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Full Length Research Paper

Stability of DNA binding with dipyrandium: A theoretical study

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Recent analysis on structure and dynamics of nucleic acid suggest that the DNA helix undergoes conformational transition as function of salt and solvent. This flexibility leads folding of DNA either smoothly or its chain direction changes abruptly by the generation of kinks. The steroid diamine binds to DNA through the minor groove at the kink site and stabilized the complex. In the present study, we reported theoretical analysis of steroid diamine, dipyrandium, binding with DNA duplex by using an amended Zimm and Bragg theory, to explain the melting behavior and heat capacity of DNA with and without dipyrandium binding. The experimental models of Marky et al. (1983) were used for the study. The sharpness of transition has been examined in terms of half width and sensitivity parameter (∆H/δ). The various 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. This theoretical analysis can be applied in order to understand bimolecular interaction and thus can be applied in the process of drug designing and development.

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

INTRODUCTION

The study of drug-DNA interactions began since 1960s but the binding of steroid-diamines to synthetic and natural nucleic acids has received considerable attention over the past several years (Mahler and Dutton, 1964; Mahler et al., 1966, 1968; Waring, 1970; Gabbay and Glaser, 1971; Waring and Chisholm, 1972; Lane, 2001; Bruylants et al., 2005; Araya et al., 2007; Islam et al., 2009; Paul et al., 2010; Gonzalez-Ruiz et al., 2011; Kunwar et al., 2011). According to Sobell et al. and other theoretical and experimental studies, steroid diamine binding may 'kink' DNA (Waring and Henley, 1975; Sobell et al., 1977; Saucier, 1977; Dattagupta et al., 1978; Patel and Canuel, 1979; Patel, 1979; Manning, 1979; Vorlickova et al., 1985; Lane, 2001; Bruylants et al., 2005; Araya et al., 2007; Islam et al., 2009; Paul et al., 2010; Gonzalez-Ruiz et al., 2011; Kunwar et al., 2011). Dipyrandium (Figure 1) is an aminosteroid neuromuscular blocking agent that revolutionized the performance of anesthesia. The model proposed by Sobell et al. (1976) for the aminosteroid-DNA complex on the basis of nuclear magnetic resonance (NMR) study 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 (Sobell et al., 1976).

Initially, the calorimetric analysis of steroid diamine binding to a DNA duplex was reported by Marky et al. (1983). According to their study 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 (Marky et al., 1983). They also concluded that binding of dipyrandium to poly d(AT) is an endothermic process and this binding increases the melting temperature of the duplex. In the present study, we have attempted to understand the effect of steroid diamine binding on a DNA duplex using the model of Marky et al. (1983) who studied the thermal and thermodynamic behaviour of dipyrandium binding to poly d(AT). To elucidate the order-disorder transition in

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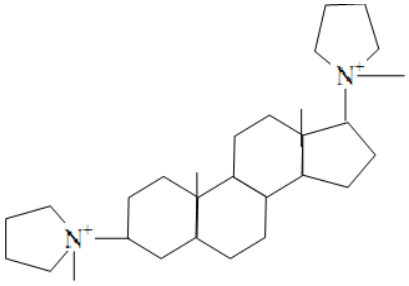

Figure 1. Structure of dipyrandium.

in dipyrandium bounded and unbounded DNA duplexes, we used amended Zimm and Bragg theory which is considered initially for the helix coil transitions in polypeptides (Zimm and Bragg, 1959; Srivastava et al., 1999, 2001). As shown later in theory, the effect of dipyrandium binding is reflected in the change in nucleation parameter, which is an inverse measure of binding strength. Once again by using amended Zimm and Bragg theory, we explained lambda point anomalies in heat capacities.

THEORY

It is clear from the above described model that Watson-Crick hydrogen bonds are intact in the neighbor-exclusion complex and every other set of base pairs partially unstacks, and the steroid diamines partially inserts at this site. Though, 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. We can use amended Zimm and Bragg (1959) theory as described in our previous publication (Srivastava et al., 2004). In brief, the theory consists of writing an Ising matrix for a two-phase system, the bounded state and unbounded state. As discussed earlier (Srivastava et al., 1999; 2001; Srivastava et al., 2004; Poklar et al., 1996; Roles and Wunderlich, 1991) and by Zimm and Bragg (1959), the Ising matrix M can be written as:

$$M = \begin{array}{c|ccc} & fr & fk & fh \\ \hline fr & fr^{1/2}fr^{1/2} & fr^{1/2}fk^{1/2} & 0 \\ fk & fr^{1/2}fk^{1/2} & 0 & fk^{1/2}fh^{1/2} \\ fh & fh^{1/2}fr^{1/2} & 0 & fh^{1/2}fh^{1/2} \end{array} \quad (1)$$

where fr, fh and fk are corresponding base pair partition functions' contributions in the three states that is ordered, disordered and boundary or nucleation. The eigenvalues of M are given by:

$$\lambda_1 = [(f_r+f_h) + \{(f_r-f_h)^2 + 4f_rf_k\}^{1/2}]/2$$

$$\lambda_2 = [(f_r+f_h) - \{(f_r-f_h)^2 + 4f_rf_k\}^{1/2}]/2$$

$$\lambda_3 = 0$$

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:

$$U = (f_r^{1/2},0,0) \quad (2)$$

where the column vector V gives the state of the last segment:

$$V = \begin{vmatrix} f_r^{1/2} \\ f_k^{1/2} \\ f_h^{1/2} \end{vmatrix} \quad (3)$$

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

$$Z = UM^{N-1}V \quad (4)$$

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

$$MX = \lambda X \quad (5)$$

where

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

By substituting the values of M from Equation 5, we get:

$$T = \begin{vmatrix} 1 & 1 & 1 \\ \dfrac{(\lambda_1 - f_r)}{(f_r^{1/2} f_k^{1/2})} & \dfrac{(\lambda_1 - f_r)}{(f_r^{1/2} f_k^{1/2})} & -(f_r^{1/2} f_k^{1/2}) \\ \dfrac{(f_h^{1/2} f_r^{1/2})}{(\lambda_1 - f_h)} & \dfrac{(f_h^{1/2} f_r^{1/2})}{(\lambda_1 - f_h)} & -(f_h^{1/2} f_r^{1/2}) \end{vmatrix} \quad (6)$$

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

$$YM = \lambda Y \quad (7)$$

where, Y = [Y1, Y2, Y3]

Again by substituting the values of M from Equation 1 in Equation 7, we get:

$$T^{-1} = \begin{vmatrix} C_1 & \dfrac{C_1(f_r^{1/2} f_k^{1/2})}{\lambda_1} & \dfrac{C_1(f_k f_r^{1/2} f_h^{1/2})}{\lambda_1 (\lambda_1 - f_h)} \\ C_2 & \dfrac{C_2(f_r^{1/2} f_k^{1/2})}{\lambda_2} & \dfrac{C_2(f_k f_r^{1/2} f_h^{1/2})}{\lambda_2 (\lambda_2 - f_h)} \\ C_3 & \dfrac{C_3(f_r^{1/2} f_k^{1/2})}{\lambda_3} & \dfrac{C_3(f_k f_r^{1/2} f_h^{1/2})}{\lambda_3 (\lambda_3 - f_h)} \end{vmatrix} \quad (8)$$

The normalization constants are:

$C_1 = (\lambda_1 - f_h)/ (\lambda_1 - \lambda_2)$, $C_2 = (\lambda_2 - f_h)/ (\lambda_2 - \lambda_1)$, $C_3 = 0$ (9)

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

$Z = UT\Lambda N^{-1}T^{-1}V$ (10)

On substituting the values from Equations 1, 2, 3, 6, 8 and 9 in Equation 10, the partition function becomes:

$Z=C_1\lambda_1^N+C_2\ \lambda_1^N$ (11)

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

$Q_r= [\delta \ln Z/\delta \ln fr]/N$

Solving the above equation, we get:

$Q_r=1/2+(1-s)(2A-1)/2P+(1+s)\{(2A-1)P-1+s\}/2P^2N$ (12)

where, $P=(\lambda_1- \lambda_2)/f_r$, $s=f_h/f_r$, $\sigma=f_k/f_r$, $A=[(f_r-f_h)^2+4f_kf_r]^{-2}$

Here, s is 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:

$A = Q_rA_r+(1-Q_r)A_h$ (13)

The extension of this formalism to specific heat (C_v) is straight forward. 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 $U = -T^2(\delta/\delta T)$ (F/T), respectively. Differentiating internal energy with respect to temperature we get the specific heat:

$Cv = \delta U/\delta T = Nk(\Delta H/RT_m)^2 (S\delta Q_r/\delta S)$ (14)

where ΔH is the molar change in enthalpy about the transition point, S is entropy which is equal to $S = \exp[(\Delta H/R)\{(1/T)-(1/T_m)\}]$, T_m is the transition temperature, and -

$\delta Qr/\Delta s = (1/2P^2)[2P(1-S)\ \delta A/\delta S-P(2A-1)\ -(1-S)(2A-1)\ \delta P/\delta S]+(1/2P^3N)[P\{(S+1)\ \{(2A-1)\ \delta P/\delta S+2P\delta A/\delta S+1\}+\{(2A-1)\ P-1+S\}\}-\{(2A-1)P-1+S\}2(S+1)]$ with $\delta A/\delta S = \{(S-\sigma\ )^N/(Z/fr^N)^2\} \times (\sigma/P^3) \times[-2+\{N(S-2\sigma\ -1)/S-\sigma\}]\ \delta P/\delta S=(S-1)/P$ and $\sigma=fk/fr$; 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 (Zimm and Bragg, 1959). The volume heat capacity C_v has been converted into constant pressure heat capacity C_p by using the Nernst-Lindemann approximation (Roles and Wunderlich, 1991):

$C_p-C_v = 3RA_0 (C_p^2T/C_vT_m)$ (15)

where A_0 is a constant often of a universal value [3.9×10^{-9} (K mol)/$^{J-1}$] and T_m is the melting temperature.

RESULTS

When dipyrandium binds to DNA, the structure of DNA still remains a highly co-operative and hence two-state theory of order-disorder transition is applicable. The Zimm and Bragg (1959) theory is amended so as to consider ordered (bounded/unbounded) and disordered states as the two states co-exist at the transition point. 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 takes all this into account. This is evident from the enthalpy change and changes in other transition parameters, such as nucleation parameter (σ) and melting point (T_m). In other words, the change in enthalpy on dipyrandium binding is maximum (7.6 Kcal/M bp) in case of nucleotide to drug ratio infinity and minimum (5.09 Kcal/M bp) at ratio of 5 to1.

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 melting temperature of the duplex increases with increasing concentration of bounded dipyrandium. At saturation, the duplex melts with a T_m 28°C above that of the free 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 sharpest in case of unbounded state and goes in order to nucleotide to dipyrandium ratio of 17 to 1, 10 to 1 and 5 to 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 given in Table 1.

The transition profiles and heat capacity for DNA duplex with different concentration of dipyrandium are shown in Figure 2. Insignificant deviation at the tail ends is primarily due to the presence of various disordered states, as this state cannot be uniquely defined. In addition, the differences could also arise due to the presence of short helical segments found in the random coil states. Figure 2A shows experimental and calculated transition curves for the duplex to single strand transition of poly d(AT) in the absence of dipyrandium. Figures 2B and C show the transition in the presence of dipyrandium at nucleotide to drug ratios of 17 to 1 and 10 to 1, respectively. Figure 2D shows the poly d(AT) transition when the duplex is saturated with drug (a nucleotide to drug ratio of 5 to 1). As expected, a cooperative transition profile is observed with calculated data. The conclusion deduced from the theoretical data is consistent with the directly measured binding enthalpy by Marky et al. (1983) determined through DSC.

The conformational and dynamical states of a macromolecular system are characterized by heat capacity which is second derivative of the free energy and has

Table 1. Transition parameters for dipyrandium binding to poly d(AT).

Parameter	Phosphate/Drug (P/D) ratio			
	∞ (unbounded)	17/1	10/1	5/1
T_m (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
Half width (Exp.)	2	3	6.5	8.5
Half width (Theo.)	2	3	6.5	8.5
Sensitivity parameter (ΔH/σ)	19000	6610	2143.33	1454.28

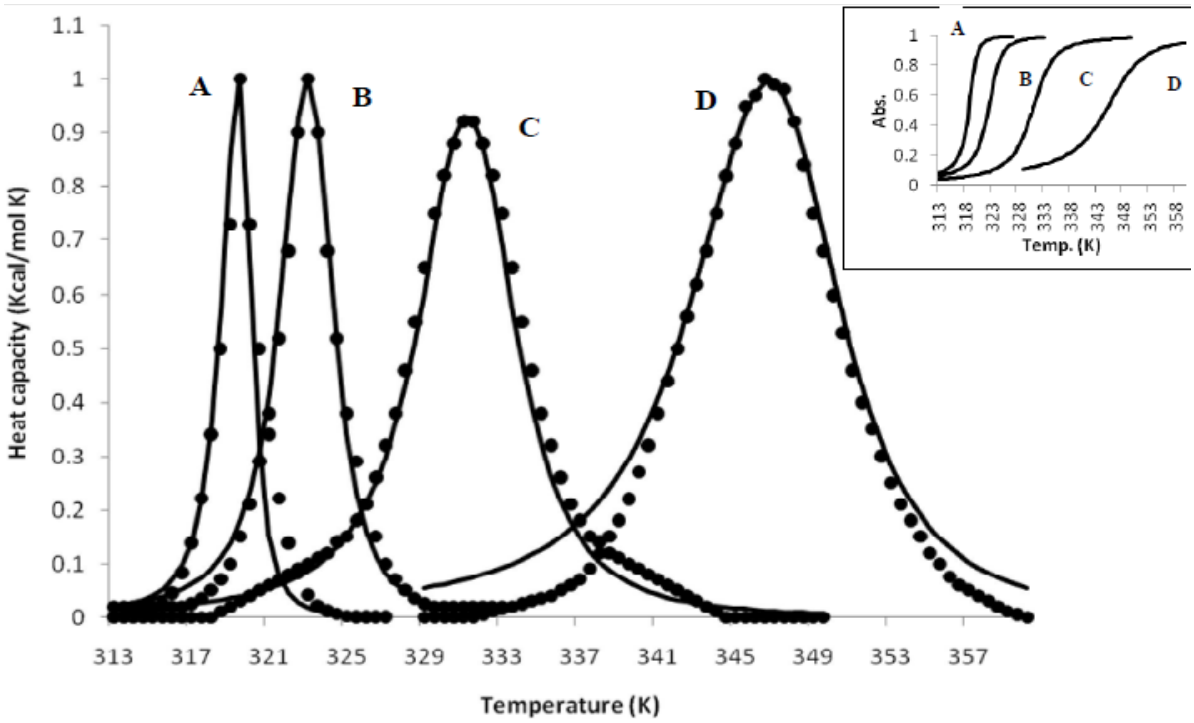

Figure 2. Heat capacity and transition profiles (inset) for dipyrandium bounded and unbounded DNA. A, Unbounded state; B, bounded state with P/D ratio 17:1; C, bounded state with P/D ratio 10:1; D, bounded state with P/D ratio 5:1. [(—) calculated and (••••) experimental values].

been calculated by using Equation 14. These heat capacity curves with λ-point anomaly are shown in Figure 2 along with their transition profile. 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 is very close to one in transition profiles and slightly higher for the heat capacity curves. The sharpness of the transition can be characterized by the half widths of the heat capacity curves that are in good agreement in both experimental and theoretical graphs.

DISCUSSION

The present study concluded that the DNA molecule is an extremely co-operative structure and when dipyrandium bind to it, the co-operativity is not so much disturbed. Therefore, the amended Zimm and Bragg theory (phase transitions theory) can be effectively applied to it. It generates the experimental transition profile and λ-point heat capacity anomaly successfully. These results will allow us to assess the thermodynamic profile of the binding process. Our theoretical studies of steroid diamine

binding are being extended to other synthetic and natural DNA polymers. Our theoretical data also demonstrate that the binding of dipyrandium to poly d(AT) is an endothermic process and that this binding increases the melting temperature of the duplex as supported by calorimetric measurement. Patel and Canuel (1979) have used NMR to investigate the binding of dipyrandium to the poly d(AT) duplex. However, Marky and et al. (1983) used DSC for the same study. Consequently, we can interpret our theoretical results in the context of the specific structural features of the complex as deduced from their NMR/DSC data. Inspection of Figures 2A and D reveals that the transition of the steroid-saturated duplex is considerably broader than the transition of the steroid-free duplex. Thus, in addition to affecting the transition enthalpy and the melting temperature, steroid binding also alters the nature of the transition as reflected by the increase in transition width in experimental and calculated both data. In recent years, an increased understanding of the role played by nucleic acids in biological systems made DNA an alternative candidate for the development of new drugs. The successful applications of molecular modeling in virtual ligand screening and structure-based design of organic and inorganic molecules that target triplex DNA are highlighted by Ma et al. (2011). The interactions of drugs and calf thymus DNA were also investigated by using non-linear fit analysis, and the binding constants are obtained (Yuan et al., 2011). Nowadays, the study of DNA interaction with a molecules is of high interest (Araya et al., 2007; Islam et al., 2009; Paul et al., 2010; Gonzalez-Ruiz et al., 2011; Kunwar et al., 2011). The present investigation can also be applied to understand bimolecular interaction and may be used in biomedical industries.

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