A THEORETICAL ANALYSIS OF DRUG-DNA INTERACTIONS: STABILITY OF POLY d(AT) BINDING WITH AMINOSTEROID DIPYRANDIUM

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Abstract. In recent years, characterization of the thermodynamics of DNA-drug interactions has acquired considerable interest in rational drug design. The number and variety of techniques committed to evidence drug-DNA interactions are continuously growing. Recent analysis on structure and dynamics of nucleic acid suggest that the DNA helix undergoes conformational transition as function of salt and solvent. The DNA accommodates the drug by changing its conformation in a novel manner that leads folding of DNA by the generation of kinks. The aminosteroid binds to DNA through the minor groove at the kink site and stabilized the drug-DNA complex. In the present study, we reported theoretical analysis of aminosteroid, dipyrandium, binding with poly d(AT) by using an amended Zimm and Bragg theory, to explain the melting behaviour and heat capacity of DNA with and without dipyrandium binding. In this study we used experimental models of Marky *et al.* [13]. The sharpness of transition has been examined in terms of half width and sensitivity parameter. The results suggested that a range of parameters such as transition profile, sharpness of the transition, heat capacity curve and half widths are in good agreement with the experimental measurements for binding of dipyrandium. An understanding of drug-DNA interactions at the molecular level is important in facilitating the design of new drugs. This theoretical study would represent a further step toward the goal of understanding the stability of nucleic acid interactions with drugs and thus can be applied in biomedical industries.

Key words: Aminosteroid, DNA binding, Dipyrandium, heat capacity, transition profile.

INTRODUCTION

Molecular interaction of the drug with DNA is a field of high topical interest and may have a great importance to its biological activity. The molecules can interact with DNA in a variety of ways such as surface binding to their minor or major grooves, intercalation between adjacent base pairs, covalent attachments to the double helix, or electrostatic binding. The study of drug-DNA interactions begins since 1960s but the binding of steroid-diamines to synthetic and natural

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nucleic acids has received considerable attention over the past several years [1, 2, 4–11, 16, 25, 26]. Thus, some more theoretical and experimental studies are conducted based on the assumption that the steroid diamine binding may 'kink' DNA as suggested by Sobell *et al.* [1–3, 5–7, 8, 12, 14–16, 19, 21, 27]. The naturally occurring steroidal diamine, dipyrandium, is amphiphilic in nature, and its biophysical features include increasing the duplex denaturation temperature and altering of the UV and CD spectra of DNA. Dipyrandium (Fig. 1) is an aminosteroid neuromuscular blocking agent that revolutionized the performance of anesthesia. The binding of dipyrandium to DNA is proposed to occur in the minor groove in conjunction with 5'-d(TA) kinks [14]. The aminosteroid-DNA complex model suggested that the Watson-Crick hydrogen bonds are intact in the neighbor-exclusion complex and that every other set of base pairs partially unstacks and the steroid diamines partially insert at this site [20].

The calorimetric analysis of aminosteroid binding with DNA duplex was reported for the first time by Marky *et al.* [13]. They recommended that increasing the concentration of bound steroid increases the thermal stability of the duplex and at saturation; the duplex melts with a T_m some 20 °C above that of the free duplex [13]. They also concluded that dipyrandium binding to poly d(AT) is an endothermic process and this binding increases the melting temperature of the duplex. In the present investigation, we have attempted to understand the effect of steroid diamine binding on a DNA duplex by using the experimental model of Marky and his coworkers [13] who studied the thermal and thermodynamic behaviour of dipyrandium binding to poly d(AT). We used amended Zimm and Bragg theory, to elucidate the order-disorder transition in dipyrandium bounded and unbounded DNA duplexes, which is considered initially for the helix coil transitions in polypeptides [22, 24, 28]. We also explained lambda point anomalies in heat capacities by using the same theory. The effect of dipyrandium binding is reflected in the change in nucleation parameter, which is an inverse measure of binding strength.

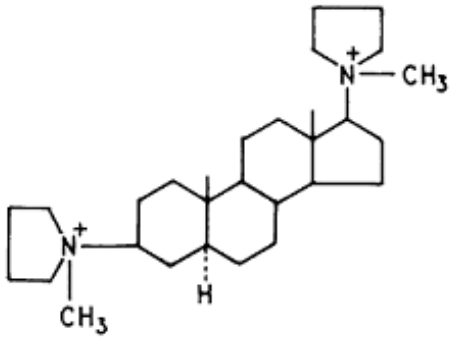

Fig. 1. Molecular structure of dipyrandium.

THEORY

The model proposed by Sobell for the aminosteroid-DNA complex on the basis of NMR study, as mentioned above, suggested that the Watson-Crick hydrogen bonds are intact in the neighbor-exclusion complex and that every other set of base pairs partially unstacks, and the steroid diamines partially inserts at this site [20]. However, the system remains a highly co-operative one therefore the co-operative transition theory could be applied to explain the melting profile and temperature dependence of thermodynamical parameter, such as heat capacity. Therefore, we can use amended Zimm and Bragg theory [28] which is described in our previous publication [23]. Briefly, the above mentioned theory consists of writing an Ising matrix for a two-phase system, the bounded state and unbounded state. As discussed earlier [17, 18, 22–24] and by Zimm and Bragg [28], the Ising matrix **M** can be written as:

$$\mathrm{M} = \begin{array}{c} \\ \mathrm{f_r} \\ \mathrm{f_k} \\ \mathrm{f_h} \end{array} \begin{array}{c} \begin{array}{ccc} f_{\mathrm{r}} & f_{\mathrm{k}} & f_{\mathrm{h}} \end{array} \\ \begin{vmatrix} \mathrm{f_r}^{\frac{1}{2}}\mathrm{f_r}^{\frac{1}{2}} & \mathrm{f_r}^{\frac{1}{2}}\mathrm{f_k}^{\frac{1}{2}} & 0 \\ \mathrm{f_r}^{\frac{1}{2}}\mathrm{f_k}^{\frac{1}{2}} & 0 & \mathrm{f_k}^{\frac{1}{2}}\mathrm{f_h}^{\frac{1}{2}} \\ \mathrm{f_h}^{\frac{1}{2}}\mathrm{f_r}^{\frac{1}{2}} & 0 & \mathrm{f_h}^{\frac{1}{2}}\mathrm{f_h}^{\frac{1}{2}} \end{vmatrix} \end{array} \tag{1}$$

where f_{r}, f_{h} and f_{k} are corresponding base pair partition functions' contributions in the three states, i.e. ordered, disordered and boundary or nucleation. The eigen values of **M** are given by:

$$\begin{aligned} \lambda_1 &= \frac{\left[(\mathrm{f_r}+\mathrm{f_h})+\left\{(\mathrm{f_r}-\mathrm{f_h})^2+4\mathrm{f_r}\mathrm{f_k}\right\}^{\frac{1}{2}}\right]}{2} \\ \lambda_2 &= \frac{\left[(\mathrm{f_r}+\mathrm{f_h})-\left\{(\mathrm{f_r}-\mathrm{f_h})^2+4\mathrm{f_r}\mathrm{f_k}\right\}^{\frac{1}{2}}\right]}{2} \\ \lambda_3 &= 0 \end{aligned} \tag{2}$$

Since we are dealing with a finite system hence the effect of initial and final states becomes important. The contribution of the first segment to the partition function is given by:

$$\mathrm{U} = (\mathrm{f_r}^{\frac{1}{2}}, 0, 0) \tag{3}$$

where the column vector **V** gives the state of the last segment,

$$\mathbf{V} = \begin{vmatrix} f_{\mathrm{r}}^{\frac{1}{2}} \\ f_{\mathrm{k}}^{\frac{1}{2}} \\ f_{\mathrm{h}}^{\frac{1}{2}} \end{vmatrix} \tag{4}$$

The partition function for an *N*-segment chain is given by:

$$Z = U\mathbf{M}^{N-1}\mathbf{V} \tag{5}$$

The matrix **T** which diagnolizes **M** consists of the column vectors given by:

$$\mathbf{M}X = \lambda X \tag{6}$$

where

$$X = \begin{vmatrix} X_1 \\ X_2 \\ X_3 \end{vmatrix}$$

By substituting the values of **M** from Eq. (6), we get:

$$\mathrm{T} = \begin{vmatrix} 1 & 1 & 1 \\ \dfrac{(\lambda_1\text{-}\mathrm{f_r})}{\left(\mathrm{f_r^{\frac{1}{2}} f_k^{\frac{1}{2}}}\right)} & \dfrac{(\lambda_1\text{-}\mathrm{f_r})}{\left(\mathrm{f_r^{\frac{1}{2}} f_k^{\frac{1}{2}}}\right)} & -\left(\mathrm{f_r^{\frac{1}{2}} f_k^{\frac{1}{2}}}\right) \\ \dfrac{\left(\mathrm{f_h^{\frac{1}{2}} f_r^{\frac{1}{2}}}\right)}{(\lambda_1\text{-}\mathrm{f_h})} & \dfrac{\left(\mathrm{f_h^{\frac{1}{2}} f_r^{\frac{1}{2}}}\right)}{(\lambda_1\text{-}\mathrm{f_h})} & -\left(\mathrm{f_h^{\frac{1}{2}} f_r^{\frac{1}{2}}}\right) \end{vmatrix} \tag{7}$$

Similarly, we get T^{-1} from the matrix equation

$$Y\mathbf{M} = \lambda Y \tag{8}$$

where $Y = [Y_1, Y_2, Y_3]$.

Again by substituting the values of **M** from Eq. (1) in Eq. (8), we get:

$$\mathbf{T}^1 = \begin{vmatrix} C_1 & \dfrac{C_1\left(f_r^{\frac{1}{2}} f_k^{\frac{1}{2}}\right)}{\lambda_1} & \dfrac{C_1\left(f_k f_r^{\frac{1}{2}} f_h^{\frac{1}{2}}\right)}{\lambda_1(\lambda_1 - f_h)} \\ C_2 & \dfrac{C_2\left(f_r^{\frac{1}{2}} f_k^{\frac{1}{2}}\right)}{\lambda_2} & \dfrac{C_2\left(f_k f_r^{\frac{1}{2}} f_h^{\frac{1}{2}}\right)}{\lambda_2(\lambda_2 - f_h)} \\ C_3 & \dfrac{C_3\left(f_r^{\frac{1}{2}} f_k^{\frac{1}{2}}\right)}{\lambda_3} & \dfrac{C_3\left(f_k f_r^{\frac{1}{2}} f_h^{\frac{1}{2}}\right)}{\lambda_3(\lambda_3 - f_h)} \end{vmatrix} \tag{9}$$

The normalization constants are:

$$C_1 = \frac{\lambda_1 - f_h}{\lambda_1 - \lambda_2}, C_2 = \frac{\lambda_2 - f_h}{\lambda_2 - \lambda_1}, C_3 = 0 \tag{10}$$

If we let $\Lambda = T^{-1}MT$ be the diagonalized form of **M**, the partition function can be written as:

$$Z = U\mathbf{T}\Lambda N^{-1}\mathbf{T}^{-1}\mathbf{V} \tag{11}$$

On substituting the values from Eqs (1), (3), (4), (7), (9) and (10) in Eq. (11), the partition function becomes:

$$Z = C_1\lambda_1^N + C_2\lambda_1^N \tag{12}$$

The fraction of the segments in the disordered form is given by

$$Q_{\mathrm{r}} = \frac{\left[\frac{\delta lnZ}{\delta lnf_{\mathrm{r}}}\right]}{N}$$

Solving the above equation, we get:

$$Q_{\mathrm{r}} = \frac{1}{2} + \frac{(1-s)(2A-1)}{2P} + \frac{(1+s)\{(2A-1)P-1+s\}}{2P^2N} \tag{13}$$

where $P = \frac{\lambda_1-\lambda_2}{f_{\mathrm{r}}},\ s = \frac{f_{\mathrm{h}}}{f_{\mathrm{r}}},\ \sigma = \frac{f_{\mathrm{k}}}{f_{\mathrm{r}}},\ A = [(f_{\mathrm{r}} - f_{\mathrm{h}})^2 + 4f_{\mathrm{k}}f_{\mathrm{r}}]^{-2}$

Here *s* is the propagation parameter, which for simplicity is assumed to be 1. In fact, in most of the systems, it is found to be close to unity. If A_{r} and A_{h} are the absorbance in disordered and ordered states, respectively, the total absorption can be written as:

$$A = Q_{\mathrm{r}}A_{\mathrm{r}} + (1 - Q_{\mathrm{r}})A_{\mathrm{h}} \tag{14}$$

The extension of this formalism to specific heat (C_{v}) is straightforward. The specific heat is related to the molar enthalpy and entropy changes in the transition from state I to II. From the well known thermodynamic relations, free energy and internal energy are $F = -KT\ln Z$ and $U = -T^2\left(\frac{\delta}{\delta T}\right)\left(\frac{F}{T}\right)$, respectively. Differentiating internal energy with respect to temperature we get the specific heat:

$$C_v = \frac{\delta U}{\delta T} = N_{\mathrm{k}}\left(\frac{\Delta H}{RT_{\mathrm{m}}}\right)^2\left(\frac{S\delta Q_r}{\delta S}\right) \tag{15}$$

where ΔH is the molar change in enthalpy about the transition point, *S* is entropy which is equal to $S = \exp\left[\left(\frac{\Delta H}{R}\right)\left\{\left(\frac{1}{T}\right) - \left(\frac{1}{T_{\mathrm{m}}}\right)\right\}\right]$, T_{m} is the transition temperature, and

$$\frac{\delta Q_{\mathrm{r}}}{\delta S} = \left(\frac{1}{2P^2}\right)\left[\frac{2P(1-S)\delta A}{\delta S} - P(2A-1) - \frac{(1-S)(2A-1)\delta P}{\delta S}\right] + \left(\frac{1}{2P^3N}\right)\left[P\left\{(S+1)\left\{\frac{(2A-1)\delta P}{\delta S} + \frac{2P\delta A}{\delta S} + 1\right\} + \{(2A-1)P - 1 + S\}\right\} - \{(2A-1)P - 1 + S\}2(S+1)\right]$$

with $\frac{\delta A}{\delta S} = \left\{\frac{(S-\sigma)^N}{\left(\frac{z}{f_r^N}\right)^2}\right\} \times \left(\frac{\sigma}{P^3}\right) \times \left[-2 + \left\{\frac{N(S-2\sigma-1)}{S} - \sigma\right\}\right]$

$\frac{\delta P}{\delta S} = \frac{S-1}{P}$ and $\sigma = \frac{f_k}{f_r}$; σ is the nucleation parameter and is a measure of the energy expanded/released in the formation (uncoiling) of first turn of the ordered/disordered state. It is related to the uninterrupted sequence lengths [28]. The volume heat capacity C_v has been converted into constant pressure heat capacity C_p by using the Nernst-Lindemann approximation [18]:

$$C_p - C_v = 3RA_0\left(\frac{C_p^2 T}{C_v T_m}\right) \quad (16)$$

where A_0 is a constant often of a universal value [3.9×10^{-9} (kmol)/J] and T_m is the melting temperature.

RESULTS AND DISCUSSION

As stated above, the DNA structure still remains very much co-operative once it binds to dipyrandium and consequently the two-state theory of order-disorder transition is applicable. The hypothesis given by Zimm and Bragg [28] is amended so as to consider ordered (bounded/unbounded) and disordered states as the two states which co-exist at the transition position. The transition is characterized mainly by the nucleation parameter and overall change of enthalpy/entropy, which are also the main thermodynamic forces driving the transition. The change in enthalpy obtained from differential scanning calorimetric (DSC) measurements and changes in other transition parameters, such as nucleation parameter (σ) and melting point (T_m), takes all this into account. This may be further elaborated that the enthalpy change (ΔH) on binding of dipyrandium is maximum (7.6 kcal/M bp) in case of nucleotide to drug ratio infinity and minimum (5.09 kcal/M bp) at the ratio of 5:1, as shown in Table 1. It may also be concluded that by increasing the drug concentration the change of enthalpy decreases gradually (Table 1). The stability of duplex may be determined by its melting temperature (T_m) and it increases with increasing concentration of bounded dipyrandium. The melting temperature for free duplex was 46.5°C and when the duplex was saturated with dipyrandium, it melts with a T_m some 28°C above that of the unbounded duplex (Table 1). The drug-induced increase in the thermal stability of the duplex is accompanied by a decrease in the overall transition enthalpy of the duplex. The sharpness of the transition can be looked at in terms of half width and a sensitivity parameter defined as ($\Delta H/\sigma$). The variation

of these parameters systematically reflects that the transition is the sharpest in case of unbounded state and goes in order to nucleotide to dipyrandium ratio of 17:1, 10:1 and 5:1. In case of λ-transition, the same trend in the sharpness of transition is seen between the dipyrandium bounded as well as unbounded curves. As expected, the sharpness is better in unbounded as compared to bounded state. The various parameters, which give transition profiles in best agreement with the experimental measurements for binding of dipyrandium, are presented in Table 1.

Table 1

Transition parameters for dipyrandium binding to poly d(AT)

Parameters	Phosphate/Drug (P/D) ratio			
	∞ (unbounded)	17/1	10/1	5/1
Melting Temp. (K)	319.5	323.2	331.57	347.5
ΔH kcal/M bp	7.6	6.61	6.43	5.09
σ	4×10^{-4}	1×10^{-3}	3×10^{-3}	3.5×10^{-3}
N	106	106	106	50
A_h	0	0	0	0
A_r	1	1	1	1
Sensitivity parameter ($\Delta H/\sigma$)	19000	6610	2143.33	1454.28

The heat capacity and transition profiles for unbounded nucleotide, poly d(AT), and with different concentrations of aminosteroid, dipyrandium, are shown in Figure 2. In case of heat capacity curve some insignificant divergences are reported at the tail ends may be due to the presence of different disordered states, as these states cannot be distinctively defined. Additionally, these variations could also arise due to the presence of short helical segments found in the random coil states. The experimental and calculated transition curves for the duplex to single strand transition of poly d(AT) in the absence of dipyrandium are shown by the graph 'A' in Figure 2. Similarly, graphs 'B' and 'C' show the transition in the presence of dipyrandium at nucleotide to drug ratios of 17:1 and 10:1, respectively. In Figure 2, graph 'D' shows the poly d(AT) transition when the duplex is saturated with dipyrandium (nucleotide to drug ratio of 5:1). There is a cooperative transition profile in between calculated and experimental data that was anticipated from the results. The results presumed from the theoretical data are in accordance with the experimental data of Marky *et al.* [13] that were directly measured binding enthalpy through a differential scanning calorimetric method. The presented data of theoretical analysis also demonstrated that dipyrandium binding to DNA duplex is an endothermic process and this binding increases the melting temperature of the duplex as supported by differential scanning calorimetric measurement. Marky and coworkers have used DSC to investigate the binding of dipyrandium to the poly d(AT) duplex [13]. However, Patel and Canuel used NMR for the same study [14].

In view of that, we can interpret our theoretical analysis results in the perspective of the particular structural features of the drug-DNA complex as deduced from their experimental data obtained through DSC/NMR study. It is evident from Figure 2 (graphs A and D) that the transition of the dipyrandium saturated DNA duplex is significantly broader than the transition of the dipyrandium free DNA duplex. Therefore, it is clear that besides affecting the enthalpy and melting temperature, binding of dipyrandium also alters the nature of the transition as reflected by the increase in transition width in experimental as well as calculated data (Fig. 2). Heat capacity is a second derivative of the free energy and has been calculated by using equation (15). By using heat capacity the dynamical and conformational states of a macromolecular system are characterized. The heat capacity curves, obtained from theoretical and experimental data, along with their transition profile are shown in Figure 2. From these curves it may be concluded that the theoretically obtained heat capacity profiles agreed with the experimentally reported ones and could be brought almost into coincidence with the use of scaling factors, which are very close to one in transition profiles and slightly higher for the heat capacity curves. In addition, the sharpness of the transition can also be characterized by the half width of the heat capacity curves. The half widths of these heat capacity curves are also calculated which is in good agreement in both theoretical and experimental graphs (Fig. 3).

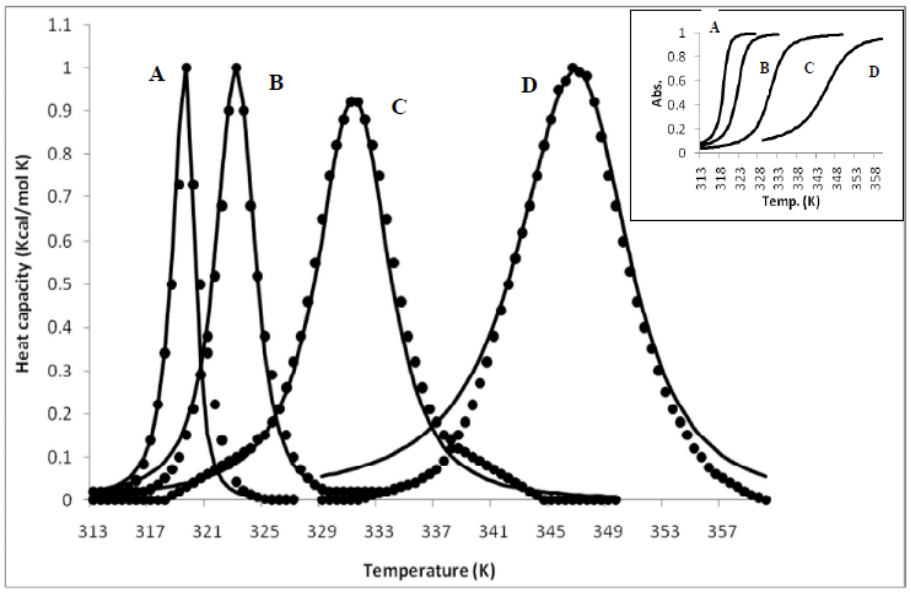

Fig. 2. Heat capacity and transition profiles (inset) for unbounded and bounded DNA with dipyrandium. (A) Unbounded state, (B) Bounded state with P/D ratio 17/1, (C) Bounded state with P/D ratio 10/1, (D) Bounded state with P/D ratio 5/1 [(—) calculated and (••••) experimental values].

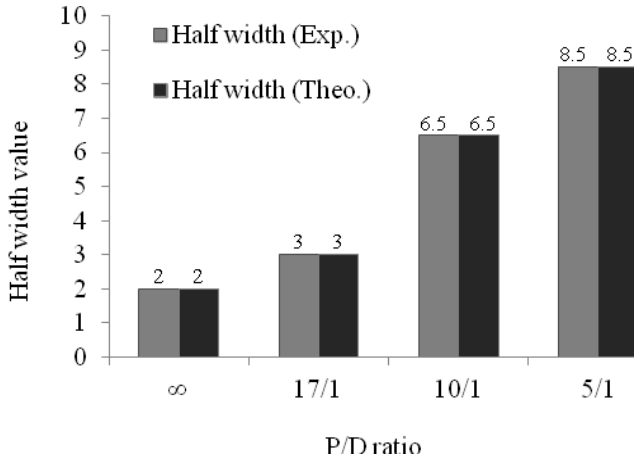

Fig. 3. Half width of the heat capacity curves.

CONCLUSION

In recent years, the study of nucleic acid interaction with molecules is of high interest [1, 5–7, 16]. The present study concluded that the DNA molecule is an extremely co-operative structure and when dipyrandium binds to it the co-operativity is not so much disturbed. Thus, the amended Zimm and Bragg theory can be effectively applied to it. It generates the experimental transition profile and λ-point heat capacity anomaly successfully. The results obtained will allow us to evaluate the thermodynamic profile of the binding process. This theoretical analysis of steroid diamine binding with nucleotide is being extended to other synthetic and natural DNA polymers. This theoretical analysis can also be useful in order to understand bimolecular interaction and thus can be applied in the process of drug design and development.

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