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DIFFERENT MODES OF BINDING OF HOECHST 33258 WITH DNA

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Spectrophotometric study of Hoechst 33258 binding to DNA has been investigated. It was shown that this ligand forms at least two types of complexes at 0.002 M and 0.02M ionic strengths of solution. One of them is strong, the other is weak. It was revealed that at 0.02M ionic strength of solution, the binding constant value by strong mode is one order higher than at 0.002M. It was obtained that at both ionic strengths of solution at weak mode of interaction the values of binding constant of Hoechst 33258 to DNA coincide with each other.

DNA-Hoechst 33258 complexes – intercalation – binding constant – binding site number

Կատարվել է ԴՆԹ-ի հետ Hoechst 33258-ի կապման սպեկտրաֆոտոմետրիկ ուսումնասիրություն: Ցույց է տրվել, որ այս լիգանդն առաջացնում է երկու տիպի կոմպլեքսներ լուծույթի 0.002 Մ և 0.02 Մ իոնական ուժերում՝ ուժեղ և թույլ: Հայտնաբերվել է, որ լուծույթի 0.02 Մ իոնական ուժում ուժեղ եղանակով կապման հաստատունի արժեքը մեկ կարգով ավելի մեծ է, քան 0.02 մոլ իոնական ուժում: Ցույց է տրվել, որ թույլ եղանակի դեպքում նշված իոնական ուժերով լուծույթներում ԴՆԹ-ի հետ Hoechst 33258-ի կապման հաստատունի արժեքները համընկնում են միմյանց հետ:

ԴՆԹ-Hoechst 33258 կոմպլեքսներ – ինտերկալյացիա –կապման հաստատուն – կապման տեղերի թիվ

Проведено спектрофотометрическое исследование связывания Hoechst 33258 с ДНК. Показано, что лиганд образует по крайней мере два типа комплексов при ионных силах раствора 0.002 М и 0.02 М. Один из них является сильным, другой – слабым. Обнаружено, что при ионной силе раствора 0.02 М значение константы связывания сильным способом на порядок больше, чем при 0.002 М. Показано, что при слабом способе значения констант связывания Hoechst 33258 с ДНК при указанных ионных силах раствора совпадают друг с другом.

ДНК-Hoechst 33258 комплексы – интеркаляция – константа связывания – число мест связывания

One of widely investigating ligands immediately binding to DNA is Hoechst 33258 which is applied as a fluorescent dye of chromosomes. This ligand penetrates through cellular and nuclear membranes, and binding to chromosomes shows a high biological activity. The molecule of Hoechst 33258 consists of piperazine, two bisbenzimidazole and one phenol group (fig. 1) and at physiological conditions it preferably binds to AT-sequences in DNA minor groove [1, 10, 17, 18].

DNA complexes with Hoechst 33258 are stabilized by electrostatic, Van-der-Waals and hydrophobic interactions and hydrogen bonds between ligand molecule and nucleotides in minor groove. Formed hydrogen bonds between ligand molecules and DNA azotic bases substitute earlier existing hydrogen bonds between solvent and DNA molecules in minor grooves [1,18]. It was revealed that the minor groove of DNA-Hoechst 33258 complexes is more hydrophobic and has□20D dielectric constant compared to water dielectric constant equal to 80D. The decreasing of dielectric permeability of the minor groove at DNA complex-formation with Hoechst 33258 enhances resistance of hydrogen bonds compared to that of these bonds between DNA and water which results in additional deposit of enthalpy at the binding [10].

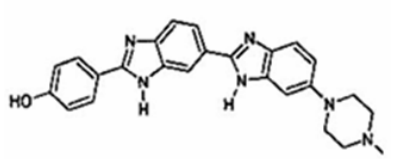

Fig. 1. Structure of groove binding compound – Hoechst 33258.

NMR investigations of Hoechst 33258 complexes with DNA showed that this ligand binds in the minor groove with nucleotides with 1:1 stoichiometry, moreover each benzimidazole interacts with two AT-pairs byforked hydrogen bond across helix and takes a region corresponding to approximately 1.5 base pairs [17]. Sequence selectivity and high affinity to AT regions are connected to both negative electrostatic potential inside of minor groove and decreasing of its width in AT-rich areas. It is conditioned by the fact that at interaction with DNA one of ends of Hoechst molecule is subjected to some changes. These structural changes in ligand molecule result in DNA minor groove width alterations [17]. Numerous investigations indicate that high affinity of H33258 to DNA AT-sequences is conditioned by ligand molecule form which is in geometric correspondence to this groove form [3-5, 12, 19]. Despite H33258 high specificity to AT-sequences of DNA it was shown that guanine exocyclic amino-group interacts with ligand molecule.

Thermodynamic investigations revealed that H33258 interacts with DNA at least by two modes – strong and weak [13]. Strong binding mode does not depend on solution ionic strength; moreover it depends on type of base pairs, while weak mode has mainly electrostatic nature and does not depend on DNA sequence in binding region [20].

The aim of this work is to reveal Hoechst 33258 binding mechanism to DNA by strong mode at low ionic strengths and to determine binding constant K value and number of bases n per one binding site.

Materials and methods. Calf thymus DNA (ultrapure) “Sigma” (USA), H33258 “Sigma” (USA), NaCl, Na-citrate (ultrapure), EDTA (ethylenediaminetetraacetate) were used in this work. All preparations were used without further purification. Concentrations of used preparations were determined by absorption spectroscopy method, using the following extinction coefficients: $\square_{260}$=6600 $M^{-1}cm^{-1}$ for DNA, $\square_{343}$=42000 $M^{-1}cm^{-1}$ for H33258. The investigations were carried out at 0.002 M and 0.02 Mionic strengths of the solution, t=25^{0}C – pH=6.95 at 0.002 M and pH=7.02 at 0.02 M.

Spectroscopic measurements were carried out on PYE Unicam-SP8-100 (England) spect-rophotometer. Huge number of theoretical [25] and experimental [8, 15, 21] studies are devoted to interaction of ligands with macromolecules (DNA, RNA). For analysis of experimental data of ligand adsorption on macromolecules it is important to achieve a state, when all binding sites on macromolecule are entirely occupied by ligands, though binding curves of ligands with DNA in Scatchard’s coordinates are constructed via an equation obtained in [8]:

$$r / C_f = K(1-nr)\left[\frac{1-nr}{1-(n-1)r}\right]^{n-1} \qquad (1)$$

In that case when ligand binds to DNA by two different modes, the binding curve is obtained in non-linear way [16]: that is why for linearization of this curve in [2] the equation (1) is modified by following way:

$$\frac{r}{c_f} = K(1-(2n-1)r) \qquad (2)$$

where r is number of ligands per one binding site, C_f – concentration of ligands in solution, K – binding constant. Comparing formula (2) with experimental data the values of K and n may be determined. For obtaining of r/C_f and r from absorption spectra of DNA-ligand complexes the concentration of non-bound ligands – C_f was determined by the following equation:

$$\frac{C_f}{C_0} = \frac{A - A_\infty}{A_0 - A_\infty} \qquad (3)$$

where A is complex absorption at given ligand concentration, A_0 and $A_{\square}$ – absorptions of thoroughly free and bound ligands respectively, where $C_0=C_f+C_b$ – total concentration of H33258 in solution; $r=C_b/C_p$; C_b – bound ligand concentration, C_p – concentration of nucleotide phosphate groups.

Results and Discussion. Despite the fact that Hoechst 33258 is a typical representative of groove binding ligands to DNA and shows a pronounced specificity to AT sequences of it, the studies of last years have shown that this ligand may bind to GC sequences as well. Moreover it is assumed that ligand molecules form stacking contacts with DNA GC-sequences [14]. To reveal H33258 binding mechanisms with DNA we have carried out a spectrophotometric titration of this ligand solutions by DNA solution at 0.002 M and 0.02 M ionic strengths.

Absorption spectra of pure H33258 (curve 1) and its complexes with DNA (curves 2-7) at 0.02 M ionic strength, $t=25^0C$ and pH=7.02 (analogous absorption spectra have been obtained at 0.002 M, because of it they are not shown) are presented in fig. 2. It is obvious from fig. 2 that absorption spectra of complexes are shifted to longer wavelength region with DNA concentration enhancement in solution, because the concentration of ligand free molecules decreases during titration. Absorption maximum of thoroughly bound ligand molecules corresponds to □=350 nm, while absorption maximum of free H33258 molecules corresponds to □=343 nm. Based on obtained absorption spectra the binding curves in Scatchard's coordinates are obtained that are presented in fig. 3 (A and B). As it is clear from presented figure the binding curves constructed on the basis of absorption spectra are non-linear. It is known that non-linearity of binding curves of low-molecular compounds with macromolecules indicates that an anti-cooperative interaction takes place or ligand binds by more than one mode [21].

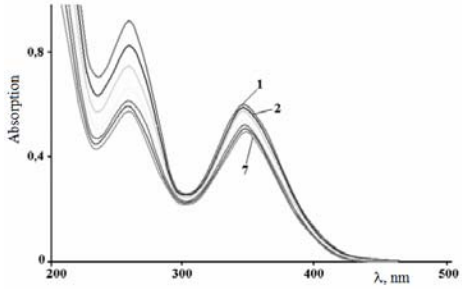

Fig. 2. Absorption spectra of pure H33258 (curve 1) and its complexes (curves 2-7) with DNA

In the statement [22] it was shown that H33258 interacts with DNA at least by two modes – strong, specific (with AT-sequences) and weak, electrostatic (with DNA phosphate groups). Based on this the obtained binding curves were analyzed taking into consideration that two interaction modes exist: one of them has a big slope and corresponds to strong binding mode, the other – to weaker mode. From adsorption curves by equation (2) the values of binding parameters were determined – K and n, and it was revealed that $K_{st}=3\cdot10^8$ M^{-1} which corresponds to strong binding mode of H33258 with DNA at 0.02 M ionic strength of solution and is in two orders higher than at weak binding mode – $K_w=2.25\cdot10^6$ M^{-1}. At 0.002 M ionic strength of solution $K_{st}=3\cdot10^7$ M^{-1}, $K_w=5\cdot10^6$ M^{-1}. Apparently a decrease of solution ionic strength by an order results in significant lowering of value of K_{st} at H33258 strong binding to DNA and approximately two-times increasing of value of K_w at weak binding mode. This experimental result may be a consequence of higher degree dependence of H33258 strong binding mode with DNA on solution ionic strength than in the case of weak binding mode. On the face of it this fact is unexpected, because at 0.02 M solution ionic strength the strong mode corresponds to specific binding of this ligand molecule with AT-sequences in DNA minor groove. Moreover, an electronegative potential of DNA minor groove in AT-rich regions plays a determining role in complex-formation [7, 12, 23]. From this point of view at H33258 specific binding to DNA AT-sequences the decrease of ionic strength should not influence on binding constant value, at weak electrostatic mode – it should. The fact that at strong binding modeK_{st} values differ by one order, most probably, indicate the qualitative change of H33258 binding mechanism with DNA, which is coordinated with data obtained in [23]. On the other hand, the fact that at weak binding mode the values of K_w differ twice is maintenance of this mode correspondence to interaction electrostatic mechanism of H33258 molecules with DNA phosphate groups. This is indicated by the fact that at weak binding mode the number of base pairs per one binding site (value of n) does not depend on solution ionic strength – $n_w\approx3$-4. In the case of strong binding mode values of n_{st} differ, because at 0.02 M $n_{st}\approx16$-17, at 0.002 M $n_{st}\approx14$-15.

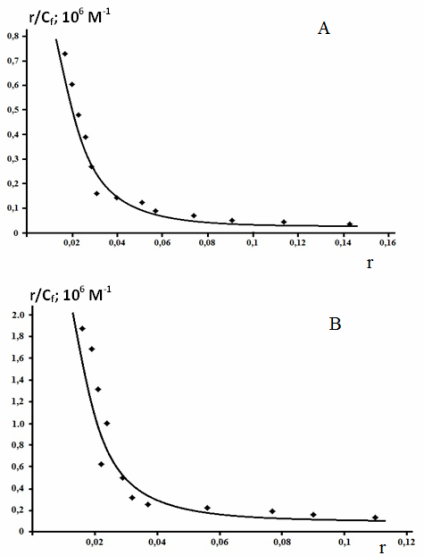

Fig. 3. Binding curves of H33258 with DNA at 0.002 M (A) and 0.02 M (B) ionic strengths of solution

Obtained experimental data indicate that H33258 specificity to DNA AT-sequences is expressed at relatively high ionic strengths of solution, consequently, at salt low concentrations this specificity disappears and this ligand besides electrostatic one binds also by other mode(s). In the literature a possibility of H33258 binding intercalation mode with DNA is discussed. Particularly, in [9, 11, 16, 24] it was shown that H33258 may bind with DNA GC-sequences by intercalation mode. Moreover, a classical intercalation in the case of this ligand is not observed. Usually, classical intercalators, particularly, ethidium bromide and acridine dyes contain a group of aromatic rings that are inserted into the plane between DNA base pairs and form stacking contacts with them [23]. From this point of view for stacking contact formation with DNA base pairs (intercalation), most probably, H33258 piperazine rings are appropriate. At the same time the group of aromatic rings of intercalators forms a flat structure which contributes to intercalation as well. H33258 molecule has semi-moon-like structure and geometrical coincidence with DNA minor groove [17]. As a consequence of such structure this ligand shows a high specificity to DNA AT-sequences in minor groove. On the other hand, this ligand shows a conformational lability in solution [17]. It is not excluded that at low ionic strengths of solution H33258 becomes more flexible, in consequence of which it may intercalate into DNA GC-rich regions.

Thus, from spectrophotometric measurements it is revealed that H33258 forms at least two types of complexes with DNA. One of them is the strong mode and characterized by high value of K (□3□108 M-1) and n (□16-17) at relatively high ionic strengths of solution (□0.02 M): this type of binding corresponds to specific interaction of ligand with AT-sequences in DNA minor groove [22]. The other mode is weaker and has an electrostatic nature with following binding parameters: K=2.25□106 M-1 and n□3-4. At low ionic strengths of solution □0.002 M H33258 binds to DNA by at least two modes: strong (K□3□107 M-1 and n□14-15) and weak (K□5□106 M-1 and n□3-4). These results are in correspondence to literature data based on which we assume that at low ionic strengths the strong mode respects to intercalation, the weak mode – to electrostatic mechanism of interaction [9, 11, 16, 24].

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