

ADSORPTION OF SHORT LIGANDS ON DNA WITH MODIFICATION  
OF ADSORPTION CENTER STRUCTURE

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The reversible adsorption of short ligands on DNA at arbitrary filling has been theoretically investigated, the kinetics of adsorption of ligands on DNA being described in a self-consistent way with the modified structure of an adsorbing center. The case when one adsorbed ligand occupied two adsorption centers on DNA was considered. It was shown that an allowance for the modification of the potential well of the adsorbing center led to the possibility of realizing various regimes of adsorption.

**Keywords:** adsorbing center, bifurcation, potential well, bistable adsorption regime.

**Introduction.** A whole range of important processes occurring in a living cell are connected with the reversible adsorption of not large molecules on DNA. The adsorption of these ligands takes place due to the interaction of the ligand with the adsorbing centers on DNA. Practically in all papers the phenomenon of adsorption is considered under the assumption that the adsorbing ligand does not affect the structure of the adsorbing center [1–4] or after the adsorption a change in the adsorbing center quickly transpires, so that the subsequent ligand adsorption (we consider the reversible adsorption) on the adsorption center occurs under conditions when the structure of adsorbing center is identical to initial one. The description of the adsorption of ligands on DNA under the conditions when changes in the structure is ignored is carried out within the framework of the classical adsorption theory. If the repeated ligand adsorption occurs on the adsorption center before the latter has relaxed to its initial structure, the description of the process of ligand adsorption on DNA shall be supplemented with a description of the modified structure of DNA adsorbing center [5]. So, together with the equation describing the change in the number of adsorbed ligands on DNA, the equation of relaxation also should be supplied, which would self-consistently describe the modification of the structure of the adsorbing center of

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DNA due to the adsorption. Just such a self-consistent description of the ligand adsorption on DNA, when in the process of the reversible adsorption a modification of the structure of the adsorbing center takes place, will be considered in the present paper. In the theory of ligand adsorption on DNA, DNA is usually represented as an one-dimensional lattice made up of adsorbing centers. At the adsorption the ligand occupies several successively located adsorbing centers called the “binding site”. Note, that the terms “adsorbing center” and “binding site” prove identical, when at adsorption one ligand occupies a single adsorbing center. In the present paper the case of one adsorbed ligand occupying two adsorption centers will be considered. At that, the case of an arbitrary filling will be considered.

**Theoretical Part.** The theoretical description of the ligand adsorption on DNA when at reversible adsorption the modification of the structure of the adsorbing center occurs, will be carried out on the basis of paper [5]. As in paper [5], the ligand adsorption and desorption on DNA is represented as a quasi-chemical reaction of binding and unbinding of the ligand with the binding center. Consider the most simple case of the adsorption of a single type ligand and of only one type of a ligand–DNA complex formation as a consequence. The quasi-chemical reaction of ligand binding ( $L$ ) with a free adsorbing site ( $M$ ) is written as



where  $(LM)$  is the ligand-adsorbing center complex;  $k_1$  and  $k_{-1}$  are the constants of formation and decay rates of  $(LM)$  complex. The process of adsorption is considered to be a transition of the ligand being adsorbed from the potential well located near the DNA to that of the free binding center on DNA. We suppose that at reversible adsorption the ligand deforms the potential energy profile of binding center in such a way that the potential well deepens by an amount of  $x$  (both here and further in this paper the energy is represented in the units of  $k_B T$ ,  $k_B$  is Boltzmann constant,  $T$  is temperature). Note, that the change in the structure of the adsorbing center can take place in the case when, on the one hand, the adsorption center is surrounded by mobile atomic groups and, on the other hand, the interaction between the adsorbing ligand and the adsorbing center is sufficiently strong. These conditions are realizable at the adsorption of a ligand on DNA since ligands, as a rule, are either charged or have a dipole moment, and their interaction with the adsorbing center can be rather strong, besides, as is well known, DNA possesses a multitude of conformational states and easily transits from one state to another. As it is shown in [5], under these conditions the constant of equilibrium  $K = k_1 / k_{-1}$  for reaction (1) equals

$$K(x) = K_0 \cdot \exp(x), \quad (2)$$

where  $K_0$  is the constant of equilibrium for reaction (1) in the case when no deformation of potential well of the binding site takes place. As is seen from (2), the equilibrium constant  $K(x)$  is an exponential function of  $x$  and a minor change in structure of the absorption center may lead to large changes in the values of equilibrium constant. The equation describing the changes in the share of absorption centers on DNA occupied by ligands (or the probability of occupation

of the adsorption center)  $r$ , in case when at an arbitrary filling one adsorbed ligand occupies  $n$  successively located centers, was derived in [6] and has the form:

$$\frac{dr}{dt} = k_1 c_f \left( \frac{1 - nr}{1 - (n-1)r} \right) (1 - (n-1)r) - k_{-1} r, \quad (3)$$

where  $c_f$  is the number (or concentration) of ligands in the solution. To escape the introduction of additional notations for the number of ligands and their concentration in solution, we assume that the binding of ligands with DNA occurs in a unit volume. In this case the concepts of concentration and number of particles coincide. To account of the deformation of the potential well of the adsorbing center, equation (3) must be supplemented with relaxation equation describing the change in the potential well depth of the adsorption center. According to [5, 7], the relaxation equation describing the dynamics of potential well deepening ( $x$ ) at receiving a ligand can be written as

$$\tau dx/dt + x = x_\infty r, \quad (4)$$

where  $\tau$  is the relaxation time of potential well deepening;  $x_\infty$  is the limiting value of the deepening that is realized after an infinitely long residency of the ligand in the potential well. The system of non-linear differential equations (3) and (4) describes the self-consistent process of adsorption and deepening of the potential well at each adsorption center. The case of small filling ( $r \ll 1$ ) was considered in [5], when the right hand side of equation (3) is a linear function of  $r$ . In the present paper we consider the general case of an arbitrary filling at  $n=2$ . In this case equation (3) has the form:

$$\frac{dr}{dt} = k_1 c_f \frac{(1-2r)^2}{1-r} - k_{-1} r. \quad (3a)$$

This case represents also an interest of its own since, there is a whole range of ligands, which at adsorption on DNA occupy two successively located adsorbing centers [8]. We can reduce the number of equations in the system (3a) and (4). We hold variable  $r$  as “fast” and variable  $x$  as “slow”. Then, due to the adiabatic principle of the exclusion of “fast” variables, we take the solution of equation (3a) for the quasi-stationary regime:

$$r(x) = \frac{1}{2} \left( 1 - \frac{1}{\sqrt{4K_0 c_f \exp(x) + 1}} \right). \quad (5)$$

Substituting equation (5) into equation (3a), the following non-linear dynamic equation describing the time dependence of variable  $x$  is obtained:

$$r \frac{dx}{dt} = -x + \beta \left( 1 - \frac{1}{\sqrt{\gamma \exp(x) + 1}} \right), \quad (6)$$

where  $\beta = x_\infty / 2$ ,  $\gamma = 4K_0 c_f$ . Equation (6) is analyzed in the standard way [9]. First, the stationary states are found and the type of their stability is investigated. To do this, the graph of the right hand side of equation (6) is plotted, which is equal to

$$f(x, \beta, \gamma) = -x + \beta \left( 1 - \frac{1}{\sqrt{\gamma \exp(x) + 1}} \right). \quad (7)$$

By definition, at the stationary point the right hand side of equation (6), that is the function  $f(x, \beta, \gamma)$ , turns into zero:

$$f(x, \beta, \gamma) = 0. \quad (8)$$

The stability of the stationary state is determined by the sign of the derivative of function (7) at the stationary point. If near the equilibrium state the function  $f(x, \beta, \gamma)$  at increasing  $x$  changes sign from plus to minus, the stationary state is stable. And if near the equilibrium state function  $f(x, \beta, \gamma)$  at increasing  $x$  is not changing the sign or changes the sign from minus to plus, the stationary state is unstable. It is easy to show that the number of stationary states and the type of their stability depends on the values of parameters  $\beta$  and  $\gamma$ . An analysis of function  $f(x, \beta, \gamma)$  shows that one, two or three stationary states may exist in the system depending on the values of  $\beta$  and  $\gamma$ . In Fig. 1 the particular case of the existence of three solutions ( $x_1, x_2$  and  $x_3$ ) of equation (8) is presented, corresponding to the three stationary states of the system. As is seen from Fig. 1, the states  $x_1$  and  $x_3$ , in which the derivative is negative, are stable, and the state  $x_2$ , in which the derivative is positive, is unstable. For understanding of bifurcation nature of system states under consideration, the dependence of  $x(\gamma)$  presented in Fig. 2 is to be investigated.

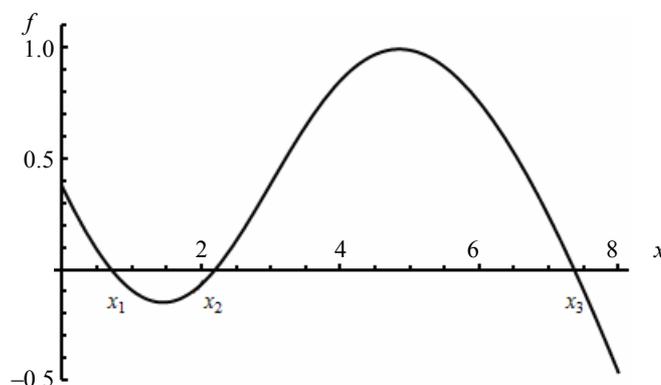


Fig. 1. Graph of function (7) at  $\beta = 8$  and  $\gamma = 0.1$ .

It is seen from in Fig. 2, that when  $\gamma$  increases from zero to the bifurcation value  $\gamma = \gamma_2$  (along AB curve), the system leaves the unstable point B and transits jumping to the upper branch of the stable states, at further increasing of  $\gamma$  the system staying at the stable (at that, unique) equilibrium state.

If  $\gamma$  decreases (along DC curve), then at reaching the bifurcation value  $\gamma_1$ , the system leaves jumping the unstable point C and transits to the lower branch of stable states and completes in this way a closed cycle of stable states and, thus, exhibits the hysteresis phenomenon.

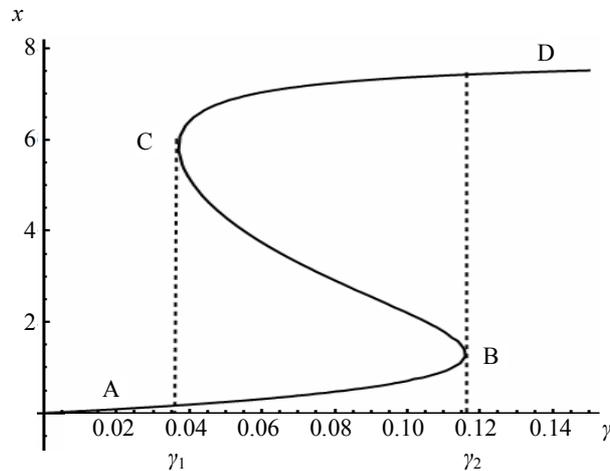


Fig. 2. The curve of the stationary states depending on the controlling parameter  $\gamma$  (at  $\beta = 8$ ).

Note also that the system under consideration possesses a trigger property, because at values of the controlling parameter  $\gamma$  between  $\gamma_1$  and  $\gamma_2$ , the system may function in either of the two stable states. Let us note that since the equilibrium constant exponentially depends on  $x$  (2), then, as follows from Fig. 2, at lower values of parameter  $\gamma$  the equilibrium with low values of equilibrium constant may be realized in the system, whereas the equilibrium with higher values of equilibrium constant may be realized at higher values of parameter  $\gamma$ . For intermediate values of  $\gamma$  the adsorption could occur in a bistable regime, when for the same value of parameter  $\gamma$  two types of equilibrium could be realized in the system with different values of the equilibrium constant.

For the determination of the adsorption isotherm, first the stationary distribution of variable  $x$  is to be found. To do this, the effective potential of  $U_{eff}(x, \gamma, \beta)$  system should be calculated with equation (6) rewritten in the form [10]

$$\tau \frac{dx}{dt} = - \frac{\partial U_{eff}(x, \gamma, \beta)}{\partial x}. \quad (9)$$

Since the right hand side of equation (9) is represented by function (7), then by integrating (7) we obtain the following expression for the effective potential:

$$U_{eff}(x, \gamma, \beta) = \frac{x^2}{2} - \beta \left( x - \ln \left( \frac{\sqrt{\gamma \exp(x)} - 1}{\sqrt{\gamma \exp(x)} + 1} \right) \right). \quad (10)$$

As was expected, the expression for effective potential essentially depends on the values of  $\beta$  and  $\gamma$  parameters. From Fig. 2 it follows that for  $\beta = 8$  and small values of  $\gamma$  parameter (in A range) there exists one stable stationary state and consequently, the effective potential must have one minimum. In the range, where  $\gamma$  is in between  $\gamma_1$  and  $\gamma_2$ , a bistable adsorption regime is realized in the system, and the effective potential must have two minima separated by a

maximum. And at lastly, for large  $\gamma$  (in D range), there exists a stable stationary state and consequently, the effective potential must have one minimum. This circumstance is illustrated in Fig. 3.

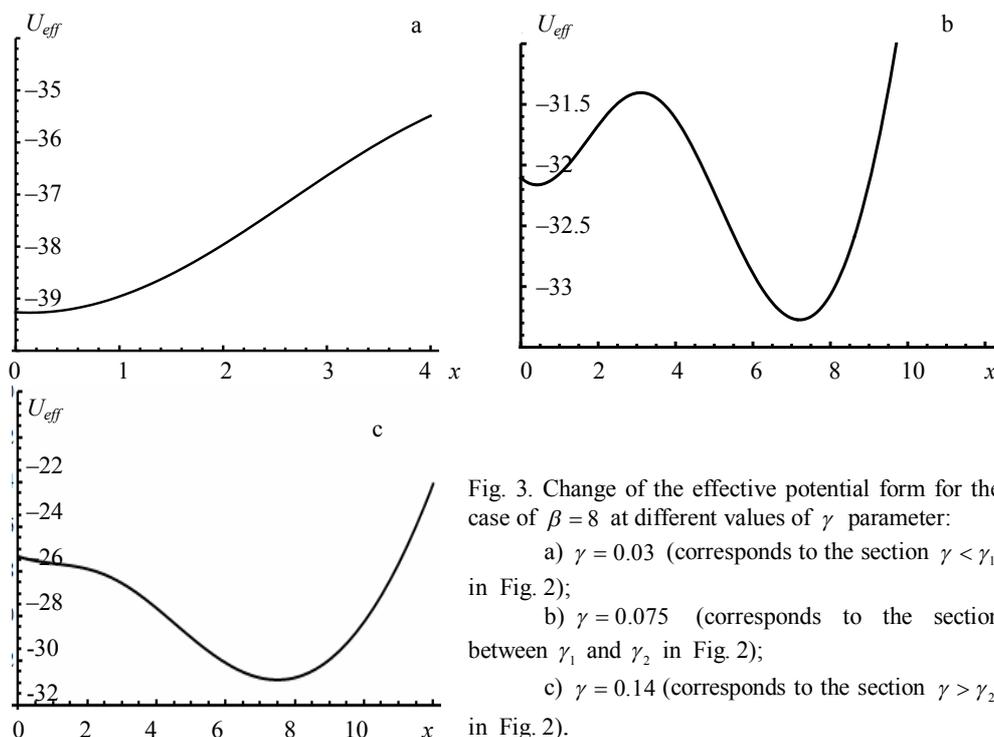


Fig. 3. Change of the effective potential form for the case of  $\beta = 8$  at different values of  $\gamma$  parameter:  
 a)  $\gamma = 0.03$  (corresponds to the section  $\gamma < \gamma_1$  in Fig. 2);  
 b)  $\gamma = 0.075$  (corresponds to the section between  $\gamma_1$  and  $\gamma_2$  in Fig. 2);  
 c)  $\gamma = 0.14$  (corresponds to the section  $\gamma > \gamma_2$  in Fig. 2).

As is seen in Fig. 3, with  $\gamma$  increasing from 0.03 to 0.075 another minimum emerges in the potential well that leads to the bistable adsorption regime. Note, that the second minimum emerges far from the first one. In the non-linear theory of the dynamic systems this phenomenon is conventionally called a catastrophe. With the further increase of  $\gamma$  from 0.075 to 0.14 the first minimum on the potential well disappears and there remains only the second minimum. Using the expression for potential well (10), one can calculate the density of the equilibrium distribution of variable  $x$  with the help of the following formulae:

$$P(x, \gamma, \beta) = Z^{-1} \exp(-U_{eff}(x, \gamma, \beta)), \tag{11}$$

$$Z = \int_0^{x_\infty} \exp(-U_{eff}(x, \gamma, \beta)) dx.$$

Then, using the distribution (11) of variable  $x$  and averaging the number of adsorbed ligands over all possible values of variable  $x$ , we obtain the following final expression for adsorption isotherm:

$$\theta(\gamma, \beta) = \int_0^{x_\infty} r(x, \gamma, \beta) \cdot P(x, \gamma, \beta) dx, \quad 0 \leq \theta(\gamma, \beta) \leq 1. \tag{12}$$

**Results and Discussion.** The important result of the present paper is the derivation of an expression (12) for the isotherm of ligand adsorption on DNA at

an arbitrary filling in the case, when one adsorbed ligand on DNA occupies two successively located adsorption centers. Here to obtain expression (12) for the adsorption isotherm, the allowance for possibility of deformation of the potential well of adsorbing center was made. The isotherm (12) was constructed as a dependence of DNA filling  $\theta(\gamma, \beta)$  on the dimensionless concentration  $K_0 c_f$ , taking into account that  $\gamma = 4K_0 c_f$ . As it is seen from (12), the adsorption regimes are controlled by  $\beta$  parameter. The analysis of expressions (7) and (10) shows that at  $\beta = 8$  in the region of intermediate values of  $\gamma$ , that is, at  $\gamma_1 < \gamma < \gamma_2$  a bistability is observed on the dependence of the effective potential on  $x$ , and at  $\beta = 4$ , in the whole range of  $\gamma$  variation, the system finds itself in a monostable state. In Fig. 4, the adsorption isotherms (12) are presented at  $\beta = 4$  and  $\beta = 8$ . From Fig. 4 it can be seen, that if the effective potential has one minimum, that is, the system is in a monostable state, the adsorption isotherm has the Langmuir form (Fig. 4, a). And if effective potential has two minima, that is, the system is in a bistable state, the adsorption isotherm has an S-form, which is characteristic for a cooperative adsorption.

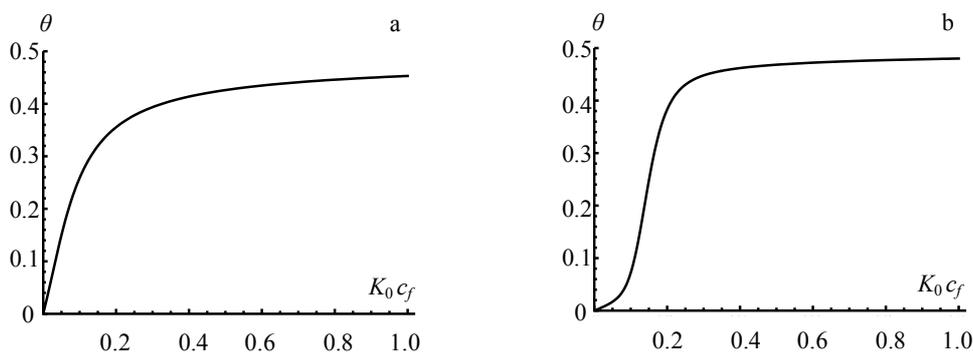


Fig. 4. The curves of adsorption  $\theta(\gamma, \beta)$  as depending on the dimensionless ligand concentration in the solution ( $K_0 c_f$ ) at various values of  $\beta$ : a)  $\beta = 4$ , b)  $\beta = 8$ .

For obtaining of cooperative adsorption isotherm, the interaction between the adsorbed particles is usually introduced [11]. As follows from the results of the present paper, the mechanisms of the S-form adsorption isotherm formation are different in these two variants. Obviously, if the average distance between the adsorbed ligands is sufficiently large, then the interaction probability of the adsorbed ligands with each other is small, and the realization of cooperative adsorption is practically excluded. In our case the origination effect of the cooperative regime of adsorption does not depend on the average distance between the adsorbed particles and is determined entirely by mobility of the atomic groups near the adsorbing center. This circumstance can serve as a basis for disclosure of the cause of the S-form of the adsorption isotherm. Let note, that if the two minima on the effective potential (Fig. 4, b) are separated by rather a high barrier (much greater than  $k_B T$ ), then the adsorption can be described with the help of a sum of two Langmuir isotherms.

Thus, if at ligand adsorption the structure of adsorbing center is changed, then this circumstance can lead in the experiment to the realization of different types of isotherms of adsorption: the conventional Langmuir adsorption, the sum of two Langmuir isotherms of adsorption and an S-form isotherm of adsorption.

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