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EFFECT OF DNA GC-CONTENT ON INTERACTION WITH METHYLENE BLUE

L. A. HAMBARDZUMYAN*

Chair of Biophysics YSU, Armenia

The melting of methylene blue (MB) complexes of DNA with different GC-content sequences at 0.02 *M* ionic strength of the solution has been carried out. It was shown that the change of melting interval width of MB complexes with DNA increases depending on GC-content enhancement within DNA sequences. It was revealed that MB is bound to DNA by semi-intercalation mode.

***Keywords*:** DNA, GC-content, methylene blue, binding semi-intercalation mode.

Introduction. Nowadays the interaction of non-covalently binding ligands with DNA remains an actual topic, since these compounds may influence on biological processes taking place in the cells. Among immediately binding to DNA ligands intercalators present a special importance, particularly, acridine dyes, including ethidium bromide (EtBr), actinomycin D (ACD), methylene blue (MB) etc., because these compounds binding to DNA effect on its biological functions [1]. MB is one of the widely spread and studied intercalators due to the fact that this ligand is a photosensibilizer and is applied in medicine especially in photodynamic therapy. It is connected with the fact that MB is bound to macromolecules (nucleic acids, proteins as well as lipids) and absorbing light quantum energy transmits to photoactive state. Photo activated molecules of MB interact with molecular oxygen which due to this transmits to singlet active state invoking damages in macromolecules [2–6]. Based on these properties of MB it is applied at the treatment of many diseases: malaria, different types of tumors etc. It is also revealed that MB may depress HIV, viruses of hepatitis B and C in human blood plasma [7]. Moreover, MB interaction mechanisms with DNA are not revealed entirely and many questions remain arguable. That is why the studies of MB interaction with DNA are very interesting.

The goal of this work is to study MB interaction mechanisms with DNA depending on its nucleotide content.

Materials and Methods. In the present work DNA (u.p.) of *Calf Thymus* with 42% average GC-content, *Clostridium perfringens* – 32%, *Micrococcus lysodecticus* – 72%, MB from "Aldrich" (USA), NaCl, Na-citrate (u.p.) were used.

* E-mail: lilit8808@mail.ru

All preparations were used without further purification. Concentrations of used preparations were determined by absorption method using the following extinction coefficients: ε_{260}=6600 $M^{-1}cm^{-1}$ for *Calf Thymus* DNA and *M. lysodecticus,* ε_{260}=7400 $M^{-1}cm^{-1}$ for *Cl. perfringens* DNA, ε_{664}=76000 $M^{-1}cm^{-1}$ for MB. The studies were carried out at 0.02 *M* ionic strength of the solution.

Melting of DNA complexes with MB was carried out in spectrophotometer PYE-Unicam-SP8-100 (England). The heating of solutions of complexes was performed with program device SP-876 Series 2. Quartz cuvettes with hermetically closed teflon covers with 3 *mL* volume and 1 *cm* optic pathway length were used for spectrophotometric measurements. The melting was realized at λ=260 *nm* wavelength. Data were displayed on PC monitor via a program elaborated in Lab VIEW medium. Melting curves were obtained as it is described in [8]. The melting curves were constructed as it is described in [9].

Results and Discussion. Many works exist dedicated to MB interaction with DNA, where binding different mechanisms are discussed, but nowadays there are not reliable data indicating the main binding mode [3]. Particularly, in [5] it was shown that MB binding mechanism to DNA depends on sequences of DNA nucleotide pairs: this ligand binds to AT-sequences as groove binding ligands (netropsin, Hoechst 33258 et al.) with GC-sequences as intercalator. Moreover, intercalation mode is performed at low ionic strengths of the solution, while the groove binding with pronounced AT-specificity practically does not depend on the solution ionic strength [4, 5]. Furthermore MB specific binding with AT-sequences in DNA minor groove still requires a precision. Based on above mentioned the studies of MB interaction with different GC-content DNA were carried out by melting method. To reveal MB specificity to certain sequences the values of ΔT were obtained from the melting curves of its complexes with different GC-content DNA. Analogous melting curves were obtained in [9] for MB complexes with Calf Thymus DNA at 0.002 *M* and 0.02 *M* ionic strengths of the solution that is why the melting curves are not presented in the present work. It should be mentioned that the change of ΔT is an appropriate parameter, by which it may be judged about ligand specificity to the certain types of DNA sequences [10]. The dependencies of the melting interval width change $\delta\Delta T$ ($\delta\Delta T$=ΔT–$\Delta_0 T$, where ΔT and $\Delta_0 T$ are the melting interval width values of DNA–ligand complexes and DNA respectively) on r (r = [ligand]/[DNA]) of DNA–MB complexes at 0.02 *M* ionic strength of the solution are presented in Fig. 1. It is obvious from Fig. 1, at MB binding to *M. lysodecticus*. DNA the biggest increasing of $\delta\Delta T$ values takes place, which, most probably, is conditioned by MB preferable binding to DNA GC-sequences.

Earlier in [9] it was shown, that the melting temperature change of *Calf Thymus* DNA–MB complexes is significantly higher at 0.002 *M* ionic strength of the solution than at 0.02 *M* ionic strength of the solution. In all appearances it is conditioned by the fact that at low ionic strengths of the solution MB preferably is bound to DNA by intercalation mode, since in these conditions DNA hydration degree is higher, as a result of which ligand molecules transmit from polar water environment to the plane between DNA base pairs, which, in its turn, is nonpolar hydrophobic environment. These data are in correspondence with the results obtained in [4, 5, 10], where it was shown that at low ionic strengths intercalation is the main binding mode of MB.

The preference of intercalation mechanism performance at MB binding to DNA at low ionic strengths of the solution is conditioned by the less twisting state of DNA helix in such surrounding and the transition of ligand molecules into the plane of two base pairs is facilitated. Moreover, at 0.02 *M* ionic strength of the solution it is necessary an additional energy for intercalation to untwist DNA helix and to distort the neighboring base pairs along DNA molecule. This fact is thermodynamically undesirable at low temperatures of the solution. At the same time, the possibility of semi-intercalation binding of MB with DNA is not excluded this is indicated by the fact that $\delta\Delta T$ change of complexes is significantly less than at the intercalation.

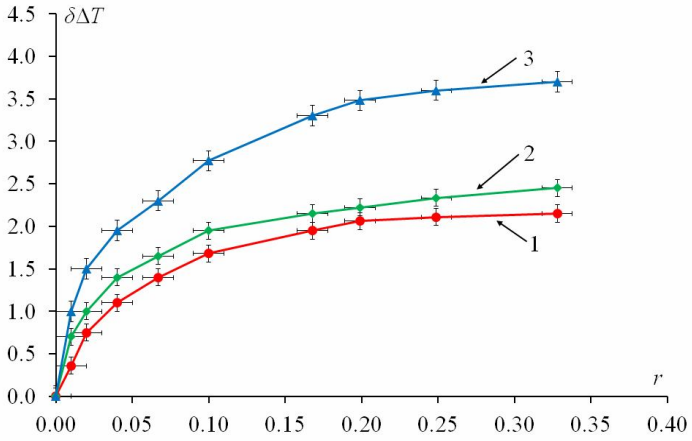

Fig. 1. The dependence of the melting interval width change $\delta\Delta T$ on r of MB complexes with different GC-content DNA at 0.02 *M* ionic strength of the solution: 1 – *Cl. perfringens,* 32%; 2 – *Calf Thymus*, 42%; 3 – *M. lysodecticus,* 72%.

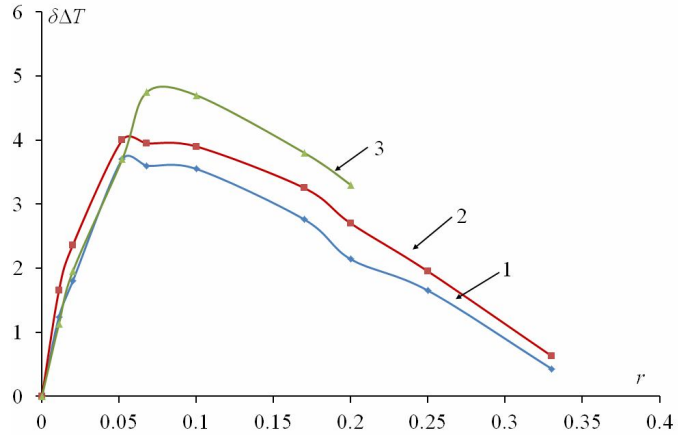

Fig. 2. The dependence of the melting interval width change $\delta\Delta T$ on r of EtBr complexes with different GC-content DNA at 0.02 *M* ionic strength of the solution: 1 – *Cl. perfringens,* 32%; 2 – *Calf Thymus*, 42%; 3 – *M. lysodecticus,* 72%.

It is necessary to mention that the analogous phenomenon is observed in the case of classical intercalator ethidium bromide (EtBr) interaction with DNA

[11–13]. Semi-intercalation mode of the binding to DNA is well studied by spectroscopic methods and it is revealed that this mode depends on DNA GC-content [14]. Taking into account this fact, for the comparing the obtained data of MB complexes with DNA the dependence of $\delta\Delta T$ on r of DNA–EtBr complexes at 0.02 M ionic strength of the solution is presented in Fig. 2. As it is obvious from presented Figure, $\delta\Delta T$ dependence values on r in the case of EtBr perform the tendency of enhancement with GC-content increasing in DNA. At the same time, in the case of EtBr $\delta\Delta T$ dependence on r has a bell-like form. It is shown in Fig. 2, that at $r > 0.1$ values $\delta\Delta T$ dependence curves on r decrease, while in the case of DNA–MB complexes these curves reach to saturation at analogous values of r. This is conditioned by the fact that EtBr forms different types of complexes with DNA simultaneously: intercalation, semi-intercalation and electrostatic [14], while in the case of MB at low concentrations one mode is mainly realized (most probably semi-intercalation), but at $r > 0.1$ values – the second mode (electrostatic) [6, 9].

It was shown in [11, 13], at low concentrations EtBr is bound to DNA mainly by intercalation in consequence of which $\delta\Delta T$ increases. At this ligand concentration increasing with intercalation, the semi-intercalation is performed and $\delta\Delta T$ decreases. At the semi-intercalation EtBr molecules are inserted into the plane of neighbor base pairs in the same strand of DNA with GC-sequence preference [14]. The dependence of intercalation mechanism (intercalation or semi-intercalation) of binding the ligands with DNA on its GC-content, is conditioned by the fact that hydration shell of these sequences is less dense compared with AT clusters, in which rigid water bridges are formed. Usually, in the regions of GC-sequences bound molecules of water form more bulky layer than in AT-rich regions, where high ordered water bridge between DNA strands opposite. This water "backbone" in the minor groove links its edges, which become less available for ligand molecule intercalation [15, 16].

Conclusion. The obtained data indicate that MB, as EtBr may be bound to DNA by intercalation mechanism. Nevertheless, in the case of EtBr the intercalation takes place at both low ($\mu \leq 0.002$ M) and relatively high ionic strengths of the solution ($\mu > 0.002$ M). In the case of MB at $\mu = 0.02$ M semi-intercalation mode is performed. These data reveal the preference of ligand molecules to GC-sequences of DNA at both intercalation and semi-intercalation modes.

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