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Development of a surface plasmon resonance sensor for coupling to capillary electrophoresis allowing affinity assessment of protein mixture components

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Surface plasmon resonance (SPR) currently is the major platform to study protein–protein interactions, but it lacks the selectivity to distinguish between binding components within one sample. Capillary electrophoresis (CE) can provide efficient separation of intact proteins under near-physiological conditions. We have hyphenated CE with SPR to achieve affinity assessment of mixture components. A microfluidic flow cell allowing straightforward coupling of CE and SPR was developed. Initial testing with non-interacting dyes showed good performance using a flow-cell channel volume of 100 nL until the detection point. Appropriate closing of the CE electric circuit was achieved using the SPR gold-sensor as grounding electrode. Division of the (bio)sensor into an electrode part (providing grounding) and a detection part (bearing the affinity surface) was crucial to avoid disturbance of the SPR signal by the CE voltage. This approach permitted CE separation and binding assessment for separation voltages up to 30 kV. Human serum albumin (HSA) or aprotinin were immobilized on carboxymethyl-dextran hydrogel-coated gold sensors and target proteins (anti-HSA, and trypsin and α-chymotrypsin, respectively) were analyzed. Efficient CE separation of the intact protein analytes was accomplished under native conditions by employing neutral and positively-charged capillary coatings. Selective binding of separated proteins to the target surface could be monitored by SPR down to 2 ng of injected protein. Regeneration of the biosensor surface was achieved by an on-line rising, allowing repeatable CE-SPR analyses of proteins with RSDs below 1% and 5% for migration time and signal intensity, respectively.

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1. Introduction

Surface plasmon resonance (SPR) currently is a major analytical technique for the study of non-covalent biomolecular interactions [1,2]. SPR measurements are based on changes in the optical reflectivity of a thin metal film – typically gold – induced by changes in refractive index at the rear metal surface [3]. This optical phenomenon has been exploited for many years to monitor binding of biomolecules to target molecules immobilized on the metal surface creating a biosensor [4–6]. SPR provides sensitive and label free detection of interacting biomolecules in solution yielding information on their affinity and binding kinetics [1,7].

SPR probes the entire sample and lacks the selectivity to distinguish between individual binding components. Protein samples, however, seldom are pure and may comprise proteoforms, degradation products and impurities exhibiting different affinities. Therefore, selective affinity assessment of protein mixture components by SPR requires separation or purification prior to detection. Coupling SPR with an efficient separation technique would provide a means to measure specific affinity of protein sample components. However, protein affinity studies require physiological-like conditions to probe proper binding, which reduces the number of separation techniques that can be directly combined with SPR. Native size-exclusion chromatography (SEC) has been coupled to SPR analysing the binding of unlabelled oligosaccharides and glycopeptides with immobilized lectins [8], SEC-SPR was also used for the analysis of carbohydrates using weakly interacting immobilized antibodies [9], and for monitoring adsorption of proteins onto
chemically modified SPR sensors [10]. More recently, our group has demonstrated the potential of the coupling of native SEC with SPR for affinity assessment of antibody samples [11]. However, separation of proteins by native SEC may lack the selectivity to resolve closely related proteins and proteoforms.

Capillary electrophoresis (CE) provides unique possibilities to achieve efficient separation of intact proteins and their variants under near-physiological conditions [12,13]. Affinity CE (ACE) has shown useful for the indirect assessment of non-covalent interactions of proteins with a target compound or ligand [14–16]. ACE is based on measuring mobility shifts of probed proteins in presence of the binding compound and in principle allows analysis of protein mixtures. However, ACE requires relatively fast on/off kinetics and addition of the target compound to the background electrolyte may adversely affect protein separation. Coupling of protein separations by CE with SPR basically would allow direct affinity-specific detection of individual protein components. Yet, CE requires application of high voltage and employs very small sample volumes and low flow rates, making the coupling of CE with SPR a challenge. Until now, only a few studies involving CE-SPR coupling have been reported [17–19]. Whelan and Zare connected the CE outlet to a miniaturized SPR sensor using a single-channel flow-cell of 400 nl [17]. CE grounding was achieved 5 cm before the end of the capillary (50 μm x 50 cm total length) by a crack in the capillary wall and assembling both capillary parts in a small piece of glass forming a scaffold. Adequate grounding was demonstrated up to 6 kV of applied voltage. The CE-SPR system was employed to detect refractive index changes induced by three separated phenols. The interaction between immobilized protein A and human IgG was also monitored by electrophoretically delivering 270-nl plugs of IgG (50 μg/mL) through the CE capillary. No protein separations were performed in this study. Gaspar and Gomez coupled a miniaturized CE system to a SPR sensor situated in a microfluidic platform fabricated from PDMS [18]. The CE outlet capillary was directly connected to the PDMS SPR cell. At high flow rates (>1 μL/s) BGE leaking occurred through the PDMS surface, allowing contact of the BGE with a grounded electrolyte reservoir. The single-channel CE-SPR microdevice was used to monitor refractive index changes of a mixture of dyes separated upon the application of +2.5 kV in a 15-cm length capillary. Liu et al. reported the combination of microchip CE with SPR [19]. The single channel SPR flow cell was integrated during the fabrication process at the end of the microchip as an overlapped region. Grounding was performed on the separation channel before the SPR flow cell. In order to transfer the sample from the grounding position to the SPR detection point, pressure-assisted separations were performed by applying and electrical field of 350 V/cm while inducing a flow of 0.5 μL/min for 100 s and 3.2 μL/min after 100 s. The fabricated device was employed to measure the refractive index changes induced by bovine serum albumin (BSA) and fluoresein. Ly et al. used surface plasmon resonance imaging (SPRi) to detect electrokinetically driven proteins in a microfluidic channel [20]. Their set-up allowed label-free tracking of protein movement. The separation and SPRi detection of BSA and glucose oxidase (15 mg/mL each) was demonstrated. No analyte binding was monitored in most of the CE-SPR studies reported so far [18–20].

The aim of the present study was to develop an on-line CE-SPR system for affinity assessment of individual proteins from mixture solutions. For this purpose we developed a dedicated microfluidic SPR flow-cell that allows coupling of CE and SPR in a straightforward way, avoiding fragile capillary parts or laborious connections, and permitting high voltage conditions (up to 30 kV). The feasibility of the new CE-SPR system was demonstrated by the assessment of antibody-antigen and enzyme-inhibitor binding in protein mixtures.

2. Materials and methods

2.1. Chemicals

All reagents employed were of analytical grade. Ammonium hydroxide (25% solution) was obtained from Fluka (Steinheim, Germany). Acetic acid was provided by Merck (Darmstadt, Germany). Formic acid was supplied by Riedel-De Haen (Seelze, Germany). Human serum albumin (HSA), anti-human albumin antibody produced in goat, trypsin, α-chymotrypsin from bovine pancreas, aprotinin (bovine lung trypsin inhibitor), phosphate buffered saline (PBS), 2-(N-morpholino)ethanesulfonic (MES) monohydrate, ethanolamine hydrochloride. N-hydroxy succinimide (NHS), N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC), Polybrene (hexadimethrine bromide, PB), dextran sulfate (DS), tris(hydroxymethyl) aminomethane hydrochloride (Tris-HCl), sodium hydroxide (NaOH), sodium chloride (NaCl), and ammonium bicarbonate were purchased from Sigma-Aldrich (Steinheim, Germany). Trypsin sequencing grade, was purchased from Roche (Mannheim, Germany). Deionized water was obtained from a Milli-Q purification system (Millipore, Bedford, MA, U.S.A.).

2.2. Capillary electrophoresis

CE analyses were performed using a Beckman PA 800 instrument (Beckman Coulter, Brea, CA, U.S.A.). Fused-silica capillaries were from Polymicro Technologies (Phoenix, AZ, U.S.A.) having an internal diameter of 50 μm and an external diameter of 360 μm. Hydrodynamic injections were performed at 5 psi for 5 s for refractive index measurements or at 1 psi for 10 s for affinity studies. In all cases, separations were performed at 20 °C. New fused-silica capillaries were rinsed with 1 M NaOH for 30 min at 20 psi and with water for 15 min at 20 psi. After this treatment, capillaries were coated with a PB–DS–PB coating. For this, solutions of 10% (w/v) PB and 0.5% (w/v) DS in deionized water were prepared. The solutions were filtered over a 0.45 μm filter type HA (Millipore, Molsheim, France) prior to use. Capillaries were coated by subsequently rinsing 30 min with 10% (w/v) PB solution at 5 psi, 10 min with deionized water at 10 psi, 30 min with 0.5% (w/v) DS solution at 5 psi, 10 min with deionized water at 10 psi, 30 min with 10% (w/v) PB solution at 5 psi, and 10 min with deionized water at 10 psi. After the final coating step, the capillary was rinsed for 10 min with 25 mM ammonium acetate (pH 8.0) at 20 psi. Before each run, coated capillaries were flushed with water for 2 min, 25 mM ammonium acetate (pH 8.0) for 2 min, and the BGE for 2 min at 20 psi. Neutral poly(vinyl alcohol) (PVA) coated capillaries with an internal diameter of 30 μm and a total length of 100 cm were purchased from Agilent Technologies (Waldbonn, Germany) and treated as indicated by the manufacturer. Overnight, coated capillaries were filled with water.

2.3. Surface plasmon resonance detection

SPR analyses were performed with a multi-parametric SPR Navi 210A instrument from Bionavis (Tampere, Finland) using the 670 nm laser. Measurements were performed at 20 °C. Gold sensors were obtained from Bionavis. Immobilization of proteins was carried out on commercially available carboxymethyl dextran (CMD5) hydrogel-coated gold sensors (Bionavis). For the immobilization 5 mM MES (pH 5.0) was employed as buffer solution and it was delivered at a flow rate of 30 μL/min at 20 °C using the conventional SPR flow-cell. After getting a stable baseline the surface was cleaned with a solution containing 2 M NaCl and 10 mM NaOH for 7 min. The surface was activated with a solution containing 0.4 M EDC and 0.1 M NHS for 7 min in both channels, followed by a 7 min injection
of 1 mg/mL HSA or 2 mg/mL of aprotinin in the sample channel and a 7 min injection of buffer in the reference channel. After immobilization, the non-reacted active esters were deactivated by injecting 1 M ethanolamine hydrochloride at pH 8 for 7 min in both channels followed by two times 1 min regeneration buffer (100 mM NaOH or 10 mM glycine–Cl pH 1.5 for HSA and aprotinin-immobilized sensors, respectively). SPR angular curves were measured between 68° and 75°. The recorded SPR curves were processed using the SPR Nav™ DataViewer software. For each biosensor, the angle of resonance measured after binding was selected and the intensity of the signal at this angle was plotted over time resulting in fixed angle sensorgrams. For concentration-dependent experiments, the shift in the resonance angle was determined and plotted against the injected concentration.

2.4. CE-SPR coupling

CE-SPR coupling was performed using a home made CE-SPR flow-cell (Fig. 1). The body of the flow-cell was designed based on the conventional Bionavis SPR flow-cell. The microfluidic part of the cell was made of PDMS. PDMS allowed perfect sealing between the flow cell and the sensor slide avoiding possible leakages. The CE capillaries were inserted into dedicated holes formed on the PDMS during casting of the flow-cell. The PDMS was sufficiently tight to provide an efficient sealed connection between the CE capillaries and the flow cell allowing easy capillary exchange. Two microfluidic channels were formed on the surface of the PDMS (cross-section 5.7 mm, 0.6 mm wide; estimated volume, 200 nL total and 100 nL until the detection point). The upper microfluidic channel was employed as a sample channel and the lower microfluidic channel as a reference channel. The SPR signal was recorded simultaneously in both channels. The outlet of the sample microfluidic channel was connected with the inlet of the reference microfluidic channel using 5 cm PEEK tubing. In the final design, a platinum electrode was integrated in the PDMS of the grounding site of the flow-cell. When the flow cell is closed, the effluent of the CE capillary is in contact with the gold surface and with the platinum electrode, allowing grounding of the CE system. In order to isolate the grounding site from the detection site of the sensor, approximately 0.5 mm of the gold was removed using a fiberglass brush (HBM Machines, Moordrecht, Netherlands). Thus, only the first 3 mm of the gold surface were in contact with the platinum electrode, thereby avoiding electric current in the following part where the SPR signal is measured.

3. Results and discussion

3.1. Design of CE-SPR flow-cell

Coupling of CE with SPR requires a dedicated flow-cell to connect the capillary outlet with the SPR sensor slide where detection takes place. The main challenges for development of the CE-SPR flow-cell are the CE voltage grounding and the small volumes employed in CE, demanding smaller dimensions/volumes of the fluidic channel. The SPR instrument employed contains two light sources allowing simultaneous detection at two flow channels. For that purpose, two parallel channels were formed in the microfluidic flow-cell (see Fig. 1). The upper channel was employed as detection channel, whereas the lower microfluidic channel was used as reference channel.

In order to establish CE grounding, three strategies were evaluated: (1) on-capillary grounding (before SPR detection), (2) outlet grounding (after SPR detection) and (3) grounding on the detection site. First attempts were focused on-capillary grounding by introducing a conductive micro-union into the last part (5 cm) of the CE capillary (i.e. prior to SPR detection). Two types of unions, viz. a commercial ‘zero-volume’ conductive union and a micro-T junction connected to a platinum electrode, were tested. With both unions, the CE electrical circuit could be closed and the expected CE current was reached. However, the current was not stable over time and current breakdowns occurred. This was most probably caused by stationary air bubbles that were formed at the electrode or conductive metal parts of the unions upon application of CE voltage. Simultaneous application of voltage and pressure (>2 psi) during the separation provided a stable current over time, however, significant band broadening was observed most probably as a consequence of dead volumes introduced by the unions. Next, outlet grounding (i.e. grounding after SPR detection) was explored. For this purpose, a grounded platinum electrode was introduced in the outlet vial which is positioned after the SPR flow cell (see Fig. 1). However, the gold SPR sensor surface (situated before the grounded platinum electrode) caused CE current leakage resulting in highly unstable currents and serious damage of the gold surface.

As a third potential grounding strategy, the gold of the SPR sensor surface was considered as grounding electrode. Actually, the usefulness of the gold sensor surface as working electrode for electrochemical analysis in conjunction with SPR detection has been demonstrated[21]. To achieve grounding of the gold surface, a platinum wire was inserted in the flow cell as shown in Fig. 1. When the flow cell is closed, the grounded platinum electrode is in contact with the gold surface, resulting in closure of the CE electrical circuit via the gold surface. With this design, stable CE currents were obtained, and when employing bare gold sensors the SPR signal was not affected by the application of voltage. Apparently, gas formed at the gold electrode surface is effectively transferred towards the outlet and does not disturb the conductivity of the solution in the CE capillary nor the SPR detection.

In order to test the CE-SPR flow-cell using sensor-surface grounding, dye solutions were injected. These solutions show relatively high refractive indices at the detection wavelength employed (670 nm), producing SPR signal when migrating along the sensor. Although the dyes do not show interaction with the gold surface, they allow evaluating the performance of the flow cell to monitor SPR responses. Bromocresol green (BCG) was analyzed using a BGE of 50 mM Tris–Cl (pH 9.0) and a voltage of +30 kV and induced a significant SPR signal. Two different microfluidic channel volumes were tested: 500 nL and 200 nL (i.e. 250 and 100 nL until the detection point, respectively). A more narrow peak was observed using the flow cell with a microfluidic flow channel of 200 nL (Fig. S1). Moreover, comparison of the peak obtained for BCG by CE-SPR using the 200 nL flow-cell with the peak obtained by CE-UV under the same conditions showed a similar peak width (Fig. S2). Therefore, the 200 nL flow-cell was selected for the CE-SPR coupling.

To further evaluate the setup, bromothymol blue (BTB) and BCG were analyzed at different concentrations (5–30 mg/mL) (Fig. 2). The CE-SPR system showed a concentration-linear response for the dyes (R², 0.944 and 0.970 for curves obtained after plotting the resonance angle shift versus concentration for BTB and BCG, respectively). Upon injection of high concentrations of dyes, adsorption of the dyes to the gold surface was observed. Adsorbed dye could be removed effectively by in-between analysis flushing of the flow cell with acetone, which was applied via the CE capillary (2 min at 20 psi). Including the flushing step, analyses were repeatable, with migration-time and signal-intensity RSDs of 0.7% and 0.9% and 10% and 9% for BTB and BCG, respectively, indicating stable performance of the microfluidic CE-SPR flow-cell.

3.2. Probing protein binding with CE-SPR

The binding of anti-HSA to HSA was used as model system to evaluate the ability of the CE-SPR flow cell to monitor protein binding. Immobilization of HSA on CMD5 sensors using amine-coupling
chemistry is well established. Injection of 20 nL of 1 μM of anti-HSA (0.02 μmol) in the CE-SPR system and delivery by pressure (5 psi) resulted in a significant SPR signal. Comparison with stand-alone SPR showed that a similar absolute signal intensity was obtained as for the injection of 250 μL of 13 nM anti-HSA (3.2 μmol) employing a conventional flow-cell. The higher mass sensitivity of the CE-SPR system may be ascribed to the fact that in the micro flow cell a relatively large part of analyte solution passes the small sensor surface area that is probed by the laser. Using the conventional cell, the same area is optically probed, whereas a much larger area is exposed to the analyte solution.

Next, CE-SPR analysis of anti-HSA was attempted using the flow cell with immobilized HSA. However, when high voltage was applied across the capillary, the SPR signal was disturbed, showing an angle-dip profile which seemed to comprise two overlapping curves (Fig. S3). A possible explanation for this effect may be that upon voltage application, the methyl dextran groups with immobilized HSA orientate differently on the surface of the biosensor [22]. Experiments performed at different CE voltages showed that the intensity of the SPR signal of anti-HSA decreased with increasing voltage and no signal was detected for voltages >10 kV as a consequence of the dip-angle distortion (Fig. 3).

In order to solve these problems, the strategy of CE-grounding was modified and the possibility to perform grounding and detection on the sensor in an independent manner was investigated. For this purpose, approximately 0.5 mm of gold was removed from the sensor immediately before the area where the laser hits the gold surface. The first part of the gold sensor was still in contact with the electrode inserted in the flow-cell providing grounding, but current was excluded from the detection site (Fig. 1). Stable CE currents and good signals for refractive index changes were obtained with bare-gold surfaces suggesting that the removal of a part of the sensor did
not affect grounding and/or detection. Also when HSA was immobilized on the second part of the sensor, the SPR signal was not altered by the application of separation voltage when the grounding and detection part were separated. Moreover, binding between anti-HSA and HSA could be detected straightforwardly and the same intensity was measured when different separation voltages were applied (Fig. 3). Fig. 4 shows the UV and SPR electropherograms obtained for the injection of an antisera sample containing 10 μM of anti-HSA and some non-interacting proteins using a voltage of +30 kV and a BGE of 25 mM Tris-Cl (pH 7.4). The UV trace shows multiple separated components. The SPR trace (Fig. 4B, blue trace) first shows a peak at 15 min, which is produced by non-binding sample components causing a transient change in refractive index, followed by a plateau as the result of the binding of anti-HSA to HSA. The developed CE-SPR flow cell comprises a second microfluidic channel which can be employed for referencing. The surface of the reference channel was treated as the detection channel (activation/deactivation), but no HSA was immobilized. The red trace in Fig. 4B shows the sensorgram detected in the reference channel. No signal due to component binding to the surface was observed confirming that specific binding of HSA to Anti-HSA is measured in the first channel. Only a single peak corresponding to non-binding components was detected by the CMD5 sensor without HSA. The additional signals observed in UV were not detected in SPR as most probably they do not produce a significant refractive index change.

After sample analysis, the SPR biosensor has to be regenerated and bound proteins should be removed without degrading the affinity surface. The manufacturer recommends using 100 mM NaOH for regeneration of HSA-immobilized sensors. Post-run regeneration of the sensor via the CE capillary was evaluated by flushing with 100 mM NaOH for different time periods: 6, 4 and 2 min (at 20 psi). Complete sensor regeneration was achieved after a 2-min rinse time allowing repeatable analyses with RSDs below 1% and 5% (n = 5) for migration time and SPR signal intensity, respectively. Different concentrations (0.1–3.0 μg/μL) of anti-HSA were analyzed by CE-SPR (Fig. 5). The SPR response increased with anti-HSA concentration showing a characteristic binding curve. Binding of anti-HSA could be monitored effectively by CE-SPR down to protein concentrations of 100 ng/μL (corresponding to 1.2 fmol or 2 ng of protein injected). For their CE-SPR system employing injection volumes of 270 nL, Whelan et al. reported similar sensitivity (2 fmol) for the binding human IgG to immobilized protein, but lower signal reproducibility (15% RSD) [17].
3.3. CE-SPR of mixtures of binding proteins

Aprotinin is a protease inhibitor with affinity towards different proteases, as for instance trypsin and α-chymotrypsin. In order to evaluate the capability of the CE-SPR system to separate and detect multiple binding proteins, a mixture of trypsin and α-chymotrypsin was analyzed using a CMD5 SPR sensor with immobilized aprotinin. Trypsin and α-chymotrypsin are positively charged at physiological pH. To avoid adsorption of positively charged proteins to the negatively charged capillary wall, normally the inner wall of the capillary is coated with a non-adsorptive agent. We tested capillaries that were precoated with a static coating in order to prevent interference of coating agents with the SPR sensor [23,24]. First, a positively charged successive-multipliconic-layer coating of PB-DS-PB was used. This coating prevents adsorption of positively charged proteins to the capillary wall and provides stable EOF in a wide pH range. For CE-SPR analysis, a voltage of −20 kV (reverse polarity) was applied in combination with a small pressure (1 psi) in order to stabilize the CE current. Fig. 6A shows the CE-SPR electropherogram obtained for the analysis of a mixture of trypsin, α-chymotrypsin (1 µg/µL each) and DMSO (5 vol%). The first SPR signal detected at 10 min can be ascribed to DMSO (EOF marker) which does not bind to the aprotinin on the sensor surface. A two-step rise of the SPR signal at 11.2 and 11.6 min indicated the migration of trypsin and α-chymotrypsin, which bind to aprotinin. Plotting the first derivative of the obtained sensorgram nicely revealed the separated peaks of trypsin and α-chymotrypsin. In addition, a third peak was discerned at 12.5 min. This signal most probably corresponds to α-chymotrypsin isofrom that is separated from the main peak of α-chymotrypsin. Trypsin and α-chymotrypsin samples often comprise different proteofoms which may have comparable affinity to aprotinin [14].

Post-run regeneration via the CE-capillary using 100 mM of NaOH did not completely remove the proteins from the sensor surface. Therefore, solutions of urea, SDS, NaCl, ethylene glycol and glycine were tested for regeneration of the aprotinin-immobilized CMD5 sensor. The use of 200 mM NaOH or 10 mM glycine (pH 1.5) provided complete regeneration of the aprotinin-immobilized CMD5 sensor without apparent loss of aprotinin binding capacity.

A neutral PVA-coated capillary was also tested in the CE-SPR system. PVA suppresses the EOF and avoids protein adsorption. In order to ensure continuous migration of proteins after the grounding point in the micro flow channel, a pressure of 1.0 psi was applied during the separation. For sensor regeneration via the PVA-coated capillary, 10 mM glycine (pH 1.5) was selected as, in contrast to NaOH, glycine is compatible with the PVA-coating. Fig. 6B shows the CE-SPR electropherogram obtained for the analysis of a mixture of trypsin and α-chymotrypsin using a voltage of +15 kV. Two main signals between 15 and 17 min were observed in the sensorgram corresponding to the binding of α-chymotrypsin and of trypsin to aprotinin. The sensorgram shows several binding events, probably produced by different proteoforms of α-chymotrypsin and trypsin. The neutral coated capillary provides enhanced protein separation and the isoforms of α-chymotrypsin and trypsin start to resolve. This is more evident from the first-derivative sensorgram, where at least three peaks can be discerned for both trypsin and α-chymotrypsin. The profile observed for trypsin resembles the profile observed previously with CE-MS [14]. However, as a positively-charge capillary coating was used in combination with a negative voltage, the migration order of the proteoforms was reverse. In the affinity CE-MS study the trypsin proteoforms also exhibited affinity for aprotinin [14].

Overall, these results demonstrate that the CE-SPR system is compatible with different CE protein separation conditions and that it can be employed for the affinity assessment of protein mixture components, including different protein isoforms.

4. Concluding remarks

We have developed a new flow cell for the coupling of CE with SPR, allowing affinity assessment of protein mixture components. The microfluidic flow-cell permits protein detection under high voltage conditions (up to +30 kV) employing flow rates (nL/min range) and injection volumes (~20 nL) that are typical for CE. Stable CE currents were achieved by using the SPR gold-sensor as grounding electrode. Division of the sensor into an electrode part (providing grounding) and a detection part (bearing the affinity surface) was crucial to avoid disturbance of the SPR detection by the high CE voltage. Moreover, the integration of an on-line
reference channel in the microfluidic SPR flow-cell allowed discrimination between specific and non-specific binding. Complete sensor regeneration was achieved via the CE capillary allowing repeatable CE-SPR binding analyses. The presented CE-SPR system has the capacity to specifically measure protein binding in presence of multiple non-interacting proteins. Moreover, mixtures of different binding proteins can be separated and individually assessed by SPR detection. The compatibility of the CE-SPR system with two commonly employed static CE-capsillary coatings was demonstrated. Overall, the results obtained in this research show the advantages of combining of efficient native protein separation with SPR for the affinity assessment of protein mixtures. Currently we are exploring the incorporation of UV detection in the CE-SPR system, which would allow direct correlation of peaks to protein binding as shown in this paper by a separate CE-UV analysis. This gives additional information with respect to the signal of the reference channel. Moreover, it is important to combine analysis by CE-SPR and CE-MS for mass assignment of binding proteins. The developed CE-SPR system shows good potential for the evaluation of the specific binding characteristics of protein variants (glycoforms, modifications, degradation products) as for example can be present in biopharmaceutical samples.

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Appendix A. Supplementary data


References


Biographies

Dr. Elena Dominguez Vega received her Ph.D. degree in 2011 at the University of Alcalá (Spain). Her Ph.D. research focused on the development of electrophoretic and chromatographic capillary methodologies for the analysis of amino acids, peptides, proteins of pharmaceutical, biomedical and nutritional interest. In 2012 she became postdoctoral researcher at Utrecht University where she concentrated on mass spectrometry (MS)-based methodologies for intact protein and peptide analysis. In 2013, she continued her work in the BioMolecular Analysis group at Vrije Universiteit Amsterdam where she develops new analytical technologies for the study of protein heterogeneity, conformation and affinity.

Dr. Rob Haselberg obtained his Ph.D. degree in 2010 at Utrecht University on the development and application of capillary electrophoresis-mass spectrometry (CE-MS) technologies for the analysis of biopharmaceuticals. Subsequent post-doctoral research at Utrecht University and Vrije Universiteit Amsterdam (VU) focused the evaluation of CE-MS for protein characterization as well as setting up affinity CE-MS methods to study protein–protein interactions. In 2013, Haselberg joined the BioMolecular Analysis group of prof. G.W. Somsen at VU, where his research focuses on the characterization of (intact) biomacromolecular compounds using a variety of analytical platforms.

Mr. Dick van Iperen is technical engineer at the Mechanical Workshop of the Faculty of Science at the Vrije Universiteit in Amsterdam. He is a specialist in the construction of (micro)fluidic parts for analytical-chemical purposes.

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