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Sensitivity of DNA Sensors in the Presence of Charged Ligands

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Abstract—Factors that influence both the thermodynamics of hybridization and the stability of the DNA–DNA duplexes are analyzed. The noncompetitive DNA hybridization cases in the presence of mono- and bivalent positively charged ligands have been investigated and the comparison is made with the case of uncharged ligands. It has been shown that the charged ligands enhance the sensitivity of the DNA chips as compared with the uncharged ones.

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1. INTRODUCTION

The DNA chips are one of the promising tools with a wide variety of applications, such as medical diagnostics, the monitoring of environmental pollutants, a protection against biological weapons, etc. [1, 2]. One of the important directions in the development of the DNA chips is the increase in their sensitivity because of the amplification of an electrical signal and the stability of the target probe of hybridization. The effectiveness of devices such as the DNA sensors and the DNA chips depends on the accuracy of the prediction of the experimental parameters responsible for the thermosstability of duplexes of nucleic acids and for the time of forming of the DNA duplexes [3].

Some factors influence the thermodynamics of hybridization, in particular, the surface density of the single-stranded DNA (25–49 nucleotides long) immobilized on the surface, and the presence of competing hybridization. The stability of the DNA–DNA and DNA–RNA duplexes is determined by two key factors: the sequence and external factors (pH, ionic strength, the concentration of low molecular weight compounds (ligands), the presence of interphase boundaries, of geometric constraints, etc.). A better understanding of the physicochemical processes underlying the hybridization of the DNA and RNA on the surface of an electrical converter is important to improve the efficiency of the DNA chips and their manufacture [4].

One of the main qualifying standards for the DNA sensors is the high sensitivity, which, in turn, requires the maximum efficiency of hybridization at the interface between the solid and liquid phases. The hybridization of nucleic acids depends largely on temperature, a salt concentration, viscosity, GC-composition, and other physico-chemical characteristics. An increase in the sensitivity of the DNA sensors can be achieved with the help of electrochemically active compounds with the higher affinity for the double-stranded than for the single-stranded DNA. This type of compounds can increase significantly

the stability of double-stranded regions, as well as 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 a planar heterocyclic structure that are placed between the nitrogenous bases and change the local structure of the double-stranded DNA [5–7].

Both in the bulk [8, 9] and on the surface [4, 10–16], the thermodynamics and kinetics of hybridization have been thoroughly studied in recent years. The spectrum of the 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 bulk [9], the isotherms of hybridization on the surface [4], etc. At the same time, the DNA–ligand interactions have been also considered in a large number of works devoted to the intercalation [5–7] and the binding of ligands in the minor groove [17, 18], their cross-docking [19], etc. However, as far as we know, the influence of the DNA–ligand interaction on the thermodynamics and kinetics of hybridization has never been previously considered. In connection to the DNA biosensors development, the theoretical analysis of the influence of the intercalation of ligands on the hybridization of DNA on the surface becomes necessary.

The present work is devoted to the study of the isotherm of the DNA hybridization on the surface in the presence of positively charged ligands that bind to the native regions of the DNA. Although the DNA chips are immersed in a target solution for a relatively short interval in practical use, where the kinetics of hybridization plays an important role. The understanding of equilibrium properties is also necessary for a comparative evaluation of the importance of the kinetic and thermodynamic factors for the efficiency of the DNA chips.

2. NONCOMPETITIVE HYBRIDIZATION IN THE PRESENCE OF LIGANDS

2.1. Free Energy

Let us consider the equilibrium isotherm of hybridization for an idealized but experimentally available situation when the DNA chip is immersed in a solution containing intercalated ligands and there are only one type of single-stranded target (Fig. 1).

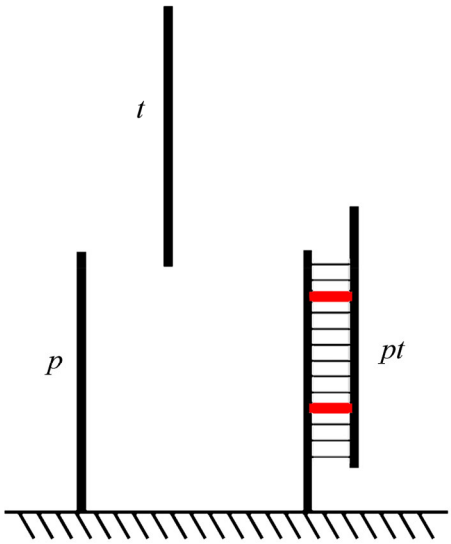

Fig. 1. Scheme of noncompetitive hybridization on the surface in the presence of ligands.

Let us consider the set of N_0 of the single-stranded DNA probe molecules p, where N_{pt} of them are hybridized with the target t. The hybridization of p and t creates a double-stranded oligonucleotide pt on the surface. In the simplest case, the surface will be covered only by free probes p and hybridized oligonucleotides for single target species consisting of the single-stranded DNA. In this case, we have one reaction

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

The reactions of competitive hybridization are absent (Fig. 1). The dependence of the degree of hybridization $x = N_{pt}/N_0$ on the concentration of targets c_t is described with the aid of the isotherm of hybridization. For intercalating ligands l, the binding reactions will have the following form:

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

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

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

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

where Σ is the area per one probe, G_0 is the free-energy density of a bare surface, μ_{pt}^0 and μ_p^0 are the chemical potentials of probes pt and p in the initial state, and γ_{el} is the electrostatic density of the free energy of the probe layer.

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

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

where m is the number of bound ligands per one hybridized probe pt, and μ_b^0 is the chemical potential of the bound ligand in the initial state. It is supposed that the available number of the binding sites pt on the duplex coincides with the length N. Thus, the free energy of the layer with probes can be 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 has the following form

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

2.2. Adsorption and Hybridization Isotherms

The equilibrium state for the reactions (1) and (2) will be determined by the conditions

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

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

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

In [11], the density of the electrostatic free energy γ_{el} of the layer with probes was estimated within the approximation of a 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. For a high content of salts, the shielding in a charged layer results in the following expression for the density of electrostatic free energy:

$$\frac{\gamma_{\text{el}}}{k_{\text{B}}T} = 4\pi\sigma^2 l_{\text{B}} \frac{r_{\text{D}}^2}{H}, \tag{8}$$

where $l_{\text{B}} = e^2/(\varepsilon k_{\text{B}}T)$ is the Bjerrum length, ε is the dielectric permeability, r_{D} is the Debye shielding length, and σ is the surface charge density. Here, H is the thickness of the layer with probes, and it is assumed that the charges are uniformly distributed in this layer. Because each chain contains the charge $-eN$, the surface charge density σ depends on the degree of hybridization x as

$$\sigma = \frac{NN_0 + NN_{pt} - zN_b}{A}, \tag{9}$$

where A is the surface area of sensor and z is the valence of a positively charged ligand.

Taking into account the dependence of the surface density of electrostatic free energy (8) on the number of hybridized probes N_{pt} and the total number of bound ligands N_b, the exchange chemical potential of the hybridized probe ($\Delta\mu_{pt} = \mu_p - \mu_t$) can be written in the form

$$\Delta\mu_{pt} = \frac{\partial G_L}{\partial N_{pt}} = \Delta\mu_{pt}^0 + N_0\Sigma\frac{\partial\gamma_{\text{el}}}{\partial N_{pt}} + k_{\text{B}}T\ln\frac{x}{1-x} + k_{\text{B}}T\ln(1-r), \tag{10}$$

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

$$\mu_b = \frac{\partial G_L}{\partial N_b} = \mu_b^0 + N_0\Sigma\frac{\partial\gamma_{\text{el}}}{\partial N_b} + k_{\text{B}}T\ln\frac{r}{1-r}. \tag{11}$$

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

$$\mu_t = \mu_t^0 + k_{\text{B}}T\ln c_t, \tag{12}$$

and the chemical potential of free ligands in the solution is equal to

$$\mu_l = \mu_l^0 + k_{\text{B}}T\ln c_l, \tag{13}$$

where the values of c_t and c_l are the volume concentrations of the targets and ligands, respectively.

Taking into account the equations (6)–(13), we obtain the hybridization isotherm

$$\frac{x(1-r)^N}{c_t(1-x)} = K_t \exp\left(-\frac{N_0\Sigma}{k_B T}\frac{\partial\gamma_{el}}{\partial N_{pt}}\right), \tag{14}$$

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

$$\frac{r}{c_l(1-r)} = K_l \exp\left(-\frac{N_0\Sigma}{k_B T}\frac{\partial\gamma_{el}}{\partial N_b}\right), \tag{15}$$

where $K_l = \exp\left(-\frac{\Delta g^0}{k_B T}\right)$ and $\Delta g^0 = \mu_b^0 - \mu_l^0$. The system of equations (14) and (15) is transformed as

$$\begin{aligned} \frac{x(1-r)^N}{1-x} &= c_t K_t \exp\left[-\Gamma(1+x-zrx)\right], \\ \frac{r}{1-r} &= c_l K_l \exp\left[\frac{\Gamma}{N}(1+x-zrx)\right], \end{aligned} \tag{16}$$

where $\Gamma = 8\pi N\sigma_0 l_B \frac{r_D^2}{H}$ and $\sigma_0 = \frac{NN_0}{A}$.

3. RESULTS

3.1. Isotherms of Adsorption and Hybridization in the Presence of Monovalent Ligands

The numerical solution of the system of equations (16) for monovalent ligands $(z=1)$ gives the hybridization and adsorption isotherms represented in Fig. 2. For uncharged ligands, the isotherms of

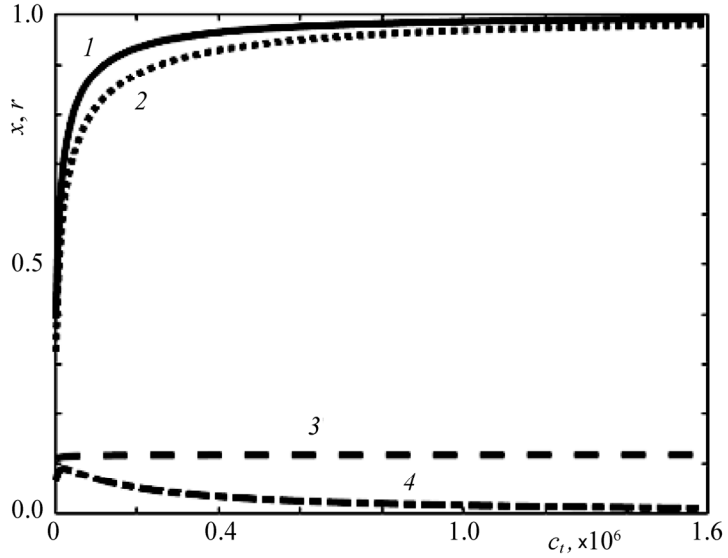

Fig. 2. Isotherms of hybridization for the monovalent positive charged ligand (*1*), for uncharged ligands (*2*), for monovalent ligands (*3*), and the shift of the isotherms of hybridization from the case of an uncharged ligand to the charged ligand (*4*). Curves are obtained for the following values of parameters: $l_B \approx 7$ Å, $r_D = 3$ Å, $N = 16$, $K_t = 10^9$ M^{-1} and $\Gamma \approx 2.57$.

hybridization were obtained in [25], where it was shown that they have the form

$$\frac{x}{1-x} = c_t \tilde{K}_t e^{-\Gamma(1+x)}, \tag{17}$$

where $\tilde{K}_t = K_t e^{-N\ln(1-r^*)}$ and $r^* = c_l K_l / (c_l K_l + 1)$ is the equilibrium degree of adsorption. The hybridization isotherm for uncharged ligands is presented in Fig. 2, which shows that the degree of adsorption of charged ligands weakly depends on the concentration of the DNA targets in the solution. At the same time, the presence of a charge significantly enhances the hybridization of the target–probe on the surface of sensor at the low concentrations c_t of targets. Perhaps, the effect is caused by the partial neutralization of the charge of the surface layer.

3.2. Isotherms of Adsorption and Hybridization in the Presence of Bivalent Ligands

In the presence of bivalent ligands, the hybridization of the probe–target on the surface of the DNA sensor is described by the system of equations (14) corresponding to $z = 2$. The degree of filling is determined by the equation

$$r(1-r) = c_l K_l (c_t K_t)^{2/N} \left(\frac{1}{x} - 1\right)^{2/N}. \tag{18}$$

Hence, the solution for the degree of filling depending on the degree of hybridization has two branches: $r \geq 1/2$ and $r \leq 1/2$. In an obvious manner, it follows from the equation (18) that the degree of hybridization satisfies the condition

$$\frac{(4c_l K_l)^{\frac{2}{N}} c_t K_t}{1 + (4c_l K_l)^{\frac{2}{N}} c_t K_t} \leq x \leq 1. \tag{19}$$

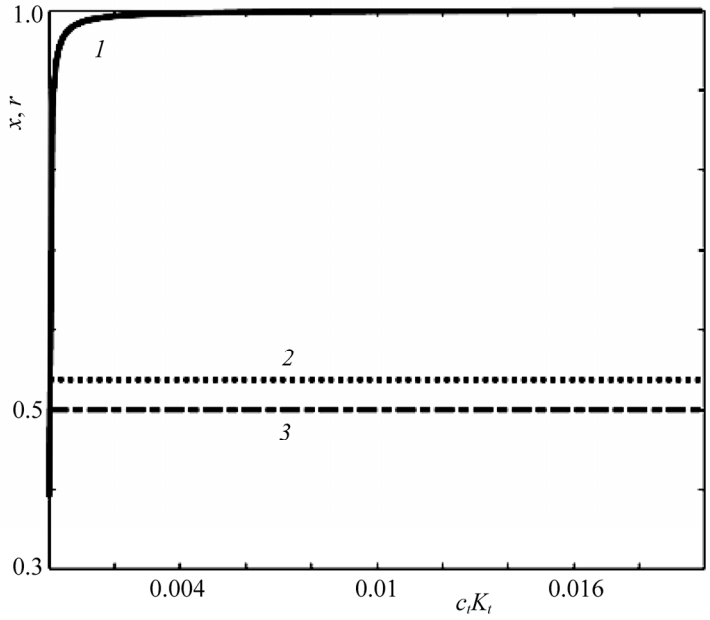

Fig. 3. Isotherms of hybridization for the bivalent positive charged ligand (*1*) and the isotherms of adsorption for the solutions $r \geq 1/2$ (*2*) and $r \leq 1/2$ (*3*). The curves are obtained for the following values of parameters: $l_B \approx 7$ Å, $r_D \approx 3$ Å, $N = 16$, $K_t = 10^9$ M^{-1} and $\Gamma \approx 2.57$.

Thus, in the presence of divalent ligands, the degree of hybridization cannot be lower than the value

$$x_{\min} = \frac{\left(4c_l K_l\right)^{\frac{2}{N}} c_t K_t}{1+\left(4c_l K_l\right)^{\frac{2}{N}} c_t K_t}.$$

At $z = 2$, the numerical solution of the system of equations (14) gives the isotherms of hybridization and adsorption shown in Fig. 3. The branches of solutions corresponding to $r \geq 1/2$ and $r \leq 1/2$ are represented by the curves 2 and 3, respectively.

These solutions do not differ from each other qualitatively and the behavior of the hybridization isotherm in both cases is almost identical. However, the ligands fill more in one case of the available binding sites for the native DNA, and less than half in the other case. Depending on the parameters of the system, either one or the other branch will correspond to the metastable state. The results represented in Figs. 2 and 3 show that the presence of a charge in the intercalating ligand enhances the hybridization on the surface of the DNA sensor as compared to that for an uncharged ligand, consequently, the sensitivity of the DNA sensor increases.

4. CONCLUSION

The thermodynamic properties of the surface of the DNA sensor with the grafted DNA probes interacting with the DNA targets and ligands in solution were investigated. Some factors which influence the thermodynamics of the DNA hybridization at the solid state – solution interface were analyzed. For cases of the noncompetitive DNA hybridization on the surface, such thermodynamic characteristics of the system as the isotherms of the DNA targets hybridization with the DNA probes and the isotherms of adsorption of intercalating ligands on the complexes of the probe–target are investigated. The analysis shows that the binding to charged intercalating ligands results in an increase of the sensitivity of the DNA sensors.

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