

EVALUATION OF BINDING PARAMETERS OF INTERCALATORS  
METHYLENE BLUE, ETHIDIUM BROMIDE AND ACRIDINE ORANGE  
WITH NUCLEIC ACIDS

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Comparison of values of the binding constant ( $K$ ) and number of binding sites ( $n$ ) of methylene blue, ethidium bromide and acridine orange with DNA and RNA has been carried out. It was revealed that values of these parameters do not depend on nucleic acid type. It was shown that in the case of binding of each of these ligands to double-stranded (ds) DNA and ds-RNA the values of  $K$  and  $n$  are practically similar.

**Keywords:** DNA, binding strong mode, weak mode, binding parameters.

**Introduction.** There are numerous works devoted to investigation of different aspects of interaction of low molecular compounds – ligands with double-stranded (ds) and single-stranded (ss) DNA, while binding of ligands to RNA (ds or ss) is less studied. Though, nowadays a positive shift in this direction is observed, since it was clear that RNA, along with DNA plays a key role in various processes of a cell cycle [1–3]. Connected with this, the role of understanding of RNA participation not only in expression of genes, but also in enzymatic processes enhances [4, 5]. Interest to RNA is conditioned by the fact, that it can be used in diagnosing of different diseases [6–8]. RNA has a big structural variety due to its ability to form secondary and tertiary structures [9–12]. However, functional aspects of RNA in ds-state do not allow to consider as totally established, that is why a necessity of various studies in this way emerges. From this point of view one of the approaches may be the comparative study of ds-DNA and ds-RNA complex-formation with different intercalators. In this case such synthetic ds polyribonucleotides are appropriate and do not form structures of higher order. One of such polyribonucleotides is poly(rA)-poly(rU), which is close to natural RNA by its structure [13].

Based on the comparison of binding constant  $K$  and number of base pairs  $n$  per binding site of methylene blue (MB), ethidium bromide (EtBr) and acridine orange (AO) with ds-DNA and ds-RNA has been carried out in this work.

**Materials and Methods.** Calf Thymus DNA, poly(rA)-poly(rU) (“Sigma”), MB and AO (“Sigma”, USA), EtBr (“Serva”, Germany) were used in experiments. Binding of ligands to nucleic acids (NA) was studied in water solution containing 0.015 M NaCl and 0.0015 M Na-citrate, with ionic strength 20 mM, pH≈7.0.

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Concentrations of DNA, poly(rA)-poly(rU), MB, EtBr and AO were determined spectrophotometrically using the following extinction coefficients ( $M^{-1}\cdot cm^{-1}$ ):  $\epsilon_{260}(P)=6600$  for Calf Thymus DNA,  $\epsilon_{260}(P)=7140$  for poly(rA)-poly(rU),  $\epsilon_{664}=76000$  for MB,  $\epsilon_{480}=5800$  for EtBr and  $\epsilon_{490}=35000$  for AO. Spectrophotometric titration was carried out on spectrophotometer PYE Unicam SP-8-100 (England), spectrofluorimetric studies were carried out on spectrofluorimeter Varian Cary Eclipse Fluorescence Spectrophotometer (Australia). Measurements were carried out in thermostating cells using quartz cuvettes with 1 cm optic pathway, 1 mL volume and hermetically closing caps.

**Results and Discussion.** Studies have been carried out by absorption and fluorescence spectroscopy methods. In all measurements concentration of ligands remains constant, concentration of ligands enhances in samples up to relation  $r = [NA] / [\text{ligand}]$ ,  $0 \leq r \leq 30$ . Along with complex-formation of the mentioned ligands with nucleic acids a change in absorption (decreasing in maxima and shift to longer wavelengths) and fluorescence (intensity increasing in the case of EtBr and AO and decreasing in the case of MB) spectra takes place in long wavelength region, in which DNA and RNA do not absorb and do not fluoresce. Consequently, these changes indicate the binding of ligands to RNA, as to DNA. This fact permits carrying out a qualitative analysis and calculating the concentration of bound molecules of ligands, based on which the binding curve of ligands with nucleic acids can be constructed. By virtue of absorption and fluorescence spectra of complexes of ligands with nucleic acids, the binding isotherms were constructed in Scatchard's coordinates according to generally accepted method. From the adsorption isotherms the binding parameters were determined as it is described in [14, 15].

Estimated changes of MB, EtBr and AO binding parameters with polynucleotides are presented in Tabs. 1 and 2. It is obvious from the table data that each of the mentioned intercalators shows almost similar affinity to ds-RNA and ds-DNA. This fact indicates that for binding ligands with nucleic acids the physical forces stabilizing complexes are determining, while chemical composition of nucleic acids (presence of additional oxygen atom in ribose and absence of methyl group in uracil) practically does not affect on this affinity.

The obtained data reveal that EtBr binding constant to ds-DNA or ds-RNA by the strong mode is higher, by an order, than in the case of AO as well as MB despite the fact that both AO and EtBr bind by intercalation and MB binds by semi-intercalation modes [15, 16].

Table 1

*Values of K and n at binding of MB, EtBr and AO with ds-RNA at 20 mM ionic strength of solution, pH $\approx$ 7.0, t=25°C*

Binding modes	ds-RNA–MB		ds-RNA–EtBr		ds-RNA–AO	
	$K, 10^5 M^{-1}$	$n$	$K, 10^5 M^{-1}$	$n$	$K, 10^5 M^{-1}$	$n$
strong	$5.0 \pm 1.0$	3.0–4.0	$25.0 \pm 2.0$	5.0–6.0	$1.5 \pm 2.0$	3.0–4.0
weak	$1.50 \pm 0.02$	1.5–2.0	$0.45 \pm 0.05$	1.5–2.0	$0.20 \pm 0.05$	1.5–2.0

At weak binding mode to ds-DNA and RNA the mentioned ligands show similar affinity, which is conditioned by electrostatic nature of this mode, which in turn is realized due to the binding of molecules of ligands with phosphate groups of

nucleic acids. The obtained results also indicate that ligands binding to DNA can modulate cellular processes through binding to RNA.

Table 2

Values of  $K$  and  $n$  at binding of MB, EtBr and AO with ds-DNA at 20 mM ionic strength of solution,  $pH \approx 7.0$ ,  $t = 25^\circ C$

Binding modes	ds-DNA–MB		ds-DNA–EtBr		ds-DNA–AO	
	$K, 10^5 M^{-1}$	$n$	$K, 10^5 M^{-1}$	$n$	$K, 10^5 M^{-1}$	$n$
strong	$6.5 \pm 1.0$	4.0	$20.0 \pm 5.0$	6.0	$2.0 \pm 0.5$	5.0–6.0
weak	$1.50 \pm 0.05$	2.0	$0.20 \pm 0.05$	1.5	$0.50 \pm 0.05$	2.0

**Conclusion.** Thus, the obtained data indicate that modulation of cellular processes may be realized via RNA complex-formation with such ligands, for which the main target is ds-DNA. This fact acquires an applicative value and may serve as a basis for screening of biologically active compounds with wider spectrum of action on nucleic acids.

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