RESEARCH PAPER

Effect of Neomycin Binding on the Stability of the DNA triple Helix Poly(dA). 2poly(dT)

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Abstract

The effect of small molecules can influence its structure abilities and also the melting characteristics. In this study, the effect of neomycin, an aminoglycoside antibiotic, on the thermal stability of the DNA triplex has been examined using a modified Zimm–Bragg theoretical framework. The influence of neomycin on DNA has been studied through the sequential structural transitions of the triplex, first into a duplex and subsequently into single-stranded DNA. The nucleation parameter and change in enthalpy contain the impacts of drug binding. A rise in value of "ΔH/σ" after binding indicates that transformation happens readily since a large ΔH indicates a strong inclination for and a large nucleation parameter indicates a high difficulty in creating stable nuclei. These findings highlight the importance of examining individual stages of DNA melting to understand how drug binding differentially influences the stability and cooperative behaviour of higher-order DNA structures.

Keywords: Transition profile, Heat Capacity, Nucleation Parameter, Order-Order transition, Order-Disorder transition

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Introduction

DNA serves as a biological instruction manual, holding the genetic code needed to create, preserve, and procreate. DNA is shaped like a double helix, which is a twisted ladder or spiral staircase. The four chemical bases that make up the "rungs" of the ladder are adenine (A), thymine (T), cytosine (C), and guanine (G). These bases are frequently denoted by their corresponding letters. These bases are always paired in a particular manner: C is always paired with G, and A is always paired with T. Groups of these base sequences make up genes, which act as recipes for specific proteins that dictate traits like eye colour, height, and biological function.

A unique nucleic acid structure known as a DNA triple helix (triple-stranded DNA) is created when a third oligonucleotide fits into the main groove of a typical Watson-Crick double helix. This third strand uses hydrogen bonds called Hoogsteen base pairs to connect exclusively to purine-rich areas. Hoogsteen bonding enables one purine to bind with two pyrimidines rather of the usual A-T and G-C pairings. For example, a protonated cytosine attaches to an already paired G-C, while a thymine binds to an already paired A-T [1]. While intermolecular triplex happens naturally when one strand of a single duplex folds back on itself at mirror-repeat sequences, intermolecular triplex is created by attaching a brief, exogenous Triplex-Forming Oligonucleotide (TFO) to a target double strand. Intramolecular triplexes are involved in recombination, DNA repair, and natural gene control [2].

Drugs alter, interact with, or damage DNA to disrupt cellular functions. They typically bind to the DNA helix or prevent enzymes from replicating and transcribing it, forcing the cell into programmed death. These mechanisms are widely utilized in medicine, while other substances cause harmful, unintended toxicity [3]. Drugs interact with DNA primarily by altering its structural integrity, mechanical stability, and ability to replicate. The effect depends heavily on the specific binding mechanism and the context of the DNA. Many drugs, especially anti-tumour agents, increase the physical and thermal stability of double-stranded DNA [4]. Some drugs weaken and destabilize the DNA duplex by slipping between adjacent base pairs, which distorts and unwinds the helix [5]. When a drug binds to DNA, it commonly induces a conformational or phase transition within the DNA's physical structure, modifying its functional capabilities. Because DNA naturally exists as a dynamic, flexible polymer, these drugs force transitions between different DNA states (such as the standard B-DNA, A-DNA, or condensed Ψ-DNA). When the drug alters the thermal stability of the DNA double helix, this transition is commonly refereed as Helix-Coil transition resulting due to melting or denaturation of DNA.

Neomycin is a broad-spectrum aminoglycoside antibiotic used to treat bacterial skin infections, reduce the risk of infection in minor cuts or wounds, and manage conditions like hepatic encephalopathy. Because it is poorly absorbed by the stomach, oral neomycin is highly effective at acting directly inside the intestines. Chemical Structure of Neomycin is shown in figure 1. It is composed of four linked sugar rings containing multiple amino (–NH_2) and hydroxyl (–OH) groups. The abundance of protonatable amino groups imparts a strong positive charge under physiological conditions, enabling electrostatic interactions with the negatively charged phosphate backbone of nucleic acids. These properties make neomycin an effective ligand for studying DNA triplex stabilization and nucleic acid recognition.

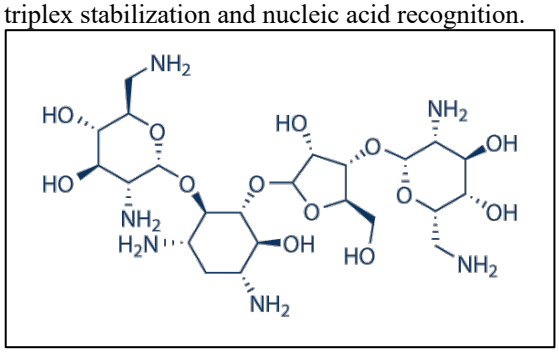

Figure 1: Chemical Structure of Neomycin

In present study, we are reporting the effect of Neomycin binding on the stability of DNA triple helix PolydA.2polydT. This triplex consists of one poly(dA) strand and two strands poly(dT). One poly(dT) is bind to poly(dA) by Watson-Crick duplex and the second poly(dT) strand binds to the major groove of duplex by Hoogesteen hydrogen bonding in order to form a triplex [6-8]. The triplex is stabilized by base-stacking interactions and hydrogen bonds between adenine and thymine bases, making it a useful model for studying ligand-induced stabilization of DNA triplexes. The transition of DNA triple helix takes place in two steps. DNA triple helix Poly(dA). 2poly(dT) to DNA double helix Poly(dA). poly(dT). This transition is referred to as Order-Order Transition because both triplex and duplex are well defined states. The second step is transition of duplex to single strands. This transition is referred as Order-Disorder transition. The experimental finding of Xi *et al.* have been used to characterize the binding of Neomycin with Poly(dA). 2poly(dT) [9].

1. Theory

The basic of all transition theories are Ising-Model designed originally to study the ferromagnetic transitions in linear chains of spin. The Zimm and Bragg theory was suitably modified to study the order-order and order-disorder transitions in triple helical DNA [10]. This theory gives a macroscopic view of phase transition. Change in enthalpy is a strong parameter that assesses the nature of intermolecular and intramolecular interactions at the transition point. This theory has been used earlier also for similar transitions in biopolymer and DNA [11-14].

2.1 Order-Order Transition:

An extended chain molecule with the ability to switch between states (h_1) and (h_2) can be expressed as follows: $h_1h_1h_1\sigma_2h_2h_2h_2h_2\sigma_1h_1h_1$ --------, where σ_1 and σ_2 denote the nucleation parameters (border state) when switching between ordered states ($h_2 \times h_1$) and vice versa ($h_1 \times h_2$). In the event that s_1 and s_2 represent the equilibrium growth parameters for the two ordered (well defined) states, the matrix for order-order can be expressed as follows:

$$M = \begin{matrix} \\ h_1 \\ h_2 \end{matrix} \begin{matrix} \begin{matrix} h_1 & h_2 \end{matrix} \\ \begin{vmatrix} s_1 & \sigma_2 s_2 \\ \sigma_1 s_1 & s_2 \end{vmatrix} \end{matrix} \quad (1)$$

For the helical coiled transition, this transition matrix is an addition to the Zimm and Bragg matrix [15]. The model is composed of two phases that can switch to each other via a border state where the initial nucleation energy expands significantly. These two states are both ordered.

We must construct the chain in the final effects in order to determine the grand partition function. The initial state vector U is expressed as follows, providing the contribution to the first segment:

$$U = (\sigma' s_1 \sigma'' s_2) \quad (2)$$

where σ ' and σ " are the parameters giving the interaction of end segments with the solvent when they are in states h_1 and h_2, respectively. The partition function for an N-segment chain is given as:

$$Z = UM^{N-1}V \quad (3)$$

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

$$V = \begin{vmatrix} 1 \\ 1 \end{vmatrix} \quad (4)$$

Going through the matrix evaluation formalism outlined by Zimm and Bragg, we obtain an expression for the fraction of segments in the state I:

$$f_1 = \frac{1}{N}\left[\frac{\delta \ln Z}{\delta \ln s_1}\right] \quad (5),$$

Solving equation 5 we obtain

$$f_1 = \frac{\left[\frac{s_1 \delta\lambda_1}{\lambda_1 \delta s_1} + B\frac{s_1 \delta\delta_2}{\lambda_2 \delta s_1} + \frac{1}{N}\left[\frac{s_1 \delta A_1}{A_1 \delta s_1} + B\frac{s_1 \delta A_2}{A_2 \delta s_1}\right]\right]}{(1+B)} \quad (6)$$

Where λ_1 and λ_2 are the given eigen values of M

$$B = \left(\frac{X_2}{X_1}\right)^N \left(\frac{A_2}{A_1}\right) \quad (7)$$

With $X_1 = s_1\lambda_1,\ X_2 = s_2\lambda_2$

$A_1 = \frac{s_1+s_2x-b\lambda_2}{\lambda_1-\lambda_2}$, $A_2 = \frac{s_1+s_2x-b\lambda_1}{\lambda_1-\lambda_2}$, $b = \frac{1+x-\sigma_2-\sigma_1 x}{1-\sigma_1\sigma_2}$ and $x = \frac{\sigma'}{\sigma''}$

$\frac{s_1}{\lambda_1}\frac{\delta\lambda_1}{\delta s_1} = \frac{X_1-1}{X_1-X_2}$, $\frac{s_1}{\lambda_2}\frac{\delta\lambda_2}{\delta s_1} = \frac{X_2-1}{X_2-X_1}$

$$\frac{s_1}{A_1}\frac{\delta A_1}{\delta s_1} = \frac{s^2(2\sigma-1+b-x-b\sigma)+s(1+x-b+b\sigma-2\sigma x)}{\{(1-s)^2+4\sigma x\}(s+x-bX_2)}$$

$$\frac{s_1}{A_2}\frac{\delta A_2}{\delta s_1} = \frac{s^2(2\sigma-1+b-x-b\sigma)+s(1+x-b+b\sigma-2\sigma x)}{\{(1-s)^2+4\sigma x\}(s+x-bX_1)}$$

Here $\sigma = \sigma_1\sigma_2$ and $s = {}^{s_1}/_{s_2}$

The overall absorbance is given b:

$$Absorbance = f_1A_1 + (1-f_1)A_2 \quad (8)$$

Where A_1, A_2 are the absorbance in 1st and 2nd state respectively.

It would be easy to extend this approach to specific heat. During step I and step II transition molar enthalpy and entropy changes that are related to the specific heat. Well-known thermodynamic relations allow us to calculate internal and free energies as follows:

$$F = KTlnZ \quad (9)$$

$$\text{and } U = -T^2\frac{\delta}{\delta T}\left[\frac{F}{T}\right] \quad (10)$$

Using expression for entropy and differentiating internal energy with regard to temperature (Eq. 11)

$$S = exp\left[\frac{\Delta H}{R}\left[\frac{1}{T}-\frac{1}{T_f}\right]\right] \quad (11)$$

We obtain the specific heat:

$$C_v = \frac{\delta U}{\delta T} = Nk(\Delta H/RT)^2\left(\frac{s\delta f_1}{\delta s}\right) \quad (12)$$

Where ΔH represents change in enthalpy per mole around the point of transition, S is the entropy and T_f represents transition temperature.

Here $\frac{\delta f_1}{\delta s} = -\frac{f_1}{(1+B)}\frac{\delta B}{\delta s} + \frac{1}{(1+B)}\left[\frac{\delta}{\delta s}\left[\frac{s_1}{\lambda_1}\frac{\delta\lambda_1}{\delta s_1}\right] + \frac{1}{N}\left[\frac{\delta}{\delta s}\left[\frac{s_1}{A_1}\frac{\delta A_1}{\delta s_1}\right]\right] + \frac{\delta B}{\delta s}\left[\frac{s_1}{\lambda_2}\frac{\delta\lambda_2}{\delta s_1} + \frac{1}{N}\frac{s_1}{A_2}\frac{\delta A_2}{\delta s_1}\right] + B\left[\frac{\delta}{\delta s}\left[\frac{s_1}{\lambda_2}\frac{\delta\lambda_2}{\delta s_1}\right] + \frac{1}{N}\left[\frac{\delta}{\delta s}\left[\frac{s_1}{A_2}\frac{\delta A_2}{\delta s_1}\right]\right]\right]$ (13)

And $\frac{\delta B}{\delta s} = NB\left[\frac{1}{X_2}\frac{\delta X_2}{\delta s} - \frac{1}{X_1}\frac{\delta X_1}{\delta s}\right] + B\left[\frac{1-b\left({}^{\delta X_1}/_{\delta s}\right)}{s+x-bX_1} - \frac{1-b\left({}^{\delta X_2}/_{\delta s}\right)}{s+x-bX_2}\right]$ (14)

$$\frac{\delta X_1}{\delta s} = \frac{1}{2} + \frac{(s-1+2\sigma)}{2[(s-1)^2+4\sigma s]^{1/2}}$$

$$\frac{\delta X_2}{\delta s} = \frac{1}{2} - \frac{(s-1+2\sigma)}{2[(s-1)^2+4\sigma s]^{1/2}} \quad (15)$$

$$\frac{\delta}{\delta s}\left[\frac{s_1}{\lambda_1}\frac{\delta\lambda_1}{\delta s_1}\right] = \frac{\sigma(s+1)}{\{(s-1)^2+4\sigma s\}^{3/2}}$$

$$\frac{\delta}{\delta s}\left[\frac{s_1}{\lambda_2}\frac{\delta\lambda_2}{\delta s_1}\right] = \frac{-\sigma(s+1)}{\{(s-1)^2+4\sigma s\}^{3/2}} \quad (16)$$

$$\frac{\delta}{\delta s}\left[\frac{s_1}{A_1}\frac{\delta A_1}{\delta s_1}\right] = \frac{[\{(1-s)^2+4\sigma s\}\{s+x-bX_2\}\{2s(2\sigma-1+b-x-b\sigma)+(1+x-b+b\sigma-2\sigma x)\}]}{[\{(1-s)^2+4\sigma s\}(s_x-bX_2)]^2} - \frac{\{s^2(2\sigma-1+b-x-b\sigma)+s(1+x-b+b\sigma-2\sigma x)\}[\{(1-s)^2+4\sigma s\}\left(1-b\frac{\delta X_2}{\delta s}\right)+(s+x-bX_2)(2s-2+4}{[\{(1-s)^2+4\sigma s\}(s_x-bX_2)]^2}$$

$$\frac{\delta}{\delta s}\left[\frac{s_1}{A_2}\frac{\delta A_2}{\delta s_1}\right] = \frac{[\{(1-s)^2+4\sigma s\}\{s+x-bX_1\}\{2s(2\sigma-1+b-x-b\sigma)+(1+x-b+b\sigma-2\sigma x)\}]}{[\{(1-s)^2+4\sigma s\}(s+x-bX_1)]^2} - \frac{\{s^2(2\sigma-1+b-x-b\sigma)+s(1+x-b+b\sigma-2\sigma x)\}[\{(1-s)^2+4\sigma s\}\left(1-b\frac{\delta X_1}{\delta s}\right)+(s+x-bX_1)(2s-2+4}{[\{(1-s)^2+4\sigma s\}(s+x-bX_1)]^2}$$

2.2 Order-Disorder Transition:

The second step takes place at a higher temperature in the process of DNA melting. The order-disorder transition is named because in this transition a duplex which is predefined and ordered state of helical DNA is changed into a stage where single strands are randomly distributed. As per the theory, the Ising matrix can be expressed in relation to partition function of segment pairs that can be expressed as helical (denoted by h) or random coil (denoted by r). A k state represents the initial sections in a series of total h number of states, i.e., the border among the helical (initial) and random coil (final) states. The corresponding partition functions of these three states are denoted by f_r, f_h, and f_k. The fundamental matrix for the issue, accounting for the interaction with the nearest neighbour, is given by:

$$M = \begin{array}{c} \\ f_r \\ f_k \\ f_h \end{array}\begin{array}{c} \begin{array}{ccc} f_r & f_k & f_h \end{array} \\ \begin{vmatrix} f_r^{1/2}f_r^{1/2} & f_r^{1/2}f_k^{1/2} & 0 \\ f_r^{1/2}f_k^{1/2} & 0 & f_k^{1/2}f_h^{1/2} \\ f_h^{1/2}f_r^{1/2} & 0 & f_h^{1/2}f_h^{1/2} \end{vmatrix} \end{array} \quad (19)$$

Where the corresponding section partition functions of the ordered (initial), disordered (final) and border or nucleation state (boundary) are represented by the characters f_r, f_h, and f_k. The Eigen values of M originate from:

$$\lambda_1 = \frac{(f_r+f_h)+\{(f_r-f_h)^2+4f_rf_k\}^{1/2}}{2}$$
$$\lambda_2 = \frac{(f_r+f_h)-\{(f_r-f_h)^2+4f_rf_k\}^{1/2}}{2}$$
$$\lambda_3 = 0$$

(20)

Considering that the system we are working with is confined, the importance of the preliminary and final states increases significantly. The partition function's impact from the first segment is provided by:

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

(21)

When V represents the state of the last section,

$$V = \begin{vmatrix} f_r^{\frac{1}{2}} \\ f_k^{\frac{1}{2}} \\ f_h^{\frac{1}{2}} \end{vmatrix}$$

(22)

The partition function representing N-section is provided by

$$Z = U\, M^{N-1} V$$

(23)

The matrix T consists only column vectors, it diagonalizes M and can be given by

$$XM = \lambda X$$

(24)

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

Matrix T can be found as follows by applying the values of M from equation (6):

$$T = \begin{vmatrix} 1 & 1 & 1 \\ \frac{\lambda_1-f_r}{f_r^{1/2}f_k^{1/2}} & \frac{\lambda_2-f_r}{f_r^{1/2}f_k^{1/2}} & -f_r^{1/2}f_k^{1/2} \\ \frac{f_r^{1/2}f_h^{1/2}}{\lambda_1-f_h} & \frac{f_r^{1/2}f_h^{1/2}}{\lambda_2-f_h} & -f_r^{1/2}f_h^{1/2} \end{vmatrix}$$

(25)

In the same way, we obtain matrix T-1 from the equation given below

$$YM = \lambda Y$$

(26)

Here, $Y = |Y_1 \quad Y_2 \quad Y_3|$

When we once more substitute the M from equation (1) in equation number (8);

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

(27)

The normalisation constants are:

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

(28)

If $\Lambda = T^{-1}MT$ is the diagonalized arrangement of M, we can write the partition function as:

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

(29)

Equations (1), (3), (4), (7), (9), and (10) are inserted into equation (11), the partition function converts to equation (30) as:

$$Z = C_1\lambda_1^N + C_2\lambda_2^N$$

(30)

In disordered form, the fraction of the segments is given by

$$Q_r = \frac{\delta lnZ/\delta lnf_r}{N}$$

The solution to the above-mentioned equation is:

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

(31)

Here

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

For the sake of simplicity, we will take s to be the propagation parameter in this instance. It turns out that in most systems, it is actually pretty near to unity.

If absorbance in the disordered and ordered states is characterized by A_r and A_h, respectively, then the entire absorption comes out to be:

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

(32)

This formalization can be easily extended to particular heat.

2.3 Heat Capacity

The specific heat is connected to the variations in enthalpy per mole in addition to entropy of system through the transition from initial state to final state. The widely recognized thermodynamic relations that provide free-energy and internally stored energy are as follows:

$$F = -KTlnZ \quad \text{and} \quad U = -T^2\left(\frac{\delta}{\delta T}\right)\left(\frac{F}{T}\right)$$

respectively. By differentiating internally stotrd energy U with regard to temperature t, one can find the specific heat.

$$C_v = \frac{\delta U}{\delta t} = N_k\left(\frac{\Delta H}{RT_m}\right)^2\left(\frac{S\delta Q_r}{\delta S}\right) \tag{33}$$

Where ΔH is the variation in enthalpy per mole around the transition point and S represents entropy. S is given by

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

Where Tm is the temperature at which transition occurs, and

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

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

Where, $\frac{\delta P}{\delta S} = \frac{S-1}{P}$

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

The energy liberated or expanded in the development of the first ordered or disordered state is measured by nucleation parameter σ.

3. Result and discussion

The denaturation of DNA triple helix takes place in two steps as stated earlier and the fact is also supported by Xi *et al.* First step is order-order transition and second step is order-disorder transition.

3.1 Order-order transition

The Triplex to duplex transition is a transition from ordered-to-ordered state since, triplex and duplex both are highly ordered states.

3.1.1 Transition Profile

The Transition profile of DNA triplex alone and bonded with DNA is shown in Figure 2a and 2b. The calculated data is in good agreement with observed experimental data. The melting temperature of unbonded DNA is 33.9˚C. When Neomycin binds to the W-H groove of DNA melting temperature increases by 15.6˚C. The various parameters characterizing the melting are given in table 1. the observed binding enthalpy (ΔH) of neomycin with triplex is primarily exothermic. Since, the binding enthalpy is negative, the formation of the neomycin–triplex complex is energetically favourable because attractive interactions are formed, such as electrostatic interactions between positively charged amino groups of neomycin and negatively charged DNA phosphates, hydrogen bonds between neomycin and DNA bases/backbone. And van der Waals contacts.

In DNA melting studies, the nucleation parameter (σ) is a measure of how difficult it is to initiate the opening of a double-stranded or triple-stranded DNA structure. DNA melting does not occur by breaking all base pairs simultaneously. Instead, a small region of base pairs first opens (nucleation). Once a melted region is formed, further strand separation (propagation) becomes easier. The nucleation parameter quantifies the energetic penalty associated with creating this first melted region. The value of nucleation parameter increases when neomycin binds to triplex which implies that the initiation of DNA melting becomes easier and hence stabilizes the DNA triplex.

Thermodynamic characteristics like enthalpy change (ΔH), which indicates the strength of intermolecular interactions, control the binding affinity and stability in drug–DNA interactions. The heat absorbed or released during a process (such as melting, solidification, or a chemical reaction) is referred to as enthalpy change [15,16]. Furthermore, nucleation parameters (σ) govern drug stability, polymorphism, and crystallization behaviour in pharmaceutical systems [17,18]. When combined, these elements show how kinetic barriers and thermodynamic driving forces are balanced in medication development. In thermodynamics and nucleation theory, the idea of "ΔH/σ" is frequently employed to comprehend the equilibrium between driving force and resistance during phase formation. This concept can be expanded to domains such as drug design crystallization. A high value of "ΔH/σ" indicates that transformation happens readily since a large ΔH indicates a strong inclination for transformation (such as crystallization or binding) and a large nucleation parameter indicates a high difficulty in creating stable nuclei. A low value of "ΔH/σ" indicates that, although thermodynamically favourable, transformation is challenging.

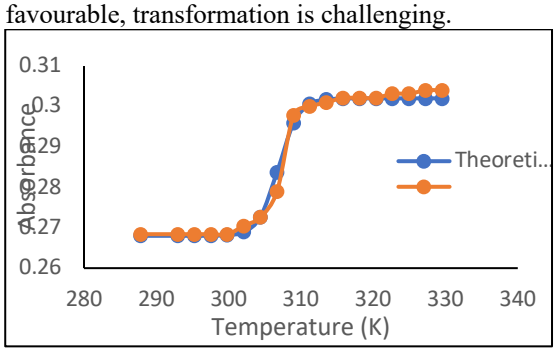

Figure 2a: Order-order Transition of Poly(dA). 2poly(dT)Triplex in the absence of Drug

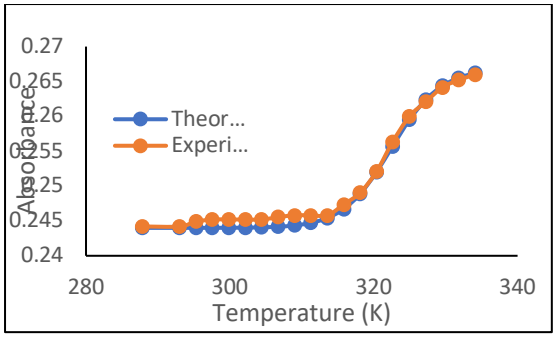

Figure 2 b: Order-order Transition Profile of Poly(dA). 2poly(dT)Triplex in the presence of Neomycin (4μM pH 6.8)

3.1.2 Heat Capacity

The variation in heat capacity of DNA with and without neomycin is shown in figure 3a and 3b respectively. In figure 3a the heat-capacity curve exhibits a sharp and narrow peak centred around 306.9 K. The transition occurs over a very small temperature range. Such a narrow peak is characteristic of a highly cooperative melting transition, where a large fraction of the triplex structure unfolds simultaneously. The maximum heat capacity is relatively low. In Neomycin bonded DNA triplex, the heat-capacity peak shifts to a higher temperature (~322.5 K). The peak becomes much broader and significantly higher (~4.5 kcal mol^{-1} K^{-1}). The transition occurs over a wider temperature range, indicating the presence of intermediate conformational states during melting. The increase in melting temperature suggests that more thermal energy is required to disrupt the triplex structure. The elevated melting temperature indicates stronger stabilization of the triplex structure, while the broadened transition suggests a reduction in melting cooperativity and the possible formation of intermediate conformational states during thermal denaturation. These observations are consistent with strong electrostatic and hydrogen-bonding interactions between neomycin and the DNA triplex, which increase the energy required for strand dissociation and alter the unfolding pathway.

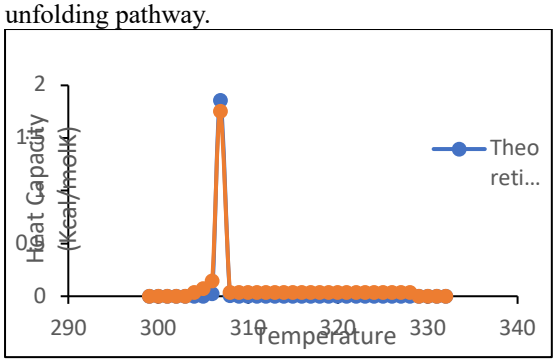

Figure 3 a: Heat Capacity of Poly(dA). 2poly(dT)Triplex in the absence of Drug

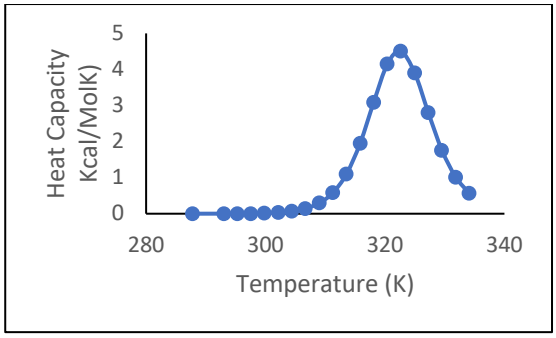

Figure 3 b: Predictive Heat capacity of Poly(dA). 2poly(dT)Triplex in the presence of Neomycin (4μM pH 6.8)

Table 1 Transition Parameters for Neomycin binding with Poly(dA). 2poly(dT) Triplex at pH 6.8

Parameters	Poly(dA). 2poly(dT)	Poly(dA). 2poly(dT) + Neomycin
T_m (K)	306.9	322.5
ΔH (Kcal/M)	-1.59	-6.4
Σ	2×10^{-13}	1.5×10^{-9}
N	202	40
A_h	0.268	0.244
A_r	0.302	0.267
Half width (experimental)	1.05	-
Half width (Calculated)	1.15	12.52
Sensitivity parameter (ΔH/σ)	0.795×10^{-13}	4.326×10^{-9}

3.1 Order-disorder transition

The Second transition step takes place when DNA double helix melts to single strands. Since, these strands are randomly distributed, therefore this transition is referred to as order-disorder transition.

3.2.1 Transition Profile

Transition curves for order-disorder transition for duplex alone and duplex-Neomycin complex are shown in figure 4a and 4b, respectively. The characteristic parameters are given in table 2.

Figure 4 a show the order–disorder transition profile for the Poly(dA). poly(dT)duplex without drug. The transition occurs around the fitted peak near ~345 K. The table also reports transition-related fit values for the absence of neomycin, including Tm = 345.1 K, and a very small σ value (narrow transition width), indicating a relatively sharp transition.

Figure 4b shows order–disorder transition with neomycin, having concentration 4 μM at pH 6.8. The transition profile is shifted to a slightly lower temperature compared with the no-drug. The table also indicates the nucleation parameter changes via $\sigma = 9.9 \times 10^{-}7$ (still small, but different from the no-drug value), implying the transition is altered when neomycin is present. Although a very small change is observed

The drug does not eliminate the order–disorder transition; it produces a subtle, modest perturbation, mainly a slight downward shift in Tm and a minor change in nucleation parameter and sensitivity parameter.

Table2 Transition Parameters for Neomycin binding with Poly(dA). poly(dT) duplex at pH 6.8

Parameters	**Poly(dA). poly(dT)**	**Poly(dA). poly(dT) with Neomycin**
T_m (K)	345.1	344.39
ΔH (Kcal/M)	-4.87	-4.87
Σ	9.5×10^{-7}	9.9×10^{-7}
N	76	24
A_h	0.3	0.26
A_r	0.37	0.328
Half width (experimental)	1	-
Half width (Calculated)	1	1.03
Sensitivity parameter (ΔH/σ)	5.12×10^{6}	4.9×10^{6}

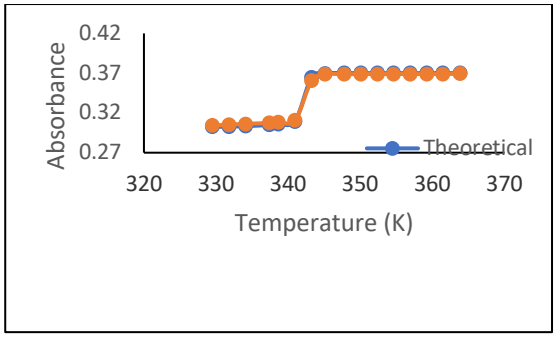

Figure 4a: Order-Disorder Transition of Poly(dA). poly(dT)Duplex in the absence of Drug

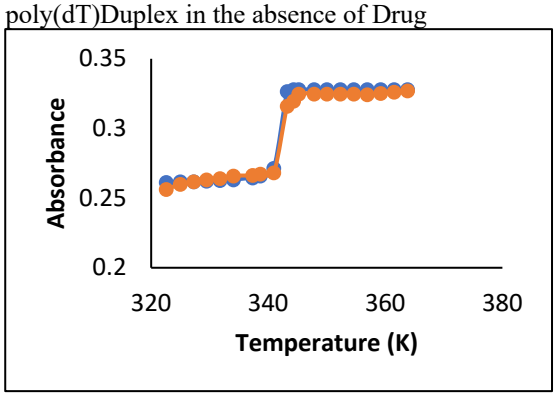

Figure 4b: Order-disorder Transition Profile of Poly(dA). poly(dT)Duplex in the presence of Neomycin (4μM pH 6.8)

3.2.2 Heat Capacity

Heat capacity variation in drug-DNA interactions is a critical thermodynamic parameter used in rational drug design to quantify the forces driving binding affinity and sequence specificity. It primarily reflects the changes in the hydration state of both the drug and the DNA double helix during complex formation. The heat capacity variation is shown in figure 5a and 5b for DNA alone and drug bonded DNA respectively. The curve for DNA alone is compared with the available experimental data. It shows a good agreement with the experimental values. The sharpness and half width of two curves show not much significant change that shows that Neomycin has a very subtle effect on DNA duplex. This fact is also supported by Xi *et al.* [9].

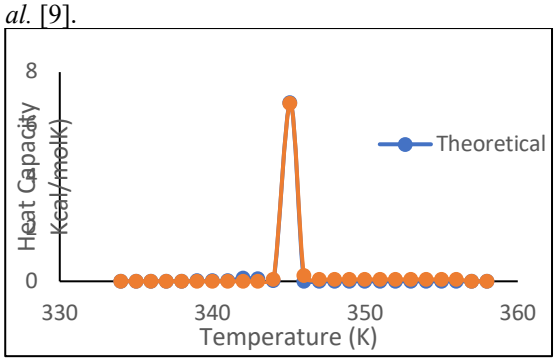

Figure 5a: Heat Capacity of Poly(dA). poly(dT)Duplex in the absence of Drug

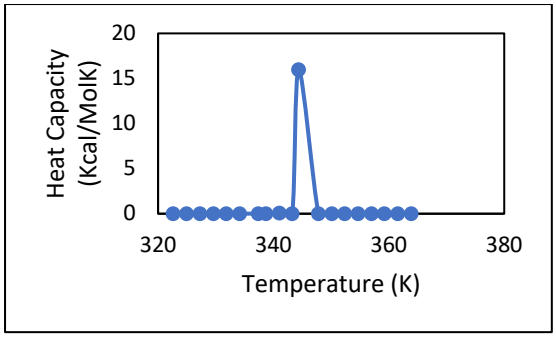

Figure 5b: Predictive Heat capacity of Poly(dA). poly(dT)Duplex in the presence of Neomycin (4µM pH 6.8)

4. Conclusions

Within the theoretical framework of Zimm and Bragg [10], the phase transition in the Neomycin-free and Neomycin-bound triple helical DNA may be effectively characterized with just two fitting parameters: the nucleation parameter and the end-effect parameter (scaling factor being extremely close to one). In every area of the transition profile, the full theoretical interpretation was tried. Half-widths show how much cooperation there is. The half-widths of the theoretical and experimental transition profiles were found to agree quite well. The nucleation parameter and change in enthalpy contain the impacts of drug binding, the redistribution of bound ligands, and the concentration dependency of the stability of the oligonucleotide complex. These different factors also depend on one another. In terms of theoretical treatment, the formalism in both order-order and order-disorder transitions is the identical, but the distinction between them is more than merely arbitrary. The Ising model formalism is its foundation. Depending on the characteristics of the two ordered states, the order-order transition has two kinds of growth factors. The analogous state in the order-disorder transition differs from the border state in the order-order transition as well.

Understanding these parameters in drug-DNA interactions is essential for rational drug design. These metrics reveal the molecular forces driving drug binding and specificity, allowing scientists to optimize a compound's clinical efficacy, prevent off-target toxicity, and maximize binding affinity.

Competing interest

There are no competing interests in this work. The manuscript's contents have been reviewed and approved by each coauthor, and there are no competing financial interests to disclose.

Data Availability statement

Data will be made available on request.

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