

The Stability of DNA binding with Bisintercalating Anthracyclin: A Theoretical Study

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ABSTRACT: The theory of cooperative transitions has been applied to explain the stability and melting behavior of bisintercalating anthracyclin (WP762) with herring sperm DNA. For implementing present study, the experimental models of Portugal et al.(2005) have been used. The transition profiles and heat capacity curves are best explained in terms of two variable parameters, namely nucleation and propagation parameters. The sharpness of transition has been examined in terms of half width and sensitivity parameter ($\Delta H/\sigma$). The conclusion of theoretical analysis recommended that the different parameters, for example, transition profile, heat capacity curve, sharpness of the transition and half widths are in good agreement with the experimental measurements

Keywords: Bisintercalating anthracyclin, Transition profile, Heat capacity, DNA, Zimm-Bragg Theory, Nucleation Parameter, Melting Temperature

1 INTRODUCTION

IN the recent years the study of small molecules interaction with DNA is an investigation area of high tropical interest. There are number of techniques dedicated to support drug-DNA interactions which are increasing continuously. Over the past several years, the interaction of aromatic heterocyclic molecules to nucleic acids has received significant interest due to their relevance in a variety of biological applications including cancer chemotherapy [7]. Thus, a number of experimental studies are conducted to understand the nature and thermodynamics of heterocyclic aromatic molecules and nucleic acid interaction [6]. A bisintercalating anthracyclin (WP762) (Fig. 1), has been studied for its intercalative interaction with DNA.

The anthracyclin antibiotics including daunorubicin and doxorubicin (Adriamycin), have broad utility in the treatment of several malignancies. These compounds are used to treat many cancers, including leukemia's, lymphomas, breast, uterine, ovarian, bladder cancer, and lung cancers. The anthracyclines are among the most effective anticancer treatment ever developed and are effective against most types of cancer

than any other class of chemotherapeutic agents. Their main adverse effect is cardio toxicity, which considerably limit their usefulness. Although, the efforts are being done to synthesize anthracyclines which have fewer toxic side effects. But the efforts have not succeeded yet and the parent compounds remain the best chemotherapeutic agents of this class [2,4]

In these daunorubicin-DNA complexes, each drug molecule was intercalated at either end of a hexanucleotide, with the daunosamine moieties of two drug molecules pointing towards one another, lining the minor groove [10]. The structural data indicate that the fit of the p-xylene linker in the minor groove was not optimal, and could be improved [13,14]. A new bisintercalating anthracycline (WP762) has been designed, in which monomeric units of daunorubicin have been linked through their amino groups on the daunosamine moieties using an m-xylene linker. M-xylene linker was designed to be of appropriate length (approximately 7Å) that might fit better into the minor groove of DNA. In present work binding of WP762 with herring sperm DNA is studied. For implementing present study, the experimental models of Portugal et al.(2005)[1] have been used.

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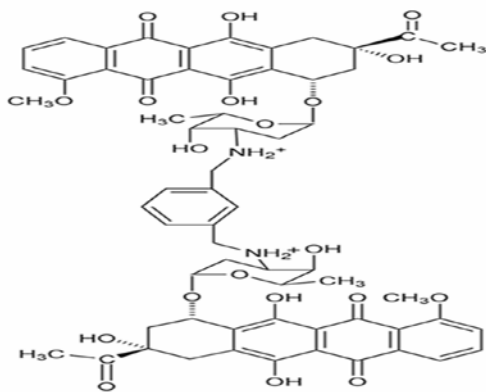


Figure 1: Chemical structure of Bisintercalating anthracyclin (WP762)

2 THEORY

On the basis of spectroscopic and calorimetric study for the complex formation between WP762 and DNA, as mentioned above, Portugal et al.(2005) [1] recommended that the drug binds extremely tightly with the herring sperm DNA that resulted in thermal stabilization of the complex. The binding of WP762 with DNA was intercalative and was non-cooperative that resulted in significant perturbation of the conformation of the DNA as well as thermal stabilization [1]. Although, the system remains a highly co-operative one hence the co-operative transition theory could be applied to elucidate the melting profile and temperature dependence of thermo-dynamical parameter, such as heat capacity. Thus, we used modified Zimm and Bragg theory [18]. All the theories are an adoption of the Ising –Model which was initially given to study the ferromagnetic transition in linear chain of spins. Similarly as a spin can exist in two states either spin up or spin down, exactly a residue can exist in ordered or disordered state in a long polymer chain. Since a one dimensional system can not show any type of phase transition due to finite nature of interactions, the Ising model was modified by introducing boundary state. This boundary state is the first ordered state in the sequence of ordered states. Once residue make a transition then its continuance in the second state becomes easier. Now, one can assign different partition function to different states.

In brief the theory consists of writing an Ising matrix for a two phase system, the fused (A+B) state (say) and disordered state. The Ising matrix M can be written as :

$$M = \begin{matrix} & \begin{matrix} f_r & f_k & f_h \end{matrix} \\ \begin{matrix} f_r \\ f_k \\ f_h \end{matrix} & \begin{bmatrix} f_r & f_k & 0 \\ f_r^{1/2} f_k^{1/2} & 0 & f_k^{1/2} f_h^{1/2} \\ f_h^{1/2} f_r^{1/2} & 0 & f_h^{1/2} f_h^{1/2} \end{bmatrix} \end{matrix}$$

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:

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

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

$$\lambda_3 = 0 \quad \dots\dots(1)$$

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:

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

Where the column vector V gives the state of the last segment,

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

The partition function for a N-segment chain is given by $Z = UM^N V$ (4)

On substituting the values from eq.1, 2, 3 and 5 the partition function becomes:

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

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

Where: $X = \begin{bmatrix} X_1 \\ X_2 \\ X_3 \end{bmatrix} \quad \dots\dots(6)$

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

$$T = \begin{bmatrix} 1 & 1 & 1 \\ \frac{\lambda_2 - f_r}{f_r^{1/2} f_k^{1/2}} & \frac{\lambda_2 - f_r}{f_r^{1/2} f_k^{1/2}} & -f_r^{1/2} f_k^{1/2} \\ \frac{f_r^{1/2} f_h^{1/2}}{\lambda_2 - f_h} & \frac{f_r^{1/2} f_h^{1/2}}{\lambda_2 - f_h} & -f_r^{1/2} f_h^{1/2} \end{bmatrix}$$

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

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

Where, $Y = [Y_1, Y_2, Y_3]$

Again by substituting the values of M from eqn. 1 in eqn. 7, we get;

$$T^{-1} = \begin{bmatrix} C_1 & \frac{(C_2 f_r^{1/2} f_k^{1/2})}{\lambda_2} & \frac{(C_2 f_r^{1/2} f_h^{1/2})}{\lambda_2 (\lambda_2 - f_h)} \\ C_2 & \frac{(C_2 f_r^{1/2} f_k^{1/2})}{\lambda_2} & \frac{(C_2 f_r^{1/2} f_h^{1/2})}{\lambda_2 (\lambda_2 - f_h)} \\ C_3 & \frac{(C_2 f_r^{1/2} f_k^{1/2})}{\lambda_2} & \frac{(C_2 f_r^{1/2} f_h^{1/2})}{\lambda_2 (\lambda_2 - f_h)} \end{bmatrix} \quad \dots(8)$$

The normalization constants are:

$$C_1 = \frac{(\lambda_2 - f_h)}{(\lambda_2 - \lambda_1)}$$

$$C_2 = \frac{(\lambda_2 - f_h)}{(\lambda_2 - \lambda_1)}$$

And $C_3 = 0$ (9)

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

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

On substituting the values from equations 1, 2, 3, 6, 8 and 9 in eqn. 10, the partition function becomes:

$$Z = C_1\lambda_1^N + C_2\lambda_2^N \quad \dots(11)$$

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

$$Q_r = \frac{\ln Z}{\ln f_r} [\delta \ln Z / \delta \ln f_r] / N$$

Solving the above equation, we get :

$$Q_r = \frac{1}{2} + \frac{(1-s)(2A-1)}{2P} + \frac{(1+s)[(2A-1)P-1+s]}{2P^2N} \quad \dots(12)$$

Where

$$P = \frac{\lambda_2 - \lambda_1}{f_r}, \quad s = \frac{f_h}{f_r}$$

$$\sigma = \frac{f_r}{f_r}$$

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

Here s is propagation parameter, which for simplicity is assumed to be one. In fact in most of the systems it is found to be close to unity.

If A_r and A_h are absorbance in disordered and ordered states respectively, the total Absorption can be written as :

$$A = Q_r A_r + (1 - Q_r) A_h \quad \dots(13)$$

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

$$C_v = \frac{\partial U}{\partial T} = Nk \left(\frac{\Delta H}{RT_m} \right)^2 \left(\frac{\partial Q_r}{\partial V} \right) \quad \dots(14)$$

where ΔH is the molar change in enthalpy about the transition point, S is entropy which is equal to

$$S = \exp \left[\left(\frac{\Delta H}{R} \right) \left(\frac{1}{T} - \frac{1}{T_m} \right) \right],$$

T_m is the transition temperature, and

$$\frac{\partial Q_r}{\partial S} = \left(\frac{1}{2P^2} \right) \left[2P(1-s) \frac{\partial A}{\partial S} - P(2A-1) - (1-s)(2A-1) \frac{\partial P}{\partial S} + (1/2P^2N) [P((S+1)((2A-1) \frac{\partial P}{\partial S} + 2P \frac{\partial A}{\partial S} + 1) + ((2A-1)P - 1 + S)) - \{(2A-1)P - 1 + S\} 2(S+1)] \right]$$

With

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

$\frac{\partial P}{\partial S} = \frac{S-1}{P}$ and $\sigma = \sigma$ 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. The volume heat capacity C_v has been converted into constant pressure heat capacity C_p by using Nernst- Lindemann approximation,

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

Where A_0 is a constant often of universal value $[3.9 \times 10^{-9} \text{ (Kmol)/J-1}]$ and T_m is the melting temperature.

3 RESULT AND DISCUSSION

3.1 Transition Profiles

The UV melting studies and Differential scanning calorimeter results show that binding constant for interaction of WP762 with herring sperm DNA is $7.3 \times 10^{12} \text{ M}^{-1}$. High value of binding constant represents that WP762 tightly bounds to DNA^[1]. As mentioned earlier each drug molecule was intercalated at either end of a hexanucleotide, with the daunosamine moieties of two drug molecules pointing towards one another, lining the minor groove^[10]. One base pair is attached to each of DNA hexagon to add stability to the double helix ends and ensure a stable realistic interaction site^[11]. When daunorubicin drug bind DNA, the structure still remains a very much co-operative. Accordingly two state theory of order-disorder transition is applicable. The Zimm-Bragg theory is modified so as to ordered and disordered states as the two co-existing states at the transition point. The transition is mainly described by nucleation parameter σ and change in enthalpy. DSC measurements take all this into account and gives change in enthalpy. The changes in enthalpy and in other transition parameters, such as nucleation parameter, melting point obtained theoretically indicate that the binding of WP762 increases the melting temperature of herring sperm DNA. At saturation the melting temperature of DNA duplex increases by 29.75K in comparison to free DNA. The sharpness of the transition can be observed in terms of half width and a sensitivity parameter as $(\Delta H/\sigma)$. The deviation in half width and sensitivity parameter revealed that the transition is sharp in case of unbound state and goes blunt with WP762 saturation. A range of parameters, which give transition profiles in best agreement with the experimental data for binding of WP762 with DNA, are given in table.

Table1. Transition parameters for WP762 binding to DNA

Parameters	DNA	Drug-DNA
$T_m(K)$	339.75	369.5
$\Delta H(Kcal/M)$	6.8	33.8
Σ	0.00058	0.004
N	34	22
A_h	0	1
A_r	0	1
Half width (Exp.)	9.40	11.40
Half width (Theor.)	9.89	10.1
$(\Delta H/\sigma)$	11.72×10^3	8.4×10^3

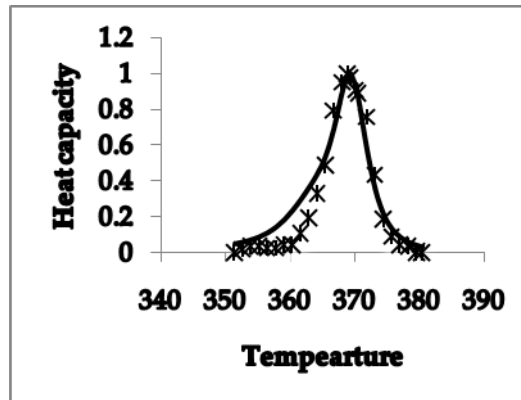


Figure 2 (b) The transition when DNA was saturated with WP762 (0.16 mol antibiotic/mol bp)

The heat capacity and transition profile for unbounded DNA and bounded with WP762 are shown in Figure2. Figure 2(a) explains experimental and calculated transition curves for herring sperm DNA at 1 mM (bp) in absence of WP762 and figure 2 (b) shows transition when DNA was saturated with WP762 (0.16 mol antibiotic/mol bp). As anticipated, a co-operative transition profile is obtained with calculated data. The conclusions of theoretical data are consistent with the measured binding enthalpy by Portugal et al.^[1].

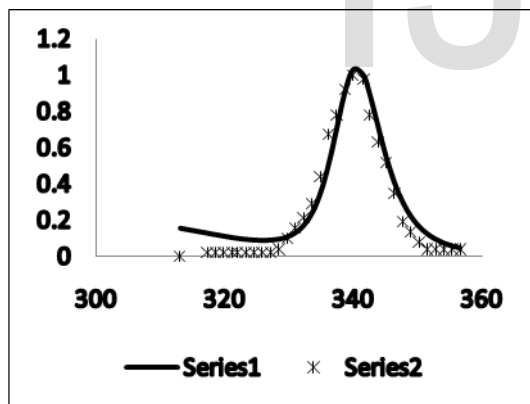


Figure 2(a) The experimental and calculated transition curves for herring sperm DNA at 1 mM (bp) in absence of WP762

3.2 Heat capacity

The second derivative of the free energy i.e. heat capacity has been calculated by using equation 14. The heat capacity curves with λ -point anomaly and along with their transition profile are shown in figure 2. The results indicate that the theoretically obtained heat capacity profile agreed with the experimentally reported ones. The theoretical data obtained also verified that binding of WP762 with herring sperm DNA is an endothermic process and this increases the melting temperature of the DNA as supported by the calorimetric measurements. The binding and intercalation of WP762 with herring sperm DNA is studied by using differential scanning calorimeter by Portugal et al.^[1]. The binding of drug stabilizes the DNA and change I melting temperature of about 30°C was obtained. Figure 2(a and b) disclosed that transition of WP762-DNA is significantly broader than the transition of unbounded DNA. Besides affecting the transition enthalpy and melting temperature, binding of WP762 with DNA also changes the nature of transition profile as reflected by the increase in transition width in experimental and calculated data. Further figure 3(a) shows Predicted absorbance of DNA solution with saturating amounts (0.16 mol antibiotic/mol bp) of WP762 added. And figure 3(b) shows absorbance at DNA concentration (20 μ M bp). The calculated data is in good agreement with the experimental results of UV melting studies performed by Portugal et al. Figure 4 represents the predicted values of heat capacity at conc. (20 μ M bp) in the range 290-380K.

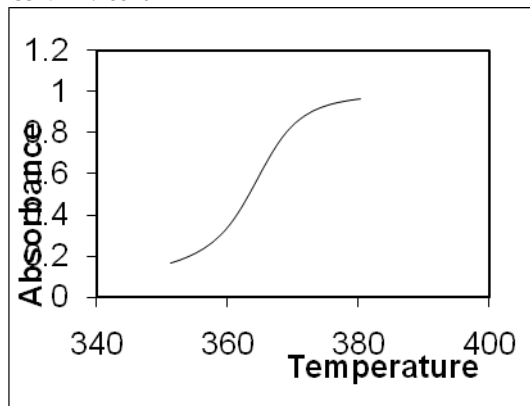


Figure 3. Predicted absorbance of DNA solution with saturating amounts (0.16 mol antibiotic/mol bp) of WP762 added

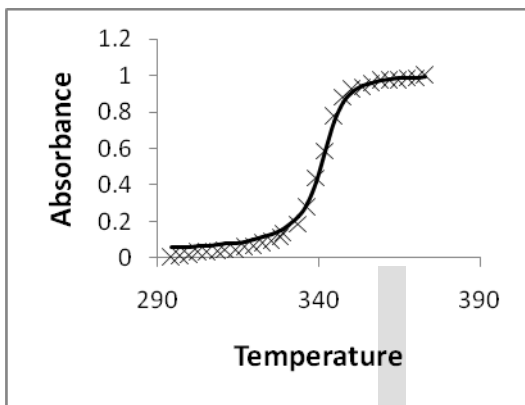


Figure 4. Absorbance at DNA conc(20 μM bp)

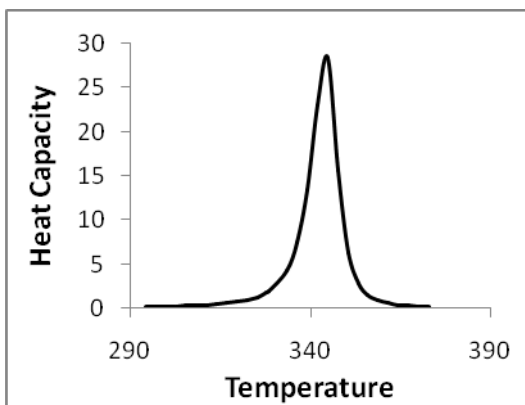


Figure 5. Predicted heat capacity at conc. (20 μM bp)

4 CONCLUSION

The present study concluded that DNA molecule is an extremely co-operative structure when WP762 binds to it, the co-operativity is not much disturbed. Therefore amended Zimm-Bragg theory can be effectively applied to it. It generates the experimental transition profile and λ point heat capacity anomaly effectively. These results allow us to assess the thermodynamic profile of binding process.

Our theoretical data also demonstrate that the binding of WP762 to DNA is an endothermic process. Therefore the theoretical analysis presented in this study can be implicated to understand bimolecular interactions and may also be applied in biomedical industry for drug design and development.

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