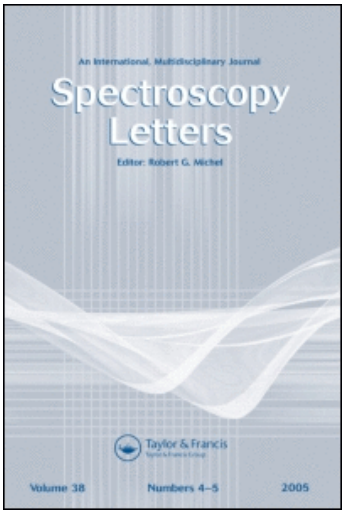

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Characterization of the Binding of Adriamycin to Dna by Spectroscopic Methods

Li Wen-You[a]; He Xi-Wen[a]; Xu Jin-Gou[b]
[a] Department of Chemistry, Nankai University, Tianjin, P.R.China [b] Department of Chemistry, Xiamen University, Xiamen, P.R.China

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CHARACTERIZATION OF THE BINDING OF ADRIAMYCIN TO DNA BY SPECTROSCOPIC METHODS

Keywords: Adriamycin, DNA, Interaction

Wen-You Li [1] *, Xi-Wen He [1], Jin-Gou Xu [2]

[1] *Department of Chemistry, Nankai University, Tianjin 300071, P.R. China*

[2] *Department of Chemistry, Xiamen University, Xiamen 361005, P.R.China*

ABSTRACT

Adriamycin(ADM) binds to the double helical DNA with a high affinity, as deduced from the absorption and fluorescence spectral data. Extensive hypochromism, red shifts, and an isosbestic point in the absorption spectra were observed when ADM binds to calf thymus DNA(CT DNA), which suggested the intercalation mechanism of ADM into DNA bases. Upon binding to DNA, the fluorescence from ADM was efficiently quenched by the DNA bases, with no shifts in the emission maximum. The large increases in the polarization upon binding to CT DNA supported the intercalation of ADM into the helix. Iodide quenching studies showed that the magnitude of K_{sv} of the

* Author to whom correspondence should be addressed.

E-mail: wyli@nankai.edu.cn

bound ADM was lower than that of the free ADM. The results of competitive binding studies showed that ethidium bromide could be displaced by ADM. Thermal denaturation experiments exhibited that the

quenching of the fluorescence from ADM by single strand(ssDNA) was smaller than that by double strand(dsDNA). The results of all further studies also proved the intercalation of ADM into DNA base stack.

INTRODUCTION

There has been considerable interest in understanding those factors that determine affinity and selectivity in the binding of small molecules to DNA [1-7]. These binding studies were driven partly by the need to understand the mechanism of anticancer drug action at the molecular level. A systematic investigation of the binding of antibiotics, heterocyclic cations, and metal complexes with DNA has revealed several structural and electronic factors that control the DNA binding affinity and sequence specificity of small molecules [4,5]. The results of these various binding studies have been very useful in designing new and promising anticancer agents for clinical use [8-10]. The DNA binding studies with drugs have also provided a molecular basis to understand the binding and DNA sequence recognition by proteins.

Small molecules bind to the double helix by two dominant modes, referred to as intercalation and groove binding. Intercalated fluorescent molecules are well protected from the aqueous solvent and water-bound fluorescence quenchers [3,11,12]. In contrast, groove-bound fluorescent molecules may not be protected from water-bound anionic fluorescence quenchers [1]. If K_{sv} (Stern-Volmer quenching constant) of the bound molecule is smaller than that of the free molecule, the interaction between this molecule and DNA should be intercalative binding [3]. In contrast, if K_{sv} of the bound molecule is

bigger than that of the free molecule, the interaction between this molecule and DNA should be groove binding [1]. Electrostatic hydrogen bonding and hydrophobic interactions contribute to the stability of groove binding [13], whereas intercalative binding of molecules is favored by stacking interactions with the adjacent DNA bases [14].

We chose adriamycin (ADM), one of the anticancer drugs, for the DNA binding studies by spectroscopic methods to evaluate its mode of interaction with DNA and the effect of DNA binding on the photophysical properties of the bound ADM. Upon binding ADM to CT DNA, an extensive hypochromic effect, appreciable red shifts, and an isosbestic point in the absorption spectra of ADM, and the efficient fluorescence quenching of ADM by the DNA bases, with no shifts in the emission maximum, were observed. The results of absorption spectra, KI quenching studies, fluorescence polarization measurements, competitive binding studies, and thermal denaturation experiments are consistent with the intercalative binding of ADM to DNA.

EXPERIMENTAL

Apparatus

All fluorescence measurements were made with a Hitachi 650-10S spectrofluorimeter equipped with a 125-W xenon lamp. Absorption spectra were recorded on a Shimadzu UV-240 ultraviolet-visible spectrophotometer. All pH measurements were made with a digital pH and temperature meter 631(Extech, Boston, U.S.A).

Reagents

Commercially prepared CT DNA, obtained from Sino-American Biotechnology Co., was directly dissolved in water at a final concentration of 100μg/ml and stored at 4°C.

The ratio of absorptions at 260 nm/280 nm is 1.9. An ADM solution (1.7×10^{-4} mol/L) was prepared by dissolving 10 mg of adriamycin (Shantou Pharmaceutical Co., China) into 100 ml of water.

A buffer solution of pH 7.0 was prepared by mixing 50 ml of 0.1 mol/L Tris and 46.6 ml of 0.1 mol/L HCl and adding water to a final volume of 100 ml.

Procedures

Experiments of Absorption Spectra

Transfer to a 10-ml standard volumetric flask, 1.0 ml of buffer solution (pH 7.0) and 1.0 ml of ADM solution (1.7×10^{-4} mol/L). Add a known volume of CT DNA standard solution. Dilute to volume with water and mix. Both the mixed solution and a reagent blank (prepared in a similar manner without ADM) are allowed to incubate for 10 min. Record the absorption spectra on a Shimadzu UV-240 ultraviolet-visible spectrophotometer, or equivalent.

Experiments of Fluorescence Spectra

Transfer to a 10-ml standard volumetric flask 1.0 ml of buffer solution (pH 7.0) and 1.0 ml of ADM solution (1.7×10^{-4} mol/L). Add a known volume of CT DNA standard solution. Dilute to volume with water and mix. Both the mixed solution and the reagent blank (prepared in a similar manner without CT DNA) are allowed to incubate for 10 min. Record the fluorescence spectra of the mixed solution and the reagent blank on a Hitachi 650-10S spectrofluorimeter, or equivalent. Measure the fluorescence intensities of the mixed solution (F) and the reagent blank (F_0) with the following settings of the spectrofluorimeter: excitation wavelength (λ_{ex}), 470 nm; excitation slit (EX), 5 nm; emission wavelength (λ_{em}), 595 nm; emission slit (EM), 10 nm.

Fluorescence Quenching Experiments

The fluorescence quenching experiments with potassium iodide were performed and the experimental data were plotted according to the Stern-Volmer equation

$$I_0 / I = 1 + K_{sv}[Q]$$

where $I_0$and I are the fluorescence intensities in the absence and in the presence of the quencher (Q). The Stern-Volmer quenching constant K_{sv} was evaluated by linear least-squares analysis of the data in terms of the above equation.

Fluorescence Polarization Experiments

The fluorescence polarization measurements were carried out on a Hitachi 650-10S spectrofluorimeter. The samples were excited at 470 nm and the fluorescence signals were monitored at 595 nm through a pair of polarizers.

Competitive Binding Experiments

The experiments of competitive binding between ADM and ethidium bromide for DNA were done by adding different quantities of ADM to the system with fixed concentrations of ethidium bromide and DNA, and monitoring the changes in the fluorescence intensity of ethidium bromide (λ_{ex} =525 nm, λ_{em} =585 nm).

RESULTS AND DISCUSSION

Studies of Absorption Spectra

The absorption spectra of ADM in the presence of increasing amounts of CT DNA showed strong decreases in the peak intensities (hypochromic effect) (Fig.1). The hypochromism was suggested to be due to a strong interaction between the electronic states of the intercalating chromophore and that of the DNA bases [15-17]. Since the

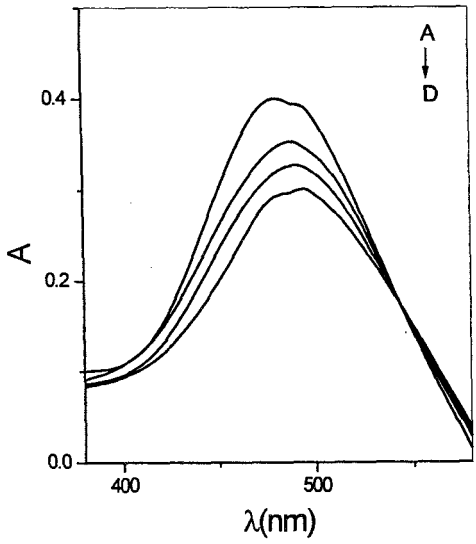

FIG. 1 Absorption spectra of ADM. (A) in the absence of CT DNA; (B)-(D) are of the solution A in the presence of CT DNA with the concentrations of 10.0, 20.0 and 40 μg/ml, respectively. ADM at 1.7×10^{-5} mol/L

strength of this electronic interaction is expected to decrease as the cube of the distance of separation between the chromophore and the DNA bases [17], the observed hypochromism suggests a close proximity of the chromophore to the DNA bases. In addition to the decrease in intensity, a small red shift and an isosbestic point at 544 nm were also observed in the spectra. These spectral changes are consistent with the intercalation of ADM into the DNA base stack [15,16].

Emission Studies

Upon binding to DNA, the fluorescence from ADM was efficiently quenched by the DNA bases with no shifts in the emission maximum. The experimental results showed

that the quenching efficiency of DNA to ADM decreased when the experimental temperature rose. Combined with the result of red shifts in the absorption spectra, the fluorescence quenching of ADM by DNA was fit to the static model.

Iodide Quenching Studies

Since groove binding exposes the bound molecules to the solvent surrounding the helix much more than does the intercalation [18], the iodide quenching experiment was chosen to further establish the DNA binding affinity of ADM. If ADM is intercalated into the helix stack, the magnitude of K_{sv} of the bound ADM should be lower than that of the free ADM [3]; in contrast, if ADM binds to DNA in the groove, the magnitude of K_{sv} of the bound ADM should be higher than that of the free ADM[1]. In aqueous solutions, iodide quenched the fluorescence of ADM very efficiently. Addition of potassium iodide to a mixture of ADM and CT DNA resulted in decreased quenching of the fluorescence intensity (Fig. 2). K_{sv} values for the free ADM and the bound ADM with CT DNA at 2μg/ml were 17.1 L/mol and 8.9 L/mol, respectively. The results showed that the magnitude of K_{sv} of the bound ADM was lower than that of the free ADM, which suggested the intercalation of ADM into the DNA bases [1].

Fluorescence Polarization Measurements

In the absence of DNA, the fluorescence of ADM was weakly polarized due to the rapid tumbling motion of the ADM molecule in aqueous media. However, if the ADM molecule intercalates into the helix, its rotational motion should be restricted, and hence, the fluorescence polarization of the bound chromophore should be increased (Fig. 3). Mere binding to the phosphate backbone or to the DNA grooves does not result in

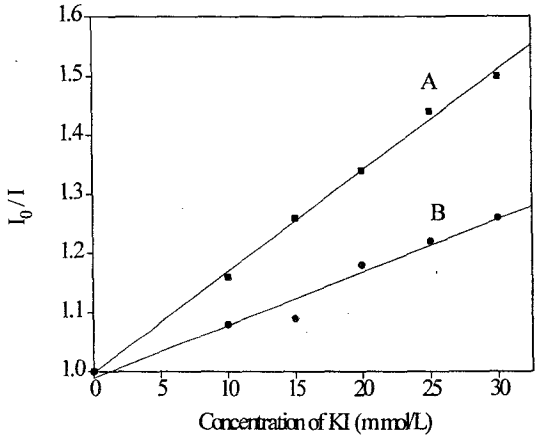

FIG. 2 Fluorescence quenching of ADM by KI in the absence of CT DNA (curve A) and in the presence of CT DNA (curve B). ADM at 1.7×10^{-6} mol/L, CT DNA at 2.0 μg/ml.

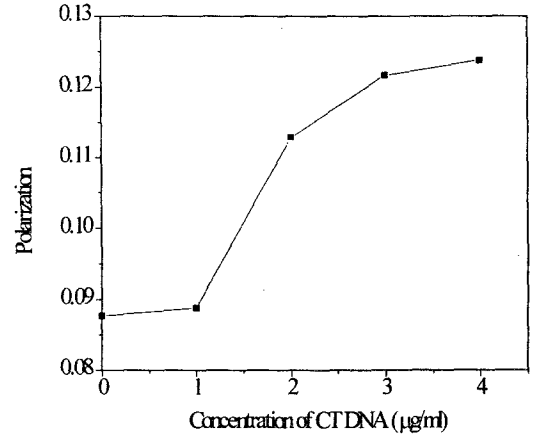

FIG. 3 Influence of CT DNA on fluorescence polarization of ADM . ADM at 1.7×10^{-6} mol/L.

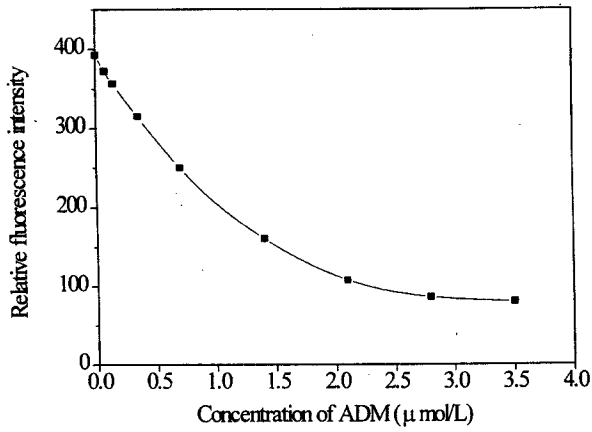

FIG. 4 Influence of ADM on the fluorescence intensity of EB-DNA complex. EB at 2.5×10^{-6} mol/L, CT DNA at 10.0 μg/ml.

enhanced fluorescence polarization [2]. The large increase of fluorescence polarization upon binding ADM to CT DNA supported the intercalation of ADM into the helix [2].

Competitive Binding Studies

Ethidium bromide(EB), a well-known intercalator, binds to DNA with intercalation. The experiment of competitive binding between ADM and EB for DNA was performed by adding different quantities of ADM to the mixture with fixed concentrations of EB and DNA(EB at 2.5×10^{-6} mol/L, CT DNA at 10.0 μg/ml). The results printed in Figure 4 showed that EB could be displaced by ADM, which supported the above intercalation mechanism of ADM into DNA bases.

Comparison between the Effects of dsDNA and ssDNA on the Quenching of ADM Fluorescence

Double strand DNA(dsDNA) was converted into single strand DNA(ssDNA) with

TABLE 1 Comparison between the effects of dsDNA and ssDNA on the quenching of ADM Fluorescence[a]

Concentration of DNA(μg/ml)	Kinds of DNA	F
0.50	dsDNA	188
	ssDNA	202
1.0	dsDNA	139
	ssDNA	164
2.0	dsDNA	66
	ssDNA	115

a: ADM at 1.7×10^{-6} mol/L

the opening of its double helix by incubating at 100°C for 10 min. and cooled in ice-water immediately. If ADM is intercalated into the helix stack, the quenching of the fluorescence from ADM by ssDNA would be smaller than that by dsDNA. The results of comparison experiments given in Table 1 exhibited that the quenching of the fluorescence from ADM by ssDNA was smaller than that by dsDNA, which also supported the intercalation of ADM into DNA helix.

CONCLUSIONS

ADM binds to the double helical DNA with a high affinity. Strong hypochromism, appreciable red shifts, and an isosbestic point in the absorption spectra can be observed when ADM binds to CT DNA. Upon binding to DNA, the fluorescence of ADM was efficiently quenched by the DNA bases, with no shifts in the emission maximum. The results of absorption spectra, KI quenching studies, fluorescence polarization

measurements, competitive binding studies, and thermal denaturation experiments suggested that the interaction between ADM and DNA is intercalative.

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