Available online at www.sciencedirect.com
SCIENCE DIRECT®
Sensors and Actuators B 101 (2004) 308–315
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

A Love-wave biosensor using nucleic acids as ligands

Marc D. Schlensog[a], Thomas M.A. Gronewold[a], Michael Tewes[a], Michael Famulok[a,b], Eckhard Quandt[a,*]

[a] *Center of Advanced European Studies and Research (Caesar), Ludwig-Erhard-Allee 2, 53175 Bonn, Germany*
[b] *Kekulé Institute of Organic Chemistry and Biochemistry, University of Bonn, Gerhard-Domagk-Strasse 1, 53121 Bonn, Germany*

Received 22 October 2003; received in revised form 9 March 2004; accepted 12 March 2004

Available online 20 May 2004

Abstract

A Love-wave biosensor array has been designed by coupling aptamers to the surface of a Love-wave sensor chip. The sensor chip consists of five single sensor elements and allows label-free, real-time, and quantitative measurements of protein and nucleic acid binding events in concentration-dependent fashion. The biosensor was calibrated for human α-thrombin and HIV-1 Rev peptide by binding fluorescently labelled molecules and correlating the mass of the bound molecules to fluorescence intensity. Detection limits of approximately 75 pg/cm^2 were obtained, and analyte recognition was specific. The sensor can easily be regenerated by simple washing steps. We further demonstrate the versatile applicability of the sensor by immobilizing single-stranded DNA (ssDNA) for the detection of the corresponding counter-strand.

Keywords: Love-wave biosensor array; Aptamer; Thrombin; Rev

1. Introduction

Acoustic wave sensors have proven to be powerful devices for the detection and quantification of different physical parameters such as mass loading [1], albeit in most examples applications were restricted to non-liquid environments. When mass is loaded onto the surface of these sensors, the propagation velocity of acoustic waves decreases, resulting in a reduction of resonance frequency or in alteration of the phase shift between output and input signal. Love-wave sensors as a special type of surface acoustic wave (SAW) sensors use shear horizontal waves guided in a layer on the surface of the sensor to reduce energy dissipation of the acoustic wave to the fluid and to increase the surface sensitivity. Therefore, they offer high enough sensitivity to detect mass loadings in liquids as low as 1.7 ng/cm^2 [2].

Several examples show that SAW Love-wave sensors can be derivatized with antibodies as receptors for cognate analytes [2,3]. An alternative class of receptor molecules with similar properties as antibodies are aptamers. They can be evolved and synthesized in vitro [4], offer high specificity in target recognition, and can be used for the detection of a wide range of target analytes. Liss et al. [5] created a biosensor by immobilizing immunoglobulin E (IgE) aptamers on a quartz crystal microbalance. This physical transducer resulted in a detection limit of 3.3 ng/cm^2. However, most analytical tasks in biological experiments require much higher sensitivity.

In this paper, we report the combination of aptamer/ligand recognition with detection by SAW Love-wave sensors. It is shown that highly sensitive analyte detection can be achieved in parallel fashion opening up the possibility of using this sensor-principle in an array format. The sensor is characterized with respect to sensitivity, selectivity, and regenerability, and the results are directly compared to the BIAcore 3000 system [6]. As an example for an aptamer/ligand pair we chose the extensively characterized DNA aptamer against the multifunctional serine protease thrombin. Playing a pivotal role in the blood coagulation and anticoagulation cascades, it is a relevant target protein for drug discovery. In order to obtain alternative efficient anticoagulants, aptamers against thrombin have been developed [7,8].

2. Experimental

Love-wave sensors were fabricated by standard physical vapour deposition (PVD) techniques and lithography.

* Corresponding author. Tel.: +49-228-9656-215; fax: +49-228-9656-116.
E-mail address: quandt@caesar.de (E. Quandt).

0925-4005/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2004.03.015

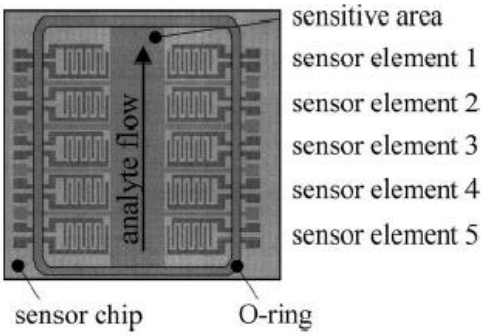

Fig. 1. Design of sensor chip and sealing.

A 500 μm ST-cut quartz substrate was covered with a Cr/Au/Cr layer (12/300/12 nm) by means of magnetron sputtering. This layer was structured by photolithography and ion beam etching (IBE) to shape the inter-digital transducers (IDTs) and contact pads. The IDT fingers were structured with a periodicity λ of 28 μm, exciting acoustic waves perpendicular to the wafer's crystallographic *X*-axes at a working frequency between 138 and 142 MHz. Each IDT consists of 25 finger-pairs and 50 reflectors. The sensors were fabricated with an IDT centre-to-centre distance of 4.48 mm (160 λ), and an acoustic aperture of 2.1 mm (75 λ). Both a SiO_2 guiding layer and a second Cr/Au layer (12/100 nm) were deposited by (reactive) magnetron sputtering. A 5.4 μm guiding layer resulted in the highest sensitivity for mass loading in water (data not shown). The upper Cr/Au layer was structured by photolithography and etched by IBE as an electric shielding. The sensors were structured with the shielding not overlapping the IDTs. In water and saline solutions, this shielding design maximally reduced interference from electromagnetic cross-talk between the sensor's input and output signal. Furthermore, the Au-surface can be used to immobilize receptor molecules by means of thiolated SAMs. Subsequently, the SiO_2 layer was structured by hydrofluoric wet etching to form the wave guiding. The final IBE step laid open the contact pads of the IDT structure. Finally, the wafer was cut with a dicing saw into 20 mm × 20 mm sensor chips.

A network analyzer (Rohde & Schwarz, ZVCE), or alternatively an I/Q-demodulator with integrated frequency synthesizer (Maxim, MAX 2312) was used for the read out of the sensors. No impedance matching electronics was used. The different sensor elements on each chip (Fig. 1) were addressed by a solid-state multiplexer circuit. An asymmetric feeding (one side of the IDT grounded) was chosen to simplify the multiplexer, although cross-talk can be greatly reduced by symmetric feeding. The network analyzer and the multiplexer circuit were monitored by a personal computer with a program developed in LabView.

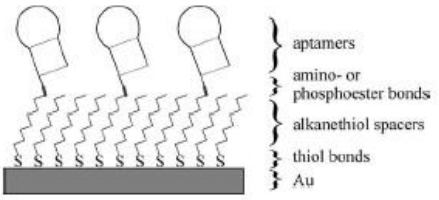

Fig. 2. Coupling of aptamers to Au-surface.

A flow-through cell (PMMA) was developed to flow liquids over the sensor elements. The liquids were sealed with a Viton seal ring in a rectangular area against the contact pads (Fig. 1). The liquids were pumped with a constant flow rate by a peristaltic pump (Ismatec, Reglo Digital), or alternatively by a HPLC pump (Sun Flow 100, Sun Chrom). Probes were injected with a probe sampler (Spark Holland, MIDAS). Due to the defined probe volume of 200 μl and constant buffer flow, the injection time of the analyte was limited. Measurements were performed at a constant temperature of 23 °C.

The sensor surface was cleaned by etching in a TePla 200 plasma system for 3 min (Power 300 W, O_2 pressure 1.0 mbar). The alkanethiol SAM was immobilized on the shielding layer by immersing the sensor for at least 12 h in an ethanolic 11-mercaptoundecanoic acid (Aldrich) solution. After rinsing the sensor with ethanol, the SAM was activated with an amine coupling kit (BR-1000-50, BIAcore) with 400 mM EDC, 100 mM NHS, and 1 M ethanolamin-hydrochloride solution (pH 8.5). Subsequently, 5 μm DNA thrombin aptamers (5′-GGT GGT GGT GGT GTT-3′), or alternatively 1 μm RNA Rev peptide aptamers (5′-GGG UGU CUU GGA GUG CUG AUC GGA CAC C-3′) were applied to the surface to couple to the SAM by carboxylamide bonds (Fig. 2). Unbound alkanethiols were blocked with 1 M ethanolamin pH 8.5.

Thrombin DNA aptamers were synthesized on an Expedite 8909 DNA Synthesizer (AME Bioscience). Rev peptide RNA aptamers were synthesized by Dharmacon. Both aptamers were diluted in water. The two serine proteases human alpha thrombin and porcine pancreatic elastase (Sigma) were solved in thrombin binding buffer (1 M Tris–HCl pH 7.2, 5 M NaCl, 3 M KCl, and 1 M $MgCl_2$). Elastase was used as a control substance in binding experiments. Thrombin was labelled with the FluoReporter FITC Protein Labelling Kit from Molecular Probes (F-6434). Rev peptide with the sequence NH_2-TRQARRNRRRRWRERQR-COOH or with the same sequence, but provided with an N-terminal 6-[Fluorescein-5(6-carboxamido]hexanoyl modification were synthesized by Metabion. Peptides were solved in Rev binding buffer (50 mM Tris–HCl pH 7.4 and 20 mM KCl).

DNA binding studies were performed with 5′-AAA AAA AAA AAA AAA AAA AAA ACC CCC CCC CCC CCC CCC CCC CCC CCT ATA GTG AGT CGT A-3′ (M = 21376 Da) and 5′-TAA TAC GAC TCA CTA

TA-3′ (M = 6125 Da), which were synthesized by MWG Biotech. The DNA strands were diluted in DNA binding buffer containing 10 mM Tris–HCl pH 8.3, 1.5 mM $MgCl_2$, and 50 mM KCl.

Reference sensors were prepared by pipeting bovine serum albumine (Sigma) on two sensor elements of a sensor chip and incubating for 30 min. The signal of the reference sensors was recorded simultaneously with the signal of the aptamer-derivatized sensors.

Control experiments were performed with a BIAcore 3000 system using chips with a Au-surface from the SIA kit AU (BR-1004-05, BIAcore). Surfaces were prepared according to the Love-wave sensors. Contrary to the Love-wave sensor, the aptamers were coupled to the BIAcore chip in flow-through. The mass loading on the BIAcore chips was calculated assuming that a signal change of one response unit equals a mass loading of 1 pg/mm^2 [6].

3. Results

3.1. Aptamer immobilization can be directly monitored

The immobilization process was monitored by rinsing the sensor with 11-mercaptoundecanoic acid (Fig. 3A). The injection of 11-mercaptoundecanoic acid resulted in a phase shift of 14.7±2.7°. Assuming, the sensitivity determined for both thrombin protein and Rev peptide (see next section) can be applied to the alkanethiol, this phase shift corresponds with a mass loading of 36 ± 11 ng/cm^2. A full-coverage phase of 11-mercaptoundecanoic acid (M = 218 Da) on Au (1 1 1) results in an area per molecule of 21.6 Å^2 [9], corresponding with a theoretical mass loading of 168 ng/cm^2. Comparing the calculated mass loading with this theoretical mass loading, the sensor surface is covered with 11-mercaptoundecanoic acid by 21%. It has been shown that the head groups of alkanethiols as is the carboxyl terminus hinder the development of a quasi-crystalline structure [9].

After the functionalization of the Love-wave sensor with the SAM and the activation with EDC–NHS, the binding of the aptamers to the sensor surface was subsequently monitored with the Love-wave sensor (Fig. 3B). Since the activated alkanethiols are instable, a slight drift can be detected during this measurement. The coupling of the thrombin aptamers to the SAM resulted in a phase shift of 2.0 ± 0.6°, which corresponds with a mass loading of 4.9 ± 1.9 ng/cm^2, assuming the sensitivity for protein can be applied to DNA molecules. Compared with a densely packed monolayer of thrombin aptamer (240 ng/cm^2, assuming a diameter of 21 Å and a molecular weight of 4964 Da), only 2% of the sensor surface are covered by the aptamer. Increasing the thrombin aptamer concentration could increase the aptamer density on the sensor surface, but results in a decreased binding constant for the thrombin (data not shown) as the steric hindrance of thrombin at the binding process increases.

3.2. Limit of detection is less than 80 pg/cm^2

The sensitivity of the sensors to biomolecules was determined by binding fluorescently labelled protein to the aptamer-derivatized Love-wave sensor. Fig. 4A shows the relative phase change of the single sensor elements of a Love-wave sensor chip coupled with thrombin aptamer as fluorescently labelled thrombin is applied to the sensor chip. The injection resulted in a phase shift of −16.2 ± 0.7°. The intensity of bound fluorescent thrombin was measured with a Fujifilm FLA-3000 phosphor imager. A calibration curve was determined by pipeting and drying of fluorescent thrombin of known concentrations to an Au-covered Si-substrate. The mass of thrombin bound to the sensor chip was derived from the calibration curve. Mass loading of 38.7 ± 5.7 ng/cm^2 was determined. Compared with a densely packed monolayer of thrombin (m = 385 ng/cm^2, calculated assuming a diameter of 4.3 nm and a molecular weight of 33.6 kDa [10]), the bound thrombin covers 10% of the surface. The sensitivity of the Love-wave biosensor for thrombin was calculated by dividing the measured phase shift through the determined mass loading, resulting in 419 ± 64° cm^2/μg. The noise of 0.01° results in a limit

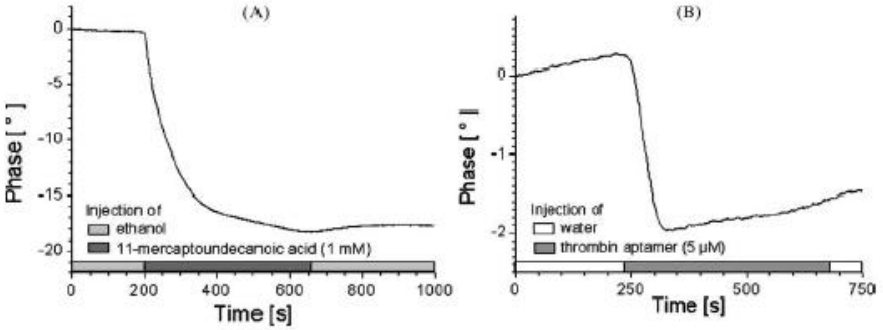

Fig. 3. Binding of 11-mercaptoundecanoic acid to sensor surface (A) and coupling of thrombin aptamer to 11-mercaptoundecanoic acid (B). Buffer solutions were pumped continuously with a flow rate of 27 μl/min.

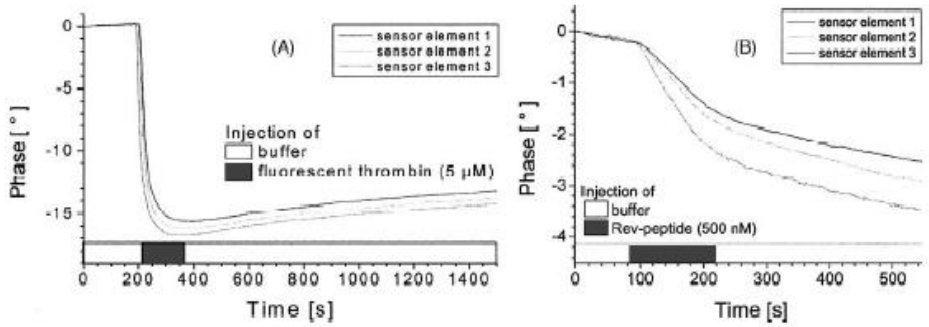

Fig. 4. Binding of fluorescent thrombin to thrombin aptamer (A) and binding of Rev-peptide to Rev aptamer (B) immobilized on Love-wave sensor chips. In each experiment, the signals of the sensor elements were measured simultaneously. Buffer solutions were pumped continuously with a flow rate of 85 µl/min.

of detection of about 72 ± 11 pg/cm^2 for biomolecules, assuming a confidence level of 3σ (99.7%).

In the same way, the sensitivity of a Love-wave sensor chip derivatized with the anti-Rev aptamer was determined using the Rev peptide epitope. Fig. 4B shows the binding of Rev peptide by a Love-wave sensor coupled with Rev aptamer. After injecting the peptide, a significant phase shift of 2.0 ± 0.6° was detected corresponding to a mass loading of 5 ± 1.5 ng/cm^2. The sensitivity was calculated as 390 ± 190° cm^2/µg which results in a limit of detection of 77 ± 36 pg/cm^2. The slight drift might result from interactions of the SAM's carboxyl-groups with the buffer solution, since the RNA aptamer is coupled with lower concentration than the thrombin DNA aptamer.

3.3. Thrombin detection is specific

Fig. 5 shows the difference of the sensor signal and the reference signal investigated over time for a Love-wave sensor (A) and for a BIAcore chip (B). The injection of elastase (5 µM) resulted in a phase shift of −0.2° on the Love-wave sensor and of a change of + 145 response units on the BIAcore chip. These results correspond with a mass loading of 14.5 ng/cm^2 (BIAcore) and of 0.5 ng/cm^2 (Love-wave sensor). When thrombin was injected, the difference of the sensor signal and the reference signal of the Love-wave sensor decreased by −7.5° and of the BIAcore chip by 744 response units corresponding with a mass loading of 74.4 ng/cm^2 (BIAcore) and of 17.9 ng/cm^2 (Love-wave sensor). Compared with the injection of elastase, the injection of thrombin resulted in a mass loading which is 36-fold higher in the Love-wave sensor system and five-fold higher in the BIAcore system. This is in the same range as results using filter binding and gel shift assays (data not shown). These data show that the specificity of the sensor system directly correlates with the specificity of the anti-thrombin aptamer for its ligand. The lower specificity of the BIAcore system might result from coupling the aptamers in flow-through. The mass loading on the BIAcore chip is generally higher than on the Love-wave sensor. This can result from the lower dead volume in the flow-through cell of the BIAcore system (0.6 µl) compared with the Love-wave system (14.5 µl). It is also notable that the mass loading of the Love-wave sensor was calculated using a calibration constant determined for

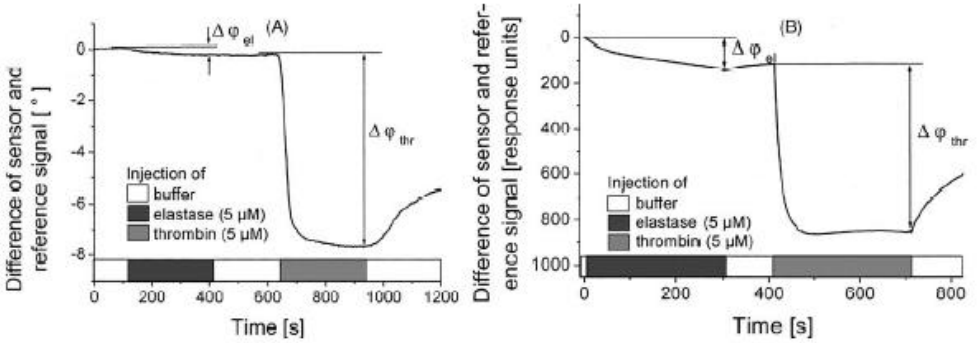

Fig. 5. Binding of elastase and thrombin to thrombin aptamer coupled to Love-wave sensor (A) and to BIAcore chip (B). Buffer solutions were pumped continuously with a flow rate of 40 µl/min.

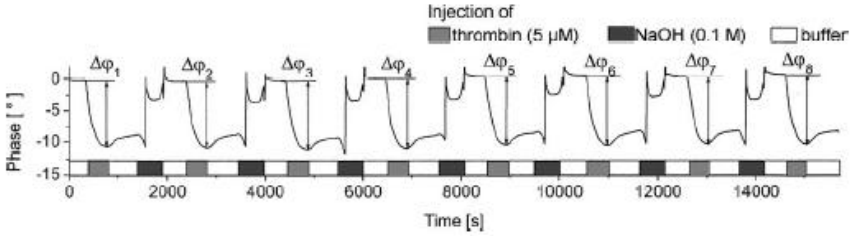

Fig. 6. Binding of thrombin and regeneration of Love-wave sensor with immobilized thrombin aptamer. Bound thrombin is washed off by pH-shift with NaOH. The buffer solution was pumped continuously with a flow rate of 40 µl/min.

thrombin (see last section). In contrast, the mass loading of the BIAcore chip was calculated using the general BIAcore conversion factor for protein. Due to the small sensitive area of the BIAcore chip and limitations of the phosphor imager, this conversion factor could not be verified for thrombin by an analogous calibration measurement.

3.4. Iterative regeneration of the sensor

Bound thrombin can be washed off at increased pH with 0.1 M NaOH. Fig. 6 shows the relative phase change of a sensor element coupled with thrombin aptamer for multiple binding and regeneration steps. The sensor signal reaches the base line after each regeneration step. Thus bound thrombin is washed off and the sensor surface is not degenerated. The evaluated data of different sensor elements of one sensor chip is shown in Table 1. Within the range of deviation, the phase shift resulting from mass loading is stable. Therefore the binding of thrombin by the aptamers is not diminished, and the sensitivity of the sensors is maintained.

The finding that the sensor can be regenerated by a simple pH-shift is an important result because it opens up the possibility to use the same sensor several times, allowing further investigations, e.g. titration of analytes. Fig. 7 shows the relative phase change of a thrombin aptamer coated Love-wave sensor for multiple binding and washing steps. An equilibrium state of the single binding processes could not be obtained, since the injection time of our experimental setup is limited. Although the thermodynamic equilibrium was not reached, an affinity-like constant of 500 nM could be derived (Fig. 8), which lies above the range of affinity constants determined by affinity chromatography (450 nM [11]), fiber-optic biosensor (300 nM [12]), and filter binding (200 nM [8]).

3.5. Immobilization and binding of DNA

Fig. 9 shows the relative phase change of a Love-wave sensor element with immobilized and activated 11-mercapto-undecanoic acid and of a reference element as ssDNA is applied. The following results are expressed as the difference of the sensor and reference elements. The first injection of ssDNA ($M = 21376$ Da) resulted in a phase shift of $-4.5 \pm 0.4°$ for the coupling of ssDNA to the activated SAM. The injection of a fragment of the second, complementary strand ($M = 6125$ Da) resulted in a phase shift of

Table 1
Relative phase change $\Delta\varphi_i$ of sensor elements at multiple injections and washing steps of thrombin, average over injections $\Delta\varphi_m$, and average over sensors

Injection	$\Delta\varphi_1$	$\Delta\varphi_2$	$\Delta\varphi_3$	$\Delta\varphi_4$	$\Delta\varphi_5$	$\Delta\varphi_6$	$\Delta\varphi_7$	$\Delta\varphi_8$	$\Delta\varphi_m$
Sensor element 1	−12.1	−12.1	−13.1	−13.0	−12.7	−13.1	−12.4	−12.7	−12.7 ± 0.4
Sensor element 2	−10.5	−10.5	−10.7	−10.9	−10.8	−11.1	−10.7	−11.0	−10.8 ± 0.2
Sensor element 3	−14.6	−15.4	−15.5	−15.6	−13.8	−15.1	−14.5	−14.7	−14.9 ± 0.6
Average	−12.4 ± 2.1	−12.7 ± 2.5	−13.1 ± 2.4	−13.2 ± 2.4	−12.4 ± 1.5	−13.1 ± 2.0	−12.5 ± 1. 9	−12.8 ± 1.9	12.8 ± 2.1

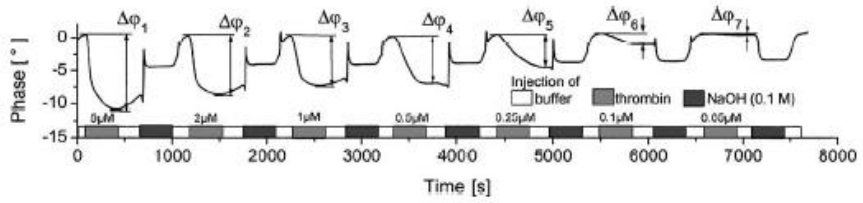

Fig. 7. Binding of thrombin with different concentrations to thrombin aptamer immobilized on Love-wave sensor and regeneration by pH-shift with NaOH. The buffer solution was pumped continuously with a flow rate of 40 µl/min.

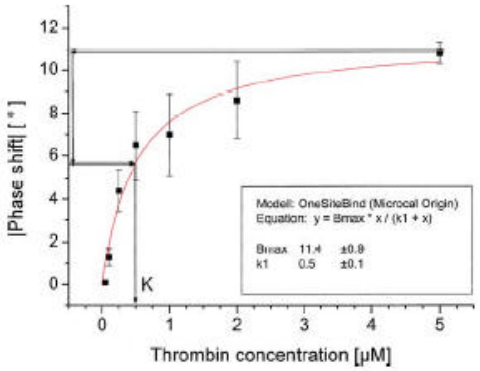

Fig. 8. Phase shift of sensor signal after injection of thrombin with different concentrations, and estimation of affinity-like constant K.

$-1.4 \pm 0.2°$. This can be explained by selective binding of the complementary strand to the previously bound ssDNA. The reference element showed a slight phase shift after the injections of DNA, however, almost all of the weakly bound DNA was washed off by DNA binding buffer.

The sensitivity for the molecular weight was calculated by dividing the phase shift through the molecular weight of the bound DNA. Within the range of deviation, both measurements resulted in the same sensitivity ($-0.22 \pm 0.03°$/kDa).

At the same analyte concentration and the same flowrate, the binding of thrombin to a Love-wave sensor derivatized with the anti-thrombin aptamer resulted in a phase shift of $-4.2 \pm 1.0°$ (data not shown). Related to the molecular weight ($M = 33600$ Da), the sensitivity for thrombin is $-0.13 \pm 0.03°$/kDa and, thus, is slightly lower than for DNA. The difference can be explained by the different shape and stiffness of the analytes, which can influence the viscosity at the sensor surface and therefore has an influence on the sensor signal. In contrast to DNA, which couples directy to the SAM, thrombin is bound to its cognate aptamer and

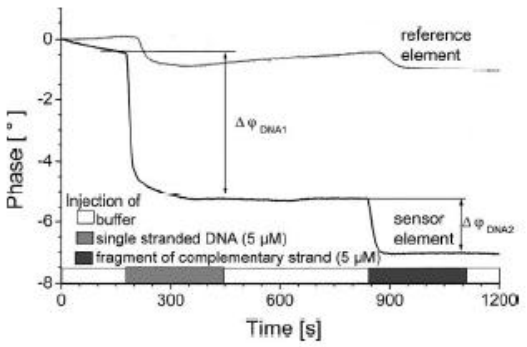

Fig. 9. Binding of DNA to SAM immobilized on Love-wave sensor and binding of fragment of complementary strand. The sensor and reference signals were measured simultaneously. The buffer solution was pumped continuously with a flow rate of 43 µl/min.

therefore the bound amount depends on the aptamer concentration on the sensor surface.

4. Discussion and summary

The combination of a Love-wave sensor with an aptamer-based sensor surface allows the detection of small molecules without limitation of antibodies such as the problems of producing antibodies against toxic substances. Additionally, aptamers can be synthesized in large scales and therefore the production cost of such aptamer-derivatized biosensors is comparably low. By using the Love-wave sensor as physical transducer, a highly sensitive biosensor is created allowing label-free detetection of binding events. Thus, no modification of the analyte molecules is necessary which is time consuming and (often) results in diminished binding.

Compared with an aptamer sensor published by Liss et al. [5], which is based on a bulk acoustic wave sensor and offers a detection limit of 3.3 ng/cm^2, the limit of detection of 72 ± 11 pg/cm^2 (thrombin) and of 77 ± 36 pg/cm^2 (Rev peptide) is significantly lower.

The rapid real-time analysis of specific binding events is comparable to measurements with the BIAcore system in terms of reproducibility and signal response but with much lesser production costs, higher reliability and lesser susceptance to failure. However, it was determined that less analyte is bound to the surface of the Love-wave sensor than to the surface of the BIAcore chip. This discrepancy could be resolved by improvements in the fluidics of the Love-wave sensor system. To verify the sensitivity ("conversion factor") of the BIAcore system, labelled protein could be bound to the surface of a chip similar to the Love-wave sensor.

Within the range of deviation, the sensitivity for thrombin ($419 \pm 64°$ cm^2/µg) corresponds with the sensitivity for Rev peptide ($390 \pm 84°$ cm^2/µg). Experiments with sensors derivatized with other aptamers could determine one sensitivity applicable to proteins in general as is being used in the BIAcore system.

The possibility to regenerate the sensors by a simple pH-shift opens up a wide range of applications, e.g. titration of analytes. In the experiments, presented in this publication, the sensor signal showed to be stable over several binding and washing steps. However, the working lifetime of the sensor surface yet has to be determined. The time limitation of the analyte injection can be overcome by improving the fluidic system. Thus it should be possible to measure equilibrium association and dissociation constants.

Shown is the possibility to easily expand the sensor to other usage, i.e. measurement of ssDNA–ssDNA binding. The sensitivity however differs between the rigid protein and the flexible ssDNA, which could be explained by viscoelastic effects due to the properties of the analyte. The Love-wave sensors can be calibrated by binding fluorescently labelled ssDNA and determining the bound mass by intensity,

enabling quantitative ssDNA–ssDNA binding measurements.

The immobilization of the SAM and the coupling of the aptamer to the SAM can be monitored. This enables the Love-wave sensor for calibration during the fabrication process, if the immobilization or coupling is reproducible. For the immobilization of the SAM, the mass loading, which was calculated with the sensitivity for thrombin and Rev peptide, differs from the theoretically expected mass loading. Even though this sensitivity could not be applied to the alkanethiols, this method might be useful for calibration of sensors relatively to each other.

Further investigations of the molecules bound to the Love-wave sensor surface are possible with a second method added externally to the sensor set up. For example, by combination with mass spectrometry, the structure of the bound analyte or of the cognate ligands can be investigated and thus the limitations of the Love-wave sensors can be overcome [13].

Acknowledgements

The authors would like to thank the German Ministry of Education and Research (BMBF) for support (Grant No. 0312708A). We also would like to thank Roland Gorski for the development of the read out electronics, Antje Baumgartner for excellent technical assistance, and Anne Kiwitz for the read out software.

References

[1] M. Hoummady, A. Campitelli, W. Wlodarski, Acoustic wave sensors: design, sensing mechanisms and applications, Smart Mater. Struct. 6 (1997) 647–657.
[2] F. Josse, F. Bender, R.W. Cernosek, Guided shear horizontal surface acoustic wave sensors for chemical and biochemical detection in liquids, Anal. Chem. 73 (2001) 5937–5944.
[3] J. Freudenberg, M.V. Schickfus, S. Hunkliner, A SAW immunosensor for operation in liquid using a SiO_2 protective layer, Sens. Actuators B 76 (2001) 147–151.
[4] A.D. Ellington, J. Szostak, In vitro selection of RNA molecules that bind specific ligands, Nature 346 (1990) 818–822.
[5] M. Liss, B. Petersen, H. Wolf, E. Prohaska, An aptamer-based quartz crystal microbalance (QCM) used as humidity sensor, Anal. Chem. 74 (2002) 4488–4495.
[6] U. Jönsson, L. Fägerstam, B. Ivarsson, B. Johnsson, R. Karlsson, K. Lundh, S. Löfas, B. Persson, H. Roos, I. Rönnberg, S. Sjölander, E. Stenberg, R. Stahlberg, C. Urbaniczky, H. Östlin, M. Malmqvist, U. Jönsson, Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology, BioTechniques 11 (1991) 620–627.
[7] L.C. Griffin, J.J. Toole, L.L.K. Leung, The discovery and characterization of a novel nucleotide-based thrombin inhibitor, Gene 137 (1993) 25–31.
[8] L.C. Bock, L.C. Griffin, J.A. Latham, E.H. Vermaas, J.J. Toole, Selection of single-stranded DANN molecules that bind and inhibit human thrombin, Nature 355 (1992) 564–566.
[9] F. Schreiber, Structure and growth of self-assembling monolayers, Prog. Surf. Sci. 65 (2000) 151–256.
[10] W. Bode, D. Turk, A. Karshikov, The refined 1.9-Å X-ray crystal structure of D-Pfe-Pro-Arg chloromethylketone-inhibited human (–thrombin: structure analysis, overall structure, electrostatic properties, detailed active-site geometry, and structure–function relationships, Protein Sci. 1 (1992) 426–471.
[11] I. German, D.D. Buchanan, R.T. Kennedy, Aptamers as ligands in affinity probe capillary electrophoresis, Anal. Chem. 79 (1998) 4540–4545.
[12] M. Lee, D.R. Walt, A fiber-optic microarray biosensor using aptamers as receptors, Anal. Biochem. 282 (2000) 142–146.
[13] B. Schmid, V. Schnaible, D. Hoffmann, E. Quandt, S. Wefing M. Tewes, M. Famulok, Verfahren zum Nachweis Biologischer Moleküle, German Patent DE 100 50 632 A 1 (2002).

Biographies

Marc D. Schlensog received his diploma in mechanical engineering from the University of Karlsruhe in 1999. He focused his studies in the field of microelectromechanical systems, especially on microstructuring of polymers by means of laser radiation. Since 1999 he has been working in the group smart materials at the Center of European Studies and Research (Caesar), Bonn, Germany. He obtained his PhD in 2003 for the development of a biosensor for the detection of chemical ligands.

Thomas M.A. Gronewold studied Biology at the Technical University Carolo Wilhelmina, Braunschweig. For his diploma he worked with Professor Karl G. Wagner at the Department of Enzymology at the German Research Center for Biotechnology (Gesellschaft für Biotechnologische Forschung, GBF), Braunschweig, Germany. He obtained his doctorate in 1996 from the TU Braunschweig for his work with Professor Hans Reichenbach at the Department of Natural Products at the GBF. After a brief thematically following postdoctoral training did he switch in 1997 to the laboratory of Dr. A. Dale Kaiser, Professor, and worked at the Departments of Biochemistry and Developmental Biology at Stanford University, Stanford, USA. In 2001, he joined the group Aptamer Biosensors at Caesar. His current research aims at the development of aptamer-based biosensors resulting in biosensor arrays.

Michael Tewes received his diploma in physics at the University of Heidelberg in 1995 where he focused his work on the development of femto second pulse lasers for surgical applications. As a PhD student he worked at the German Cancer Research Centre in Heidelberg on the development of new techniques for biochemical analysis, especially fluorescence-cross-correlation-spectroscopy. His main research topics at caesar are physical/biological sensors and the high frequency characteristics of thin magnetic films.

Michael Famulok studied chemistry at the University of Marburg (Germany) where he obtained his Doctoral degree in 1989. After postdoctoral training at the Massachusetts Institute of Technology, Cambridge (USA) and Harvard Department of Genetics, Boston (USA), he worked at the Ludwig-Maximilians-University Munich (Germany) as an independent researcher. In 1999 he became Professor of Biochemistry/Bioorganic Chemistry at the University of Bonn (Germany). The major focus of his current research is on combinatorial chemistry of nucleic acids and protein libraries, ribozymes, functional genomics, and synthesis of modified nucleic acids.

Eckhard Quandt received his diploma in physics and his doctorate Ing. at the Technical University Berlin in 1986 and 1990, respectively, working on solid-state physics and electron microscopy. Since 1991 he has worked on thin film smart materials and their applications in microsystem technology at the "Institute of Materials Research" of the

Forschungszentrum Karlsruhe. For his research and development on this field he was awarded the "Georg-Sachs-Preis 1995" of the Deutsche Gesellschaft für Materialkunde (DGM). He has arranged and successfully carried out European projects as well as bilateral cooperative projects with industrial partners on different applications. Furthermore, he has been granted a lectureship on "Smart materials and their Applications" at the "Faculty of Mechanical Engineering" of the University Karlsruhe. In April 1999, he joined the Stiftung caesar setting up the project "smart materials" that concentrates on the engineering aspects related with smart materials in thin film form. He is member of different scientific societies as the Deutsche Physikalische Gesellschaft (DPG), Deutsche Gesellschaft für Elektronenmikroskopie (DGE), and Deutsche Gesellschaft für Materialkunde (DGM), currently being the head of the working group "Dünnschichten in der Mikrosystemtechnik" of the DGM.