

The study of surface properties of an IgE-sensitive aptasensor using an acoustic method

M. Šnejdárková · L. Svobodová · V. Polohová ·
T. Hianik

Received: 17 September 2007 / Revised: 7 November 2007 / Accepted: 9 November 2007
© Springer-Verlag 2007

Abstract We applied the acoustic transverse shear mode (TSM) method for study of the surface properties of a DNA aptasensor that specifically binds human immunoglobulin E (IgE). The biotinylated 45-mer DNA aptamers were immobilized on the surface of a self-assembled layer composed of a mixture of polyamidoamine dendrimers of the fourth generation with 1-hexadecanetiol covered by neutravidin. Using the TSM method, we studied the kinetics of changes of the series resonant frequency, f_s , and the motional resistance, R_m , of a quartz crystal transducer, used as a support for formation of the sensing layer. We have shown that attachment of the biotinylated DNA aptamers onto the surface covered by neutravidin results in a decrease of f_s , but in an increase of R_m . Similar changes of f_s and R_m were observed following addition of IgE. This suggests the contribution of friction forces to the crystal oscillation, which was taken into account in the calculation of the mass changes at the sensor surface following binding processes.

Keywords DNA aptamer · Immunoglobulin E · Dendrimers · Transverse shear mode method · Biosensor

Introduction

DNA aptamers are single-stranded oligonucleotides that specifically bind various compounds with high specificity comparable to that of antibodies [1]. DNA aptamers are, however, more stable than antibodies and can be selected in vitro by means of a systematic evolution of ligands by exponential enrichment (SELEX) procedure. The synthesis and chemical modification of aptamers is well established, which makes them an ideal tool for practical applications in biosensors as artificial receptors [2]. So far aptamer-based technology has been used mostly for detection of proteins, using various physical methods (see [3] for a recent review). The mass-detection method based on a quartz crystal microbalance (QCM) belongs to most popular methods owing to its relative simplicity and good sensitivity comparable with methods based on optical detection, such as surface plasmon resonance [4]. The QCM has been extensively used with aptasensors for detection of human immunoglobulin E (IgE) [5] or human thrombin [6]. The QCM method is based on the linear dependence of the frequency of oscillation of a quartz crystal on its mass [7]; however, the corresponding Sauerbrey equation is exactly valid only for a dry crystal. In a solution friction between the sensing layer and the buffer may result in damping of the oscillations [8, 9]; therefore, for exact determination of the mass changes the contribution of the viscous forces to the frequency should be estimated.

In this work we studied the surface properties of an aptasensor specific to human IgE. For this purpose we used the transverse shear mode (TSM) method. This method allowed us to estimate the contribution of intermolecular friction between the sensor surface and the buffer to the frequency of crystal oscillation in all stages of sensor preparation.

M. Šnejdárková · L. Svobodová · V. Polohová
Institute of Animal Biochemistry and Genetics,
Slovak Academy of Sciences,
Moyzesova 61,
900 28 Ivanka pri Dunaji, Slovak Republic

T. Hianik (✉)
Department of Nuclear Physics and Biophysics,
Comenius University,
Mlynská dolina F1,
842 48 Bratislava, Slovak Republic
e-mail: hianik@fmph.uniba.sk

Materials and methods

Chemicals

Polyamidoamine dendrimer of the fourth generation (G4), 1-hexadecanethiol (HDT), human serum albumin (HSA), glutaraldehyde (GA; 50% aqueous solution), chloroform, methanol (99%), absolute ethanol (99.9%), sodiumborohydride, sodium dodecyl sulfate, HCl, ethylenediaminetetraacetic acid and NaOH were purchased from Sigma-Aldrich (USA). Glycine and acetone were from Slavus (Slovakia). Neutravidin was from Pierce Biotechnology (Rockford, IL, USA).

We used the optimized DNA aptamer that selectively binds human IgE (D17.4ext) reported in [5] with the following composition: 5' biotin GCG CGG GGC ACG TTT ATC CGT CCC TCC TAG TGG CGT GCC CCG CGC 3'. The aptamer was purchased from Thermo Electron (Germany). Human IgE, clone HE1, was purchased from Diatec (Norway). All solutions and chemicals were of analytical grade. The Millipore water was produced by an Elix 5 instrument (Millipore, El Paso, USA). We used phosphate-buffered saline (PBS) of the following composition 138 mM NaCl, 1.6 mM KH_2PO_4 , 2.7 mM KCl, 8.1 mM Na_2HPO_4 , pH 7.4, and PBS*, which contained PBS and 1 mM MgCl_2 . Before use the buffers were briefly boiled and then slowly cooled to ambient temperature (25 ± 1 °C). For sensor regeneration, 0.2 M glycine-HCl buffer, pH 2.3 was used.

Preparation of the aptasensor

For preparation of the aptasensor we used AT-cut quartz with a fundamental frequency of 8 MHz (CH Instruments, USA), covered on both sides by thin gold layers that served as electrodes (working area 0.2 cm^2). The HDT-dendrimer layers were formed on a clean surface of the crystal as described elsewhere [6]. The crystal was mounted between two silicon O-rings in the flow-through cell [9]. The analyte was introduced into the cell by means of a Genie Plus syringe pump (Kent Scientific, USA).

The neutravidin layer was formed on a surface of G4 HDT by cross-linking with GA as described elsewhere [10]. The proper folding of the aptamer into the native structure was performed prior to its immobilization by heating the stock solution of aptamer in PBS* (0.2 μM) to 95 °C. The solution was then quickly cooled in ice for 5 min and then slow equilibration to the desired temperature of 25 ± 1 °C took place. Immobilization of the biotinylated-DNA aptamer (0.2 μM in PBS*) onto the neutravidin layer was performed by flow of the aptamer solution over the neutravidin-modified surface for 30 min. All noncovalently attached compounds were washed out with the 0.2 M glycine-HCl buffer, pH 2.3 for 30 s. The aptasensor was finally rinsed

with PBS* at a flow rate of $50 \mu\text{l min}^{-1}$ until a stable baseline was reached. The IgE dissolved in PBS* in various concentrations was injected into the flow cell with a flow rate $50 \mu\text{l min}^{-1}$. After binding of IgE, the surface of the aptasensor was regenerated with 0.2 M glycine-HCl buffer, pH 2.3 for 20 s at a flow rate of $50 \mu\text{l min}^{-1}$. After this step the crystal was rinsed with PBS* at the same flow rate and was ready for further addition of IgE. As a negative control, HSA dissolved in PBS* was used.

Detection of IgE-aptamer interactions using the TSM method

The TSM method is based on application of a high frequency ac voltage to an AT-cut quartz crystal. Owing to the piezoelectric effect an acoustic shear wave is generated and propagates through the sensing layer into the liquid. This acoustic wave can be characterized by storage and dissipation processes. The measured electrical impedance of the sensor is related to the complex acoustic impedance. The imaginary part of the impedance represents energy storage and is proportional to a change in the series resonant frequency, f_s , while the real part is connected with energy dissipation and is related to motional resistance, R_m . The viscous forces result in friction between the surrounding liquid and the layer immobilized at the surface of the crystal. This is reflected in an increase of R_m and a decrease of f_s [8]. An 8712ES network analyzer (Agilent Technologies, USA) was used to measure the impedance properties of the sensor. The analyzer measured the complex scattering parameter S_{11} (reflection magnitude and phase). From S_{11} the admittance spectra were determined as follows:

$$Y(f) = \frac{1}{Z_0} \frac{1 - S_{11}(f)}{1 + S_{11}(f)}, \quad (1)$$

where $Z_0 = 50 \Omega$. The analyzer was connected to an IBM Pentium PC. The experimental setup is shown in Fig. 1a. The spectra $Y(f)$ obtained were fitted using the Butterworth-van Dyke model electrical circuit (Fig. 1b) [11–13]. Each element of the circuit represents a physical property of the crystal. The resistance R_1 represents the loss arising from internal friction, L_1 is the mass/motional inertia of the system and C_1 is the mechanical elasticity of the quartz. C_0 is the electrical capacitance of the crystal. The sensing layer deposited on the top of the crystal caused the appearance of additional elements: L_m is related to addition of the mass and R_m is the motional resistance that represents the dissipation of acoustic energy due to the friction between the biolayer and the surrounding buffer [11, 12]. The basic parameters studied were f_s that corresponds to the maximum of the admittance [11] and R_m . All experiments were performed at 25 ± 1 °C.

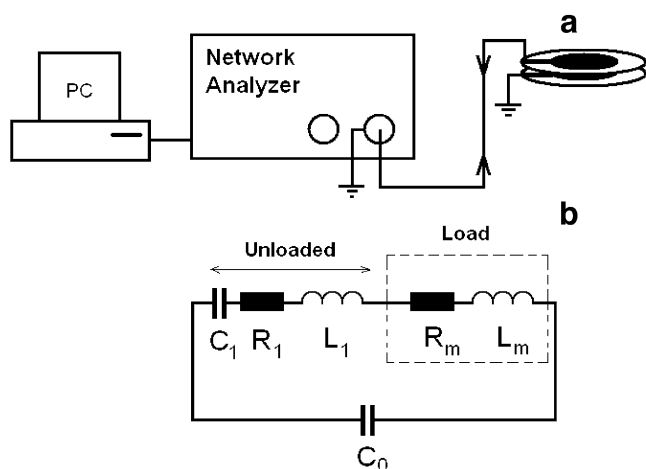


Fig. 1 **a** The transverse shear mode method experimental setup [13]. **b** The Butterworth-van Dyke model electrical circuit of a quartz crystal transducer [12]

Results and discussion

In the first series of experiments we studied the changes of the series resonant frequency, Δf_s , and motional resistance, ΔR_m , following various modifications of the quartz crystal surface. As seen from Fig. 2, addition of GA resulted in a decrease of f_s by 24 Hz and an increase of R_m by 3 Ω . However, subsequent washing of the surface by water caused recovery of R_m to the initial value (prior to addition of GA), while f_s became slightly higher (by 9 Hz). The increase in f_s indicates increasing storage of elastic energy.

Probably the layer becomes more rigid after addition of GA [14]. Addition of neutravidin caused a sharp decrease of the frequency. After the sensor surface had been washed with water the steady state value of Δf_s was -198 Hz. The

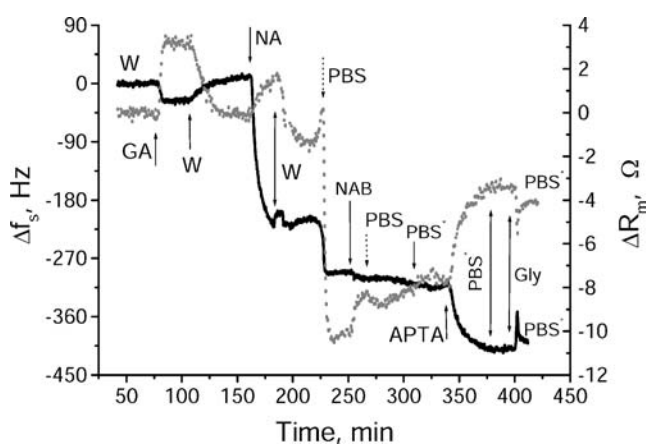


Fig. 2 Representative plot of the changes of the series resonant frequency, Δf_s (black), and motional resistance, ΔR_m (gray), following various modification of the quartz crystal surface. *W* Millipore water, *GA* glutaraldehyde, *NA* neutravidin, *PBS* phosphate-buffered saline, *PBS** PBS + 1 mM MgCl_2 , *NAB* 0.01% sodiumborohydride in PBS, *APTA* aptamer, *Gly* 0.2 M glycine-HCl, pH 2.3

washing of the surface with PBS, sodiumborohydride and PBS^* produced a further decrease of the resonant frequency and caused rather complex changes of R_m . This phenomenon is related to the effect of salts that may change the surface properties and modify the molecular slippage between the biolayer and the buffer [8]. Addition of the aptamer resulted in further decrease of f_s and increase of R_m .

According to the Sauerbrey equation [7] from changes of Δf_s it is possible to estimate the mass changes at the device surface:

$$\Delta f_s = -2.26 \times 10^{-6} f_0^2 (\Delta m / A), \quad (2)$$

where A is the area of the working electrode (0.2 cm^2) and f_0 is the fundamental frequency of the crystal (7.98 MHz). However, a decrease of the frequency is particularly due to changes of the friction between the biolayer and surrounding buffer. In order to estimate the contribution of the viscous forces to the frequency changes we can use the approach reported earlier [8]. The changes of the friction should result in changes of the resonance width, $\Delta \Gamma$, which is proportional to the changes of motional resistance as follows:

$$\Delta \Gamma = 8K^2 C_0 f_0^2 \Delta R_m / \pi, \quad (3)$$

where C_0 is the electrical capacitance of the TSM device (Fig. 1b), f_0 is the fundamental resonant frequency, and $K^2 = 7.74 \times 10^{-3}$ is an electromechanical coupling coefficient of quartz [13]. In our experiment $C_0 = 4.1 \text{ pF}$ and $f_0 = 7.98 \text{ MHz}$. As seen from Fig. 2, addition of neutravidin to the surface of the crystal and subsequent washing resulted in a decrease of motional resistance by 7.64 Ω . This suggests an increase of the rigidity of the biolayer, or an increase of molecular slippage between the biolayer and the buffer. According to Eq. 3 this corresponds to a decrease of the resonance width $\Delta \Gamma = -39.3 \text{ Hz}$. Therefore, the resonant frequency change should be corrected as $(\Delta f_s)_{\text{cor}} = \Delta f_s + \Delta \Gamma = -237.3 \text{ Hz}$, which corresponds to a change of mass $\Delta m = 330 \text{ ng}$. Having the Δm value, we can estimate the number of neutravidin molecules at the surface: $N_{\text{neutravidin}} = (\Delta m / \text{MW}) \times N_A = 3.3 \times 10^{12}$ (MW = 60 kDa is the molecular weight of neutravidin, $N_A = 6.02 \times 10^{23} \text{ molecules mol}^{-1}$ is Avogadro's number). Addition of biotinylated aptamer resulted in a decrease of the series resonant frequency: $\Delta f_s = -87 \text{ Hz}$. However, the motional resistance increased by 4.26 Ω . This suggests the appearance of friction between the biolayer and the buffer; therefore, the frequency shift measured should be corrected. Using Eq. 3, we obtain $\Delta \Gamma = 21.9 \text{ Hz}$. Thus, the corrected value of the resonant frequency change is -65.1 Hz . This corresponds to mass changes of 91 ng. The number of aptamer molecules immobilized on a surface is 3.9×10^{12} (in the calculation we used MW = 14,166 Da for the aptamer). Thus, there are about 1.18 times more aptamer molecules at the surface than neutravidin molecules. Considering the fact

that neutravidin has four binding sites, of which at least two are unavailable owing to immobilization onto the dendrimer surface, the estimated number of aptamer molecules seems to be lower than suggested by the binding ratio of neutravidin and biotin. Probably the surface is not fully saturated by aptamers.

Addition of IgE to the sensor surface resulted in a decrease of the resonant frequency and in an increase of motional resistance (Fig. 3). The changes of motional resistance allow us to estimate the contribution of friction forces to the frequency changes. In analogy with the approach applied above we determined the corrected series resonant frequency for binding of IgE to the aptamers. Figure 3 shows the plot of the Δf_s , ΔR_m , $\Delta \Gamma$ and the changes of the corrected series resonant frequency due to the effect of the friction. We can see that the viscous effect, expressed by an increase of motional resistance, is significant and resulted in an increase of $\Delta \Gamma$.

The value $(\Delta f_s)_{\text{cor}}$ represents the estimated contribution of the mass to the Δf_s ; therefore, the corrected resonant frequency can be used for calculation of the mass changes using Eq. 2. For example, $\Delta R_m = 0.8 \Omega$ due to addition of $1 \mu\text{g ml}^{-1}$ IgE corresponds to $\Delta \Gamma = 4.1 \text{ Hz}$. Thus, only $(\Delta f_s)_{\text{cor}} = -13.0 + 4.1 \text{ Hz} = -8.9 \text{ Hz}$ is connected with changes of the mass of the sensor, while the rest, 4.1 Hz , is connected with the increased friction between the biolayer and the surrounding buffer. Having the value of the frequency changes that correspond to the mass loading, we can determine the surface density of IgE. The change of the series resonant frequency of 8.9 Hz corresponds to a change of mass $\Delta m = 12.3 \text{ ng}$ and the number of IgE molecules at the surface is 3.9×10^{10} (MW = 190 kDa for IgE). Thus, the surface density of IgE at a concentration of $1 \mu\text{g ml}^{-1}$ is one IgE molecule per 513 nm^2 , i.e., the distance between IgE molecules is on average 25.6 nm .

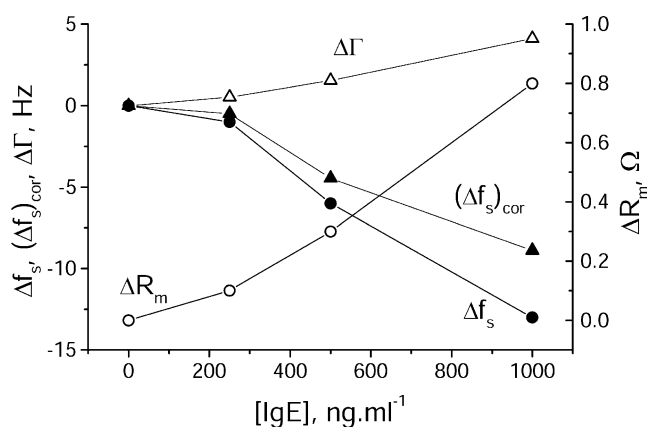


Fig. 3 The changes of the series resonant frequency, Δf_s , the resonance width, $\Delta \Gamma$, corrected changes of the series resonant frequency, $(\Delta f_s)_{\text{cor}} = \Delta f_s + \Delta \Gamma$, and changes of motional resistance, ΔR_m , as a function of immunoglobulin E (IgE) concentration

Using 0.2 M glycine-HCl solutions, we could regenerate the biosensor. An example of the changes of the series resonant frequency after addition of $1 \mu\text{g ml}^{-1}$ IgE and sensor regeneration is shown in Fig. 4. We can see that addition of IgE resulted in a decrease of f_s .

The flow of glycine caused a substantial increase of the resonant frequency owing to changes of the surface properties of the sensor. Subsequent washing of the sensor surface by PBS*, however, resulted in recovery of the baseline to a level comparable with that prior to addition of IgE. (Addition of IgE and sensor regeneration resulted also in changes of motional resistance. These changes had the opposite sign in comparison with those typical for the series resonant frequency. These results are not shown.) After the regeneration the sensor sensitivity was practically the same as for a freshly prepared sensor. The nonspecific interactions were negligible. Addition of HSA at rather the high concentration of 3 mg ml^{-1} , which is close to the physiological concentration of HSA in blood ($3.2\text{--}3.5 \text{ mg ml}^{-1}$) resulted in negligible changes of the series resonant frequency as well as motional resistance (Fig. 4) (changes of motional resistance are not shown).

Thus, interaction of IgE with the DNA aptamer resulted in a decrease of the series resonant frequency of the quartz crystal transducer and in increase of motional resistance. The changes in the series resonant frequency are connected not only with changes of the mass, but also with increased friction between the sensor surface and the surrounding buffer. This conclusion is in good agreement with the viscosity effect on affinity interactions between antigens and antibodies at the solid surface reported earlier [10, 15].

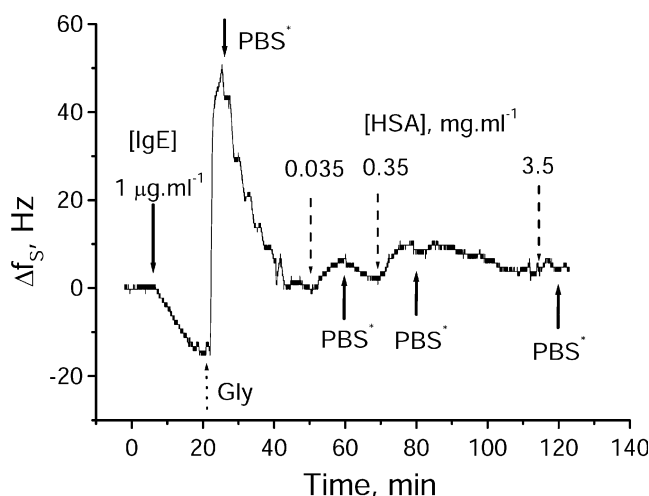


Fig. 4 The kinetics changes of the series resonant frequency following addition of IgE ($1 \mu\text{g ml}^{-1}$) to a sensor surface with immobilized DNA aptamer, following sensor regeneration by Gly, washing with PBS* and subsequent addition of human serum albumin (HSA) at various concentrations. The moment of addition of IgE, Gly, PBS* and HSA is shown by arrows

Acknowledgements This work was supported by European Commission 6 FP program NoE NeuroPrion (contract no. FOOD-CT-2004-506579), by the Agency for Promotion Research and Development under contract no. APVV-20-P01705 and by the Slovak Grant Agency (projects no. 2/7134/7 to M.S. and 1/4016/07 to T.H.). We are grateful to M. Thompson for the generous gift of the flow cell.

References

- Jayasena SD (1999) Clin Chem 45:1628–1650
- Mayer G, Andreas J (2004) Biodrugs 18:351–359
- Mairal T, Cengiz Özalp V, Sánchez PL, Mir M, Katakis I, O'Sullivan CK (2007) Anal Bioanal Chem (in press)
- Tombelli S, Minunni M, Luzi E, Mascini M (2005) Bioelectrochemistry 67:135–141
- Liss M, Petersen B, Wolf H, Prohaska E (2002) Anal Chem 74:4488–4495
- Hianik T, Ostatná V, Sonlajtnerová M, Grman I (2007) Bioelectrochemistry 70:127–133
- Sauerbrey G (1959) Z Phys 155:1546–1551
- Ellis JS, Thompson M (2004) Phys Chem Chem Phys 6:4928–4938
- Tassew M, Thompson M (2003) Biophys Chem 106:241–252
- Svobodová L, Šnejdárková M, Polohová V, Grman I, Rybár P, Hianik T (2006) Electroanalysis 18:1943–1949
- Martin SJ, Grandstaff VE, Frye GC (1991) Anal Chem 62:2272–2281
- Holloway AF, Nabok A, Thompson M, Ray AK, Crowther D, Siddiqi J (2003) Sensors 3:187–191
- Balantine DS, White RM, Martin SJ, Ricco AJ, Zellers ET, Frye GC, Wohlthen H (1997) In: Stern R, Levy M (eds) Acoustic wave sensors. Academic, San Diego, pp 331–395
- Wang X, Ellis JS, Lyle E-L, Sundaram P, Thompson M (2006) Mol Biosyst 2:184–192
- Zhang J, Su XD, O'Shea SJ (2002) Biophys Chem 99:31–41