Pergamon

PII: S0277-5387(97)00042-9

Polyhedron Vol. 16, No. 19, pp. 3395–3398, 1997

0277–5387/97 $17.00+0.00

Synthesis and DNA binding studies of Co^{III} mixed-ligand complex containing dipyrido [3,2-a : 2′,3′-c]phenazine and phen

Lan Jin[a] and Pin Yang[b]*

[a] State Key Laboratory of Coordination Chemistry, Nanjing University, Nanjing, P.R. China

[b] Institute of Molecular Science, Shanxi University, Taiyuan 030006, P.R. China

(Received 28 October 1996; accepted 16 January 1997)

Abstract—The complexion $[Co(phen)_2dppz]^{3+}$ (where phen is *o*-phenanthroline, and dppz is dipyrido[3,2-a : 2′,3′-c] phenazine) has been synthesized. This complex (as perchlorate salt) was characterized by elemental analysis, molar conductivity and IR spectra. The interaction of the complex with calf thymus DNA has been studied using absorption spectra and fluorescence spectra. The complex is also shown to be a more efficient photosensitiser for single strand breaks in plasmid DNA. © 1997 Elsevier Science Ltd

Keywords: Co^{III}; mixed-ligand complex; DNA; absorption spectra; photochemistry; binding mode.

The interaction of transition metal polypyridyl coordination compounds with DNA has been extensively studied in the past few years, as their unusual binding properties, combined with their general photoactivity, make them suitable candidates as DNA secondary structure probes, photocleavers and antitumor drugs [1].

Most metal polypyridyl coordination compounds are positively charged and may thus bind electrostatically to single or double stranded DNA at low ionic strength. In the case of double stranded DNA, some coordination compounds may also bind in the major groove with one ligand that inserts between two base pairs of DNA. The effect of size, shape, hydrophobicity, and the charge on the binding of the complex to DNA has been studied by changing the type of heteroaromatic ligand or metal center [2,3]. In order to make mixed-ligand coordination compounds intercalate in DNA, the intercalated ligand needs to be flat, with a large surface area and have a special geometry that permits overlapping between the aromatic ring of intercalated ligand and the base pairs in DNA.

Recently, a ruthenium(II) polypyridyl complexion $Ru(phen)_2dppz^{2+}$ was shown to be a remarkable luminescence light switch for DNA [4]. In aqueous solutions, this compound lacks luminescence but it shows intense luminescence in the presence of DNA. Studies of the interaction of this complex with DNA have been concerned largely with characterization of its luminescence properties in the presence and absence of DNA. In contrast, the investigations about the complex as a DNA photocleavage reagent have been relatively fewer. In this paper, we described the synthesis of the mixed-ligand coordination compound of cobalt(III) containing dipyrido[3,2-a : 2′,3′-c]phenazine (Fig. 1) and phen, then extend our studies to the complex-DNA binding system by using a variety of physical methods. The ability of $[Co(phen)_2dppz]^{3+}$ to induce DNA cleavage upon photoexcitation has also been researched. To our knowledge, this type of

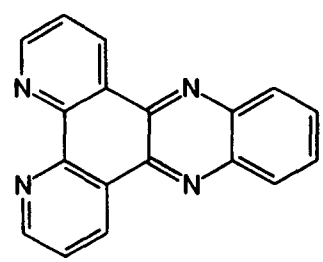

Fig. 1. dppz.

* Author to whom correspondence should be addressed.

cobalt(III) mixed-ligand complex photocleaving DNA is still unknown. The results should be valuable in understanding the mode of the complex binding to DNA, as well as laying the foundation for the rational design of DNA structure probes and antitumor drugs.

EXPERIMENTAL

Materials and methods

$[Co(phen)_2Cl_2]Cl$ was prepared and purified according to the literature [5]. Calf thymus DNA was obtained from Sigma Chemical Company. The solution of DNA was prepared by dissolving DNA in aqueous solution and dialyzing several times against buffer until the UV absorbance ratio A_{260}/A_{280} is greater than 1.90. The concentration of the prepared DNA stock solution was expressed as DNA (p), and was determined according to its absorbance at 260 nm using $\varepsilon_{260} = 6.6\ mM^{-1}\ cm^{-1}$. Ethidium bromide (EthBr) was obtained from Fluka (Buchs, Switzerland), plasmid pBR322 DNA, agarose DNA grade (high gel strength), and Tris were purchased from Beijing Sino-American Biotechnology Company. All other reagents and solvents were analytical reagent grade, and were used as received.

Carbon, hydrogen, and nitrogen contents were determined by a Perkin–Elmer 240c instrument, IR spectra were recorded on a Perkin–Elmer-1700 spectrophotometer with KBr as disks. Absorbance spectra were recorded on a Shimadzu UV-365 spectrophotometer. Fluorescence measurements were made with a Hitachi Model-850 fluorescence spectrophotometer, excitation and emission slits were 5 nm. The electrophoresis experiments were carried out on a set of electrophoresis systems (Beijing Liu Instrument Company) by using TAE buffer (pH = 7.2), the gel was stained with EthBr (0.5 $\mu g/cm^3$) for 0.5 h after electrophoresis, and then photographs under UV light were taken.

Synthesis of $[Co(phen)_2dppz](ClO_4)_3$

$[Co(phen)_2Cl_2]Cl$ (0.526 g) was dissolved in water–methanol (20 cm^3, 1 : 2 V/V), then, dppz (0.449 g), prepared by the literature method [4], was added. The solution was stirred under reflux. After 3 h, the reaction mixture was cooled to room temperature and filtered. To the filtrate was added 30% $NaClO_4$ solution, and a yellowish precipitate formed. The precipitate was washed with ice water, methanol, and then dried *in vacuo* (65%). Found: C 50.2, H 2.5, N 10.8, Calc. for $C_{42}H_{26}N_8CoCl_3O_{12}$: C 50.4, H 2.6, N 11.2%. IR data (cm^{-1}): 1522 m (ν ring), 1506 m ($\delta_{C=C}$), 1429 s (δ_{CCH}), 1091 vs ($\nu_{ClO_4^-}$), 847 s (δ phen), 723 s (δ phen), 625 s ($\delta_{ClO_4^-}$).

RESULTS AND DISCUSSION

Characterization of the Co^{III} *complex*

The conductivity of the soluble complex was recorded in 10^{-3} M DMF solution on a conductivity meter. The molar conductance value of the complex in DMF is in the range of 200–400 suggesting that it is a 1 : 3 electrolyte [6]. The absorption bands δ (C—H) (852 cm^{-1}, 737 cm^{-1}) of phen and of the phen ring at 1558 cm^{-1} are red-shifted to 847 cm^{-1}, 723 cm^{-1}, and 1506 cm^{-1} (phen ring) after coordination, which proves that phen coordinates the Co ion through N.

Absorption studies

The electronic absorption of $[Co(phen)_2dppz]^{3+}$ in the presence of increasing amounts of CT DNA showed strong decreases in the peak intensities (Fig. 2). Hypochromism was suggested to be due to a strong interaction between the electronic state of the intercalating chromophore and that of the DNA bases [7]. In addition to the decrease in intensity, a small red shift and an isosbestic point at 388 nm were also observed in the spectra. These various spectral changes are consistent with the intercalation of $[Co(phen)_2dppz]^{3+}$ into the DNA base stack. These features are equivalent to those observed with $[Ru(phen)_2dppz]^{2+}$ and suggest that cobalt complex binds by intercalation in a manner that parallels $[Ru(phen)_2dppz]^{2+}$ [7].

Fluorescence studies

Binding of the complex to DNA was found to increase the fluorescence intensity. For the $[Co(phen)_2dppz]^{3+}$, no detectable emission is observed in aqueous solution due to quenching by

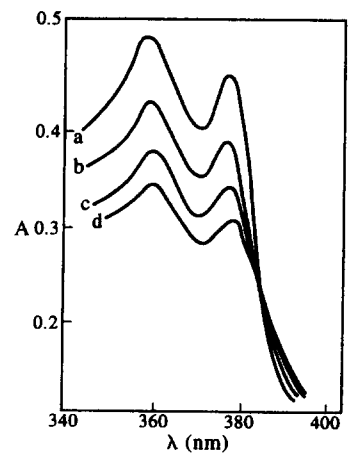

Fig. 2. Absorption spectra of $Co(phen)_2dppz^{3+}$ (10 μM) in the absence and presence of DNA. R_t = [DNA]/[Co] = 0, 0.5, 1, 2 for a–d, respectively.

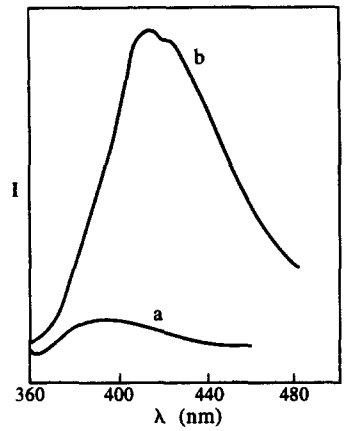

Fig. 3. Fluorescence spectra of $Co(phen)_2dppz^{3+}$ (50 μM) in the absence (a) and presence (b) of DNA, R_t = [DNA]/[Co] = 44. Sample excitation was at 330 nm.

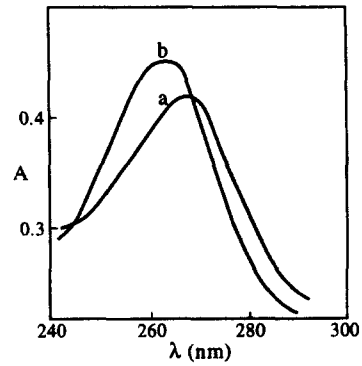

Fig. 4. Absorption spectra of the $Co(phen)_2dppz^{3+}$–DNA system (C_{DNA} = 16 μM, R_t = [DNA]/[Co] = 2) without light (a) and after irradiation at 254 nm for 20 min (b).

hydrogen bonding between water and the phenazine nitrogens of the dppz ligand [8]. Binding to DNA, however, protects the phenazine nitrogens from water through preferential intercalation of the dppz ligand and leads to intense photoluminescence. Figure 3 shows the steady-state of $[Co(phen)_2dppz]^{3+}$ in buffered solution both in the presence and absence of double-stranded DNA.

Photochemistry reaction of $[Co(phen)_2dppz]^{3+}$ *with CT DNA*

Some transition metal polypyridyl coordination compounds can cleave DNA when irradiated at 254 nm light [9]. The irradiation of calf thymus DNA in the presence of $[Co(phen)_2dppz]^{3+}$ was studied so as to determine the efficiency with which it sensitises DNA cleavage. This can be achieved by monitoring the absorption spectra of the complex-DNA system under 254 nm light. Figure 4 shows the change of absorption spectra of the complex-DNA system (the background spectra involving buffer was deducted automatically by the instrument). Without irradiation, the decrease in A_{260} of the complex-DNA system compared to the total sum of absorbance of the complex, and DNA alone, suggested the reaction between the cobalt and DNA. In the presence of light, the A_{260} of the system increased obviously. Meanwhile, we tried observing the absorbance change of $[Co(phen)_2dppz]^{3+}$ or DNA with identical concentration during the same time under 254 nm light, and found that DNA did not change, while the $[Co(phen)_2dppz]^{3+}$ had a small enhancement in absorption. The net spectral change is a "hyperchromic effect". This spectral change process reflected the corresponding change of DNA in its conformation and structures after the drug bound to DNA. The hyperchromism effect resulted from the damage of the DNA double-helix structure. Therefore, the above process reflected the secondary structural damage to DNA. This result will be further supported by the electrophoresis experiment.

*Photoactivated cleavage of pBR*322 *DNA by* $[Co(phen)_2dppz]^{3+}$

The cleavage reaction on plasmid DNA can be monitored by agarose gel electrophoresis. When circular plasmid DNA is subjected to electrophoresis, relatively fast migration will be observed for the intact supercoiled form (Form I). If scission occurs on one strand (nicking), the supercoils will relax to generate a slower-moving open circular form (Form II) [9]. If both strands are cleaved, a linear form (Form III) will be generated that migrates between Form I and II. Figure 5 shows gel electrophoretic separation of pBR 322 DNA after incubation with cobalt complexes and irradiation for variable times. Figure 5(A) reveals the conversion of Form I to Form II after a 40 min

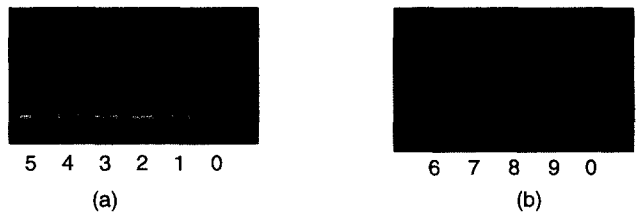

Fig. 5. (A) Cleavage of pBR 322 DNA in the presence of $Co(phen)_2dppz^{3+}$ and light. DNA alone (lane 0), the concentration of $Co(phen)_2dppz^{3+}$ was 2.5, 5, 7.5, 10, 15 μM (lanes 1–5) (B) pBR 322 DNA was incubated with $Co(phen)_2dppz^{3+}$ (10 μM) and irradiated for 5, 10, 20, 30 min (lanes 6–9).

irradiation in the presence of varying concentrations of $[Co(phen)_2dppz]^{3+}$. From Fig. 5(B), it can be seen that with extended irradiation times, Form I of pBR 322 DNA diminishes gradually, whereas the amount of Form II increases. This is the result of single-stranded cleavage of pBR322 DNA. Neither irradiation of the DNA at 254 nm without cobalt nor incubation with cobalt without light yielded significant strand scission. It is likely that the reduction of Co^{III} is the important step leading to DNA cleavage. Here, the Co^{III} complex is photoreduced, with perhaps concomitant hydroxide oxidation that is responsible for cleavage [9]. The mechanism of photoactivated cleave DNA with $[Co(phen)_2dppz]^{3+}$ may be similar to that of $[Co(phen)_3]^{3+}$ [10].

CONCLUSION

Absorption properties of $[Co(phen)_2dppz]^{3+}$ change dramatically as a result of binding to DNA. Strong hypochromism and emission enhancement were observed when $[Co(phen)_2dppz]^{3+}$ binds to DNA. Hypochromism and red-shifted absorption spectra strongly support intercalation of the complex. From the fluorescence, it is concluded that the dppz ligand intercalates into the helix. When irradiated at 254 nm, $[Co(phen)_2dppz]^{3+}$ is capable of inducing single-strand scissions of DNA. The above results clearly indicate that it is possible to design molecular systems that can bind to DNA easily and achieve photoactivated DNA damage. This compound should continue to be useful as tools for probing DNA.

Acknowledgement—This work is supported by the National Science Foundation of China.

REFERENCES

1. Barton, J. K., Danishefsky, A. T. and Goldberg, J. M., *J. Am. Chem. Soc.*, 1984, **106,** 2171.
2. Barton, J. K., *Science*, 1986, **233,** 727.
3. Carter, M., Rodriguez, M. and Bard, A. J., *J. Am. Chem. Soc.*, 1989, **111,** 8901.
4. Hiort, C., Lincoln, P. and Norden, B., *J. Am. Chem. Soc.*, 1993, **115,** 3448.
5. Grassini-Strazza, G. and Isola, S., *J. Chromatogr.*, 1978, **154,** 127.
6. Geary, W. J., *Coord. Chem. Rev.*, 1971, **7,** 81.
7. Tysoe, S. A., Baker, A. D. and Strekas, T. C., *J. Phys. Chem.*, 1993, **97,** 1707.
8. Holmlin, R. E. and Barton, J. K., *Inorg. Chem.*, 1995, **34,** 7.
9. Barton, J. K. and Raphael, A. L., *J. Am. Chem. Soc.*, 1984, **106,** 2466.
10. Fleisher, M. B., Waterman, K. C., Turro, N. J. and Barton, J. K., *Inorg. Chem.*, 1986, **25,** 3549.