh^{III}(TMPyP)^{5+}$ when compared with other metalloporphyrins. This may be due to involvement of Rh(III)/Rh(II) species for enhanced DNA cleavage. A possible DNA cleavage mechanism is discussed.

2. Experimental

2.1 Materials and Methods

Rhodium (III) chloride trihydrate, sodium chloride, sodium hydroxide, L-ascorbic acid, sodium acetate, ethanol was purchased from Wako Chemicals Co. (Osaka, Japan). 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) was purchased from Dojindo laboratories Ltd., Japan. Moreover, $H_2(TMPyP)^{4+}$ was purchased from Dojindo Laboratories and its Rh(III)- porphyrins were prepared by a standard procedure (Golovina et al., 1998). The pBluescript II plasmid DNA was prepared from a plasmid bearing *Escherichia coli* strain using a standard procedure and then dissolved in sterilized water. The concentration of base pairs of DNA was determined by absorbance measurements using $\epsilon_{260} = 1.32 \times 10^4 \text{ mol}^{-1} \text{ dm}^{-3} \text{ cm}^{-1}$ at the absorption maximum of 260 nm. Doubly deionized water was used throughout the experiments (Milli-QPS TOC, Nippon Millipore Lit. Japan).

Unless otherwise mentioned all experiments were carried out at 25 °C.

2.2 UV-visible Measurements

The UV-visible spectra were acquired on a Shimadzu UV 2100 spectrophotometer. The titrations were made by addition of the DNA stock solution directly to the Rh(III)TMPyP solution at increasing concentrations in base pairs, and the added volume of pBluescript II plasmid DNA was no more than 15 μL to avoid complications due to dilution effects within titration (total volume is 1 mL). The mixture was shaken thoroughly, and then equilibrated for 30 minutes.

2.3 Studies on DNA Damage by Gel Electrophoresis

The standard reaction mixture (in a microtube; 1.5 ml; Eppendorf) contains a buffer agent (HEPES), various concentrations of Rh-porphyrins and plasmid DNA. The mixture was incubated at 37 °C for 60 min in a constant

temperature bath (Yamato, Japan). After incubation, the samples were stained with 1.0×10^{-3} ml of a loading buffer (Orange G) and then run in 1 % neutral agarose slab horizontal gel containing Tris, acetic acid and EDTA of pH, 7.4 in 500 ml doubly deionized water for 30 min. The gel was stained by stirring in a solution of $0.5 \mu\text{g l}^{-1}$ of ethidium bromide for 60 min. Gel electrophoresis was performed by means of a Mupid-2 Cosmo Bio Company apparatus (Japan) and DNA bands were photographed with a Polaroid MP-4 land camera using a Polapan black and white coatless film. Moreover, similar gel electrophoresis experiments were carried out with HEPES in the presence of different concentrations of Rh(III)-porphyrins with ascorbic acid as a reducing agent.

2.4 Electron Spin Resonance Measurement

ESR spectra were recorded to detect radicals of Rh species in the presence of ascorbic acid. The spectra were measured at room temperature using a JES-TE 300 (JEOL, Tokyo, Japan) spectrometer. The spectra were recorded with a microwave power of 2 mW and modulation amplitude of 0.63 mT. Moreover, a series of ESR experiments was carried out with different concentrations of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ and $\text{Rh}^{\text{III}}(\text{Br}_8\text{TMPyP})^{5+}$ in the presence of ascorbic acid.

2.5 Circular Dichroism Measurements

Circular dichroism (CD) measurements were conducted with a Jasco J-720 spectropolarimeter (Japan). After each addition of Rh(III)-porphyrin, the spectra were scanned five times at room temperature and then averaged. All experiments were conducted in the presence of 0.10 M sodium chloride. The reagents were added in the order of NaCl, HEPES, DNA, ascorbic acid and Rh(III)-porphyrins. Since the radical formation occurred after the addition of Rh(III)-porphyrin, it was added as a final reagent into the solution.

2.6 Electrochemical Measurements

Electrochemical measurements were carried out in a single-component three-electrode glass cell. A glassy carbon electrode, an Ag/AgCl electrode and a platinum electrode were used as a working electrode, reference electrode and auxiliary electrode, respectively. The glassy carbon (GC) electrode was purchased from Bioanalytical System (area 0.07 cm^2) and was pretreated by sequential polishing with 1 and $0.05 \mu\text{m}$ of alumina/water slurries on felt pads, followed by rinsing with double distilled water prior to use. Solutions were thoroughly deoxygenated, unless otherwise indicated, by bubbling with nitrogen. During the data acquisition, a nitrogen atmosphere was maintained over the solution in the cell. The supporting electrolyte in this research was 0.1 M tetrabutylammonium hexafluorophosphate solution. CV was performed with an 802A Electrochemical Analyzer (CH Instruments, TX).

3. Results and Discussion

3.1 UV-Visible Spectrum Studies

The binding of porphyrin complexes to DNA causes a red/blue shift, hypo/hyper chromism, or a broadening of the Soret band in UV-Vis spectra. These changes depend on the nature of the DNA, the porphyrin and the binding mode. In general, the spectral changes are large for intercalation and small for groove binding or stacking mode. The absorption spectra of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ recorded in the presence of increasing amounts of pBluescript plasmid DNA in HEPES buffer, are shown in Figure 1. Upon addition of pBluescript plasmid DNA to $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$, the Soret band of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ was blue shifted from 435 to 431 nm with 12 % hypochromicity. A similar observation has been previously made for $\text{Co}^{\text{II}}(\text{TMPyP})$ following addition of plasmid DNA. The intrinsic binding constant, as evaluated from Benesi-Hildebrand plots, was $2.0 \times 10^5 \text{ M}^{-1}$ for $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ (See Figure S1, supporting information). Since the spectral shifts, hypochromicities and binding constant were not in the range of associated with intercalation, this suggested that $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ binds to DNA via an outside binding mode (Pratviel et al., 1989; Zhao et al., 2008; Gibbs et al., 1988). The axial ligand in $\text{Rh}^{\text{III}}(\text{Br}_8\text{TMPyP})^{5+}$ create steric difficulties for intercalating $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ into DNA under the present experimental conditions.

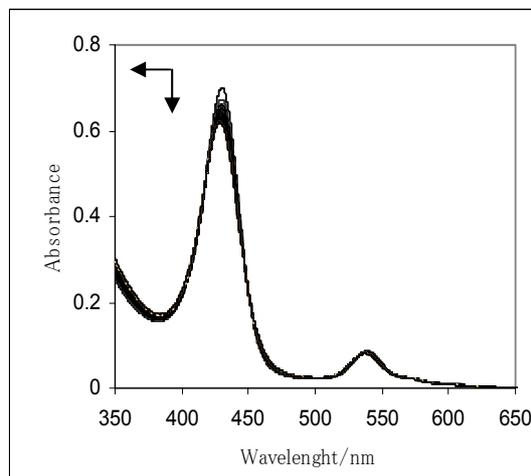


Figure 1. UV-visible spectrum of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ on increasing addition of pBluescript plasmid DNA (0.003 μM each addition) and 10-fold ascorbic acid in HEPES buffer of pH 7.5 and 0.1 M NaCl. Concentration of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ is 3.0 and 3.6 $\times 10^{-6}$ M. Cell path length is 10 mm

3.2 Luminescence Studies

The luminescence of metalloporphyrins is related to the presence/absence of d-d transitions lying between the porphyrin $\pi\text{-}\pi^*$ triplet and the ground state (Vasil'ev et al., 2003). Metalloporphyrin complexes that form with 3d transition metals having low energy excited state of the fields of ligands or charge transfer states do not show luminescence. However metalloporphyrins belonging to the platinum group (Pd(II), Pt(II), Rh(III)) emit luminescence. The luminescence properties of Pd(II), Pt(II) and Rh(III) complexes have been previously studied, along with the generation of singlet oxygen under various conditions. Rh(III)-porphyrins have been demonstrated to show fairly long triplet lifetimes at room temperature; hence, these complexes can be used in photodynamic therapy (Nyarko et al., 2001; Vasil'ev et al., 1999).

Excitation of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in aqueous solution gives rise to very weak fluorescence at 631 nm and moderate phosphorescence at 871 nm. The fluorescence peak is broad whereas the phosphorescence peak is quite sharp. The spectrometric titration of a $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ solution with increasing amounts of pBluescript plasmid DNA provides good information about the interactions of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ with DNA. As shown in Figure 2, addition of DNA decreased the fluorescence intensity of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$. It also caused peak splitting in the spectra, yielding two new peaks at 631 and 649 nm. The relative disappearance of initial fluorescence was 37 %. This quenching may be attributed to the self stacking of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ along the DNA structure. The same type of behavior has been observed previously for $\text{Au}^{\text{III}}(\text{TMPyP})$ with DNA. However, the relative intensity of the phosphorescence peak at 871 nm was increased during the titration of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ with DNA. As described previously, in the absence of oxygen or other quenchers of phosphorescence, the principle paths of degradation of the energy of electronic excitation are radiative and nonradiative deactivation. However, in an aqueous solution, the phosphorescence of the $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ is effectively quenched by molecular oxygen. During titration of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ with DNA, the marked enhancement of phosphorescence could be attributed to the shielding of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ by DNA. This shielding would prevent porphyrin from reacting with the dissolved molecular oxygen in water. Thus, enhancement of phosphorescence is widely accepted as an indication of the interaction between DNA and $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ via outside binding. Previous studies have been shown that the quantum yield for luminescence varies in the following order: intercalation complex < free porphyrin < external, groove bound complex (Rodriguez et al., 1990). Our results agreed well with reported phosphorescence enhancements and confirmed an out-side binding mode for metalloporphyrins with DNA.

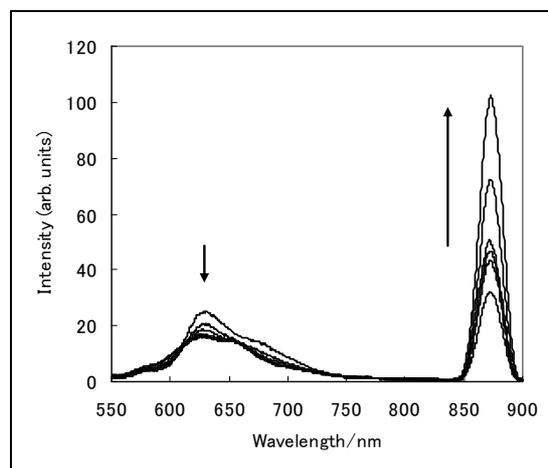


Figure 2. Luminescence spectra of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in the presence of increasing amounts of (1) 0, (2) 2.6, (3) 3.4, (4) 6.8, (5) 12.5, (6) 25.0×10^{-6} M DNA in 0.1 M NaCl solution. Concentration of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ is 4.0×10^{-6} M. Cell path length is 10 mm

3.3 Circular Dichroism Studies

The CD spectra of porphyrins in the Soret region have been reported as well-defined indicators of the binding modes of porphyrins with DNA. In the absence of DNA, the $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ itself does not display CD peaks in the Soret region. However, after interacting with DNA, the achiral porphyrins show different modes in CD spectra. A positive peak in the CD spectrum can be seen when porphyrins bind to the DNA groove; a negative peak can be seen due to intercalation; and a conservative peak can be ascribed to outside binding (Drexler et al., 1998; Pratviel et al., 1989). Figure 3 illustrates the CD spectrum for $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ bound to DNA. The ellipticities observed for $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in the presence of DNA are predominantly positive in character and centered at wavelengths ranging from 440 to 460 nm. This positive character became stronger with the increasing amounts of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$. In addition, weak negative peak was also observed in the CD spectrum in the range of 420 to 440 nm. These observations suggest that the DNA binding mode for $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ is primarily through an external binding, accompanied by partial intercalation or aggregation of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ at a high $[\text{DNA}]/[\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}]$ ratio.

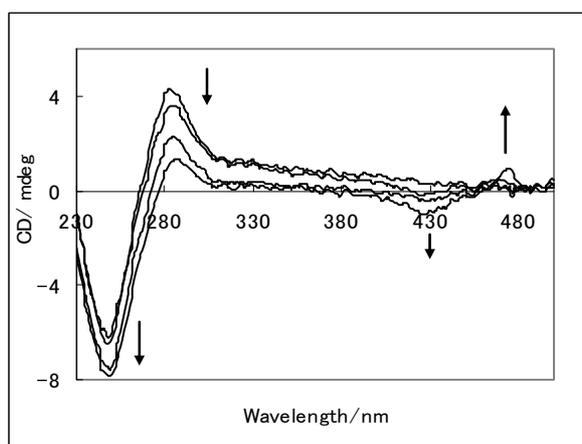


Figure 3. CD spectra of plasmid DNA (1.0×10^{-4} base pairs) upon the addition of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ (1) 0, (2) 6.0, (3) 9.0, (4) 12.0×10^{-6} M. Cell path length is 1 mm

3.4 Electron Spin Resonance Studies

ESR studies can be successfully used to understand the oxidation state, and the site of oxidation or reduction of both the porphyrin macro-cycle and/or the metal center of the metalloporphyrin complexes. ESR studies revealed

that among the various oxidation states of Rh-porphyrins, only Rh(0) (d9), Rh(II) (d7) and Rh(IV) (d5) states are paramagnetic, with $S=1/2$. The low-spin Rh(III) (d6), does not show an ESR signal. Since hyperfine splitting in the EPR spectra depends on the interaction of the electrons with the 4 nitrogen atoms of the porphyrin macro-cycle and the metal center, it can also be used to analyze the strength of interactions of metalloporphyrins (Kadish et al., 1985; Kadish et al., 1993; Hoshino et al., 1984; Nakamura, et al., 2006). As expected, a fresh sample of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ having the Rh(III) oxidation state was ESR silent under the present experimental conditions. However, addition of ascorbic to $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ showed a new peak in ESR spectra indicating formation of new $\text{Rh}^{\text{II}}(\text{TMPyP})^{5+}$ species (See Figure S2, supporting information). These results agreed well with the formation of $\text{Rh}^{\text{II}}(\text{TMPyP})^{5+}$. The $\text{Rh}^{\text{II}}(\text{TMPyP})^{5+}$ eventually generate a hydroxyl anion radical under the experimental conditions. As shown in Scheme-2, this reduction process enhances DNA scission in aqueous media.

3.5 Gel Electrophoresis Studies

As discussed in materials and methods section, samples containing pBluescript plasmid DNA with $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in a HEPES buffer, pH 7.5, were electrophoresed at 37 °C for one hour at different [DNA]/[porphyrin] ratios. The influence of a reducing agent, ascorbic acid, on the reactivity of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ was also examined. The cleavage position can be analyzed by agarose gel electrophoresis experiments. After gel electrophoresis experiments, no DNA cleavage was observed with $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in the absence of ascorbic acid (data not shown). Ascorbic acid does not cleave DNA itself. This non-cleaving behavior of DNA indicates the inability to generate DNA cleavage species by $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ or ascorbic acid alone under the present experimental conditions. However, as shown in Figure 4, DNA cleavage was induced by $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in the presence of ascorbic acid. In Figure 4, lane 1 is a DNA control and lanes 2-6 indicate the decreasing concentrations of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ from 10^{-4} - 10^{-8} mol dm^{-3} . Lane-2, where $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ concentration is 10^{-4} mol dm^{-3} , shows a complete conversion of form I of DNA (the supercoiled form) to form II (the relaxed circular form). The intensity of form II band decreased with decreasing the $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ concentration. As described in Scheme-2, the reduction of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ to generate hydroxyl radicals is an important process for DNA scission.

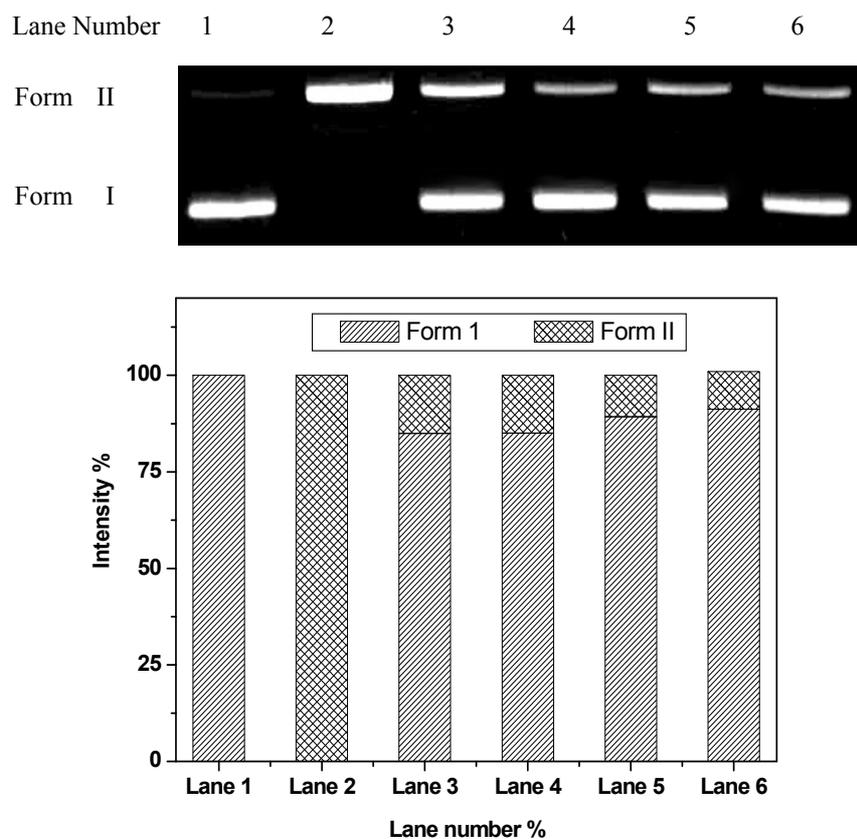


Figure 4. Gel-electrophoresis results showing cleavage of DNA by $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$; Lane 1, DNA control; lane 2-6, 1×10^{-4} to 1×10^{-8} M $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ with 0.1 M ascorbic acid. Concentrations of buffering agents and Sodium chloride were maintained at 1×10^{-2} M and 1×10^2 M, respectively

To understand more about their reduction potential we then measured CV of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in DMF solution. The potential corresponding to the reduction process of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ is located at $E_{1/2} = -0.72$ V vs Ag/AgCl. This indicates that $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ could be more readily reduced than other reported metal-TMPyP complexes.

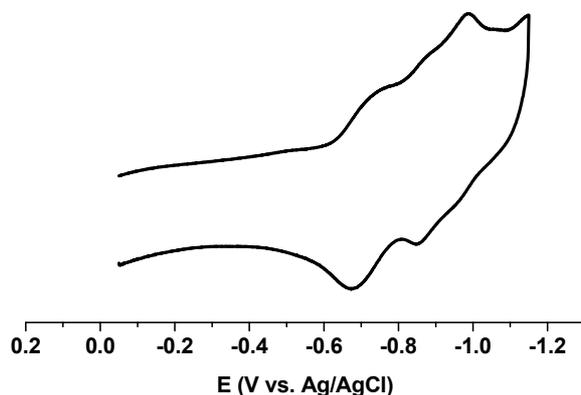
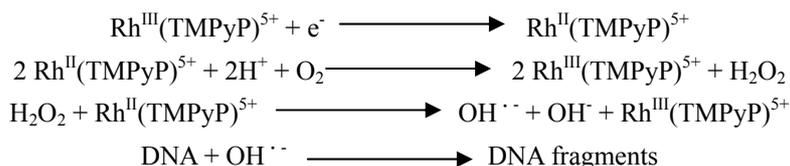


Figure 5. Cyclic voltammogram of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$. Scan rate 0.2 V/s. Reference electrode is Ag/AgCl (3.3 M KCl)

The proposed DNA cleavage mechanism for $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ with ascorbic acid is shown in Scheme-2. According to the proposed mechanism, the initial step involves reduction of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ to a reduced state, $\text{Rh}^{\text{II}}(\text{TMPyP})^{5+}$, in the presence of ascorbic acid. The CV and ESR data confirmed formation of this intermediate. The reduced rhodium porphyrin then binds to dioxygen and catalytically reduces it to hydrogen peroxide. The resulting hydrogen peroxide further reacts with the reduced rhodium porphyrin to generate hydroxyl radicals, which in turn cleave the DNA.



Scheme 2. Proposed Mechanism of Reductively Induced $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ for DNA Cleavage

4. Conclusion

We were able to demonstrate the interactions of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ with plasmid DNA. UV-vis and CD studies were carried out to determine the nature of binding between $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ and plasmid DNA. The relative shift and percent hypochromicity in the Soret band and the binding constant indicate the external binding mode, which was further supported by CD and luminescence studies. The proposed mechanism indicates that catalytic activity is a result of the generation of $\text{OH}^{\cdot\cdot}$ radicals by reduced form of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$, $\text{Rh}^{\text{II}}(\text{TMPyP})^{5+}$. This mechanism is further supported by gel-electrophoresis results that indicate the occurrence of DNA damage only under reducing conditions. In addition ESR data suggest that the reduced form of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$, $\text{Rh}^{\text{II}}(\text{TMPyP})^{5+}$, is responsible for generating $\text{OH}^{\cdot\cdot}$ radicals that enhance the DNA cleaving ability.

Abbreviations

HEPES: 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid

ESR: electron spin resonance

CD: circular Dichroism

CV: cyclic voltammogram

SOD: superoxide dismutase

$\text{OH}^{\cdot\cdot}$: hydroxyl radical

EDTA: ethylenediaminetetraacetic acid

Tris: tris(hydroxymethyl)aminomethane

Rh^{III}(TMPyP)⁵⁺Rh(III) tetrakis-*N*-methylpyridyl porphyrin

References

- Drexler, C., Hosseini, M. W., Pratviel, G., & Meunier B. (1998). Design, Synthesis and Cleaving Activity of an Abiotic Nuclease Based on a Manganese(III). *Chem. Comm.*, 1998(13), 1343-1344. <http://dx.doi.org/10.1039/A801095A>
- Gibbs, E. J., Maurer, M. C., Zhang, J. H., Reiff, W. M., Hill, D. T., Malicka-Blaszkiwick, M., Mckinnie, R. E., Liu, H. Q., & Pasternack, R. F. (1988). Internations of porphyrins with purified DNA and more highly organized structures. *J. Inorg. Biochem.*, 32, 39-46. [http://dx.doi.org/10.1016/0162-0134\(88\)80014-X](http://dx.doi.org/10.1016/0162-0134(88)80014-X)
- Habib, A., & Tabata, M. (2004). Oxidative DNA damage induced by HEPES 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid) in the presence of Au(III). *J. Inorg. Biochem.*, 98(11), 1696-1702. <http://dx.doi.org/10.1016/j.jinorgbio.2004.07.005>
- Hoshino, M., Yasufuku, K., Konishi, S., & Imamura, M. (1984). Optical absorption and ESR spectra of monomeric rhodium(II) tetraphenylporphyrin in 2-methyltetrahydrofuran solution at 77 K. *Inorg. Chem.*, 23, 1982-1986. <http://dx.doi.org/10.1021/ic00181a040>
- Kadish, K. M., Hu, Y., Boschi, T., & Tagliatesta, P. (1993). Factors influencing the site of the electroreduction in rhodium-porphyrins. *Inorg. Chem.*, 32, 2996-3002. <http://dx.doi.org/10.1021/ic00066a007>
- Kadish, K. M., Yao, C. L., Anderson, J. E., & Cocolios, P. (1985). Electrochemical and spectrochemical studies of monomeric rhodium(III) porphyrins in nonaqueous media. *Inorg. Chem.*, 24, 4515-4518. <http://dx.doi.org/10.1021/ic00220a020>
- McMillin, D. R., Shelton, A. H., Bejune, S. A., Fanwick, P. E., & Wall, R. K. (2005). Understanding Binding Interactions of Cationic Porphyrins with B-form DNA. *Coord. Chem. Rev.*, 249(13-14), 1451-1459. <http://dx.doi.org/10.1016/j.ccr.2004.11.016>
- Mező, G., Herényi, L., Habdas, J., Majer, Z., Myśliwa-Kurdziel, B., Tóth, K., & Csík, G. (2011). Syntheses and DAN binding of new cationic porphyrin-tetrapeptide conjugates. *Biophysical Chemistry*, 155(1), 36-44. <http://dx.doi.org/10.1016/j.bpc.2011.02.007>
- Moser, H. E., & Dervan, P. B. (1987). Sequence-specific cleavage of double helical DNA by triple helix formation. *Science*, 238, 645-650. <http://dx.doi.org/10.1126/science.3118463>
- Nyarko, E., & Tabata, M. J. (2001). Interactions of tetracationic mercury(II), cadmium(II) and lead(II) porphyrins with DNA and their effects on DNA cleavage. *J. Porp. Phat.*, 5(12), 873-880. <http://dx.doi.org/10.1002/jpp.557>
- Nyarko, E., Hanada, N., Habib, A., & Tabata, M. (2004). Fluorescence and phosphorescence spectra of Au(III), Pt(II) and Pd(II) porphyrins with DNA at room temperature. *Inorg. Chim. Acta*, 357(3), 739-745. <http://dx.doi.org/10.1016/j.ica.2003.08.023>
- Pasternack, R. F. (2003). Circular dichroism and the interactions of water soluble porphyrins with DNA - A minireview. *Chirality*, 15, 329-332. <http://dx.doi.org/10.1002/chir.10206>
- Pratviel, G., Bernadou, J., Ricci, M., & Meunier, B. (1989). Origin of the Oxygen Atom in C-H Bond Oxidations Catalyzed by a Water-Soluble Metalloporphyrin. *Biochem. Biophys. Comm.*, 160(3), 1212-1218. [http://dx.doi.org/10.1016/S0006-291X\(89\)80132-9](http://dx.doi.org/10.1016/S0006-291X(89)80132-9)
- Romera, C., Bombarde, O., Bonnet, R., Gomez, D., Dumy, P., Calsou, P., Gwan, J-F., Lin, F-H., Defrancq, E., & Pratviel, G. (2011). Improvements of Porphyrins for G-quadruplex DNA targeting. *Biochimie*, 93(8), 1310-1317. <http://dx.doi.org/10.1016/j.biochi.2011.06.008>
- Tabata, M., Kumar, S. A., & Nyarko, E. (2003). Enhanced conformational changes in DNA in the presence of mercury(II), cadmium(II) and lead(II) porphyrins. *J. Inorg. Biochem.*, 94(1-2), 50-58. [http://dx.doi.org/10.1016/S0162-0134\(02\)00635-9](http://dx.doi.org/10.1016/S0162-0134(02)00635-9)
- Tabata, M., Sarker, A. K., & Watanabe, K. (1998). Enhanced DNA Photocleavage by a Subnanomolar Amount of Mercury(II) Porphyrin. *Chem. Lett.*, 27, 325-326. <http://dx.doi.org/10.1246/cl.1998.325>
- Vasil'ev, V. V., Blinova, I. A., Golovina, I. V., & Borisov, S. M. (1999). Photophysical and photochemical properties of the water-soluble porphyrin complexes of metals of the platinum group. *J. Applied Spectroscopy*, 66(4), 583-587. <http://dx.doi.org/10.1007/BF02675390>

- Vasil'ev, V. V., Borisov, S. M., & Golovina, I. V. (2003). Luminescence of Water-Soluble Rh(III) Porphyrins. *Optics and Spectroscopy*, 95(1), 29-34. <http://dx.doi.org/10.1134/1.1595208>
- Yamamoto, S., Hoshino, M., Yasufuku, K., Konishi, S., & Imamura, M. (1984). Wavelength-dependant photochemical reaction of (μ -tetraphenylporphinato)bis[dicarbonylrhodium(I)]. Locking of metal ion into ligand. *Inorg. Chem.*, 23, 195-199. <http://dx.doi.org/10.1021/ic00170a016>
- Yellappa, S., Seetharamappa, Y., Rogers, L. M., Chitta, R., Singhal, R. P., & De Souza, F. (2006). Binding, electrochemical activation, and cleavage of DNA by cobalt(II) tetrakis-N-methylpyridyl porphyrin and its beta-pyrrole brominated derivative. *Bioconjugate Chem.*, 17(6), 1418-1425. <http://dx.doi.org/10.1021/bc060153x>
- Zhao, P., Xu, L. C., Huang, J. W., Zheng, K. C., Liu, J., Yu, H. C., & Ji, L. N. (2008). DNA binding and photocleavage propertoes of a novel cationic porphyrin-anthaquinone hybrid. *Biophys. Chem.*, 134, 72-83. <http://dx.doi.org/10.1016/j.bpc.2008.01.009>

Supporting Information

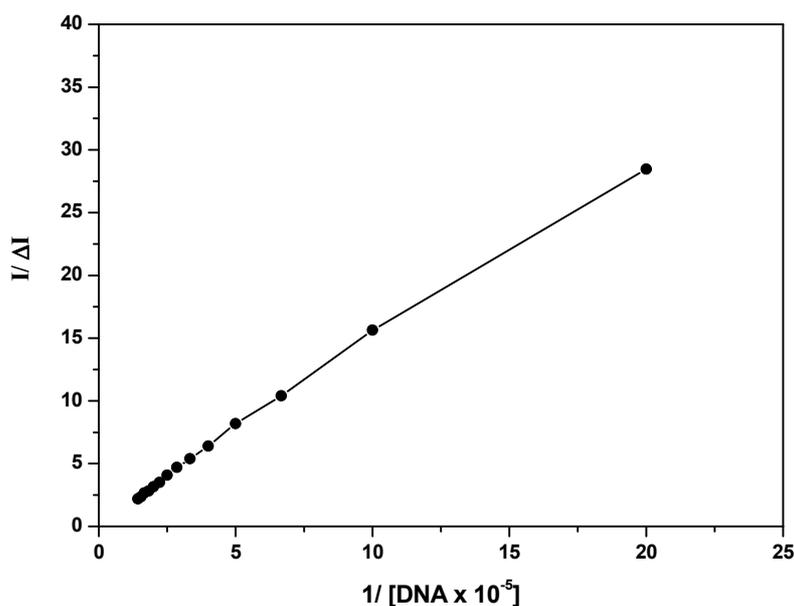


Figure S-1. Benesi-Hildebrand plot of $I/\Delta I$ Versus $1/C$ constructed for the determination of binding constant of $Rh^{III}(TMPyP)^{5+}$ with DNA

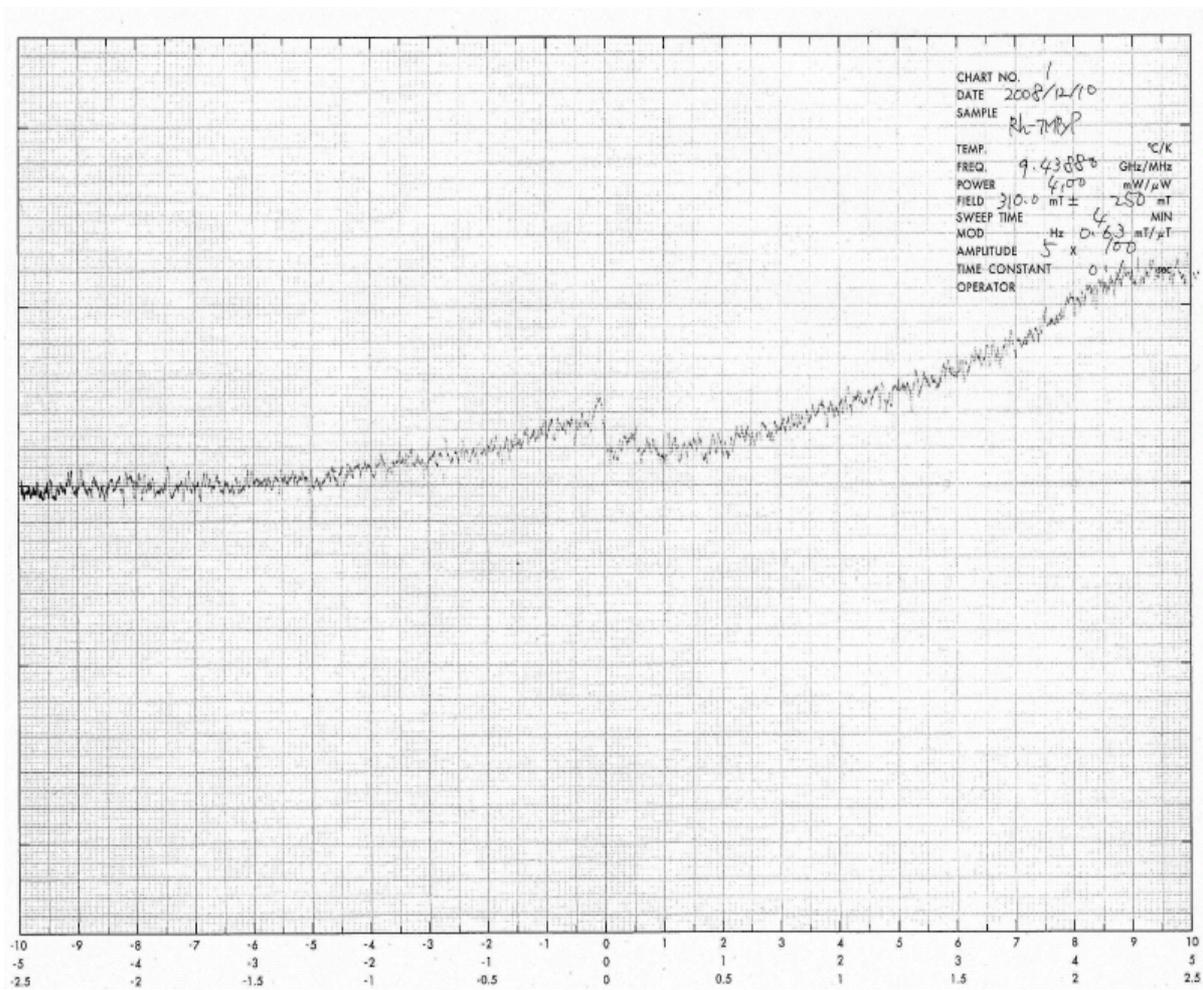


Figure S-2. ESR spectrum of $\text{Rh}^{\text{III}}(\text{TMPyP})^{5+}$ in DMF solution. Concentration of solutions is 1×10^{-5} M. Concentration of ascorbic acid is 1.0 M