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“Tie Calorimetry” as a Tool for Determination of Thermodynamic Parameters of Macromolecules

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Additional information is available at the end of the chapter

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Abstract

Determination of free energy of double helix formation from two single-stranded polynucleotides and estimation of energetics of different low-molecular compounds binding to nucleic acids provide valuable tools for understanding of mechanisms that govern noncovalent binding of ligands to their receptor targets. In order to completely understand the molecular forces that drive and stabilize double helix formation and its complexes with ligands, thermodynamic studies are needed to complement the structural data. Structural characterization of a number of DNA-ligand complexes by X-ray and high-resolution NMR method provides key insight relating to the properties of complex formation, but structural data alone, even when coupled with the most sophisticated current computational methods, cannot fully define the driving forces for binding interactions (or interactions) or even accurately predict their binding affinities. Thermodynamics provides quantitative data of use in elucidating these driving forces and for evaluating and understanding at a deeper level the effects of substituent changes on binding affinity.

Keywords: free energy, double helix, helix-coil transition, transition thermodynamic parameters, DNA-ligand complexes, binding parameters

1. Introduction

The 3D structure of solids by the change of environmental conditions may convert to a phase with quite different physical parameters describing the resulting state of matter. Transitions from one phase to another are accompanied by absorption or release of heat and sharply defined changes of energetic characteristics of the matter. At the fifties of last century, the biologically important molecules, nucleic acids and proteins, have been discovered — the structures of which were like one-dimensional linear aperiodic crystals [1]. The phase transition

in linear crystals was theoretically treated at the twenties of last century [2]. According to this theory, the thermodynamic equilibrium is impossible for two homogeneous phases sharing common frontiers. Proper demonstration of the theorem efficacy was given much later, when the linear crystal to coil (helix-coil) transition of proteins and nucleic acid was investigated [3].

The unique feature of nucleic acid chains is their folding manner that encloses functional groups, i.e., purine and pyrimidine bases, so as to protect them inside a rigid and monotonous double-helix structure. At present, it is well established that DNA, the “major” molecule in the living cells, is polymorphous, and while functioning, the biopolymer may be in several forms: B-, A-, Z-, coil, etc., of which only Z-form was found to be a left-handed helix [4, 5]. There are two different types of structural transitions in DNA one of which (helix-coil, A-coil, Z-coil) is accompanied by unwinding of double helix (translation and replication, etc.). The second type of transitions (B-B¹, B-Z, B-A, A-Z, etc.) is realized by certain structural changes in sugar-phosphate backbone and base-pairs (bp) of DNA without unwinding the helix of the biopolymer. To understand the biological role of the existence of various forms of DNA, it is important to know the thermodynamic parameters of the phase transitions, particularly the value of free energy changed (ΔF), which is very difficult to obtain directly from the experiment. To estimate the ΔF value, enthalpy (ΔH) and entropy (ΔS) of transitions as usual are experimentally determined that are the constituents of free energy. We shall discuss below the experimental ways of estimating the values of these major thermodynamic parameters.

2. Main body

2.1. Theory

Along with genetic information realization in vivo (replication, transcription, translation), the molecule of DNA is being subjected to different conformational transitions. Moreover, there are no conformational transitions in “pure” molecule: it is always surrounded and interacts with huge number of various low-molecular compounds, which in turn, interacting with DNA, can stabilize or destabilize different conformational states of polymer molecule. To judge if this or other ligands stabilize or not different conformations of DNA, DNA conformational transition in the complex under any external factor inducing this transition should be studied (temperature, pH, chemical effect, etc.) and compared with the pure molecule transition.

Nowadays, it may be strictly established that these transitions (B-coil, A-coil, B-A, B-Z, Z-A, etc.) carry a cooperative character. The transition cooperativity is a direct consequence of the fact that the transition occurs in quasi-one-dimensional aperiodic crystal: in this case, the real phase transition is excluded.

Analysis of numerous experimental data, as well as some general representations about helix-coil transition, condition the possibility to formulate DNA main model, which is applied for theoretical observation of its melting. The model is sufficiently simple – DNA is one dimensional system that forms pairs of bases and each of them may be only in two states: helical and coil-like. Lengthening of the helical region per pair is accompanied by free energy value change ΔF . The value of ΔF determines the constant of this process:

$$\sigma = \frac{[\sigma_{i+1}]}{[\sigma_i]} = \exp(\Delta F/RT) \quad (1)$$

where R is gas constant, T is temperature, and σ_i and σ_{i+1} are concentrations of molecules containing helical regions from i and $i + 1$ pairs of bases, respectively. In the transition point T_0 $\sigma = 1$, consequently, ΔF turns to zero. In the vicinity of this point, ΔF linearly depends on the temperature:

$$\Delta F = \Delta H + T\Delta S \quad (2)$$

where ΔH and ΔS are changes of enthalpy and entropy, respectively.

Formation of new melted region in helical part is connected to appearing of additional boundaries between helical and melted regions and requires additional changes of free energy.

Value of ΔF determines the cooperativity of the system and

$$\sigma = \exp(-F_0/2RT) \quad (3)$$

is called a cooperativity factor. If $F_0 = 0$, the cooperativity is absent. When $F_0 \rightarrow \infty$, the system is exposed to phase transition. At $0 < F_0 < \infty$, the transition carries a cooperative character and the higher is F_0 , the more favorable are long helical and melted regions and correspondingly the melting interval decreases.

Observed model, known in statistical physics as Ising model, physically corresponds to a case of single-stranded homopolymer. Let us observe this model applying the method of more probable distribution [6].

It is known that equilibrium values of physical magnitudes are corresponded to their most probable values at the given energy of the system. They can be found from the condition of "nonequilibrium" free-energy minimum:

$$F = E - T \ln W \quad (4)$$

where W is the number of states corresponding to the given energy of E .

Linear homopolymer consisting of N rings is observed. Each of these rings may be in one of these two states: melted – coil-like and helical. Macroscopic state of such system at the certain T temperature is given by three parameters: N_2 is number of helical rings (in the second state), N_1 is the number of rings in coil-like state (1), and n is the number of regions consisting of rings 1 or 2. It is clear that $N_1 + N_2 = N$; moreover, the case of infinite homopolymer is observed $N \rightarrow \infty$. If F_1 and F_2 are free energies of rings being in melted and helical states, respectively, $F_0/2$ is the free energy of boundary between helical and coil-like rings, the whole energy of the system is:

$$F = F_1 N_1 + F_2 N_2 + F_0 n \quad (5)$$

Number of microstates corresponding to given values of N_1 , N_2 , and n will be equal to

$$W = W_1 - W_2 \quad (6)$$

where W_2 and W_1 are numbers of modes by which helical and coil-like (melted) rings at the given values of N_1 , N_2 , and n may be distributed:

$$W_1 = \frac{(N_1 - 1)!}{(n - 1)!(N_1 - n)!} \quad (7)$$

$$W_2 = \frac{(N_2 - 1)!}{(n - 1)!(N_2 - n)!} \quad (8)$$

In the observed case in Eqs. (7) and (8), the unit can be neglected ($N \rightarrow \infty$). In this case,

$$W(N_1, N_2, n) = \frac{N_1! N_2!}{n!(N_1 - n)! n!(N_2 - n)!} \quad (9)$$

Replacing (5) and (9) in (4) and applying Stirling's formula, we will obtain

$$F = F_1 N_1 + F_2 N_2 + F_0 n - T [N_1 \ln N_1 - (N_1 - n) \ln (N_1 - n) + N_2 \ln N_2 - (N_2 - n) \ln (N_2 - n) - 2n \ln n] \quad (10)$$

Equilibrium values of N_1 , N_2 , and n are determined from conditions

$$\left| \frac{\partial F}{\partial n} \right|_{N_1 N_2} = 0 \quad (11)$$

and

$$\left| \frac{\partial F}{\partial N_1} \right|_n = 0 \quad (12)$$

If to mark $\xi = \exp(-F_0/RT)$ and $\sigma = \exp(\Delta F/RT)$, where $\Delta F = F_1 - F_2$ is a free energy change at helix-coil transition, from Eqs. (11) and (12), we will obtain

$$\frac{1}{\xi} = \left(\frac{N_1}{n} - 1 \right) \left(\frac{N_2}{n} - 1 \right) \quad (13)$$

$$\sigma = \frac{1 - \frac{n}{N_2}}{1 - \frac{n}{N_1}} \quad (14)$$

at the condition of total ring number constancy ($N_1 + N_2 = N$):

The obtained equations have dependences of N_1 , N_2 , and n on σ . Jointly solving Eqs. (13) and (14), the equation of ring part being in helical state $\theta = N_2/N_1$ is

$$\frac{1 - 2\theta}{\sqrt{(1 - \theta)\theta}} = \frac{1}{\sqrt{\xi}} \cdot \frac{1 - \sigma}{\sqrt{\sigma}} \quad (15)$$

The Eq. (15) describes the helix-coil transition curve.

From definition of the transition interval width, we will obtain:

$$\Delta T = 4\sqrt{\xi} \frac{T_0^2}{\Delta H} \quad (16)$$

where ΔH is enthalpy and T_0 is the transition temperature.

T_0 is determined from the condition that in transition point, the free energy change is equal to zero

$$\Delta F = 0 \quad (17)$$

From the Eq. (2), we will obtain

$$T_0 = \frac{\Delta H}{\Delta S} \quad (18)$$

where ΔS is the difference of entropy in melted and helical states.

It should be mentioned that in the case of $F_0 = 0$, i.e., at the absence of interaction between rings ($\xi=1$), the formula (16) transmits to Boltzmann's distribution. This case responds to cooperativity absence. At boundary energy increasing (decreasing of ξ) the system "becomes" cooperative; the melting interval decreases Eq. (16). In the threshold case when $\xi \rightarrow 0$, the system is entirely cooperative, but the transition is sharp: $\Delta T \rightarrow 0$.

One of the fundamental predictions is that in the transition interval, polynucleotide chain is divided into alternate helical and coil-like regions, the length of which depends on the value of ξ . The average length of the helical region is equal to

$$v_2 = \frac{N_2}{n} = \frac{1}{\sqrt{\xi}} \cdot \sqrt{\frac{\theta}{1-\theta}} \quad (19)$$

In the transition point, $\theta = 1/2$ and the average length of helical (and coil-like) region is equal to:

$$v_0 = \frac{1}{\sqrt{\xi}} \quad (20)$$

One of the first attempts to estimate the cooperativity factor value was presented in [7] by comparison of experimentally obtained value of ξ for homopolymer to the theory. It was shown that the value is in interval 10^{-4} – 10^{-5} . For heteropolymer, the estimation is less precise since the melting interval width dependence on ξ in this case is logarithmic [6]. Uncertainty in values of ΔT depending on the cooperativity factor in the cases of different models shows that it is necessary to calculate and compare to experiment such characteristics of the helix-coil transition, which do not depend on ξ in wide change interval of this parameter.

Such invariant values are changes of melting temperature and melting interval width invoked by DNA binding to low-molecular compounds (ligands) [6, 8–11]. From the point of view of the effect on DNA double-helix stability, ligands that are able to form complexes with polymers may be divided into stabilizers and destabilizers. Comparison of the melting curves of

“pure” and ligands bound to >DNA can give information about the character of ligand binding to DNA: if the complex melting temperature (T_m) is higher than T_0 for pure DNA, stabilization occurs, and if T_m decreases, then, destabilization occurs. Independently on the chosen model, molecules, possessing high affinity to double-helical polynucleotide, will stabilize the native structure and molecules, well binding to coil-like DNA — destabilize polymer double helix. What concerns to the melting interval, in both cases it increases as compared to that of pure polymer.

One of the predictions of the theory is that the melting interval width dependence on ligand concentration should have bell-like shape. It is explained by the fact that at small concentrations of ligands, ΔT of complexes increases due to the redistribution of ligands between helical and coil-like regions, which takes place during denaturation process with ligand concentration enhancement in accordance to their affinity to those regions. This redistribution results in additional stabilization of remained helical (or formed denatured) regions, and the melting process is extended. Due to confinement of number of the binding sites on DNA, the further increasing of concentration of ligands leads to difficulties of redistribution process and the melting interval width again decreases. In the boundary case when all binding sites are occupied by ligands, the melting interval width increment tends to zero. In the observed case, it is assumed that each pair of bases in polymer may be a binding site for ligand [12].

It is followed from the above-mentioned case that maximum of bell-like curve of the melting interval width increment dependence on ligand concentration corresponds to concentration of the ligand on DNA equal to half of the binding sites. The treated theory was compared with the experiment of complex melting, where as a ligand acridine dyes and actinomycin [13], native (destabilizer) and denatured (stabilizer), RNAase, heavy metal ions were used.

The effect of ligands on the helix-coil transition in polynucleotide in the case of random number of the binding sites has been studied. The chosen model in [14] is the following. We will assume that in solution, there are polymer molecules with fixed values of N_1 (number of rings in coil-like state) and N_2 (number of helical regions), the total number of rings N remains constant:

$$N_1 + N_2 = N \quad (21)$$

Let add ligands into solution with polymer that can bind both with coil-like and with native regions of DNA and can be in solution in nonbound state as well. If K_2 and K_1 are numbers of ligands bound to helical and coil-like regions, respectively, and K_0 is the number of nonbound ligands, it is obvious that total number of ligands K per molecule satisfies the condition:

$$K_1 + K_2 + K_0 = K \quad (22)$$

Let us mark the number of pairs of bases per binding site for denatured and native parts of the molecules as r_1 and r_2 , respectively. In this case, the number of binding sites for the respective regions will be equal to N_1/r_1 and N_2/r_2 . Taking this fact into consideration for nonequilibrium free energy, we will have:

$$F = F_1 N_1 + F_2 N_2 + F_0 n + \psi_1 K_1 + \psi_2 K_2 + \psi_0 K_0 - TS_0 \\ + \ln W_1 \left(\frac{N_1}{r_1}, K_1 \right) + \ln W_2 \left(\frac{N_2}{r_2}, K_2 \right) + \ln W_0(N_0, K_0) \quad (23)$$

where Ψ_1 and Ψ_2 are free energies of ligand bond with coil-like and helical parts of polymer, Ψ_0 is the free energy, N_0 is the number of binding sites in solution for free, nonbound ligand to polymer, $S_0(N_1, N_2, n)$ is the entropic member bound to pure polynucleotide, $W(N, K)$ function is determined by:

$$W(N, K) = \frac{(N-1)!}{(K-1)!(N-K)!} \quad (24)$$

Taking into account the Eq. (24) and neglecting the unit (when $N \rightarrow \infty$ case is observed) for additional entropic member in (23) responsible for redistributing entropy of ligands, we will obtain:

$$S_{add.} = \frac{\frac{N_1}{r_1}! \frac{N_2}{r_2}! N_0!}{K_1! \left(\frac{N_1}{r_1} - K_1 \right)! \left(\frac{N_2}{r_2} - K_2 \right)! K_2! (N_0 - K_0)! K_0!} \quad (25)$$

It is obvious that the equation obtained from the condition $(\partial G / \partial n) = 0$ remains as it was in the absence of ligand. It means that the average length of helical region $v_2 = N_2/n$ at given denaturation degree does not change when the ligand is added. In its turn, it means that ligand does not change the boundary energy. On the other hand, the equation obtained from $\partial G / \partial N_1 = 0$ condition does not change:

$$\frac{1 - \frac{n}{N_2}}{1 - \frac{n}{N_1}} = \sigma \frac{(1 - c_1 r_1)^{1/r_1}}{(1 - c_2 r_2)^{1/r_2}} \quad (26)$$

where $c_1 = K_1/N_1$, $c_2 = K_2/N_2$ are concentrations of ligands for denatured and coil-like parts of polymer, respectively.

The developed theory gives dependencies of the experimentally observed transition parameters (the melting interval width ΔT and melting temperature T_m) on the binding parameters of a ligand with DNA (the binding constant K and the binding site r_q) and the concentration of ligands [15].

$$\delta \frac{1}{T_m} = \frac{1}{\Delta H} \ln \left[\frac{\prod_{j=l+1}^m (x_0 + p_j)^{1/r_j}}{\prod_{i=1}^l (x_0 + p_i)^{1/r_i}} x_0^{\left[\sum_{i=1}^l \frac{1}{r_i} - \sum_{j=l+1}^m \frac{1}{r_j} \right]} \right] \quad (27)$$

$$\delta \frac{\Delta T}{T_m^2} = \frac{1}{x_0 \Delta H} \left[\sum_{j=l+1}^m \frac{p_j}{r_j (x_0 + p_j)} - \sum_{i=1}^l \frac{p_i}{r_i (x_0 + p_i)} \right] \frac{\partial x}{\partial \theta} \Big|_{\theta = 1/2} \quad (28)$$

where

$$\delta \frac{1}{T_m} = \frac{1}{T_m} - \frac{1}{T_0} \quad \delta \frac{\Delta T}{T_m^2} = \frac{\Delta T}{T_m^2} - \frac{\Delta_0 T}{T_0^2} \quad (29)$$

where T_0 and $\Delta_0 T$ are the melting temperature and melting interval width for DNA in the absence of the ligand, T_m and ΔT are the same parameters for DNA-ligand complexes; ΔH is the enthalpy of the transition; $p_q = K_q/K_1$, where K_1 is the binding constant for the first type (arbitrary chosen) of interaction of the ligand with one of the DNA forms; K_q is the binding constant for the q -th binding type ($q = 2, \dots, m$), which is expressed by the following equation:

$$K_q = \frac{c_q \cdot r_q}{c_0(1 - c_q \cdot r_q)}, \quad (q = 1, \dots, m) \quad (30)$$

$c_i = \frac{k_i}{N_1}$ ($i = 1, \dots, l$), $c_j = \frac{k_j}{N_2}$ ($j = l+1, \dots, m$) are the concentrations of the ligand bound to the corresponding forms of DNA and $c_0 = k_0/N_0 < 1$ is the concentration of unbound ligand, where x_0 is the equation solution at $q = 0.5$ ($q = N_f/N$ ($f = 1, 2$) is the fraction of either forms of the polynucleotides (B, Z, A, coil, etc.) within the melting interval).

$$(1 - \theta) \sum_{i=1}^l \frac{p_i}{r_i(x + p_i)} + \theta \sum_{j=l+1}^m \frac{p_j}{r_j(x + p_j)} = c \quad (31)$$

$c = 2D/P$, where D is the total concentration of ligand in solution and P is that of bases of DNA.

2.2. Experiment

DNA is a one-dimensional aperiodic crystal [1]. Therefore, as it was mentioned above, the true phase transition in such molecules could not occur. The two phases formed during the transition will tend to be mixed as continuously decreasing parts of the system. Such conversion is known as cooperative phase transition, two thermodynamic parameters of which are characterized by temperature of transition T_0 and width of transition ΔT , on the contrary of real phase transition, which is realized at fixed temperature.

2.2.1. Helix-coil transition

All nucleotides in the native state of DNA are in helix form, which has much lower free energy, i.e., high stability, than any other states that DNA assumes to be at room temperature and other ordinary physiological conditions. In the nonbound state, the nucleotide chain to which the nucleotide base pairs are attached has freedom of motion. The bound or nonbound states may be classified in terms of "helix" and "coil" states, respectively, and the transition from one phase to another is called helix-coil transition or melting.

Unfolding of the double helix of DNA is produced as an effect of temperature (T), pH, ionic strength (μ), and denaturants [16, 17]. The process is accompanied by the transition of the bound state of bp to nonbound state, which propagates from more stable to less stable groups.

It has been found experimentally that the transition of DNA occurs in a very sharp manner (the transition is highly co-operative), which is characterized by two physical parameters: the melting temperature, T_0 and the width of transition, ΔT . The sharpness of the transition depends on the value of junction free energy, F_j . The true phase transition (transition of crystal structures) occurs only at the case of $F_j \rightarrow \infty$ (the junction energy is infinitely large). These parameters change for complexes of DNA with "low-weight" compounds (ligands). Integration of the structural, kinetic, and thermodynamic data of ligand-nucleic acids interaction is necessary to clearly understand the mechanisms of ligand-nucleic acid complex formation. Such investigations are much important to characterize the binding mode, sequence specificity, and understanding in detail designing new generation of drugs affecting the gene expression. Structural data obtained by X-ray crystallography and NMR for many drug-nucleic-acid complexes were successfully used for estimating ligands that attempt to correlate structure with binding affinity. It was established that upon binding, the ligands interact with substrate as a rigid compound, which is advantageous for revealing thermodynamic contribution from structural data [18]. Data of the structures of ligand-DNA complexes obtained by X-ray crystallography and NMR methods showed the more possible way to much ligand shape with the receptors of substrates and represent only one aspect of the complex formation. That is, the binding site will be occupied by ligand complemented it in terms of shape, charge, and other binding components [19], neglecting the energetic characteristics of binding process. So, the structural data alone cannot define the driving forces for binding and predicting the binding affinities. To understand the molecular mechanism and energetics of ligand-nucleic acid interaction, knowledge of thermodynamic parameters provide data elucidating the driving forces of complex formation process [20]. A complete thermodynamic profile for a system of interest requires determination of the free energy, enthalpy, and entropy.

The free energy ΔG is the key thermodynamics parameter, dictating the direction of biomolecular equilibria. If its sign is negative, the binding reaction or conformational transition will proceed spontaneously to an extent governed by the magnitude of ΔG . If its sign is positive, the magnitude of ΔG specifies the energy needed to drive the reaction to form product. The free energy is a balance between enthalpy and entropy. The enthalpy change reflects the amount of heat energy required for achievement a particular state, and the entropy measures how easily that energy might be distributed among various molecular energy levels. For binding reactions, negative enthalpy values are common (but not omnipresent), reflecting a tendency for the system to fall to lower energy levels by bond formation. Positive entropy values are common for binding reactions, reflecting a natural tendency for disruption of order. All binding reactions must overcome inescapable entropic penalties resulting from the loss of rotational and translational degrees of freedom.

The binding enthalpy (ΔH) can be detected using isothermal titration calorimetric (ITC) or differential scanning calorimetric (DSC) methods [18–21]. The methods have several advantages for measuring binding energetic parameters at the same time having distinct difficulties, the dominant of which is high concentration of nucleic acids that require large quantities of expensive products, and besides, the possible aggregation makes very difficult to explain the experimental results [21–23]. DSC and ITC are laborious and time-consuming methods that often relegate calorimetric ones to be used as a secondary screening method. To overcome these limitations, several attempts have been made to improve the throughput of calorimetric

and thermodynamic measurements. Mentioned difficulties for detecting the thermodynamic parameters of ligand-nucleic acid interaction may be overcome by applying methods, which are experimentally easy to perform, where very low concentrations of nucleic acids are used, which exclude the very unwanted process of aggregation [24].

The quantitative analyses of the effect of different substances (ligands) such as ions, antibiotics, dyes, proteins, etc. made it possible to suggest a simple method named "tie calorimetry" to estimate ΔH of conformational transitions [25–28]. It has been shown that the enthalpy of helix-coil transition or melting (per base pair) could be determined from the experiments on DNA melting with ligands by the following general formula (32) (This formula is valid for all known types of conformational transition in one-dimensional crystals and for all types of ligands):

$$\Delta H = R \cdot \lim_{c \rightarrow 0} \left\{ (\delta \Delta T / \delta T_m)^2 T_0^2 \right\} \cdot c, \quad (32)$$

where $\delta T_m = T_m - T_0$ and $\delta \Delta T = \Delta T - \Delta_0 T$, T_m and ΔT are the melting temperature and width of transition for DNA when the ligand is added to the solution, T_0 and $\Delta_0 T$ are the same quantities for DNA without ligand, $c = 2D/P$ is the total number of ligand molecules in solution (D) divided by the total number of DNA base pairs ($2P$), and R is the gas constant. Eq. (32) is absolutely general, and its validity does not depend on the values of the thermodynamics parameters of complexes such as binding constants of ligand with DNA, the number of binding sites on the biopolymer, etc. This was covered comprehensively in [25–28]. On the other hand, obtained data showed that if the ligand complexes preferably bind with one of the conformations of DNA, the calculations become very simple [21–23], and for calculations, it is enough to compare the theoretical formula with the experiment either for δT_m or $\delta \Delta T$ [29]. Ethidium bromide, a very well-known ligand, binds preferably with the helix DNA [26, 27]. This enabled us at very low ligand concentration ($c \rightarrow 0$) with the combination of the area method [28, 29] to estimate ΔH for DNAs of two different GC contents at different Na^+ concentrations with very high accuracy.

It was shown that at very small concentration of the ligand, the shift of the melting temperature (δT_m) and widening of the melting curve ($\delta \Delta T$) are represented by the following equations:

$$\delta T_m = \Lambda \frac{RT_0^2 c}{\Delta H} \quad (33)$$

$$\delta \Delta T = \Lambda^2 \frac{RT_0^2 c}{\Delta H} \quad (34)$$

For the coefficient Λ , the following formula is obtained:

$$\Lambda = 2 \frac{(r_1/r_2)p - 1}{(r_1/r_2)p + 1} \cdot \frac{(K_1/r_1)P + (K_2/r_2)P}{4 + (K_1/r_1)P + (K_2/r_2)P} \quad (35)$$

where r_2 and r_1 , are the number of binding sites on the duplex and single-stranded DNA, respectively, K_2 and K_1 are the binding constants of ligand with helix and coil states of DNA, respectively, and P is the concentration of phosphate groups of DNA: $p = K_2/K_1$.

The only condition for validity of Eqs. (33) and (34) is $c \rightarrow 0$.

From Eq. (35), it follows that if

$$(K_1/r_1)P \ll 4 \quad (K_2/r_2)P \gg 4 \quad (36)$$

$$\delta T_m = 2 \frac{RT_0^2 c}{\Delta H} \quad (37)$$

$$\delta \Delta T = 4 \frac{RT_0^2 c}{\Delta H}. \quad (38)$$

These formulas show that if $\delta \Delta T$ is twice greater than δT_m , the binding constant of ligand with one of the conformation of DNA is much greater than that of the other conformation. In this case, ΔH may be estimated on by Eqs. (34) or (35). The accuracy of ΔH value depends on the accuracy of experimental estimations of the $\delta \Delta T$ and δT_m values at different concentrations of ligand (different c). Therefore, the error is large (1.5–2 kcal/mol) when ΔH is calculated by Eq. (32) [28].

The accuracy of ΔH estimation is much higher if the "area" method is used for obtaining δT_m . The method may be explained as following. The DNA melting temperature T_0 may be defined as the first moment of the differential melting curve ($-d\vartheta/dT$):

$$T_0 = \int_{T_{AT}}^{T_{GC}} \left(-\frac{d\vartheta}{dT} \right) T dT \quad (39)$$

After integration, we have the following expression:

$$T_0 = T_{GC} - \int_{T_{AT}}^{T_{GC}} (1 - \vartheta) dT \quad (40)$$

Here,

$$s = \int_{T_{AT}}^{T_{GC}} (1 - \vartheta) dT \quad (41)$$

is numerically equal to the square limited by the melting curve ($1-\vartheta$), the temperature axis, and the $T = T_{GC}$ vertical line. It follows from Eq. (39) that T_0 varies if the shape and place of melting curve change. Both the shape and the place of the melting curve change if ligand is added to the DNA solution. In this case, melting temperature of the complex T_m is found as:

$$T_m = T_{GC} - \int_{T_{AT}}^{T_{GC}} (1 - \vartheta^*) dT, \quad (42)$$

where T_m is the temperature and $(1-\vartheta^*)$ is the melting curve of DNA-ligand complex.

It follows from Eqs. (39) and (40) that the variation in temperature can be expressed as:

$$\delta T = \delta s = \int_{T_{AT}}^{T_{GC}} (1 - \vartheta) dT - \int_{T_{AT}}^{T_{GC}} (1 - \vartheta^*) dT, \quad (43)$$

where δs is the area limited by melting curves of DNA (left curve) and DNA-ligand complex (right curve) (**Figure 1**).

Substituting Eq. (43) to Eq. (36) for the enthalpy of helix-coil transition, one gets:

$$\Delta H = 2 \frac{RT_0^2 c}{\delta s} \quad (44)$$

It should be noted that measuring of δs should be done at very small concentrations of ligand ($c < 3 \cdot 10^{-2}$) [23], where ΔH is independent of the chosen concentrations of ligand. The dependence of ΔH (in kcal/mol) on Na^+ is shown in **Figure 2**.

The values of ΔH obtained here agree excellently with calorimetric data [30]. The ΔS value may be calculated taking into account that at the transition mid-point (T_0), the free energies of the phases (for example, helix and coil) are equal to each other. Therefore,

$$\Delta G = \Delta H - T_0 \Delta S = 0. \quad (45)$$

Knowing the value of transition point T_0 , one can calculate the value of ΔS by equation

$$\Delta S = \frac{\Delta H}{T_0}. \quad (46)$$

The averaged values of ΔH , T_0 , ΔS at different concentrations of Na^+ are presented in **Table 1**.

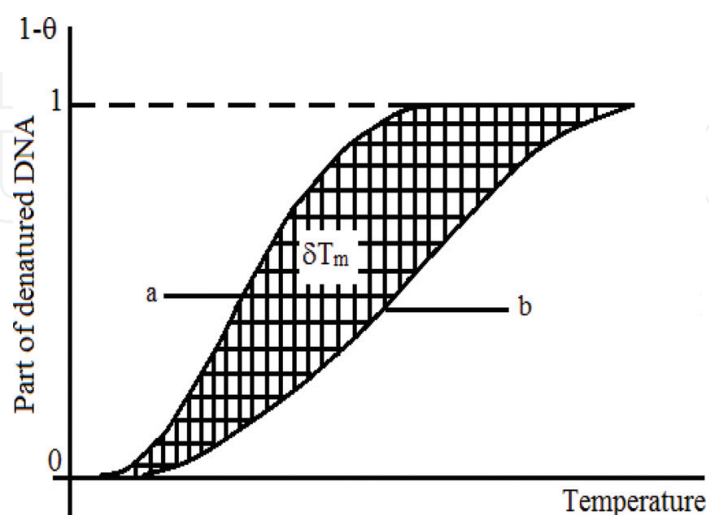


Figure 1. The area limited by melting curves (δs) of DNA (left curve) and DNA-ligand complex (right curve) is numerically equal to the shift of the melting temperature (δT_m).

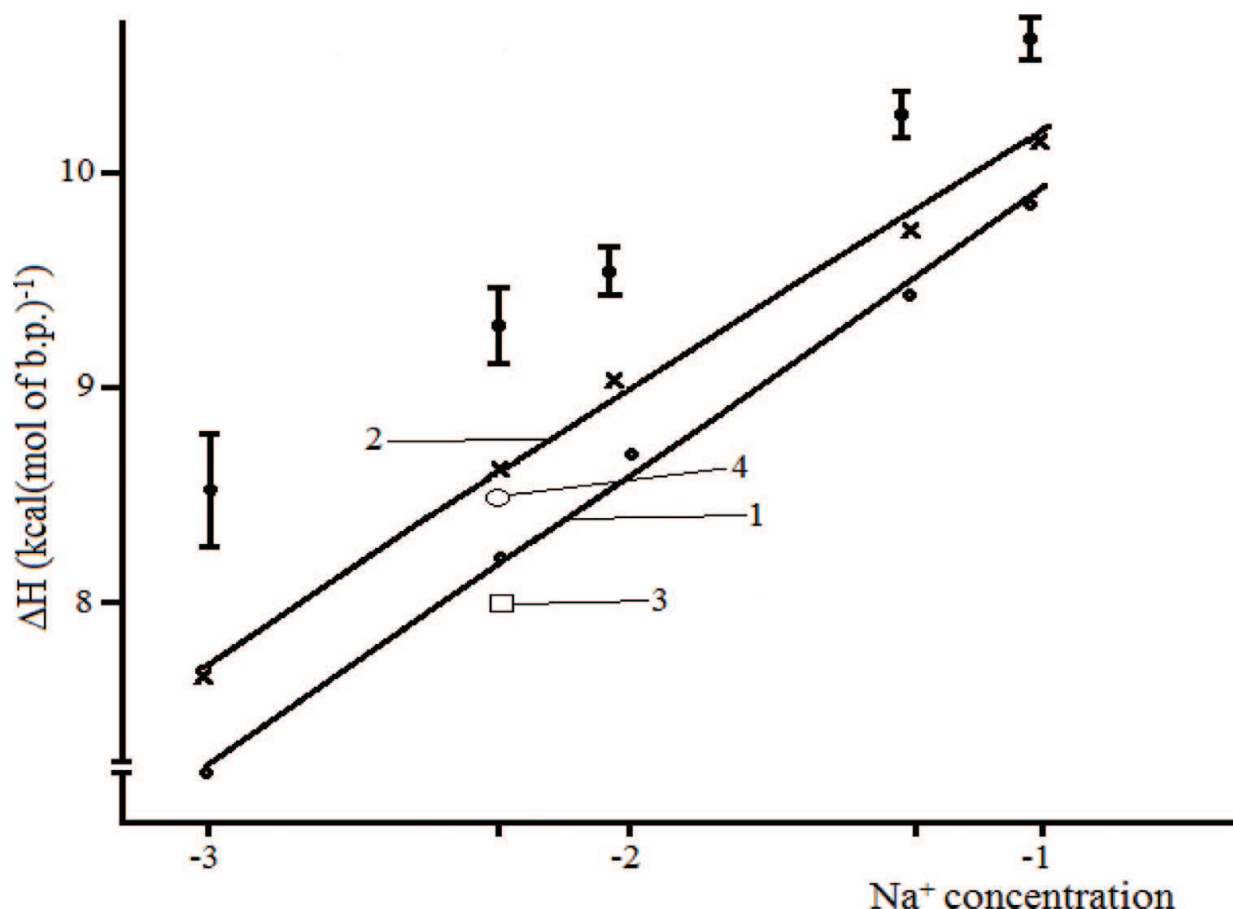


Figure 2. Dependence of ΔH on concentration of Na^+ . 1,3 Cl.Perfringens DNA; 2,4 M. lysodeikticus DNA. 1 and 2 are our data. 3 and 4 data are of Klump and Ackermann [30]. Error bars are shown separately above the experimental points.

	$\text{Na}^+ \text{ M}$	$-\lg \text{Na}^+$	$T_0 \text{ } ^\circ \text{C}$	Cl.perfr. $\Delta H \text{ kcal/mol}$	$\Delta S \text{ ent. unit}$	$T_0 \text{ } ^\circ \text{C}$	M. Lysod. $\Delta H \text{ kcal/mol}$	$\Delta S \text{ ent. unit}$
1	10^{-3}	3.0	45.1	7.2 ± 0.5	22.6	68.4	7.7 ± 0.4	22.5
5	10^{-3}	2.3	56.3	8.2 ± 0.3	24.9	77.6	8.6 ± 0.3	24.5
1	10^{-2}	2.0	61.0	8.7 ± 0.2	26.0	81.8	9.0 ± 0.2	25.4
5	10^{-1}	1.0	77.3	9.8 ± 0.2	28.0	94.9	10.1 ± 0.2	27.4

Table 1. The averaged values of ΔH , T_0 , ΔS at different concentrations of Na^+ .

2.2.2. B-A transition

Investigations show that B-A conformational transition is cooperative, and it is realized in big amount of nucleotides conversion from B-form to A-form. Since the transition is independent of temperature and GC content of biopolymer, the Ising model has been employed for theoretical description of the process. The B-A transition initiated by any external factor a is represented by:

$$\frac{1 - 2\vartheta}{\sqrt{(1 - \vartheta)\vartheta}} = \frac{1 - s}{\sqrt{s}\sqrt{\sigma_{AB}}} \quad (47)$$

where $\vartheta=f(a)$ is the dependence of part of A-form on $-a$ factor, s -form is the equilibrium constant of transition, $\sigma = (\exp - \varepsilon_0 T)$, ε_0 is the energy of junction, and T is the absolute temperature. Eq. (47) gives

$$\Delta a = \left| \frac{\partial \vartheta}{\partial a} \right|_{a=a_0}^{-1} = \frac{4Q}{v_0} \quad (48)$$

where $v_0 = (\sqrt{\sigma_{AB}})^{-1}$ is the length of cooperativity and Q is a constant showing the steepness of free energy ΔG of A- and B-forms at the transition region. Knowing Q and Δa , the ΔG of A-B transition can be determined. It was shown that for pure water (100%) environment, $\Delta G_{AB} = 1 \text{ kcal/mol}$ [31, 32]. The obtained data coincide with the experimental results of [33], where the junctions of A- and B-forms are considered as a tie, which stabilizes the duplex.

2.2.3. B-Z transition

Poly[d(G-C)] in a 55% ethanol solution exhibits the B-Z transition when the temperature increased [15, 33]. A polyamine, AEPDA, stabilizes Z-form and binds to it much stronger than to the B-form (**Figure 3**). Results show the temperature effect on the B-Z equilibrium without the polyamine (**Figure 3a**) and in its presence at a concentration of one molecule per 50 base pairs (**Figure 3b**). Obviously, the B-form of the polymer is stabilized by the rise of temperature in both cases. The pattern of CD spectra and the presence of a distinct isodichroic point at 301 nm show that only B- and Z-forms are involved in the equilibrium.

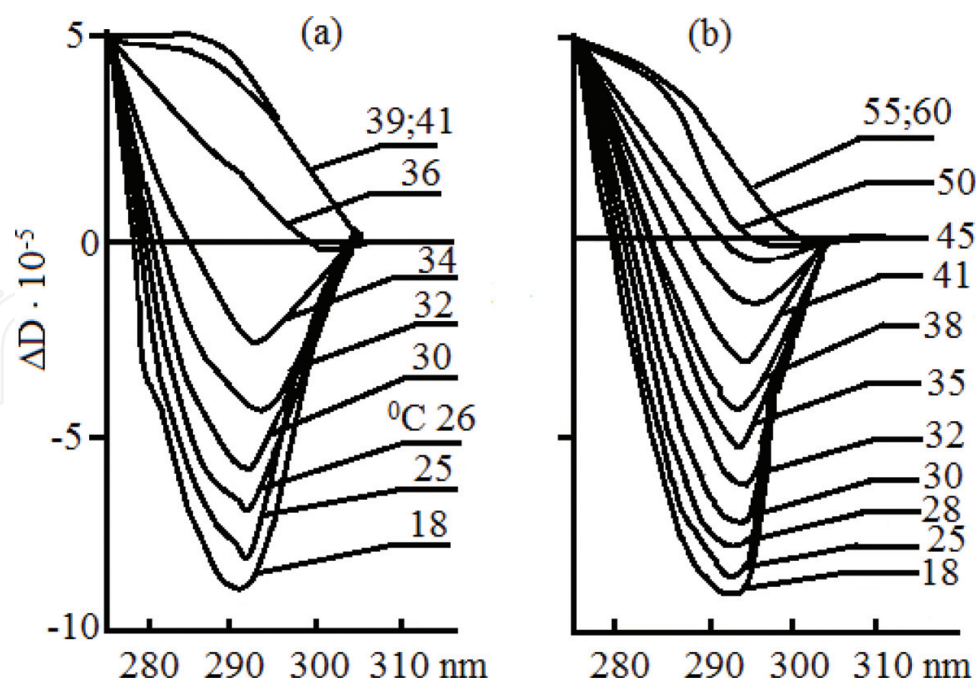


Figure 3. A family of equilibrium circular dichroism (CD) spectra of poly[d(G-C)] at different temperatures in the absence (a) and presence (b) of polyamine (1 molecule per 50 base pairs). Conditions: ethanol: 55% v/v, NaCl: $5 \cdot 10^{-4}$ M, and EDTA: $5 \cdot 10^{-5}$ M.

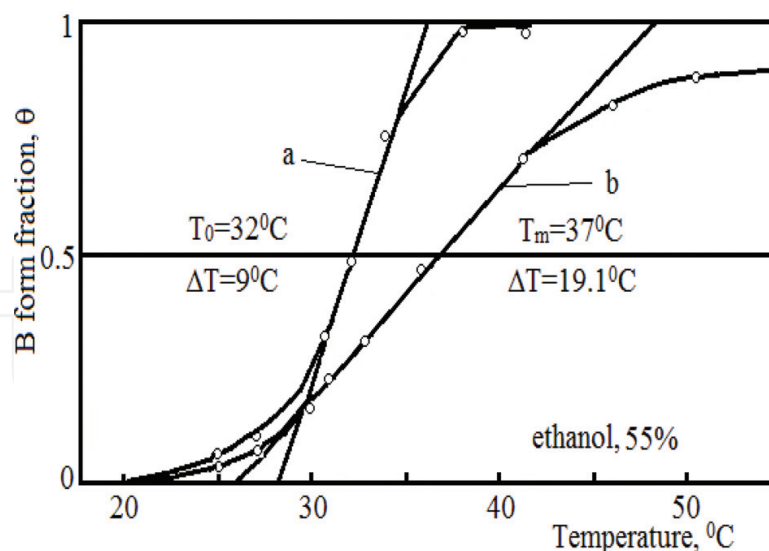


Figure 4. Profiles of the Z-B transition of free poly[d(G-C)] and its complex with polyamine (1 molecule per 50 base pairs). θ is for the B-form fraction from the CD data of **Figure 3**.

The transition profiles (**Figure 4**) show that, when the polyamine is added to the Z-form of poly[d(G-C)], the Z-form is stabilized and also the transition interval ΔT significantly widens. So, the polyamine is a "tie" for the Z-form. In this case, the transition enthalpy can be calculated by measuring the shift of the transition point (δT_0) and the widening of the transition curve $\delta \Delta T$ (see the Eqs. (36)–(38)).

Experiments showed that the ratio $\delta \Delta T / \delta T = 2$, which is independent on ionic strength [15, 34–36]. Thus, the ΔH value for the poly[d(G-C)] in 55% ethanol, $\Delta H_{BZ} = -1.4$ kcal/mol, is independent on the ionic strength.

Eqs. (37) and (38) are restricted to the only condition of $c \rightarrow 0$. $K_Z \gg 1$, $K_B \gg 1$, and at this case, we obtain Eqs. (6) and (7), which show that if the widening of the transition curve is twice as great as the shift of the transition point, ΔH_{BZ} can be determined independently by each equation.

Figure 5 shows that for the polyamine, the ratio $\delta \Delta T / \delta T_0 = 2$; therefore, this ligand is perfect for thermodynamic investigations of a B-Z transition. **Table 2** presents the data on the polyamine action calculated within the range of ionic strength from 0.5–2 mM NaCl. These data show that δT_0 and $\delta \Delta T$ values do not depend on NaCl concentration. Consequently, ΔH_{BZ} is independent of ionic strength under these conditions. **Figure 5** shows the $\delta \Delta T$ and δT_0 of a B-Z transition plotted as a function of $c = 2D/P$.

Since the slopes of the lines are related as 2:1, the enthalpy of the transition may be obtained using Eqs. (37) or (38):

$$\Delta H_{BZ} = -2 \frac{RT_0^2}{\delta T_0} \cdot c = -(1.4 \pm 0.2) \text{ kcal/mol}$$

$$\Delta H_{BZ} = -4 \frac{RT_0^2}{\delta \Delta T} \cdot c = -(1.4 \pm 0.2) \text{ kcal/mol}$$
(49)

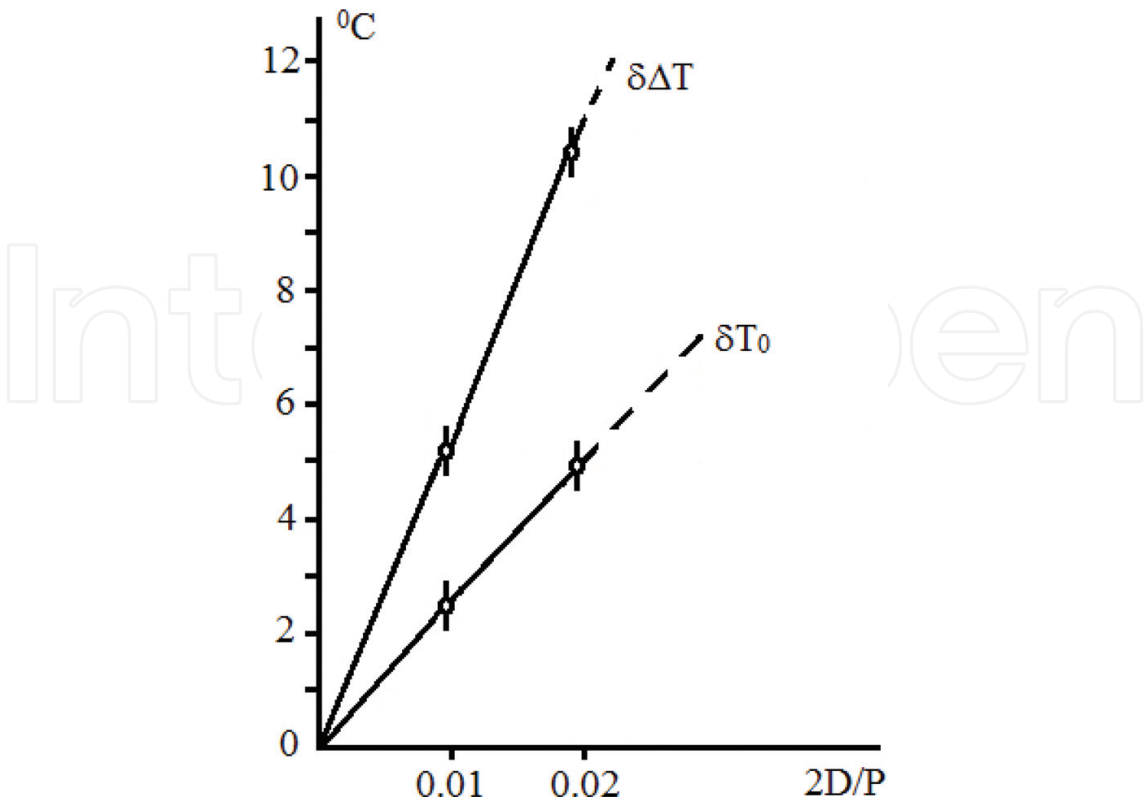


Figure 5. Dependents of widening $\delta\Delta T$ and shift δT_0 of the B-Z transition on “tie” centration.

$c = 2D/P$	$c = 0.01$		$c = 0.02$	
NaCl, M	δT_0	$\delta\Delta T$	δT_0	$\delta\Delta T$
5×10^{-4}	3.0	4.6	5.6	11.6
	2.7	5.2		
	2.0	4.6	5.0	10.1
	2.6	4.8	5.3	10.8
10^{-3}	2.1	5.0		
	2.6	4.4		
	2.0	6.0		
	2.2	5.1		
1.4×10^{-3}	2.7	6.6		
2×10^{-3}	3.0	5.6		
	3.0	5.0		
	3.0	5.3		

Table 2. Changes in the parameters of the Z to B transition induced by a temperature increase in the presence of the polyamine (AEPDA) at two $c = 2D/P$ and different ionic strengths.

We consider the values of ΔH_{BZ} obtained in this work reliable, which is in good agreement with the recently obtained same value of ΔH_{BZ} using an independent method based on the poly-electrolyte theory of the B-Z transition [37].

2.2.4. Thermodynamic parameters of binding: binding constants (K and binding site size r , the number of DNA base pairs corresponding to a binding site)

A traditional method of obtaining the interaction thermodynamic parameters is the Scatchard's analysis of the ligands binding data, which consists of plotting the r/c_f value versus r , where r is the ratio of the bound ligand to DNA base pair concentration and c_f is the free ligand concentration [37]. This method has two major drawbacks. The first is the uncertainty in the c_f value [37, 38], and the second is the existence of two different models of interaction of the ligands with DNA in the case of nonlinear Scatchard plots. One model assumes the presence of more than one type of independent binding sites, and the other model suggests interaction between bound ligands [34].

Our theory suggests another method for obtaining the binding parameters of the ligands interacting with DNA [15].

The binding parameters (K and r) are the parameters of the theory. They can be evaluated from comparison of the theory with experiment. The shape of the curves of dependencies of the inverse melting temperature $\delta(1/T_m)$ and of the melting range width $\delta(\Delta T/T_m^2)$ on the concentration of ligands is very different and sensitive to different values of r : the binding site size and $p_q = K_q/K_1$, where K_1 is the binding constant for the first type (arbitrarily chosen) of interaction of the ligand with one of DNA forms, K_q is the binding constant for the q -th binding type ($q = 2, \dots, m$, m types of bending are considered). **Figure 6** shows that the effect of the value of p_q is very significant on the shape of the dependence of $\delta(\Delta T/T_m^2)$ on c . Therefore, the parameters may be determined, so as to provide the best fit between the theory and experiment. Major criteria for fitting are the position, the shape, and the size of maximum of the experimentally obtained $\delta(\Delta T/T_m^2)$ on c or $\delta(1/T_m)$.

We applied the conjugated gradient method for the theoretical analysis of the obtained experimental data of helix-coil transition of the complexes EtBr and AMD with DNA. The binding parameters were determined to provide the best fit between the calculated dependence of $\delta(\Delta T/T_m^2)$ on c and the observed one (**Figure 7**). The analysis of the obtained data shows that EtBr and AMD may form at least five types of complexes of which three types with helix DNA and two types with coiled DNA at 10^{-2} M Na^+ (**Table 3**). Another theoretical parameter is the ratio of the binding constants $p_q = K_q/K_t$ ($q = 2, \dots, m$). K_q values are readily calculated if one of the binding constant is known.

The calculated values of K_q for EtBr and AMD are presented in **Table 3**. The values of n and K_q obtained agree with the values determined from independent experiments [15].

Index "s" corresponds to the "strong" binding mode and "w" to the "weak" binding mode.

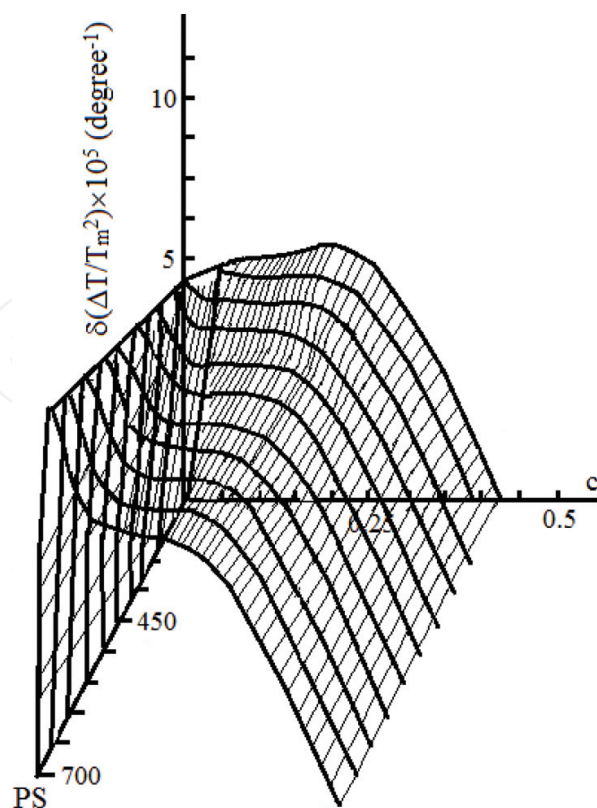


Figure 6. Dependence of $\delta(\Delta T/T_m)$ on the concentration of the ligand (c) and p_q [39]. Parameters of the theory are: $m = 5$; $l = 3$; $r_1 = 3$; $r_2 = 10$; $r_3 = 5$; $r_4 = 6.75$; $r_5 = 12$; $p_1 = 1$; $p_2 = 4.2$; $p_3 = 5$; $p_4 = 5.5$; $p_5 = 15$; $p_6 = 120$.

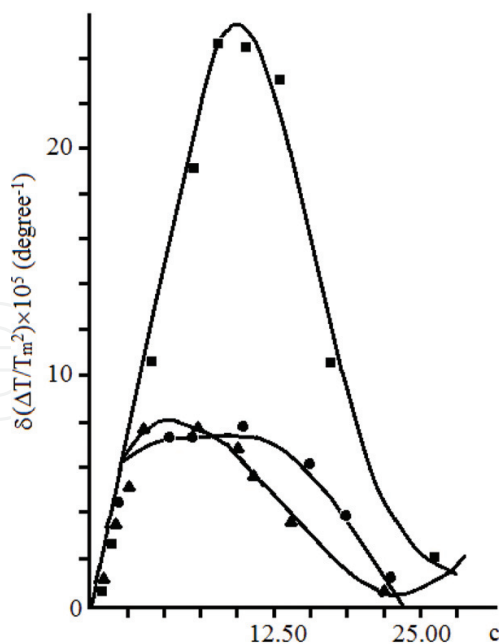


Figure 7. Dependence of $\delta(\Delta T/T_m^2)$ on c [39]. Parameters of the theory are: (■) EtBr at 2.2×10^{-3} M Na⁺ $m = 7$; $l = 4$; $\Delta H = 7.6$ kcal/mol and $r_1 = 3$; $r_2 = 10$; $r_3 = 2$; $r_4 = 6.75$; $r_5 = 12$; $p_1 = 1$; $p_2 = 1.2$; $p_3 = 0.01$; $p_4 = 10$; $p_5 = 15$; $p_6 = 120$; $p_7 = 0.02$. (▲) AMD at 2.2×10^{-3} M Na⁺ $m = 6$; $l = 4$; $\Delta H = 7.6$ kcal/mol and $r_1 = 3.0$; $r_2 = 6.0$; $r_3 = 6$; $r_4 = 7$; $r_5 = 12$; $r_6 = 4$; $p_1 = 1$; $p_2 = 1.5$; $p_3 = 4.2$; $p_4 = 5.5$; $p_5 = 160$; $p_6 = 0.1$. (●) EtBr at 2.2×10^{-2} M Na⁺ $m = 5$; $l = 3$; $\Delta H = 8.5$ kcal/mol and $r_1 = 3$; $r_2 = 10$; $r_3 = 5$; $r_4 = 6.75$; $r_5 = 12$; $p_1 = 1$; $p_2 = 5$; $p_3 = 4.2$; $p_4 = 5.5$; $p_5 = 200$. Points stand for experimental values.

Experimental conditions	Ligand	Binding site size n					Binding constant $K_q \times 10^{-3}$				
		Helix		Coil			Helix		Coil		
		n_s	n_{w1}	n_{w2}	n_s	n_{w1}	K_s	K_{w1}	K_{w2}	K_s	K_{w1}
10^{-2} M Na ⁺	EtBr and AMD	6	3.3	2.5	5	1.5	2.7	0.07	0.06	0.07	0.013
2.2×10^{-3} M Na ⁺	EtBr	6	3.3	2.5	5	1.5	5.0	0.6	0.4	0.05	0.04

Table 3. Binding parameters of EtBr and AMD with helix and coil DNA as estimated by the theory-experiment comparison by conjugated gradient method.

3. Conclusion

In this work, it has been shown that "tie calorimetry" possesses a number of advantages. The measurements can be carried out in such concentrations that the intermolecular interactions and denaturation effect on medium pH are neglected. From the above mentioned, it is concluded that the helix-coil transition enthalpy can be calculated by the "tie calorimetry" according to the formula (32) and the only condition is that $c \ll 1$. On the other hand, determining the value of ΔH , the respective value of ΔS may be calculated by the formula (18). Calorimetry is a direct technique especially suitable when ΔH_{BZ} is high. By contrast, the "tie" calorimetry is most suitable at low ΔH_{BZ} values. It follows from Eqs. (33), (34) or (36), (37) that the low ΔH_{BZ} value results in a great change in the position of the transition point or in the transition width. Therefore, these methods are complementary.

Besides, it may be said that the "tie" calorimetric method is simple and very easy to be performed. It is absolute and no graduation is required for it. The method is based on the measuring of differential experimental values, which excluded systematic errors. Therefore, combination of both "area" and "tie" calorimetric methods makes it possible to establish the energetic parameters of phase transitions with very high accuracy.

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