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Effect of Ligands Binding on the Isotherm of Hybridization of the DNA-Chip

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Abstract—The factors are analyzed, which influence both the hybridization thermodynamics and the DNA/DNA-duplex stability. The non-competitive hybridization of the DNA as well as the competitive hybridization on the surface are investigated. It is shown that the binding to the intercalating ligands results to an increase in the selectivity and sensitivity of the DNA chips.

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1. INTRODUCTION

The DNA-chips are promising tools with a wide variety of applications such as the medical diagnostics, the environmental monitoring, the protection from biological weapons, and so on [1, 2]. An important direction in the development of the DNA-chips is an increase in the selectivity and sensitivity at the expense of the amplification of the electrical signal and the stability of the probe-target hybridization. The effectiveness of such devices as the DNA-sensors and the DNA-chips depends on the accuracy of determining the experimental parameters responsible for the thermosstability of duplexes of nucleic acids and for the time of formation of the DNA-duplexes [3]. Some of the factors influence the hybridization thermodynamics, in particular: the surface density of the single-stranded DNA (length of 25–49 nucleotides) immobilized on the surface, and the presence of competing hybridization. The stability of the DNA–DNA- and the DNA–RNA-duplexes is determined by two key factors: a sequence and external factors such as *pH*, the ionic strength, the concentration of low molecular weight compounds (ligands), the presence of interphase boundaries, the geometric limitations, etc. A better understanding of the physical and chemical processes underlying the hybridization of the DNA and the RNA on the surface of the electrical transducer is important for improving the efficiency of the DNA-chips and their manufacture [4].

The requirements for the DNA-sensors are the high sensitivity and selectivity, which, in turn, require the maximum efficiency of hybridization and the minimal non-specific adsorption at the interface between the solid and liquid phases. To a great extent, the hybridization of nucleic acids depends on temperature, the concentration of salts, the viscosity, the GC-composition and other physical and chemical characteristics.

An increase in the selectivity and sensitivity of the DNA-sensors can be achieved by the use of electrochemically active compounds with a higher affinity to the double-stranded DNA than to the single-stranded DNA. This type of compounds can increase essentially the stability of double-stranded regions and, simultaneously, the amplitude of the generated signal, which in turn will increase the sensitivity of

the DNA-sensor. For example, such ligands are the intercalators, the molecules with the planar heterocyclic structure that are placed between the nitrogenous bases and change the local structure of the double-stranded DNA [5–7].

In recent years, the thermodynamics and kinetics of hybridization both in the volume [8, 9] and on the surface [4, 10–16] have been studied thoroughly. For example, the spectrum of problems under consideration includes the kinetics of hybridization at the surface [12, 14], the influence of salts on the hybridization of the DNA in the volume [9], the hybridization isotherms on the surface [4], and etc. At the same time, the DNA–ligand interactions have also been examined in a large number of works devoted to intercalation [5–7] and the binding of ligands in a minor groove [17, 18], their cross-docking [19], and etc. However, the influence of the interaction of the DNA–ligand on the thermodynamics and kinetics of hybridization has never been considered before. In the context of the development of the DNA–sensors, the theoretical analysis of the influence of ligands intercalation on the DNA–hybridization on the surface becomes necessary.

The present work deals with the study of the DNA–hybridization isotherm on the surface in the presence of ligands that bind to the double-stranded regions of the DNA. In practice, the DNA–chips are immersed into the target solution for a relatively short period of time, and the kinetics of hybridization plays a decisive role. However, an understanding of equilibrium properties is also necessary for a comparative estimation of the importance of kinetic and thermodynamic factors for the productivity of the DNA–chips.

2. NON-COMPETITIVE HYBRIDIZATION

2.1. Free Energy

Consider the equilibrium hybridization isotherms for two idealized but experimentally realizable situations, when the DNA–chip is immersed in a solution containing intercalating ligands. As a result, we have only one type of single-stranded target (Fig. 1a) or the solution contains the targets of two different types that do not hybridize in volume but are capable to hybridize with the same probe on the surface (Fig. 1b).

Consider a set N_0 of p single-stranded probe molecules of the DNA, where N_{pt} of them are hybridized to the target t. On the surface, the hybridization of p and t creates a double-stranded

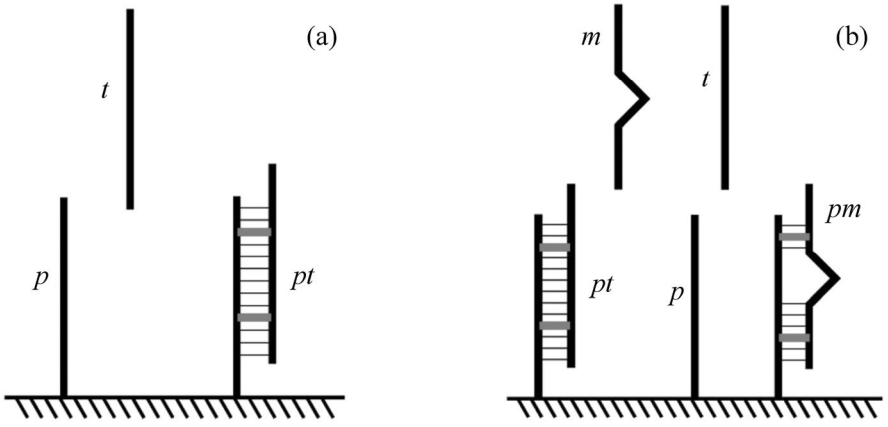

Fig. 1. Diagrams of (a) non-competitive and (b) competitive hybridization on the surface in the presence of ligands.

oligonucleotide *pt*. In the simplest case, the surface will be covered only by the free probes *p* and the hybridized oligonucleotides *pt* for one type of target consisting of the single-stranded DNA. Then, we have one reaction

$$p + t \rightleftharpoons pt\,. \tag{1}$$

In this case, the reactions of non-competitive hybridization are absent (Fig. 1a). The dependence of the degree of hybridization $x = N_{pt}/N_0$ on the concentration of targets c_t is described with the use of the isotherm of hybridization. The binding reactions for intercalating ligands *l* will have the following form:

$$\begin{aligned} &pt + l \rightleftharpoons pt_1,\\ &pt_1 + l \rightleftharpoons pt_2,\\ &\dots\\ &pt_{N-1} + l \rightleftharpoons pt_N, \end{aligned} \tag{2}$$

where *pt* is the free duplex, and pt_j a target–probe duplex coupled to a ligand *l*.

In the absence of ligands, the free energy of the layer with probes will have the following form [11]:

$$G = G_0 + N_{pt}\mu_{pt}^0 + (N_0 - N_{pt})\mu_p^0 + N_0\Sigma\gamma_{\rm el} + k_{\rm B}T\left[N_{pt}\ln\left(\frac{N_{pt}}{N_0}\right) + \left(N_0 - N_{pt}\right)\ln\left(\frac{N_0 - N_{pt}}{N_0}\right)\right], \tag{3}$$

where Σ is the area per one probe, G_0 the density of the free energy of the bare surface, μ_{pt}^0 and μ_p^0 the chemical potentials of the *pt* and *p* probes in the initial state, and $\gamma_{\rm el}$ the electrostatic density of the free energy of the probe layer.

If the intercalation is the only mechanism of ligands binding, the formation of the DNA–ligand complex will be restricted only by the double-stranded regions, and the free energy of the probe layer is

$$G_L = G + N_{pt}\left\{m\mu_b^0 + k_{\rm B}T\left[m\ln\left(\frac{m}{N}\right) + \left(N - m\right)\ln\left(\frac{N - m}{N}\right)\right]\right\}, \tag{4}$$

where *m* is he number of bound ligands per hybridized probe *pt*, and μ_b^0 the chemical potential of the bound ligand in the initial state. It is assumed that the available number of binding places on the duplex *pt* coincides with the length *N*. Thus, the free energy of the layer with probes is written as a function of independent quantities: the number of hybridized probes N_{pt} and the number of bound ligands $N_b = mN_{pt}$. The free energy is as follows

$$G_L\left(N_{pt}, N_b\right) = G\left(N_{pt}\right) + N_b\mu_b^0 + k_{\rm B}T\left[N_b\ln\left(\frac{N_b}{NN_{pt}}\right) + \left(NN_{pt} - N_b\right)\ln\left(\frac{NN_{pt} - N_b}{NN_{pt}}\right)\right]. \tag{5}$$

2.2. Adsorption and Hybridization Isotherms

For the reactions (1) and (2), the equilibrium state will be determined by the conditions

$$\mu_{pt} = \mu_p + \mu_t\,, \tag{6}$$

$$\mu_b = \mu_l\,, \tag{7}$$

where μ_{pt} is the chemical potential of the hybridized probe *pt*, μ_t the chemical potential of the target, μ_p the chemical potential of the probe, and the quantities μ_b and μ_l the chemical potential of the bound and unbound ligands, respectively [20].

The exchange chemical potential of the hybridized probe ($\Delta\mu_{pt} = \mu_p - \mu_t$) is written in the form

$$\Delta\mu_{pt} = \frac{\partial G_L}{\partial N_{pt}} = \Delta\mu_{pt}^0 + N\frac{\partial\gamma_{\text{el}}}{\partial\sigma} + k_{\text{B}}T\ln\frac{x}{1-x} + k_{\text{B}}T\ln(1-r), \tag{8}$$

where $r = N_b/NN_{pt}$ describes the degree of adsorption of l ligands in the double-stranded DNA. The density of electrostatic free energy is considered as a function of the charge density at the surface σ. At the same time, the chemical potential of bound ligands [20] is

$$\mu_b = \frac{\partial G_L}{\partial N_b} = \mu_b^0 + k_{\text{B}}T\ln\frac{r}{1-r}. \tag{9}$$

In the weak-solution approximation, the chemical potential of the target has the following form

$$\mu_t = \mu_t^0 + k_{\text{B}}T\ln c_t \tag{10}$$

and the chemical potential of free ligands in solution

$$\mu_l = \mu_l^0 + k_{\text{B}}T\ln c_l, \tag{11}$$

where the quantities c_t and c_l are the volumetric concentrations of targets and ligands, respectively. With allowance of (6)–(11), we obtain the isotherm of hybridization

$$\frac{x(1-r)^N}{c_t(1-x)} = K_t\exp\left(-\frac{N}{k_{\text{B}}T}\frac{\partial\gamma_{\text{el}}}{\partial\sigma}\right), \tag{12}$$

where $K_t = \exp\left(-\Delta G^0/(k_{\text{B}}T)\right)$ and $\Delta G^0 = \mu_{pt}^0 - \mu_p^0 - \mu_t^0$. The equilibrium distribution l between the bound and free states will be described by the adsorption isotherm

$$\frac{r}{c_l(1-r)} = K_l, \tag{13}$$

where $K_l = \exp\left(-\Delta g^0/(k_{\text{B}}T)\right)$ and $\Delta g^0 = \mu_b^0 - \mu_l^0$.

3. COMPETITIVE SURFACE HYBRIDIZATION

Consider the second scenario, in which the solvent contains targets of two different types t and m that do not hybridize in the volume, however, both are capable of hybridizing to one and the same probe p on the surface (Fig. 1b). Here, t is a sequence completely complementary to probe p, and m is an inconsistent sequence that is only partially complementary to the probe p. It is assumed that the available number of binding places for intercalating ligands at the duplex pm is M, where $M < N$.

According to the approach developed in [11], the free energy of the layer of probes free of ligands can be written in the following form:

$$\begin{aligned} G_C = {} & G_0 + N_{pt}\mu_{pt}^0 + N_{pm}\mu_{pm}^0 + (N_0 - N_{pt} - N_{pm})\mu_p^0 + N_0\Sigma\gamma_{\text{el}} \\ & + k_{\text{B}}T\left[N_{pt}\ln\left(\frac{N_{pt}}{N_0}\right) + N_{pm}\ln\left(\frac{N_{pm}}{N_0}\right) + (N_0 - N_{pt} - N_{pm})\ln\left(\frac{N_0 - N_{pt} - N_{pm}}{N_0}\right)\right], \end{aligned} \tag{14}$$

where N_{pm} is the number of duplexes pm, and μ_{pm}^0 the chemical potential of these duplexes in the initial state.

If the intercalation is the only binding mechanism, the formation of the DNA–ligand complexes will be limited only to the double-stranded regions, and the free energy of the probe layer will be

$$G_{CL} = G_C + N_{pt}\left\{n\mu_b^0 + k_{\mathrm{B}}T\left[n\ln\left(\frac{n}{N}\right) + (N-n)\ln\left(\frac{N-n}{N}\right)\right]\right\} + N_{pm}\left\{m\mu_b^0 + k_{\mathrm{B}}T\left[m\ln\left(\frac{m}{M}\right) + (M-m)\ln\left(\frac{M-m}{M}\right)\right]\right\}, \quad (15)$$

where n is the number of ligands related to the probe *pt*, and m the number of ligands associated with the probe *pm*. Thus, the free energy of the layer with the probes depends on the number of completely complementary hybridized probes N_{pt}, the number of not completely complementary hybridized probes N_{pm}, and the number of ligands related to the duplexes *pt* and *pm* (for N_1 and N_2, respectively):

$$G_{CL}(N_{pt}, N_{pm}, N_1, N_2) = G_C(N_{pt}, N_{pm}) + (N_1 + N_2)\mu_b^0 + k_{\mathrm{B}}T\left[N_1\ln\left(\frac{N_1}{NN_{pt}}\right) + (NN_{pt} - N_1)\ln\left(\frac{NN_{pt} - N_1}{NN_{pt}}\right)\right] + k_{\mathrm{B}}T\left[N_2\ln\left(\frac{N_2}{MN_{pm}}\right) + (MN_{pm} - N_2)\ln\left(\frac{MN_{pm} - N_2}{MN_{pm}}\right)\right]. \quad (16)$$

Here $N_1 = nN_{pt}$ and $N_2 = mN_{pm}$. The chemical potential of the ligands related to the complementary duplex *pt* will be

$$\mu_b^1 = \frac{\partial G_{CL}}{\partial N_1} = \mu_b^0 + k_{\mathrm{B}}T\ln\frac{r_1}{1-r_1}, \quad (17)$$

where the quantity $r_1 = N_1/(NN_{pt})$ characterizes the degree of adsorption of ligands on the duplex *pt*. The chemical potential of the ligands related to the partially complementary duplex *pm* will be

$$\mu_b^2 = \frac{\partial G_{CL}}{\partial N_2} = \mu_b^0 + k_{\mathrm{B}}T\ln\frac{r_2}{1-r_2}, \quad (18)$$

where $r_2 = N_2/(NN_{pm})$.

At the same time, the chemical potentials for the exchange of hybridized probes *pt* and *pm* will have the form:

$$\Delta\mu_{pt} = \Delta\mu_{pt}^0 + N\frac{\partial\gamma_{\mathrm{el}}}{\partial\sigma} + k_{\mathrm{B}}T\ln\frac{x}{1-x-y} + Nk_{\mathrm{B}}T\ln(1-r_1),$$
$$\Delta\mu_{pm} = \Delta\mu_{pm}^0 + N\frac{\partial\gamma_{\mathrm{el}}}{\partial\sigma} + k_{\mathrm{B}}T\ln\frac{y}{1-x-y} + Nk_{\mathrm{B}}T\ln(1-r_2), \quad (19)$$

where $y = N_{pm}/N_0$.

The equilibrium state between the ligands, targets, incompatible sequences in solution, and the hybridized probes will be described by the following conditions:

$$\mu_b^1 = \mu_l,$$
$$\mu_b^2 = \mu_l,$$
$$\Delta\mu_{pt} = \mu_t,$$
$$\Delta\mu_{pm} = \mu_m, \quad (20)$$

where μ_m is the potential of free incompatible sequences *m*. Within the weak-solution approximation, the value of μ_m can be estimated as $\mu_m = \mu_m^0 + k_B T \ln c_m$, where the c_m value is the concentration of the *m* sequences.

4. RESULTS AND DISCUSSION

The isotherm of targets hybridization in the absence of ligands $x_0(c_t)$ [11] is reproduced by substitution the value $r = 0$ in equation (12). Consider the shift of the hybridization isotherm $\delta x = x - x_0$ caused by the ligands. The equilibrium degree of adsorption r^* is

$$r^* = \frac{c_l K_l}{1 - c_l K_l}. \tag{21}$$

Thus, the effect of adsorption of intercalating ligands reduces to the renormalization of the binding constant K_t:

$$\tilde{K}_t = K_t \exp\left(-N \ln\left(1 - r^*\right)\right). \tag{22}$$

The density of the electrostatic free energy of the layer with the probes γ_{el} was estimated in [11], in the approximation of two-component box [21–24]. In this approximation, the stepped profile of the distribution of monomers allows one to consider the polyelectrolytes on the surface as a continuous region with a uniform charge distribution. With a high salts content, the screening in a charged layer results to the following expression for the density of electrostatic free energy:

$$\frac{\gamma_{el}}{k_B T} = 4\pi\sigma^2 l_B \frac{r_D^2}{H}, \tag{23}$$

where $l_B = e^2/(\in k_B T)$ is the Bjerrum length, $\in$ the dielectric permeability, r_D the Debye shielding length, and *H* the thickness of the layer with probes. It is assumed that in this layer the charges are distributed uniformly. Because each chain contains the charge –*eN*, the charge density σ depends on the

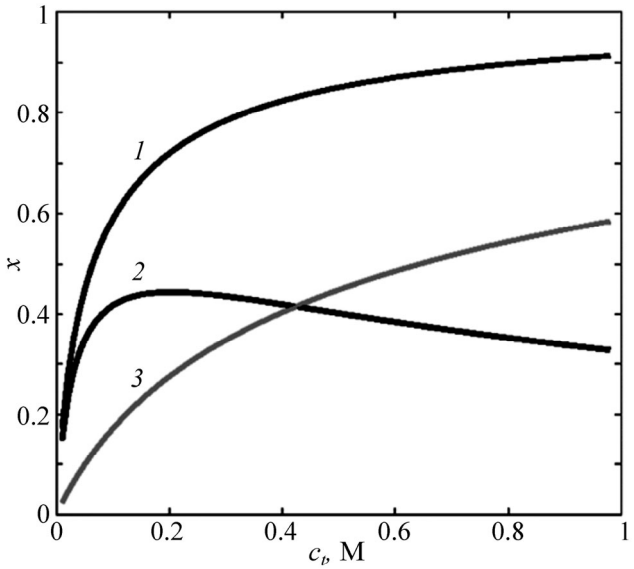

Fig. 2. Dependence of the degree of hybridization on the concentration of target: *1* – in the presence of ligands, *2* – the shift of the hybridization isotherm, and *3* – without ligands. The results are obtained for the values of the parameters $l_B \approx 7$ Å, $r_D \approx 3$ Å, $K_t = 10^9$ M^{-1} and $\Gamma \approx 2.57$.

degree of hybridization x as

$$\sigma = \sigma_0 (1 + x), \tag{24}$$

where $\sigma_0 = NN_0 / A$ and A is the area with the probes.

The hybridization isotherms obtained with the aid of equations (12), (21), (22), and (24) are shown in Fig. 2.

Thus, intercalating ligands cause a significant increase in the degree of hybridization. One of the important parameters responsible for the sensitivity of the DNA-chip is the concentration of targets, which results to the half-hybridization c_{50}^t [4–11]. The shift of concentration c_{50}^t as compared to the case without ligands δc_{50}^t is

$$\delta c_{50}^t = \frac{e^{3\Gamma/2}}{K_t} \left[\left(\frac{c_l K_l}{c_l K_l + 1} \right)^N - 1 \right], \tag{25}$$

where $\Gamma = 8\pi N \sigma_0 l_B r_D^2 / H$. The shift of concentrations for half-hybridization δc_{50}^t depends on the concentration of ligands l (Fig. 3).

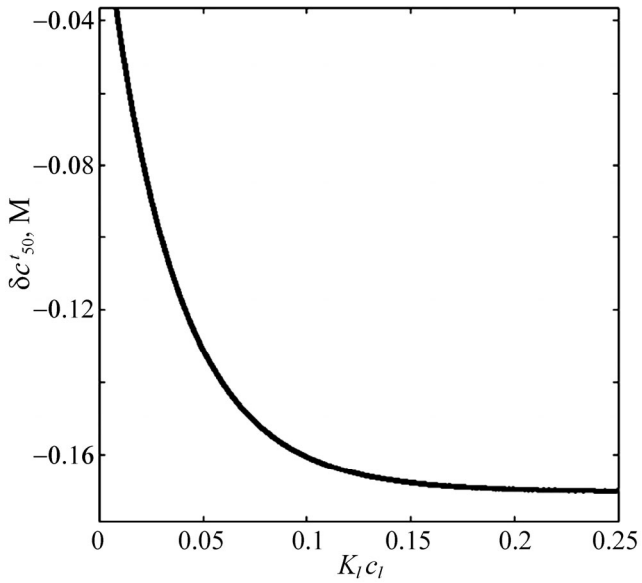

Fig. 3. Dependence of the shift of concentration for half-hybridizations on $K_l c_l$.

Thus, the intercalating ligands related to the hybridized probes *pt*, reduce the concentration of half-hybridization essentially, and then influence the sensitivity of the DNA-chip in the case of the non-competitive hybridization.

From the system of equations (20) we obtain the hybridization isotherm in the case of competitive hybridization on the surface:

$$\begin{aligned} \frac{x}{1-x-y} &= c_t \tilde{K}_t \exp\left(-\frac{N}{k_B T} \frac{\partial \gamma_{el}}{\partial \sigma} \right), \\ \frac{y}{1-x-y} &= c_m \tilde{K}_m \exp\left(-\frac{N}{k_B T} \frac{\partial \gamma_{el}}{\partial \sigma} \right), \end{aligned} \tag{26}$$

where $\tilde{K}_t = K_t\left(1-r_1^*\right)^{-N}$ and $\tilde{K}_m = K_m\left(1-r_2^*\right)^{-M}$ are the renormalized constants of binding similar to the results obtained for the without the ligands case [11]:

$$K_t = \exp\left(-\frac{\mu_{pt}^0 - \mu_p^0 - \mu_t^0}{k_B T}\right),$$
$$K_m = \exp\left(-\frac{\mu_{pm}^0 - \mu_p^0 - \mu_m^0}{k_B T}\right). \tag{27}$$

In the initial state, if the chemical potential of the bound ligands μ_b^0 is the same for the duplexes *pt* and *pm*, then the equilibrium degree of helicity is $r_1^* = r_2^*$. As a result, we obtain that

$$r^* = \frac{c_l K_l}{c_l K_l + 1}, \tag{28}$$

where the constant of ligand binding is equal to

$$K_l = \exp\left(-\frac{\mu_b^0 - \mu_l^0}{k_B T}\right). \tag{29}$$

Thus, in the case of competitive hybridization at the surface, the ligand intercalation increases essentially the binding constants of targets and the incompatible sequences, thereby increasing the sensitivity of the DNA-chip.

At the same time, the influence of ligands is more expressed for the target sequences *t* due to the exponential dependence on the number of binding places *N* and *M* for binding constants

$$\tilde{K}_t = K_t\left(1-r^*\right)^{-N},$$
$$\tilde{K}_m = K_m\left(1-r^*\right)^{-M}. \tag{30}$$

This effect is especially important at high values of the parameter $K_l c_l$, which corresponds to the high concentration of ligands and/or the large values of the binding constant K_l. If the number of binding sites *M* for the incompatible duplexes *pm* differs essentially from the number of binding places of complementary duplexes *pt*, an essential increase in the selectivity can be obtained.

5. CONCLUSION

The thermodynamic properties of the surface of the DNA-chip with the DNA-probes attached to it interacting with the DNA-targets and ligands in solution are investigated. Some factors that influence the thermodynamics of the DNA hybridization at the interface between the solid and solution are analyzed. For the non-competitive and competitive hybridization of the DNA on the surface, such thermodynamic characteristics of the system as the hybridization isotherms and the concentration of the DNA targets corresponding to the half-hybridization of the DNA targets with the probes were studied. A comparative analysis is carried out of the hybridization isotherms of the DNA-probe with the DNA-targets and the DNA with sequences that only partly complementary to the DNA-probes, which shows that the binding to intercalating ligands results to an increase in the selectivity and sensitivity of the DNA-chips.

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