Taxol Anticancer Activity and DNA Binding

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Abstract: The interaction of taxol with DNA has major biological importance since it is shown the presence of higher concentration of taxol in the nucleus, than in the human lung tumor cell. Therefore, in this report we examine the interaction of taxol with calf-thymus DNA in aqueous solution at physiological pH, using constant DNA concentration (25 or 1.25 mM phosphate) and various taxol/DNA (phosphate) ratios 1/200 to 1/2. Capillary electrophoresis and Fourier transform infrared (FTIR) difference spectroscopic methods are used to characterize the nature of drug-DNA interaction and to determine the taxol binding site, the binding constant, sequence selectivity, helix stability and biopolymer secondary structure in the taxol-DNA complexes *in vitro*.

Structural analysis showed that taxol is an external DNA binder with no affinity towards DNA intercalation. The major target of taxol is A-T, G-C bases and the backbone PO_2 groups. Two bindings were observed for taxol-DNA complexes with $K_1 = 1.4 \times 10^4\ M^{-1}$ and $K_2 = 3.5 \times 10^3\ M^{-1}$. The taxol-DNA interaction is associated with a partial helix stabilization and no major alterations of B-DNA structures.

Keywords: DNA, taxol, binding site, binding constant, conformation, helix stability, electrophoresis, FTIR, UV-visible spectroscopy.

INTRODUCTION

Paclitaxol (**structure 1**), first isolated from the yew tree Taxus brevifolia [1,2], acts oncologically by increasing the stability of microtubules and preventing mitosis [3,4]. It interacts with microtubules, important cellular structural proteins, in a manner that catalyzes their formation from tubulin and stabilizes the resulting structure [3-6]. In cells this phenomenon leads to an altered morphology with microtubules, forming stable bundles and the cell being unable to assemble a normal mitotic spindle [7]. Cells treated with taxol normally arrest the transition between interphase and mitosis and die. The elucidation of this unique mechanism of action during the late 1970s and 1980s promoted taxol development as an anticancer drug. Taxol has shown unusual efficacy as a clinical agent, experiencing rapid development for the treatment of breast, ovarian, skin, lung, head and neck cancers [8]. In 1993, taxol was approved by the FDA to be used in USA for the treatment of breast and ovarian cancers.

In recent years, to further elucidate the mechanism of action of taxol antitumor activity, the structural analysis of several taxol-protein and taxol-lipid complexes were investigated [9,10]. On the other hand, it was demonstrated that the presence of nucleotide strongly affects the taxol-tubulin complexation [11]. It has also been demonstrated the presence of higher paclitaxol concentration (two fold) in the nucleus than in the human lung tumor cell [12]. Recently, it was reported that the paclitaxol exhibits major interaction with poly(dA-dT) and not with poly(dG-dC), inducing partial helical stability of the oligonucleotides[13]. This prompted our present investigation related to the taxol complexation with DNA *in vitro.*

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We report the capillary electrophoresis and FTIR difference spectroscopic results on the interaction of taxol with calf-thymus DNA, in aqueous solution at physiological pH with taxol/polynucleotides/ (P) molar ratios of 1/80 to 1/4 with final DNA (P) concentration of 12.5 mM for infrared, and 1/200 to 1/12 with final DNA (P) concentration of 1.25 mM for capillary electrophoresis. Structural analyses regarding the drug binding site, the binding constant, sequence preference, helix stability and DNA conformation are provided here. The structural evidence presented here, provides major information for the taxol-DNA complexation and helps elucidating the nature of this biologically important complex formation at molecular level.

EXPERIMENTAL

Materials

Highly polymerized type I calf-thymus DNA sodium salt (7% Na content) was purchased from Sigma Chemical Co., and was deproteinated by the addition of $CHCl_3$ and isoamyl alcohol in NaCl solution. Taxol was obtained from Biolyse Pharmacopée (Port-Daniel, Québec) and recrystallized from methanol. Other chemicals were of reagent grade and used without further purification.

Preparation of Stock Solutions

Sodium-DNA was dissolved to 2% w/w (0.05 M DNA/phosphate) in 0.1 M NaCl and 1 mM sodium cacodylate (pH 7.30) at 5^{o}C for 24 h with occasional stirring to ensure the formation of a homogeneous solution. The appropriate amount of taxol (0.3 to 25 mM) was prepared in methanol-water mixture (methanol/H_2O=20/80%). Taxol is not soluble in H_2O or D_2O but soluble in methanol-water mixture. The drug solution then was added dropwise to DNA solution to attain desired taxol/DNA(P) molar ratios of 1/80, 1/40, 1/20, 1/10, and 1/4 at a final DNA concentration of 1% w/w or 12.5 mM (phosphate) for infrared

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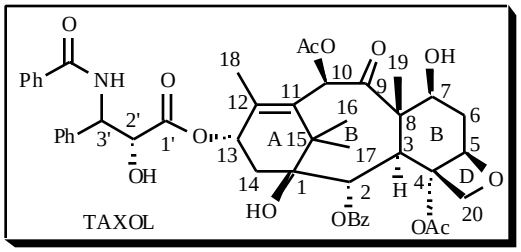
measurements. For capillary electrophoresis, the taxol-DNA ratios were 1/200 to 1/12 with a final 1.25 mM DNA(phosphate). The pH solution was adjusted to 6.5-7.5, using NaOH solution. The infrared spectra were recorded 2h after incubation of taxol with DNA solutions.



Capillary Electrophoresis

A P/ACE System MDQ (Beckman) with PDA (photodiode array) detector was used to study taxol-nucleic acids complexation. Uncoated fused silica capillary of 75 um i.d. and 57 cm length was used. The capillary was initially conditioned by washing with 1 N sodium hydroxide for 30 min, followed by a 15 min wash with 0.1 M sodium hydroxide. Then, it was extensively rinsed with deionized water and running buffer before use. Samples were injected using a voltage injection at 10 kV for 5 s. Electrophoresis was carried out at a voltage of 25 kV for 10 min using normal polarity. All runs were carried out at 25ºC. Stock solution of taxol (2 mM) was prepared in 20 % methanol. The capillary inlet and outlet vials were replenished after every run. The taxol binding experiments were performed in a sample buffer containing 1.5 mM Tris-HCl, pH 7.2 (3 mM NaCl and 20% methanol) using constant concentrations of DNA and various concentrations of taxol. Calf-thymus DNA was dissolved in 3 mM Tris-HCl, pH 7.2 and 6 mM NaCl, at a DNA(P) concentration of 1.25 mM. The taxol solution (20% methanol) was added to DNA solutions to attain desired drug/polynucleotides (P) molar ratios of 1/200 to 1/12. Each sample was allowed to equilibrate for 30 min and tested with two separate runs for the same stock solution. The electropherograms were monitored at 260 nm for taxol-DNA complexes.

Data Analysis

The binding constants for drug-DNA complexes can be determined by capillary electrophoresis, using Scatchard analysis [14,15]. The saturation (R_f) of the DNA was determined from the change of the peak heights of DNA in the presence of various concentrations of taxol by the following equation

$$R_f = (h - h_0)/(h_s - h_0) \quad (1)$$

where h is the change in the peak height of DNA measured for any added taxol concentration, while h_0 and h_s correspond to the peak heights of free DNA and drug saturated, respectively.

Using the equation for binding constant

$$K_b = [\text{taxol-DNA}]/[\text{DNA}][\text{taxol}] \quad (2)$$

The binding constant K_b was then computed by fitting the experimental values of R_f and taxol to the equation

$$R_f = K_b[\text{taxol}]/(1 + K_b[\text{taxol}]) \quad (3)$$

The last equation gives a convenient form for Scatchard analysis:

$$R_f/[\text{taxol}] = K_b - K_b R_f \quad (4)$$

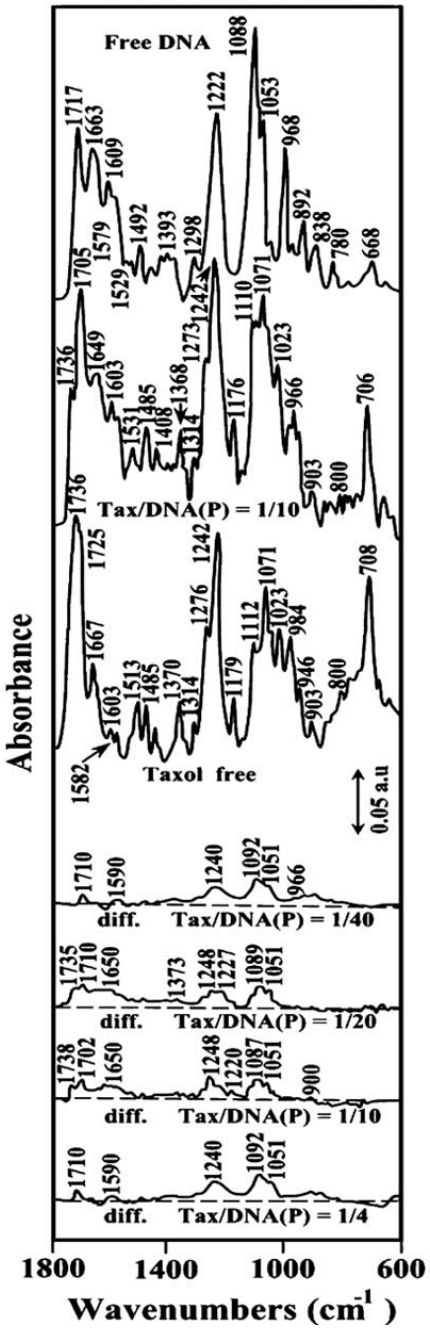


Fig. (1). FTIR spectra (top three spectra) and different spectra (bottom four spectra) for the free calf-thymus DNA and its taxol adducts in aqueous solution at pH=6.5-7.5, in the region of 1800-600 cm^{-1}.

FTIR Spectra

Infrared spectra were recorded on a Bomem DA3-0.02 FTIR spectrometer equipped with a nitrogen cooled HgCdTe detector and KBr beam splitter. The solution spectra are taken using AgBr windows with resolution of 2 to 4 cm^{-1} and 100-500 scans. Each set of infrared spectra were taken

(three measurements) on three identical samples with the same DNA and drug concentrations. The water subtraction was carried out with 0.1 M NaCl solution used as a reference at pH 6.5-7.5 [16]. A good water subtraction is achieved as shown by a flat baseline around 2200 cm^{-1}, where the water combination mode is located. This method is a rough estimate, but removes the water content in a satisfactory way [16]. Due to the insolubility of taxol in D_2O, we did not run the same experiments in D_2O solution. The difference spectra [(DNA solution + taxol solution) - (DNA solution)] are produced, using a sharp DNA band at 968 cm^{-1} as internal references. These bands, due to sugar C-C and C-O stretching vibrations, exhibit no spectral changes (shifting or intensity variations) on taxol-complexation and they were cancelled upon spectral subtraction. The intensity ratios of several DNA in-plane vibrations related to A-T and G-C base pairs and the PO_2 stretchings are measured with respect to the reference band at 968 (DNA) as a function of taxol concentrations with an error of ±3%.

RESULTS AND DISCUSSION

FTIR Spectra of Drug-DNA Complexes

Evidence related to the taxol-DNA complexation comes from the infrared spectroscopic results shown in (Fig. **1** and **2**). At low taxol concentration (r=1/80), no drug-DNA interaction occurs. Evidence for this comes from a minor decrease in the intensity of several polynucleotides in-plane vibrations related to the G-C, A-T and the backbone phosphate modes such as 1717 (G), 1663 (T), 1609 (A), 1529 (C), 1492 (C) and 1222 cm^{-1} (asymmetric PO_2 stretching vibration) [16-27], in the presence of taxol (Fig. **2**). As drug concentration increased, major increase in the intensities of the bands at 1717 (G) 1663 (T), and 1222 cm^{-1} (phosphate) were observed (Figs. **1** and **2**). The increase in intensity of DNA in-plane vibrations was characterized by the positive features at 1710 (G), 1650 (T) and 1659 (A) and 1220-1227 cm^{-1} (PO_2) in the difference spectra of the taxol-DNA complexes (Fig. **1**). It is worth mentioning that the other positive features centered at 1743-1735, 1240, 1080-1092, 1070-1050 and 708 cm^{-1}, in the difference spectra of drug-DNA complexes are coming from the taxol vibrational frequencies and are not due to DNA vibrations (Fig. **1**). These intensity variations of DNA vibrational frequencies were also associated with the shifting of the bands at 1717 (G) to 1713; 1663 (T) to 1658 and 1609 (A) to 1605 cm^{-1} for DNA bases, upon taxol complexation (Fig. **1**). The observed spectral changes are indicative of major taxol bindings to the guanine, thymine, adenine bases and the backbone PO_2 groups. The band at 1492 cm^{-1} (mainly cytosine) showed no spectral changes upon drug interaction, which is indicative of no participation of cytosine bases in taxol-DNA complexation (Fig. **2**).

At high taxol concentration (r>1/10), a partial helix stabilization occurs for DNA, which is associated with the decrease in the intensities of the bands at 1717, 1663, 1609 and 1222 cm^{-1}(Fig. **2**). Similar infrared spectral changes were observed for DNA vibrations, where metal cation interaction induced helical stability [28-30]. Recent calorimetric titration of several synthetic DNAs by pacilitaxol also showed major increase of helical stability for poly (dA-dT) oligonucleotides [13].

Further evidence regarding taxol-DNA complexation comes also from the major shifts of drug vibrational frequencies. A strong band at 1736 cm^{-1} related to the drug exocyclic carbonyl C=O stretching was shifted to 1733 cm^{-1}, the spectra of taxol-DNA complexes (Fig. **1**). A band with

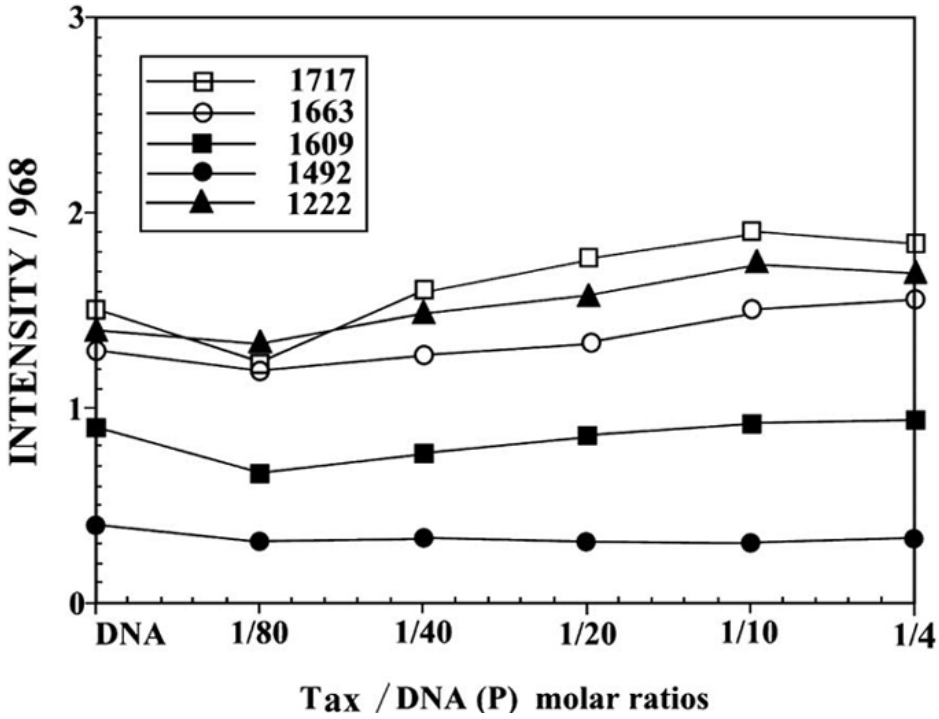


Fig. (2). Intensity ratio variations for several DNA in-plane vibrations at 1717 (G,T), 1663 (T,G,A,C), 1609 (A), 1492 (C,G) and 1222 cm^{-1} (PO_2 stretch) as a function of taxol concentrations [different drug)/DNA(P) molar ratios].

medium intensity at 1667 cm^{-1} related to the free taxol OH bending and C-N stretching vibrations also shifted towards a lower frequency in the spectra of the drug-DNA complexes. However this band was overlapped by the strong DNA vibration at 1656 cm^{-1} in the spectra of the drug-DNA complexes (Fig. **1**). The observed spectral changes are indicative of taxol-DNA complexation through drug polar groups.

DNA Confirmation

It is important to note that taxol-DNA interaction leads to no major biopolymer conformational changes with DNA remaining in the B-family structure. Evidence for this comes from no major shifts of the B-DNA marker bands [21,24,28] at 1717 (G), 1222 (PO_2) and 836 cm^{-1} (phosphodiester) (Fig. **1**). When B to A transition occurs, the DNA marker bands are shifted to 1710-1700 cm^{-1}, 1225-1240 cm^{-1} and 825-800 cm^{-1}, respectively, and a new band appears at about 870-860 cm^{-1} [21,24,28]. In the B to Z conformational transition, the sugar-phosphate band at 836cm^{-1} appears at 800-780 cm^{-1}, and the guanine band displaces to 1690 cm^{-1}, while the phosphate band shifts to 1216 cm^{-1} [21,24,28]. Since no such spectral changes occurred for infrared marker bands here, DNA remains in the B-conformation upon taxol interaction. The shifting of the band at 1717 (G) to 1713 cm^{-1} is due to a major perturbation of the G-C base pair by drug interaction. (Fig. **1**).

Capillary Electrophoresis of Taxol-DNA Complexes

The bindings of the taxol-DNA complexes were also studied by capillary electrophoresis. Mixtures containing various concentrations of taxol and either DNA with molar ratios of 1/200 to 1/12 were prepared and subjected to the electrophoresis, using uncoated silica capillary 75 cm i.d. (50 cm effective length) at 25 kV. The electropherogram was monitored at 260 nm for DNA and RNA in a run buffer (7.5 mM Tris-HCl pH 7.2, 15 mM NaCl) at 25 ^oC. The peak heights of taxol-DNA complexes gradually increased as the taxol concentrations increased and reached nearly maximum at a taxol concentration of 1 mM (Fig. **3A**). Based on these results, binding constants of the drug-DNA complexes were determined by Scatchard analysis as described in Materials and Methods. As shown in Fig. **3A**, (saturation curves), the slope for the taxol-DNA is biphasic, suggesting the presence

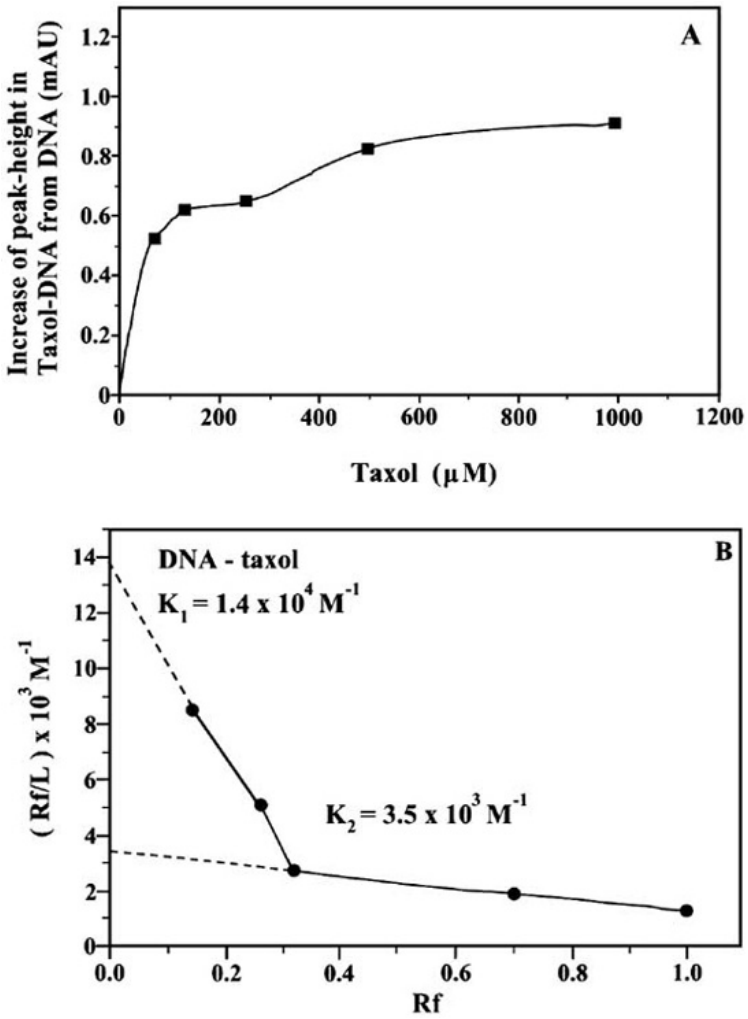


Fig. (3). Plots for taxol concentrations *vs*. increase of peak-heights in taxol-DNA complexes. The increase of peak-heights in taxol-DNA was determined by subtracting the peak-height of the free DNA from each taxol-DNA complex. Scatchard plots (**B**) for taxol-DNA complexes.

of low affinity and high affinity binding sites for the drug-DNA complexes. Scatchard analysis showed binding constants for the high and low affinity sites for the taxol-DNA adducts with K_1= 1.4 x 10^4 M^{-1} and K_2=3.5 x 10^3 M^{-1}, respectively (Fig. **3B**). These results suggest that taxol has two distinct binding sites on A-T and G-C base pairs.

SUMMARY

Our capillary electrophoresis and spectroscopic results on taxol-DNA complexes showed. (**a**) Taxol binds to major and minor grooves of DNA and the backbone phosphate group. (**b**) The two bindings on A-T and G-C bases are with K_1=1.4 x 10^4 M^{-1} and K_2=3.5 x 10^3 M^{-1}. (**c**) Taxol-DNA complexation results in a partial helix stabilization with no alterations of the B-DNA structure.

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ABBREVIATIONS

Tax	=	Taxol
DES	=	Diethylstilbestrol
FTIR	=	Fourier Transform Infrared
A	=	Adenine
C	=	Cytosine
G	=	Guanine
T	=	Thymine
r	=	Taxol/DNA(P) molar ratio

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