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Thermodynamics of the binding of cytotoxic protoberberine molecule coralyne to deoxyribonucleic acids

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Abstract

The binding thermodynamics of the interaction of protoberberine molecule coralyne to various DNAs have been investigated. Thermodynamic data revealed that the binding was enthalpy driven in GC rich DNA and GC polynucleotides while the same was favored by both negative enthalpy and positive entropy changes in the AT rich DNA and AT polymers. Parsing the free energy change of the binding in terms of polyelectrolytic and nonpolyelectrolytic contribution showed the involvement of major contributions from the later. The heat capacity change (ΔC_p°) for the binding of coralyne to calf thymus DNA and *Micrococcus lysodeikticus* DNA was − 147 and − 190cal/(mol K) respectively. The binding data in these systems also showed significant enthalpy–entropy compensation confirming the involvement of multiplicity of weak non-covalent interactions in agreement with the negative heat capacity data. Circular dichroic studies revealed that the binding was accompanied by moderate conformational change of B-form structure and more importantly the achiral alkaloid molecules acquired strong induced optical activity. These results contribute to the understanding of energetics of coralyne-DNA complexation that will guide synthetic efforts of medicinal chemists for developing better therapeutic agents.

Keywords: Coralyne; DNA; Thermodynamics; Melting studies

1. Introduction

Polycyclic aromatic molecules that can intercalate into DNA helix have been the focus of considerable interest in the recent past. DNA complexation of such molecules was thought to be the molecular basis of their biological activities [1–3]. Particular importance was attributed to plant products that can bind to the DNA structure [4,5] due to their natural availability. Isoquinolines that include benzylisoquinolines, protopines, benzophenantridines, protoberberines are one of the largest group of alkaloids that have been endowed with extensive biological activities [6,7]. These alkaloids were investigated since long for DNA binding activities. Protoberberines represent one of the exhaustively studied groups, of which, berberine, palmatine and synthetic coralyne are especially noteworthy. They have extensive and wide-ranging biological properties like anti-inflammatory, antimicrobial, antiviral, anticancer and anticholesterol effects [8–12]. Large numbers of studies have been done on the DNA and RNA binding properties of the extensively distributed berberine and the next widely distributed palmatine [13–21]. It has been suggested that both berberine and palmatine intercalates to DNA either partially or fully with specificity towards AT base pairs [13,21]. Besides, they also have DNA linked topoisomerase I and II inhibition activities [22–24]. Studies on the derivatives and dimers of these alkaloids have also yielded encouraging results in terms of improved biological activities, topoisomerase poisoning and DNA binding [25,26]. On the other hand, studies on the binding of the synthetic protoberberine, coralyne (Fig. 1) to DNA and RNA are scanty [27–30] and there is no data on the thermodynamics of coralyne DNA complexation in the literature. Unlike buckled berberine and palmatine, coralyne is a planar molecule with potential biological applications particularly

Abbreviations: DNA, deoxyribonucleic acid; CP, Citrate–Phosphate; bp, base pair; ITC, isothermal titration calorimetry; DSC, differential scanning calorimetry; Tm, thermal melting; CD, circular dichroism; D/P, alkaloid/DNA molar ratio; P/D, DNA /alkaloid molar ratio

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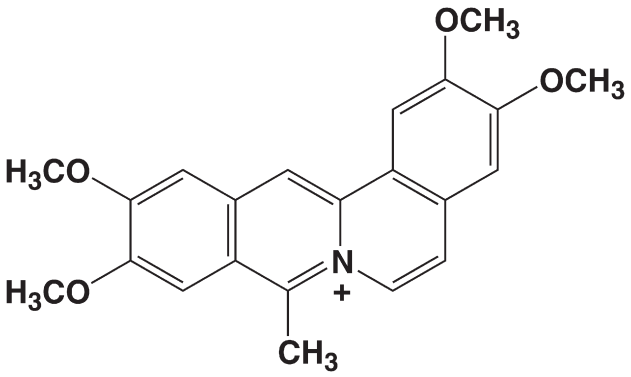

Fig. 1. Chemical structure of coralyne.

known for its potential antileukemic activity [31]. The low toxicity and its pronounced ability to act as a poison to human topoisomerase I [32] make coralyne a promising lead compound for further investigation as an anticancer agent. A previous study from our laboratory [33] had revealed the ability of coralyne to intercalate to DNA with GC base pair specificity. Further, recently coralyne has been shown to exhibit an unusually high affinity to poly(A) and inducing self structure formation in this RNA [34]. Although DNA intercalation of coralyne is thought to be a possible mechanism by which it exerts its antitumoral effects, the energetics and conformational aspects of intercalation remained unexplored and is still a lacuna in the literature. Since structural and energetic aspects are complementary and the latter knowledge is an essential element for the further development of coralyne based antitumor agents, in the present study we investigate the thermodynamics of interaction of coralyne to B-form DNAs of various base composition and sequences using sensitive biophysical techniques like isothermal titration calorimetry, thermal melting, differential scanning calorimetry and circular dichroism.

2. Materials and methods

2.1. Materials

All the natural DNAs and polynucleotides samples were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA) and used as such. Concentrations of the nucleic acid samples in terms of base pairs were determined by UV absorbance measurements utilizing known extinction values. Coralyne chloride (Sigma-Aldrich) concentration in aqueous buffers was determined by visible absorbance measurements using molar extinction coefficient $\varepsilon_{420} = 14{,}500 M^{-1} cm^{1}$. All other reagents were of analytical grade or better. All stock solutions were made in 10mM citrate-phosphate (CP) buffer pH 7.0, containing 5mM Na_2HPO_4. pH was adjusted using citric acid. pH measurements were made on an Cyberscan 2100 high precision bench pH meter with an accuracy of $> \pm 0.001$ units (Eutech Instruments Pte. Ltd., Singapore). All buffer solutions were passed through 0.45 μm syringe filters (Millipore India Pvt. Ltd., Bangalore, India) to remove any particulate matter. Salt dependent studies were performed in CP buffer, pH 7.0 containing different amounts of $[Na^+]$. Circular dichroic studies of drug–DNA complexation were performed in CP buffer, pH 7.0, containing 8% methanol.

2.2. Absorption spectra

A Shimadzu Pharmaspec 1700 unit equipped with a thermoelectrically controlled cell holder and temperature controller (TCC 240A) (Shimadzu Corporation, Tokyo, Japan) was used for absorption measurements as described previously [18,19,21].

2.3. UV optical melting study

Absorbance versus temperature profiles (melting curves) of DNA and DNA–drug complexes were measured on the Shimadzu Pharmaspec 1700 unit equipped with the peltier controlled TMSPC-8 model accessory (Shimadzu Corporation) as described earlier [35].

2.4. Isothermal titration calorimetry and analysis of coralyne binding to DNA

Isothermal titration calorimetry (ITC) experiments were performed on a Microcal VP-ITC microcalorimeter (MicroCal, Inc., Northampton, MA, USA) at various temperatures as reported earlier [21,35]. The graphics software Origin 7.0 (MicroCal Inc), supplied by the manufacturer was used for data acquisition and analysis. Aliquots of DNA/polynucleotide (5 μL each form a stock of 65 μM) were injected from a 299 μL rotating syringe (290 rpm) into the isothermal sample chamber equilibrated at the desired experimental temperature containing 1.4235 mL of coralyne (15 μM) solution. Corresponding control experiments to determine the heat of dilution of the DNA/polynucleotide to buffer were also performed. All the solutions used for ITC experiments were degassed prior to use under vacuum (140 mbar, 8 min) on the Microcal's Thermovac unit to eliminate air bubbles. The duration of each injection was 14s and the delay time between each injection was 240s. Other experimental and analysis details were as reported previously [21,35].

2.5. Differential scanning calorimetry

DSC experiments were done on a Microcal VP-differential scanning calorimeter (MicroCal, Inc., Northampton, MA, USA). In a series of DSC scans, both the cells were loaded with buffer solution, equilibrated at 20°C for 15min and scanned from 20°to 110°C at a scan rate of 50°C/h. The buffer scans were repeated till reproducible and on cooling, the sample cell was rinsed and loaded with DNA or DNA–drug complex and scanned. DSC thermograms of excess heat capacity versus temperature plots were analyzed using Origin 7.0 software. ΔH_m values for the helix coil transitions for each DNA/polymer were obtained from DSC experiments.

2.6. Circular dichroism studies

Circular dichroism (CD) spectra were recorded on a Jasco J715 spectropolarimeter (Japan Spectroscopic Ltd., Japan) in rectangular quartz cells of 1cm path length as reported earlier [21]. Intrinsic CD (210–400nm region) and extrinsic CD (300–500nm region) were measured by keeping a fixed concentration of DNA (60μM) and increasing the coralyne concentration and by keeping a fixed concentration of coralyne (25 μM) and varying the concentration of polynucleotide/DNA. The molar ellipticity values $[\theta]$ were expressed in terms of either per base pair (210–400 nm region) or per bound alkaloid (300–500 nm region).

3. Results and discussion

3.1. Binding of coralyne to DNA: isothermal titration calorimetry

Thermodynamic analysis of drug–DNA binding provides valuable insights into the nature of the molecular forces that are involved in the complexation. ITC is one of the most powerful, reliable and precise techniques currently available to characterize the energetics of the binding of small molecules to macromolecules [36–38]. ITC provides thermodynamic parameters as well as the binding affinity in one experiment. Fig. 2A–H (upper panels) shows the representative raw ITC profiles resulting form the titration of coralyne to the eight DNA samples studied. In Fig. 2, lower panel (I–P), the resulting corrected injection heats are plotted against the respective molar ratios. In