Chinese Chemical Letters Vol. 16, No. 6, pp 805-808, 2005 805
http://www.imm.ac.cn/journal/ccl.html

Electrochemical Studies on the Interactions of Dipyridophenazine Complex of Ruthenium and Herring Sperm DNA

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Abstract: Cyclic voltammetry (CV) and single-step chronocoulometry were used to study the interaction of $[Ru(phen)_2dppz]^{2+}$ (phen=1,10-phenanthroline; dppz=dipyrido[3,2-a:2′,3′-c]phenazine) with herring sperm DNA. The addition of DNA caused a diminution in the peak current and a positive shift in the peak potential of the complex of $[Ru(phen)_2dppz]^{2+}$. The 12 mV positive shift in the peak potential of $[Ru(phen)_2dppz]^{2+}$ indicates that $[Ru(phen)_2dppz]^{2+}$ binds 2.6 times more strongly to DNA than its reductive form. In addition, by using fluorimetric and UV-spectrophotometric methods and studies of denatured DNA and the effect of NaCl solution, it was also found that the binding mode was intercalation. The decrease of peak current is proportional to the concentration of DNA, which can be applied to estimate DNA concentration.

Keywords: Ru complex, electrochemistry, DNA.

Binding studies on small molecules with DNA play an important role in the development of new therapeutic reagents and DNA molecular probes[1]. $[Ru(phen)_2dppz]^{2+}$, as molecular "light switch", binds extremely strongly to ds-DNA and displays an increase in luminescence owing to emission from the metal-to-ligand charge-transfer excited state[2,3]. To date, the complexes containing the dppz ligand are noted for DNA intercalation by luminescence spectroscopy[4-5], UV-Vis spectroscopy[6], linear dichroism (LD) spectro-scopy[7], circular dichroism spectroscopy[8], viscosity measurements[9], resonance Raman[10], unwinding[11], and NMR spectroscopies[12] studies. While electro- chemical investigations of metal DNA interactions[13] can provide a useful complement to spectroscopic methods, *e.g.* for information about interactions with both the reduced and oxidized form of the metal. To our knowledge, it is the first time to investigate the mechanism of $[Ru(phen)_2dppz]^{2+}$-DNA interactions by using mercury electrode. The results described here supported and extended earlier structural models derived from luminescence studies that the $[Ru(phen)_2dppz]^{2+}$ binds to DNA by intercalation between base pairs[2-5].

Experimental

Cyclic voltammetric experiment and the linear sweep second derivatived polarographic wave were obtained by JP-303 single-sweep oscillopolarography instrument. The chronocoulometric experiment was carried out with CHI660 electrochemical analyzer. The Shimadzu Model UV-2550 spectrophotometer and F3010 spectrofluorometer were used for spectrophotometric determinations.

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Results and Discussion

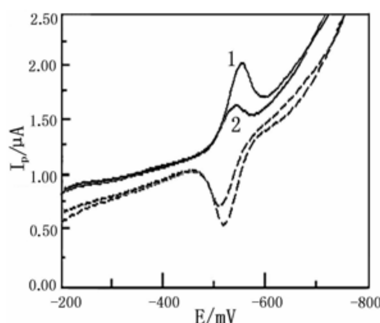
$[Ru(phen)_2dppz](BF_4)_2$ $3.5H_2O$ was synthesized according to the literature[14] and identified by H^1 NMR. Typical CV behavior of $[Ru(phen)_2dppz]^{2+}$ in the absence (curve 1) and presence (curve 2) of herring sperm DNA is shown in **Figure 1**. The $[Ru(phen)_2dppz]^{2+}$ complex had one pair of well-defined redox peaks (ΔE=32 mV, $E_{1/2}$ = -552 mV *vs* SCE) in Tris-HCl buffer (Curve 1). The reductive response near –568 mV *versus* SCE is far away from the redox potential of ruthenium (Ⅱ) to ruthenium (Ⅳ), as well as the same redox peak at the potential of –576 mv and –540 mV in the same conditions showed by the complex of $[Ru(bipy)_2dppz]^{2+}$ and according to the π-acceptor ability of the ligand, the dppz ligand is stronger than phen ligand[17] due to the low energy π^* orbital of the phenazine moiety, so the redox peaks at the potential of $E_{1/2}$ = -552 mV is not assigned to phen ligand but is assigned to redox potential of dppz ligand. According to equation ΔE_p=32=58/n (mV), we come to the conclusion that this electrode process involves a $2e^-$ transfer. Moreover the plot of $E_{1/2}$ *vs* pH is linear over the range pH 7.25~ 10.30 with a slope of 68 mV/pH unit, consistent with a two-electron, one-proton reactions. The addition of 7 μg/mL DNA causes diminution of the peak currents of the reduction of $[Ru(phen)_2dppz]^{2+}$ and the anodic wave on the reverse scan. It is due to variation of the binding state and the slower mass transfer of complexes bound to DNA fragments respectively. In addition, as a sharp decrease takes place in pH 9.40, so Tris-HCl buffer of pH 9.40 was used as optimum medium.

It has been shown that binding of the metal complex to DNA can bring about a shift in the redox potential if one redox state is more strongly bound than the other [15-16] because the intercalative condition affects the equilibrium of Kred/Kox, *i.e.* the $E_{1/2}$ value of the ruthenium complex, so the net shift in $E_{1/2}$ can be used to estimate the ratio of equilibrium constants for the binding of the oxidative and reductive ions to DNA according to the literature [15]:

$$E_b^o - E_f^o = RT/nF\log(K_{red}/K_{ox})$$

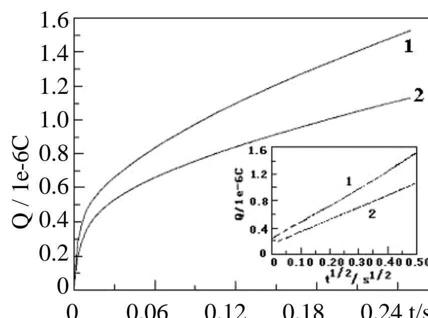
By substituting appropriate values to suit the electrochemistry of $[Ru(phen)_2dppz]^{2+}$ and

Figure 1 Cyclic voltammograms of 1.0×10^{-5} mol/L $[Ru(phen)_2dppz]^{2+}$



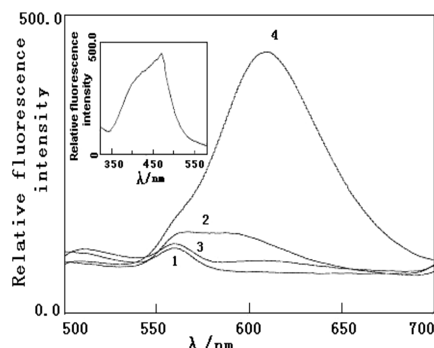
In the absence (curve 1) and presence (curve 2) of 7 μg/mL herring sperm DNA, Tris-HCl (pH=9.40), scan rate 250 mV/s; Accumulation time:5 s; Accumulation potential E_0: -0.20V

Figure 2 Q-t curves of 5.0×10^{-6} mol/L $[Ru(phen)_2dppz]^{2+}$



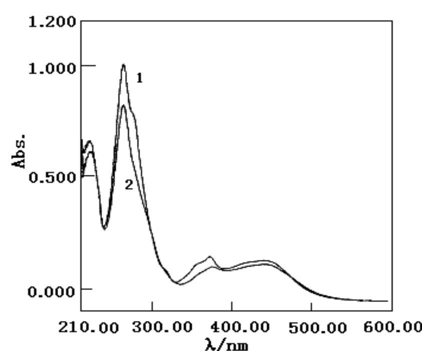
In the absence (curve 1) and presence of 3.5 μg/mL herring sperm DNA (curve 2), inset is the Q-$t^{1/2}$ curve respectively. Bubbling N_2 for 5 min.The other conditions are the same as in **Figure 1**

Figure 3 Excitation and emission spectrum of $[Ru(phen)_2dppz]^{2+}$-DNA



(λex=469.0 nm, λem=611.2 nm) excitation slit (EX) 10 nm; emission slit (EM) 10 nm; Energy. (1) pH= 9.40, Tris-HCl buffer solution. (2) 1+1×10^{-5} mol/L $[Ru(phen)_2dppz]^{2+}$. (3) 1+7 μg/mL DNA. (4) 2+7 μg/mL DNA

Figure 4 UV spectra of the complex



(1) 1×10^{-5} mol/L$[Ru(phen)_2dppz]^{2+}$
(2) 1+7 μg/mL DNA

from a maximum shift of 12mV, it can be calculate that K_{red}/K_{ox} =2.6, thus the reductive species of Ru-complex binds to DNA less strongly than $[Ru(phen)_2dppz]^{2+}$, this result is in agreement with the known intercalative ability of the $[Ru(phen)_2dppz]^{2+}$ [2-3]. The negative E_s value for complex indicates that the complex tend to interact with the negatively charged region of the sugar phosphate backbone in DNA by electrostatic interaction, while the positive E_s value in the present paper indicates that the $[Ru(phen)_2dppz]^{2+}$ bind more favorably with hs-DNA *via* intercalation.

The differences in diffusion coefficient between free and bound forms of the complex have been used to quantitate the extent of binding to the DNA strand [15, 18]. As expected, the covalent attachment of the oligonucleotide to the ruthenium metal complex significantly affects the rate of diffusion. The apparent diffusion coefficient value was calculated by chronocoulometry (**Figure 2**). The diffusion coefficients were determined using the Anson equation:

$$Q = \frac{2nFAD_0^{1/2}C_0^*}{\pi^{1/2}}t^{1/2} + Q_{dl} + nFAF_0$$

Inset in **Figure 2** shows a plot of C *vs* $t^{1/2}$ for the $[Ru(phen)_2dppz]^{2+}$ form and the $[Ru(phen)_2dppz]^{2+}$-DNA form. According to the slopes of these lines, the diffusion coefficients were found to be 3.11×10^{-10} and 1.34×10^{-10} cm^2/s in the absence and presence of DNA respectively, so the decrease in current upon the addition of DNA may be interpreted in terms of diffusion of an equilibrium mixture of free and DNA-bound ruthenium complex to the electrode surface, which suggests that $[Ru(phen)_2dppz]^{2+}$ bounds to DNA so that its equilibrium concentration decreases when DNA was mixed. To show that the decrease in I_{pc} is due to binding of $[Ru(phen)_2dppz]^{2+}$ to the large , slowly diffusing DNA and not to an increase in solution viscosity, the effect of denatured DNA on I_{pc} was investigated. ΔIp caused by denatured DNA was smaller than that by native hs-DNA (not shown) and the impact of salt effect [19] on the system was also examined, a addition of 0.05~0.125 mol/L NaCl solution has little influence on the reaction of hsDNA with the complex (not shown). From above results it can be proposed that the complex intercalated in DNA.

The linear sweep second derivative polarographic waves of cathodic current (I_{pc}) were employed to determine the binding site (m). With reference to the method of Li *et al.*[20], the binding site m = 1.8 was obtained, which is in agreement with the references [21-22].

The absorption of DNA was removed by adding equal amounts of the DNA solution to both the sample and reference cells. With regard to peak at 373.0 nm, a percent hypo-chromicity (defined as $(A_{free} - A_{bound})/A_{free}$) of 31.0% and a bathochromic shift of 2 nm. The "molecular light switch" effect (**Figure 3)** and the bathochromic effect and hypochromicity of UV spectra (**Figure 4)** indicate that the complex of $[Ru(phen)_2dppz]^{2+}$ binds with DNA duplex *via* intercalation in the condition proposed in present paper.

The cathodic current decreases linearly with the concentration of the targeted herring sperm DNA in the range 1~50 μg/mL, and the linear equation is y=8.195-0.07638C_{DNA} (μg/mL), r=0.998. The relative standard deviation was less than 5%, and the detection limit was 0.5 μg/mL by the general procedure, which can be applied to estimate DNA concentration. Many substrates had no interference on the detection of DNA and the applications of determination of DNA in three synthetic samples were satisfactory (**Table 1**). In a word, the proposed method herein is applicable.

Table 1 Determination of DNA in the presence of some coexisting substances

DNA contained in the samples (μg/mL)	Interferences	Amount found	Recovery (%)
10.0	BSA(2μg/mL), Mg^{2+}, Co^{2+}	9.86	98.6
15.0	BSA (2μg/mL), Al^{3+}, Ni^{2+}	15.5	103
20.0	A, K^{+}, Ag^{+}, Zn^{2+}	20.2	101

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Received 29 June, 2004