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Review

Thermal analysis of ligand-DNA interaction: determination of binding parameters

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Abstract: The review discusses the methods of thermodynamic analysis of reactions of non-covalent binding of biologically active compounds with DNA, which is a key constituent of cell chromatin. Knowledge of thermodynamic profile of ligand binding with nucleic acids is important for understanding the mechanism of medico-biological action of the currently existing drugs and for designing of new drugs with improved medical effect. Thermodynamic analysis of ligand binding with DNA is based on analysis of experimentally measured changes of Gibbs free energy (ΔG), enthalpy (ΔH), entropy (ΔS) and heat capacity (ΔC_p). Right selection of the methods of measurement of these parameters and understanding of limitations of currently existing approaches for numerical data analysis are crucially important for correct interpretation of the obtained results and getting insight into the mechanism of drug-DNA interaction. In the present work the currently existing methods of determination of thermodynamic parameters of ligand binding with DNA were divided into two main groups. The first group is associated with the approaches used in cases when the heating of the system does not cause melting of the biopolymer. The main focus is given to the second group of the methods which are based on description of helix-coil transition of DNA-ligand complexes with further comparison of the same transition for «free» biopolymer. The methods of computation of the binding parameters utilizing the Ising-type models and models based on the equations of chemical equilibrium are discussed.

Keywords: DNA; ligand; melting thermodynamics; binding parameters; calorimetry; UV-spectroscopy

1. Introduction

DNA is the main part of the chromatin and it carries the very important functions of storage, transfer and implementation of genetic information. Due to significant role of chromatin and DNA in living organisms the development of methods for targeting them with an aim to achieve the desired medical and biological effect is of great importance. One of the most effective ways to implement such effect is the modification of the genetic material by biologically active compounds, particularly, antibiotics and mutagens, which promote disruption and suppression of vital processes, viz. replication, transcription and translation [1–4].

Both, the protein part of chromatin (histones) [5–7] and DNA [8–11], can be the target for small molecules (ligands). However, numerous studies have shown that the interaction of anticancer drugs occurs mostly with the DNA molecule, and the primary role of histones is to reduce the DNA regions, which are available for ligand binding [10–14]. Thus, the DNA-ligand system serves as a good model to study the action of biologically active compounds on chromatin.

Since the therapeutic effect of many DNA-binding drugs correlates with thermodynamic parameters of complex formation [15–23], one of the important tasks of modern molecular biophysics is to elucidate the thermodynamics of such interaction. New data about the thermodynamics of molecular interactions of ligands with DNA are necessary for understanding the mechanism of medical and biological action of existing drugs and for creation of new more effective ones.

This review focuses on critical analysis of modern theoretical approaches for determination of the thermodynamic parameters of DNA-ligand binding.

2. General Approach to Determination of Thermodynamic Parameters of DNA-ligand Binding

The first stage of the thermodynamic analysis of DNA-ligands interaction is the determination of the equilibrium binding constants K_{bind} (Gibbs free energy (ΔG_{bind})). The general approach to obtain K_{bind} is measuring of the dependence of a certain experimentally observed parameter ξ_{exp} (e.g., absorption in spectroscopy, chemical shift in NMR, etc.) on the concentration of solution components [24–33]. To analyze the experimental titration curve the binding models are used [34–39]. Such models provide theoretical functional relationship of ξ_{exp} with quantitative characteristics of dynamic equilibrium in solution. These characteristics are the DNA and ligand concentrations, and the parameters of their interaction, viz. the binding constant and some other characteristics, which depend on the model selected (e.g., the binding site size n, degree of cooperativity, etc.).

Knowledge of enthalpy (ΔH_{bind}) and entropy (ΔS_{bind}) contributions to the free energy of binding is also very important for understanding the driving forces of complex formation. The heat capacity change upon binding, $\Delta C_{p,bind}$, allows one to get insight into the mechanism of complex formation.

All these parameters can be determined by isothermal titration calorimetry (ITC) from direct measurements of thermal effect of DNA-ligand binding [40–46]. To analyze the calorimetric titration curve one of the following binding models is usually used: 1) the ligand binds with one-type site; 2) the ligand binds with two-type sites independently; 3) the ligand saturates three sites sequentially [46].The values of K_{bind}, n and ΔH_{bind} are determined by comparison of theoretical and experimental dependences of thermal effect on the DNA and ligand concentrations. The $\Delta C_{p,bind}$ value is calculated from experiments carried out at different temperatures [47–53]. Although ITC allows obtaining the full set of binding parameters, it is not applicable to DNA-ligand systems characterized by very high or low

binding constants. In such cases the appropriate titration curve cannot be obtained because the heat of reaction commensurate with the noise of calorimeter [46].

To determine the enthalpy and entropy of the ligand binding with DNA an indirect method can be also used. Such method is based on van't Hoff equation, which sets relationship between the equilibrium binding constant, K_{bind}, and temperature [43,54,55]:

$$lnK_{bind} = -\frac{\Delta H_{bind}}{RT} + \frac{\Delta S_{bind}}{R}. \quad (1)$$

The input data for analysis are the values of equilibrium constants, $K_{bind}(T)$, is commonly obtained from titration experiments at different temperatures [56–58]. An approximation of the temperature dependence of logarithm of the binding constant allows to determine coefficients of linear regression and to calculate the binding enthalpy and entropy.

It should be noted that this method can be used only if the enthalpy and entropy dependences on temperature are neglected. This assumption is valid only for narrow temperature range and/or in case of negligible heat capacity change of the binding reaction. However, the binding of small molecules with DNA may be characterized by a significant change of the $\Delta C_{p,bind}$ value [59,60], and the dependence of lnK_{bind} on $1/T$ is not linear [54,55].

Also the DNA-ligand binding enthalpy can be estimated by van't Hoff formalism directly from the temperature changes of the experimental value, ξ_{exp}, when the concentrations of the interacting species are known. In this case, the calculation of the binding constant is not required. The value of equilibrium constant is replaced with the temperature dependence of K_{bind} according to equation (1). Hence, the function $\xi_{exp}(T)$ contains two unknown parameters, viz. ΔH_{bind} and ΔS_{bind}, which can be determined by minimization of the deviations of the calculated from experimental values of ξ. This method was successfully applied to determine the enthalpy and entropy changes of ligand binding to short DNA oligonucleotide sequences by means of NMR spectroscopy [61,62].

It is worth noting that the strong exponential relationship between K_{bind} and the search parameters, ΔH_{bind} and ΔS_{bind} (equation 1), causes significant errors in their determination. Another source of large error in the values of binding enthalpy and entropy in equation (1) is the fact that ξ_{exp} experiences little changes in the temperature range up to the complex melting. By these reasons this method is rarely used in thermodynamic analysis of the DNA-ligand binding reactions.

An alternative approach for calculation of the thermodynamic parameters of DNA-ligand binding is the analysis of ξ_{exp} changes in the temperature range where a helix-coil transition (or melting) of DNA occurs. During the melting the DNA double helix dissociates into individual strands which is accompanied by breakage of hydrogen bonds between complementary base pairs, distortion of interplanar stacking interactions and changings of ion-hydration environment [63]. Therefore, significant alteration of some physical properties of DNA and its complexes, and, consequently, of experimental ξ_{exp} values, is observed [64,65]. By that reason analysis of experimental data measured in the melting range allows determining the values of the fundamental thermodynamic parameters of DNA-ligand systems more accurately. Let us consider these approaches in more detail.

3. Methods of Determination of the Binding Parameters Based on Analysis of DNA-ligand Melting

The basic experimental methods extensively used now for the study of the melting of DNA-ligand complexes and for determination of the thermodynamic parameters are UV-spectroscopy and differential scanning calorimetry (DSC).

At helix-coil transition an increase of the absorption of the DNA molecule by 30–40% in the ultraviolet region of the spectrum (λ = 250–270 nm) is observed [66,67]. This allows determining the melting temperature and melting range of DNA with high degree of accuracy. Back in the 60-ies of the last century it was shown that the addition of the ligand leads to significant changes in these parameters [68–71]. To determine the binding constants and binding types, analysis of the melting temperature shift and the change of melting range width on DNA-ligand interaction are commonly used [72–75].

Another method of studying the helix-coil transition is a differential scanning calorimetry. This method allows to determine directly the thermal effects, which accompany the conformational transitions of biological macromolecules and their complexes [76–81].

However, in order to calculate thermodynamic parameters of DNA-ligand interaction from experimental data of complex melting the modeling approaches describing the helix-coil transition in these systems are needed.

Majority of the existing approaches the melting of DNA-ligand complexes is considered as two independent equilibrium processes, viz. ligand dissociation from the double-stranded DNA and the denaturation of the biopolymer (fig. 1).

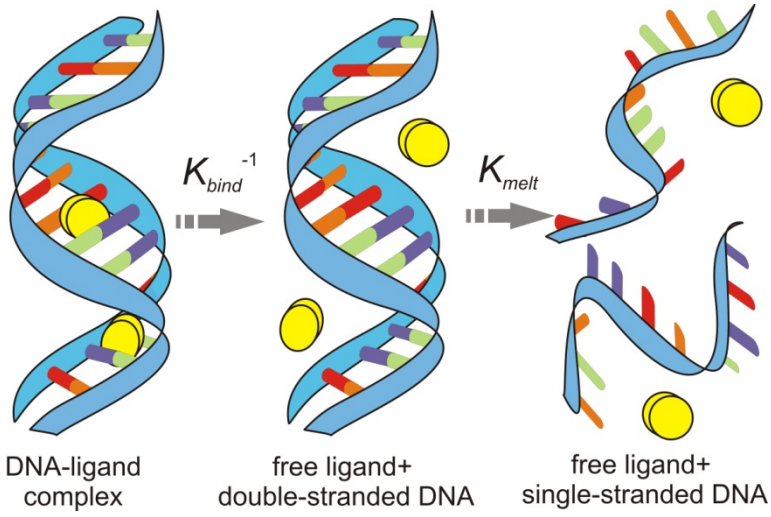

Figure 1. Schematic representation of the melting of DNA-ligand complex.

These approaches differ in DNA site size for which the melting and binding equations are written. Thus, in the Ising-like models the melting is described for each base pair. Alternative approach is based on equations of chemical equilibrium and considers the melting of extended DNA region. Both approaches are discussed in detail below.

3.1. Description of the melting of DNA-ligand complexes using Ising-like models

In early theoretical models of the melting of DNA-ligand complexes the DNA molecule was represented as an infinitely long homogeneous polynucleotide, wherein each unit may be in open or closed state. The energy required for base pair opening is determined only by the state of neighboring pairs. Since such description appears to be similar to the Ising model of ferromagnetism [82], these phenomenological approaches applied in various forms for DNA melting investigation [63,83–88], were called the Ising-like models. They include, for example, the model of "zipper", in which the helical and the coil regions of DNA may exist in the chain simultaneously during the helix-coil transition, but only one helical region is formed in each chain [89]. In the matrix method of the Ising model it is assumed that the coil and the helical regions may be located anywhere in the chain and in any combination. Their sizes are determined by the cooperativity factor σ. Each unit in the chain is associated with a matrix of statistical weights of states. The most simple and straightforward polymer chain melting theory based on Ising model was developed by Zimm and Bragg [90].

Ligand molecules interact with helical and coil regions of the polymer in solution altering their free energy. To describe the process of DNA-ligand melting the series is written in which each term corresponds to a single act of one base pair opening and the single act of ligand binding to a base pair. Most complete theoretical analysis of the DNA-ligand melting using Ising-like models was given by McGhee, Frank-Kamenetskii and Crothers [68,69,71,91,92].

The difference between these approaches is displayed only when the discrete approach for mathematical description of the system is replaced with a continuous. Thus, Frank-Kamenetskii used the most probable distribution method [68,69,71], Crothers used direct thermodynamic approach [91], McGhee used the method of generating functions [92]. These methods are physically equivalent, but utilize different mathematical tools. The obtained equations are written in general and most strict form, allowing to calculate theoretical melting curve for DNA-ligand complexes at any concentration of the ligand using as an input the experimental melting curve of free DNA.

Theoretical description of DNA-ligand melting by the Ising-like models allows taking into account structural variety of the lattice and the whole set of complex interactions in the system. For example, within the framework of Frank-Kamenetskii's approach melting of DNA with arbitrary primary structure on non-specific ligand binding [68,69,71], melting of DNA complexes with selectively binding ligands [93,94] and melting with account for the interaction between the adsorbed ligands [95,96], were analyzed. The effect of ligands' redistribution during the melting process [97] and the existence of two binding types [98] were also taken into account with respect to the parameters of helix-coil transition. As a result the theoretical dependences of the melting temperature shifts (ΔT_{melt}) and the changes of melting range width ($\delta\Delta T$) on the relative ligand concentration were obtained. It allowed to carry out a qualitative analysis of experimental dependences and to suggest possible binding mechanisms in the DNA-ligand systems.

Quantitative analysis of experimental data within the framework of Ising-like models was carried out using McGhee's equations [92]. The parameters of cooperative binding of extended ligands with DNA were calculated by this method. Subsequently, these equations form the basis of a computer optimization program which allows calculating the DNA-ligand binding parameters by fitting the melting curves [99,100,101]. However, the large number of search parameters results in significant error in their estimation. Especially it becomes apparent for small values of the binding constant and large binding site size. Unambiguous solution of this problem can be achieved only if

the values of some search parameters are determined independently by additional experimental method [100].

In summary it may be concluded that the Ising-like models have been developed mainly for theoretical analysis of the influence of binding parameters on the shape of DNA-ligand melting curves. Nevertheless, statistical-thermodynamic description of the melting of DNA-ligand complexes leads to the problem of big number of search quantities in the final expressions. It limits the application of such models for calculation of binding parameters from experimental melting curves.

3.1.1. Calculation of DNA-ligand binding parameters at full saturation of polymeric lattice by ligand molecules

In practice the Ising-like models are extensively used to calculate the DNA-ligand binding parameters from experimental melting curves under condition of nearly full saturation of lattice by ligand molecules. In such case there no statistical distribution of the ligands along the polymer lattice needs to be accounted for [102–105]. It is also assumed that the ligands interact with DNA in noncooperative and unspecific manner. In this case the McGhee's, Frank-Kamenetskii's and Crothers's equations [68,69,71,91,92] are transformed into similar analytical expression for the dependence of DNA melting temperature shift on the ligand concentration:

$$\frac{1}{T^0_{melt}} - \frac{1}{T_{melt}} = \frac{R}{\Delta H_{melt}} ln\left[\frac{(1 + K_{bind,T_{melt}} L_{free,T_{melt}})^{1/n}}{(1 + K_{bind_{ss},T_{melt}} L_{free,T_{melt}})^{2/n_{ss}}}\right], \quad (2)$$

where T^0_{melt} and ΔH_{melt} are melting temperature and enthalpy change on melting of free DNA, respectively; T_{melt} is the melting temperature of DNA-ligand complex; K_{bind} and n are the equilibrium constants and site sizes of ligand binding to double-stranded DNA, respectively; $K_{bind_{ss}}$ and n_{ss} are the equilibrium constants and site sizes of ligand binding to single-stranded DNA, respectively; $L_{free,T_{melt}}$ is the concentration of free ligand at T_{melt}.

To explain the experimentally observed high temperature shift it is assumed that the ligand does not bind to single-stranded polynucleotide and the equation (2) is simplified to:

$$\frac{1}{T^0_{melt}} - \frac{1}{T_{melt}} = \frac{R}{\Delta H_{melt}} ln\left[(1 + K_{bind,T_{melt}} L_{free,T_{melt}})^{1/n}\right]. \quad (3)$$

The theoretical approaches of McGee, Frank-Kamenetskii and Crothers [68,69,71,91,92] were initially developed for the analysis of the DNA-ligand melting curves measured spectrophotometrically in the ultraviolet region of spectrum. Nevertheless, the equation (3) is written for the full saturation of lattice by the ligand and it can also be applied for binding parameters calculation from DSC data [50,101].

Calculation of the DNA-ligand binding parameters from melting data using equation (3) can be carried out as follows. T^0_{melt} and T_{melt} values are determined from the melting curves measured by means of UV spectroscopy or DSC. The ΔH_{melt} value is calculated from heat absorption curves of free DNA.

To determine the free ligand concentration at melting temperature it is assumed that the half of bound ligand (L_{bind}) at T_{melt} had dissociated from the polymer. Many authors use to calculate the value of $L_{free,T_{melt}}$ as $C_{L,tot}/2$ [72,106,107]. However, at high relative concentration of the ligand this estimation becomes too approximate because $C_{L,tot} >> L_{bind}$ especially at low values of the binding constants [92].

The more accurate approach is to compute $L_{free,T_{melt}}$ assuming that at room temperature certain fraction of the ligand is unbound [80]:

$$L_{free,T_{melt}} = C_{L_{tot}} - \frac{1}{2} L_{bind,298} \,. \tag{4}$$

Using the values of T^0_{melt}, T_{melt}, ΔH_{melt}, n and $L_{free,T_{melt}}$ it is easy to obtain the binding constant at melting temperature from equation (3).

The binding enthalpy can be determined from DSC data according to the Hess's law [55,108]. If the difference between the enthalpy of DNA complex melting ($\Delta H_{DNA\text{-}L}$) and the enthalpy of free DNA melting (ΔH_{DNA}) is caused only by the ligand, L, interaction with double-stranded regions, the binding enthalpy at melting temperature, $\delta\Delta H_{bind,T_{melt}}$, can be defined as:

$$\delta\Delta H_{bind,\,T_{melt}} = \Delta H_{DNA-L} - \Delta H_{DNA} \,. \tag{5}$$

The value of $\delta\Delta H_{bind,\,T_{melt}}$ is expressed in mole of bases. It is convenient to normalize it per mole of ligand:

$$\Delta H_{bind,T_{melt}} = \delta\Delta H_{bind,T_{melt}} / r \,. \tag{6}$$

The value of r is defined as the ratio of the bound ligand concentration to the total DNA concentration.

Usually the binding parameters at room temperature are of major interest. The binding enthalpy at T = 298 °C is calculated by the equation:

$$\Delta H_{bind}(T) = \Delta H_{bind,T_{melt}} + \Delta C_{p,bind}\left(T - T_{melt}\right). \tag{7}$$

The $\Delta C_{p,bind}$ value can be determined by ITC [47-53] or estimated by van't Hoff's equation from the dependence of the logarithm of binding constant on reverse temperature [42,49,50,51,58].

The binding constant at T = 298 °C is determined taking into account the equation (7):

$$K_{bind,T_{melt}} = K_{bind,298} exp\left[-\frac{1}{R}\Delta H_{bind,298}\left(\frac{1}{T_{melt}} - \frac{1}{298}\right) - \frac{1}{R}\Delta C_{p,bind}\left(1 - \frac{T_{298}}{T_{melt}} - \ln\left(\frac{T_{melt}}{298}\right)\right)\right] \tag{8}$$

.

The method of calculation of the binding parameters reviewed above allows to evaluate the DNA-ligand binding constant and enthalpy quite easy and it is therefore used extensively [72,101,106,107,109].

Nevertheless, it has several disadvantages and limitations:

- Equation (3) is not applicable in a wide range of DNA/ligand concentration ratios. It can be used only at full saturation of lattice by ligand or if the ligand binds to one DNA base pair. This is due to the fact that on derivation of this equation the mixing energy upon ligand binding and the energy change on ligand redistribution during the melting process were ignored. However, the condition of full lattice saturation is often not the case in practice, e.g. when the binding constants have low values or binding site size is large. Furthermore, the melting temperature of DNA on addition of large amounts of ligand usually becomes rather high and the end of the melting cannot be observed in spectrophotometric and DSC experiment.
- Equation (3) ignores the binding constant dependence on temperature. It causes an error of determination of the free ligand concentration at melting temperature.
- This calculation method neglects the dependence of DNA melting enthalpy on temperature whereas the binding of ligand results in substantial increase of the DNA melting temperature. The neglect of the DNA heat capacity changes during the melting leads to overestimated values of ΔH_{bind} and K_{bind} (equations (5) and (8)).
- In order to calculate the binding parameters according to equations (3)–(8) the information from different independent experimental methods is required (for example, DSC, UV-spectroscopy and ITC). These data, however, cannot be obtained under the same experimental conditions, for example, at similar concentrations.

3.2. Description of the DNA-ligand melting process by the equations of chemical equilibria

The binding parameters of ligands with biopolymers can be determined from DSC melting study using the equations of chemical equilibria (the law of mass action and the mass balance equations). Originally such approach was proposed and widely used for analysis of heat absorption curves of some protein complexes with ligands [110–113]. This is because the denaturation of single domain proteins follows two-state model, and the complexes with ligands are formed on independent binding sites. Later this approach was extended for calculation of the ligand binding parameters with high-polymer DNA molecules [114,115].

According to statistical thermodynamics the average excess enthalpy of equilibrium system can be represented as [77,114–118]:

$$\langle \Delta H \rangle = \sum_{j=0}^{l} P_j(T) \Delta H_j(T), \tag{9}$$

where $P_j(T)$ is the relative population of the ***j*** state on transition and $\Delta H_j(T)$ is the enthalpy change associated with the transition between the reference state and the ***j*** state.

The average excess heat capacity $<\delta C_p>$ measured in DSC experiment is a derivative from the average excess enthalpy $<\Delta H>$ at constant pressure:

$$\left\langle \delta C_p \right\rangle = \sum_{j=0}^{l} \Delta H_j(T) \frac{dP_j(T)}{dT} + \sum_{j=0}^{l} P_j(T) \frac{d\Delta H_j(T)}{dT}. \quad (10)$$

To describe calorimetric behaviour of the DNA-ligand system it is necessary to obtain the expressions for P_j and ΔH_j. In this model the melting of complexes is considered as two independent equilibrium processes: dissociation of the ligand from the double-stranded DNA and the helix-coil transition of biopolymer.

Generally the double-stranded state of DNA is defined as a reference state, with respect to which all thermodynamic parameters of the system are calculated [113]. In this case, the DNA transition from the double-stranded to single-stranded state is characterized by the melting enthalpy ΔH_{melt}, and the ligand binding with double-stranded DNA is characterized by the binding enthalpy ΔH_{bind}.

The chemical equilibria equations are used for calculation of the relative populations of single-stranded DNA and the DNA-ligand complexes.

The theory of chemical equilibria is applicable only for the DNA regions which melt by two-state model [76,119–122]. Therefore, in this approach the high-polymer DNA molecule is considered as an assembly of cooperative units and their melting can be described by the equation:

$$dsDNA_m \rightarrow 2ssDNA_m \qquad K_{melt} = \frac{[ssDNA_m]^2}{[dsDNA_m]}, \quad (11)$$

where $dsDNA_m$ is the cooperative unit of the double-stranded DNA, which consists of m base pairs; $ssDNA_m$ is a fragment of the single-stranded DNA, which consists of m bases; K_{melt} is a macroscopic equilibrium melting constant of the DNA cooperative units.

The cooperative unit size is determined from the heat absorption curve as a ratio of model-dependent van't Hoff enthalpy, ΔH_{vH}, to calorimetric enthalpy, ΔH_{melt}, calculated as an area under experimental curve ($\Delta H_{vH} = 2m \cdot \Delta H_{melt}$) [121,123,124].

The temperature dependence of the melting constant is given as:

$$K_{melt} = \frac{C_{DNA}}{m} exp\left(\frac{1}{R} \left[-2m \times \Delta H_{melt}(T_{melt}) \left(\frac{1}{T} - \frac{1}{T_{melt}} \right) - 2m \times \Delta C_{p,melt} \left(1 - \frac{T_{melt}}{T} - ln \frac{T}{T_{melt}} \right) \right] \right) \quad (12)$$

,

where $\Delta C_{p,melt}$ is the heat capacity change upon DNA melting; C_{DNA} is the total DNA concentration.

The binding site size for the majority of known ligands (n) is substantially smaller than the size of DNA cooperative unit. Hence, the DNA-ligand interaction can be presented in terms of stepwise saturation:

$$dsDNA_m - L_{i-1} + L \rightarrow dsDNA_m - L_i \qquad K_{bind,1} = \frac{[dsDNA_m - L]}{[dsDNA_m][L]}, \quad (13)$$

where L is the free ligand; $dsDNA_m$–L_i is the complex of DNA cooperative unit with i ligand molecules ($1 \le i \le m / n$); $K_{bind,i}$ is the macroscopic association constant for each binding step.

The relationship between the microscopic (k_{bind}) and the macroscopic binding constants is determined taking into account the statistical distribution of the ligands on finite one-dimensional lattice [35]:

$$K_{bind,i} = \frac{(m - i(n-1))!}{(m - n \times i)!\, i!} k_{bind}^{i} \ . \tag{14}$$

According to the melting and binding equations given above, the relative populations of single-stranded DNA and DNA-ligand complexes are defined as:

$$P_{ssDNA_m} = \frac{[ssDNA_m]}{C_{DNA}/m} \qquad P_{dsDNA_m - L_i} = \frac{[dsDNA_m - L_i]}{C_{DNA}/m}. \tag{15}$$

To calculate the concentrations of single-stranded DNA and $dsDNA_m$–L_i complexes, the mass balance equations for DNA (C_{DNA}) and ligand (C_L) molecules are used:

$$C_{DNA} = 2m[dsDNA_m] + m[ssDNA_m] + 2m\sum_{i=1}^{m/n}[dsDNA_m - L_i], \tag{16}$$

$$C_L = [L] + \sum_{i=1}^{m/n} i[dsDNA_m - L_i]. \tag{17}$$

The concentrations of $dsDNA_m$–L_i and $dsDNA_m$ components are expressed in terms of the single-stranded DNA concentration using the mass action laws (equations (11) and (13)) and the statistical distribution of the ligands (equation (14)):

$$[dsDNA_m - L_i] = \frac{(m - i(n-1))!}{(m - n \cdot i)!\, i!} k_{bind}^{i}(T)[L]^i [dsDNA_m], \tag{18}$$

$$[dsDNA_m] = \frac{[ssDNA_m]^2}{K_{melt}}. \tag{19}$$

The concentration of single-stranded DNA [$ssDNA_m$] at any temperature is determined by solving equations (16)–(19) with the account for the temperature dependence of the binding constant by equation (8).

Employing the temperature dependences of the melting and binding enthalpies (equation 7), the expression for the heat capacity change upon complex melting can be written as:

$$\begin{aligned} \delta C_p = {} & \frac{m}{C_{DNA}}\left(\Delta H_{melt}(T)\frac{d[ssDNA_m]}{dT} + \sum_{i=0}^{m/n} i\Delta H_{bind}(T)\frac{d[dsDNA_m - L_i]}{dT}\right) + \\ & + \frac{m}{C_{DNA}}\left([ssDNA_m]\Delta C_{p,melt} + \sum_{i=1}^{m/n} i[dsDNA_m - L_i]\Delta C_{p,bind}\right) \end{aligned} \tag{20}$$

where $\Delta C_{p,bind}$ is the heat capacity change upon binding.

The system of equations (7), (8), (12), (16)–(20) allows obtaining the full set of binding parameters in the DNA-ligand system by fitting experimental heat absorption curves.

In the first step of calculations the melting parameters of the DNA in the absence of ligand ($i = 0$) are determined from the heat absorption curve of free DNA. The calculated values of T_{melt}, $\Delta H_{melt}(T_{melt})$, m, $\Delta C_{p,melt}$ are maintained constant while the heat absorption curves of the DNA-ligand

complexes are being fitted. The adjustable parameters are k_{bind}, n, ΔH_{bind}, $\Delta C_{p,bind}$ as well as the concentration of free ligand. These parameters are determined by minimizing the standard deviation of the theoretical and experimental heat absorption curves at different DNA and ligand concentration ratios [115].

The correct use of this method requires the value of the size of cooperative unit to substantially exceed the binding site size. In cases of low-cooperative DNA helix-coil transition and/or DNA binding of large ligands, the influence of end-effects leads to errors in binding parameters determination.

It should also be noted that the parameters, K_{bind} and n, as well as ΔH_{bind} and $\Delta C_{p,bind}$, are interdependent. Nevertheless, these parameters can be determined uniquely by analysis of the excess heat capacity function in wide temperature range, since K_{bind} and ΔH_{bind} are temperature dependent, whereas n and $\Delta C_{p,bind}$ are temperature independent. Therefore, the necessary condition in order to apply this method is the presence of significant temperature shift upon ligand binding to DNA.

4. Conclusion

The paper has reviewed two basic methods of calculation of DNA-ligand binding parameters from melting data.

The first method is based on Ising-like models. The corresponding equations describing the melting of the DNA-ligand complexes are written in general form, they are rigorous and do not contain significant limitations in their application. However, the final expressions are difficult to implement in computations and, by that reason, this approach has not got wide application for calculation of the binding parameters from experimental temperature measurements in DNA-ligand systems. The most frequent application of the Ising-like method was noted only in melting curve analysis at full saturation of lattice by ligand. In this case the final equations are becoming simple. However, the set of limitations introduced during the derivation of these equations lead to errors in the binding parameters determination.

In the second method the DNA-ligand melting is described by the equations of chemical equilibria. The main limiting factors for application of this approach are the low-cooperativity of the DNA helix-coil transition and/or a large value of the ligand binding site. In these cases, the end-effects are playing significant role in the binding parameters calculation.

The usefulness of this approach is the possibility of easy modification of the basic equations enabling to describe more complex DNA-ligand interactions, e.g. multimode and competitive binding [125]. In addition, the proposed system of equations allows calculating the concentrations of free and bound ligand at any temperature. In this approach it is easy to obtain the functional dependence of any experimental parameter, $\xi_{exp}(T)$, on L_{bind}. The value of $\xi_{exp}(T)$ can, in turn, be determined by common methods, e.g. spectrophotometry, luminescence, etc.

In summary it is worth noting that the choice of experimental methods for thermal investigations and numerical data processing for each specific DNA-ligand system are depended on many factors, such as the type of DNA used, the size of the ligand, the DNA/ligand concentration ratio, the types of complexes formed in the system, etc. It is always necessary to keep in mind the existing limitations imposed by the properties of the objects under study and the introduced model assumptions. These factors are very important for accurate calculation of the binding parameters and subsequent interpretation of the results.

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Conflict of Interest

The authors declare no conflict of interests.

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