Study of the Interaction between Novel Ruthenium(II)–Polypyridyl Complexes and Calf Thymus DNA

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Abstract

Four new complexes $L_2$Ru$L'$$_2^{2+}$, where $L = 2,2'$-bipyridine (bpy) and 1,10-phenanthroline (phen), $L'$ are Schiff bases or phenylhydrazones derived from 4,5-diazafluoren-9-one (AFO), were prepared. They were characterized by elemental analyses, absorption, IR, and emission spectra. The binding of these complexes to calf thymus DNA has been investigated by absorption, circular dichroism spectroscopy, and viscosity measurement. The experimental results show that the complexes bind to calf thymus DNA by three kinds of binding modes, respectively—electrostatic mode, nonintercalative binding mode, and intercalative binding mode.

Introduction

The binding of DNA of cationic tris-chelates of ruthenium(II) in which the ligands are bidentate diimines with aromatic ring structures has been extensively studied in recent years. An intercalative binding mode, in which a portion of one of the three chelated aromatic rings intercalates between adjacent base pairs of the DNA structure via a major groove, has been proposed to rationalize the observation that enantioselectivity is a feature of the binding of many such complex ions to DNA.

For some complexes (e.g., (phen), Ru(dppez)$$^2$$+, phen = 1,10-phenanthroline; dppez = dipyrido[3,2-a-2',3':c]phenazine), the intercalative binding mode has been accepted by most of the researchers [1–3]. But for Ru(phen)$_2^{2+}$, the most well-studied compounds of the group, there are still several different explanations about the binding mode of this complex. Barton et al. studied the binding of $\Delta$ and $\Lambda$-Ru(phen)$_2^{2+}$ to DNA using equilibrium dialysis, NMR, and photophysical methods [4–6]. It is concluded that $\Delta$, $\Lambda$-Ru(II) complexes use both an intercalative and surface binding mode in interacting with DNA; $\Delta$-Ru(II) complex prefers intercalation, while $\Lambda$ enantiomer prefers surface binding; intercalative binding is proposed to occur in the major groove of DNA, while surface binding occurs in the minor groove.

Experimental

Materials

Calf thymus DNA was purchased from Shanghai Changyang Pharmaceutical Factory. DNA concentrations per nucleotide were determined spectrophotometrically by employing an extinction coefficient of 6600 cm$$^{-1}$$ at 260 nm.

4,5-diazafluoren-9-one (AFO) was prepared according to the literature [10]. Ru(bipy)$_2$Cl$_2$•3H$_2$O, Ru(phen)$_2$Cl$_2$•3H$_2$O were synthesized as described by Meyer et al. [11].

General Methods

Absorption spectra were recorded on a Shimadzu 2000 UV–vis spectrophotometer. IR spectra were obtained with a Nicolet 170SX FT-IR spectrophotometer (KBr pallet). Emission spectra were recorded on a Hitachi Model 850 fluorescence spectrophotometer; samples in CH$_3$CN were not deoxygenated prior to data collection.

Circular dichroism spectra were measured on a Jasco J-20C spectropolarimeter. Equilibrium dialysis experiments were conducted according to the literature [12]. The buffer used was 5 mM tris, 50 mM NaCl at pH 7.0 (for complexes 3, 4, the buffer contained 10% DMSO to improve the solubility). A 5 mL sample of calf thymus DNA was dialyzed against the buffer.
DNA (0.8 mMbp) was dialyzed against 10 mL of buffer plus Ru(II) complex (0.5 mM) for 48 h with continuous agitation. CD spectra were obtained from the dialysate.

Viscosity experiments used a Ubbelodhe viscometer, immersed in a thermostated water both maintained at 27(±0.1)°C. Relative viscosities for DNA in the presence and absence of Ru(II) compounds were calculated from the relation \( \eta = (t - t^0)/t^0 \), where \( t^0 \) is the buffer flow time and \( t \) is the observed flow time [9]. The buffer (5 mM tris-HCl, 50 mM NaCl) contained 10% DMSO to enhance the solubility of the samples for viscosity measurement [13].

Preparation of [Ru(bpy)₂(AFO)](ClO₄)₂

cis-Ru(bpy)₂Cl₂ (1 mmol, 0.52 g) and 4,5-diazafluoren-9-one (1 mmol, 0.182 g) were refluxed in 50% ethanol/50% water under Ar for 8 h. The red solution was filtered hot, and NaClO₄ (10 mmol, 1.4 g) was added. After the solution stood overnight at 0°C, crystals of the product precipitated, which can be used directly in the following preparations. By a slow vapor diffusion of diethyl ether into the acetonitrile solution (in a closed system), the compound crystallized out in a pure form: brownish red, microcrystalline solid.

Preparation of [Ru(phen)₂(AFO)](ClO₄)₂

It was synthesized as described above, using Ru(phen)₂Cl₂ rather than Ru(bpy)₂Cl₂ as the starting material.

Results and Discussion

Characterization of the Complexes

Data of UV-vis, luminescent, and IR spectra are listed in Table 2.
IR data provide the evidence for formation of C=N. One can easily find that in the IR spectra Ru(bpy)$_2$(AFO)$^{2+}$, Ru(phen)$_2$(AFO)$^{2+}$, which contain a carbonyl group, there is a peak at 1740 cm$^{-1}$ corresponding to the C=O vibration band. In the IR spectra of complexes 1, 2, 3, 4, there appears a new peak (ca. 1600 cm$^{-1}$) instead of the peak at 1740 cm$^{-1}$. We assign this new peak to C=N vibration. This clearly shows that new complexes have been formed.

As for the absorption spectra of these compounds, we find from Table 2 that all of them show an absorption near 450 nm, which is the metal-to-ligand charge transfer transition (MLCT). After they reacted with aromatic amine or phenylhydrazine, MLCT absorption shifts red. Nondeoxygenated solution of these complexes in CH$_3$CN at room temperature is found to be luminescent. But in buffer, these complexes are nonemissive whether DNA was added or not.

Absorption Spectra on Binding to DNA

From Table 3, only very weak hypochromism and a spectral shift were found after complexes 1, 2 were mixed with DNA. The optical changes of Ru(II) complexes 1, 2 upon DNA binding are unlike those observed for proven intercalators (e.g., Ru(phen)$_2$dppz$^{2+}$) [15], but very similar to that of Ru(bpy)$_2$$^{2+}$. For the latter compound, DNA has no effect on its absorption spectrum. But for complexes 3, 4, we observed pronounced hypochromism and a large red shift. Because the magnitude of the red shift and hypochromism are found to correlate with the strength of the intercalative interaction [12], we conclude that the complexes 1, 2 synthesized by us do not intercalate into DNA base pairs, while complexes 3, 4 bind to DNA, probably by intercalation.

CD Spectra

For complexes 1, 3 no CD spectra signals were observed, but for complexes 2, 4, CD spectra were developed after 48 h dialysis versus calf thymus DNA (see Fig. 2). Equilibrium dialysis experiments offer the opportunity to examine any enantiomeric selectivities associated with binding. After dialysis of the DNA against the racemic mixture, optical activity observed in the dialysate reflects an enrichment of the less favored enantiomer in the dialysate. So we think that coordination compounds 2, 4 can bind to calf thymus DNA stereoselectively.

Viscosity Measurements

The effect of the complexes on the viscosity of DNA is shown in Fig. 3. Three different kinds of behavior can be distinguished by this experiment. Complex 1 actually has no effect on the viscosity of DNA. For compound 2, at a low concentration of Ru(II) complex, a decrease in DNA viscosity is observed, but as the concentration [Ru] increases, a viscosity increase is seen. The behavior of 2 is very much like that of Δ-Ru(phen)$_2$$^{2+}$ [13], Chaires et al. proposed that such a phenomenon can be considered as evidence of DNA kinking or bending by the compounds. For complexes 3, 4, the viscosity of DNA increases with the increase of the concentration of the complexes, which is similar to that of proven DNA intercalator (e.g., Ru(phen)$_2$dppz$^{2+}$, ethidium) [9, 15].
**Binding Mode**

According to experimental results, we think that there are three kinds of binding modes for the interaction between these complexes and calf thymus DNA.

1. Electrostatic binding mode: Weak hypochromism, no CD spectra, and viscosity measurement show that complex 1 may bind to DNA only by electrostatic force.

2. Nonintercalative binding mode: Although complex 2 has weak hypochromism, it binds to DNA with some enantiomeric selectivity, and can bend or kink DNA. Norden et al. have proposed a binding mode to explain NMR and LD data for the interaction of Ru(phen)$_2^{2+}$ binding to calf thymus DNA [12, 13]. Barton et al. think that besides intercalation, there exists another binding mode—"surface" interaction for the binding of Ru(phen)$_2^{2+}$ with DNA. With our knowledge, we cannot assert which mode is more suitable to explain the experimental results about this complex, but we think the binding of complex 2 to DNA is not classical interaction; it should belong to nonintercalative binding.

   The Schiff base ligand of complex 2 has a substituent (NH$_2$) which would prevent this ligand from intercalating into DNA base pairs. At the same time, the Schiff base is so large that it may impart steric hindrance if phenanthrolines of complex 2 were to intercalate into DNA base pairs. This may be the reason why complex 2 binds to DNA by the nonintenca-vative mode with some degree of enantioselectivity. From these results, we conclude that enantioselectivity associated with binding to DNA has no direct relation to intercalation. It is more likely that the shape of the molecule plays an important role in which binding mode can be adopted, and in whether or not the binding is of enantioselectivity.

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3. Intercalative binding mode: Complexes 3 and 4 show pronounced hypochromism and a large spectral red shift. Both complexes increase the relative viscosity of DNA in a manner consistent with the behavior expected from classical intercalation. We conclude that these two complexes bind to DNA by intercalation.

Complex 3 shows no enantioselectivity on binding to DNA, although it intercalates into DNA base pairs; meanwhile, complex 2 binds to DNA by a nonintegra-tive mode with some degree of enantioselectivity. From these results, we conclude that enantioselectivity associated with binding to DNA has no direct relation to intercalation. It is more likely that the shape of the molecule plays an important role in which binding mode can be adopted, and in whether or not the binding is of enantioselectivity.

*This work was supported by the National Natural Science Foundation of China, the RSC Research Fund, and the Can-An Ma Fund.*

**References**


Received May 25, 1996; accepted September 15, 1996