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Simple Method for Determining the Thermodynamic Parameters of Ligand with Nucleic Acids Interaction

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Abstract. Simple and easily accessible spectrophotometric method for estimating the thermodynamic parameters of the ligands-nucleic acid interaction is suggested. The method has been used to determine the binding constant (K), Gibbs free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) changes for mitoxantrone (MTX)-DNA and Ethidium bromide (EtBr)-poly(A)poly(U) complexes at different temperatures and at 10^{-2}M Tris buffer containing 0.1 M NaCl. Estimated negative sing of ΔG for both intercalators MTX and EtBr interaction with A- and B- forms of nucleic acids pointed to the driving the spontaneously going binding process. Negative values of ΔH=-9.7 kcal/mol for MTX-DNA and – 7.6 kcal/mol for EtBr-poly(A)·poly(U) were found, which indicated to the low energy levels of complexes. Positive entropy values of ΔS=5.2cal/(mol·K) for MTX-DNA and ΔS=3.8cal/(mol·K) for EtBr-poly(A)·poly(U) complexes reflected a structural changes due to the intercalation.To illustrate the general applicability of the suggested simple method the thermodynamic parameters of two intercalators interaction with nucleic acids have been determined which are consistent with the existing in literature binding data of well known ligand EtBr and MTX binding with nucleic acids.

Keywords: mitoxantrone, ethidium bromide, absorption, DNA, enthalpy and entropy binding

Introduction

Significant progress has been made in learning of design principles for small biological molecules (ligands) interacting with nucleic acids. These biomolecules are the target for several ligands that bind to them and control the gene expression.

Interest in these area based on the facts that many nowadays available ligands bind to DNA exhibit significant therapeutic effect. They are used in treatment of genetic, oncogenic and viral diseases [1-4]. On the other hand, ligand binding to nucleic acids forms a simple physical system for understanding the general principles of complex formation particularly suggesting a model providing information about the molecular forces contributions to binding free energy, which are the significant factors for understanding the principles of complexing.

Integration of the structural, kinetic and thermodynamic data of ligand-nucleic acids interaction will make it possible clearly understand the mechanisms of stable ligand-nucleic acid complexes. Such investigations are much important to characterize the binding mode, sequence specificity and understanding in detail of designing new generation of drugs affecting the gene expression. Structural data obtained by X-ray crystallography or NMR for many drug-nucleic-acid complexes were successfully used for estimating that ligands attempt to correlate structure to binding affinity. It was established that upon binding the ligands interact with substrate as a rigid compound, which is an advantageous for revealing thermodynamic contribution from structural data [5]. Obtained by X-ray crystallography and NMR structures of ligands-nucleic acids complexes showed the more possible way to much ligand shape with the receptors of

substrates but 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 [6] neglecting the energetic characteristics of binding process. So the structural data alone cannot define the driving forces for binding and predict the binding affinities. To understand the molecular mechanism and energetic of ligand-nucleic acid interaction knowledge of thermodynamic parameters which provide data elucidating the driving forces of binding and for deeper understanding the complex formation process [4]. Key role here belongs to free energy, which dictates to the direction of a system equilibrium. The free energy is a sum of two thermodynamic parameters of the reaction: enthalpy, which is considered as a measure of bond formation (hydrogen and van der Waals bonding) and entropy is responsible of the nonspecific hydrophobic forces [7, 8].

The binding enthalpy (ΔH) can be detected using isothermal titration calorimetry (ITC) or differential scanning calorimetry (DSC) [4, 10, 11].

Analyses of the existing in literature a considerable body of experimentally determined thermodynamic data for variety of ligand-nucleic acid complexes shows that observed binding free energy arises from a balance of opposing forces [9], one of which is enthalpy of interaction.

These methods provide several advantages for measuring binding energetic parameters at the same time having distinct difficulties the dominant of which is high concentration of nucleic acids ($\sim 10^{-2} M(P)$) and at the titration the air bubbles limit the accuracy of the obtained experimental results. Besides the use of big amount of nucleic acids require large quantities of expensive products and the possible aggregation makes very difficult to explain the results [2, 10, 12]. Mentioned difficulties for detecting the thermodynamic parameters of ligand-nucleic acid interaction may be overcome applying methods, which are experimentally easy to perform, in particularly spectrophotometric methods, where very low concentrations of nucleic acids are used (10^{-5}-$10^{-4}\, M(P)$), which exclude the very unwanted process of aggregation [13,14] and may admit above mentioned difficulties.

The goal of this article is to offer an easily accessible and simple method for obtaining ligand-nucleic acid complexes thermodynamic parameters (free energy, enthalpy, entropy and binding constant) by spectrophotpmetric measurements. Suggested simple method has applied for obtaining thermodynamic parameters of binding to nucleic acid of a known intercalator ethidium bromide (EtBr). The results are in good agreement with existing in literature data of well-known ligands (EtBr and MTX) binding with nucleic acids.

Materials and Methods

Calf thimus (CT) DNA, poly(A)·poly(U), mitoxantrone (MTX) and ethidium bromide (EtBr) were purchased from Sigma. All chemicals used without further purification. Details of the complexion, the binding isotherms and binding parameters have been described in [10, 15]. The molar absorbance values used are: $\varepsilon_{478} = 5680\, M^{-1}cm^{-1}$for EtBr,$\varepsilon_{260} = 7140\, M^{-1}cm^{-1}$ for poly(A)·poly(U) [16], $\varepsilon_{659} = 25.900\, M^{-1}cm^{-1}$ for MTX [17].

Binding isotherms were determined spectrophotometrically using Unicam-SP8-100 (England). The heating of solutions of complexes was performed with Temperature Promamme Controller SP-876 Series 2. The quartz cuvets with hermetically closed teflon plug of 1 cm optical pathway length were used for spectrophotometric measurements.

All titration experiments have been performed at constant ligand concentrations (see legends for corresponding Figures) with increasing amounts of polynucleotides. All experiments have been performed in a 10^{-2} M Tris buffer containing 0.1 M NaCl, pH 7.5.

Results and Discussion

A complete structural and thermodynamic data are require revealing the important insights the role of known nowadays several ligands interacting with nucleic acids and understanding of how they bind to the genetic materials regulating cell replication and gene expression. To reveal the distinctive characteristics of their functions and thus to make it possible to develop more effective novel therapeutics drugs, in complement to structure-based design strategies the energetic data are necessary to integrate for establishing the driving forces stabilizing the ligand-nucleic acid complexes [9]. Complete binding thermodynamic parameters consist of free energy, enthalpy and entropy. The dominant parameter is the free energy ΔG showing the direction of equilibria and it is the balance between enthalpy (ΔH) and entropy (ΔS). For the most of intercalating ligands, ΔH is negative pointing to the lower energy levels by bond formation. Rupture of ordered hydration shell of the nucleic acid and release of bound water to form the bound site make common positive entropy (ΔS) values for ligand-nucleic acid complexes. Thermodynamic parameters of the complex formation provide important information, which are independent on general molecular processes of complexing [4, 8, 9, 16]. The key thermodynamic parameter ΔG is expressed by equation

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

Enthalpy changes (ΔH) can be obtained by calorimetric methods (there are differential canning calorimetry (DSC) and Isothermal scanning calorimetry (ISC)) [4], or by van't Hoff relationship using equilibrium temperature dependence of standard free energy change ΔG by means of the Gibbs equation

$$\Delta G = -RT \ln K \tag{2}$$

where R-is a gas constant, T-is a temperature in Kelvin and K-is the equilibrium constant. Equations (1) and (2) for $\ln K$ give:

$$\ln K = -\frac{\Delta H}{R} \cdot \frac{1}{T} + \frac{\Delta S}{R} \tag{3}$$

Obtained equation (3) shows that if the dependence of $\ln K$ on $\frac{1}{T}$ is linear function, then the intersection of the line with the axis of ordinate give $\frac{\Delta S}{R}$ value, and the tangent of the angle of the line with abscissa numerically is equal to $-\frac{\Delta H}{R}$. To determine the binding constant K dependence on temperature, the absorption spectroscopy has been used, plotting the dependences of absorption of the complexes of EtBr-poly(A)·poly(U) versus wavelength (Figure 1) and the temperature (obtained spectra at 25^0 C, 35^0 C and 45^0 C) are not brought

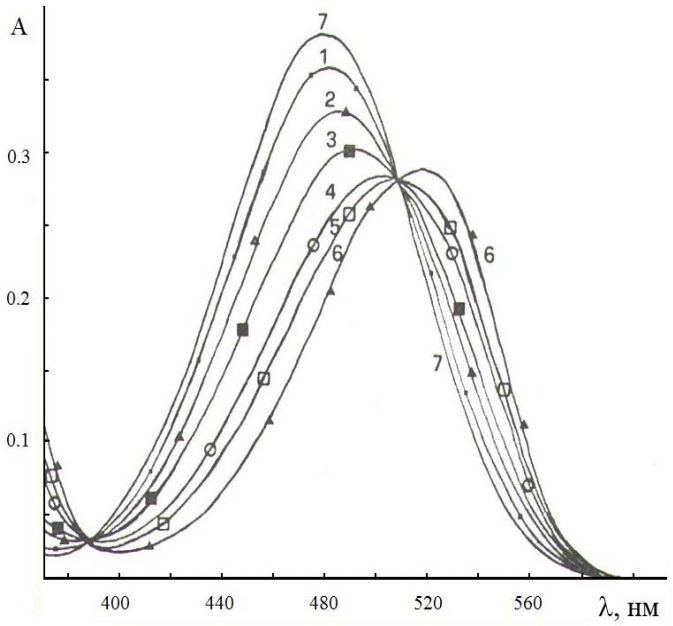

Fig. 1. Spectrophotometric titration of EtBr with ds-poly(A)poly(U) at 25^{0}C and 10^{-2} M Tris buffer and 0.1 M NaCl, pH 7,5. Titration was made at the constant concentration of EtBr (1.4×10^{-4} M) (7) and poly(A)poly(U) concentrations of 7.5×10^{-5} M (1), 2.1×10^{-4} M (2), 3.3×10^{-4} M (3), 5×10^{-4} M (4), 6×10^{-4} M (5) and 1×10^{-3} M (6). Titration was performed at 25^0 C, where poly(A)poly(U) is in duplex A-form [18].

but for the complex of EtBr-poly(A)poly(U) at 25^{0}C. Absorption spectra for MTX-DNA at different temperatures (35^0 C, 50^0 C and 60^0 C) had analogy to the spectra of Figure 1 (they are not presented). Spectroscopic titration curves of constant concentration of EtBr in the absence and presence of different concentrations of double stranded poly(A)poly(U) (see the legend for Figure 1) were recorded in the absorption band of EtBr in the visible region (350-600 nm) as poly (A)·poly(U) absorbs efficiently only in UV region. Thus, all registered spectral changes were governed by the ligand-nucleic acid interaction. Figure 1 shows that with the increasing addition of ds-poly(A)poly(U) to EtBr well pronounced hypochromicityas well as bathochromicity shift, driving the absorption maximum (λ_{max}=480 nm) for the free ligand (curve 7, Figure 1) up to ~520 for the bound EtBr-ds-poly(A)poly(U) at the ratio of 0.14. These spectral shifts are the characteristic of staking interaction between the ligand chromofore and the bases of ds-poly(A)poly(U) indicating the intercalating mode of binding of EtBr to A-form of ribonucleic acid. Existing isobestic points in the absorption spectrum of EtBr (λ_1=388 nm and λ_2=508 nm, Figure 1) indicate the stoichiometric binding of the ligand to poly(A)poly(U) [14, 15].

The comparison of our spectroscopic results with those obtained from studies on DNA make possible to suggest the intercalationmode of EtBr into ds-poly(A)·poly(U). To describe the complete thermodynamic characteristics of EtBr binding to ds-poly(A)·poly(U) we apply the absorption properties of well known intercalatior EtBr binding to ds-RNA [20, 21] (absorption spectra at different temperatures) to find the binding constants dependences on temperature, which will provide data for calculating ΔH and ΔS (see eq. 3). Significant changes in the absorption properties of EtBr upon binding to ds-poly(A)·poly(U) (Figure 1) have been used to obtain the binding isotherms by mixing of different amounts of poly(A)·poly(U) with a fixed amount of EtBr (see the legend of Figure 2 and text below).

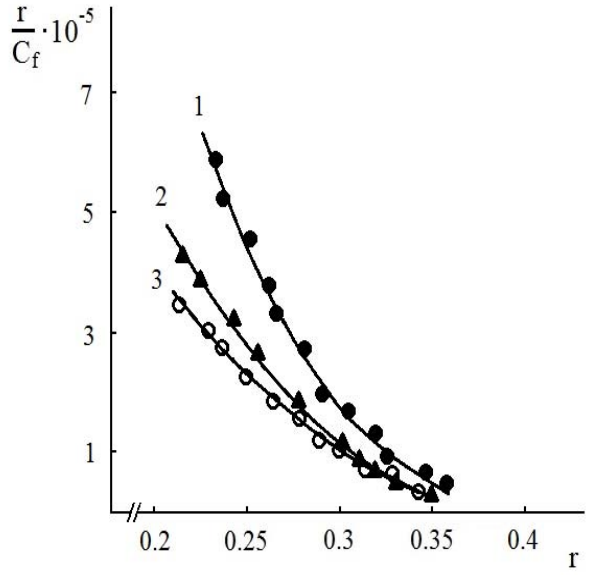

Fig. 2. Binding isotherms at various temperatures 25⁰C (1), 35⁰C (2) and 45⁰ C (3). The concentration of EtBr was 1.4×10^{-4} M. Concentration of poly (A)·poly(U) as in the Figure 1.

Binding isotherms obtained at different temperatures are shown in Figure 2, which were treated by McGhee and von Hippel equation [19]

$$\frac{r}{C_f} = \frac{K(1-nr)^n}{[1-(n-1)r]^{n-1}}, \tag{4}$$

where K-is binding constant, n-is the stoichiometry of the complex, c_f-is the unbound ligands concentration and $r = \frac{C_b}{C_p}$, where C_b and C_p are bound ligand and the nucleic acid concentrations, respectively. For obtaining the magnitudes of ΔS and ΔH, the dependences of $\ln K$ on $\frac{1}{T}$ have been constructed by mean-square method, which are represented on the Figure 3.

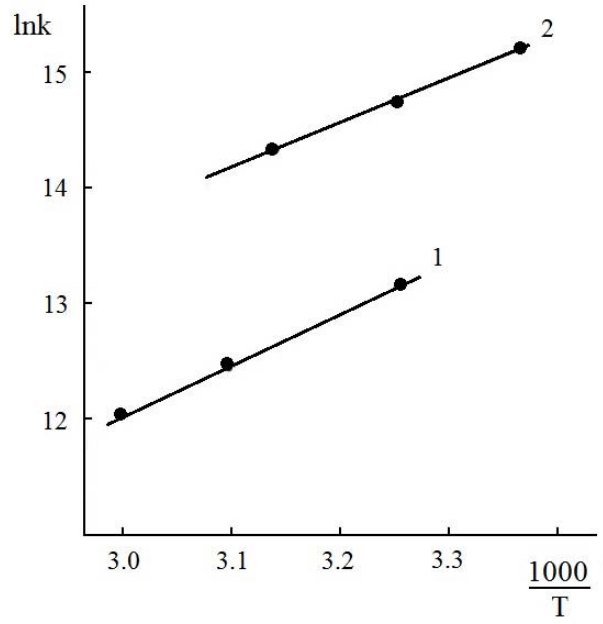

Fig. 3. Dependes of lnK on 1/T for the DNA·MTX(1) poly(A)poly(U)·EtBr (2) complexes . See text above.

The results of estimated values of the thermodynamic parameters are presented in the Table below.

Table. The thermodynamic parameters of ligand-nucleic acids complexes at different temperatures and 0.11 M NaCl ionic strength.

Complexes	t,°C	K,M^{-1}	-ΔG kcal/mol	-ΔH kcal/mol	ΔS cal/(mol·K)
DNA-mitoxantrone	35	$(5,2\pm0,4)10^5$	8,0±0,2	9,7±0,8	5,2±0,3
	50	$(2,5\pm0,5)10^5$	8,0±0,2		
	60	$(1,6\pm0,5)10^5$	8,0±0,2		
poly(A)poly(U)-EtBr	25	$(3,8\pm0,3)10^6$	8,7±0,2	7,6±0,5	3,8±0,3
	35	$(2,5\pm0,3)10^6$	8,8±0,3		
	45	$(1,7\pm0,3)10^6$	8,8±0,3		

Conclusion

Data of Table show that for both intercalators the binding with nucleic acids is driven by enthalpy (the enthalpies of interaction are negative and independent on temperature). Obtained results show a good fit to the data revealed by the plotting of the binding enthalpy against binding entropy for 26 drug-DNA interactions [22-23], which make it possible to suggest with the assurance this simple method for estimating the complete thermodynamic parameters of ligand-nucleic acid interaction.

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