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MITOXANTRONE INTERACTION WITH POLY(G)

A. T. KARAPETYAN[1] *, Yu. S. BABAYAN[1] **, M. A. TOROSYAN[1], M. A. SHAHINYAN[2]

[1] *Chair of Physics YSUAC, Armenia*
[2] *Chair of Biophysics YSU, Armenia*

In the present work the thermodynamic studies of mitoxantrone complex-formation with four-stranded poly(G) have been carried out. The binding thermodynamic parameters, the binding constant K and the number of nucleotides corresponding to one binding site n, were determined. It was shown that mitoxantrone is bound stronger with four-stranded poly(G), then it is bound with double-stranded helix. It was also shown that due to difficulties of the intercalation of mitoxantrone molecules into four-stranded structure is bound mainly by external binding mode with $[poly(G)]_4$ and at the interaction saturation mitoxantrone bound to much more guanines ($n \cong 10$) per one molecule.

***Keywords*:** four-stranded structure, $[poly(G)]_4$, mitoxantrone, complex-formation, absorption spectrum, thermodynamic binding parameters.

Investigations show that besides single-stranded and double-stranded (ds) helical structures, guanine-rich nucleic acids may form intramolecular four-stranded (fs) structures (G-quadraplex) [1–4], the stability of them is mainly conditioned by Na^+ and K^+ ions bound by specific mode [4–6].

Fs structures represent a big interest due to their biological role particularly they may be a target for anti cancer drugs [7–9].

Fs structures may be formed *in vivo*, particularly in areas of chromosome telomeres and promoters, where large amount of guanines is accumulated [5, 7, 8]. In vitro investigations show that a classical intercalator ethidium bromide (EtBr) facilitates G-quadraplex structure formation and increases the melting temperature [10]. Netropsyn and distamycin anti-tumorous compounds, interacting with DNA by non-intercalation mechanism and binding with AT-rich regions, form complexes with G-quadraplex in the minor groove and the binding constant estimated for this interaction is three orders lower ($\sim 8 \cdot 10^4\ M^{-1}$) than that of double-helical DNA. Complex-formation is exothermic process, as a result of which the big exothermic binding enthalpy (~ -10.8 *kcal*/*mol*) is observed [11].

The interaction of classical intercalator EtBr with $[poly(G)]_4$ has been studied, and it was shown that the system complex-formation thermodynamic parameter (ΔH, ΔS, K) differs significantly from the respective parameter of EtBr

* E-mail: karapetyanat@gmail.com ** E-mail: babayanyura@gmail.com

complex-formation with double-helical structure. Particularly, at the interaction saturation one molecule of EtBr binds to n=5 guanines of $[poly(G)]_4$ [12], while for EtBr interaction with double helix n=4 [13, 14].

Mitoxantrone (MTX) is an anti-tumor drug, which has a big application in the therapy of different cancers [15, 16].

It was proven that MTX effect is realized due to the binding with cellular DNA [17], but the existence of one or other type of any binding mode is arguable. Particularly it was not studied MTX interaction with fs structure. Spectroscopic investigations have shown that MTX interacts with double-helical nucleic acids by partial intercalation mode [18].

The present work is devoted to the thermodynamic studies of MTX complex-formation with $[poly(G)]_4$. The experimental data obtained in UV interval of absorption spectrum make it possible to determine the binding thermodynamic parameters such as the binding constant (K) and the number of nucleotides corresponding to one binding site (n).

Materials and Methods. Poly(G) ("Sigma", USA), MTX ("Farmatalia"), NaCl and Tris buffer were used in experiments. The concentrations of poly(G) and MTX were determined spectrophotometrically using the following extinction coefficient values: ε_{260}=9900 $M^{-1}\cdot cm^{-1}$ for poly(G), ε_{665}=20900 $M^{-1}\cdot cm^{-1}$ for MTX. The interaction of poly(G) with MTX was studied in 0.1 M NaCl, 0.01 M Tris buffer, pH 7.5, temperature interval was equal to 290.15–310.15 K. It should be mentioned that in external conditions poly(G) forms mainly fs structure [6].

For obtaining absorption spectra, Unicam SP8-100 spectrophotometer (UK) was used.

MTX maximal concentration that was used in the experiment was $C_0 \approx 5\cdot 10^{-6}$ M. MTX self association may be neglected at the mentioned concentrations [19]. The solutions were prepared by double distilled and deionized water.

Results and Discussion. The behavior of absorption spectra change of MTX complexes with $[poly(G)]_4$ in visible interval has been studied. It is known that in the visible interval nucleic acids do not absorb, that is why the change of absorption spectra of MTX in this interval is conditioned by only complex-formation with poly(G). It is obvious from Fig. 1, that at the addition of $[poly(G)]_4$ to MTX solution a hypochromic effect and a shit of the curve maximum to the long wavelength side are observed. In the studied ionic strength and temperature interval an isosbestic point at λ=676 nm is clearly appeared on absorption spectra, which remains unchanged until a certain concentration of MTX in the solution. Consequently in the mentioned external conditions MTX–$[poly(G)]_4$ bound state is observed by only one type of spectroscopic absorption spectra. It

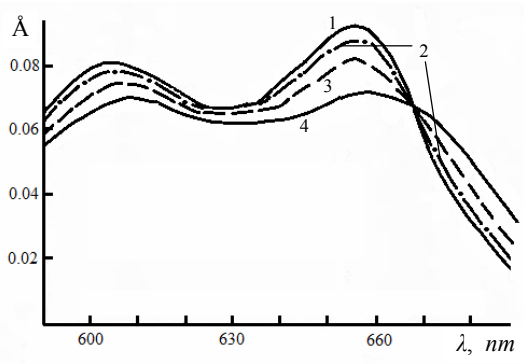

Fig. 1. Mitoxantrone absorption spectra change at the interaction with $[poly(G)]_4$ at 300.15 K temperature. During titration MTX concentration remains constant ($4.1\cdot 10^{-6}$ M); $[poly(G)]_4$ concentration, M: 1 – 0; 2 – $7.4\cdot 10^{-5}$; 3 – $1.72\cdot 10^{-4}$; 4 – $4.2\cdot 10^{-4}$.

is followed from experimental data that beginning from the certain values of C_p/C_0 relative concentration (C_p/C_0=4.5), where C_p is $[poly(G)]_4$ molar concentration by guanine, C_0 is MTX molar concentration the absorption spectra of complexes stop altering: all MTX molecules in the solution are in bound state.

The main quantitative parameters characterizing the complex-formation, K and n, were determined from absorption spectra. From the presented spectra free (C_f) and bound (C_b) MTX concentrations were determined by formulae:

$$\frac{C_f}{C_0}=\frac{A-A_b}{A_f-A_b}, \qquad C_b=C_0-C_f, \tag{1}$$

where A_f and A_b are free and bound MTX absorption values respectively at λ=665 *nm*, which corresponds to spectrum absorption maximum; A is absorption value of MTX–$[poly(G)]_4$ complexes in intermediate states. A_b is determined via linear extrapolation of $A=f\left(1/C_p\right)$ dependence, when $1/C_p \to 0$.

Taking into account the values of C_f and C_b calculated via formula (1), the binding isotherm of MTX with $[poly(G)]_4$ in Scatchard coordinates was constructed (r/C_f dependence on r, where $r=C_b/C_p$).

The binding isotherms were described by (2), which describes the binding of biologically active compounds with nucleic acids more precisely [20]:

$$\frac{r}{C_f}=K\left(1-nr\right)\left[\frac{1-nr}{1-\left(n-1\right)r}\right]^{n-1}. \tag{2}$$

In the formula (2) n is equal to number of guanines, with which MTX one molecule binds at the interaction saturation.

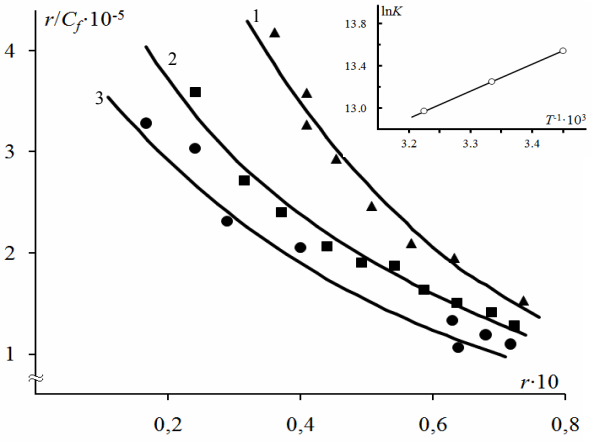

Fig. 2. The binding isotherms of mitoxantron with $[poly(G)]_4$ at temperatures:
1 – 290.15 *K*;
2 – 300.15 *K*;
3 – 310.15 *K*.
In the right angle ln *K* dependence on 1/T, calculated from obtained isotherms is presented.

On the Fig. 2 the binding isotherms of $[poly(G)]_4$–MTX complexes are presented at 0.11 *M* ionic strength of the solution and 290.15, 300.15, 310.15 *K* temperatures. The solid lines present the theoretical curve passed throughout experimental points according to formula (2) by the smallest square method and the values of K and n of $[poly(G)]_4$–MTX complexes at three different temperatures were determined (see Table).

Using the obtained values of K it is possible to calculate Gibbs free energy change, which takes place as a consequence of complex-formation:

$$\Delta G = -RT \ln K, \tag{3}$$

where R is gas universal constant.

Thermodynamic parameters of [poly(G)]$_4$–MTX interaction

Polynucleotide	T, K	K, 10^5 M^{-1}	$-\Delta G$, *kcal/mol*	n
poly(G)	290.15	7.75	7.79	10
	300.15	5.8	7.88	10
	310.15	4.34	7.98	10

The changes of entropy ΔS and enthalpy ΔH may be determined from Eq. (4)

$$\ln K = -\frac{\Delta H}{R} \cdot \frac{1}{T} + \frac{\Delta S}{R}. \tag{4}$$

According to (4), if ln$K$ dependence on $1/T$ is linear, the tangent of direct line angle with abscise axis is equal to $-\frac{\Delta H}{R}$ and the ordinate of direct line with ordinate axis is equal to $\frac{\Delta S}{R}$.

From the obtained data using (2), ln$K$ dependence on $1/T$ was constructed (Fig. 2, right angle). A direct line was passed by average square method and the values of ΔS and ΔH were determined.

Calculations show that for [poly(G)]$_4$–MTX complexes $\Delta H \approx 5.0$ *kcal / mol* and $\Delta S \approx 9.6$ *cal/mol·K*. From the values of experimentally determined thermodynamic parameters it is followed that MTX interaction with fs structure differs significantly from MTX interaction with ds RNA. Most probably at the mentioned ionic strength of the solution, [poly(G)]$_4$ has very dense twisting, as a result of which the intercalation of MTX molecules becomes sufficiently difficult.

Calculations also show that for MTX interaction with [poly(G)]$_4$ $n \cong 10$ (see Table). It is known that at MTX binding with ds poly(G)·poly(C) ribonucleotide at $T = 308.15$ K temperature $K \cong 10^5$ M^{-1} and $n \cong 6$ [19]. Consequently in spite of ds helical ribonucleotide, in the case of [poly(G)]$_4$ MTX one molecule binds to much more guanines when the interaction saturates. It means that despite the fact, that each strand of [poly(G)]$_4$ in the solution forms a structure belonging to A-family and characterizing by special conformation parameters, its fs structure certainly interferes the intercalation process, in result of which MTX one molecule binds to approximately 10 guanines instead of 6 at the interaction saturation. Most probably, at MTX interaction with fs poly(G) ligand molecules even partially do not insert into the planes of base pairs in the way as it occurs at ds nucleic acids. MTX binds to negatively charged phosphate groups by its side groups that are positively charged at neutral pH, as it takes place at many low-molecular compounds containing long side groups [21].

It is followed from Table, that at MTX binding with fs poly(G) and ds poly(G)·poly(C) the values of ΔG do not change significantly in error frame consequently, it may be considered to be constant $\Delta H \approx -5.0$ *kcal/mol* [19]. The

obtained value for $[poly(G)]_4$–MTX complex exceeds the obtained value for partial intercalation ($\Delta H \approx -(2–3)$ *kcal/mol*) by its absolute value [19], but is lower from ΔH value obtained at ligand binding by intercalation mode ($\Delta H \approx -(7–8)$ *kcal/mol*) [22].

Concerning to entropy change it should be mentioned that ΔS=9.6 *cal/mol·K* for fs $[poly(G)]_4$–MTX complex and ΔS=12.1 *cal/mol·K* for ds poly(G)·poly(C)–MTX complex [19].

Consequently generalizing the values of changes of system thermodynamic parameters due to complex-formation of MTX with fs poly(G), it may be assumed that MTX binds stronger with fs poly(G). Due to this binding fs structure change is less compared to double helix poly(G). It is probable that in result of compact arrangement of strands within fs structure, the intercalation of MTX molecules is impeded, which in its turn results in mainly external binding of MTX molecules with $[poly(G)]_4$ and binding with much more guanines ($n \cong 10$) of one MTX molecule at the interaction saturation.

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