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Study on the Interaction of Mitomycin C with ct-DNA by Pd-Porphin Room Temperature Phosphorescence Probe

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Abstract: Anticancer drug Mitomycin C (MMC) quenches remarkably phosphorescence and reduces lifetime of phosphorescence probe, Pd-meso-tetrakis-(4-trimethylaminophenyl)porphin (Pd-TAPP), in the presence of calf thymus DNA. These results may be attributed to the site competition of MMC with the probe and electron transfer between MMC and probe. MMC also increases polarization degree of the probe by covalent drug-DNA or DNA-drug-DNA crosslinking.

Keywords: Phosphorescence probe, mitomycin C, ct-DNA.

Study of the interaction of drugs with calf thymus DNA (ct-DNA) in molecular level[1-4] is helpful to understand the fundamental aspects of activation of anticancer drugs and provide the valuable information for designing and developing new antitumor agents. Mitomycin C (MMC) is an anticancer drugs being widely used in clinic with high activity and effectiveness for many cancers. DNA has been proved to be the main target molecule of MMC in the body. The activated MMC can hinder the replication of DNA by the alkylation cross-linking and thus effectively inhibit the growth of cancer cells. In the past years, the interaction mechanisms of MMC with DNA have been an interested topic[2]. In the present paper we first use palladium mesotetrakis-(4-trimethylaminophenyl)porphin as room temperature phosphorescence probe to study the interaction of MMC with ct-DNA.

Experimental

Phosphorescence and fluorescence spectra were recorded on Perkin-Elmer LS-50B spectrofluoremeter. Delay time and gate time were set as 0.02 ms and 2 ms respectively. Excitation and emission wavelength were set 416 nm and 686 nm respectively for measuring phosphorescence signal. All experiments were completed at 20°C.

The meso-tetrakis-(4-trimethylaminophenyl)porphin (TAPP) iodide was purchased from ACROS Co. and its palladium complex was synthesized *in situ* and its concentration was adjusted as 5×10^{-4} mol/L. The ct-DNA was purchased from the Sino-American Biological Co. and dissolved in phosphate buffer at pH 7 with 0.02 mol/L

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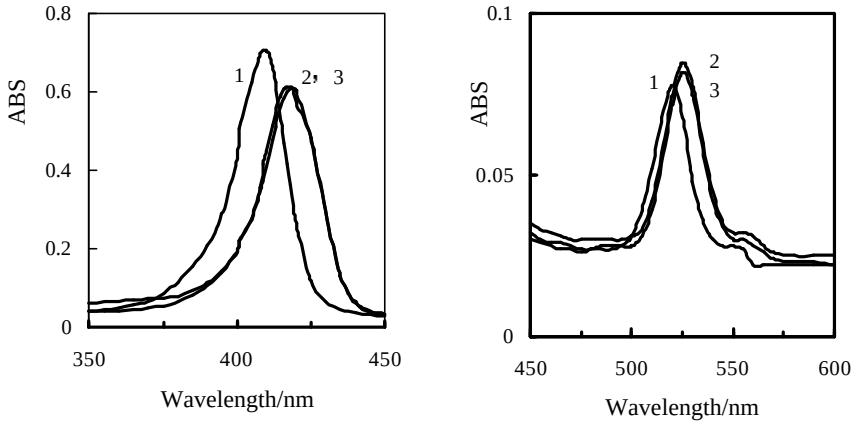
NaCl, the concentration of DNA was determined by ε_{260} 6600 $Lmol^{-1}cm^{-1}$ at 260 nm. MMC was purchased from Sigma Co. and prepared as 1.2×10^{-3} mol/L aqueous solution. The doubly distilled water was used throughout. Other reagents were of analytical grade.

Results and discussion

Absorption of Pd-TAPP in the presence of ct-DNA

Figure 1 shows that the Soret and Q bands of Pd-TAPP are at 410 nm and 520 nm respectively in aqueous solution (curve1) and shifted bathochromically to 420 nm and 525 nm respectively in the presence of ct-DNA (curve 2 and 3). The hypochromicity corresponding to Soret band and hyperchromicity corresponding to Q band were observed, which implying typically the formation of complex between probe and ct-DNA. In the presence of 2.4 μmol/L MMC peak position of both soret and Q band does not change remarkably except for little increase in absorbance.

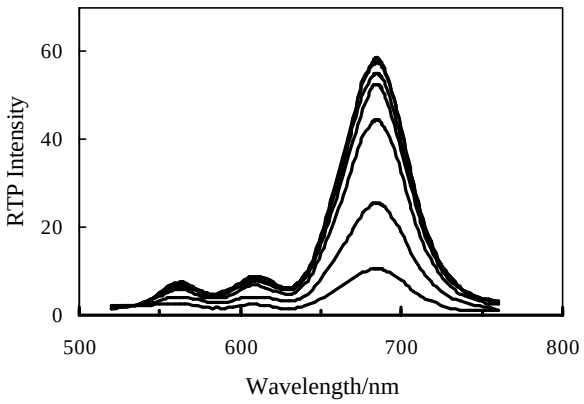
Figure 1 Absorption spectra of Pd-TAPP in the presence of ct-DNA and MMC



Effect of MMC on phosphorescence of Pd-TAPP/ct-DNA system

Activated MMC by reductive agents such as $Na_2S_2O_4$ acts as an anticancer drugs. Here sodium sulfite was used as both reductive activation agent and chemical deoxygenation agent. **Figure 2** shows that phosphorescence of Pd-TAPP was quenched gradually with increase of the concentration ratio R_f, C_{MMC}/C_{DNA}, with fixed DNA concentration. The quenching data can be fitted as exponential form $I_0/I = 0.953 \exp(0.349 [R_f])$ with relation coefficient 0.9965. It also is noticed the data can be fitted in Stern-Volmer equation $I_0/I = 0.994 + 0.319 [R_f]$, with relation coefficient 0.9989, in the presence of lower MMC (R_f from 0 to 1.0). These imply the multipathway of quenching under various MMC concentration.

Figure 2 Quenching of phosphorescence of Pd-TAPP/ct-DNA system by MMC

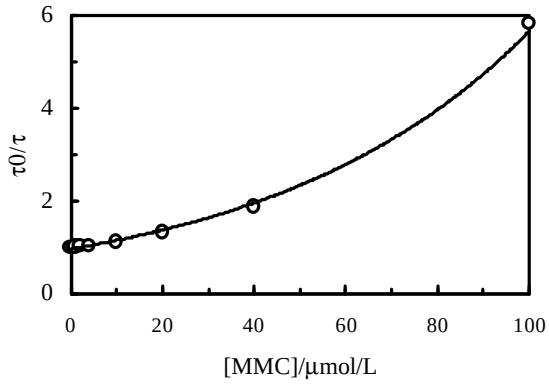


[Pd-TAPP] 5 μmol/L; [ct-DNA] 100 μmol/L. R_f 0, 0.1, 0.2, 0.4, 1.0, 2.0, 4.0 from top to bottom

Effect of MMC on phosphorescence lifetime of Pd-TAPP/ct-DNA system

Figure 3 shows that lifetime ratio τ_0/τ increases with increase of MMC concentration and the results are similar to that in **Figure 2**. The plots of τ_0/τ *vs* [MMC] also can be fitted as exponential form.

Figure 3 Effect of MMC on phosphorescence lifetime of Pd-TAPP/ct-DNA system

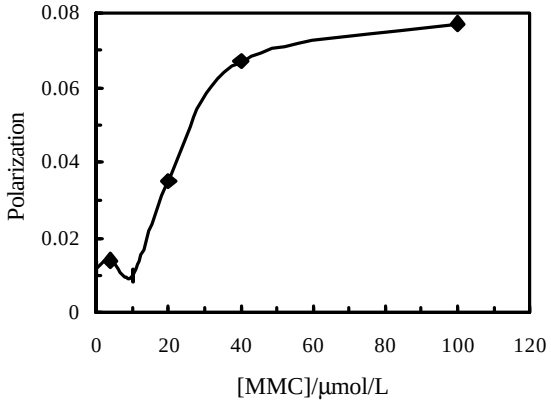


[Pd-TAPP] 5 μmol/L; [ct-DNA] 100 μmol/L

Effect of MMC on phosphorescence polarization of Pd-TAPP/ct-DNA system

Figure 4 shows that phosphorescence polarization degree of probe increases as the concentration of activated MMC increases. This may imply change of the viscosity of microenvironment in which probe is located because of drug-DNA cross-linking.

Figure 4 Phosphorescence polarization of probe with increasing MMC



In summary, MMC caused the decrease of both phosphorescence intensity and lifetime of probe and the quenching data can be fitted as dynamic Stern-Volmer equation under lower MMC concentration and as exponential form in whole concentration range investigated from 0 to 100 μmol/L MMC. The dynamic quenching process should be attributed to the competition of MMC with probe molecules to binding sites. In addition, the electron transfer may be another important factor in quenching of probe phosphorescence between benzoquinone group of MMC and Pd-TAPP probe, which is studying in our laboratory.

Acknowledgments

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