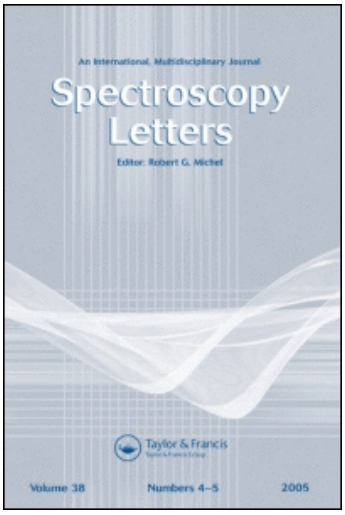

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A SPECTROSCOPIC STUDY ON THE DNA BINDING BEHAVIOR OF THE ANTICANCER DRUG DACARBAZINE

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A SPECTROSCOPIC STUDY ON THE DNA BINDING BEHAVIOR OF THE ANTICANCER DRUG DACARBAZINE

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ABSTRACT

The selective binding of some drug to DNA has been explored in this study to elucidate the structure-function relationship of the anticancer agent. In the case of the binding behavior of the anticancer drug dacarbazine [5-(3,3-dimethy-1-triazenyl)imidazole-4-carboxamide; DTIC], it was found that DNA causes significant decrease of the absorption intensity of DTIC without any shift of the peak position. Besides, nuclear magnetic resonance (NMR) study shows that only slight upfield shift was observed upon addition of DNA. These results as well as our nanostructural study by atomic force microscopy (AFM) illustrate the DNA recognition specificity of DTIC though this anticancer drug interacts with DNA by using non-intercalation mode.

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Key Words: Dacarbazine; DNA recognition; Spectroscopic study

INTRODUCTION

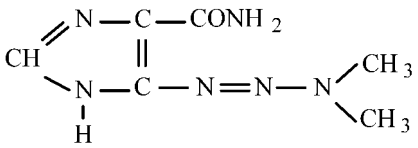
Binding studies of small molecules with nucleic acids have been the focus of considerable interest[1–10] in view of their potential uses to search the key structural determinants pertaining to the underlying binding mechanisms of the action of antitumor or antiviral components. A variety of modern analysis approaches may enable us to selectively and sensitively determine molecular models of interactions as well as the functional molecular groups of individual agents, which is not only valuable to provide new insight into biologically important non-covalent binding mechanisms but also useful to afford a molecular basis to understand the interaction and sequence recognition by promising gene drugs.

Since the antitumor and antimetastatic effects of dacarbazine [5-(3,3-dimethy-1-triazenyl)imidazole-4-carboxamide; DTIC],[11] singly and/or together with other drugs, DTIC has been widely used in clinical treatment of some malignant diseases including malignant melanoma, metastatic melanoma, and so on. As an efficient anticancer drug, it may inhibit DNA repair as a means of the increasing antitumor activity when combined with other drugs in chemotherapy.

Although DTIC have been clinically utilized as an antitumor agent, yet the mechanism of its antitumor action at the molecular level is still unknown and need to be explored. Therefore, in this work we have studied the potential binding properties of DTIC to DNA by using spectroscopic techniques including electronic absorption spectroscopy, NMR, atomic force microscopy (AFM) and so on. The present study illustrates that the relative spectroscopic studies can facilitate new and independent insight as well as reliable information in both binding affinity and binding mode of DTIC to DNA. The results show that these spectral changes of DTIC in the presence of DNA are in contrast to the behavior observed with many intercalators, indicating that non-intercalation is a typical mode of the binding of DTIC to DNA.

EXPERIMENTAL SECTION

Calf thymus DNA (ct-DNA) was purchased from Signal Chemical Co and used directly without further purification. All other chemicals used were of analytical grade. The water used in experiments was double-distilled and



Scheme 1.

the specimen was prepared in TE buffer (10 mM Tris, 1mM EDTA, pH 7.5). Fresh solutions were prepared every day to achieve reliable measurements. To prepare the complexes, ct-DNA was mixed with the ligand DTIC with different concentration ratio. Scheme 1 illustrates the structure of DTIC.

UV-Vis absorption titrations were carried out by using a Shimadzu UV-2001 spectrophotometer. Fluorescence spectra were obtained with a Shimadzu fluorescence spectrophotometer RF-5000. A Nanoscope III (Digital Instruments) was utilized for AFM measurements which were performed in the tapping mode in air at 20°C, while the specimen was prepared in TE buffer (10 mM Tris, 1 mM EDTA, pH7.5) and deposited onto pre-soaking freshly cleaved mica with a Mg^{2+} containing buffer, then rinsed several times and dried by evaporation in ambient air before the observation by AFM. Each sample was imaged in different places and many times in order to obtain reliable measurements.

The ^{1}H NMR spectra were recorded with a Bruker AM-500 nuclear magnetic resonance spectrometer. Chemical shifts were measured with respect to an external reference (DSS in D_2O). Deuterium oxide (Beijing, 99.8% atom D) was used as the solvent in the NMR studies. Solutions were prepared in D_2O containing 20 mM sodium phosphate, and the pD was adjusted to a value of 7.2. Sample temperature for the NMR experiments was maintained at 20 ± 0.1°C. Proton chemical shifts were reproducible within 0.001 ppm.

RESULTS AND DISCUSSION

Initially, evidence for the interaction of DTIC with DNA comes from the electronic absorption study. It is found from the absorption titrations that in the presence of increasing amounts of calf thymus DNA (ct-DNA), strong decreases of absorption intensity appeared in UV-Vis spectroscopy. Figure 1 is a representative absorption titration for the DTIC upon addition of ct-DNA, which shows remarkable decrease in the peak intensities of DTIC at 329 nm (up to nearly 25% hypochromicity even at the 1:1 concentration ratio of the DNA to DTIC) accompanying with little shift of the

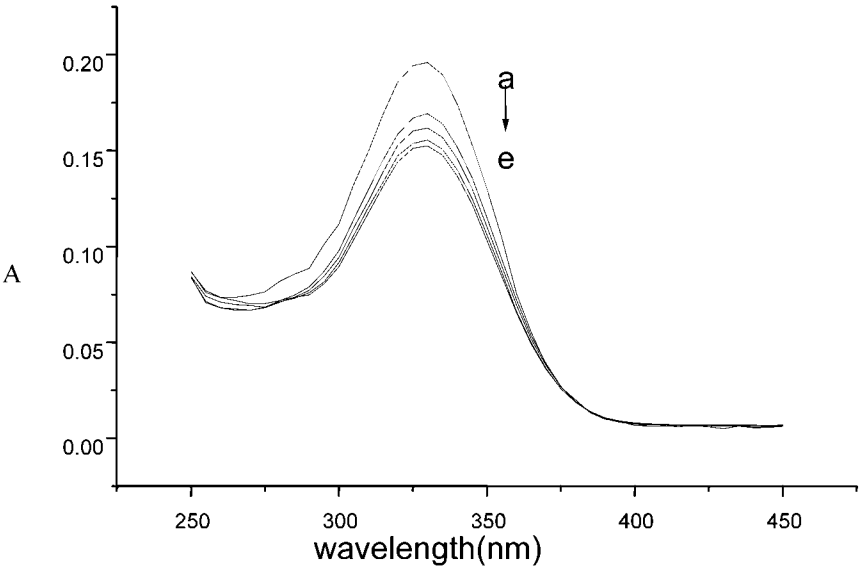


Figure 1. Absorbance spectra of DTIC with increasing concentration of ct-DNA. DNA concentrations were as follows: a) 0.0, b) 0.2, c) 2.0, d) 5.0, e) 9.0 μM.

peak position. This phenomenon suggests that groove binding may occur between DTIC and DNA.

Additional evidence for the interaction of DTIC with DNA can be obtained by comparison with NMR study, which could afford an effective means of conformational analysis of the interaction manner. As shown in Fig. 2, some relative information upon DNA binding was obtained from the ^{1}H NMR spectra at 500 MHz of D_2O solutions. It was observed that the principal proton signals of DTIC in the presence of up to 5 mM ct-DNA only leads to slight shielding effects. In D_2O phosphate buffer solution the peak of HDO is located at δ4.72. Upon addition of DNA the proton signal in the ring of DTIC at δ7.6 is shifted upfield by about 0.02 ppm, and the peaks of the methyl groups, centered at δ3.55 and δ3.24, move upfield by ca. 0.02 ppm and 0.03 ppm, respectively. This phenomenon indicates that the side-chains of DTIC also interact with DNA, though the binding of DTIC to DNA results in only slight upfield shifts of proton signal. These spectral changes are in contrast to the behavior observed with the intercalators, suggesting that non-intercalation is a typical mode of the binding of DTIC to DNA.

In comparison, the results of our atomic force microscopy (AFM) and fluorescent study also afford further information for the DNA molecular

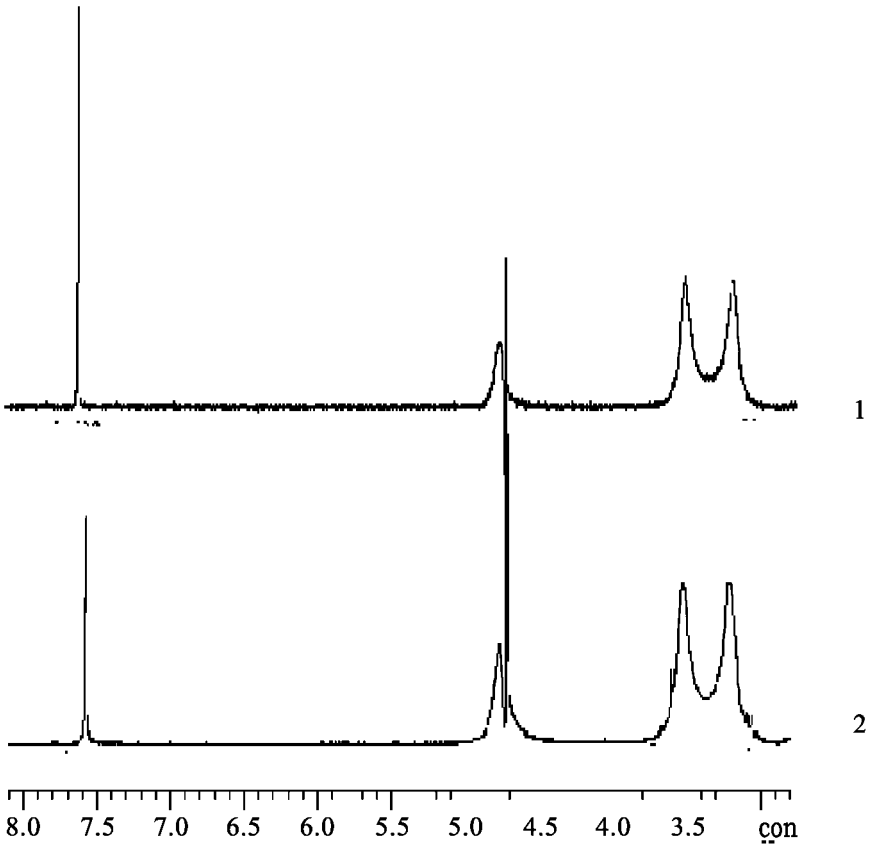


Figure 2. ^{1}H NMR spectra of DTIC in D_2O. 1) without DNA, 2) in the presence of ct-DNA. [DTIC] = 1.0 mM, [bp] = 2.5 mM.

interaction with DTIC. The fluorescence competition measurements with ethidium bromide, conducted in identical ways as previous studies in the literature,[7] clearly indicates the strong binding affinity of DTIC to ct-DNA. Moreover, the nanostructural information obtained by AFM topographical study illustrates that specific interaction occurs upon complexation of DTIC with DNA. Besides, in the nanostructural image of the complexes, the apparent packing particles appeared at some specific site of the DNA sequence directly provide additional binding evidence for the recognition specificity of DTIC to ct-DNA.

Further binding studies of DTIC with some single-stranded DNA indicate that DTIC shows poor binding affinity to single-stranded

DNA, suggesting its high recognition selectivity for the double-stranded DNA. This high binding discrimination towards single and double stranded DNA is vitally important for the future genetic diagnosis.

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