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HOECHST 33258 COMPOUND INTERACTION WITH DIFFERENT GC-CONTENT DNA

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The interaction of Hoechst 33258 (H33258) compound with different GC-content DNA has been studied using the melting method. The obtained data show that at low ionic strengths of the solution (~0.002 *M*) H33258 does not display specificity for DNA AT sequences. Moreover, the effect was revealed not to depend on DNA nucleotide sequence. It has been shown that the increase in the ionic strength by an order of magnitude results in the development of a pronounced specificity of H33258 for DNA AT-sequences. H33258 was also found to exhibit a higher affinity for DNA having a block structure.

Keywords: GC-content of DNA, Hoechst 33258, minor groove, AT-specificity, melting parameters.

Introduction. Studies of the interactions of low molecular weight compounds (ligands) with DNA are currently of great interest, since the most important cell viability processes are regulated by DNA, which, being surrounded by multimodal low-molecular weight compounds (ligands), may undergo various conformational alterations during these processes. Among these compounds, Hoechst 33258 (H33258) has a relevant value, since this ligand is applied as a fluorescent dye of chromosomes, as well as possesses antitumor properties [1–5]. This ligand preferably binds to B-form DNA in its minor groove and exhibits high specificity for AT-rich regions. Though, not all AT-pairs are binding sites, as this ligand was shown to bind to central $(AT)_n$ pairs ($n = 4 \div 6$), which are adjacent to GC-pairs on both sides [6–10].

H33258 and its derivatives are bis-benzimidazole compounds. Fluorescence studies of H33258 complexes with Calf Thymus DNA at various ligand-to-DNA ratios show that at low concentrations this ligand binds to DNA minor groove, while at high concentrations partially intercalates into GC-sequences [6, 7–14]. Moreover, given that native DNA usually contains a large amount of damages and breaks, the possibility of inserting H33258 into these flexible regions cannot be

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ruled out. By binding to DNA, H33258 blocks DNA replication during cell division process, so this ligand may be applied as an antibiotic or antitumor preparation [1–5].

Thermodynamic studies have revealed that the H33258 compound interacts with DNA by at least two modes – strong and weak. Meanwhile, it was shown that the strong binding mode does not depend on the ionic strength of the solution, but depends on the types of pairs of bases, while the weak mode, which has an electrostatic nature, does not depend on the types of pairs [15–17]. Furthermore, it was later revealed that the strong binding mode does not depend on the solution ionic strength [15]. Recently obtained data have shown that H33258 can interact with DNA GC-sequences as well. However, the ligand molecules were assumed to display stacking interaction with these sequences [9]. Some studies have shown that part of H33258 molecules intercalates, another part is localized in DNA major groove – GC-rich regions [9].

From this point of view, the work is aimed at studying the peculiarities of the interaction of H33258 with different GC-content DNA and an assessment of the degree of affinity for these sequences.

Materials and Methods. The following preparations were used in this work: ultrapure DNA from Calf Thymus (average GC-content – 42%), from *Clostridium perfringens* (average GC-content – 31%), from *Micrococcus luteus* (average GC-content – 71%), H33258 (all preparations were obtained from "Sigma Chemical Company" (USA)), NaCl, sodium citrate trisubstituted, ethylenediaminetetraacetic acid (EDTA) (u.p.), bidistilled water. All preparations were used without additional purification.

The concentrations of the used preparations were determined spectrophotometrically, assuming the following extinction coefficients: ε_{260}=6600 $M^{-1}cm^{-1}$ for Calf Thymus DNA; ε_{260} = 7400 $M^{-1}cm^{-1}$ for *Clostridium perfringens* DNA; ε_{260}= 6600 $M^{-1}cm^{-1}$ for *Micrococcus luteus* DNA; ε_{343} = 42000 $M^{-1}cm^{-1}$ for H33258. The experiments were carried out at the following ionic strengths of the solution, μ : 0.002 and 0.02 *M*.

Spectrophotometric measurements were performed on Unicam SP8-100 (England) ultraviolet-visible spectrophotometer. In spectral measurements, quartz cuvettes with optical pathway length of 1 *cm* and hermetically closed caps were used. The melting of DNA–H33258 complexes was conducted at λ=260 *nm* wavelength. Absorbance values at a wavelength of λ=260 *nm* were displayed on a monitor in the LabVIEW 7.0 software environment. During the melting of DNA–H33258 complexes, the solutions were placed in thermostabilizing cells and heated by continuous mode at heating rate of 0.25°*C*/*min* via SPX-876 Temperature Programme Controller (UK) device. The temperature of the solutions changed from 28 to 95°*C*.

Results and Discussion. Studies of H33258 interaction with DNA have shown that DNA hydration degree and polyelectrolyte make an important contribution to the interaction of this ligand with DNA, since at relatively high ionic strengths of the solution and high concentrations of ligand there appears an electrostatic binding mode of this ligand [10–13]. Enhancement of the ionic strength results in increasing of density of electronegative charges of AT-rich regions in DNA minor groove and decreasing of hydration degree, as a

consequence of which H33258 binding specificity for DNA becomes more expressed [10–13]. Furthermore, the role of GC-sequences during complexation, as well as their effect on thermodynamic parameters have not yet been fully identified. Therefore, studies on the interaction of H33258 with DNA GC-rich regions are important within the framework of disclosure of the above problems.

With this aim, the melting of H33258 complexes with different GC-content DNA was carried out in a wide range of the ratio r = ligand / DNA ($0 < r \leq 0.33$). It should be mentioned that an increase in the ligand concentration leads to an enhancement of T_m of the complexes regardless of the average GC-content of DNA. In particular, T_m is increased not only in the cases of DNA from *Clostridium perfringens* and Calf Thymus (average GC-content was 32% and 42%, respectively), but also in the case of DNA from *Micrococcus luteus* (average GC-content was equal to 72%). Based on the values of T_m and ΔT for pure DNA and its complexes with H33258, curves of the dependence of $\delta(1/T_m)$ and $\delta(\Delta T/T_m^2)$ on r were constructed.

The curves of $\delta(1/T_m)$ as a function of r at ionic strengths of μ=0.002 M and μ=0.02 M are presented in Fig. 1. It turns out that the value of $\delta(1/T_m)$ increases with increasing values of r. But in the case of high concentrations of H33258, no destabilization effect is observed and $\delta(1/T_m)$ curve tends to saturation, especially in the cases of DNA from Calf Thymus or *Clostridium perfringens*. It means that this ligand does not bind to single-stranded regions of DNA. At the same time, the comparison of the corresponding curves shows that the value of $\delta(1/T_m)$ is higher at μ=0.002 M than at 0.02 M (Fig. 1, curves 1 and 2). Due to electrostatic interaction of this ligand with DNA, the complex thermal stability enhances. As the ionic strength increases, the contribution of this interaction mode decreases and, as a result, $\delta(1/T_m)$ decreases. Interestingly, the addition of GC-content in DNA (mainly in the case of DNA from *Micrococcus luteus*, 72%) does not contribute to quick saturation of the H33258 binding sites, due to which the melting point monotonously increases, as in the case of DNA from Calf Thymus and *Clostridium perfringens*.

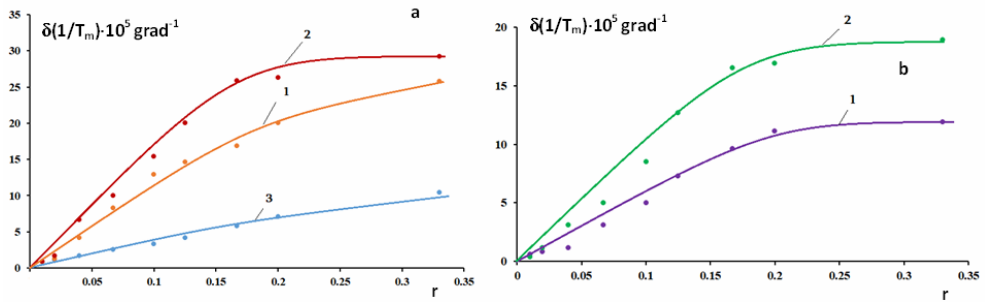

Fig. 1. Curves of the dependence of $\delta(1/T_m)$ on r for H33258 complexes with DNA from *Clostridium perfringens* (1), Calf Thymus (2), and *Micrococcus luteus* (3), at the ionic strength of 0.002 M (a) and 0.02 M (b).

Obtained data indicate that H33258, being an AT-specific and groove-binding ligand, interacts with high GC-content DNA. Despite the fact that in this DNA the specific binding sites for this ligand are strongly limited, at small ligand-

to-DNA ratio, saturation is not revealed. Therefore, we assume that under conditions of low ionic strengths of the solution, H33258 does not display any specificity for DNA AT-sequences. At the ionic strength of 0.02 *M*, H33258 complexes with DNA practically do not melt in the temperature range from 25 to 95°*C*. So that, with such an ionic strength, the curve of the dependence of $\delta(1/T_m)$ on *r* for the H33258 complexes with DNA from *Micrococcus luteus* was not obtained in this work.

The curves of $\delta(\Delta T/T_m^2)$ as a function of *r* at the ionic strengths of 0.002 *M* and 0.02 *M* are presented in Fig. 2. It can be seen that the curves at low ionic strength increase up to certain value of *r* ($r \approx 0.1$), after which they remain almost constant, while with an increase in the ionic strength by an order of magnitude, the changes in the width of the melting interval turn out to be negative (Fig. 2, b). This is due to the fact that at high ionic strengths, H33258 exhibits pronounced specificity for AT-sequences of DNA and stabilizes these regions. As a result, the differences between thermal stabilities of the AT- and GC-rich regions are reduced, which results in a decrease in the melting interval of the DNA–ligand complexes as compared to that of DNA. This leads to negative values of $\delta(\Delta T/T_m^2)$ for DNA–H33258 complexes at the ionic strength of 0.02 *M*, in case of DNA originates from *Clostridium perfringens* or Calf Thymus [18].

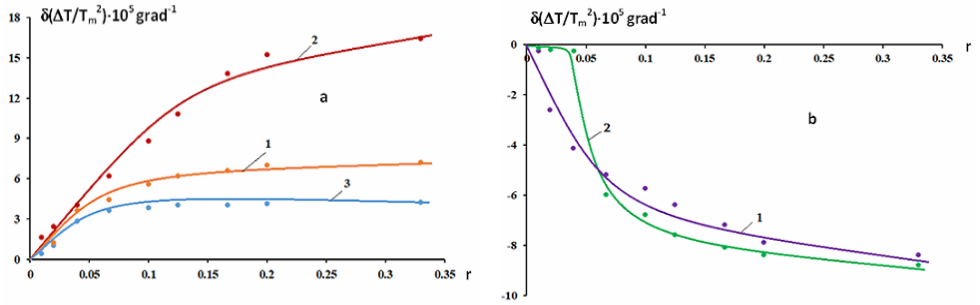

Fig. 2. Curves of the dependence of $\delta(\Delta T/T_m^2)$ on *r* for H33258 complexes with DNA from *Clostridium perfringens* (1), Calf Thymus (2) and *Micrococcus luteus* (3) at the ionic strength of 0.002 *M* (a) and 0.02 *M* (b).

Obviously, with an ionic strength of 0.002 *M*, the increase in $\delta(\Delta T/T_m^2)$, when ligand-to-DNA ratio is small, is due to the alteration of the H33258 binding mechanism and is most likely the result of the intercalation of this ligand into DNA GC-rich sequences. From this point of view, the highest increase in ΔT was not expected to be observed in the case of DNA from supreme organism (Calf Thymus), while in the cases of DNA from bacteria, which significantly differ from each other in GC-content, the increase of ΔT is less. Moreover, in the case of H33258 complexes with DNA from *Clostridium perfringens* and *Micrococcus luteus*, the curves of $\delta(\Delta T/T_m^2)$ vs. *r* differ slightly from each other (Fig. 2, a, curves 1 and 3). This fact indicates that the change in the width of the melting interval of H33258 complexes with DNA does not depend on GC-content, as in the case of the ethidium bromide intercalator [19], despite the opinion that H33258 is AT-specific ligand.

Conclusion. Thus, the data obtained show that at low ionic strengths (~0.002 *M*) H33258 does not express binding specificity for AT-sequences of DNA, since under these conditions DNA double-helix has a structure that deflects the geometric correspondence between the ligand and DNA. On the other hand, high hydration degree of the ligand and DNA is considered to be the main factor for non-specific binding of H33258. Since $\delta(\Delta T/T_m^2)$ increases along with an increase in the ligand-to-DNA ratio, it can be assumed that during the melting a redistribution of ligand molecules from denatured parts of DNA to still non-denatured parts occurs.

This effect does not depend on DNA nucleotide sequence, whereas, in order to reduce the contact of the hydrophobic molecules of the ligand with the surrounding water, H33258 molecules preferably intercalate into planes of DNA bases. It should be noted that in the case of Calf Thymus DNA the dependence of $\delta(\Delta T/T_m^2)$ on the ligand-to-DNA ratio (Fig. 2, a, curve 2) is more sensitive as compared with those obtained for DNA released from *Clostridium perfringens* and *Micrococcus luteus* (Fig. 2, a, curves 1 and 3), which is a result of DNA block structure. Such behavior is characteristic of ligands that interact specifically or non-specifically with DNA GC-sequences [17]. With an increase in ionic strength, H33258 exhibits a pronounced affinity for DNA AT-sequences, which is inherent in this ligand. At the ionic strength of 0.02 *M*, $\delta(\Delta T/T_m^2)$ takes on negative values, which indicates that the difference between melting points of AT- and GC-rich regions decreases. The preferred interaction occurs between this ligand and two AT- and one GC-sequences of double-stranded DNA, distributed alternatively, with the result that the value of $\delta(\Delta T/T_m^2)$ for the H33258 complexes with DNA from Calf Thymus and *Clostridium perfringens* decreases monotonously throughout the ligand concentration range.

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