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Effect of thionine binding on stability of herring testes DNA: A theoretical study

Ghazala Yunus*, Seema Srivastava
Department of Physics, Integral University, Kursi Road, Lucknow-226026, (INDIA)
E-mail : ghazala_kuddus@yahoo.com

ABSTRACT

The recent findings from the study on binding of small molecules with DNA duplex suggested that the intercalation of a tricyclic heteroaromatic molecule, Department of Physics, Integral University, Kursi Road, Lucknow-226026, IndiaDepartment of Physics, Integral University, Kursi Road, Lucknow-226026, India, with natural double stranded DNA provided thermal stabilization to the complex. In this study, we reported theoretical analysis of thionine binding with herring testes DNA (Type XIV, 41 mol% GC) by using an amended Zimm and Bragg theory, to reveal the melting behaviour and heat capacity of herring testes DNA with and without thionine binding. We used experimental models of Paul *et al.* (2010) for the study. The sharpness of transition has been examined in terms of half width and sensitivity parameter ("H/σ). The results of theoretical analysis concluded that the various parameters such as heat capacity curve, transition profile, half widths and sharpness of the transition are in good harmony with the experimental measurements for interaction of thionine. Accordingly, the theoretical analysis proposed in this study may be useful to understand interaction of small molecules with DNA. This approach may also be applied to design DNA binding therapeutic molecules and in drug innovation.

KEYWORDS

Thionine;
Herring testes DNA;
Heat capacity;
Transition profile;
DNA binding.

INTRODUCTION

The study of small molecules interaction with DNA has attracted worldwide attention in recent years and verity of techniques devoted to evidence drug-DNA interactions are increasing continuously. Accordingly, since last several years, the interaction of heterocyclic molecules to DNA has received considerable attention due to their significance in cancer chemotherapy and a range of biological applications[1-7]. Therefore, a number of experimental studies are conducted to understand the nature and thermodynamics of heterocyclic aromatic molecules and DNA interaction[6, 8-14]. Thionine (3,7-Diamino-5- phenothiazinium), a tricyclic heteroaromatic molecule (Figure 1), has been studied for its intercalative binding with DNA duplex[10] and its photoinduced mutagenic actions on binding to DNA[12]. As per the satellite hole spectroscopy study, thionine binds specifically with guanine–cytosine (GC) contents of DNA duplex[15] but it was not very marked[10]. The

other study suggested that thionine exists in two different tautomeric forms *viz.* amino form and imino form and only amino form is intercalated[16-17]. Another study carried out through pressure tuning hole burning spectroscopy has exposed an external stacking mode of thionine binding with quadruplex structures[16]. Recently Paul *et al.*[6] reported the experimental analysis of thionine binding to a DNA duplex along with the structural and thermodynamic aspects of thionine binding to natural DNAs of varying base composition. Paul *et al.*[6] concluded strong binding of thionine with herring testes DNA (HT DNA) that increases the thermal stability of the HT DNA duplex, and at saturation the duplex melts with 7.8°C above that of the free duplex[6]. They also suggested that binding of thionine with HT DNA is an exothermic process. In the present study, we have attempted to understand the effect of thionine binding with a native DNA duplex through the theoretical analysis using the experimental model of Paul *et al.*[6] who studied the thermal and thermodynamic behaviour of thionine binding to HT DNA. We used amended Zimm and Bragg theory, initially considered for helix coil transitions in polypeptides, to explain lambda point anomalies in heat capacities and order-disorder transition in thionine bounded and unbounded HT DNA[18-20].

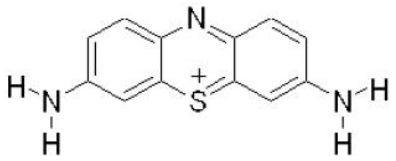

Figure 1 : Chemical structure of thionine

THEORY

The biophysical and calorimetric study carried out by Paul *et al.*[6] for the complex formation between thionine and DNA concluded that thionine binds robustly with the herring testes DNA (Type XIV, 41 mol% GC) which resulted in thermal stabilization of the complex. The binding of thionine to the HT DNA was intercalative and was non-cooperative that resulted in significant perturbation of the conformation of the DNA as well as thermal stabilization[6]. However, the system remains a highly co-operative one; consequently the co-operative transition theory could be applied to explain the melting profile and temperature dependence of thermodynamical parameter. Therefore, amended Zimm and Bragg theory[18] may be used which is explained in our earlier publication[21]. Briefly, the Zimm and Bragg theory consists of writing an Ising matrix for a two-phase system *viz.* the bounded state and unbounded state. As discussed previously[19,20, 22-24] and by Zimm and Bragg[18], the Ising matrix M can be written as:

$$\mathbf{M} = \begin{array}{c} \\ \mathbf{F_r} \\ \mathbf{f_k} \\ \mathbf{f_h} \end{array} \begin{array}{c} \begin{array}{ccc} \mathbf{f_r} & \mathbf{f_k} & \mathbf{f_h} \end{array} \\ \begin{vmatrix} \mathbf{f_r^{1/2} f_r^{1/2}} & \mathbf{f_r^{1/2} f_k^{1/2}} & \mathbf{0} \\ \mathbf{f_r^{1/2} f_k^{1/2}} & \mathbf{0} & \mathbf{f_k^{1/2} f_h^{1/2}} \\ \mathbf{f_h^{1/2} f_r^{1/2}} & & \mathbf{f_h^{1/2} f_h^{1/2}} \end{vmatrix} \end{array} \quad \mathbf{(1)}$$

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:

$$\lambda_1 = [(f_r + f_h) + \{(f_r - f_h)^2 + 4f_r f_k\}^1]$$
$$\lambda_2 = [(f_r + f_h) - \{(f_r - f_h)^2 + 4f_r f_k\}^1] \quad \mathbf{(2)}$$
$$\lambda_3 = 0$$

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:

$$\mathbf{U} = (f_r^{1/2}, 0, 0) \quad \mathbf{(3)}$$

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

$$\mathbf{V} = \begin{vmatrix} f_r^{1/2} \\ f_k^{1/2} \\ f_h^{1/2} \end{vmatrix} \quad \mathbf{(4)}$$

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

$$\mathbf{Z = UM^{N-1}V} \quad \mathbf{(5)}$$

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

$$\mathbf{MX = \lambda X} \quad \mathbf{(6)}$$

Where, $\mathbf{X} = \begin{vmatrix} X_1 \\ X_2 \\ X3 \end{vmatrix}$

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

Full Paper

$$\mathbf{T}=\begin{vmatrix} 1 & 1 & 1 \\ \dfrac{(\lambda_1-f_r)}{(f_r^{1/2}f_k^{1/2})} & \dfrac{(\lambda_1-f_r)}{(f_r^{1/2}f_k^{1/2})} & -(f_r^{1/2}f_k^{1/2}) \\ \dfrac{(f_h^{1/2}f_r^{1/2})}{\lambda_1-f_h} & \dfrac{(f_h^{1/2}f_r^{1/2})}{\lambda_1-f_h} & -(f_h^{1/2}f_r^{1/2}) \end{vmatrix} \quad (7)$$

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

$$\mathbf{YM=\lambda Y} \quad (8)$$

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

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

$$T^1=\begin{vmatrix} C_1 & \dfrac{C_1(f_r^{1/2}f_k^{1/2})}{\lambda_1} & \dfrac{C_1(f_kf_r^{1/2}f_h^{1/2})}{\lambda_1(\lambda_1-f_h)} \\ C_2 & \dfrac{C_2(f_r^{1/2}f_k^{1/2})}{\lambda_2} & \dfrac{C_2(f_kf_r^{1/2}f_h^{1/2})}{\lambda_2(\lambda_2-f_h)} \\ C_3 & \dfrac{C_3(f_r^{1/2}f_k^{1/2})}{\lambda_3} & \dfrac{C_3(f_kf_r^{1/2}f_h^{1/2})}{\lambda_3(\lambda_3-f_h)} \end{vmatrix} \quad (9)$$

The normalization constants are:

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

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

$$\mathbf{Z = UT\Lambda N^{-1}T^{-1}V} \quad (11)$$

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

$$\mathbf{Z = C_1\lambda_1^N + C_2\lambda_1^N} \quad (12)$$

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

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

Solving the above equation, we get:

$$Q_r=\frac{1}{2}+\frac{(1-s)(2A-1)}{2P}+\frac{(1+s)\{(2A-1)P-1+s\}}{2P^2N} \quad (13)$$

Where $P=\frac{\lambda_1-\lambda_2}{f_r}$

$$s=\frac{f_h}{f_r}$$

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

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

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:

$$\mathbf{A = Q_rA_r + (1 - Q_r)A_h} \quad (14)$$

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 = -KTlnZ and U = - $T^2(/\delta T)$ (F/T), respectively. Differentiating internal energy with respect to temperature we get the specific heat:

$$\mathbf{CV = \delta U/\delta T = N_k(\Delta H/RT_m)^2 (S\delta Q_r/\delta S)} \quad (15)$$

Where ΔH is the molar change in enthalpy about the transition point, S is entropy which is equal to S = exp[(ΔH/R){(1/T) – (1/Tm)}], T_m is the transition temperature, and

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

with

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

$\delta P/\delta S = (S - 1)/P$ and $\sigma = f_k/f_r$; 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[18]. The volume heat capacity C_v has been converted into constant pressure heat capacity C_p by using the Nernst-Lindemann approximation[22]:

$$\mathbf{C_p - C_v = 3RA_o(C_p^2T/C_vT_m)} \quad (16)$$

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

RESULTS

Transition profiles

The spectroscopic studies of thionine binding with herring testes DNA (Type XIV, 41 mol% GC) showed strong intercalative binding and enabled the assumption of two state systems consisting of bound and free thionine[6]. When thionine binds with natural HT DNA duplex, the structure of duplex still remains a very much co-operative and so two-state theory of order-disorder transition is applicable. The Zimm and Bragg[18] theory is amended so as to consider ordered (bounded/unbounded) and disordered states as the two states which 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 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 (TABLE 1).

TABLE 1 : Transition parameters for thionine binding to HTDNA

Parameters	Unbounded HT DNA	HT DNA saturated with thionine
T_m (K)	353	362
ΔH Kcal/M bp	$6.41x10^3$	$9.72x10^3$
σ	$9x10^{-3}$	$2x10^{-2}$
N	28	16
A_h	0	0
A_r	1	1
Half width (Exp.)	11	12
Half width (Theo.)	11	12
Sensitivity parameter ($\Delta H/\sigma$)	$712x10^3$	$486x10^3$

The results obtained from theoretical analysis recommended that the binding of thionine increases melting temperature of HT DNA at saturation point. As shown in TABLE 1, the melting point of HT DNA duplex was increased with 9°C in comparison to free duplex. The sharpness of the transition may be defined in terms of half width and a sensitivity parameter which is defined as $\Delta H/\sigma$. The deviations in half width and sensitivity parameter ($\Delta H/\sigma$) scientifically revealed that the transition is sharp in case of unbounded state and goes blunt with thionine saturation. In case of λ-transition, the same trend in the sharpness of transition is seen between the thionine bounded as well as unbounded curves. As expected, the sharpness is better in unbounded state, as compared to bounded state. The various parameters, which give transition profiles in best agreement with the experimental measurements for binding of thionine to HT DNA are provided in TABLE 1. The heat capacity and transition profile for unbounded HT DNA duplex and bounded with thionine are shown in Figure 2. As per profile there are slight insignificant deviation at the tail ends that may be primarily due to the presence of various disordered states and short helical segments found in the random coil states. Figure 2 (A) shows experimental and calculated transition curves for the HT DNA in the absence of thionine and (B) shows the transition when the HT DNA was saturated with thionine (thionine/DNA=0.5). As expected, a cooperative transition profile is obtained with calculated data obtained through theoretical analysis. The results obtained from the theoretical analysis are in good agreement with the binding enthalpy determined through DSC by Paul *et al.*[6].

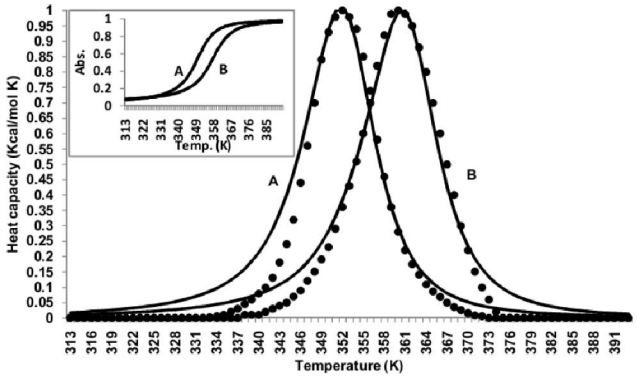

Figure 2 : Heat capacity and transition profiles (inset) for thionine bounded and unbounded HT-DNA. (A) unbounded state, (B) bounded state at saturation. [(—) calculated and (••••) experimental values]

Heat capacity

The conformational and dynamical states of a macromolecular system are characterized by heat capacity and have been calculated by using equation[14]. These heat capacity curves along with their transition profile are shown in Figure 2 which recommended that the theoretically calculated heat capacity profile agreed with the experimentally reported

ones and could be brought almost into agreement 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 width of the heat capacity curves that are in good agreement in both experimental and theoretical graphs (Figure 2).

The findings of theoretical analysis also demonstrated that the binding of thionine to HT DNA is an endothermic process and this binding increases the melting temperature of the HT DNA as suggested by Paul *et al.*[6] through calorimetric measurement. The specific binding and intercalation of thionine with DNA have been studied by using different spectroscopic methods[10,15]. Nevertheless, Paul *et al.* used optical melting and DSC techniques to understand the interaction of small molecule, thionine, with native HT DNA[6]. The binding of thionine stabilized HT DNA and change in melting temperature of 9°C was obtained under saturating condition. Consequently, we can interpret our theoretical analysis results in the background of the specific structural features of the thionine-DNA complex as supposed from their DSC/spectroscopic data. Figures 2 revealed that the transition of the thionine-saturated HT DNA is significantly broader than the transition of the thionine-free HT DNA. Thus, in addition to affecting the transition enthalpy and the melting temperature, binding of heterocyclic aromatic molecules also alters the nature of the transition as revealed by the increase in transition width in experimental and calculated (theoretical) both data. Recently, an augmented understanding of the role played by nucleic acids in biological systems made DNA as an alternative candidate for the development and formulation of new drugs. The successful applications of molecular modeling in virtual ligand screening and structure-based design of organic and inorganic molecules that target specific DNA are highlighted by Ma *et al.*[25]. The significance of DNA interaction with a small molecules is also reviewed by other literatures[6, 26-29].

CONCLUSIONS

This theoretical analysis concluded that the natural herring testes DNA molecule is a highly co-operative structure and when thionine bind to it the co-operativity is not so much disturbed. Thus, the amended Zimm and Bragg theory can be successfully applied to it. These results will allow us to calculate the thermodynamic profile of the binding process. Our theoretical studies of small molecules binding are being extended to other synthetic and natural DNA also, and can be applied to understand bimolecular interactions in biomedical industries for drug formulation.

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