

MELTING PARAMETERS OF DNA COMPLEXES
WITH SEVERAL INTERCALATORS

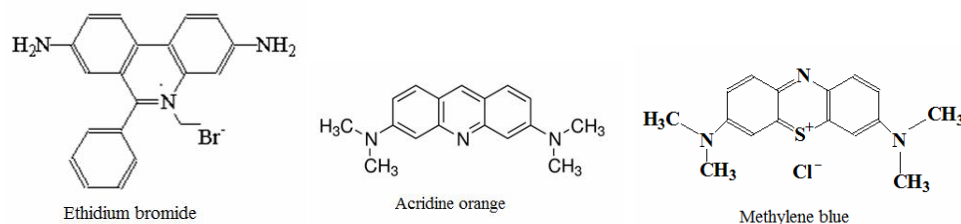
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Comparative study of DNA complexes with acridine orange (AO), ethidium bromide (EtBr) and methylene blue (MB) has been carried out at 0.02 M ionic strength of solution in $0 < r \leq 0.33$, $r = [\text{ligand}] / [\text{DNA}]$ range. The values of changes of melting parameters ($\delta(1/T_m)$ and $\delta(\Delta T/T_m^2)$) of these complexes were determined. From the obtained data it was revealed that the dependence of $\delta(1/T_m)$ enhances in the whole interval of change of r for three ligands, while the dependence of $\delta(\Delta T/T_m^2)$ rises at relatively low values of r and comes out to plateau in the case of AO and MB; in the case of EtBr this dependence takes bell-like form.

Keywords: DNA, acridine orange, ethidium bromide, intercalation, methylene blue, melting curve.

Introduction. Studies of interaction of ligands-intercalators with DNA represent a big interest since these compounds show some important properties. Particularly, acridine compounds and among them acridine orange (AO) (see Scheme) have anti-bacterial, mutagen activity [1–6].



Scheme. Chemical structures of intercalators EtBr, AO and MB.

AO shows metachromism (dye color changes, usually to red, when it binds to certain cellular components), which conditions its wide application for analytic aims, particularly in cytochemistry [2]. AO binds to those structures of a cell, in which nucleic acids exist, for example in chromosomes, and invokes structural damages in them [2]. It was revealed that this ligand preferably binds to double-stranded (ds) DNA by intercalation mode, moreover, it can bind with single-stranded (ss) DNA as well, but the mechanism of the latter case is not established yet [2]. AO is a photosensibilizer and along with another acridine preparation methylene blue (MB) is used in photodynamic therapy

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(PDT) [1–6]. Recent studies have indicated that ligands, belonging to intercalators or groove binding preparations, binding to DNA can bind by more than one mode, in consequence of which their effect on DNA structure and function may be radically opposite [7–11]. Particularly, both ethidium bromide (EtBr) and AO may bind not only with ds- but also with ss-DNA consequently, depending on affinity to these regions of DNA, they can stabilize or destabilize its native structure [7–11]. MB binding molecular mechanisms to DNA continue to remain an object of discussion, despite the fact that, literature data indicate the intercalation mechanism of this ligand to DNA [9].

Materials and Methods. Calf Thymus DNA (“Sigma”, USA), AO and MB (“Sigma”, USA), EtBr (“Serva”, Germany), NaCl, Na-citrate, EDTA (ethylenediaminetetraacetate, chemically pure) were used without additional purification in experiments. Concentrations of DNA and ligands were determined spectrophotometrically using the following values of extinction: $\varepsilon_{260}=6600 M^{-1}cm^{-1}$ for DNA; $\varepsilon_{490}=35000 M^{-1}cm^{-1}$ for AO; $\varepsilon_{664}=76000 M^{-1}cm^{-1}$ for MB; $\varepsilon_{480}=5800 M^{-1}cm^{-1}$ for EtBr. Measurements were realized at $T=298 K$ and pH 6.96, the ionic strength of the solution $\mu=0.02 M$.

Melting of DNA complexes with ligands as well as spectrophotometric measurements of absorption of preparation of solutions were realized on PYE Unicam-SP8-100 spectrophotometer (England). Heating of complexes were carried out through program device SP 876 Series 2. For spectrophotometric measurements quartz cuvettes with hermetic closing Teflon caps, 3 mL volume and 1 cm optic pathway length were used. Melting was carried out at $\lambda=260 nm$, which corresponds to DNA maximal absorption. Values of complexes absorptions at melting were recorded on PC monitor via program elaborated in LabVIEW medium. Melting curves of complexes were constructed as described in [10].

DNA complexes with ligands were prepared taking into account the concentration ratio $r=[\text{ligand}]/[\text{DNA}]$. Values of r change in $0 < r \leq 0.33$ interval. Error of experimental data does not exceed 5%.

Results and Discussion. From melting curves of DNA and its complexes with the mentioned ligands at r concentration ratio, it is obtained that the melting curves of complexes are shifted to high temperatures compared with DNA curve by enhancement of values of r (data are not presented). This fact indicates that AO (as well as EtBr and MB) binding to DNA stabilizes its native ds structure. In the case of AO and EtBr it is

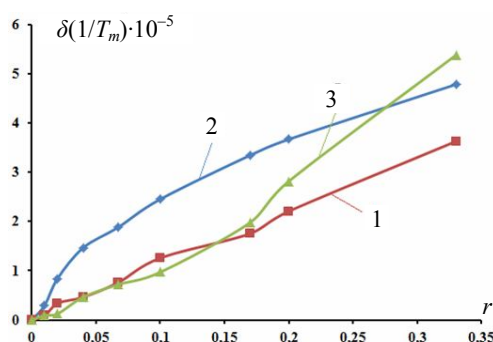


Fig. 1. $\Delta(1/T_m)$ dependence curves on r of DNA–AO (1); DNA–EtBr (2); DNA–MB (3) complexes at 0.02 M ionic strength of the solution, pH 6.96.

the result of preferable binding with ds-DNA by intercalation mode, in the case of MB the main binding mode is semi-intercalation. For revelation of binding peculiarities with DNA the determination of melting parameter changes depending on $r-\Delta(1/T_m)$ and $\Delta(\Delta T/T_m^2)$ is informative, (where $\Delta(1/T_m)=1/T_0-1/T_m$, T_0 and T_m are melting temperatures of DNA and DNA–ligand complexes respectively, $\Delta(\Delta T/T_m^2)=\Delta T/T_m^2-\Delta T_0/T_0^2$, ΔT_0 and ΔT_m are melting interval widths of DNA and DNA–ligand complexes).

Dependence curves of $\Delta(1/T_m)$ on r for DNA–AO, DNA–EtBr, DNA–MB complexes are presented in Fig. 1. It is obvious from Fig. 1, that $\Delta(1/T_m)$ dependence curves on r rise within all interval of r change for all complexes. This fact is the result of stabilizing effect of these ligands on DNA ds structure. These data are in correspondence

with the results obtained in the statements [7, 10]. It should be mentioned that it is theoretically established [11] and experimentally shown [7, 10] that classical intercalator EtBr binds to DNA by several modes simultaneously, which is reflected on the melting parameters of its complexes with DNA. Particularly, it is theoretically calculated that $\delta(1/T_m)$ increases up to certain values of r , then with the further enhancement of EtBr concentration in the solution, $\delta(1/T_m)$ starts decreasing [11]. It was shown that this effect is conditioned by the fact that EtBr being a stabilizer of DNA ds structure, at high concentrations begins to show an affinity to its ss regions, i.e. transforms from DNA native structure stabilizer to destabilizer [10, 11]. However, in interval $0 < r \leq 0.33$ in the case of DNA complexes with the mentioned three ligands $\delta(1/T_m)$ dependence curves increase with r , which indicates that in these conditions these ligands perform only stabilizing effect on DNA ds structure, despite the fact that EtBr and AO can bind to ss-DNA as well [2].

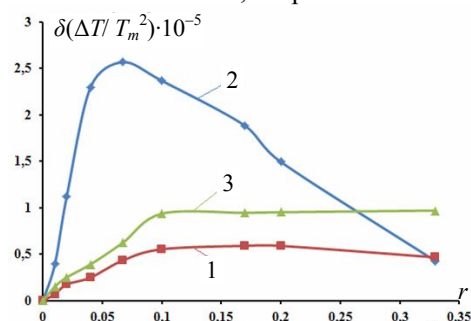


Fig. 2. Dependence curves of $\delta(\Delta T/T_m^2)$ on r for DNA-AO (1); DNA-EtBr (2); DNA-MB (3) complexes at 0.02 M ionic strength of the solution, pH 6.96.

Change of another parameter $\delta(\Delta T/T_m^2)$ on r (Fig. 2) enhances with increasing of values of r up to $r \approx 0.1$ in the case of AO and MB, with the further enhancement of concentration of these ligands in the solution, the dependence curve of $\delta(\Delta T/T_m^2)$ on r comes out on plateau. At the same time in the case of DNA-EtBr complexes this dependence is bell-like. Enhancement of values of $\delta(\Delta T/T_m^2)$ at low concentrations of ligands in the solution is conditioned by preferable binding with ds-DNA by intercalation mode in the cases of AO and EtBr and by semi-intercalation mode in the case of MB. This fact is in correspondence to results obtained in the statement [7]. Enhancement of values of $\delta(\Delta T/T_m^2)$ on r is conditioned by the fact that along with DNA melting the bound molecules of AO and EtBr by intercalation mode and MB bound by semi-intercalation mode are redistributed from denatured to still non-denatured regions, which result in widening of the melting interval of complexes. However, at $r \approx 0.1$ the intercalation centers for AO and semi-intercalation centers for MB on ds-DNA are saturated, in the result of which redistribution of bound ligand molecules by these modes stops. In the case of the absence of these binding modes the dependence curve $\delta(\Delta T/T_m^2)$ on r should come out on plateau, as it takes place for DNA-AO and DNA-MB complexes (Fig. 2, curves 1 and 3). Moreover, in this case the dependence curve of $\delta(1/T_m)$ for these ligands on r should come out on plateau as well which, by the way, does not occur. This fact indicates the existence of one more binding mode, which does not invoke changes of ΔT values of the complexes. The majority of ligands, including EtBr, MB and AO, can also bind to DNA by electrostatic mode, because they are in cationic form in solution [12–15]. Consequently we assume that at 0.02 M ionic strength of the solution AO and MB bind to DNA by electrostatic mode along with intercalation and semi-intercalation ones. At the same time it has been shown [7, 11], that besides the intercalation and electrostatic modes EtBr binds to DNA by semi-intercalation mode, which results in acquiring of bell-like form of $\delta(\Delta T/T_m^2)$ dependence curve on r . It should be mentioned that EtBr binds to both ds- and ss-DNA by semi-intercalation mode [7, 10, 11]. In the case of AO the semi-intercalation in the case of MB intercalation modes are not revealed, despite the fact that MB and AO are analogs and differ from each other by

the fact that MB contains *S* atom in acridine ring (see Scheme). Most probably, the positive charge of *S* atom is the factor, which interferes the entire intercalation of this ligand into DNA at relatively high ionic strengths of solution. At the same time, it was shown that at ten-fold decreasing of the solution ionic strength (at 0.002 *M*) along with semi-intercalation and electrostatic modes, MB binds to ds-DNA by one more intercalation mode, due to which the dependence curve of $\delta(\Delta T/T_m^2)$ on *r* becomes bell-like [7].

Conclusion. The obtained data indicate that AO, as EtBr, being intercalator stabilizes ds structure of DNA, moreover, this effect is conditioned both by intercalation and by electrostatic modes of the binding; MB also stabilizes ds structure of DNA and binds to it by semi-intercalation and electrostatic modes. Moreover, the stabilizing effect of MB on ds structure of DNA is bigger than that of AO. This fact indicates that in the case of AO contribution of electrostatic mode to ds-DNA stabilization is much weaker than in the case of MB. In the case of EtBr, DNA ds structure stabilization is also revealed (Fig. 1, curve 2), despite the fact that semi-intercalation type of binding with DNA ss structures becomes more relevant and induces destabilization of its native structure.

This work was supported by the RA MES State Committee of Science, in the frame of research project № 15T-1F105.

Received 24.09.2016

REFERENCES

1. **Kusuzaki K.** et al. Clinical Outcome of a Novel Photodynamic Therapy Technique Using Acridine Orange for Synovial Sarcomas. // *Photochemistry and Photobiology*, 2005, v. 81, p. 705–709.
2. **Kapuscinski J., Darzynkiewicz Z., Melamed M.R.** Interactions of Acridine Orange with Nucleic Acids. // *Biochemical Pharmacology*, 1983, v. 32, № 24, p. 3679–3693.
3. **Lantukh Yu.D., Pashkikh S.N.** et al. Time-Dependent Holographic Recording in Biopolymer DNA–Acridine Orange Films. // *Optics and Spectroscopy*, 2013, v. 114, № 2, p. 283–287.
4. **Lantukh Yu.D., Pashkeich S.N.** et al. Spectroscopic Properties of DNA–Acridine Orange Biopolymer Films. // *Optics and Spectroscopy*, 2011, v. 110, № 6, p. 880–884.
5. **Lai Sh.** et al. Fluorometric Determination of DNA Using Nano-SiO₂ Particles as an Effective Dispersant and Stabilizer for Acridine Orange. // *Microchim. Acta*, 2007, v. 156, p. 225–230.
6. **Rawtani D., Agrawai Y.K.** A Study of the Behavior of HNT with DNA Intercalator Acridine Orange. // *BioNanoSci.*, 2013, v. 3, p. 52–57.
7. **Vardevanyan P.O.** et al. Thermodynamic Analysis of DNA Complexes with Methylene Blue, Ethidium Bromide and Hoechst 33258. // *Biopolymers and Cell*, 2013, v. 29, № 6, p. 515–520.
8. **Rohs R., Sklenar H.** Methylene Blue Binding to DNA with Alternating AT Base Sequence: Minor Groove Binding is Favored over Intercalation. // *J. Biomol. Struct. & Dyn.*, 2004, v. 21, p. 699–711.
9. **Hossain M., Giri P., Kumar G.S.** DNA Intercalation by Quinacrine and Methylene Blue: a Comparative Binding and Thermodynamic Characterization Study. // *DNA and Cell Biology*, 2008, v. 27, № 2, p. 81–90.
10. **Vardevanyan P.O.** et al. Study of Ethidium Bromide Interaction Peruliarities with DNA. // *Exp. and Mol. Medicine*, 2001, v. 33, № 4, p. 205–208.
11. **Karapetian A.T., Grigoryan Z.A.** et al. Theoretical Treatment of Helix-Coil Transition of Complexes DNA with Two Different Ligands Having Different Binding Parameters. // *J. Biomol. Struct. Dyn.*, 2016, v. 34, № 1, p. 198–202.
12. **Gowda K.R.S., Blessy B.M., Sadhamani C.N., Naik H.S.B.** Mechanism of DNA Binding and Cleavage. // *Biomedicine and Biotechnology*, 2014, v. 2, № 1, p. 1–9.
13. **Lane A.N., Jenkins T.C.** Thermodynamics of Nucleic Acids and Their Interactions with Ligands. // *Q. Rev. Biophys.*, 2000, v. 33, № 3, p. 255–306.
14. **Nafisi Sh., Saboury A.A.** et al. Stability and Structural Features of DNA Intercalation with Ethidium Bromide, Acridine Orange and Methylene Blue. // *Journal of Molecular Structure*, 2007, v. 827, p. 35–43.
15. **Xinhui H., Wang Q., Pingang H., Fang Y.** Spectroelectrochemistry Study on the Electrochemical Reduction of Ethidium Bromide. // *Analytical Sciences*, 2002, v. 18, p. 645–650.