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Effect of substituent of terpyridines on the DNA-interaction of polypyridyl ruthenium(II) complexes

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ABSTRACT

An octahedral complexes of ruthenium with 2,9-dimethyl-1,10-phenanthroline (dmphen) and substituted terpyridine have been synthesized. The Ru^{II} complexes have been characterized by elemental analyses, thermogravimetric analyses, magnetic moment measurements, FT-IR, electronic, ^1H NMR and FAB mass spectra. The binding strength and mode of interaction of the complexes with Herring Sperm DNA has been investigated using absorption titration and viscosity measurement studies. Results suggest that the substituent on terpyridine ligand affects the binding mode and binding ability of the complexes. Effect of time and ionic strength on DNA cleavage ability of complex has also been studied by gel electrophoresis. Results suggest that more than 200 mM concentration of NaCl decreases the cleavage ability of complex.

1. Introduction

Metal complexes binding with nucleic acid are currently investigated because of their utility as DNA structural probes, DNA foot printing and specific cleavage agents, and potential anticancer drug [1]. Studies of the interaction of transition metal complexes with DNA have been a pet subject of researchers in the field of bioinorganic chemistry [2]. Transition-metal complexes are well suited for application as artificial nucleases, because of their diverse structural features, and the possibility to tune their redox potential through the choice of proper ligands [3]. Synthetic routes to 2,2′:6′,2″-terpyridines have attracted considerable interest because these heterocycles are used extensively in both coordination chemistry and supramolecular chemistry [4,5].

Dipyridyl complexes of ruthenium have been reported to show high excited state potential but are not very efficient DNA intercalators, while phenanthroline complexes of ruthenium are reported to bind DNA via intercalation. The ruthenium complexes of terpyridine ligands are more rigid compared to bipyridine ligands, and have not been investigated systematically. The complexes of terpyridine moieties have several synthetic and structural advantages over the bipyridine complexes [2]. As far as Ru^{II} complexes with tridentate ligands, the concerned studies have received a limited degree of attention, because interest in such complexes is restricted by the absence of room temperature luminescence of $[Ru(tpy)_2]_2$ [6], and their exact mode and extent of DNA-binding still remain unknown. Therefore, extensive studies on structurally different tridentate ligands are necessary to further evaluate and understand the factors that determine the DNA-binding mode and extent [7]. The octahedral polypyridyl Ru^{II} complexes bind to DNA in three dimensions, but the ancillary ligands can also play an important role in governing DNA-binding of the complexes. By varying substitutive group or substituent position in the ancillary ligand creates some interesting differences in the space configuration and the electron density distribution of Ru^{II} polypyridyl complexes, which will result in some differences in spectral properties and the DNA-binding behaviors of the complexes, and will be helpful to understand the binding mechanism of Ru^{II} polypyridyl complexes to DNA [8].

In this article, we report the synthesis and characterization of three complexes: $[Ru^{II}(4\text{-cptpy})(dmphen)Cl]ClO_4$ (**1**), $[Ru^{II}(ptpy)(dmphen)Cl]ClO_4$ (**2**) and $[Ru^{II}(4\text{-ttpy})(dmphen)Cl]ClO_4$ (**3**), where 4-cptpy = 4′-(4-chlorophenyl)-2,2′:6′,2″-terpyridine, ptpy = 4′-phenyl-2,2′:6′,2″-terpyridine, 4-ttpy = 4′-(4-tolyl)-2,2′:6′,2″-terpyridine, dmphen = 2,9-dimethyl-1,10-phenanthroline. Binding behaviors of the synthesized complexes towards Herring Sperm DNA have been investigated using absorption titration and viscosity measurement methods. Cleavage of pUC19 DNA by the complexes has also been studied by gel electrophoresis technique.

2. Experimental

2.1. Materials and chemicals

2-Acetyl pyridine, 4-chlorobenzaldehyde, benzaldehyde and 4-methylbenzaldehyde were purchased from Spectrochem

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(Mumbai, India). Ruthenium trichloride and sodium perchlorate were purchased from Chemport (Mumbai, India). Luria Broth, agarose, ethidium bromide (EB), TAE (Tris–Acetyl–EDTA), bromophenol blue and xylene cyanol FF were purchased from Himedia, India. Herring Sperm DNA was purchased from Sigma Chemical Co., India. 2,9-Dimethyl-1,10-phenanthroline was purchased from Loba Chemie (India). Culture of pUC19 bacteria (MTCC 47) was purchased from Institute of Microbial Technology, Chandigarh, India.

2.2. *Physical measurements*

Infrared spectra were recorded on Fourier transform IR (FTIR) Shimadzu spectrophotometer as KBr pellets in the range 4000–400 cm^{-1}. The ^1H NMR and ^{13}C NMR were recorded on a Bruker Avance (400 MHz). The fast atomic bombardment mass spectra (FAB MS) were recorded on Jeol SX 102/Da-600 mass spectrometer/data system using Argon/Xenon (6 kV, 10 mA) as the FAB gas. The accelerating voltage was 10 kV and spectra were recorded at room temperature. The electronic spectra were recorded on a UV-160A UV–Vis spectrophotometer, Shimadzu (Japan). TGA was carried out using a 5000/2960 SDTA, TA instrument (USA) operating at a heating rate of 10 °C per minute in the range of 20–800 °C under N_2. C, H and N elemental analyses were performed with a model 240 Perkin Elmer elemental analyzer. The magnetic moments were measured by Gouy's method using mercury tetrathiocyanatocobaltate(II) as the calibrant ($\chi_g = 16.44 \times 10^{-6}$ cgs units at 20 °C), with a Citizen Balance.

2.3. *Synthesis of ligands*

2.3.1. *Synthesis of 4′-(4-chlorophenyl)-2,2′:6′,2″-terpyridine (4-cptpy)*

2-Acetylpyridine (2.42 g, 20.0 mmol) was added to 70 mL ethanolic solution of 4-chlorobenzaldehyde (1.4 g, 10.0 mmol). KOH pellets (1.4 g, 26 mmol) and aqueous NH_3 (30 mL, 25%, 0.425 mol) were added to the solution and was then stirred at room temperature for 8 h (Scheme 1). An off-white solid was formed which was collected by filtration and washed with H_2O (3× 10 mL) and ethanol (2× 5 mL). Recrystallization from $CHCl_3$–MeOH gave white crystalline solid. Yield: 1.48 g, 43%, mp: 168–169 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ/ppm 8.753–8.744 (m, 4H, $H_{3,3',5',3''}$), 8.697 (d, 2H, $H_{6,6''}$), 7.939–7.859 (m, 4H, $H_{4,4''}$, $H_{ph2,6}$), 7.495 (d, 2H, $H_{ph3,5}$), 7.390 (dd, 2H, $H_{5,5''}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 156.24 ($C_{2',6'}$), 155.74 ($C_{2,2''}$), 149.24 ($C_{4'}$), 148.81 ($C_{6,6''}$), 137.4 ($C_{4,4''}$), 136.82 (C_{ph1}), 135.3 (C_{ph4}), 129.23 ($C_{ph2,6}$), 128.65 ($C_{ph3,5}$), 123.96 ($C_{5,5''}$), 121.65 ($C_{3,3''}$), 118.83 ($C_{3',5'}$). Anal. Calc. for $C_{21}H_{14}ClN_3$: C, 73.36; H, 4.10; N, 12.22. Found: C, 73.12; H, 4.24; N, 12.06. UV–Vis (DMSO): λ/nm (ε_{max}/$dm^3\ mol^{-1}\ cm^{-1}$) 280.5 (3.1×10^4).

2.3.2. *Synthesis of 4′-phenyl-2,2′:6′,2″-terpyridine (ptpy)*

The ligand was prepared by the same method as described above, but using benzaldehyde (1.06 g, 10 mmol) instead of 4-chlorobenzaldehyde. Yield: 1.11 g, 36%, mp: 202–204 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ/ppm 8.802 (s, 2H, $H_{3',5'}$), 8.771 (d, 2H, $H_{3,3''}$), 8.72 (d, 2H, $H_{6,6''}$), 7.959–7.91 (m, 4H, $H_{4,4''}$, $H_{ph2,6}$), 7.556–7.456 (m, 3H, $H_{ph3,4,5}$), 7.395 (dd, 2H, $H_{5,5''}$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 155.88 ($C_{2',6'}$), 155.55 ($C_{2,2''}$), 150.47 ($C_{4'}$), 148.78 ($C_{6,6''}$), 138.31 (C_{ph1}), 137.32 ($C_{4,4''}$), 129.11 ($C_{ph2,6}$), 127.39 ($C_{ph3,4,5}$), 123.94 ($C_{5,5''}$), 121.6 ($C_{3,3''}$), 119.2 ($C_{3',5'}$). Anal. Calc. for $C_{21}H_{15}N_3$: C, 81.53; H, 4.89; N, 13.58. Found: C, 81.32; H, 4.71; N, 13.41. UV–Vis (DMSO): λ/nm (ε_{max}/$dm^3\ mol^{-1}\ cm^{-1}$) 279.5 (3.7×10^4).

2.3.3. *Synthesis of 4′-(4-tolyl)-2,2′:6′,2″-terpyridine (4-ttpy)*

The ligand was prepared by the same method as described above but using 4-methylbenzaldehyde (1.2 g, 10 mmol) instead of 4-chlorobenzaldehyde. Yield: 1.26 g, 39%, mp: 151–152 °C. ^1H NMR ($CDCl_3$, 400 MHz) δ/ppm 8.798 (s, 2H, $H_{3',5'}$), 8.776 (d, 2H, $H_{3,3''}$), 8.721 (d, 2H, $H_{6,6''}$), 7.924 (dd, 2H, $H_{4,4''}$), 7.869 (d, 2H, $H_{ph2,6}$), 7.404 (dd, 2H, $H_{5,5''}$), 7.344 (d, 2H, $H_{ph3,5}$), 2.453 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ/ppm 156.26 ($C_{2',6'}$), 155.75 ($C_{2,2''}$), 150.18 ($C_{4'}$), 148.98 ($C_{6,6''}$), 139.1 (C_{ph1}), 136.92 ($C_{4,4''}$), 135.45 (C_{ph4}), 129.66 ($C_{ph3,5}$), 127.15 ($C_{ph2,6}$), 123.75 ($C_{5,5''}$), 121.42 ($C_{3,3''}$), 118.7 ($C_{3',5'}$), 21.25 (CH_3). Anal. Calc. for $C_{22}H_{17}N_3$: C, 81.71; H, 5.30; N, 12.99. Found: C, 81.96; H, 5.13; N, 13.12. UV–Vis (DMSO): λ/nm (ε_{max}/$dm^3\ mol^{-1}\ cm^{-1}$) 283.0 (3.5×10^4).

2.4. *Synthesis of complexes*

2.4.1. *Synthesis of $[Ru^{II}(4\text{-cptpy})(dmphen)Cl]ClO_4$ (**1**)*

The $[Ru^{III}(4\text{-cptpy})Cl_3]$ (5 mmol) was synthesized by stirring 4-cptpy in ethanol (500 mL) with gentle heating till dissolution followed by addition of $RuCl_3{\cdot}3H_2O$ (5 mmol) to it and the solution was refluxed for 3 h with stirring. The mixture was allowed to cool at room temperature resulting in the formation of brown precipitate. The precipitate was filtered off and was washed with ethanol and ether followed by drying in air [9]. The complex $[Ru^{III}(4\text{-cptpy})Cl_3]$ (280 mg, 0.5 mmol), 2,9-dimethyl-1,10-phenanthroline (114 mg, 0.55 mmol), excess LiCl (122 mg, 2.94 mmol) and NEt_3 (0.9 mL) were taken in 50 mL ethanol and the mixture was refluxed for 2 h under a dinitrogen atmosphere (Scheme 1). In this reaction, NEt_3 functions as a reducing agent and facilitates dissociation of the Ru–Cl Bond. The initial dark brown color of the solution gradually changed to a deep purple. The solvent was then removed under reduced pressure. The dry mass was dissolved in a minimum volume of acetonitrile and an excess saturated aqueous solution of $NaClO_4$ was added to it. The precipitate was filtered off and washed with cold ethanol followed by ice-cold water. The product was dried in vacuum and purified using a silica column. The complex was eluted by 2:1 CH_2Cl_2/CH_3CN. Yield: 0.279 g, 71%, mol. wt. 788.04. IR (KBr): ν 3066 w,br; 2920 sh; 1603 m,sh; 1498 m,sh; 1088 s,sh; 756 s,sh; 626 vs,sh; 492 w,sh cm^{-1}. ^1H NMR [dimethyl sulfoxide-d_6 (DMSO-d_6), 400 MHz] δ/ppm 9.512 (s, 2H, $T_{3',5'}$, where T = Terpyridine), 9.117 (d, 2H, $T_{6,6''}$), 8.491 (d, 1H, P_7, where P = Phenanthroline), 8.402 (d, 2H, $T_{3,3''}$), 8.083 (t, 2H, $T_{4,4''}$), 7.875 (d, 2H, $T_{ph3,5}$), 7.864 (s, 2H, $P_{5,6}$), 7.792–7.775 (m, 2H, $P_{4,8}$), 7.757 (d, 1H, P_3), 7.557 (d, 2H, $T_{ph2,6}$), 7.295 (t, 2H, $T_{5,5''}$), 3.321 (s, 3H, CH_3), 2.747 (s, 3H, CH_3). Anal. Calc. for $C_{35}H_{26}Cl_3N_5O_4Ru$: C, 53.34; H, 3.33; N, 8.89%. Found: C, 53.55; H, 3.16; N, 9.05%. FAB MS: m/z = 789 $[M]^+$, 691 $[M-ClO_4+H]^+$, 653 $[M-ClO_4-Cl]^+$, 345 $[4\text{-cptpy}+2H]^+$, 209 $[dmphen+H]^+$.

2.4.2. *Synthesis of $[Ru^{II}(ptpy)(dmphen)Cl]ClO_4$ (**2**)*

The complex was synthesized in a manner identical to that described for $[Ru^{II}(4\text{-cptpy})(dmphen)Cl]ClO_4$, with $[Ru^{III}(ptpy)Cl_3]$ (258 mg, 0.5 mmol) in place of $[Ru^{III}(4\text{-cptpy})Cl_3]$. Yield: 0.233 g, 62%, mol. wt. 753.6. IR (KBr): ν 3072 w,br; 2924 sh; 1596 m,sh; 1496 m,sh; 1083 s,sh; 757 s,sh; 623 vs,sh; 496 w,sh cm^{-1}. ^1H NMR (DMSO-d_6, 400 MHz) δ/ppm 9.481 (s, 2H, $T_{3',5'}$), 9.119 (d, 2H, $T_{6,6''}$), 8.44 (d, 2H, $T_{3,3''}$), 8.34 (d, 1H, P_7), 8.078 (t, 2H, $T_{4,4''}$), 7.864 (s, 2H, $P_{5,6}$), 7.798–7.778 (m, 2H, $P_{4,8}$), 7.698 (d, 1H, P_3), 7.619 (d, 2H, $T_{ph2,6}$), 7.584–7.571 (m, 3H, $T_{ph3,4,5}$), 7.298 (t, 2H, $T_{5,5''}$), 3.354 (s, 3H, CH_3), 2.77 (s, 3H, CH_3). Anal. Calc. for $C_{35}H_{27}Cl_2N_5O_4Ru$: C, 55.78; H, 3.61; N, 9.29%. Found: C, 55.93; H, 3.49; N, 9.42%. FAB MS: m/z = 753 $[M]^+$, 655 $[M-ClO_4+H]^+$, 619 $[M-ClO_4-Cl]^+$, 311 $[ptpy+2H]^+$, 209 $[dmphen+H]^+$.

2.4.3. *Synthesis of $[Ru^{II}(4\text{-ttpy})(dmphen)Cl]ClO_4$ (**3**)*

The complex was synthesized in a manner identical to that described for $[Ru^{II}(4\text{-cptpy})(dmphen)Cl]ClO_4$, with $[Ru^{III}(4\text{-ttpy})Cl_3]$ (265 mg, 0.5 mmol) in place of $[Ru^{III}(4\text{-cptpy})Cl_3]$. Yield: 0.284 g, 74%, mol. wt. 767.62. IR (KBr): ν 3071 w,br; 2925 sh; 1594