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STUDY OF POLY(dA) STRUCTURE IN THE ACIDIC MEDIA

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The study of EtBr (ethidium bromide) interaction with poly(dA) at different pH of solution has been carried out. The obtained data revealed that poly(dA) depending on pH may be in different structural states – double-stranded at 4.0 > pH > 3.0 and single-stranded in neutral (pH≈7.0), weak acidic (6.0 > pH ≥ 5.5) and strong acidic (pH < 3.0) conditions. It was shown that EtBr binds to poly(dA) by intercalation at pH≈3.5 as well as semi-intercalation at pH≈7.0 and pH≈5.5 modes. From the obtained data it was also revealed that EtBr binding mechanisms are universal and practically do not depend on medium conditions.

Poly(dA) – ethidium bromide –absorption spectrum –intercalation – semi-intercalation – electrostatic binding

Իրականացվել է poly(dA)-ի հետ ԷԲ-ի (էթիդիում իբրոմիդ) փոխազդեցության ուսումնասիրություն լուծույթի տարբեր pH-ների դեպքում: Ստացված տվյալները ցույց են տվել, որ poly(dA)-ն, կախված pH-ից, կարող է գտնվել տարբեր կառուցվածքային վիճակներում՝ երկշղթա 4.0 > pH > 3.0 և միաշղթա չեզոք (pH≈7.0), թույլ թթվային (6.0 > pH ≥ 5.5) և ուժեղ թթվային (pH < 3.0) պայմաններում: Հայտնաբերվել է, որ ԷԲ-ն poly(dA)-ի հետ կապվում է ինտերկալյացիոն), ինչպես նաև կիսաինտերկալյացիոն (pH≈7.0) և (pH≈5.5) եղանակներով: Ստացված տվյալները նաև ցույց են տալիս, որ ԷԲ-ի կապման մեխանիզմները ունիվերսալ են և գործնականում կախված չեն միջավայրի պայմաններից:

Poly(dA) – էթիդիում իբրոմիդ – կլանմանսպեկտր – ինտերկալյացիա – կիսաինտերկալյացիա – էլեկտրաստատիկ կապում

Исследовалось взаимодействие БЭ (бромистый этидий) с poly(dA) при различных pH раствора. Полученные данные выявили, что poly(dA), в зависимости от pH раствора может находится в различных структурных состояниях – двухцепочечном при 4.0 > pH > 3.0 и одноцепочечном при нейтральном (pH≈7.0), слабокислом (6.0 > pH ≥ 5.5) и сильнокислом (pH < 3.0) условиях. Обнаружено, что БЭ с poly(dA) связывается интеркаляционным при pH≈3.5 и полуинтеркаляционным при pH≈7.0 и pH≈5.5 способами. Полученные данные свидетельствуют о том, что механизмы связывания БЭ являются универсальными и практически не зависят от условий среды.

Poly(dA) – бромистый этидий –спектр абсорбции –интеркаляция – полуинтеркаляция – электростатическое связывание

The studies of interaction of different biological active low-molecular compounds – ligands with canonic forms of nucleic acid are actual topics and they represent a big interest since these compounds significantly effect on structural transitions and functional activity of these macromolecules, particularly DNA. From this point of view some ligands including

ethidium bromide (EtBr), proflavine (PF), acridine orange (AO) have a wide application. The biological activity of these compounds is conditioned by intercalation mode of binding with DNA which invokes structural reconstructions in DNA. Moreover these compounds bind to DNA by several modes. It was revealed that EtBr may bind to single-stranded DNA as well by more than one mode [2, 4, 10, 12, 14, 19, 20, 23, 25]. That is why many theoretical and experimental works are dedicated to EtBr interaction with DNA [2, 4, 10, 12, 14, 19, 20, 23, 25], but the binding of this ligand with different non-canonic forms of DNA is not entirely studied.

After the finding out of DNA structural organization, it becomes an object of numerous investigations in consequence of which it has been revealed that besides canonic Watson-Crick double helixes there are also double helixes consisted of one-type nucleotides [7,16-18].Incidentally depending on the type of azotic bases, single-stranded ordered structures may be formed. Furthermore from one-type polynucleotides in some cases (depending on ionic strength, pH of solution or existence of different mixtures) double-, three- or four-stranded structures are formed [1, 8, 11, 24]. From this point of view poly(dA) represents a certain interest because its structure at acidic values of pH of solution is not sufficiently studied [16]. In the solution it may be in both single-stranded (ss-) and double-stranded (ds-) states depending on pH of solution.

The goal of the present work is to study EtBr binding to poly(dA) at different values of pH of solution and to evaluate structural peculiarities of this polynucleotide as well as to determine values of the binding constant K and the number of bases n per one binding site for EtBr on this polynucleotide.

Materials and methods. Poly(dA) "Sigma" (USA), EtBr "Serva" (Germany), NaCl, Na-citrate, ethylenediaminetetraacetate (EDTA) (ultrapure) 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:$\varepsilon_{260}=10.1\cdot10^3$ $M^{-1}cm^{-1}$ for poly(dA), $\varepsilon_{480}=5800$ $M^{-1}cm^{-1}$ for EtBr. The investigations were carried out in 0.1×SSC (standard salt-citrate solution containing 0.015 M NaCl, 0.0015 M Na-citrate (three-substituted), 10^{-5} M EDTA),the ionic strength was equal to 0.02 M. All measurements were carried out at room temperature 25^0C and neutral (pH≈7.0) and acidic (pH≈5.5; pH≈3.5; pH≈2.5) values of solution pH. The mentioned values of pH are chosen because poly(dA) at pH≈7.0 and in alteration interval $6.0 > pH \geq 5.5$; $3.0 > pH \geq 2.5$ was in ss-state, at alteration interval $4.0 > pH \geq 3.0$ – at ds-state.

Compounds containing a group of aromatic chromophore rings, including EtBr, may form dimers at high concentrations (>1.5·10-4 M). Taking into account this fact at investigation of EtBr complexes with polynucleotides it is necessary to work with such concentrations of dye that make the dimerization negligible. EtBr total concentration in our experiments was equal to 5·10-5 M, and the concentration of dimers composes almost ≈0.1% and it may be ignored.

Spectrophotometric measurements were carried out on spectrophotometer PYE UnicamSP8-100 (England). For these measurements quartz cuvettes with 0.5 ml volume and 1 cm optic pathway length were used. Titration of solutions of studying samples was carried out by micropipette with 10 μl volume ("Hamilton", USA). PH of solutions was measured by universal ionomer EV-74 (USSR) with measuring electrode ESL 63-07.

To obtain the absorption spectra, EtBr concentration remains constant in the solution and polynucleotide concentration enhances with titration. The spectra of EtBr complexes with polynucleotides and pure EtBr were obtained in $400\leq\lambda\leq600$ nm wavelength interval. Maximum of pure EtBr absorption corresponds to λ=480 nm wavelength. With spectrophotometric titration of poly(dA) solution the absorption maximum of EtBr solution decreases and is shifted to longer wavelength interval. Moreover isosbestic point is observed at λ=510 nm and pH=3.5, at other values of pH isosbestic point is revealed at λ=505 nm. From some value of C_p/C_0, where C_p is polynucleotide concentration in the solution, C_0 – EtBr concentration when a maximum shifting to longer wavelength interval stops (at 520-525 nm) and spectra deviate from isosbestic point which indicates that EtBr is in thoroughly bound state.

For determination of r/C_f and r from the absorption spectra of DNA-EtBr complexes, the concentration of non-bound ligand Cf was calculated:

$$\frac{C_f}{C_0} = \frac{A - A_\infty}{A_0 - A_\infty} \qquad (1)$$

where A is complex absorption at certain ligand concentration, A0 and A∞ – absorption of fully free and bound ligands respectively, $C_0=C_f+C_b$ – total concentration of EtBr in the solution, $r=C_b/C_p$, C_b – bound ligand concentration, C_p – concentration of nucleotide phosphate groups.

To plot ligand binding curve with DNA the following equation is usually used:

$$r / C_f = K(1-nr)\left[\frac{1-nr}{1-(n-1)r}\right]^{n-1} \qquad (2)$$

where K is binding constant, n – number of bases per one binding site [2]. For more precise description of binding of ligands with nucleic acids and determination of K and n, in [2] there was suggested a method which permits linearization of equation (2), and as a result the following expression is obtained:

$$\frac{r}{c_f} = K(1-(2n-1)r) \qquad (3)$$

EtBr binding curves with poly(dA) were obtained by equation (3) at the above indicated pH and the values of K and n were calculated as it is described in [21]. To receive the above mentioned values of pH the solutions were titrated by 0.2 N HCl, adding 2 μl acid each time and mixing on magnetic stirrer. Error of final values of pH of solutions does not exceed ±0.02. Experimental error does not exceed 10-15%.

Results and Discussion. Many theoretical and experimental works are devoted to EtBr binding studies with DNA. It has been shown that this ligand forms different types of complexes with DNA – intercalation, semi-intercalation, electrostatic etc. [10, 20, 23]. Moreover it was shown that EtBr binding mechanisms with DNA are universal and are realized independently on different factors of medium (ionic strength, pH, nucleotide sequence) [3, 15]. Based on this EtBr was chosen as an appropriate ligand for poly(dA) structure investigation. This polynucleotide possesses several structural peculiarities. At alkaline, neutral or strong acidic pH values of the solution poly(dA) is mainly in single-stranded state but in 4.0 > pH > 3.0 interval this polynucleotide protonating transmits to double-stranded state since separate chains form pairs in type of poly(dAH^+)-poly(dAH^+) as in case of polyriboadenilic acids [7, 16-18]. At higher acidic solutions this polynucleotide again transmits to single-stranded state. Consequently, EtBr binding investigation with poly(dA) at different values of pH may allow to evaluate its structural peculiarities in the solution.

EtBr complex-formation with poly(dA) was investigated by spectrophotometric titration method in the following conditions: pH≈7.0; pH≈5.5; pH≈3.5 and pH≈2.5. The absorption spectra of EtBr and its complexes at pH≈3.5 (A) and pH≈7.0 (B) are presented on fig. 1. As it is obvious from fig. 1A, at pH≈3.5 the absorption spectra of poly(dA)-EtBr complexes decrease in maximums and are shifted to long wavelength interval. Besides, an isosbestic point is formed on spectra at λ=510 nm. These changes of the absorption spectra of EtBr and its complexes with poly(dA) are similar with those of EtBr complexes with double-stranded DNA at pH neutral values. Moreover, it is obvious from fig. 1B that despite EtBr absorption spectra with poly(dA) at pH≈7.0 are subjected to analogous changes as in the case of pH≈3.5, they differ from those since decreasing of maximums of the absorption spectra of poly(dA)-EtBr complexes in this case is not big, the shifting to the long wavelength interval is less as well than at pH≈3.5. On the other hand, the isosbestic point is formed at λ=500 nm. The mentioned changes of the absorption spectra of poly(dA)-EtBr complexes at pH≈7 are analogous to those obtained for EtBr complexes

with single-stranded DNA [20, 22]. The absorption spectra of poly(dA)-EtBr complexes at pH≈5.5 and pH≈2.5 are obtained as well. At pH≈5.5 the absorption spectra of poly(dA)-EtBr complexes coincide with those of these complexes obtained at pH≈7.0 (spectra are not presented), while the changes of spectra at pH≈2.5 are insignificant.

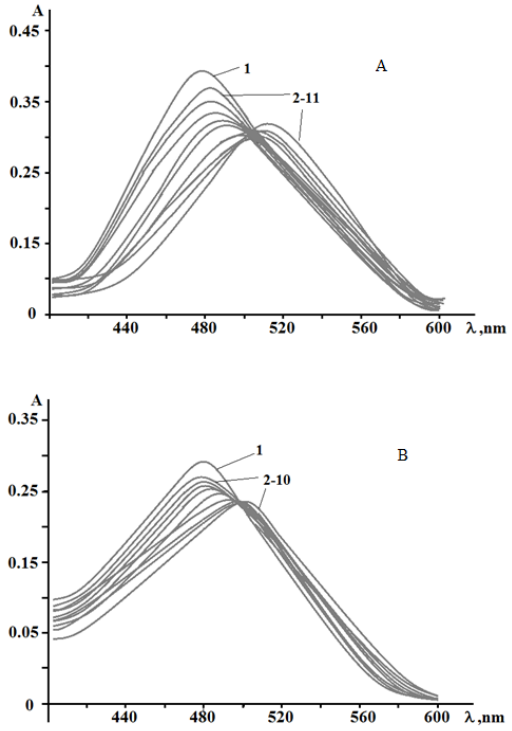

Fig. 1. The absorption spectra of EtBr (1) and its complexes (2-11) with poly(dA) at pH≈3.5 (A) and pH≈7.0 (2-10) (B). EtBr concentration was equal to $6.9 \cdot 10^{-5}$ M/L at pH≈3.5 and $5.2 \cdot 10^{-5}$ M/L at pH≈7.0. Concentration ratio of C=poly(dA)/EtBr changes in $0 < C \leq 2$ interval.

Based on the absorption spectra of EtBr and its complexes with poly(dA) the bin ding curves are constructed in Scatchard's coordinates. The binding curve of EtBr with poly(dA) obtained at pH≈3.5 is presented on fig. 2A, the curves at pH≈7.0, pH≈5.5 and pH≈2.5 – on fig. 2B. The experimental points obtained at pH≈7.0 and pH≈5.5 practically coincide with each other.

It is obvious from presented figure that a slope on the binding curve obtained at pH≈3.5 is bigger than on the other curves at the rest of pH. The binding curve at pH≈2.5 practically does not have a slope. Most probably it is conditioned by the fact that at pH < 3.0 all ionizing groups of not only poly(dA) but also EtBr are in totally protonated state. This results in radical changes of charge density of polyadenilic acid, which has a total electro-negative charge at neutral values of pH (is poly-anion in solutions).EtBr is in cationic form in neutral solutions. At acidic values of pHan increasing of total electropositive charge of EtBr takes place in consequence of protonation. Based on this it may be assumed that EtBr interaction with poly(dA) at pH≈2.5 is insignificant or EtBr molecules in these conditions do not bind to polydeoxiadenilic acid.

At neutral values of solution pH (pH≈7.0) as well as at pH≈5.5,EtBr binds to poly(dA) by weaker mode than at pH≈3.5. It is indicated by the binding constant value (K)

obtained from EtBr binding curves with this polynucleotide. Particularly, at pH≈3.5 $K=9.2\cdot10^5$ M^{-1}, at pH≈7.0 and pH≈5.5 – $K=8.6\cdot10^4$ M^{-1}. It is obvious that EtBr binding constant value with poly(dA) at pH≈3.5 is higher by one order than at pH≈7.0 and pH≈5.5. Moreover the value of n is much higher at pH≈3.5 – n=10.5-11.0 than at pH≈7.0 and pH≈5.5 – n=3.5-4.0. This value of n is higher at EtBr binding with double-stranded DNA by strong mode (n=5-6) [20].

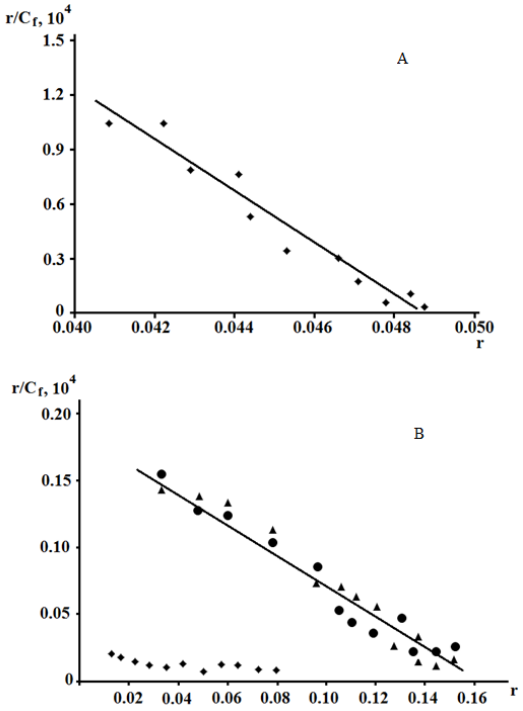

Fig. 2. EtBr binding curves with double-stranded poly(dA) at pH≈3.5 (A) and single-stranded poly(dA) at pH≈7.0, pH≈5.5 and pH≈2.5 (B). • corresponds to experimental points obtained at pH≈7.0; ▲– at pH≈5.5, ♦ – pH≈2.5.

It is known that at pH<4.0, when protonation of adenine bases takes place [16], polyriboadenilic acid (poly(A)) transmits to double-stranded state due to formation of hydrogen bonds and forms poly(AH^+)-poly(AH^+).Poly(dA) structure in acidic solutions is studied less than poly(A), but it should not be excluded the fact that poly(dA) may transmit to double-stranded state at protonation of adenilic bases. Taking into the consideration this fact we assume that the obtained values of K and n for EtBr binding to poly(dA) differ from those for DNA-EtBr complexes but at pH≈3.5 correspond to intercalation binding mode of this ligand. It is indicated also by strong confinement of binding site number on this polynucleotide. The fact that the value of K at EtBr intercalation into poly(dAH^+)-poly(dAH^+) differs from that obtained at this ligand binding to double-stranded DNA, most probably, is conditioned by decreasing of electro-negative charge of poly(dAH^+)-poly(dAH^+) due to protonation, in consequence of which non-canonic helix acquires more embedded packing compared to DNA. On the other hand, EtBr molecules are also protonated in these conditions (pKa≈5.5 for EtBr) and their total electro-positive charge increases (at neutral solutions EtBr is in cationic state) [9]. As a result of mentioned effects at EtBr intercalation into poly(dAH^+)-poly(dAH^+) electrostatic interaction plays a

significant role which at neutral solutions promotes an intercalation which in its turn is realized by two phases: during the first phase positively charged ligand molecules bind from external side of DNA (with negatively charged sugar-phosphate skeleton), during the second phase these molecules intercalate into the plane between neighbor base pairs [6, 12]. From this point of view in the case of poly(dAH^+)-poly(dAH^+), electrostatic interaction obstructs intercalation process since the binding constant value decreases. Incidentally it was shown that EtBr preferably intercalates into pyrimidine-purine sequences compared to purine-pyrimidine sequences [5]. Poly(dAH^+)-poly(dAH^+) is double-stranded helix consisted of purine bases in result of which, in all appearances, intercalation sites for EtBr molecules become confined.

At pH≈7.0 and pH≈5.5 the value of K is less by one order than at pH≈3.5. This fact is conditioned by the fact that this polynucleotide in these conditions is in single-stranded state and the entire intercalation of EtBr molecules is impossible. Nevertheless we assume that EtBr binds to ss-poly(dA) as in the cases of ss-DNA and ss-poly[d(A-T)] by semiintercalation mode. This fact is maintained by good coincidence of the values of K with the same values obtained for semi-intercalation complexes of this ligand with ss-DNA and ss-polynucleotides [20, 22, 23]. As it is obvious from fig. 2B, EtBr binds to ss-poly(dA) by semi-intercalation mode with similar affinity in both neutral and weak acidic conditions, despite at pH≈5.5 ligand molecules are protonated.

Therefore, the obtained data indicate that poly(dA) depending on solution pH may be in different structural states – double-stranded at pH≈3.5 and single-stranded at neutral (pH≈7.2), weak acidic (pH≈5.5) and strong acidic (pH≈2.5) conditions. It was also shown that EtBr binds to poly(dA) by intercalation or semi-intercalation modes depending on structural state of this polynucleotide. These data may become a fundament for evaluation of structures of different polynucleotides that do not have canonic structure. From the obtained data it is also revealed that EtBr binding mechanisms are universal and practically do not depend on medium conditions as it was shown earlier in [15].

REFERENCES

1. *Adrian M., Heddi B., Phan A.T.* NMR spectroscopy of G-quadruplexes.Methods, *57*, 1, p. 11-24, 2012.
2. *Arakelyan V.B., BabayanS.Yu., Tairyan V.I., Arakelyan A.V., Parsadanyan M.A., Vardevanyan P.O.* Kinetics of ligand binding to nucleic acids. J. Biomol. Str. Dyn., *23*, 4, p. 479-484, 2006.
3. *Antonyan A.P.* Acidic denaturation of ethidium bromide complexes with DNA. Biolog. Journ. of Armenia, *65*, 2, p. 31-35, 2013.
4. *Borisova O.F., Shcholkina A.A., Karapetian A.T., Surovaya A.N.* Heterogeneity of strong binding sites of ethidium bromide on DNA. Fluorescence and non-fluorescence complexes. Mol. Biol., *32*, p. 855-862, 1998.
5. *Cantor Ch., Shimmel P.* Biophysical chemistry. M., Mir, 1985.
6. *Chaires J.B.* A thermodynamic signature for drug-DNA binding mode. Arch Biochem. Biophys., *1*, 453, p. 26-31, 2006.
7. *Eisenberg H., Felsenfeld G.* Studies on the temperature-dependent conformation and phase separation of polyriboadenilic acid solutions at neutral pH, J. Mol. Biol., 30, p. 17-37, 1967.
8. *Holder I.T., Drescher M., Hartig J.S.* Structural charachterization of quadruplex DNA with in-cell EPR approaches.Bioorg Med Chem., *21*, 20, p. 6156-61, 2013.
9. *Jones R.L., Wilson W.D.* Effect of ionic strength on the pKa of ligands bound to DNA. Biopolymers, 20, p. 141-154, 1981.

10. *Karapetian A.T., Mehrabian N.M., Terzikian G.A., Vardevanian P.O., Antonian A.P., Borisova O.F., Frank-Kamenetskii M.D.* Theoretical treatment of melting of complexes of DNA with ligands having several types of binding sites on helical and single-stranded DNA. J.Biomol. Struct. Dyn., *14*, 2, p. 275-283, 1996.
11. *Kim B.G., Shek Y.L., Chalikian T.V.* Polyelectrolyte effects in G-quadruplexes: Biophys Chem., *184*, 31, p. 95-100, 2013.
12. *Lane A.N., Jenkins T.C.* Thermodynamics of nucleic acids and their interactions with ligands. Q. Rev. Biophys., *33*, 3, p. 255-306, 2000.
13. *Minasyan S.H., Tavadyan L.A., Antonyan A.P., Davtyan H.G., Parsadanyan M.A., Vardevanyan P.O.* Differential pulse voltammet-ric studies of ethidium bromide binding with DNA. Bioelectrochemistry, *68*, p. 48-55, 2006.
14. *Monaco R.R.* A Novel Major Groove Binding Site in B form DNA for EthidiumCation. J. Biomol. Struct. Dyn, 25, 2, p. 119-125, 2007.
15. *Mukhaelyan Zh.H., Antonyan A.P., Minasyants M.V.* Investigation of acidic denaturation of ethidium bromide complexes with DNA. Proceedings of the Yerevan state University, 2, p. 48-53, 2014.
16. *Saenger V.* Principles of structural organization of nucleic acids. M., Mir, 1987.
17. *Saenger W., RieckeJ., Suck D.* A structural model for the polyadenilic acid single helix. J. Mol. Biol., 93, p. 529-534, 1975.
18. *Scovell W.M.* Structural and conformational studies of polyribonucleic acid in neutral and acid solution. Biopolymers, 17, p. 969-984, 1978.
19. *Suh D.* Environments of ethidium binding to allosteric DNA: II. Accessibility, mobility and mode of binding. Exp. and Mol. Medicine, *32*, 4, p. 204-209, 2000.
20. *Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., Davtyan H.G., KarapetianA.T.* The binding of ethidium bromide with DNA: interaction with single- and double-stranded structures. Experimental and Molecular Medicine, *35*, 6, p.527-533, 2003.
21. *Vardevanyan P.O., Arakelyan V.B., Parsadanyan M.A., Antonyan A.P., Hovhannisyan G.G., Shahinyan M.A.* Analysis of experimental binding curves of EtBr with single- and double-stranded DNA at small fillings. Modern Physics Letters B, *28*, 22, p. 1450178 (1-11), 2014.
22. *Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., Davtyan H.G., Boyajyan Z.R., Karapetian A.T.* Complex-formation of ethydim bromide with poly[d(A-T)]-poly[d(A-T)]. J. Biomol. Struct. Dyn., *22*, 4, p. 465-470, 2005.
23. *Veselkov A.N., Baranovskii S.F., Dymant L.N., Petrenko N.V., Veselkov D.A., Taker A., Devis D.B.* Complex-formation of ethidium bromide with single-stranded non-complementar deoxitetranucleotide5'-d(ApApGpC). Mol Biol., *31*, 2, p. 263-273, 1997.
24. *Vorlickova M., Kejnovska I., Sagi J., Renciuk D., Bednarova K., Moltova J., Kypr J.* Circular dichroism and guanine quadruplexes. Methods, *57*, 1, p. 64-75, 2012.
25. *Xinhui H.,Wang Q., Pingang H., Fang Y.* Spectroelectrochemistry study on the electrochemical reduction of ethidium bromide. Analytical Sciences, *18*, p. 645-650, 2002.

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