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Combination of a SAW-biosensor with MALDI mass spectrometric analysis

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Abstract

A S-sens® K5 surface acoustic wave biosensor was coupled with mass spectrometry (SAW-MS) for the analysis of a protein complex consisting of human blood clotting cascade factor α-thrombin and human antithrombin III, a specific blood plasma inhibitor of thrombin. Specific binding of antithrombin III to thrombin was recorded as a function of time with a S-sens® K5 biosensor. Two out of five elements of the sensor chip were used as references. To the remaining three elements coated with RNA *anti*-thrombin aptamers, thrombin and antithrombin III were bound consecutively. The biosensor measures mass changes on the chip surface showing that 20% of about 400 fmol/cm^2 thrombin formed a complex with the 1.7-times larger antithrombin III. Mass spectrometry (MS) was applied to identify the bound proteins. Sensor chips with aptamer-captured (1) thrombin and (2) thrombin–antithrombin III complex (TAT-complex) were digested with proteases on the sensor element and subsequently identified by peptide mass fingerprint (PMF) with matrix assisted laser desorption/ionization time-of-flight (MALDI-ToF) mass spectrometry. A significant identification of thrombin was achieved by measuring the entire digest with MALDI-ToF MS directly from the sensor chip surface. For the significant identification of both proteins in the TAT-complex, the proteolytic peptides had to be separated by nano-capillary-HPLC prior to MALDI-ToF MS. SAW-MS is applicable to protein interaction analysis as in functional proteomics and to miniaturized diagnostics.

Keywords: MALDI-ToF MS; Surface acoustic wave sensor; Thrombin; Aptamer; Protein-complexes

1. Introduction

Biosensor research and development has grown rapidly over the years. In parallel, technological advances were achieved in protein analytical and biochemical methods, in structural and molecular biology, electronics, optics, micro fluidics and chemical approaches to surface modification. Biosensors have potential applications not only in basic research, but also in medical diagnostics, explosives detection, food quality determination, genetic screening and environmental monitoring. Mass spectrometry, especially matrix assisted laser desorption/ionization time-of-flight (MALDI-ToF), has become a common laboratory technique in proteomics, protein characterization, protein visualization in cells and even in clinical research. By the combination of both techniques, it is feasible to gain more information on proteins and protein–protein interactions even for clinical applications, useful for detecting biomarker proteins (Nedelkov and Nelson, 2001a; Nedelkov et al., 2006) in diagnostics (Nedelkov and Nelson, 2003).

The S-sens® K5 surface acoustic wave sensors in Love-geometry are highly sensitive towards surface effects and are used to detect and quantify mass and viscosity changes due to biomolecular interactions. Selectivity of the sensor surface for defined analyte molecules can be achieved by adding specific ligands, e.g. antibodies or aptamers. Previously, a S-sens® K5 biosensor with a detection limit of <1 pg/mm^2 (Perpeet et al., 2006; Schlensog et al., 2004) was used to characterize human thrombin, a serine protease acting as a component of the blood-coagulation cascade and by use of a sensor-immobilized *anti*-thrombin RNA-aptamer (White et al., 2001). The main role of thrombin as the last protease in the clotting cascade (Holland et al., 2000) is to process numerous enzymatic actions on other blood proteins and on receptors (Coughlin, 2005). Aptamers are nucleic acid-based receptor molecules that are isolated from combinatorial libraries of synthetic oligonucleotides by *in vitro*

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selection on nearly every class of ligand molecules. The resulting oligonucleotides rival antibodies in selectivity and affinity (Ellington and Szostak, 1990; Robertson and Joyce, 1990; Tuerk and Gold, 1990). Aptamers have been used in basic research or drug development (Proske et al., 2005) for aptamer-based biosensor arrays for detection and quantification of different biological macromolecules (McCauley et al., 2003), especially for aptamer-based quartz crystal protein biosensors (Liss et al., 2002) and for many other analytical applications (Tombelli et al., 2005). We have evaluated und quantified the complex formation of aptamer-immobilized thrombin with thrombin-specific inhibitors and natural anticoagulants (Olson and Chuang, 2002) antithrombin III (ATIII) and heparin with a S-sens® K5 biosensor (Gronewold et al., 2005). Analysis of the generated data have shown that heparin activated the formation of tighter ternary inhibitory complexes of antithrombin III with thrombin 2.7-fold of up to 74% of bound thrombin. The immobilization of the RNA aptamer did not inhibit its binding ability to their cognate analytes (Gronewold et al., 2005). Furthermore, affinity captured thrombin has been detected by its molecular mass with MALDI-ToF MS, even from biological fluids (Dick and McGown, 2004).

We describe the combination of the biosensor with MALDI-ToF MS (Schmid et al., 2002). An RNA thrombin-binding aptamer (Jeter et al., 2004) was covalently coupled to an activated self-assembled monolayer (SAM) on a gold surface. Binding of molecules was quantified from the sensor signal. Proteins bound to the sensor surface were digested on the chip and either measured directly with MALDI-ToF MS on-chip, thus avoiding further regeneration procedures. Alternatively, the bound proteins were eluted from the sensor surface, digested the resulting peptides separated by HPLC and further analyzed with MALDI. We here demonstrate the first identification of a thrombin–antithrombin III complex from an aptamer-based SAW-MS biosensor.

2. Materials and methods

2.1. Reagents

α-Cyano-4-hydroxycinnamic acid was from Bruker Daltonik (Bremen, Germany). Bombesin, human adrenocorticotropic hormone fragments 18–39 (ACTH 18–39), human angiotensin II, Tris(2-carboxyethyl)phosphine (TCEP) and iodoacetamide (IAA) were obtained from Sigma–Aldrich (Taufkirchen, Germany). Human α-thrombin and human antithrombin III were purchased from Haematologic Technologies (Essex Junction, VT, USA). Endoproteinase LysC (sequencing grade) was bought from Roche Diagnostics (Mannheim, Germany). Somatostatin-28 was purchased from Bachem (Basel, Switzerland). Disodium-hydrogenphosphate was bought from J.T. Baker (Deventer, Netherlands). *N*-Octyl-b-D-glucopyranoside (nOGP), trifluoroacetic acid (TFA), NH_4HCO_3 and acetonitrile (HPLC gradient grade) were delivered from Merck (Darmstadt, Germany). Ethylenediamine-tetraaceticacid-disodium-dihydrate (EDTA) was purchased from Roth (Karlsruhe, Germany).

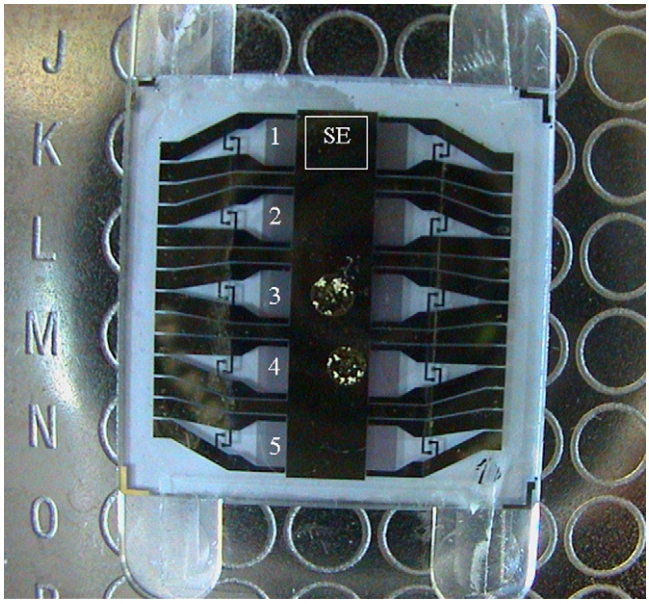

Fig. 1. S-sens(R) K5 chip with five sensor elements (SE) mounted onto a MALDI target plate. Each element is flanked by transducer elements and connectors for electric contact. On the third and fourth sensor element HCCA matrix was added to the dried protein digest mixture for MALDI measurements. On the top sensor element matrix with calibration peptides were applied for MS calibration.

2.2. Love-wave sensor set-up and immobilization of aptamers

For preparation of the sensor, an AT cut quartz crystal was processed by photolithography as described previously (Perpeet et al., 2006). The obtained sensor chips of Love-wave sensor type with five sensor elements (Fig. 1) were placed into an S-sens® K5 read out system (S-sens analytics, Nanofilm, Germany), and the detected signals were recorded in real-time using a standard PC. A self-assembled monolayer of 11-mercaptoundecanoic acid (Sigma) was formed on the gold shielding. Its carboxyl groups were activated using 50 mM *N*-hydroxysuccinimide (NHS) and 200 mM *N*-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide (EDC). RNA aptamers with the sequence 5′-NH_2–$(CH_2)_3$–GGG AAC AAA GCU GAA GUA CUU ACC C-3′ (Jeter et al., 2004), synthesized by Dharmacon Inc. (Lafayette, CO, USA), were coupled to the activated SAM via a 5′-amino linker (Gronewold et al., 2005).

2.3. Aptamer-analyte binding experiments

Binding experiments with human thrombin (33,600 Da) and antithrombin III (58,000 Da) were performed at 23 °C in binding buffer (20 mM Tris–HCl, pH 7.4, 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$). Displayed are the phase shifts measured with the Love-wave sensors. First, the difference between sensor and reference signal was evaluated. Bound masses were calculated using the sensitivity of the Love-wave sensor of 515° ($cm^2/\mu g$), under the assumption that the value is generally applicable. Additional sensitivity values have been determined for proteins in the range from 2000 to 200,000 g/mol, showing variations of

less than 8%. Concentrations were calculated from the molecular weights.

2.4. MALDI-ToF mass spectrometry

After biosensor experiments, the sensor chips with aptamer-captured thrombin, were removed from the S-sens® K5 biosensor, and typically 2–4 μl of an aqueous digestion mixture (2.5 mM *n*-octyl-β-D-glucopyranoside, 40 ng LysC in 50 mM NH_4HCO_3) was applied on one of the sensor elements. In a control experiment, a sensor chip coupled covalently with human α-thrombin on the SAM without aptamer was used. Proteolysis was performed at 37 °C for about 8–10 h in a humid atmosphere. After air drying of the digestion mixture, 0.5 μl matrix solution (saturated HCCA matrix solution in 33% acetonitrile/0.1% TFA) was spotted onto the array. Additionally, 0.5 μl of a mixture of calibration peptides (bombesin, ACTH 18–39, human angiotensin II and somatostatin-28) was added on a different area of the chip, and 0.5 μl of the matrix solution was applied. The chip was mounted onto a conventional Bruker alloy target plate in microtiter plate format with an in-house made cavity suitable for the array to be introduced into the ion source (Fig. 1). MALDI spectra were manually recorded in positive reflective mode on a Bruker Reflex III (Bruker Daltonik, Germany) mass spectrometer. The nitrogen laser power was adjusted to the minimum intensity necessary to obtain signal. The acceleration voltage was maintained at 20 kV, the reflective voltage at 28 kV. MALDI and MALDI-post-source-decay (PSD) measurements were performed in positive mode. External calibration was performed by singly charged monoisotopic masses of calibration peptides bombesin (*m/z* 1619.832), ACTH 18–39 (*m/z* 2465.199), human angiotensin II (*m/z* 1046.542) and somatostatin-28 (*m/z* 3147.472). Signal interpretation of MALDI and MALDI-PSD spectra were performed with Biotools 2.1 software (Bruker Daltonik, Bremen, Germany). Database search for evaluation of protein identification was performed with Mascot MS ion search (Matrix Science, London, UK). Assignment of signals for thrombin proteolytic fragments was performed with SearchXLinks® (www.searchxlinks.de), being able to additionally assign disulfide linked as well as multi-chain protein fragments.

2.5. On-chip digest of human thrombin antithrombin III complex and LC-MS measurement

Reduction and alkylation of the array-bound protein-complexes (α-thrombin/antithrombin III) were performed according to Happersberger et al. (2000); 8 μl of a mixture of 5 μl 25 mM TCEP, 5 μl of iodoacetamide (IAA) in 50 mM phosphate buffer, pH 8.0, 5 μl acetonitrile and 10 μl of 50 mM phosphate buffer, pH 8.0 were applied evenly onto the sensor chip surface. 2 μl of an aqueous solution of 10 mM nOGP was added and the chip was incubated for 2 h at 37 °C in a humid atmosphere. To remove TCEP and excessive IAA, the spot was washed three times with 10 μl of ultra pure water. Proteolysis was performed with 8 μl of a digestion mixture (2.5 mM nOGP, 80 ng LysC in 50 mM phosphate buffer, 1 mM EDTA, pH 8.0) at 37 °C in a humid atmosphere for about 8 h. The digest solution was removed from the chip surface, and the digest eluted four times with 0.1% TFA in ultra pure water. The solutions were combined and dried in a speed vacuum. Peptides resulting from the digest were redissolved in 3 μl 0.1% TFA in 5% acetonitrile/water. 1 μl of the solution was separated on a RP-18 PepMap column (75 μm i.d., 15 cm in length, 3 μm RP-18 stationary phase) at 30 °C with a nano-HPLC system (LC Packings Ultimate, Dionex, Idstein, Germany). Gradient elution was performed with 5% acetonitrile/water with 0.1% TFA (solvent A), and 80% acetonitrile/water and 0.1% TFA (solvent B), at a flow rate of 200 nl/min. After 5 min of equilibration with 2% solvent B, a linear gradient from 2% to 50% solvent B in 30 min was used. Fractions were collected directly on an anchor target with spots of 600 μm in diameter (Bruker Daltonik GmbH, Bremen, Germany). 0.6 μl of matrix solution (saturated HCCA in 33% acetonitrile/0.1% TFA diluted 1–8 in 80% acetonitrile/0.1% TFA) was added simultaneously to each spot. Fraction collection and target preparation was performed with a b.a.i. Probot (Dionex, Idstein, Germany). MALDI MS measurement of the fractions was performed automatically with the conditions described above. Mass lists of the fractions of each sample were combined for database search. Proteins were identified by a Mascot Search (Matrix Science, London, UK) with Bruker Biotools 2.1 software (Bruker Daltonik GmbH, Bremen, Germany). PSD spectra interpretation was performed with the Biotools software. Additionally, peak assignment to thrombin and antithrombin III sequence was performed with SearchXLinks software.

3. Results and discussion

It has previously been shown that the aptamers used are fully functional with respect to thrombin binding when linked to the chip surface. Both the chosen aptamer density and the length of the 5′-NH_2–$(CH_2)_3$– linker do not hinder immobilized RNA aptamer to specifically interact with its analyte as compared to filter binding experiments in solution (Proske et al., 2005; Gronewold et al., 2005). In the current set-up, thrombin is bound by the RNA *anti*-thrombin aptamers. Thus, the amount of thrombin bound highly depends on the amount of immobilized aptamers. In 15 measurements with comparable amounts of bound RNA thrombin aptamer, an average phase shift of about $\Delta\varphi_{\text{thrombin}} = 6.8 \pm 0.7^{\circ}$ was measured. The standard deviation was equal to about 10% of the average phase shifts. Sensor elements on the same chip mostly showed standard deviations as low as 5–7%.

3.1. Antithrombin III complexation with aptamer-bound thrombin

Further experiments were performed to monitor the formation of the complex of antithrombin III with thrombin (TAT-complex) bound to the RNA aptamer on the sensor. Fig. 2 shows the results of consecutive injections in one single experiment on the same sensor chip for about 1700 s. Subsequent binding of thrombin and inhibitor antithrombin III

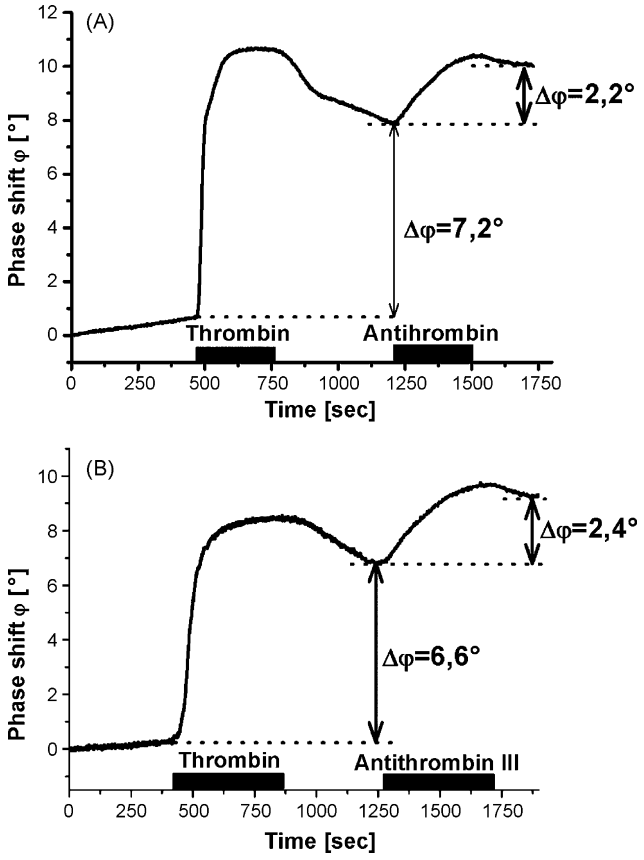

Fig. 2. Examples of two independent sensor measurements showing the sequential binding of proteins to immobilized RNA *anti*-thrombin aptamer. The difference of sensor and reference signal is shown. RNA thrombin aptamer specifically binds human thrombin with a fitted K_d-value of 181 nM. Injections of 1 μM thrombin binding to the aptamer surface and 300 nM human antithrombin III binding to captured thrombin. Concentration and amount of each bound analyte were calculated. (A) Thrombin $7.2 \pm 0.4°$, 14.3 ± 0.8 ng/cm^2, 425.2 ± 75 pmol/cm^2; antithrombin III $2.2 \pm 0.3°$, 4.4 ± 0.6 ng/cm^2, 75.3 ± 10.3 pmol/cm^2. (B) Thrombin $6.6 \pm 0.4°$, 13.1 ± 0.8 ng/cm^2, 389.7 ± 23.6 pmol/cm^2; antithrombin III $2.4 \pm 0.22°$, 4.8 ± 0.44 ng/cm^2, 82.1 ± 7.5 pmol/cm^2.

were investigated by direct injections. From the equilibrium phase shifts, concentration and amount of each bound analyte were calculated. Injection of 1 μM of the cognate analyte thrombin, which ensured saturation of the sensor-immobilized RNA aptamer sites, resulted in phase shift of $7.2 \pm 0.4°$ and $6.6 \pm 0.4°$, corresponding to a concentration of about 425 ± 24 and 390 ± 24 pmol/cm^2 due to specific binding to its ligand. Antithrombin III added at a concentration of 300 nM, bound to 75 ± 12 and 82 ± 8 pmol/cm^2 ($0.5 \pm 0.35°$ and $4.8 \pm 0.4°$), which is attributed to a complexation of antithrombin III to the aptamer-captured thrombin of 17.7% and 21.1%. These results are in the same range as previously shown (Gronewold et al., 2005).

3.2. Detection of bound proteins and thrombin–antithrombin III protein complex

Fig. 1 shows an S-sens® K5 biosensor chip mounted onto a MALDI target plate. The chip can be easily removed from the biosensor and prepared for mass spectrometric analysis with MALDI-MS. The chip is either fixed with screws or with tape onto the target. To guarantee the same surface height as the standard spots on the target, a cavity was drilled into the target bearing the chip. Each chip has five sensor elements. One sensor element usually is covered with a calibrating peptide solution and at least one element was left without immobilized aptamers as control. In the current set-up, all preparative steps as digestion, reduction, alkylation and application of MALDI-MS matrix are performed manually. As shown in Fig. 2, consecutive binding of thrombin to the *anti*-thrombin aptamer, and then binding of antithrombin III to the bound thrombin can be measured. The first binding event of thrombin to the *anti*-thrombin aptamer, and the identification of thrombin were demonstrated in a separate experiment (Fig. 3). Proteins such as thrombin were analyzed directly on the chip without separation and on-chip identification with high significance (MOWSE score above 70). Peptides resulting from an on-chip digest of thrombin with LysC were identified with the proprietary software SearchXLinks (www.searchxlinks.de). The results are summarized in Table 1.

The TAT protein complex could not be detected from the chip. Best results were achieved when the peptides resulting from a digest on the chip were separated with nano-HPLC prior to MALDI-MS and then prepared on a separate MALDI-MS target. The presence of antithrombin III bound to thrombin unambiguously was demonstrated by simultaneous identification of thrombin and antithrombin III with MALDI-MS and peptide mass fingerprint after separation with nano-HPLC. Fig. 4 shows a mass spectrum of peptides resulting from an on-chip LysC digest of captured proteins thrombin and antithrombin III. Resulting peptide matches from a Mascot database search are given in Table 2. The MOWSE scores were always significant (e.g. 107 for thrombin, 74 for antithrombin III). To further validate these results, we analyzed a putative antithrombin III peptide with a PSD analysis. The fragment of amino acids 365–380 was submitted for PSD in the MALDI-MS. The corresponding PSD-spectrum is shown in Fig. 5. Antithrombin III was identified with PSD as the corresponding protein. Identification of the whole thrombin protein directly from the chip was possible (data not shown), but not for the tight complex of thrombin–anti-thrombin III. This might be due to the very strong interaction of both proteins and to the aptamer. Aptamers are just one class of affinity capture molecules, others as antibodies or interacting proteins are feasible.

The combination of biosensor measurements with MS has advanced in recent years. The combination of surface plasmon

Table 1
Matched peptides from MALDI-ToF-MS measurement of covalently coupled thrombin on SAM digested with LysC assigned with SearchXLinks

Peptide	*m/z* (exp)	*m/z* (calc)	Residues
1	1087.668	1087.601	172–181
2	1580.872	1580.792	24–36
3	1588.886	1588.831	102–113
4	2393.411	2393.199	237–57
5	3481.034	3480.744	58–88

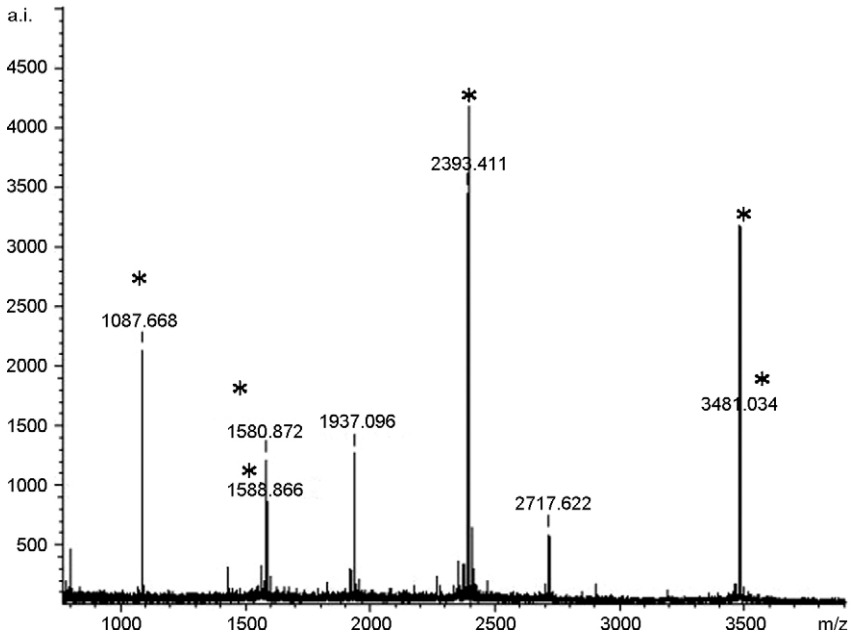

Fig. 3. MALDI-ToF-MS measurement of covalently coupled thrombin on SAM digested with LysC. Identified thrombin fragments are indicated with asterisk.

resonance (SPR) and MS, especially MALDI-MS has progressed in functional genomics (Nedelkov and Nelson, 2006, 2003, 2001a; Nelson et al., 2000; Buijs and Franklin, 2005; Larsericsdotter et al., 2006). Recently, the development of a high-throughput system has been shown (Nedelkov et al., 2006; Nedelkov, 2007). We here show the first time the combination of a SAW based biosensor with MALDI-MS.

Different strategies for the identification of proteins with SPR-MS or biosensor-MS in general can be distinguished. In principle, the captured protein or protein-complexes are either analyzed by MS directly from the chip after application of matrix (MALDI), or they are eluted prior to detection. In both cases, the choice to digest the samples with proteases for peptide-mass-fingerprint is kept. Some of the Biacore-systems offer the option to automatically transfer the sample from the chip to for example a MALDI target, which is suitable for ligand fishing experiments (Buijs and Franklin, 2005; Zhukov et al., 2004).

For detailed biochemical protein analysis, the combination of SPR with LC-MS has been shown (Bouffartigues et al., 2007). The authors have directly coupled the integrated fluidic cartridge of a Biacore2000^{TM} to a HPLC system, which itself was linked to an electrospray mass spectrometer. Additionally, they coupled the SPR to ProteinChip^{TM}-based mass spectrometry SELDI^{TM}. We have used the separation with nano-HPLC of proteolytic peptides resulting from the digest of captured proteins and a protein complex prior to MALDI-MS to decrease the complexity of the sample and take full advantage of the highly sensitive MALDI-MS. A direct coupling of the SAW-sensor with nano-HPLC attached to an electrospray mass spectrometer might be useful and is technically not challenging.

The elucidation of the nature of protein-complexes is of high interest not only in functional proteomics. Biosensor-MS offers an interesting and promising tool for this task.

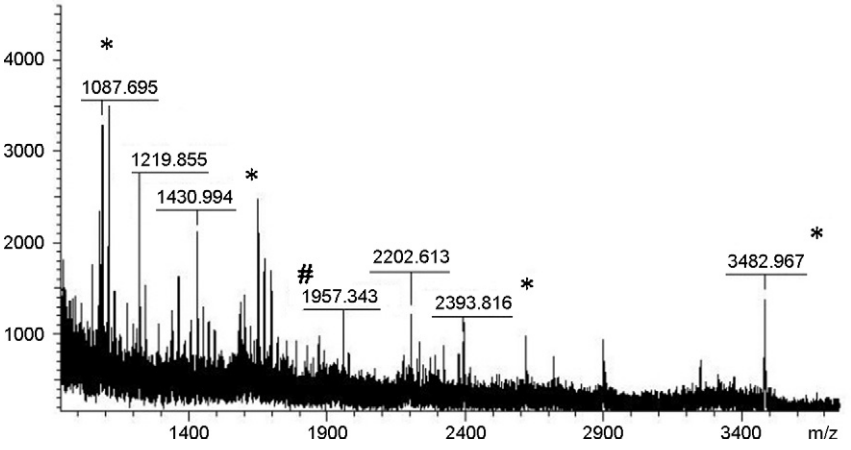

Fig. 4. MALDI mass spectrum of peptides from human thrombin (*) and antithrombin III (#) proteolyzed with LysC on a sensor element. Human thrombin was captured by RNA-aptamers on a microbalance. Then a solution of antithrombin III was delivered onto the array and proteins digested.

Table 2
Matched peptides from MALDI-ToF-MS measurement of thrombin and antithrombin III captured by RNA-aptamers on a S-sens® K5 biosensor chip

Obs. *m/z*	M_r (expt)	M_r (calc)	Start	End	Peptide sequence
(A) 1AWHB mass: 30,118, score: 107, alpha thrombin (EC 3.4.21.5), chain B—human					
1087.55	1086.54	1086.59	136	145	GRVTGWGNLK
1362.61	1361.60	1361.70	249	259	WIQKVIDQFGE
1430.73	1429.72	1429.75	237	247	YGFYTHVFRLK
1558.77	1557.76	1557.85	237	248	YGFYTHVFRLKK
2232.37	2231.37	2231.26	155	174	GQPSVLQVVNLPIVERPVCK
2393.19	2392.18	2392.19	1	21	IVEGSDAEIGMSPWQVMLFRK
2574.20	2573.20	2573.21	175	196	DSTRIRITDNMFCAGYKPDEGK
2716.52	2715.51	2715.41	84	104	IYIHPRYNWRENLDRDIALMK
3246.84	3245.83	3245.67	107	135	KPVAFSDYIHPVCLPDRETAASLLQAGYK
(B) XHHU3 mass: 53,025, score: 74, antithrombin III precursor [validated]—human					
1340.61	1339.60	1339.66	147	157	TSDQIHFFFAK
1698.84	1697.83	1697.92	72	85	IPEATNRRVWELSK
1848.86	1847.85	1847.90	365	380	EQLQDMGLVDLFSPEK
1866.89	1865.89	1865.91	124	139	LGACNDTLQQLMEVFK
1956.96	1955.95	1955.95	86	102	ANSRFATTFYQHLADSK
2202.16	2201.15	2201.11	383	402	LPGIVAEGRDDLYVSDAFHK
2299.15	2298.14	2298.12	103	123	NDNDNIFLSPLSISTAFAMTK
3447.78	3446.77	3446.78	403	435	AFLEVNEEGSEAAASTAVVIAGRSLNPNRVTFK

Captured proteins were digested using the protease LysC. (A) Matched peptides of the first hit (thrombin B-chain) and (B) antithrombin III hit (antithrombin III precursor) of Mascot database search.

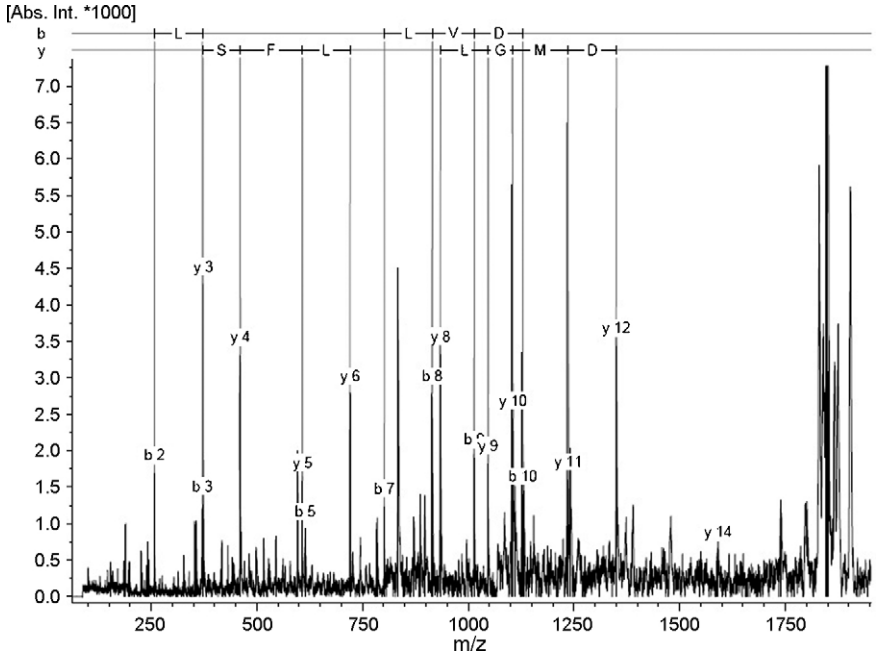

Fig. 5. Post-source-decay (PSD) spectrum of fragments 365–380 of antithrombin III with annotated b- and y-ions of analyzed peptide sequence EQ**LQDMGLVDLFS**PEK (identified residues in bold letters).

The combination of aptamer enhanced affinity binding and MALDI-MS has been shown with blood-coagulation protein-complexes (Dick and McGown, 2004). Nedelkov has shown the detection of multi-protein-complexes from biological fluids with biomolecular interaction analysis mass spectrometry (Nedelkov and Nelson, 2001b). SAW-MS offers another set-up for the detection of protein-complexes from difficult samples. The system is robust as the detection principle is probably less negatively influenced as SPR.

4. Conclusions

It has been shown that the S-sens® K5 sensor is a sophisticated and reliable device for following ligand/analyte interactions in real-time. The biosensor allows coupling of various biomolecules and can easily be used for interaction studies. Efficient binding is reproducible, with low background of non-specific binding, and the sensor surfaces allow good regeneration in order to repeat the cycle of measurements, but is limited by the specificity of

surface-bound receptors for certain binding regions of the analyte.

The results show that the supplemental MALDI-MS method gives additional information about whole proteins or of the binding partners in protein-complexes. For the sensor chip modified with *anti*-thrombin aptamer, the analyte human α-thrombin was identified from the chip by MALDI-MS with a high MOWSE score of 107, but also the 1.7-times larger natural plasma thrombin inhibitor antithrombin III (score 74), which only binds to thrombin, but not to the aptamer itself. It can be deduced that the combination of the SAW-biosensor with mass spectrometry is a very useful tool in proteomics and pharmacology for the determination of protein–protein interactions, and for the detection and verification of binding partners and ligands, allowing the distinction of binding sites of any given analyte. The set-up of the presented design is easy and opens up a number of possibilities and technical options for the analysis of bound proteins or ligands, especially protein-complexes. Very likely can this method be applied to the identification of certain post-translational modified proteins with distinct binding capacities. The use of MALDI-ToF MS can be complemented by other techniques, as electrospray coupled with ion traps, or even FTICR to enhance sensitivity and broaden information about the bound analytes. The process of preparing the chip for MALDI-MS is automatable with low effort for high-throughput applications.

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