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A Novel Optical Properties of Molecular "Light Switch" and its Analytical Application

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Abstract: Because of the high affinity ($K_B \geq 10^6$ L mol^{-1}) between $Ru(phen)_2(dppz)^{2+}$ and DNA, the adding of DNA in the solution of $Ru(phen)_2(dppz)^{2+}$-SDS makes the dissociation of $Ru(phen)_2(dppz)^{2+}$-SDS, and results in decrease of the resonance light scattering (RLS) signals and increase of the absorbance. Based on this, a novel method is proposed for DNA assay.

Keywords: $Ru(phen)_2(dppz)^{2+}$, SDS, DNA, resonance light scattering.

Over the past decade there has been substantial interest in the binding properties of a number of ruthenium(Ⅱ) complexes. One of the most interesting observations is the discovery of the molecular "light switch" complexes[1], which are not photoluminescent in water but do emit in nonaqueous solvents or in the presence of DNA. The studies about molecular "light switch" complexes focused on three aspects: 1. Searching for new molecular "light switch" complexes[2-5]; 2. Depicting the charge tendency of fluorescence intensity of molecular "light switch" complexes when they interact with DNA and investigating the interaction mode between molecular "light switch" complexes and DNA[6-10]; 3. Studying the properties and utility of molecular "light switch", such as DNA assay[11] which include single-mismatch DNA detection[12] and triplex DNA study[13]; function of nonaqueous environment[14] and the determination of low concentration of water in nonaqueous solution [15].

Based on the enhancement of RLS signals, many resonance light scattering (RLS) methods were established for DNA assay in recent years[16-18]. Hereby we report an interesting phenomena of decrease of RLS signals after adding DNA in $Ru(phen)_2dppz^{2+}$-SDS-DNA system. Based on this, a novel method for DNA assay is proposed.

Experimental

All RLS measurements were performed using a Perking Elmer Model LS-55 spectrometer with a quartz cuvette (1 × 1 cm). A Shimadzu Model UV-1601 double-beam

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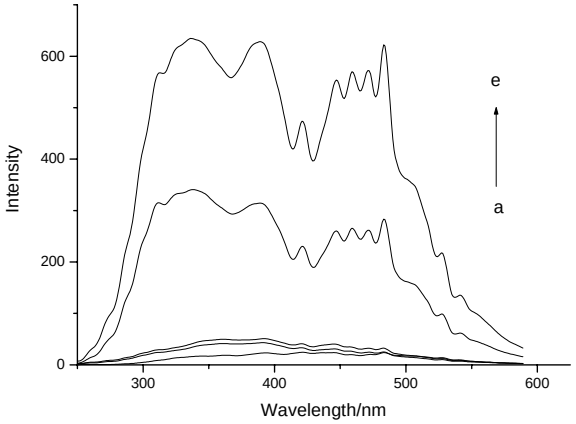
spectrophotometer was used for recording the absorption spectra. The pH was measured with a Model pHS-3C meter (Shanghai Leici Equipment Factory, China).

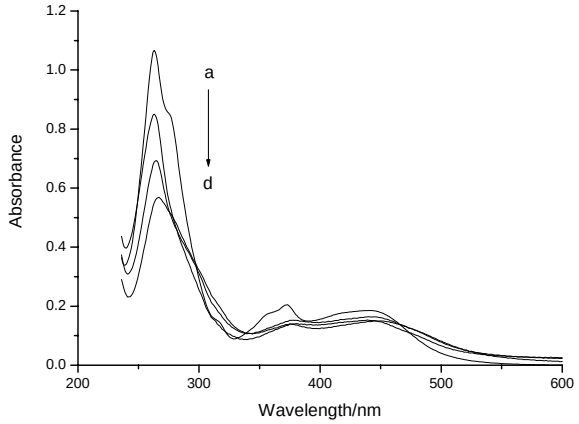
All chemicals were analytical reagents of the best grade commercially available. All stock solutions were prepared using doubly distilled water. The calf thymus DNA was purchased from HuaMei Biochemical Co. $Ru(phen)_2(dppz)(BF_4)_2 \cdot 2H_2O$ was synthesized according to the reference [1] and identified by ^1H NMR[11].

Figure 1 RLS spectra



a. $Ru(phen)_2dppz^{2+}$, b. DNA, c. SDS, d. $Ru(phen)_2dppz^{2+}$+SDS+DNA and e. $Ru(phen)_2dppz^{2+}$+SDS, $Ru(phen)_2dppz^{2+}$: 2×10^{-6} mol L^{-1}, SDS: 2.5×10^{-4} mol L^{-1}, DNA: 0.5 μg mL^{-1}.

Figure 2 Absorption spectra



a. $Ru(phen)_2dppz^{2+}$, b. a+SDS+2.0 μg mL^{-1} DNA, c. a+SDS+1.0 μg mL^{-1} DNA, d. a+SDS, $Ru(phen)_2dppz^{2+}$: 1×10^{-5} mol L^{-1}, SDS: 2.5×10^{-4} mol L^{-1}.

Results and Discussion

The RLS spectra of molecular "light switch" are shown in the **Figure 1**. Reagent blank RLS signals of $Ru(phen)_2dppz^{2+}$ (**Figure 1a**), DNA (**Figure 1b**) and SDS (**Figure 1c**) are low. Both the mixture of SDS and DNA and the mixture of $Ru(phen)_2dppz^{2+}$ and DNA have low RLS signals(not shown in **Figure 1**). However, the RLS signals of the mixture of $Ru(phen)_2dppz^{2+}$ and SDS increase dramatically due to $Ru(phen)_2dppz^{2+}$ assemble on the surface of the SDS micelle (**Figure 1e**). But when DNA was added, the RLS signals decrease greatly (**Figure 1d**), which may be due to that $Ru(phen)_2dppz^{2+}$ has stronger interaction with DNA, intercalating into the base pairs of DNA with the binding constant more than 10^6 L mol^{-1} [1]. So SDS - $Ru(phen)_2dppz^{2+}$ was dissociated and resulted in decrease of RLS signal.

Another interesting phenomenon was the absorption spectra of the system. Previous study showed that strong hypochromic effect and red shift appeared with the absorption spectra of $Ru(phen)_2dppz^{2+}$, owing to the intercalation of $Ru(phen)_2dppz^{2+}$ into the base pairs of DNA[19-21]. But the absorbance value of $Ru(phen)_2dppz^{2+}$ decreased greatly in the presence of SDS, and when DNA was added, the hyperchromic effect instead of hypochromic effect was observed.

The effect of pH, reaction time, concentration of $Ru(phen)_2dppz^{2+}$ and SDS have been studied. Similar phenomenon occurs in the pH range of 1.0 -12.0, and the RLS decreased greatly at pH 9.0 - 10.0. The experiments show that the reaction was complete in 3 minutes and the RLS signals could be stable more than one hour. The concentration of SDS is one of the most important conditions to observe the phenomena of decrease of RLS signals by adding DNA. When the SDS concentration exceeded the range from 5×10^{-5} mol L^{-1} to 5×10^{-4} mol L^{-1} , no decrease can be observed. When the concentration of SDS is lower than 5×10^{-5}mol L^{-1}, no apparent increase of the RLS signals occured by adding SDS into $Ru(phen)_2dppz^{2+}$ solution, and no decreasing of RLS signals were observed by adding DNA. However, the RLS signals will increase by adding DNA while the SDS concentration is higher than 5×10^{-4}mol L^{-1}. $Ru(phen)_2dppz^{2+}$ concentration does not interfere the phenomena of decrease of RLS signals by adding DNA, and the RLS signals changed greatly by adding 0.5 μg mL^{-1} ctDNA while the concentration of $Ru(phen)_2dppz^{2+}$ was 4×10^{-6} mol L^{-1} .

It was also found that the decrease of RLS signals of $Ru(phen)_2(dppz)^{2+}$- SDS-DNA is in proportion to the concentration of DNA and could be used to determine DNA. Under the optimum conditions of pH 9.0(B-R buffer), 4.0×10^{-6} mol L^{-1} $Ru(phen)_2(dppz)^{2+}$ and 2.5×10^{-4} mol L^{-1} SDS, the linear regression equation of ctDNA is I_{RLS}=504.8-348.8 c (c: μg mL^{-1}) and the correlation coefficient is 0.9992, the linear range and the detect limit for ctDNA are 0.018-1.26 μg mL^{-1} and 8.6 ng mL^{-1}, respectively. The proposed method was successfully applied to determine the extracted *colibacillus* plasmid DNA.

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