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Berberine–DNA complexation: New insights into the cooperative binding and energetic aspects

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ABSTRACT

The equilibrium binding of the cytotoxic plant alkaloid berberine to various DNAs and energetics of the interaction have been studied. At low ratios of bound alkaloid to base pair, the binding exhibited cooperativity to natural DNAs having almost equal proportions of AT and GC sequences. In contrast, the binding was non-cooperative to DNAs with predominantly high AT or GC sequences. Among the synthetic DNAs, cooperative binding was observed with poly(dA).poly(dT) and poly(dG).poly(dC) while non-cooperative binding was seen with poly(dA–dT).poly(dA–dT) and poly(dG–dC).poly(dG–dC). Both cooperative and non-cooperative bindings were remarkably dependent on the salt concentration of the media. Linear plots of ln K_a versus [Na^+] for poly(dA).poly(dT) and poly(dA–dT).poly(dA–dT) showed the release of 0.56 and 0.75 sodium ions respectively per bound alkaloid. Isothermal titration calorimetry results revealed the binding to be exothermic and favoured by both enthalpy and entropy changes in all DNAs except the two AT polymers and AT rich DNA, where the same was predominantly entropy driven. Heat capacity values (ΔCp^o) of berberine binding to poly(dA).poly(dT), poly(dA–dT).poly(dA–dT), *Clostridium perfringens* and calf thymus DNA were −98, −140, −120 and −110 cal/mol K respectively. This study presents new insights into the binding dependent base pair heterogeneity in DNA conformation and the first complete thermodynamic profile of berberine binding to DNAs.

1. Introduction

Berberine (Fig. 1) is a natural isoquinoline plant alkaloid endowed with diverse pharmacological and biological activities [1–12]. Berberine has inhibitory effect against telomerase activity [13], induces apoptosis and necrosis [14–16] and prevents the invasion of human cancer cells [17]. The wide ranging biological activities of berberine in general and the anticancer activities in particularly have generated considerable interest to correlate its mechanism of action to the biophysical parameters of DNA binding, topoisomerase I and II poisoning and related events [18–20]. Several studies from our and other laboratories have characterized the interaction of berberine to DNA [18–27]. It is now well established that the alkaloid binds to DNA by partial intercalation with remarkable adenine–thymine (AT) base pair specificity [19,24,26,27]. Nevertheless, two aspects of berberine–DNA complexation remained unexplored; evidences of any heterogeneity in DNA sequences and conformations on binding and the energetics of the interaction. In all the previous studies where spectroscopic analysis was employed for elucidating the binding characteristics, the data analysis was limited to narrow regions of bound drug [18,19,24,26,27]. Such analysis, inadvertently excluded the details of the binding phenomena at low levels of the bound drug. In several drug–DNA interaction studies [28–33], at low bound drug concentrations cooperativity of binding has been revealed. Cooperativity is an important aspect in drug–DNA complexation as it may influence the biological activity of a drug binding to a continuous array of potential binding sites. Although so far it is not clearly known why such cooperativity arises in some sequences and with some compounds and what role it has to play from the standpoint of a potential drug, it is essential to know what sequences and or structures can give rise to such cooperative binding and what are the conformational and energetic aspects of such binding. Further, although indeed a wealth of information on various aspects of berberine–DNA complexation is available, the thermodynamic aspects of the interaction have remained unexplored all these years. In order to gain more insight into these aspects, this work focuses on the heterogeneity of DNA binding sites and energetics of berberine interaction with DNAs.

2. Materials and methods

2.1. General

Clostridium perfringens (CP) DNA (30 mol% GC), calf thymus (CT) DNA (42 mol% GC), *Escherichia coli* (EC) DNA (50 mol% GC), *Micrococcus lysodeikticus* (ML) DNA (72 mol%

Abbreviations: DNA, deoxyribonucleic acid; CP, Citrate–Phosphate; ITC, isothermal titration calorimetry

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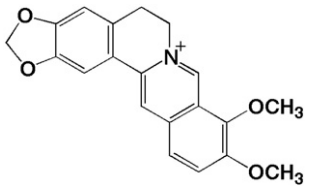

Fig. 1. Chemical structure of berberine.

GC) and synthetic polynucleotides, poly(dA).poly(dT), poly(dA–dT).poly(dA–dT), poly (dG).poly(dC) and poly(dG–dC).poly(dG–dC) were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA) and used as such. Each natural DNA/synthetic polynucleotide (hereafter DNA in general) concentration in terms of base pairs was determined spectrophotometrically using molar extinction coefficient values reported previously [34]. Berberine chloride was purchased from Sigma-Aldrich and was prepared according to the reported protocol [24]. All the experiments were performed in citrate–phosphate (CP) buffer containing 5 mM anhydrous Na_2HPO_4, PH 7.0 of various $[Na^+]$ concentrations obtained by adding required volumes of NaCl solution from a known concentrated stock. All buffer solutions were filtered through Millipore filters of 0.45 μM (Millipore India Pvt., Ltd. Bangalore, India).

2.2. Spectrophotometric titrations and analysis of binding data

The absorption spectra of berberine–DNA complexes were recorded on a Shimadzu Pharmaspec 1700 spectrophotometer (Shimadzu Corporation, Tokyo, Japan) in quartz cells of 1 cm path length at 20 °C using the methodology of Chaires [30] described previously [35]. A known concentration of the DNA solution was kept in the sample and reference cell and small aliquots of a known concentrated stock solution of the alkaloid was titrated into the sample cell. After each addition, the solution was exhaustively mixed and allowed to reequilibrate before noting the absorbance values at the wavelength maximum and isosbestic point. The data obtained from such spectrophotometric titrations were cast into Scatchard plots of r/C_f versus r, where r is the number of alkaloid molecules bound per mole of DNA base pair and C_f is the molar concentration of the free alkaloid; r is calculated from the relation $r = C_b/P$, where P is the DNA concentration in base pairs and C_b is the bound alkaloid concentration. The resulting plots were analyzed as follows. Scatchard plots

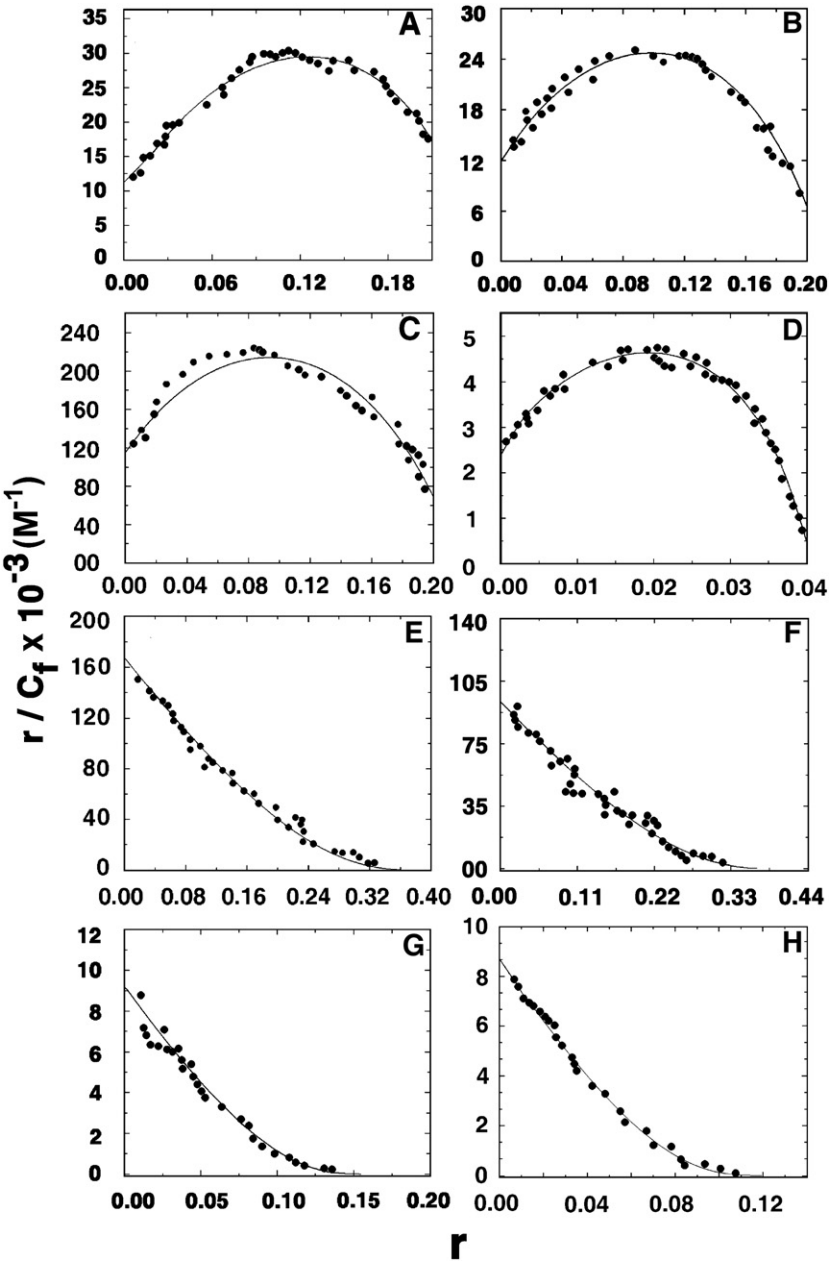

Fig. 2. Scatchard plots for the binding of berberine to (A) CT DNA (B) EC DNA (C) poly(dA).poly(dT) (D) poly(dG).poly(dC) (E) poly(dA–dT).poly(dA–dT) (F) CP DNA (G) ML DNA and (H) poly(dG–dC).poly(dG–dC) obtained from spectrophotometric analysis as described in [34]. The solid lines represent the non-linear least square best fit of the experimental points to the McGhee–von Hippel equation. In case of (A–D) the data were fit to the cooperative binding model while in (E–H) the non-cooperative binding model was employed. The best-fit values of values of K_a, n and ω are presented in Table 1.