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Thermodynamics of the interaction of berberine with DNA

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Abstract—The interaction of berberine [7,8,13,13*a*-tetrahydro-9-10-dimethoxy-2,3-(methylene dioxy)-berberinium] with calf thymus DNA has been studied by spectrophotometry in buffers of various salt concentrations and temperatures. Binding parameters obtained are best fit by the neighbour exclusion model. The salt and temperature dependence of the binding constants are used to estimate thermodynamic parameters involved in the complex formation of berberine with DNA. The binding process is exothermic over the entire range and the values of enthalpy and entropy change are strongly dependent on the salt concentration. The negative enthalpy and positive entropy changes compensate one another to produce a relatively small Gibbs' free energy change. Possible molecular contribution to the enthalpy and entropy changes is discussed.

Berberine chloride [7,8,13,13*a*-tetrahydro-9-10-dimethoxy-2,3-(methylene dioxy)-berberinium chloride] is one of the most widely distributed plant alkaloids and is known to occur in at least nine botanical families. Berberine and several of its derivatives have diverse biological activities [1] of which antimalarial, antiplatelet and anticancer activities are the most significant [2]. DNA binding is thought to be crucial for its biological activities. In fact, our earlier studies [3–7] established that (i) berberine intercalates to DNA exhibiting AT base pair preference, (ii) it shows a surprising affinity for single stranded poly (A) chains over DNA and tRNA and (iii) it transforms the conformation of poly (dI-dC).poly(dI-dC) to an A-form on binding. To substantiate the strong affinity of berberine for DNA and to understand the reasons for the stability and forces of interaction, this report describes the thermodynamic aspects of the binding of berberine to DNA.

Materials and Methods

Calf thymus DNA (type 1) and berberine chloride were from the Sigma Chemical Co. (St Louis, MO, U.S.A.). The concentrations of DNA and berberine were determined using extinction coefficients (ε) of 6600 M^{-1} cm^{-1} at 260 nm (in terms of nucleotide phosphate) and 22,500 M^{-1} cm^{-1} at 344 nm, respectively. All the experiments were performed in BPES buffer (pH 6.9 ± 0.1) containing 1.5 mM of Na_2HPO_4, 0.5 mM NaH_2PO_4 and 0.25 mM EDTA with different Na^+ molarity obtained by the addition of required volumes of known concentrated sodium chloride solution.

Spectroscopic binding studies were performed at 15°, 25°, 35° and 45° on a Shimadzu UV 260 spectrophotometer equipped with a temperature programmer (KPC-5) and temperature controller using matched cuvettes as reported earlier [8, 9]. Thermodynamic parameters were elucidated from a complete titration at a particular temperature or by increasing the temperature of a sample containing a fixed ratio of DNA/berberine (P/D) as described [8] allowing an equilibrium period of 10 min. before recording the spectrum. Comparison of values obtained by either method showed excellent agreement. The titration data obtained at the absorbance maximum 344 nm of berberine were used in Scatchard plots [10]; r/C versus r, where r is the

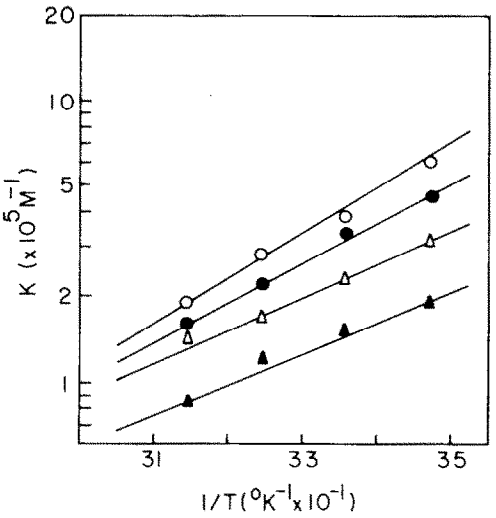

Fig. 1. van't Hoff plot of berberine–calf thymus DNA interaction at various [Na^+] concentrations: 6 mM BPES (○), 10 mM BPES (●), 20 mM BPES (△) and 50 mM BPES (▲). In all cases the correlation coefficient value varies between 0.962 and 0.986.

Table 1. Binding parameters for berberine–DNA complexation*

[Na^+] (M)	Temp. (°C)	K ($\times 10^4 M^{-1}$)	n
0.006	15	57.0 ± 3.0	3.8 ± 0.09
	25	37.5 ± 2.0	4.0 ± 0.10
	35	28.0 ± 1.5	4.0 ± 0.10
	45	19.0 ± 1.0	4.0 ± 0.11
0.010	15	45.0 ± 2.3	5.7 ± 0.13
	25	33.0 ± 1.8	6.0 ± 0.14
	35	22.0 ± 1.1	6.4 ± 0.16
	45	16.0 ± 0.8	6.5 ± 0.16
0.020	15	32.0 ± 1.6	10.7 ± 0.26
	25	22.5 ± 1.2	10.7 ± 0.25
	35	16.5 ± 0.8	11.5 ± 0.27
	45	14.0 ± 0.7	12.6 ± 0.29
0.050	15	19.0 ± 1.0	18.5 ± 0.37
	25	15.0 ± 0.8	20.0 ± 0.40
	35	12.0 ± 0.6	22.0 ± 0.50
	45	8.5 ± 0.40	22.5 ± 0.55

* Values are means of five determinations.

Table 2. Thermodynamic parameters for berberine–DNA complexation*

[Na^+] (M)	ΔG^0 (25°) (kcal/mol)	ΔH^0 (25°) (kcal/mol)	ΔS^0 (25°) (cal/deg/mol)
0.006	−7.68 ± 0.07	−6.78 ± 0.15	1.13 ± 0.09
0.010	−7.56 ± 0.07	−6.37 ± 0.14	3.99 ± 0.32
0.020	−7.36 ± 0.06	−5.38 ± 0.12	6.67 ± 0.53
0.050	−7.09 ± 0.06	−5.03 ± 0.11	6.91 ± 0.55

* Values are means of five determinations.

number of molecules of berberine bound per nucleotide and C is the free concentration of berberine. The data fit well to the neighbor exclusion model of McGhee and von Hippel [11] for non-cooperative binding according to the following equation:

$$r/C = K(1 - nr)[(1 - nr)/(1 - (n - 1))r]^{n-1}$$

where K and n represent the intrinsic binding constant to an isolated site and the exclusion parameter, respectively.

The analysis of van't Hoff plots, LnK versus $1/T$ obtained over the temperature range of the study, enabled the calculation of ΔH^0, since the gradient is equal to $-\Delta H^0/R$. ΔG^0 and ΔS^0 were calculated in turn from the relationships, $\Delta G^0 = -RT\text{Ln}K$ and $\Delta G^0 = \Delta H^0 - T\Delta S^0$, as described earlier [8, 9].

Results and Discussion

The binding of berberine with increasing concentrations of calf thymus DNA was studied spectrophotometrically. The binding parameters determined from these measurements by Scatchard analysis according to an excluded site model [11], are presented in Table 1 and show that binding affinity decreases with increasing temperature or NaCl concentration. The 5-fold difference in n value on raising the ionic strength from 0.006 to 0.05 M suggests that the electrostatic force plays an important role [8, 12]. Figure 1 illustrates the van't Hoff plots of berberine–DNA interaction at different salt concentrations. The linear relationship indicates small values of heat capacity change, analogous with that observed for other intercalators [8, 12]. The values of the thermodynamic parameters as a function of sodium ion concentration obtained at 25° are given in Table 2. As the ionic strength is increased the enthalpy value slightly decreases from 6.78 to 5.03 kcal/mol, while the value of entropy of binding increases from 1.3 to 6.91 cal/deg/mol revealing the binding to be mostly entropy driven. Hopkins and Wilson [13] and Breslauer *et al.* [14] have shown that the electrostatic interaction between ethidium, DAPI and netropsin contributes little towards overall binding. At the highest ionic strength studied here, the contributions of electrostatic forces are negligible and the positive contributions to the entropy suggest the importance of the hydrophobicity of the compound in its transfer from the bulk solvent to the intercalation site. Similar positive entropic contributions to DNA binding are observed for intercalators like ellipticine, *o*-AMSA, *m*-AMSA and ethidium [15, 16]. Berberine with partial saturation in the ring has a buckled structure and it is likely that its intercalation results in a transient base pair unstacking [17], resulting in the disruption of the spine of the water structure and an altered drug–DNA complex all, of which could contribute to positive entropy.

Another interesting feature was observed when the data of Table 2 were plotted in terms of enthalpy change versus entropy change (not shown). This plot yields a compensation temperature of 320°K with a linear correlation coefficient of 0.963 showing a similar enthalpy–entropy compensatory behaviour to other intercalators [12]. It is likely that positive contribution to the binding entropy can be envisioned as an endothermic process, thereby reducing the exothermicity of the observed binding enthalpy, resulting in enthalpy–entropy compensation. However, we have ascertained that our data on compensation behaviour are not artifactual, in that the observed compensation temperature, 320°K, is significantly different from the harmonic mean temperature of 303°K at which the data were gathered. Also, more importantly, a plot of enthalpy change versus free energy change again retains a linear relationship (not shown) implying that the observed compensation phenomenon reflects a true chemical causality. In conclusion, the thermodynamic parameters of berberine–DNA interaction determined here indicate the binding to be an exothermic and entropy-driven process. The negative enthalpy and the positive entropy values characterize the gross changes in the overall binding process.

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