PROCEEDINGS OF THE YEREVAN STATE UNIVERSITY

Chemistry and Biology 2018, **52**(1), p. 63–66

Biology

DETERMINATION ERROR OF NUCLEIC ACID–LIGAND BINDING THERMODYNAMIC PARAMETERS CONDITIONED BY DETERMINATION ERROR OF PHYSICAL CHARACTERISTICS OF COMPLETELY BOUND STATE OF LIGAND

S. N. HAKOBYAN *

Department of Machine Science, National Polytechnic University of Armenia

To construct the binding isotherms of nucleic acid–ligand it is necessary to determine physical characteristics of completely bound state of ligand (A_b) experimentally. It is often impossible to determine A_b experimentally, due to which as A_b the value of the parameter is accepted, which almost does not change in the experiment error framework. Calculations show that in this case the binding constant determination error does not exceed 10%.

Keywords: poly(G)·poly(C), ethidium bromide, mitoxantrone, binding parameters, determination error.

Introduction. In external conditions (solution ionic strength, pH, etc.) ligands mainly bind to nucleic acids (NA) by more than one mode [1, 2]. Due to ligand binding physical-chemical properties of NA–ligand complexes change. It is useful to investigate quantitatively NA–ligand interaction in such conditions, when only one type of binding dominates. In this case the binding constant (K) and complex stoichiometry determining parameter (n) are calculated throughout the study of change of the physical parameters characterizing the complex [3]. If the behavior of change of the system optic properties due to the complex-formation is studied (particularly, the absorption spectra), which is relatively easy to realize, it becomes appropriate to investigate the behavior of change of the absorption spectra in visible range due to the NA–ligand interaction [1–3]. Isosbestic points often emerge in NA–ligand complex absorption spectra, after which any other regularities in the spectra change are not observed [4]. It may be conditioned by a number of reasons. Particularly, the studying ligand since the certain relative concentration up to this concentration may interact with NA by more than one mode [2, 4], or depending on the ligand relative concentration the value of K of complex-formation may change [5], consequently, the complex physical characteristics may change (for example, the absorption spectra). It is also possible that the ligand binding to NA may be much weaker, due to which the monotonous change

* E-mail: 1977poi@gmail.com

of the complex physical characteristics may be observed and even for NA high relative concentration (twice or much more higher than the ligand concentration) it will be impossible to attain the ligand completely bound state experimentally [3].

In all mentioned cases the physical parameters of the ligand completely bound state are determined with approximation, due to which the main parameters characterizing the complex-formation (K and n) are determined by a certain error.

In the present work the errors of the values of K and n have been determined via investigation of double-stranded NA–ligand complex absorption spectra change. This error is conditioned by absorption value determination error of the ligand completely bound state.

Materials and Methods. Double-stranded poly(G)·poly(C) polyribonucleotide, mitoxantrone (MTX) and ethidium bromide (EtBr) were used. All preparations were bought from "Sigma" and used without further purification. Measurements were carried out in water solutions, which contain 10 *mM* Tris buffer and 0.1 *M* NaCl, pH 7.4. The concentration of preparations was determined spectrophotometrically using the following extinction coefficients, $M^{-1}\cdot cm^{-1}$: ε_{260}(p)=7900 for poly(G)·poly(C), ε_{659}=25090 for MTX and ε_{480}=5850 for EtBr.

Titration curves were obtained using Unicam SP-8-100 spectrophotometer (England). Quartz cuvettes with 1 *cm* optic pathway length and hermetically closing Teflon caps were used. Spectrophotometric titration was carried out at the following concentrations: $C_0 \leq 10^{-4}$ *M* for EtBr and $C_0 \leq 3\cdot 10^{-6}$ *M* for MTX. It is known that for EtBr [6] and MTX [7] mentioned concentration interval and their self-association may be neglected. Absorption spectra were used for poly(G)·poly(C)–EtBr complex for calculations [3].

Results and Discussion. The interaction of MTX and EtBr with poly(G)·poly(C) polyribonucleotide in visible range has been studied through the ligand absorption spectra change, when poly(G)·poly(C)–ligand complex is formed. MTX and EtBr absorb electromagnetic waves in ultraviolet and visible ranges (λ<320 *nm*). Consequently, in the visible interval of the spectrum the ligand absorption spectrum change is conditioned only by ligand–polynucleotide interaction.

The behavior of MTX absorption spectra is presented in Fig. 1, when poly(G)·poly(C) was added to MTX solution. It is followed from Fig. 1, that in the case of MTX constant concentration when poly(G)·poly(C) was added to the solution a hypochromism and deviation to longer wavelengths (red shift) in the absorption maximum (λ=665 *nm*) take place. As it is seen from Fig. 1 an isosbestic point at λ=676 *nm* for the absorption spectra is observed. Consequently, in the mentioned external conditions poly(G)·poly(C) interacts with MTX by only one – semi-intercalation mode [1, 8]. In some relative concentrations of MTX, the complex absorption spectra have been changed insignificantly. Consequently, in this case the absorption size of completely bound ligand MTX (A_b) may be considered to be the absorption value. Usually, the completely bound state of the ligand is realized, when nucleic acid concentration exceeds by an order of the ligand concentration value. Simultaneously, taking into consideration that at addition of poly(G)·poly(C) to MTX solution A_b carries little change, it may be calculated from $A(1/C_p)$ dependence (C_p is poly(G)·poly(C) concentration) through linear extrapolation [9]. Sometimes ligand–nucleic acid bond is so weak (for example, for poly(G)·poly(C)–EtBr [3]) that even at twice high concentration of the ligand all

molecules are not in bound state. For all cases A_b is determined via $A(1/C_p)$ dependence linear extrapolation, when $1/C_p \to 0$, despite the fact that such determination of A_b, in turn, contains a certain error.

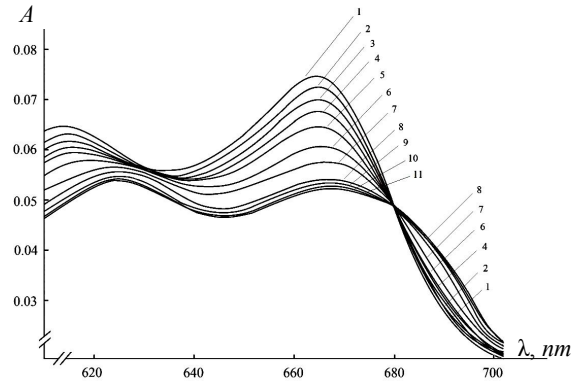

Fig. 1. Behavior of MTX absorption spectra change in visible range due to the complex-formation with poly(G)·poly(C) polyribonucleotide at 0.11 *M* NaCl ionic strength and 35°*C* temperature. As a result of titration the MTX concentration remains constant (C_0=2.9·10^{-6} *M*). The concentration of poly(G)·poly(C) C_p is: 1 – 0; 2 – 11.51·10^{-5}; 3 – 4.62·10^{-5}; 4 – 9.05·10^{-5}; 5 – 1.18·10^{-4}; 6 – 2.08·10^{-4}; 7 – 2.61·10^{-4}; 8 – 3.78·10^{-4}; 9 – 4.91·10^{-4}; 10 – 5.32·10^{-4}; 11 – 6.45·10^{-4} (*M*).

In the presented work for poly(G)·poly(C)–MTX and poly(G)·poly(C)–EtBr complexes the value of A_b is determined by different modes, then the binding isotherms are constructed [3, 9] as were described by McGhee, von Hippel formula [10]

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

where $r = C_b/C_p$, C_b is the bound ligand concentration. In Fig. 2 the optic density dependence on reciprocal value of the polynucleotide concentration is presented, which has been determined from the absorption spectra brought in Fig. 1 under the wavelength corresponding to the absorption maximum. The value of A_b was determined by linear extrapolation from $A(1/C_p)$ dependence (Fig. 2). As A_b the absorption value of the last measurement was taken for calculations (when there is no any change observed in the absorption spectrum) (A_b=0.0525) and the obtained value via linear extrapolation was chosen (A_b=0.049). The binding isotherm for poly(G)·poly(C)–MTX interaction was constructed for two mentioned values of A_b and the values of K and n were determined.

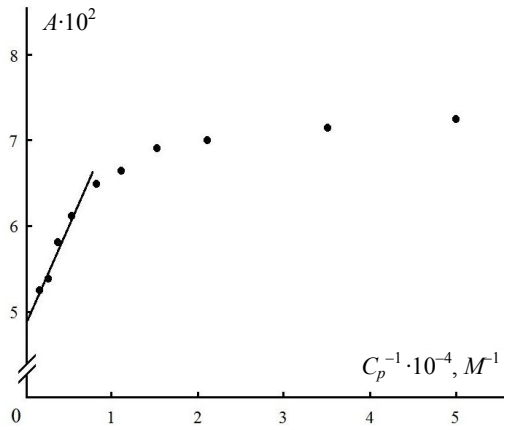

Fig. 2. Optic density (A_{665}) dependence of poly(G)× ×poly(C)–MTX complex under λ=665 *nm* wavelength on reciprocal value of polyribonucleotide concentration.

The interaction of poly(G)·poly(C)–EtBr was also studied by aforementioned mode, from the titration absorption spectra the dependence of $A(1/C_p)$ was constructed and the value of A_b through the linear extrapolation (I mode) and the last measurement (II mode) was determined. The values of K and n determined by these modes are presented in Table.

The ligand adsorption characterizing parameters to poly(G)·poly(C) polyribonucleotide at 0.11 M ionic strength and 35°C temperature

Ligand type	Determination mode of A_b	A_b	K, M^{-1}	n
Mitoxantrone	I	0.049	$(8.9 \pm 0.2)\ 10^4$	6.2 ± 0.2
	II	0.0525	$(9.6 \pm 0.3)\ 10^4$	6.0 ± 0.3
Ethidium bromide	I	0.237	$(0.9 \pm 0.2)\ 10^3$	2.2 ± 0.2
	II	0.245	$(1.1 \pm 0.2)\ 10^3$	2.1 ± 0.2

One can compare the values of K and n obtained by various determination modes for poly(G)·poly(C)–MTX and poly(G)·poly(C)–EtBr complexes (see Table). It is followed from Table, that through the linear extrapolation of A_b the value of K is less and the value of n remains unaltered in the error framework. Calculated values of the binding constant were compared to those in literature [1, 3, 6, 8]. The comparison showed that a good coincidence for K is observed when A_b is determined by linear extrapolation (I mode). What concerns to the values of K of the same complexes determined by the II mode, the difference does not exceed 10% (see Table). Consequently, if it is not necessary to determine precisely the value of K (usually it is necessary, if the binding thermodynamic parameters are determined though it), it is possible to take as A_b the absorption value at which the complex physical characteristics do not change at addition of biopolymer. By the same mode the value of K calculated from the absorption isotherm differs by 10%.

Received 02.11.2017

REFERENCES

1. **Awasthi P.** et al. Multispectroscopic Methods Reveal Different Modes of Interaction of Anticancer Drug Mitoxantrone with poly(dG-dC)-poly(dG-dC) and poly(dA-dT)-poly(dA-dT). // J. Photochem. Photobiol. B: Biology, 2013, v. 127, p. 78–87.
2. **Vardevanyan P.O.** et al. Complex-Formation of Ethidium Bromide with poly[d(A-T)]-poly[d(A-T)]. // J. Biol. Struct. Dyn., 2005, v. 22, p. 465–470.
3. **Babayan Y.** et al. Base Specificity in the Interaction of Ethidium Bromide with Synthetic Polynucleotides. // Nuclear Acids Res., 1987, v. 15, № 14, p. 5803–5812.
4. **Hakobyan S.N.** et al. Interaction of poly(I) with Ethidium Bromide. // Proceedings of the YSU. Chemical and Biological Sciences, 2017, v. 51, № 1, p. 38–43.
5. **Babayan Y.S.** et al. Thermostability of DNA Complexes with Mitoxantrone at Small Fillings. // Biophys. Reviews and Letters, 2017, v. 12, № 3, p. 1–9.
6. **Vardevanyan P.O.** et al. Ethidium Bromide Interaction with poly(G). // Biophys. Reviews and Letters, 2014, v. 9, № 3, p. 239–247.
7. **Enache M.**, **Volanschi E.** Spectral Characterization of Self-Association of Antitumor Drug Mitoxantrone. // Rev. Roum. Chim., 2010, v. 55, № 4, p. 255–262.
8. **Agarwal Sh.** et al. Spectroscopic Studies of the Effects of Anticancer Drug Mitoxantrone Interaction with Calf-Thymus DNA. // J. Photochem. Photobiol. B: Biology, 2013, v. 120, № 5, p. 177–182.
9. **Hakobyan S.N.** Error in Determination of Binding Thermodynamic Parameters Appeared Due to Adsorption Linear Isotherm Description. // Proceedings of the YSU. Chemical and Biological Sciences, 2015, № 2, p. 40–44.
10. **McGhee J.D., von Hippel P.H.** Theoretical Aspects of DNA-Protein Interactions. // J. Mol. Biol., 1974, v. 84, № 3, p. 469–489.