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THERMODYNAMICS OF THE INTERACTION OF ARISTOLOLACTAM-β-D-GLUCOSIDE WITH DNA

IONIC STRENGTH DEPENDENCE OF ENTHALPY AND ENTROPY

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Abstract—The interaction of aristololactam-β-D-glucoside with calf thymus DNA has been studied by measuring the changes in the absorbance of the alkaloid over a wide range of temperatures and sodium chloride concentrations. The binding parameters obtained are best fit by the neighbour exclusion model. The salt and temperature dependence of the binding constants are used to estimate the thermodynamic parameters involved in the interaction of the alkaloid with DNA. It is observed that aristololactam-β-D-glucoside binding to DNA is an exothermic process over the entire range of salt and temperature, and the estimated values of enthalpy and entropy change are strongly dependent on the ionic strength of the solution. The enthalpy and entropy changes compensate one another to produce a relatively small Gibbs' free energy change. The possibility that aristololactam-β-D-glucoside exists as a monovalent cation at neutral pH and the possible molecular contribution to the enthalpy and entropy changes of the aristololactam-β-D-glucoside–DNA complex are discussed.

We have learned a great deal about the intercalator–DNA interaction, particularly directed towards understanding the fundamental binding equilibria involved, the structures of intercalated complexes, and the structural alterations in DNA that result from intercalation [1, 2]. It is known that thermodynamic and structural studies are mutually complementary and both are necessary for complete elucidation of the molecular details of the binding process for the delineation of the molecular interaction involved at the intercalator–DNA binding site [3–5].

Aristolochia group of alkaloids and their glucoside derivatives have attracted recent attention for their antimicrobial, antitumour and various other biological properties [6]. Among this group of alkaloids, aristololactam-β-D-glucoside (Structure I) has recently been shown in our laboratory to form a molecular complex with DNA by the mechanism of intercalation [7]. However, the thermodynamic parameters of its intercalation phenomenon have remained unexplored.

The present study has been undertaken in order to examine the enthalpy and entropy values involved in the intercalation process over a wide range of temperature and sodium chloride concentration from the measurement of absorption spectrophotometric studies.

MATERIALS AND METHODS

Chemicals.

Aristololactam-β-D-glucoside (ADG) was extracted from *Aristolochia indica* and was crystallized from ethanol. Its purity was checked as described previously [7, 8]. The alkaloid concentration was determined spectrophotometrically using molar extinction coefficient (ε) of 10930 M^{-1} cm^{-1} at 398 nm in dimethyl sulphoxide (DMSO). Calf thymus (CT) DNA (type I, 42 mole % GC) and DMSO were obtained from the Sigma Chemical Co. (St Louis, MO) and were used as such. DNA concentration in terms of nucleotide phosphate was determined spectrophotometrically using ε values of 6600 M^{-1} cm^{-1} at 260 nm. Deionized glass distilled water and analytical grade reagents were used throughout. The DNA binding experiments were performed in a BPES–DMSO buffer containing 1.5 mM Na_2HPO_4, 0.5 mM NaH_2PO_4, 0.25 mM EDTA, 240 mM DMSO, pH 7.0 ± 0.05 and different Na^+ molarity obtained by addition of required volumes of sodium chloride solution from a known concentrated stock.

Binding studies. The binding studies were performed at 15°, 25° and 40° on a Shimadzu UV-260 automatic recording spectrophotometer (Shimadzu Corporation, Japan) using procedures described in Ref. 7. Thermodynamic parameters were determined either from a complete titration at the given temperature or by increasing the temperature of a sample containing a fixed ratio of alkaloid/DNA as described [3] allowing an equilibration period of 10 min before each spectrum was recorded. Binding data were cast into the form of a Scatchard plot [9] of r/C vs r, where r is the number of moles of ligand bound per mole of DNA phosphate and C is the concentration of free ligand, and were fitted to the neighbour exclusion model [10, 11]:

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

where K is the binding constant to an isolated DNA binding site and n is the exclusion parameter.

Estimation of thermodynamic parameters. The Gibbs' free energy change (ΔG) was determined from the binding constant according to the relation

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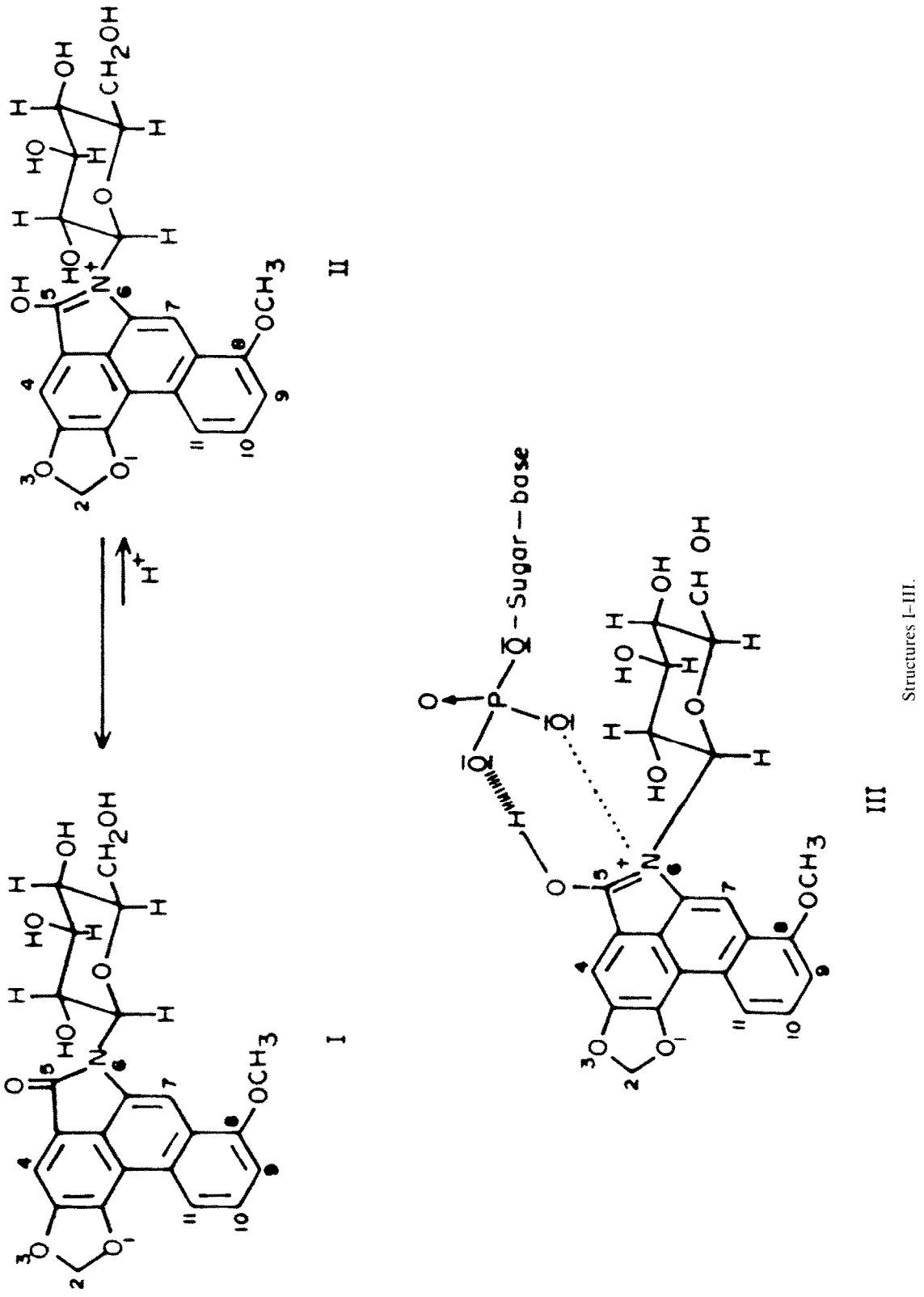

Structures I–III.

$$-\Delta G = RT \ln K \quad (2)$$

The binding enthalpy change (ΔH) was determined from plots of the temperature dependence of the binding constant according to the van't Hoff relationship

$$[\delta \ln K/\delta(1/T)] = -\Delta H/R \quad (3)$$

The entropy change (ΔS) was estimated from the Gibbs' free energy and the enthalpy as

$$\Delta S = -(\Delta G - \Delta H)/T \quad (4)$$

RESULTS

The effect of progressive increment in the concentration of calf thymus DNA on the absorption spectrum of ADG was studied at three different temperatures in buffers of different molarities of sodium ion at pH 7.0. The spectrophotometric measurements in buffer of a particular sodium molarity were obtained at 15°, 25° and 40°. The spectral changes involve essentially a red shift and hypochromicity in complexes until saturation is reached. However, hypochromicity for a particular ADG/DNA ratio decreases with increase in temperature.

The spectrophotometric titration data for the interaction of ADG with DNA at various salt molarities and temperatures is shown in Fig. 1. The fact that the hypochromicities of ADG–DNA complexes are also significantly dependent on the salt molarity of the solution is observed in Fig. 1.

Binding isotherms for the interaction of ADG with calf thymus DNA at different sodium concentrations and temperatures are shown in Fig. 2. Data of the above isotherms are fit to the neighbour exclusion model (Eqn 1) and the results are presented in Table 1. It can be seen from Fig. 2A and B that the binding affinity of ADG to DNA significantly increases with decreasing sodium molarity and also with decreasing temperature of the reaction mixtures. However, significant deviation from neighbour exclusion predictions are observed in low ionic strength (below 0.02 M).

The dependence of the binding constant K on the ionic strength of the buffer medium expressed as total positive ion concentration, $[Na^+]$ at different temperatures is shown in Fig. 3. The nature of this relationship has been stated by Record *et. al.* [12] and it is expressed as

$$\ln K = m'\psi \ln[Na^+] + \ln K_0 \quad (5)$$

where m' is the charge on the alkaloid molecule, and ψ is the fraction of counter ions associated with each DNA phosphate group (for double stranded DNA–ligand complex $\psi = 0.82$ as reported by Wilson and Lopp [13]). The quantity $m'\psi$ is the number of counter ions released upon binding of a ligand with charge m' and K_0 is the value of K at 1 M positive ion concentration which is assumed to be free of electrostatic components. The possibility of ADG existing as a monovalent cation under the conditions studied here is discussed (see discussion section below). The slopes of the straight lines (Fig. 3) indicate release of 0.57, 0.49 and 0.38 Na^+ ions per bound ADG molecule at 15°, 25° and 40°, respectively. Extrapolation of the data to 1 M $[Na^+]$ion concentration yields K_0 values of 6.5×10^4, 4×10^4 and 2.31×10^4, respectively, at the three different temperatures studied.

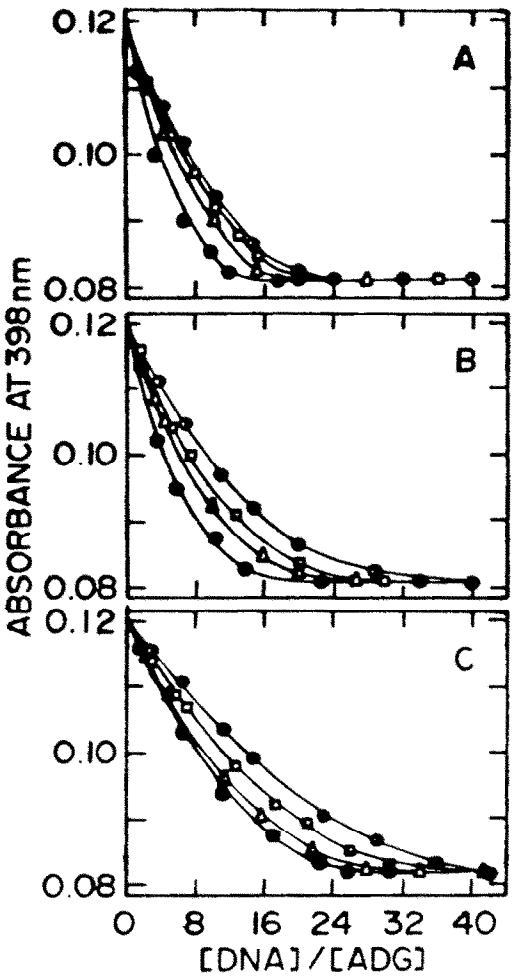

Fig. 1. Spectrophotometric titration data on the binding of ADG (11.16 μM) to calf thymus DNA in BPES–DMSO buffer of $[Na^+]$ 0.02 M (●—●), 0.05 M (△—△), 0.1 M (□—□) and 0.2 M (◑—◑) at 15° (A), 25° (B), and 40° (C) respectively.

Figure 4 shows van't Hoff plots used to estimate ΔH and ΔS of the ADG binding reaction as a function of salt concentration. The data fits to a straight line, indicating a small value of heat capacity change in analogy with that observed for several other drug–DNA interactions [3, 4]. The values of binding and thermodynamic parameters of the ADG–DNA interaction as a function of sodium ion concentration are presented in Table 1. It can be seen from Table 1 that there is very little variation in the values of ΔG with increasing salt concentration while the values of ΔH and ΔS lower considerably under identical conditions. Finally, Fig. 5 shows that the values for the ΔH and ΔS are linearly correlated, indicating a general feature for the intercalation phenomenon.

DISCUSSION

In this paper, we have analysed the binding of

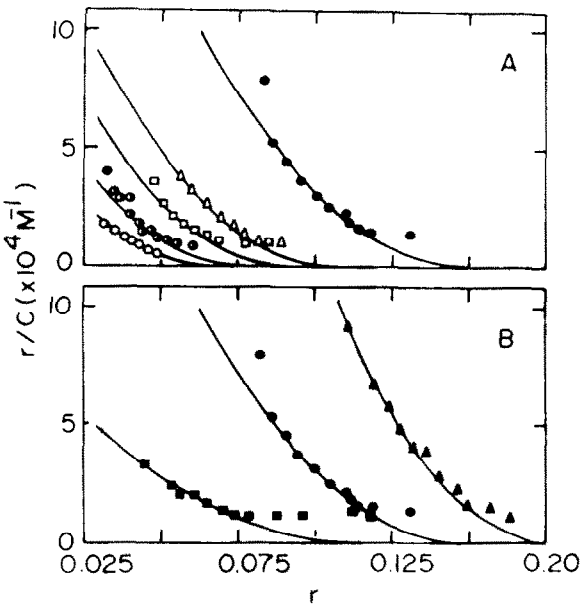

Fig. 2. Representative Scatchard plots for ADG binding to calf thymus DNA in (A) BPES–DMSO buffer of varying [Na^+] 0.02 M (●—●), 0.05 M (△—△), 0.1 M (□—□), 0.2 M (◑—◑), 0.5 M (○—○) and in (B) BPES–DMSO buffer of [Na^+] 0.02 M at 15° (▲—▲), 25° (●—●) and 40° (■—■).

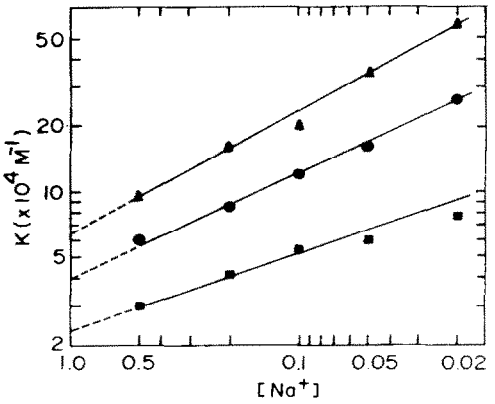

Fig. 3. Plot of K as a function of ionic strength [Na^+] for ADG binding to calf thymus DNA at 15° (▲—▲), 25° (●—●) and 40° (■—■).

ADG to calf thymus DNA over a wide range of temperatures and ionic strengths. The interaction is sensitive to both of these variables. The importance of electrostatic forces with regard to the formation of the complex is evident from the results obtained from spectrophotometric studies when the ionic strength of the medium is increased. The binding parameters, K and n (Table 1) suggest that the electrostatic force is an important criterion for the interaction of ADG with DNA. It is thus reasonable to believe that ADG may exist as a monovalent cation and this can be explained as follows. In aqueous buffer solutions under neutral pH the equilibrium is predominantly in the neutral form (structure I). However, the equilibrium may shift gradually in favour of structure II when ADG is intercalated to DNA [14]. In this context, it can be noted that ADG undergoes a pH dependent structural change with a pK value of 12.7 [15] whereas we assume N6 of ADG is protonated around neutral pH. Again it is probable that the initiating step for the formation of an intercalated complex involves an electrostatic attachment of the cationic form of the ligands to the negatively charged phosphate groups outside the DNA helix axis. In case of ADG this could be achieved by the formation of an ion-pair with the quaternary N6 of ADG and the phosphate oxygen (structure III). Further stabilization may be

Table 1. Binding and thermodynamic parameters for the ADG–DNA interaction

Na^+ (M)	Temp (°C)	K ($\times 10^4$ M^{-1})	n	$-\Delta G$ (25°) kcal/mol	$-\Delta H$ kcal/mol	$-\Delta S$ (25°) cal/degree/mol
0.02	15	58.0 ± 4.0	5.2 ± 0.10	7.43 ± 0.04	13.80 ± 0.25	21.50 ± 0.80
	25	26.0 ± 2.0	6.1 ± 0.15			
	40	8.0 ± 0.5	7.7 ± 0.17			
0.05	15	35.0 ± 2.2	7.2 ± 0.15	7.18 ± 0.02	11.89 ± 0.26	15.8 ± 0.69
	25	16.0 ± 0.8	8.5 ± 0.20			
	40	6.0 ± 0.4	10.3 ± 0.24			
0.10	15	20.0 ± 2.1	9.2 ± 0.12	6.98 ± 0.03	10.04 ± 0.36	10.20 ± 0.97
	25	12.0 ± 0.5	9.8 ± 0.2			
	40	5.5 ± 0.35	12.0 ± 0.25			
0.20	15	16.0 ± 1.4	9.3 ± 0.23	6.82 ± 0.02	8.86 ± 0.53	6.88 ± 1.45
	25	8.5 ± 0.5	11.5 ± 0.18			
	40	4.2 ± 0.2	13.5 ± 0.26			
0.50	15	9.5 ± 0.5	10.0 ± 0.24	6.56 ± 0.02	8.54 ± 0.56	6.68 ± 1.53
	25	6.0 ± 0.4	13.0 ± 0.26			
	40	3.0 ± 0.2	15.0 ± 0.3			

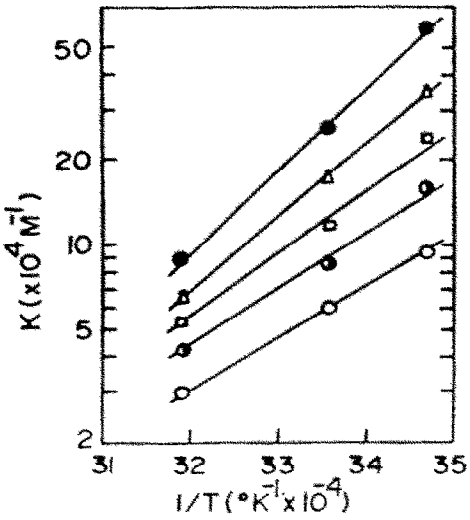

Fig. 4. Representative van't Hoff plots of the ADG–DNA interaction. Symbols are same as Fig. 2A.

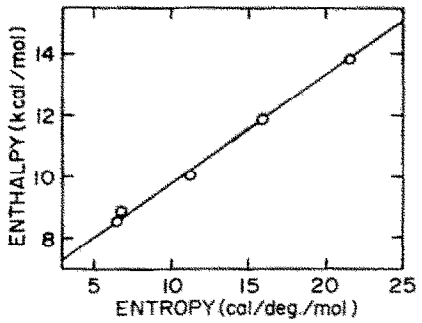

Fig. 5. Plot of ΔH vs ΔS for binding of ADG to calf thymus DNA over range of [Na^+] ion concentration (data from Table 1). The data is fitted to a straight line with a slope of 355°K and correlation coefficient of 0.941.

obtained via H-bond formation of the C5-OH group of ADG with DNA phosphate oxygen. However, H-bond formation may also be possible with the base oxygen or base nitrogen which are also accesible to the alkaloid.

The possibility was considered that the ionic strength dependence on binding might be artifactual perhaps arising from aggregation of alkaloid molecules at higher salt concentration, but no evidence for such a phenomenon was found. Moreover, no deviation from Beer's Law was noted over the entire range of concentrations studied. The ionic strength dependence on binding of ADG to DNA is in good agreement with other intercalating ligands like ethidium [16], anthracyclines [17], quinacrine [13] and sanguinarine [18], all of which are positively charged. This is again in support of the above postulation.

Record's expression (Eqn 5) can also be used to predict the expected changes in the Gibbs' free energy on the basis of polyelectrolyte theory. Solving Eqn 5 for the different concentrations of salts it has been found that such predictions are in agreement with the free energy changes calculated from the salt dependence of the neighbour exclusion model's binding constants. Over the entire range of sodium ion concentration studied, ADG binding process is exothermic (Table 1). Both ΔH and ΔS of the binding reaction are strongly dependent on the ionic strength, while ΔG is less dependent, due to compensating effects in the enthalpy and entropy. The enthalpy and entropy (Fig. 5) compensation is characterized as a general feature for intercalation phenomenon [3, 4]. The compensation temperature 355°K (slope of Fig. 5) is significantly different from the harmonic mean temperature of 299°K at which the data were collected. Again, a plot of ΔH and ΔG retains linear relationship (data not shown) with correlation coefficient of 0.997 implying that the observed compensation behaviour reflects true chemical causality [3, 4]. Similar enthalpy and entropy compensatory phenomenon was reported for daunomycin [3, 4] and ethidium [16] binding to calf thymus DNA.

Taking analogy with other intercalators, the possible contributions for negative enthalpy indicates the molecular interactions at the intercalation site, while negative entropy value is due to the decreased flexibility in the DNA helix following intercalation [3]. It may, therefore, be concluded that (i) ADG can exist as a monovalent cationic ligand at neutral pH, (ii) the ADG–DNA interaction involves a large favourable non-electrostatic part and (iii) it shows a similar enthalpy–entropy compensatory behaviour like many other intercalators.

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