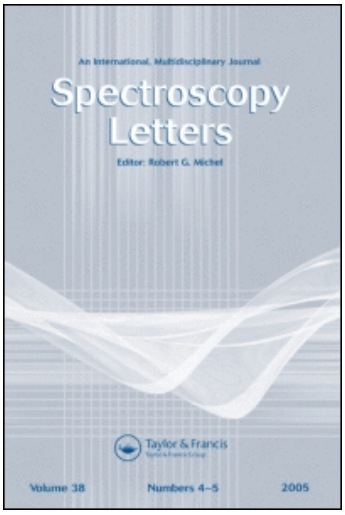

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Spectral Studies on the Interaction of $Ru(phen)_2(dppx)^{2+}$ (phen=1.10-phenanthroline, dppx=7,8-dimethyldipyrido [3,2-a:2',3'-c] phenazine) and DNA

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Spectral Studies on the Interaction of $Ru(phen)_2(dppx)^{2+}$ (phen=1.10-phenanthroline,dppx=7,8-dimethyldipyrido [3,2-a:2`,3`-c]phenazine) and DNA

Keywords: $Ru(phen)_2(dppx)^{2+}$, DNA, Absorption spectroscopy, Fluorescence, Intercalate binding

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Abstract

The interaction of $Ru(phen)_2(dppx)^{2+}$ (phen=1.10-phenanthroline,dppx=7,8-dimethyl dipyrido[3,2-a:2`,3`-c]phenazine) and the calf thymus DNA were studied with fluorescence and ultraviolet visible absorption spectroscopy. $Fe(CN)_6^{4-}$ and NaCl can not quench the fluorescence of $Ru(phen)_2(dppx)^{2+}$-DNA,which indicate that $Ru(phen)_2(dppx)^{2+}$ intercalate into the double helix of DNA. The ultraviolet visible absorption spectrum of $Ru(phen)_2(dppx)^{2+}$, calf thymus DNA, and the results of their interaction reveal that $Ru(phen)_2(dppx)^{2+}$intercalate into the double helix of DNA via the ligand dppx.

1 Introduction

The binding of Ru(II) complex to DNA has been the subject of intense investigation ,owing to their stereo and sequence specific interaction with the double helix [1].In the last decades, people took great interest in studying the molecular "Light Switch" complexes, it mainly referred to $Ru(phen)_2(dppz)^{2+}$ and $Ru(bipy)_2(dppz)^{2+}$ (phen=1,10-phenanthroline, bipy=2,2-bipyridine, dppz=dipyrido [3,2-a:2`,3`-c]phenanthroline)[2-3]. The binding mode between DNA and "Light Switch" complexes have been studied with Linear dichroism [4,5], viscometry[6], unwinding[2], photoluminescence[2,3,7], NMR[8,9] and Tm[9],etc. These studies revealed that the "Light switch" complexes intercalated into the double helix of DNA. Only the method of using NMR clearly showed that $Ru(phen)_2(dppz)^{2+}$ intercalated into the double-helical DNA via

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the ligand dppz. Recently, we found that $Ru(phen)_2(dppx)^{2+}$ (dppx=7,8-dimethyldipyrido[3,2-a:2',3'-c] phenazine) had the same "Molecular Light Switch Effect" for DNA[10], in order to study the binding mode between $Ru(phen)_2(dppx)^{2+}$ and DNA, the ultraviolet-visible spectral changes were used to study the interaction mode between $Ru(phen)_2(dppx)^{2+}$ and DNA, the results clearly showed that $Ru(phen)_2(dppx)^{2+}$ intercalated into the double helix of DNA via the ligand dppx. The results of fluorescence quenching and salt effect also supported the intercalating binding mode.

2 EXPERIMENT SECTION

Materials. Double distilled water was used to prepare all solutions. Unless stated, all the chemicals were of analytical-reagent grade or better. Calf thymus DNA was purchased from Baitai Biochemical Co. (Chinese Academy of Sciences, Beijing, China) .$Ru(phen)_2(dppx)(BF_4)_2.1.5H_2O$ was synthesized according to the reference[11]. $Ru(phen)_3Br_2$ was synthesized in our laboratory .

Instrumentation. Ultraviolet-visible absorption spectra were recorded on a Perkin-Elmer Lambda 17 Spectrophotometer or UV-240 spectrophotometer using 1cm path length cells. The fluorescence intensity was measured with a Shimadzu RF - 540 spectrofluorimeter with a quartz cell (1×1cm cross-section) equipped with a xenon lamp and dual monochrometer. The pH was measured with a Model PHB-4pH meter (Shanghai Leici Equipment Factory, China).

3 RESULTS AND DISCUSSION

3.1 Anionic quenching.

Although $Fe(CN)_6^{4-}$ quenches the groove bound DNA and ruthenium complexes with efficiency less than that of free ruthenium complexes, it does not quench the closely intercalated bound form. Therefore, $Fe(CN)_6^{4-}$ can be used to discriminate the groove binding mode and intercalated binding mode[17]. So the effect of the $Fe(CN)_6^{4-}$ on the fluorescence of $Ru(phen)_3^{2+}$-DNA and $Ru(phen)_2(dppx)^{2+}$-DNA was studied (fig. 1). It showed that the value of I_0/I increased and reached a plateau when the concentration of ferrocyanide increased for the $Ru(phen)_3^{2+}$-DNA system, which was consistent with the results reported previously. As for the $Ru(phen)_2(dppx)^{2+}$-DNA system, the value of I_0/I remained at 1.0 and unchanged with the increase of the concentration of $Fe(CN)_6^{4-}$. It showed that DNA can protect $Ru(phen)_2(dppx)^{2+}$ well from water-bound quencher. Which revealed that there existed intercalate binding mode between $Ru(phen)_2(dppx)^{2+}$ and DNA.

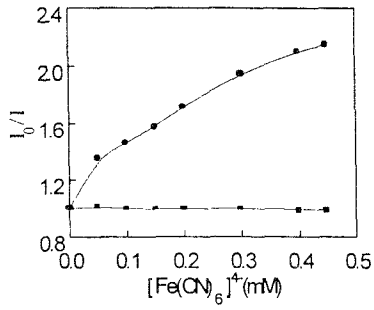

Fig.1.Effect of $[Fe(CN)_6]^{4-}$ on the fluorescence intensity of $Ru(phen)_3^{2+}$-DNA (circle) and $Ru(phen)_2(dppx)^{2+}$-DNA (square)
$Ru(phen)_3^{2+}$:1.0×10^{-5} mol/L
$Ru(phen)_2(dppx)^{2+}$:1.0×10^{-5} mol/L
DNA:15.0 μ g/mL pH=8.0

3.2 Salt effect.

Since the groove bound molecules can be released from the helix by increasing the ionic strength, while it was difficult for the intercalating bound molecules [12], the effect of ionic strength is an efficient method to distinguish binding mode between molecules and DNA. So the effect of NaCl on the fluorescence of $Ru(phen)_3^{2+}$-DNA and $Ru(phen)_2(dppx)^{2+}$-DNA were studied in the range of 0 ~ 0.125mol/L. As shown in fig.2, the I_0/I of $Ru(phen)_3^{2+}$ - DNA increased and reached a plateau with the increase of the concentration of NaCl. Which was consistent with the partial insertion of one phenanthroline between the base pairs [13]. However, the I_0/I of $Ru(phen)_2(dppx)^{2+}$-DNA remained at 1.0 and was independent of the concentration of NaCl. $Ru(phen)_2(dppx)^{2+}$ was very sensitive to the surroundings, and the fluorescence can be thoroughly quenched by water. The results showed that NaCl could not make $Ru(phen)_2(dppx)^{2+}$ release from the double helix of DNA, it revealed that $Ru(phen)_2(dppx)^{2+}$ intercalated into the double helix of DNA.

3.3 The ultra-violet visible absorption spectra of $Ru(phen)_2(dppx)^{2+}$

The absorption spectrum of $Ru(phen)_2(dppx)^{2+}$ was studied by comparing it with that of $Ru(phen)_3^{2+}$,1.10-phenanthroline and dppx. As shown in fig.3, 1.10-phenanthroline had two peaks at the wavelengths of 262.0nm and 227.2nm. While dppx had four peaks, 376.0nm, 357.6nm, 267.2nm and 208.4nm, respectively. The absorption peaks of $Ru(phen)_3^{2+}$ was at the wavelengths of 445.2nm and

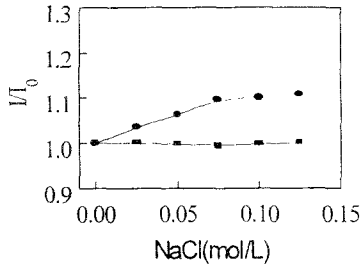

Fig.2.Effect of NaCl on the fluorescence intensity of $Ru(phen)_3^{2+}$-DNA(up) and $Ru(phen)_2(dppx)^{2+}$-DNA(down)
$Ru(phen)_3^{2+}$:1.0×10^{-5} mol/L
$Ru(phen)_2(dppx)^{2+}$:1.0×10^{-5} mol/L
DNA:15.0 μ g/mL pH=8.0

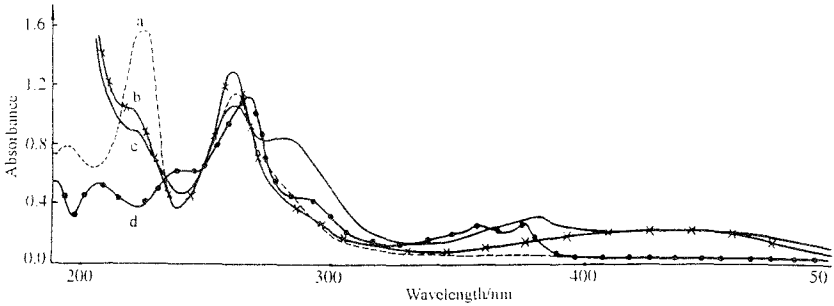

Fig.3 Ultraviolet visible absorption spectrum of $Ru(phen)_2(dppx)^{2+}$, 1.10-phenanthroline, $Ru(phen)_3^{2+}$ and dppx
a. 2.0×10^{-5}mol/L 1.10-phenanthroline
b. 1.0×10^{-5}mol/L $Ru(phen)_3^{2+}$
c. 2.0×10^{-5}mol/L dppx
d. 1.0×10^{-5}mol/L $Ru(phen)_2(dppx)^{2+}$

262.0nm. A peak at 445.2nm was produced by Metal to Ligand Charge Transfer(MLCT) band, and the absorption peak of 262.0nm was attributed to the absorption of 1.10-phenanthroline. But $Ru(phen)_2(dppx)^{2+}$ had four absorption peaks, 441.2nm, 383.0nm, 285.0nm (shoulder peak) and 262.0nm, respectively. Compared with that of $Ru(phen)_3^{2+}$, in addition to the peaks of 1.10-phenanthroline and MLCT band, there appeared two new absorption peaks at 383.0nm and 285.0nm, which were almost at the same site with that of ligand dppx. Therefore, the absorption peaks of 383.0nm and 285.0nm were the contribution of ligand dppx.

3.4 The absorption spectra of $Ru(phen)_2(dppx)^{2+}$ - DNA complexes

The ultraviolet-visible absorption spectra of $Ru(phen)_2(dppx)^{2+}$ - DNA complexes were studied (fig.4).the results showed that the absorption spectra of $Ru(phen)_2(dppx)^{2+}$ - DNA complexes were not the simple accumulation of the single spectrum of $Ru(phen)_2(dppx)^{2+}$ and DNA. Compared to that of free $Ru(phen)_2(dppx)^{2+}$, the absorption intensity increased only at the peak of 262.0nm; while the absorption intensity decreased severely at wavelengths of 383.0nm and 285.0nm, which belong to the absorption of the ligand dppx; the absorption of MLCT band also decreased slightly. It indicated that there existed strong interaction between $Ru(phen)_2(dppx)^{2+}$ and DNA, and the interaction might have occurred between the ligand dppx of $Ru(phen)_2(dppx)^{2+}$ and DNA.

3.5 Effect of DNA on the absorption Spectra of $Ru(phen)_2(dppx)^{2+}$

Great changes in electronic spectra of $Ru(phen)_2(dppx)^{2+}$ were detected when 24.0μg/mL DNA was added to the 10μmol/L $Ru(phen)_2(dppx)^{2+}$ solution (fig.5).The absorption spectra of $Ru(phen)_2(dppx)^{2+}$ with and without DNA clearly showed that there existed strong hypochromism and red shift in the presence of DNA. In the absence of DNA, $Ru(phen)_2(dppx)^{2+}$ had absorption peaks at wavelengths 441.2nm, 383.0nm, 285.0nm and 262.0nm,respectively. When 24.0μg/mL DNA was added, the absorption intensity at these wavelengths decreased by 10.5%, 28.4%, 39.6% and 14.6%, respectively. Red shift occurred at the peak of 383.0nm(5.0nm). Strong hypochromism and red shift was shown to accompany intercalation of the molecules into the base stack [14,15]. The results revealed that there existed intercalate interaction between $Ru(phen)_2(dppx)^{2+}$ and DNA. From the fig.5, It can be seen that the absorption peak belonging to the ligand dppx decreased much more than that of other peaks. It revealed that $Ru(phen)_2(dppx)^{2+}$ may intercalate into the double helical-DNA via the ligand dppx. When the denatured DNA was added to the $Ru(phen)_2(dppx)^{2+}$ solution, there also existed hypochromism and red shift, but the hypochromism attenuated compared to that of adding native DNA. The results showed that DNA denature was unfavorable to the hypochromism of $Ru(phen)_2(dppx)^{2+}$ and hypochromism was greatly related to the double helix of DNA, which was in agreement with the intercalate binding mode.

3.6 Determination of intercalated binding constant.

Since $Ru(phen)_2(dppx)^{2+}$ may intercalate into the double helical-DNA, the apparent intercalate binding constant (K_{ap}) can be calculated with the equation: $C/\Delta\varepsilon_a = C/\Delta\varepsilon + 1/\Delta\varepsilon K_{ap}$ [16]. Where C is the concentration of DNA in mol/L, $\Delta\varepsilon_a = |\varepsilon_a - \varepsilon_F|$, $\Delta\varepsilon = |\varepsilon_B - \varepsilon_F|$, and ε_a, ε_F, and ε_B are

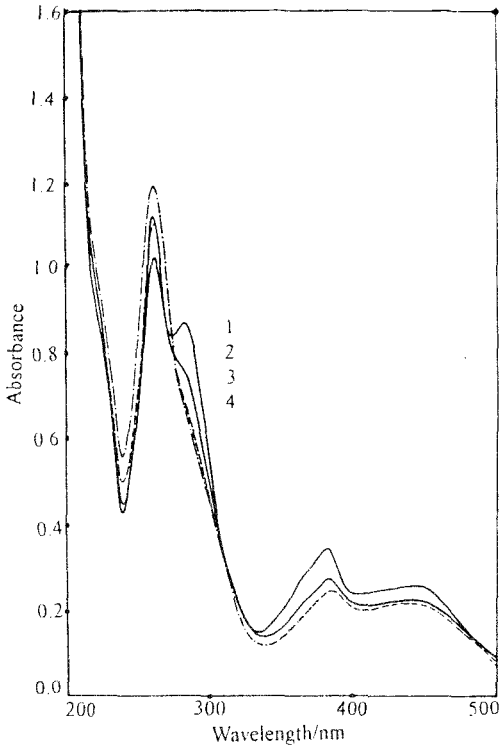

Fig.4 Ultraviolet visible absorption spectrum of $Ru(phen)_2(dppx)^{2+}$ - DNA complex

1. 1.0×10^{-5}mol/L $Ru(phen)_2(dppx)^{2+}$
2. 1+4.0 μ g/mL DNA
3. 1+8.0 μ g/mL DNA
4. 1+12.0 μ g/mL DNA

the apparent , free and bound extinction of $Ru(phen)_2(dppx)^{2+}$, respectively. A plot of $C/\Delta\ \varepsilon_a$ versus C attained the equation as following: $C/\Delta\ \varepsilon_a=1.128\times10^{-11}+3.97\times10^{-5}C$.The correlation coefficient is 0.9989. The apparent intercalate binding constant (K_{ap}) is 3.5×10^5 L/mol was calculated from this equation.

3.7 The effect of $Ru(phen)_3^{2+}$and $Ru(phen)_2(dppx)^{2+}$ on the absorption spectrum of DNA

The effect of $Ru(phen)_3^{2+}$ and $Ru(phen)_2(dppx)^{2+}$ on the absorption spectrum of DNA were shown in fig.6.The spectrum of DNA has an absorption peak at the wavelength of 260nm. The results showed that the

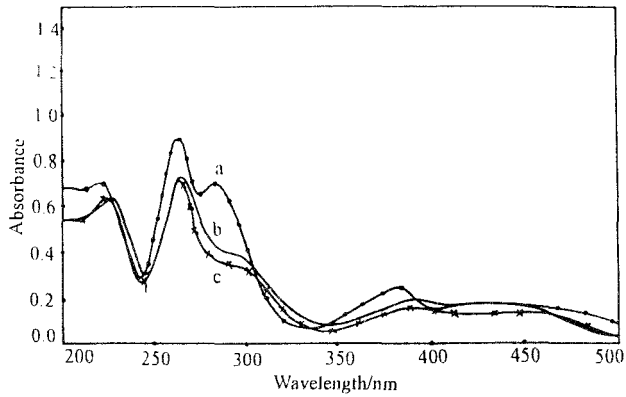

Fig.5 Effect of DNA and denatured DNA on the absorption spectrum of $Ru(phen)_2(dppx)^{2+}$
a.1.0×10^{-5}mol/L $Ru(phen)_2(dppx)^{2+}$
b. a+24.0 μ g/mL denatured DNA
c. a+24.0 μ g/mL native DNA

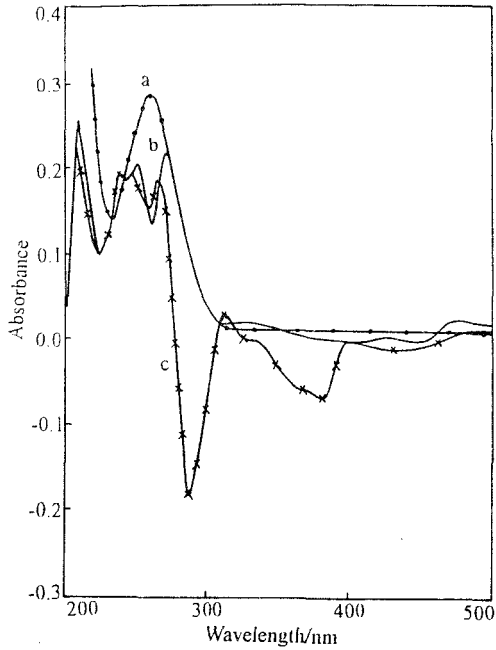

Fig.6 Effect of $Ru(phen)_3^{2+}$ and $Ru(phen)_2(dppx)^{2+}$ on the absorption spectrum of DNA
a. 24.0 μ g/mL DNA
b.a+1.0×10^{-5}mol/L $Ru(phen)_3^{2+}$
c.a+1.0×10^{-5}mol/L $Ru(phen)_2(dppx)^{2+}$

absorption spectrum of DNA changed slightly with the addition of $Ru(phen)_3^{2+}$, the absorption intensity decreased at the wavelength of 260nm, and two new peaks appeared at the wavelengths of 252nm and 272nm, and a wide negative absorption band appeared around the wavelength of 440nm,which may be due to the stack of 1.10-phenanthroline with DNA. But when 1.0×10^{-5} mol/L $Ru(phen)_2(dppx)^{2+}$ was added to the DNA solution, the absorption spectrum changed greatly, compared with that of adding $Ru(phen)_3^{2+}$, in addition to the same change around 260nm and 440nm, there appeared two strong negative peaks at wavelengths of 382.2nm and 288.0nm, which belong to the absorption band of dppx . It well indicated that $Ru(phen)_2(dppx)^{2+}$ intercalated into the double helix of DNA via the ligand dppx.

In conclusion, the interaction between the $Ru(phen)_2(dppx)^{2+}$ and DNA was studied with spectroscopic methods. There are four phenomenon to support the intercalate binding mode: Firstly, $Fe(CN)_6^{4-}$ could not quench the fluorescence of $Ru(phen)_2(dppx)^{2+}$-DNA . Secondly, NaCl had no effect on the fluorescence of $Ru(phen)_2(dppx)^{2+}$-DNA. Thirdly, when DNA was added to the solution of $Ru(phen)_2(dppx)^{2+}$, there exist strong hypochromism and red shift in the absorption spectra of $Ru(phen)_2(dppx)^{2+}$. Fourthly, there appeared two strong negative peaks in the absorption of DNA when $Ru(phen)_2(dppx)^{2+}$ was added in it. Furthermore, the different extents of hypochromism at the different peaks of $Ru(phen)_2(dppx)^{2+}$and the two strong negative peaks also revealed that $Ru(phen)_3(dppx)^{2+}$ intercalated into the double helix of DNA via the ligand dppx.

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