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THERMODYNAMIC INVESTIGATION OF METHYLENE BLUE COMPLEXES WITH DNA

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The melting of DNA–methylene blue (MB) complexes has been carried out at 0.002 *M* and 0.02 *M* Na^+ concentration. The values of melting parameters – melting temperature and melting interval width were obtained in concentration ratio change interval r=[ligand]/[DNA] – $0<r\leq0.33$. It was revealed that melting interval width change of complexes depending on ligand concentration arises at 0.02 *M* Na^+ and $r\leq0.1$ and gets out on plateau at $r>0.1$, while at 0.002 *M* Na^+ this dependence acquires bell-like form. The increase of the interval of melting temperature was revealed at mentioned ionic strengths of solution depending on ligand concentration.

Keywords: DNA, methylene blue, intercalation, semi-intercalation, electrostatic binding.

Introduction. Investigations of interaction peculiarities of non covalently binding ligands with DNA are actual as far as they give the possibility to find out different aspects of DNA functioning, as well as the effect of low molecular compounds, including drug compounds, on its structure and functions. This fact is also important from the point of view of synthesis of new drug preparations that bind to DNA and effect on its functioning [1, 2].

Based on interaction mechanism of low molecular compounds (ligands) they are divided into intercalators and groove binding compounds. Both intercalators and groove binding compounds represent wide class of compounds possessing high biological activity and having practical importance. Intercalators contain a group of planar aromatic rings (for example, acridines, phenantridines, phenothyasines), due to which these molecules prefer to become embedded in space between DNA base pairs (intercalate) [3–8]. Moreover, the stacking contacts are formed between ligand molecules and DNA base pairs while the lengthening of DNA occurs due to untwisting of helix [1].

Groove binding ligands have crescent-shaped form of molecule that is in good correspondence with geometry of DNA minor groove. Ligand molecule is localized along DNA basic axis at groove binding and hydrogen bonds are formed between ligand molecules and base pairs [3–8]. Moreover, structural changes in DNA practically do not take place, while DNA helix acquires an additional rigidity in binding regions [8].

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Reversibly binding ligands may interact with DNA electrostatically in case when they are in cationic form in solutions, which is characteristic for majority of ligands [1–8]. Ligand molecules bind to negatively charged phosphate groups of DNA from its external side at electrostatic interaction.

Among above mentioned ligands photosensibilizators represent special interest, particularly methylene blue (MB) that may directly bind with proteins, lipids and DNA [2, 9]. Binding with DNA MB intensively absorbs the light and transfers to excited state and transmits the excitation energy to oxygen, which in its turn transfers to singlet state and invokes damages of DNA (photooxidative damage – POD) [5]. MB also possesses anti-malarial property, may inactivate viruses including HIV, hepatitis B and C in human blood plasma [10, 11].

Numerous investigations of MB interaction to DNA reveal that this ligand may bind to DNA by different modes, occurrence of that depends on solution ionic strength, DNA sequence and concentration ratio – [ligand]/[DNA] [3, 5, 12–14]. Despite this fact, thermodynamic aspects of MB interaction with DNA are not sufficiently investigated. The goal of present work is the investigation of MB interaction with DNA and determination of changes of melting parameters – melting temperature and melting interval width of complexes depending on both Na^+ ion and ligand concentration.

Materials and Methods. The following preparations were used in present work: Calf Thymus DNA – "Sigma" (USA), MB – "Aldrich" (USA). All preparations were used without additional purification. Concentrations of used preparations were determined by absorption method using the following extinction coefficients: Calf Thymus DNA – $\varepsilon_{260} = 6600\ M^{-1} \cdot cm^{-1}$, MB – $\varepsilon_{664} = 76000\ M^{-1} \cdot cm^{-1}$. The investigations were carried out at solution ionic strengths – $\mu = 0.002$ and 0.02 *M* Na^+, pH ≈ 7.0.

Instruments. The melting of DNA complexes with MB, as well as spectrophotometric measurements, were carried out on spectrophotometer PYE Unicam-SP8-100 (England). Heating of solutions of complexes was carried out by program device SP-876 Series 2. Quartz cuvettes with hermetic closed teflon plugs and volume of 3 *ml*, optic pathway length 1 *cm* were used for spectrophotometric measurements. The melting was carried out at wavelength $\lambda = 260\ nm$ corresponding to DNA maximal absorption. During melting process, absorption values of complexes were taken out on PC monitor in LabVIEW program environment. The melting curves of complexes were constructed as it is described in [8].

Results and Discussion. Reference data indicate that MB is bound to DNA by both intercalation and non intercalation modes, moreover, the occurrence of one of them depends on external factors. For revealing the conditions that are necessary to express either one or the second mode, the melting of MB complexes with DNA were carried out at following Na^+ concentrations – 0.002 and 0.02 *M*. The melting curves of DNA (curve 1) and its complexes with MB (curves 2 and 3) are represented on Fig. 1 at μ=0.02 *M* Na^+. Analogous curves were obtained at μ=0.002 *M* Na^+ (data are not represented).

As it is obvious from Fig. 1, melting curves shift to the interval of higher temperatures compared with the same pure DNA. This indicates that this ligand preferably binds to double-stranded (ds-) DNA and stabilizes its structure, moreo-

ver, this effect is revealed in change interval $0 < r \le 0.5$, where r=[MB]/[DNA]. The values of melting temperature (T_m) and melting interval width (ΔT) of DNA and its complexes with MB were determined from these curves at μ=0.02 M Na^+. It is revealed from obtained data that values of T_m monotonously increase at ligand concentration enhancement. The values of ΔT also increase at ligand low concentrations, but at further increase of its concentration the values of ΔT change insignificantly at μ=0.02 M Na^+. It is also revealed from represented data that at decreesing of Na^+ concentration about an order, T_m and ΔT significantly increase with arising of ligand concentration compared with them obtained at μ=0.02 M Na^+.

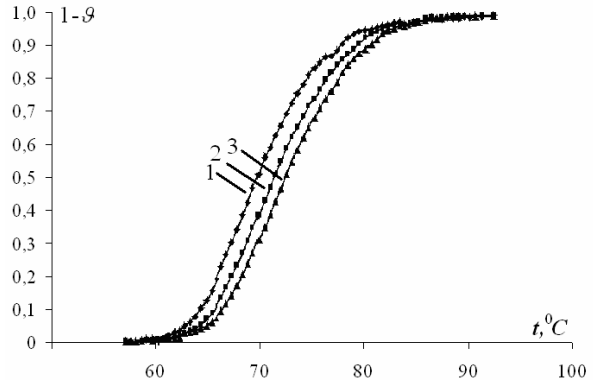
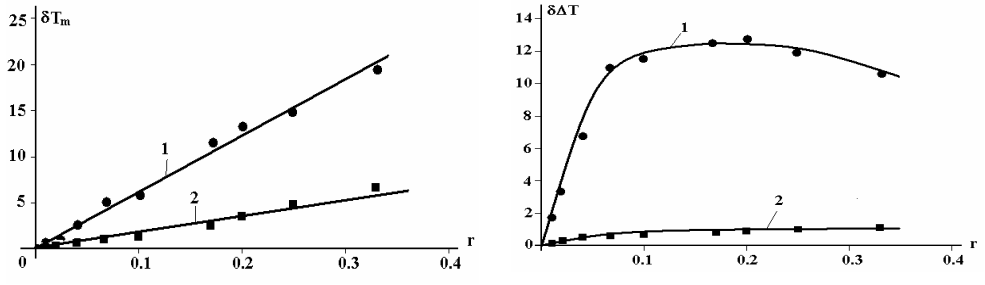


Fig. 1. The melting curves of DNA (1) and its complexes with MB at μ = 0.02 M Na^+ and r= 0.1 (2) and 0.167 (3).

The values of δT_m and $\delta\Delta T$ were calculated form melting parameters ($\delta T_m = T_m - T_0$, where T_m is the melting temperature of DNA–MB complexes, T_0 – that of pure DNA, and $\delta\Delta T = \Delta T - \Delta_0 T$, where ΔT is melting at the interval width of DNA–MB complexes, $\Delta_0 T$ – that of pure DNA). Dependences of δT_m (a) and $\delta\Delta T$ (b) on r are represented on Fig. 2.

It is obvious from Fig. 2, that at μ=0.002 M Na^+ ion melting temperature change is significantly higher than at 0.02 M Na^+. Most probably this fact is conditioned by binding of MB to DNA via intercalation mode at low ionic strengths of solution, when ligand molecules transfer from polarized water surrounding to space between DNA base pairs, the latter is non-polarized hydrophobic environment.



A B

Fig. 2. Dependence of melting temperature change δT_m (a) and melting interval width $\delta\Delta T$ (b) of MB complexes with DNA on r: μ = 0.002 M Na^+ (1); 0.02 M Na^+ (2).

These data are in accordance with results obtained in [5, 6] works, where it has been shown that the main mode of MB binding is intercalation at low ionic strengths. Besides intercalation MB molecules may also bind to DNA by electrostatic mode, since at low ionic strengths of solution Na^+ ions screen negatively charged phosphate groups of DNA from positively charged MB molecules are less than at high ionic strengths. This fact may be maintained by bell-like form

having dependence of $\delta\Delta T$ (Fig. 2, b, curve 1) on r obtained at 0.002 M Na^+, while at 0.02 M Na^+ this curve arises at low values of r and reaches saturation at increasing values of r. It was shown earlier that the dependence of $\delta\Delta T$ on r of complexes of ethidium bromide (EtBr) with DNA has a bell-like shape [7, 8, 15]. Moreover, it was shown that EtBr is bound to DNA by several modes, and such form of dependence of $\delta\Delta T$ on ligand concentration is conditioned by simultaneous occurrence of these modes [7, 8, 15, 16]. It was shown that EtBr is bound to DNA by intercalation, semi-intercalation and electrostatic mechanisms. Besides it was shown that EtBr is bound to single-stranded DNA by semi-intercalation and electrostatic modes [16, 17]. Based on absence of bell-like dependence at 0.02 M Na^+, we suppose that in these conditions MB does not bind to DNA by several modes. However, increasing of $\delta\Delta T$ on r at low concentrations of ligands may be conditioned by two binding modes. It is indicated by the fact that one of binding modes is electrostatic, which is nonspecific and always takes place at complex formation of MB with DNA. The second mechanism is more specific and takes place at low ionic strengths of solution and corresponds to limited binding sites and at saturation of these sites MB molecules become unable to bind with DNA by this mode. In this case when all binding sites corresponding to mentioned mode are filled, the dependence of $\delta\Delta T$ on r has hyperbolic form (Fig. 2, b, curve 2). It is impossible to determine the mechanism of nonspecific binding from this curve. There exist opposite conclusions in literature. Particularly, in work [5] groove binding is discussed in AT-rich regions, in work [6] intercalation mode of binding is discussed. Most probably additional investigations should be carried out for revealing the binding mechanism in these conditions.

In μ=0.002 M Na^+ the bell-like form of dependence of $\delta\Delta T$ on r indicates that MB is bound to DNA by intercalation, semi-intercalation and electrostatic mechanisms. This is proved by the fact that at low concentrations of ligand the dependence of $\delta\Delta T$ on r arises in $0 < r \le 0.1$ interval. These data coincide with analogous results obtained in [16], where it was shown that at low concentrations, when EtBr interact with DNA by intercalation mechanism, the stabilization of double-stranded structure of DNA takes place during melting. Meanwhile, at melting the redistribution of ligand molecules takes place from denatured regions to still non denatured ones, owing to which ΔT complexes increase compared with analogous values of pure DNA. There is no $\delta\Delta T$ change practically in interval $0.1 < r \le 0.25$. In these conditions MB is bound to DNA by semi-intercalation as well as electrostatic mechanisms, since binding sites on DNA by these modes are not saturated. Most probably semi-intercalated MB molecules have destabilizing effect on ds-DNA in spite of MB molecules are bound by intercalation or electrostatic mechanisms in mentioned interval of r changes of $\delta\Delta T$ remains practically constant. At further increasing of ligand concentration decreasing of $\delta\Delta T$ is observed, which in all appearances, is conditioned by the fact that increasing the number of semi-intercalated MB molecules in DNA results in domination of destabilizing effect of this ligand on ds-structure. This effect may be more expressed at higher values of r $(r \ge 0.4)$, but in these conditions ligand concentration is so high that it becomes experimentally impossible to obtain certain effects. On the other hand, comparison of above discussed data with similar results

obtained in case of EtBr reveals a good coincidence that makes possible to suggest that at higher concentrations MB starts binding to single-stranded DNA. This is maintained by the fact that, as it was shown in [6], MB may bind to single-stranded DNA by intercalation mechanism.

Therefore, obtained data indicate that MB as some ligands, particularly EtBr, as well as Hoechst 33258, may bind to DNA by different modes and each of them takes place depending on external factors, particularly ionic strength of solution.

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