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Theoretical study on effect of binding of Netropsin on thermal denaturation of synthetic oligo-nucleotides

Onkar Prasad[1*], Leena Sinha[1], Neeraj Misra[1], R.C. Agnihotri[2], Jitendra Pathak[1],

[1]*Physics Department, University of Lucknow, Lucknow, India*
[2]*S.I.E.T., Lucknow, India.*

Abstract

The multiphasic nature of transition in Netropsin free and Netropsin bound in virgin reference mixture as well as in oligomers $(dA)_{12}$-X-$(dT)_{12}$-X-$(dT)_{12}$ with and without salt surroundings has been successfully interpreted within the theoretical framework of Zimm and Bragg theory of helix to coil transition. The phenomenon of destabilization and stabilization of triplex and duplex due to the binding of Netropsin, in all cases has been successfully explained in terms of the nucleation parameter σ and enthalpy changes ΔH. The Netropsin binding to the triplex leads to the decrease in the cooperativity of triplex → duplex and duplex → random coil melting and the increase in the enthalpy of both triplex → duplex and duplex→ random coil transition

Key Words: Cooperativity, Nucleation parameter, Enthalpy, Triplex, Duplex.

Introduction

The importance of short synthetic oligonucleotides stems from the fact that, these can be designed to form local triple helices on long double-stranded DNA target sequences and have many potential applications including the regulation of gene expression [1-5]. The triple helix forming oligonucleotides [T.F.O.] are highly sequence specific DNA – binding ligands and present the possibility of producing the designer molecules with extremely high degree of specificity towards recognition of binding sites at the target. In biotechnology, The DNA helices are found to undergo order to order and order to disorder reversible transitions under different environmental conditions and also when bound to drugs like Netropsin, Ethidium bromide, Actinomycin ligands with known sequence preferences [6-14]. Netropsin (Net) is an oligomer isolated from streptomyces netropsis and has been the focus of various antifungal, antibacterial and antiviral targeted studies. The study reported by Srivastava et al.[15], regarding the effect of

Netropsin on DNA triplex of varying length using the spectroscopic and calorimetric data published by Plum et.al [16] and Park et. al. [17] seems to be incomplete as it lacks to explain the attributes of short synthetic oligonucleotides. Maurice and others [18] have reported the thermal denaturation and circular dichroism spectroscopic studies on interaction of Net, a minor groove binding drug with triple helix and double helix and found that Net always destabilizes triplex whereas it stabilizes duplex. The present communication deals with the theoretical interpretation of the experimental data using the Zimm and Bragg model [19] of helix ↔ coil transition (modified suitably), the work reveals that Net binding to the triplex leads to the decrease in the cooperativity of triplex → duplex and duplex → random coil melting and the increase in the enthalpy of both triplex → duplex and duplex→ random coil transition. This work is in continuation to our ongoing research work on phase transition, vibrational analysis, phonon dispersion and DFT technique [20-39] in a variety of macromolecules,

Results and Discussion

We report here an extension of the Zimm and Bragg model [19] to explain the temperature induced order → order transition in DNA triplex and order → disorder transition in DNA duplex. The melting is represented by the following steps:

triplex ↔ duplex + strand (order ↔order transition)

duplex ↔ strand +strand (order ↔disorder transition)

Theoretical transition curves for a polymer chain of length N have been obtained from equation (6) to equation (8) [given in theory section]. The expression for the calculation of crystallinity (degree of order), is given by equation (6). These curves are found to be linear in the transition region. In general the sharpness of the transition depends upon the value of enthalpy change, and the fluctuations around the transition point. The holistic effect of these is reflected in the magnitude of 'σ' and the half width of transition profile. The smaller the value of σ, sharper is the transition.

In contrast to the significant variation in values of growth parameter 's' with temperature, the nucleation parameter 'σ' has a weak dependence on it, hence for all theoretical purposes the values of nucleation parameter are assumed to be constant and independent of temperature as well as other surrounding interactions.

Order - Order Transition

The denaturation of DNA triplex being a highly cooperative process, is characterized by a large number of segments of DNA chain that undergo transition together. During the melting of Net-bound DNA triplex, it is only the third strand that gets expelled and the Netropsin remains bound to the Watson-crik duplex. The values of nucleation parameters and enthalpy changes which result in the best fit to the experimental data for the Net-free reference mixture $(dA)_{12}$ - $2(dT)_{12}$ and in case of Net-bound modified-oligomer $(dA)_{12}$-X-$(dT)_{12}$-X-$(dT)_{12}$ both in 1M NaCl salt are listed in the Table 1.

Table 1. Various input parameters for Order-Order transition in DNA triplex in 1 M NaCl Salt

S. No	Transition	Transition temp. (K)	ΔH in Kcal-mol^{-1} base pair	x	σ_1	σ_2	$\sigma = \sigma_1\sigma_2$
01.	Net-free reference mixture $(dA)_{12}$ - $2(dT)_{12}$	293	3.3	1.5	$1X10^{-4}$	$1X10^{-1}$	$1X10^{-5}$
02.	Net-bound modified-oligomer $(dA)_{12}$-X-$(dT)_{12}$-X-$(dT)_{12}$	308	3.9	1.5	$1X10^{-4}$	$6X10^{-1}$	$6X10^{-5}$

Theoretical transition curves corresponding to N=36 have been obtained by using equations (6) to (8) and are drawn in fig. 1 and 2.

Fig.1. Schematic representation of degree of order as a function of temperature
(Triplex →Duplex transition).

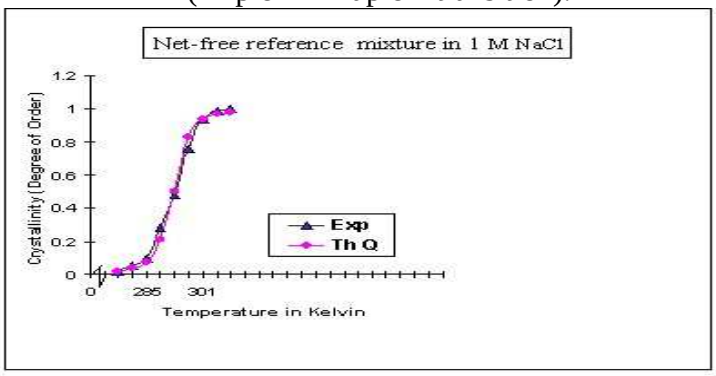
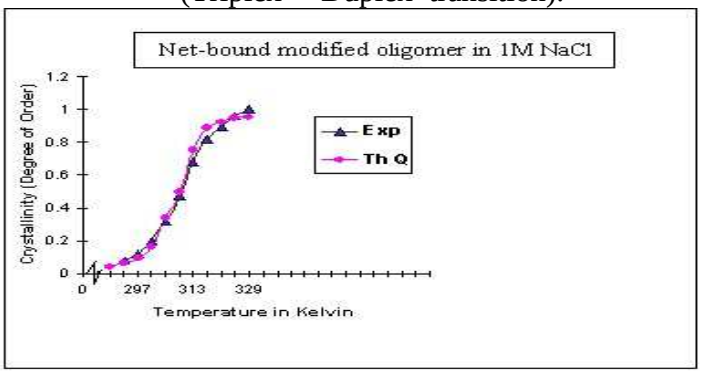


Fig.2. Schematic representation of degree of order as a function of temperature
(Triplex →Duplex transition).



The consequence of the requirement of $\sigma_2 > \sigma_1$ for best fit implies that within the chain, the nucleation of form II (DNA duplex) is more probable than the nucleation of form I (DNA

triplex). The role of end parameter 'x' and its importance in case of the finite chains is manifested by the deviation of its value from unity for an infinite chain. The assignment of value of end parameter 'x' (x=1.5) for best fit also implies that nucleation of duplex at the ends is more probable than the nucleation of triplex. As obvious from the figures 1 and 2 that order-order transition in reference mixture Net-free $(dA)_{12}$ - $2(dT)_{12}$ is sharper than case of Net-bound modified DNA triplex $(dA)_{12}$-X-$(dT)_{12}$-X-$(dT)_{12}$ both in 1M NaCl. The above experimental observation is supported by the order of the theoretically calculated values of the nucleation parameters in the two cases, σ Net-free [$(dA)_{12}$ - $2(dT)_{12}$] ⟨ σ Net-bound [$(dA)_{12}$-X-$(dT)_{12}$-X-$(dT)_{12}$].

The smaller the value of σ, the larger will be the free energy penalty in creating the transition/boundary interface, consequently smaller value of σ reflects larger cooperativity as well as greater sharpness. The increase in value of σ (refer table 1), attributed to netropsin binding in case of the Net-bound reference $(dA)_{12}$ - $2(dT)_{12}$ mixture, concomitantly results in decrease of both the co-operativity of the DNA triplex melting event and the sharpness in transition profile. As evident from the table 1, it is clear that the Net-bound DNA triplex → duplex transition requires a roughly 20% greater enthalpic cost (3.90 kcal for Net-bound vs 3.30 kcal for Net-free). This result suggests that the Netropsin binding increases the enthalpy of DNA triplex → duplex transition (more endothermic).

The ionic interactions arising due to the presence of salt seem to reduce the degree of destabilization of DNA triplex and thereby compensates up to a certain extent for the destabilization of triplex form induced by Netropsin. This is also reflected by the fact that Net-bound DNA triplex to duplex transition in 1 M salt solution takes place at higher temperature (308 K) as compared to the melting temperature of salt-free Net-bound DNA triplex → duplex melting temperature (well below 293 K).

The implications of the nature of Net binding to the minor groove of DNA triplex along with its structural details are given by Haq et al.[40], Nunn et al.[41], and Tabernero et al. [42]. The destabilization of DNA triplex due to Netropsin binding, occurs possibly due to the repulsion between non bonded atoms and the weakening of H bonds arising from redistribution of charges and also due to the change in base pair propeller angles [38,39].

Order-Disorder transition

The order → disorder transition (duplex → random coil) has also been explained by same theory. The transition curves in these cases are obtained by using equations (12) and (13). The order→ disorder transitions occurring at higher temperatures are found to be sharper as compared to order→ order transition (triplex to duplex), as these transition involve the thermal disruption of the duplex into its constituents in the random state and theoretically correspond to a single σ, whereas order-order transitions are generated by two σ values, namely σ_1 and σ_2 .

The enthalpy changes in Net-bound reference mixture as compared to Net-free reference mixture (refer table 2), are indicative of the stability, arising due to the binding of Netropsin at the DNA minor groove site. The stability is further reflected in the increase of transition temperature of duplex to coil transition from 315K to 335K. The monophasic nature of order-disorder transition in Net- bound reference mixture, could be related to the fact that Netropsin molecule when

bound to its minority groove destabilizes the DNA triplex consequently the DNA triplex → duplex transition takes place well below the lower limit of temperature range.

Table 2.Various input parameters for Order-disorder transition in DNA duplex in the different environmental surroundings

S.No.	Transition	Transition temp. (K)	ΔH in Kcal-mol^{-1} base pair	σ
01	Net-free reference mixture $(dA)_{12}$ - $(dT)_{12}$ in 1 M NaCl Salt	315.0	06.0	$1.50X10^{-2}$
02	Net-bound reference mixture $(dA)_{12}$ -$(dT)_{12}$ in 1 M NaCl Salt	335.0	11.0	$2.80X10^{-2}$
03	Net-free modified-oligomer $(dA)_{12}$-X-$(dT)_{12}$ in 1 M NaCl Salt	344.0	08.0	$1.00X10^{-2}$
04	Net-bound modified-oligomer $(dA)_{12}$-X-$(dT)_{12}$ in 1 M NaCl Salt	358.0	14.0	$2.50X10^{-2}$
05	Net-free modified-oligomer $(dA)_{12}$-X-$(dT)_{12}$ in Salt free medium	310.8	05.5	$1.75X10^{-2}$
06	Net-bound modified-oligomer $(dA)_{12}$-X-$(dT)_{12}$ in Salt free medium	361.0	16.0	$2.00X10^{-2}$

The values of enthalpy changes in case of Net-free and Net-bound modified oligomer $(dA)_{12}$-X-$(dT)_{12}$ in salt free case are also given in the table [2]. The transitions are characterized by monophasic duplex to coil, with complete absence of triplex to duplex thermal transition. Increase in enthalpy changes, reflecting the stabilization of Net-bound duplex, has been calculated theoretically. The theoretically predicted stability is further manifested by the shift in transition temperature i.e. 361K with Net-bound triplex as compared to 310.8 K in case Net-free duplex.

Fig.3. Schematic representation of degree of disorder as a function of temperature
(Duplex → Coil transition).

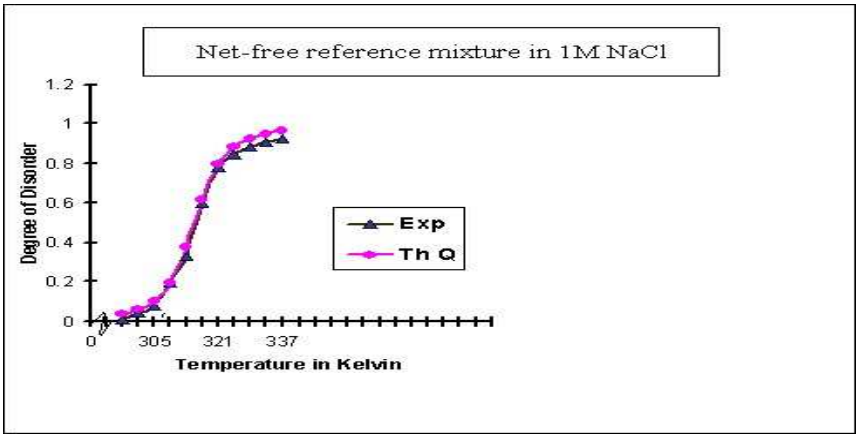


Fig.4. Schematic representation of degree of disorder as a function of temperature
(Duplex → Coil transition).

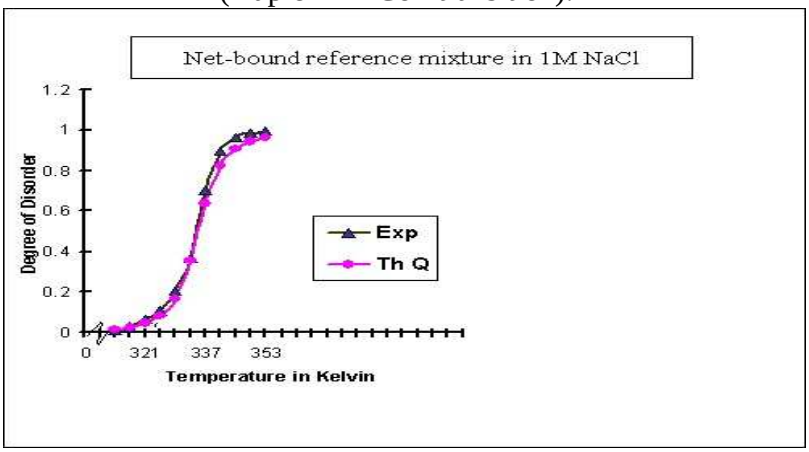


Fig.5. Schematic representation of degree of disorder as a function of temperature
(Duplex → Coil transition).

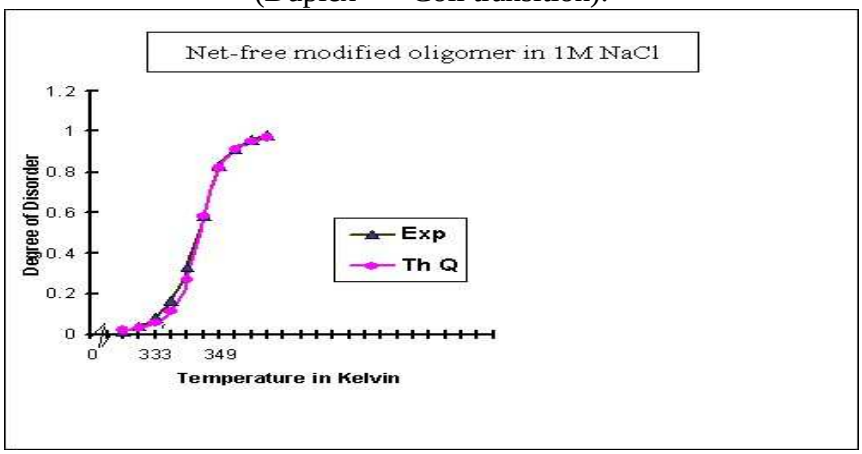


Fig.6. Schematic representation of degree of disorder as a function of temperature
(Duplex → Coil transition).

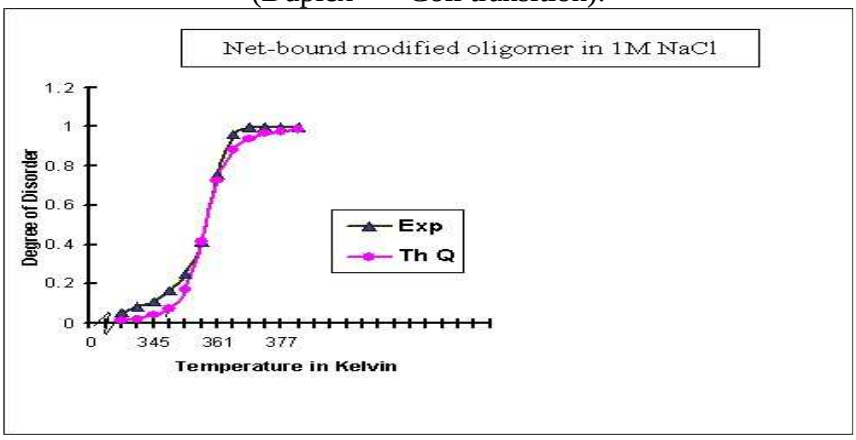


Fig.7. Schematic representation of degree of disorder as a function of temperature
(Duplex → Coil transition).

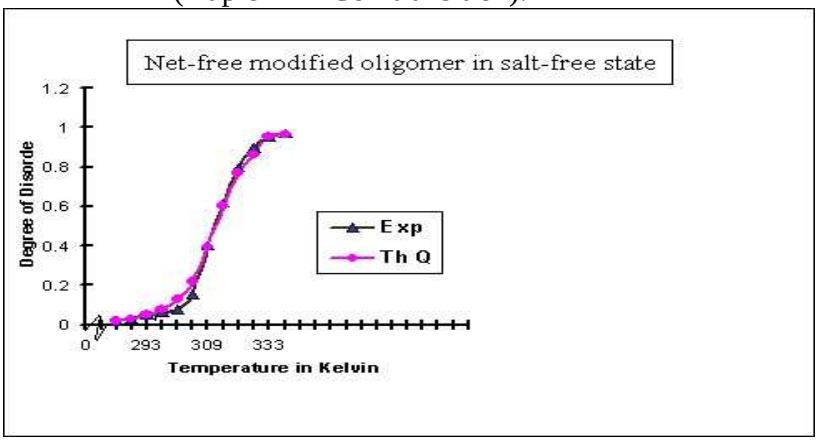
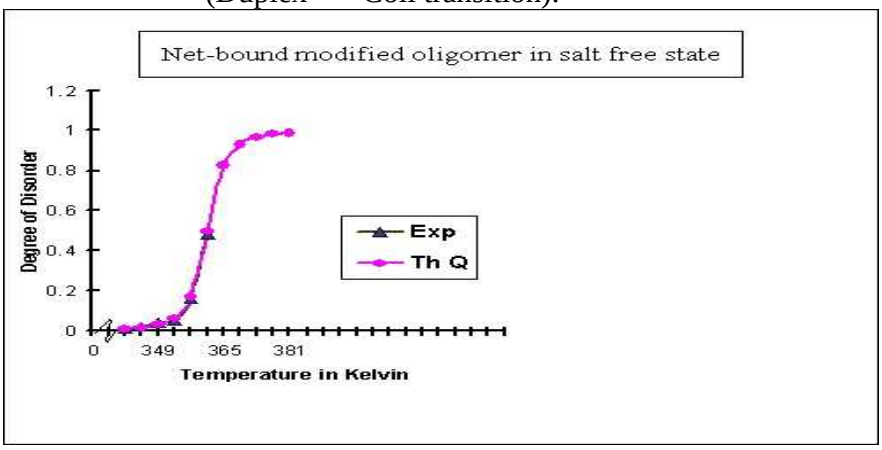


Fig.8. Schematic representation of degree of disorder as a function of temperature
(Duplex → Coil transition).



In case of all the Net-bound DNA Duplex oligomers, it is found that irrespective of their environmental surroundings the thermal stability increases and the sharpness of the order → disorder transitions decreases as compared to the corresponding pair of Net-free oligomers (refer to table 2). Further the relative degree of stability in Net-bound cases increases in the reverse order of corresponding nucleation parameters, i.e. σ Net-bound [$(dA)_{12}$ - $(dT)_{12}$ reference mixture in salt] > σ Net-bound [$(dA)_{12}$ -X- $(dT)_{12}$ in salt] > σ Net-bound [$(dA)_{12}$-X-$(dT)_{12}$ salt-free] and is also reflected in the shift of transition temperatures. The theoretically calculated values (refer table 2) are well supported by the experimental data (Fig. 3-8). For the sake of comparison, table 2 comprises of nucleation parameters and enthalpy data for Net free DNA duplex as well as their corresponding Net bound DNA duplex under the same environmental surroundings. As evident from theoretically calculated transition parameters that like the Net binding in DNA triplex, the Net binding in DNA duplex also causes the decreases in cooperativity of the transition and increase in enthalpic input as a consequence of commensurate rise in both the values of nucleation parameters and the enthalpy changes (refer table 2) during the melting of DNA duplex. The similar results have been reported by Marky et. al. in their calorimetric and spectroscopic investigation of drug DNA interactions [13].

Materials and Methods

Thermal denaturation of triplex has been treated here as two state phase system and involves both the order-order as well as order-disorder transitions. The Zimm and Bragg model [19] for helix ↔ coil (order ↔ disorder) transition has been modified to explain the melting of triplex. The present theoretical approach has earlier been used by our group to explain an order ↔ order transition in the case of PBLAsp and it's copolymer [Copoly(SLAsp-BLAsp)] as well as in poly-L-proline [31,34] and order ↔ disorder transition in the case of collagen, polystyrene-polybutadiene and polyethylene [31-33,36]. The method involves the construction of grand partition function for the entire chain which gives an expression for degree of order 'Q' in terms of nucleation parameter σ.

Order-order transition

Taking into account the nearest - neighbor interactions, the basic transition matrix 'M' in case of order-order transition is given below:

$$M = \begin{array}{c|cccc} & h_1 & k_1 & k_2 & h_2 \\ h_1 & s_1 & 0 & \sigma_2 s_2 & 0 \\ k_1 & s_1 & 0 & 0 & 0 \\ k_2 & 0 & 0 & 0 & s_2 \\ h_2 & 0 & \sigma_1 s_1 & 0 & s_2 \end{array}$$

The segments in form I and the form II have been represented by h_1 and h_2. Whereas k_1 and k_2 are the boundary states which is the first of the sequence of the segments in states I and II respectively. The nucleation and growth parameters have been represented by σ_1 and σ_2 and s_1 and s_2 respectively. The variation of σ_1 and σ_2 means the variation of the probabilities of nucleation of form I in a sequence of segment of II and the nucleation of form II in a sequence of segment of I respectively

The eigen roots are given by secular equation,

$$| M-\lambda I | = 0 \qquad (1)$$

$$\lambda^2 (\lambda-s_1)(\lambda-s_2) - \sigma_1 \sigma_2 s_1^2 s_2^2 = 0 \qquad (2)$$

The four eigen roots of the Eq. (2) depend only on the product $\sigma_1 \sigma_2$ (= σ say). If $\sigma_1 \sigma_2 = 0$, the eigen roots are s_1, s_2 0,0 and if product $\sigma_1 \sigma_2 \langle\langle 1$, the two smaller eigenroots are of the order of $\sqrt{(\sigma_1 \sigma_2)}$ and the larger eigenroots are of the order of s_1, and s_2 that is unity in the transition range. Thus the contribution of smaller eigenroots to the partition function is negligible and the main contribution comes from the larger eigenroots denoted by λ_1 and λ_2. These eigenroots can be obtained on iteration and are given by the following relations:

$$\lambda_1 = s_1 + [\sigma_1 \sigma_2 s_1^2 s_2^2 / \lambda^2 (\lambda-s_2)] \qquad (3)$$

and

$$\lambda_2 = s_2 + [\sigma_1 \sigma_2 s_1^2 s_2^2 / \lambda^2 (\lambda-s_1)] \qquad (4)$$

The partition function Z of a chain of N segments is then given by

$$Z = \sigma' \sum A_i \lambda_i^N \qquad (i=1 \text{to } 4) \qquad (5)$$

$A_i = \{ \lambda_i^2(1+x) - \lambda_i (s_2 + s_1 x) + s_1 s_2(\sigma_1 x+\sigma_2)\} / \{4\lambda_i^2 - 3 \lambda_i (s_1 + s_2) + 2 s_1 s_2\}$

With $x = \sigma'' / \sigma'$

Factor x is defined as the end parameter. The parameter σ' and σ'' give the interaction of the end segments in the states h_1 and h_2 with the surroundings.

Hence Q_I, the fraction of the state in form I can thus be calculated as following;

$$Q_I = (1/N)(\partial \text{In } Z / \partial \text{In } s_1)$$
$$= (1/N)(s_1/Z) (\partial Z / \partial s_1)$$
$$\approx [(s_1/\lambda_1) (\partial\lambda_1 / \partial s_1) + (s_1/\lambda_2) (\partial\lambda_2 / \partial s_1)B +$$
$$(1/N)\{(s_1/ A_1) (\partial A_1 / \partial s_1) + (s_1/ A_2) (\partial A_1 / \partial s_1)B\}] / (1+B) \qquad (6)$$

where $B = (A_2/ A_1)(\lambda_2//\lambda_1)^N$

The equilibrium constant s and nucleation parameter σ for transition are given by

$s = s_1/ s_2$, $s_1 s_2 = 1$ and $\sigma = \sigma_1 \sigma_2$ (7)

where

Since the growth parameter s and subsequently s_1 ($s_1 = \sqrt{s}$) depend on the temperature T as given by eq. (8). The variation in values of s or s_1 parameter is reflected by the corresponding change in temperature T. Hence the variation of one parameter takes care of the other. ΔH is the molar change in enthalpy, T_f is the transition temperature and R is the constant given by 2 cal/ mol.

Order-disorder transition

The basic transition matrix 'M' in case of order-disorder transition is given below:

$$M = \begin{array}{c|ccc} & r & k & h \\ r & \sqrt{f_r}\sqrt{f_r} & \sqrt{f_r}\sqrt{f_k} & 0 \\ k & \sqrt{f_k}\sqrt{f_r} & 0 & \sqrt{f_k}\sqrt{f_h} \\ h & \sqrt{f_h}\sqrt{f_r} & 0 & \sqrt{f_h}\sqrt{f_h} \end{array}$$

Where r, k and h are segments, whereas f_r, f_k and f_h denote the segment partition functions in disordered, boundary and ordered regions of the macromolecular chain respectively.

The eigenroots of M, determined by the secular equation

$$| M-\lambda I | = 0 \qquad (9)$$

are as follows:

$$\lambda_1 = (1/2)[(f_r + f_h) + \sqrt{\{(f_r - f_h)^2 + 4 f_r f_k\}}]$$
$$\lambda_2 = (1/2)[(f_r + f_h) - \sqrt{\{(f_r - f_h)^2 + 4 f_r f_k\}}]$$
$$\lambda_3 = 0 \qquad (10)$$

where I is a 3x3 unity matrix.

The grand partition function 'Z' for a chain of N segments is

$$Z = C_1 \lambda_1^N + C_2 \lambda_2^N$$
$$= Z_1 + Z_2 \quad (11)$$

The fraction of disordered 'r' segments $< n_r > = (n_r)/ N$

$$< n_r > = (1/N)[(\partial \ln Z)/ (\partial \ln f_r)]$$
$$= [f_r /(NZ)][(\partial Z)/ (\partial f_r)] \quad (12)$$

For an infinite chain the fraction of disordered segments is calculated by the following equation:

$$< n_r > = (\lambda_1 - f_h)/ (\lambda_1 - \lambda_2)$$

The fraction of segments in the ordered phase denoted by 'Q' is given by

$$Q = 1 - < n_r >$$
$$= (1/2)[(s-1) + \sqrt{\{(s-1)^2 + 4\sigma s\}}]/ [\sqrt{\{(s-1)^2 + 4\sigma s\}}] \quad (13)$$

where $s = f_h / f_r$ and $\sigma = f_k / f_h$ are the growth and nucleation parameters respectively.

Conclusion

The multiphasic nature of transition in Net free and Net bound in virgin reference mixture as well as in oligomers $(dA)_{12}$-X-$(dT)_{12}$-X-$(dT)_{12}$ with and without salt surroundings has been successfully interpreted within the theoretical framework of Zimm and Bragg theory of helix to coil transition and the phenomenon of destabilization and stabilization of triplex and duplex in all cases has been successfully explained in terms of the nucleation parameter σ and enthalpy changes ΔH. It seems that the ionic interactions arising due to the presence of salt reduces the degree of destabilization of triplex and thereby compensates up to a certain extent for the destabilization of DNA triplex produced by binding of the drug Netropsin. In this regard further experiments need to be performed to ascertain the role of different salt surroundings on the attributes of Netropsin binding or any other drugs with the biological systems. The evolution of new tools for the study of sequence and structural-selective ligand binding are important for the better understanding of drug-receptor binding mechanism and as a whole may lead to the efficient drug discovery.

Acknowledgement

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