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ERROR IN DETERMINATION OF BINDING THERMODYNAMIC PARAMETERS APPEARED DUE TO ADSORPTION LINEAR ISOTHERM DESCRIPTION

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In the present work the description of absorption spectra of ethidium bromide complexes with synthesized polyribonucleotide by several modes has been carried out. The binding constant value and the values of changes of thermodynamic parameters were determined. It was shown that at the description by linear binding isotherm the value of enthalpy and the number of nucleotides corresponding to one ligand molecule at interaction saturation are approximately the same as real ones, but the values of the binding constant, Gibbs free energy change and entropy change differ from real values.

***Keywords*:** synthesized polyribonucleotide, ethidium bromide, description modes of binding isotherm, linear mode, thermodynamic parameters.

Introduction. At the reversible binding of ligands with nucleic acids (NA), different models of description are used. Let's consider that in investigating conditions ligands interact with NA by only one mode. To carry out a quantitative investigation of experimental data of ligand binding to NA it is necessary to achieve experimentally the state when all binding centers on NA are occupied by ligands. If it is possible to realize this state experimentally, the adsorption isotherm often is described by McGhee and von Hippel formula [1], where NA is observed as a linear, infinitive structure composed of one-type and non-interacting binding elements. For such non-cooperative model the binding isotherm is described by equation

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

where K is ligand binding constant to NA; n is the number of NA bases becoming unavailable when one ligand binds; C_f is the free ligand concentration in the solution; $r = C_b/C_p$, where C_b is the bound ligand concentration; C_p is the NA concentration by azotic bases.

If the interaction of ligands with each other is taken into account, a cooperativity parameter (ω) is inserted and in the last case the formula (1) takes the following form:

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$$\frac{r}{C_f} = K(1-nr)\left[\frac{(2\omega-1)(1-nr)+r-R}{2(\omega-1)(1-nr)}\right]^{n-1}\left[\frac{1-(n+1)r+R}{2(1-nr)}\right]^2, \qquad (2)$$

where $R = \left([1-(n+1)r]^2 + 4\omega r(1-nr)\right)^{\frac{1}{2}}$, the ω parameter can be bigger from 1 (ligands "attract" each other) and less from 1 (ligands "repulse" each other).

Most frequently due to the weak interaction of ligands with NA (or by other reasons) it is impossible to obtain experimentally the physical characteristics of the thoroughly bound state [2]. It should be mentioned simultaneously for experimental realization of the thoroughly bound state, sufficiently big amount of NA should be used. By this reason in many cases it is appropriate to evaluate the adsorption parameters from titration curves obtained at small fillings. In this case the adsorption isotherm has a linear form [3] and is determined by equation

$$\frac{r}{C_f} = K[1-(2n-1)r]. \qquad (3)$$

It is obvious that the determination of K and n parameters by the Eq. (3) is easier and does not demand a big amount of NA.

The aim of the present work is to describe the adsorption spectra by Eqs. (1)–(3), to determine the binding constant value in each case and to calculate the values of change of thermodynamic parameters occurred by binding. For each thermodynamic parameter the error value by linear Eq. (3) in the consequence of adsorption isotherm description is determined.

Materials and Methods. Poly(A)poly(U) and ethidium bromide ("Sigma", USA) were used in the experiment. Polyribonucleotide was used without further purification. Concentrations of double-stranded poly(A)poly(U) and ethidium bromide (EtBr) were determined spectrophotometrically, using the following values of extinction coefficients: ε_{260}=7140 $M^{-1}cm^{-1}$ for poly(A)poly(U) and ε_{480}=5800 $M^{-1}cm^{-1}$ for EtBr. The interaction of EtBr with poly(A)poly(U) was studied in buffer containing 0.1 *M* NaCl and 0.01 *M* Tris, pH 7.5 at 290.15 and 300.15 *K* temperatures. It is known that in the mentioned conditions EtBr interacts with poly(A)poly(U) by only one intercalation mode [4, 5].

Binding isotherms were constructed from the absorption spectra of poly(A)poly(U)–EtBr complexes. Absorption spectra were obtained via Specord UV VIS spectrophotometer.

EtBr molecules form auto associates in the solution. Calculations showed [6], that if EtBr concentration $C_0 \le 1.4 \cdot 10^{-4}\, M$, their auto association may be neglected.

Results and Discussion. Usually the binding isotherm is constructed during titration process from absorption, cyclic dichroic and fluorescence spectra in the visible interval, because in this interval NA does not absorb and any change of spectra is conditioned by NA–ligand interaction. If the binding process is studied via change of absorption spectra due to complex-formation, at the ligand constant concentration, absorption spectra of NA–ligand complexes are obtained, and by Eq. (4) the concentrations of free and bound ligands in the solution are calculated accepting that the relative change of spectra due to the binding is directly proportional to bound ligand concentration.

$$\frac{C_f}{C_0} = \frac{A - A_b}{A_f - A_b}, \qquad C_b = C_0 - C_f, \tag{4}$$

where A_f and A_b are absorption values of free and bound ligands respectively under the wavelength corresponding to absorption maximum (for example λ=480 *nm* for EtBr). Usually the determination of spectral characteristics of bound ligands is connected with many complications. The two of them are: it is almost impossible to obtain the absorption spectrum of bound state of NA–ligand studying complex, because at first NA big amount is demanded, and simultaneously at NA relatively high concentrations $(C_p/C_0 >> 1)$. a redistribution of bound ligands between those binding by different mechanisms takes place in consequence of which isosbestic points disappear. In the present work it is suggested to determine approximate physical characteristics of ligand bound state via linear extrapolation from several absorption spectra of poly(A)poly(U)–EtBr complexes that were obtained at relatively low concentrations of poly(A)poly(U) $(C_p / C_0 << 1)$. Particularly, in our experiments the bound ligand absorption was determined under the wavelength corresponding to spectrum absorption maximum:

$$A_b = \lim_{1/C_p \to 0} A\left(1/C_p\right).$$

Using the values of C_f and C_b calculated by Eq. (4), the binding isotherm in Scatchard's coordinates (r/C_f vs r) was constructed and described by Eq. (3).

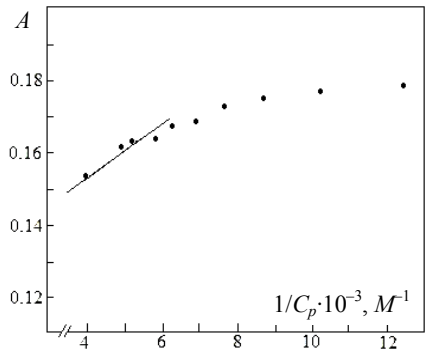

Fig. 1. Optic density (A_{480}) dependence of poly(A)poly(U)–EtBr complex under λ=480 *nm* wavelength on reciprocal value of polyribonucleotide concentration.

In this work the experimental data of binding isotherm of EtBr with double-stranded synthetized polyribonucleic acid were used by prof. Yu. Babayan [2, 4]. Optic density dependence (A_{480}) on $1/C_p$ corresponding to absorption maximum wavelength was constructed from these data (Fig. 1) and A_b was determined via linear extrapolation. It is natural that A_b determination by the mentioned mode comprises some mistake due to which C_b and C_f values differ a little from real values. The binding isotherms of poly(A)poly(U)–EtBr complexes are presented in Fig. 2 at 290.15 *K* temperature. Curve 1 is taken from [2], the curve 2 is the binding isotherm passed through experimental points, which is described by Eq. (1), and

curve 3 is the linear binding isotherm constructed from absorption spectra by the mentioned mode.

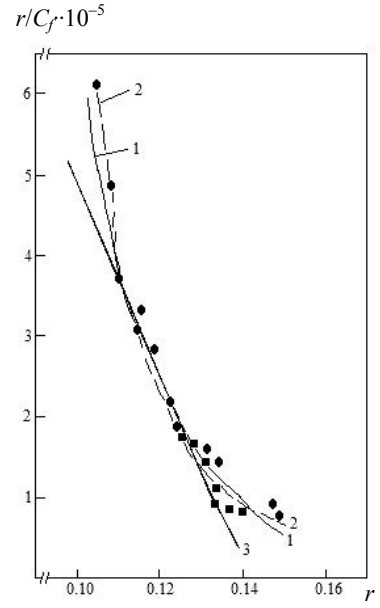

Fig. 2. Binding isotherms at 290.15 *K*, constructed by Eqs. (1)–(3) via experimental points at poly(A)poly(U)–EtBr adsorption spectrophotometric description.

The values of *K* and *n* parameters determined from Eqs. (1)–(3) at 290.15 and 300.15 *K* temperatures are presented in Table. Having the binding constant values at least in two different temperatures the change of thermodynamic parameters (Gibbs free energy, enthalpy and entropy) may be determined.

If K_1 and K_2 are binding constant values at T_1 and T_2 temperatures, the enthalpy change ΔH of the system due to binding will be determined by equation

$$\Delta H = -R\frac{\ln(K_1/K_2)}{(1/T_1)-(1/T_2)}. \qquad (5)$$

The values of Gibbs free energy (ΔG) and entropy (ΔS) change due to binding are determined by equations

$$\Delta G = -RT\ln K, \qquad (6)$$

$$\Delta S = \frac{1}{T}\left(\Delta G - \Delta H\right), \qquad (7)$$

where R is gas universal constant.

Some thermodynamic parameter values of poly(A)poly(U)–EtBr interaction

T, *K*	Equation	*K*, $10^{-6}M^{-1}$	*n*	$-\Delta G$, *kcal/mol*	$-\Delta H$, *kcal/mol*	ΔS, *cal/mol·K*
290.15	(1)	1.8 ± 0.2	4.2	8.34 ± 0.03	7.0	4.5
300.15		1.2 ± 0.2	4.1	8.36 ± 0.05		
290.15	(2)	2.5 ± 0.3 [2]	4.0 [2]	8.50 ± 0.05	7.2	4.4
300.15		1.65 ± 0.2	4.0	8.54 ± 0.05		
290.15	(3)	0.61 ± 0.03	4.1	7.69 ± 0.02	6.9	2.7
300.15		0.41 ± 0.02	4.0	7.71 ± 0.03		

The values of ΔH, ΔG and ΔS, determined by the Eqs. (5)–(7) respectively, are presented in the Table. It is followed from the Table, that at description of binding isotherm by Eqs. (1)–(3), sufficient differences of *K* binding constant values are observed. The binding stoichiometry describing *n* parameter values may be considered the same in error framework, i.e. it does not almost depend on the binding isotherm description formula. At the binding isotherm nonlinear description the same values are obtained for ΔH and ΔS. It should be mentioned that at nonlinear description of binding isotherms ΔH value well coincides with that obtained for poly(A)poly(U)–EtBr complexes by microcalorimetric method [2]. At the description of binding isotherm by the linear Eq. (3) it is obtained a value of *K*, which is 3–4 times less than the real value, due to which the values of ΔH, ΔG

and ΔS calculated by Eq. (3) noticeably differ. If we take into account that the error of microcalorimetric measurements is equal to 5% in best cases, the value of ΔH= –6.9 *kcal*/*mol* determined from Eq. (3) may be considered to be true in the experiment error framework. It is followed from Eq. (3) that the description by Eq. (3) gives approximately twice less value of ΔS.

Consequently, generalizing the binding isotherm description modes for poly(A)poly(U)–EtBr interaction, it may be assumed that the linear isotherm may be used, if it should be determined by enthalpy change system (ΔH) occurred due to interaction as well as the number of nucleotides bound with one ligand molecules at the interaction saturation (n).

The values of K, ΔG and ΔS parameters determined from linear isotherm significantly differ from their real values.

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