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COMBINED INFLUENCE OF COMPETITIVE BINDING AND MASS TRANSFER ON RESPONSE OF AFFINITY-BASED BIOSENSORS

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Abstract

Binding of target and competitor molecules to the functionalized surface of affinity-based biosensors is analyzed, considering also mass transfer processes of both molecular species. It is shown that the mentioned processes have a significant influence on the sensor's transient and equilibrium time response.

Introduction

Detection of target biomolecules in solutions is an important task in medicine and environmental protection, while investigation of biomolecular interactions is of great significance for the fundamental biological and pharmaceutical research. One class of biosensors for such applications use surface-based detection methods, where highly specific binding of target analyte to probe molecules (called receptors) immobilized on the sensing surface is converted to the sensor's output signal. The number of bound target molecules determines the sensor's response. Biological samples often contain other molecular species (competitors) which also bind to the same receptors with a certain affinity. For correct interpretation of biosensor response it is necessary to analyze all the processes relevant for generation of the sensor's output signal. The mass transfer (MT) influence on the kinetics of binding of molecules is investigated in (1, 2), while (3) deals with binding of the target and competitor molecules. In this paper we analyze the influence of both competitive binding (CB) and MT on the biosensor response, assuming that the distribution of the analyte concentration in the sensor's reaction chamber can be approximated using the two-compartment model (4). This is typically the case in surface plasmon resonance (SPR), quartz crystal microbalance (QCM) and thin film bulk acoustic resonator (FBAR) sensors (1, 4).

Theoretical considerations

In the affinity-based detection methods it is desirable that only the target molecules bind to the receptors and that transport of the target molecules to the sensing surface is fast compared to the binding reaction speed. Assuming one binding site per receptor and equivalence of all binding sites, a reversible simple one-to-one binding reaction between the target and the receptor molecules is described by the equation:

$$dN_T/dt = k_{fT} C_T (N_m - N_T) - k_{rT} N_T \qquad (1)$$

thus, the number of bound target molecules N_T exponentially reaches the equilibrium value $N_{Te1}=k_{fT}C_T N_m/(k_{rT}+k_{fT}C_T)$ with the time constant $t_T=1/(k_{rT}+k_{fT}C_T)$. Here C_T, k_{fT}, and k_{rT} are the concentration in the sample, the association rate constant and the dissociation rate constant of the target molecules, respectively, and $N_m=n_m A$ is the number of receptors on the surface of area A (n_m is the receptor surface density).

When transport of the target molecules is taken into account, Eq. 1 changes: instead of C_T it contains the concentration of the target molecules in the immediate vicinity of receptors, C_{TS}. In biosensors in which a thin layer depleted of target molecules is formed close to the sensing surface (4), C_{TS} can be determined by the two-compartment model as $C_{TS}=(k_{mT}AC_T+k_{rT}N_T)/(k_{mT}A+k_{fT}(N_m-N_T-N_C))$, where k_{mT} is the MT coefficient of target molecules. Then, the change of the number of bound molecules in time is nearly exponential with the time constant $t_{Tm}=(k_{rT}+k_{fT}C_T+k_{fT}k_{rT}n_m/k_{mT})/(k_{rT}+k_{fT}C_T)^2$ only if $N_{Te1}k_{fT}/(\tau_{Tm}k_{mT}A)<<1$. In other cases, the dependence $N_T(t)$ is determined by the Lambert special function (2).

We analyze the situation when only one species of competitor molecules exists in the sample solution, and we assume: 1) reversible binding reactions between each molecular species (target and competitor) and receptors, without altering any species of reacting molecules, 2) each receptor has one type of binding sites for one molecular species, and all receptors are equivalent, 3) only one molecule can be bound to a receptor at any time. If we include in the consideration the MT of both molecular species through the sample solution to and from the receptor sites, which can be slow compared to the binding reactions, the rates of change of the numbers of bound molecules will then be described by the system of two nonlinear differential equations

$$dN_T/dt = k_{fT}C_{TS}(N_m - N_T - N_C) - k_{rT}N_T \quad (2)$$

$$dN_C/dt = k_{fC}C_{CS}(N_m - N_T - N_C) - k_{rC}N_C \quad (3)$$

where N_C, k_{fC}, k_{rC} and C_C are the parameters for competitor molecules, which correspond to previously mentioned target molecules parameters N_T, k_{fT}, k_{rT}, and C_T. Assuming also the validity of the two-compartment model for the competitor concentration, C_{CS} can be expressed as $C_{CS}=(k_{mC}AC_C+k_{rC}N_C)/(k_{mC}A+k_{fC}(N_m-N_T-N_C)$, where k_{mC} is the MT coefficient of the competitor molecules.

If neglecting the mass transfer effect, Eqs. 2 and 3 can be solved analytically, yielding the time evolution of the number of bound target and competitor molecules

$$N_T(t) = N_{Te} + K_I \exp(-t/r_I) + K_{II}\exp(-t/r_{II}) \quad (4)$$

$$N_C(t) = N_{Ce} + K_{III}\exp(-t/r_I) + K_{IV}\exp(-t/r_{II}) \quad (5)$$

After the transient period, whose duration is determined by the time constants τ_I and τ_{II} ($\tau_{I,II}=2[\tau_T^{-1}+\tau_C^{-1}\mathrm{m}((\tau_T^{-1}-\tau_C^{-1})^2+4N_{Te1}N_{Ce1}(\tau_T\tau_C N_m^2)^{-1})^{1/2}]^{-1}$, $N_{Ce1}=k_{fC}C_C N_m/(k_{rC}+k_{fC}C_C)$, $t_C=1/(k_{rC}+k_{fC}C_C)$), the equilibrium is established, characterized by the equilibrium numbers of bound molecules $N_{Te}=N_m k_{fT}C_T/k_{rT}/D$ and $N_{Ce}=N_m k_{fC}C_C/k_{rC}/D$, $D=1+k_{fT}C_T/k_{rT}+k_{fC}C_C/k_{rC}$. The coefficients K_I-K_{IV} are determined by the initial conditions $N_T(0)=N_C(0)=0$ and Eqs. 2-5 must be satisfied for every t. However, if MT is considered, Eqs. 2 and 3 become too complex to be solved analytically. We obtain $N_T(t)$ and $N_C(t)$ by solving Eqs. 2 and 3, numerically.

The biosensor's signal depends on the number of bound molecules of both kind. For example, it can be determined by the total bound mass $m_b=M_T N_T+M_C N_C$ (M_T and M_C are the molecular masses of the target analyte and the competitor, respectively).

Results and Discussion

The presented theory is used for the analysis of the separate influences of CB and MT, and also of their combined effects on the biosensor's response, for the parameter values realistic for biomolecules and biosensors (1-4): $k_{fT}=10k_{fC}=8\cdot10^7$ $(\mathrm{Ms})^{-1}$, $k_{rT}=4k_{rC}=0.08$ s^{-1}, $C_T=C_C/2=1$ nM, $M_T=10M_C=5$ kDa, $k_{mT}=k_{mC}=20$ μm/s, $n_m=1\cdot10^{11}$ Mm, $A=1\cdot10^{-9}$ m^2. Fig. 1a shows the sensor signal (the mass of bound molecules) in time, for four cases. The solid line refers to the case when only the target molecules

exist in the sample and their transport to binding sites is fast ("T only" case). The dashed line corresponds to the binding of two molecular species to the receptors, with neglected mass transfer effects ("CB" case). The dotted line is for the case when only the target molecules bind to the receptors and their MT is taken into account ("MT" case). The dashed-dotted line shows the combined influence of the competitive binding and mass transfer, ("CB+MT" case). The diagram shows that binding of competitor molecules influences both the equilibrium value of the sensor's signal and the transient response. In the "CB" case the total equilibrium bound mass is even lower compared to binding of only the target molecules, and the response rate is also lower. Mass transfer also causes deviation of binding kinetics from the ideal case by decelerating the sensor's response. The combination of CB and MT processes changes the equilibrium value. It also decreases the sensor's response rate more than CB or MT does. Fig. 1b (the parameters that differ from Fig. 1a: $k_{fC}=k_{fC}/100$, $k_{rC}=k_{rT}/8$, $M_C=2M_T$) shows that in "CB" case the equilibrium bound mass can be greater than in the "T only" case. Fig. 1c shows that the time needed for the equilibrium state to be reached is significantly affected by slow mass transfer.

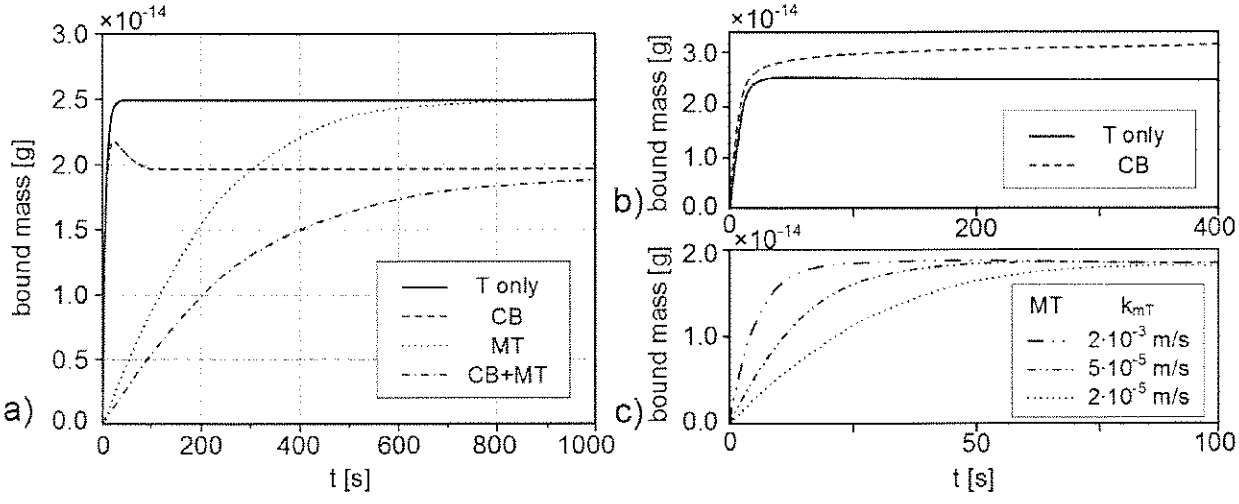

Figure 1. a) *The change of the sensor's signal in time for four different cases,* ***b)*** *influence of CB on the sensor's response,* ***c)*** *influence of MT on the sensor's response.*

The presented analysis shows that the influences of CB and MT must be considered in order to correctly interpret the response of affinity-based biosensors when time domain measurements are used for determination of target molecules concentration or for characterization of bimolecular reactions. Apart from its applicability in bimolecular affinity studies and also in investigations of competitive adsorption of biomolecules, the analysis can be used to provide the guidelines for improvement of biosensor sensitivity, selectivity and response rate. It is also useful for development of methods for simultaneous detection of multiple analyte specimens.

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