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ANALYSIS OF ISOTHERMS WITH TWO BINDING SITES IN THE REGION OF SMALL FILLINGS

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The adsorption isotherm of ligands on DNA in the region of small fillings which describes the isotherm with pronounced linear areas has been obtained in this research. It has been shown that experimental data description by such isotherm permits receiving not only more precise values of - both binding constant and number of adsorption centers with which one ligand molecule binds but also new parameter value-value of the portion of adsorption centers on DNA, having different constants of binding.

DNA – ligand – adsorption isotherm – small fillings – binding constant – number of adsorption centers

Աշխատանքում ստացվել է ԴՆԹ-ի հետ լիգանդների կապման կորը փոքր հագեցումների տիրույթում, որը նկարագրվում է երկու հստակ արտահայտված գծային տեղամասերով: Ցույց է տրվել, որ փորձարարական տվյալների նկարագրությունն այս կորի միջոցով հնարավորություն է ընձեռում ոչ միայն կապման հաստատունի և լիգանդի մեկ մոլեկուլի հետ կապվող կապման կենտրոնների թվի ավելի ճշգրիտ արժեքների ստացման համար, այլև նոր պարամետրի՝ ԴՆԹ-ի վրա տարբեր հաստատուններ ունեցող կապման կենտրոնների մասնաբաժնի արժեքի որոշման համար:

ԴՆԹ – լիգանդ – կապման կոր – փոքր հագեցումներ – կապման հաստատուն – կապման կենտրոնների թիվ

В работе получена изотерма адсорбции лигандов на ДНК в области малых заполнений, которая описывается с двумя ярко выраженными линейными участками. Показано, что описание экспериментальных данных такой изотермой позволяет не только получать более точные значения константы связывания и число адсорбционных центров, с которыми связывается одна молекула лиганда, но и определять значение нового параметра, доли адсорбционных центров на ДНК, имеющих разные константы связывания.

ДНК – лиганд – изотерма адсорбции – малые заполнения – константа связывания – число адсорбционных центров

The main processes of cell life ability are conditioned by interaction of different low molecular compounds – ligands with macromolecules: at first proteins and nucleic acids. This fact lies under the base of numerous studies on interaction of ligands with DNA by different experimental methods [1, 3, 7, 10-12, 14, 15]. Mostly of fully filled bound situation is not received due to weak binding of ligand to DNA as well as very small amount of adsorbing ligands. That is why the reasonable way of carrying out the experiment is the condition of small fillings when the ratio of molecules of ligands bound to DNA to number of base pairs of DNA is less from unit, in other word the estimation of adsorption

parameters within frame of simplified linear binding isotherm of ligands to DNA molecule [2,4,5]. It should be mentioned that small fillings permit not only describing adsorption process in relatively easy way but also having an independent interest. In vast majority of cases the filling of macromolecules by ligands may be considered to be small in conditions of real cellular system. Small fillings represent an interest due to the fact that the quantity of ligands on macromolecule in other equal conditions weakly excites the system and the obtained results describe the system adequately.

The statistic properties of such systems significantly differ from those when one adsorbed ligand takes one adsorption center [8, 9, 13, 16]. It should be noted that mostly ligands bind to DNA by several modes. This circumstance additionally complicates the analysis of experimental results. Despite adsorption isotherm has some characteristic peculiarities facilitating the processing of experimental isotherm analysis of binding of ligands to DNA, the problem of experimental data analysis of ligand binding to DNA may not be considered finally solved.

In the present work the adsorption isotherm of binding of ligands to DNA is obtained which describes all experimental points in single way (by one curve), i.e. describes experimental points that have two pronounced linear areas.

Theoretical part: It is known that in the region of small fillings the isotherm of Crothers-Gurskii may be approximated by line [9, 13]. Let's examine the case of binding of ligands to DNA when in the region of small fillings two types of centers of binding exist and ligand may bind independently to these adsorption centers. These centers are characterized by different binding parameters i.e. have different values of K binding constant and different number of n adsorption centers with that one ligand molecule binds. Therefore assuming that DNA molecule is characterized by two pairs of parameters – (K_1, n_1) and (K_2, n_2) the portion of adsorption centers on DNA that has binding parameters (K_2, n_2), should be indicated. Let's indicate this portion via p. It is obvious that the portion of adsorption centers on DNA that has (K_1, n_1) binding parameters will be equal to $q = 1 - p$. Let's represent DNA as one-dimensional crystal with N adsorption centers of binding and ligand having much less linear sizes at binding takes n adsorption centers on DNA disposed alternately. At first let's observe the case when one type of adsorption centers with (K, n) parameters exists on DNA. In this case the average number of bound ligands with DNA is x at small fillings, i.e. adsorption isotherm according to [2] is determined by formula:

$$x(t) = \frac{k_1 c_f N}{k_{-1} + (2n-1)k_1 c_f}, \qquad (1)$$

where k_1 and k_{-1} are rate constants of formation and dissociation of ligand complex with DNA, c_f – number of free ligands. If there are two types of adsorption centers on DNA with (K_1, n_1) and (K_2, n_2) binding parameters and they are filled independently the total number of adsorbed ligands on DNA will be:

$$x = \frac{K_1 c_f N_1}{1 + (2n_1 - 1)K_1 c_f} + \frac{K_2 c_f N_2}{1 + (2n_2 - 1)K_2 c_f}, \qquad (2)$$

where N_1 and N_2 are numbers of adsorption centers that are characterized by (K_1, n_1) and (K_2, n_2) binding parameters respectively, K_1 and K_2 – binding constants of ligands with adsorption centers of the first and the second types. To describe the experimental data let's rewrite (2) as:

$$\begin{aligned} 1 &= \frac{1-p}{Y + \alpha_1 X} + \frac{p \cdot \gamma}{Y + \alpha_2 \gamma \cdot X} \\ Y &= \frac{r}{K_1 c_f}, \quad X = r, \quad \gamma = \frac{K_2}{K_1} \\ \alpha_1 &= 2n_1 - 1, \quad \alpha_2 = 2n_2 - 1, \quad p = \frac{N_2}{N_1 + N_2}, \end{aligned} \qquad (3)$$

In formula (3) the "p" has the meaning of adsorption center portion on DNA that have K_2 binding constant and number of adsorption centers with which one ligand molecule binds is equal to n_2. Equation (3) gives the dependence of Y on X not clearly, that is actually the adsorption isotherm in Scatchard's coordinates. Comparing theoretical adsorption isotherm (3) with experimental data the values of n_1, K_1, n_2, K_2, p parameters may be determined. For comparison of theoretical adsorption isotherm (3) with experimental data, it is appropriate to represent (3) isotherm as:

$$\frac{r}{c_f} = K_1 \frac{1-\alpha_1 r-\alpha_2\gamma r-p(1-\gamma)}{2} + \\ +K_1\sqrt{\frac{(1-\alpha_1 r-\alpha_2\gamma r-p(1-\gamma))^2}{4}-\gamma r(\alpha_1\alpha_2 r-\alpha_2-p(\alpha_1-\alpha_2))}\,, \qquad (3a)$$

Results and Discussion. Let's apply above obtained theoretical results to experimental data analysis on isotherms of which two linear areas are clearly appeared. An example of such isotherm is represented on fig. 1.

If experimental binding isotherms of ligands to DNA have two pronounced linear areas it may be considered that these ligands bind to DNA at least by two modes. Through these experimental points two lines may be lined. The line with big slope angle corresponds to strong binding mode and the line with small slope angle corresponds to weak binding mode. In vast majority of cases the analysis of curve with pronounced linear areas is carried out in following way. Experimental linear areas of binding isotherm are compared with theoretical linear binding isotherm separately [2, 6].

$$\frac{r}{c_f} = K(1-(2n-1)r)\,, \qquad (4)$$

As a result of such comparison two important binding parameters – ligand binding constant to DNA K and number of adsorption centers on DNA n, with which one ligand molecule binds are determined by the least square method. The determined binding parameters for experimental points presented on fig. 1, are equal to $n_1 = 6$, $K_1 = 2 \times 10^6$ M^{-1}, $n_2 = 3.6$, $K_2 = 2.7 \times 10^5$ M^{-1} (for EtBr).

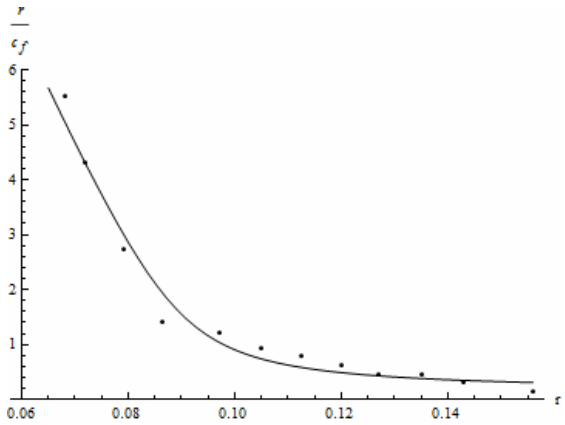

Fig.1. The binding curve of EtBr with DNA in Scatchard's coordinates. The binding curve is composed of two directly linear areas corresponding to two binding modes. Ionic strength of solution is 2×10^{-2}M. Points correspond to experimental data and curve is constructed by the least square method according to (3a) equation.

Moreover, this mode of binding isotherm analysis of ligands to DNA is approximated since the obtained experimental results artificially are divided into two groups and the analysis of each group is carried out separately. In each group only those experimental points remain that approximately are on the line. It is obvious that such mode of experimental data treatment for determination of binding parameters is incorrect. The more substantiated treatment mode of experimental data is that one, which would take all these data (i.e. experimental points). Experimental data analysis by new (3a) binding isotherm of ligands with DNA permits not only obtaining the precise values of K and n parameters but also determining new parameter value – the portion of adsorption centers of each type of p adsorption centers. It should be mentioned that theoretical binding isotherm comprises five varying parameters. This results in ambiguous determination of varying parameters. To obtain unique values of parameters let's fix several parameters hence their values practically are known for certain from both our and numerous literature data. Such parameters are $n_1 = 6$, $K_1 = 2 \times 10^6$ M^{-1}, for strong binding mode of EtBr to DNA. Giving values to these parameters the adsorption isotherm (3a) may be constructed along all experimental points by the least square method (fig. 1) and the values of $n_2 = 0.5$, $K_2 = 5.3 \times 10^5$ M^{-1}, $p = 0.025$ parameters may be determined. Comparing the values of K and n parameters obtained at experimental data description by one curve and two lines it is obvious that the value of n_2 is decreased about seven-times and K_2 is increased about two-times. It should be mentioned that the approximated description method of experimental data allows to determine the value of new parameter p, the portion of adsorption centers on DNA having K_2 binding constant that in our experiments is equal to $p = 0.025$.

REFERENCES

1. *Antonyan A.P., Davtyan A.G., Vardevanyan P.O.* Formation of semi-intercalatong complexes at EtBr binding to DNA. Scientific Reports YSU, (In Russian), 3, c. 97-102, 2003.
2. *Arakelyan V., Babayan Yu., Potikyan G.* Determination of constant rates of adsorption of ligand on DNA: Analysis of Correlation Functions. J. of Biomol. Struct. & Dyn., 18, 2, p. 231-235, 2000.
3. *Aroutyunyan S.G., Mamasakhlisov E.Sh., Morozov V.F., Dalyan Y.B., Khachikyan R.E., Vardevanyan P.O., Karapetian A.T., Orioli P., Bruni B.* Modification of DNA dimensional structure in the complex with cis-platine. Biophysics, (In Russian), 42, 2, c. 372-377, 1997.
4. *Babayan Yu.S., Manzini G., Xodo L.E., Quadrifoglio F.* Base specificity in the interaction of ethidium with synthetic polyribonucleotides. Nucleic Acids Res., *15*, 14, p. 5803-5813, 1987.
5. *Babayan Yu.S., Manzini O., Kuadrofolio F.* Interaction of ethidium bromide with synthetic double helix polyribonucleotides. Molecular Biology, (In Russian), *22*, 4, c. 989-910, 1988.
6. *Bloomenfeld V., Crothers D., Tinoco I.* Nucleic Acids. University Science Books Sausalito, California, 789 p., 1999.
7. *Borisova O.F., Shchelkina A.K., Karapetian A.T., Surovaya A.N.* Heterogeneity of ethidium bromide strong binding sites on DNA. Fluorescent and non fluorescent complexes. Molecular Biology, (In Russian), *32*, c. 855-862, 1998.
8. *Cantor Ch., Shimmel P.* Biophysical Chemistry. (In Russian), M., Mir, *3*, 534 p., 1985.
9. *Crothers D.M.* Calculation of binding isotherms for heterogeneous polymers. Biopolymers, *6*, p. 575-584, 1968.
10. *Dalyan E.B., Haroutiunyan T.S., Haroutiunyan S.G., Vardevanyan P.O.* Melting of complexes of DNA-*cis*-DDP in acidic environment. Exp. and Mol. Medicine, *35*, 6, p. 534-537, 2003.
11. *Vardevanyan P.O., Antonyan A.P., Manukyan G.A., Karapetian A.T., Shchelkina A.K., Borisova O.F.* Binding of ethidium bromide with native and denaturized poly(dA)-poly(dT). Mol. Biol., (In Russian), *34*, 2, c. 310-315, 2000.

12. *Minasyan S.G., Tavadyan L.A., Antonyan A.P., Davtyan H.G., Parsadanyan M.A., Vardevanyan P.O.* Differential pulse voltammetric studies of ethidium bromide binding with DNA. Bioelectrochemistry, *68*, p. 48-55, 2006.
13. *McGhee J.D., Von Hippel P.H.* Theoretical aspects of DNA-protein interactions: cooperative and noncooperative binding of large ligands to a one-dimensional homogeneous lattice. J. Mol. Biol., *86*, p. 469-489, 1974.
14. *Tang T.Ch., Huang H.J.* Electrochemical studies of the intercalation of ethidium bromide to DNA. Electroanalysis, *11*, 16, p. 1185-1190, 1999.
15. *Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., Davtyan H.G., Markaryan A.Sh., Kazaryan R.S., Babayan Yu.S.* Disperse of bound ligands method in study of adsorbtion isotherm. J. of Biomol. Struct. and Dyn., *20*, 6, p. 857, 2003.
16. *Zasedatelev A.S.,Gurskii G.V., Volkenstein M.V.* Theory of one-dimensional adsorption. Adsorption of small molecules on homopolymer. Mol. Biol., (In Russian), *5*, 2, c. 245-251, 1971.

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