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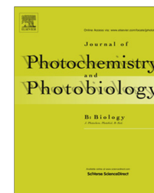

Multispectroscopic methods reveal different modes of interaction of anti cancer drug mitoxantrone with Poly(dG-dC).Poly(dG-dC) and Poly(dA-dT).Poly(dA-dT)

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A B S T R A C T

The interaction of mitoxantrone with alternating Poly(dG-dC).Poly(dG-dC) and Poly(dA-dT).Poly(dA-dT) duplex has been studied by absorption, fluorescence and Circular Dichroism (CD) spectroscopy at Drug to Phosphate base pair ratios D/P = 20.0–0.04. Binding to GC polymer occurs in two distinct modes: partial stacking characterized by red shifts of 18–23 nm at D/P = 0.2–0.8 and external binding at D/P = 1.0–20.0 whereas that to AT polymer occurs externally in the entire range of D/P. The binding constant and number of binding sites is 3.7×10^5 M^{-1}, 0.3 and 1.3×10^4 M^{-1}, 1.5 in GC and AT polymers, respectively at low D/P ratios. CD binding isotherms show breakpoints at D/P = 0.1, 0.5 and 0.25, 0.5 in GC and AT polymers, respectively. The intrinsic CD bands indicate that the distortions in GC polymer are significantly higher than that in AT polymer. Docking studies show partial insertion of mitoxantrone rings between to GC base pairs in alternating GC polymer. Side chains of mitoxantrone interact specifically with base pairs and DNA backbone. The studies are relevant to the understanding of suppression or inhibition of DNA cleavage on formation of ternary complex with topoisomerase-II enzyme and hence the anti cancer action.

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1. Introduction

The synthetic antitumor drug mitoxantrone (Fig. 1) 1,4-dihydroxy-5,8-bis{[2-[(2-hydroxyethyl) amino]-ethyl]amino]-9,10-anthracenedione has shown promising anticancer activity against a variety of cancers [1–3]. It has a similar activity spectrum but a less severe cardiotoxic effect than anthracyclines e.g. daunomycin, adriamycin [4]. It interferes with topoisomerase-II-mediated DNA cleavage and causes multiple DNA strand breaks, large changes in chromatin structure [5] and DNA compaction [6]. There is strong evidence that the interaction of mitoxantrone (MTX) with cellular DNA contributes towards its cytotoxic action [6,7]. The molecular relationship of structural changes in DNA upon interaction with MTX and binding modes is still doubtful.

Footprinting experiments [8,9] and in vitro transcription assay [10] on 117 bp and 497 bp fragments of DNA, respectively showed that MTX intercalates in DNA with preferred sites being 5′-(A/T) CG and 5′-(A/T) CA. However in vitro DNA cleavage assay in ternary complex DNA–drug–topoisomerase-II showed [9] that its sequence dependence effects were different from that of daunomycin. In view of preference of MTX in binding to CG sequences, several studies were initiated with Poly(dG-dC).Poly(dG-dC) and Poly(dA-dT).Poly(dA-dT). Preliminary results of measurements of absorbance in visible region showed that on binding of MTX to polynucleotide, the 660 nm absorption peak shifted towards red [11]. The absorption maxima of 660 nm peak in free mitoxantrone shifted to 678 nm and 683 nm on binding to Poly(dA-dT).Poly(dA-dT) and Poly(dG-dC).Poly(dG-dC), respectively; the corresponding number of bases covered on polynucleotides by the MTX molecule or the binding size was 2.4 and 2.0, respectively [12]. Stopped flow kinetics measurements showed that the dissociation rate from its DNA complexes was biphasic and MTX had much slower dissociation rate from Poly(dG-dC).Poly(dG-dC) than Poly(dA-dT).Poly(dA-dT) [13].

Circular Dichroism (CD) studies on MTX binding to Poly(dG-dC).Poly(dG-dC) at Drug to Phosphate base pair (D/P) ratios 0–0.3 showed [14] reversal of 251 nm band, large increase in ellipticity of 274 nm band and induced positive band at 690 nm. Two positive induced bands at 640 and 690 nm, were observed in another study [15] on binding to Poly(dG-dC).Poly(dG-dC) and Poly(dA-dT).Poly(dA-dT) but the stoichiometry and intrinsic DNA bands were not monitored. It was further shown that 251 and 274 nm bands changed differently at two different ionic concentrations but measurements were done only in UV region for D/P = 0–0.3 and induced bands were not monitored [16]. A systematic theoretical study

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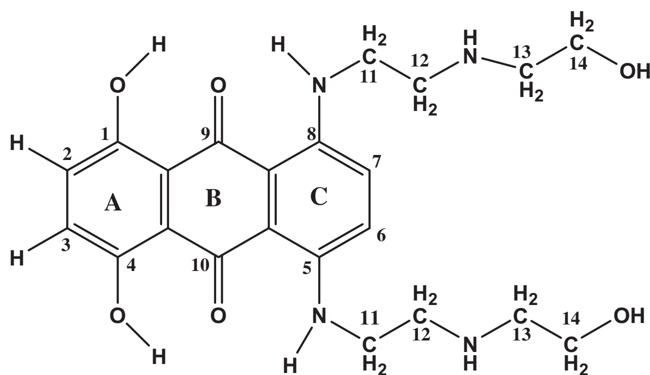

Fig. 1. Molecular structure of mitoxantrone (MTX).

has shown that the induced CD band of a groove bound molecule is one order of magnitude stronger than that of an intercalated molecule, on binding to both Poly(dA-dT).Poly(dA-dT) as well as Poly(dG-dC).Poly(dG-dC) [17,18].

In order to get a complete picture of the mode of binding, we have carried out absorption and fluorescence spectral studies on binding of MTX to Poly(dG-dC).Poly(dG-dC) and Poly(dA-dT).Poly(dA-dT) at Drug to Phosphate (D/P) base pair ratios 0.2–20.0. The CD spectra have been recorded in the wavelength range 200–700 nm in order to monitor changes in intrinsic and induced bands at D/P = 0.04–0.8 and obtain stoichiometry of the drug–DNA complex. The results have been coupled with studies by Docking in order to get structural details of the bound complex. The results indicate different mode of binding in the two polydeoxynucleotides.

2. Materials and methods

2.1. Chemicals

The DNA sequences Poly(dA-dT).Poly(dA-dT), Poly(dG-dC).Poly(dG-dC), mitoxantrone and other chemicals like Sodium Di hydrogen Phosphate, Di Sodium Hydrogen Phosphate, and Sodium Chloride, used for buffer preparation were purchased from Sigma Chemical Co., USA. Mitoxantrone and DNA sequences were used without further purification. DNA sequences Poly(dA-dT).Poly(dA-dT), Poly(dG-dC).Poly(dG-dC) were dissolved in 20 mM sodium phosphate buffer (pH 7.0) containing 70 mM NaCl, which was stored at 273–277 K (277 K is abbreviated form of temperature reading 277.15 K) and shaken gently when needed. The concentration of DNA sequences Poly(dA-dT).Poly(dA-dT) and Poly(dG-dC).Poly(dG-dC) were determined using molar extinction coefficient $\varepsilon = 8300\ M^{-1}\ cm^{-1}$ and $7100\ M^{-1}\ cm^{-1}$, respectively at 260 nm. The stock solution of MTX was prepared by dissolving 1 mg of MTX into 1 mL of phosphate buffer (pH 7.0). Similarly the concentration of mitoxantrone was determined using extinction coefficient $\varepsilon = 20{,}900\ M^{-1}\ cm^{-1}$ at 665 nm [16].

2.2. Apparatus and measurements

Absorbance measurements were carried out by using CARY-100 Bio-spectrophotometer (Varian, USA) equipped with a thermostatic cell holder and quartz cuvette having optical path length = 1 cm. 10 μM mitoxantrone was incubated individually in the absence and presence of various concentrations of DNA sequence in phosphate buffer (pH 7.0) at 298 K. Drug to phosphate base pair (D/P) ratio was varied and spectra were recorded at D/P values of 20.0, 2.0, 1.8, 1.6, 1.4, 1.2, 1.0, 0.8, 0.6, 0.4 and 0.2 for Poly(dA-dT).Poly(dA-dT) and 20.0, 16.0, 12.0, 2.0, 1.8, 1.6, 1.4, 1.2, 1.0, 0.8, 0.6, 0.4 and 0.2 for Poly(dG-dC).Poly(dG-dC) at 298 K.

Fluorescence experiments were performed by using Fluorolog-3 Spectrofluorimeter LS55 (make HORIBA Jobin Yvon Spex®). Mitoxantrone showed an emission maxima at 685 nm on using excitation wavelength, $\lambda_{exc} = 610$ nm. Emission spectra were recorded for the samples used in absorbance measurements.

CD measurements were carried out on a PC controlled Applied Photophysics (Model Chirascan, UK) spectro-polarimeter equipped with a Peltier temperature controller system (make Quantum Northwest Peltier) accessory using a rectangular quartz cell of 0.2 cm path length. All spectra were acquired in the wave length range 200–700 nm. The instrument was purged continuously with clean nitrogen using High Purity Nitrogen Generator (make SAS F-DGS, Every, France model HPNG10/1) prior to igniting the xenon arc lamp to prevent formation of ozone. Titrations were performed by keeping the concentration of DNA duplex constant (75 μM) while the concentration of mitoxantrone was varied in a solution containing phosphate buffer at pH 7.0 at 298 K. CD spectra were recorded at D/P ratios of 0.04, 0.08, 0.16, 0.2, 0.24, 0.32, 0.36, 0.4, 0.48, 0.56, 0.6 and 0.8 for Poly(dA-dT).Poly(dA-dT) sequence and 0.04, 0.08, 0.16, 0.2, 0.24, 0.32, 0.36, 0.4, 0.48, 0.56, 0.6, 0.72 and 0.8 for Poly(dG-dC).Poly(dG-dC).

2.3. Docking studies

The structure of mitoxantrone is built using MOE software tool and optimized with MMF94× force field. Auto Dock Tools (ADT) (version 1.4.5) were used for DNA and ligand preparation. For DNA sequences, all hydrogen, including non-polar, Kollman charges, and solvation parameters were added to all atoms. After adding charges, the non-polar hydrogen atoms were merged. Autogrid was used to generate the grid maps. The docking area was defined using Autogrid $50 \times 50 \times 50$–3D affinity grid centered on the binding site of receptor and a 0.375 Å grid point space was identified. Gasteiger charges were assigned and then nonpolar hydrogen atoms were merged for MTX. Lamarckian genetic algorithm (LGA) was employed for ligand conformational searching because it has enhanced performance relative to simulated annealing or the simple genetic algorithm. For each complex, we used the default docking parameters with the exception of the following: initial population of 150 randomly placed individuals, maximum number of 2.5×10^6 energy evaluations and maximum number of 2.7×10^4 generations. The mutation rate and crossover rate were set to 0.02 and 0.80, respectively. The elitism value was set to 1 and the local search frequency to 0.06. Fifty independent docking runs were carried out for the ligand using these parameters for rapid screening. The best docked position was determined by comparing docking poses and taking total energy value into consideration. Among several similar docking poses, the more energetically favorable conformation was selected. The docking results were clustered on the basis of root-mean-square deviation between the Cartesian coordinates of the atoms using 2.0 Å cut off and were ranked on the basis of the binding free energy ΔG and inhibitory constant K_i.

3. Results

3.1. Absorption spectra

The mitoxantrone shows four absorption bands in the UV–Visible region, that is, at 242, 276, 610 and 660 nm. We have used absorption spectroscopy to validate the binding mode between mitoxantrone and polydeoxynucleotides Poly(dG-dC).Poly(dG-dC) and Poly(dA-dT).Poly(dA-dT) by using the visible bands as DNA absorbs in the UV region efficiently. With the increasing addition of

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