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Surface plasmon resonance for real-time study of lectin–carbohydrate interactions for the differentiation and identification of glycoproteins

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ABSTRACT

A study of specific interactions between lectins and glycoproteins has been carried out using surface plasmon resonance (SPR) in a flow-injection mode. Lectins were covalently immobilised on the surfaces of the microfluidic sensor chip via amine coupling and serum glycoproteins were injected into the flow channels. Specific lectin–glycoprotein interactions caused the shift of refractive index proportional to the mass concentration accumulated on the channel surface. Lectins showed different affinity to the tested glycoproteins and each glycoprotein displayed its own lectin-binding pattern. It is possible to distinguish and identify even glycoproteins with similar sugar structures by simple and quick screening. The working conditions of the assay were optimised. The lectin-based SPR made it possible to carry out the label-free detection of glycoproteins within a broad concentration range with a good linearity. Regeneration conditions for the surface of the sensor chip were found and optimised. Combination of 10 mM HCl and 10 mM glycine–HCl (pH 2.5) removes the bound glycoproteins from the lectin surface without damaging it. The kinetic and affinity parameters of lectin–glycoprotein binding were evaluated. The proposed method was tested on human glycosylated serum. Combination of the lectin panel with SPR is suitable both for specific screening and for sensitive assay of serum glycoproteins.

1. Introduction

Glycoproteins (GPs) are proteins that contain oligosaccharide chains (glycans) covalently attached to polypeptide side chain. They play a key role in a number of biological processes involving cell-to-cell communication, cell proliferation, recognition and cell death, nervous functions, biosynthesis and tumor metastasis [1–4]. The majority of the proteins targeted by therapeutic compounds are glycoproteins. This includes pharmaceutical products for heart diseases, cancer, neurodegenerative disorders and diabetes [5]. Some GPs are used as markers to recognise infections and to monitor the adherence of the bacteria to tissues [6]. It has been reported that protein glycosylation, the linking of carbohydrates to amino acids of proteins, is related to cancer, inflammations, chronic, immunological and cardiovascular disorders [7–12]. Thus, the development of sensitive and reliable methods to detect glycoproteins and to monitor the binding of carbohydrates to proteins becomes the important task in the fields of clinical chemistry and medicine.

The methods of glycoproteins detection described in the literature, such as gel electrophoresis [13,14], mass-spectrometry [15–18], high-resolution NMR [19], fluorescent spectroscopy and radioactive methods are often time-consuming, require skilled personnel, sometimes usage of labelling agents [20–22]. This often makes them unsuitable for routine analysis. Label-free methods based on a direct interaction of glycoproteins with specific receptors in a combination with sensitive detection techniques have a great potential to become new powerful tools for GPs identification and determination. One group of such receptors that recognise, interact and reversibly bind sugar moieties of GPs is lectins. Each lectin recognises a unique specific carbohydrate epitope and binds the carbohydrate reversibly through hydrophobic interactions [23].

Application of lectins as recognition molecules for the discrimination of glycans is well known and described elsewhere [24]. Some lectin-based label-free methods for the determination of carbohydrates with optical and mass-sensitive detection are reported in [25–28]. They are highly sensitive, however, they do not provide the information about affinity and kinetic parameters of the biospecific interaction.

Surface plasmon resonance is one of the examples of label-free techniques for real-time studying and monitoring of biospecific

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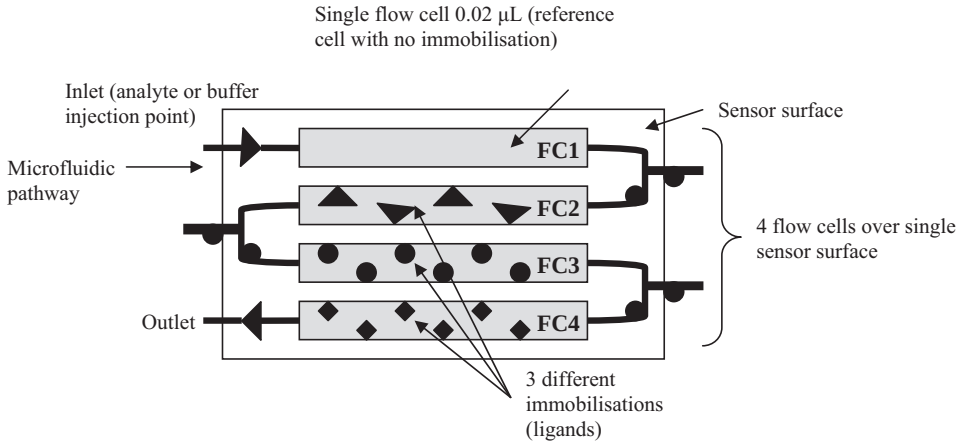

Fig. 1. Four-channel microfluidic sensor chip with three different ligands individually immobilised on the flow cells, FC (drawn schematically). One flow cell (FC1) contains no ligand and serves as a reference channel. The symbol ● represents valve.

interactions, which also provides additional information about their affinity and kinetic parameters. SPR has been intensively developed during the last decade and has received the increasing attention for different applications, from surface studies to biosensors [29–31]. Surface plasmon resonance is used to monitor interactions occurring in a biospecific surface on a metal layer by measuring changes in the analyte surface concentration as a result of the interactions. In order to study an interaction the ligand is immobilised onto the sensor surface. The analyte is injected in aqueous solution (sample buffer) through the flow cell, under continuous flow. Analyte binding to the ligand causes a change of refractive index in the vicinity of the working surface and a corresponding shift of the SPR frequency measured in real time. For most biological molecules the shift in the resonance index is proportional to mass of the adsorbed analyte.

Here we show the possibility to carry out the rapid screening and sensitive quantitative analysis of serum glycoproteins based on their different binding to the lectin panels using surface plasmon resonance biosensor system (Biacore 3000).

2. Materials and methods

2.1. Materials

2.1.1. Supplies

Four-channel CM5 sensor chip coated with carboxymethylated dextran layer, amine coupling kit containing EDC (*N*-ethyl-*N*′-(di-methylaminopropyl)carbodiimide) and NHS (*N*-hydroxysuccinimide), ethanolamine hydrochloride were obtained from Biacore (Uppsala, Sweden). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), Tween P20, EDTA (ethylenediaminetetraacetic acid), glycine, hydrochloric acid, $MgCl_2$, $MnCl_2$, $CaCl_2$ purchased from Sigma (Stockholm, Sweden) were of analytical grade. All the solutions were prepared using Milli-Q water (Millipore 18.2 MΩ cm).

2.1.2. Glycoproteins

Glycoproteins transferrin, fetuin, asialofetuin, ribonuclease A (RNAase), thyroglobulin, fibrinogen and human glycosylated serum were purchased from Sigma (Stockholm, Sweden). Stock solutions of the glycoproteins and serum were prepared at concentration 1 mg mL^{-1} in 10 mM HEPES buffer (pH 7.4) containing 1 mM of $CaCl_2$, $MnCl_2$, $MgCl_2$ and 0.005% Tween P20 which was a running buffer as well. The prepared solutions were stored at 4 °C if not used. The working concentrations were prepared by further dilution of stock solution with HEPES buffer.

2.1.3. Lectins

The freeze-dried native lectins from Lens culinaris (LCA), Maackia amurensis (MAL), Sambucus nigra (SNA), Aleuria aurantia lectin (AAL), concanavalin A (ConA), peanut agglutinin (PNA) were purchased from Vector Laboratories (Burlingame, CA, USA). Lectins were dissolved in HEPES buffer (pH 7.4) containing 1 mM of $CaCl_2$, $MnCl_2$, $MgCl_2$ and 0.005% Tween P20 at concentration 1 mg mL^{-1}. Further dilutions of lectins solutions were made in HEPES buffer.

2.2. Instrumentation

All measurements were carried out in flow-injection mode on a Biacore 3000 biosensor system (Biacore AB, Uppsala, Sweden) operated with the Biacore software package version 3.2 RC1.

2.2.1. Immobilisation of lectin in the flow cell of the microfluidic sensor chip

Lectins were immobilised on the surface of CM5 sensor chip via amine coupling. The sensor chip contains four linked flow channels (flow cells), which are formed inside the integrated microfluidic cartridge (Fig. 1). Flow cells may be used separately (individually), in series or as two pairs. If four flow channels are used individually (as in this work), then simultaneous monitoring of four different ligand–analyte interactions in a single injection becomes possible.

First, the carboxymethylated dextran layer on the chip surface was activated by derivatization with freshly prepared mixture of aqueous solutions of 0.4 M EDC and 0.1 M NHS (1:1, v/v). The mixture was injected into the chip flow channels sequentially at a flow rate 5 μL min^{-1} during 30 min. Then, 200 μL of lectin solution at concentration 0.1 mg mL^{-1} in 10 mM HEPES (pH 7.4) was injected into each flow cell at a flow rate 5 μL min^{-1}. Finally, the unoccupied sites were blocked by injection of 150 μL of 1 M ethanolamine hydrochloride (pH 8.5) for the duration of 30 min. Each step was followed by buffer rinses for a few min. Both activation and immobilisation procedures were performed at room temperature.

2.2.2. Analysis of glycoproteins

The working concentrations of the tested GPs were prepared by dilution of their stock solutions with running buffer. 30 μL of the analyte were injected at a flow rate of 5 μL min^{-1} into the flow channel.

The stock solution of the human glycosylated serum with concentration 1 mg mL^{-1} was diluted 100 times with running buffer prior to injection into the flow channel. The sample was injected during 6 min at a flow rate of 5 μL min^{-1}.

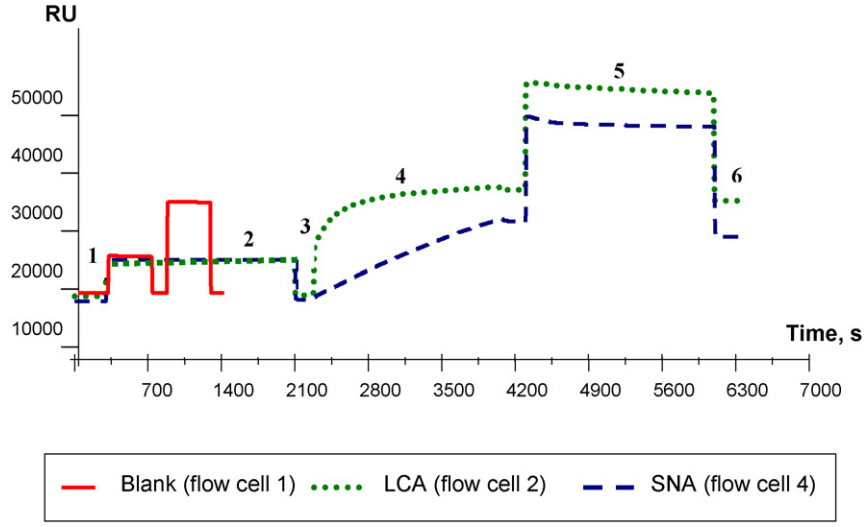

Fig. 2. On-line immobilisation of LCA and SNA lectins on the flow channels of the sensor chip via amine coupling. Parts of the sensorgrams: 1 – baseline, 2 – activation of the carboxymethylated dextran layer with EDC and NHS, 3 – rinse with running buffer; 4 – immobilisation of the lectin, 5 – blocking of the unoccupied sites with ethanolamine.hydrochloride (pH 8.5), 6 – rinse with the running buffer. Solid red line shows the activation of the channel just by EDC and NHS followed by blocking with ethanolamine hydrochloride (pH 8.5), consists no lectin (reference channel). Dotted green line shows the immobilisation of LCA and dashed blue line – the immobilisation of SNA. Running buffer: 10 mM HEPES (pH 7.4) containing 1 mM of Ca^{2+}, Mg^{2+} and Mn^{2+}. Flow rate: 5 μL min^{-1}. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussions

The assay of the glycoproteins was based on their binding with lectins followed by SPR detection. In order to achieve the interaction ligand (lectin) was immobilised on the surface of the sensor chip while the injected analyte (GP) was passing through the flow channel under continuous flow. The accumulation of the analyte bound to the surface results in an increase in refractive index, which serves as an analytical signal. This change in refractive index measured in real time and plotted as a response in resonance units (RU) vs. time (min) is called a sensorgram.

Usually the whole assay procedure includes three steps: modification of the surface of the sensor chip with ligand, binding/detection of the analyte and regeneration of the chip surface. All steps are described successively in the following part.

3.1. Modification of the surface of the sensor chip

In order to carry out the assay with high specificity and sensitivity the appropriate surface and the most suitable immobilisation approach must be found. In this work CM5 sensor chip was used as a support to immobilise lectins. The chip represents an integrated microfluidic cartridge containing four linked microchannels (flow cells or flow channels), which may be used separately or in sequence, liquid channels to deliver buffer and sample solution and computer-controlled pneumatic valves. The pneumatic valves can be opened and closed in user-defined protocol in order to guide the injected solution over the sensor surface. Each flow cell has its own liquid delivery channel and control valve, so the injected liquid can be simultaneously directed to different addressable detection spots. Individually addressable four flow cells in one sensor chip increase the potential number of simultaneous measurements. The schematic sketch of the sensor chip containing three different ligands immobilised on its separated flow channels is shown in Fig. 1.

The volume of each flow channel facing the chip is 0.02 μL. The CM5 sensor chip contains carboxymethylated dextran attached to the gold surface. The surface is highly stable and can be easily derivatized. No additional pre-treatment of the surface was needed prior its activation. The whole procedure of the surface modification contains three steps: activation, immobilisation and blocking. Four flow cells were modified in sequence. The procedure is automated and can be programmed by the user. So, opening and closing the pneumatic valves allow introducing the reagents and lectins into the chosen channel without affecting the other. The pathway for introducing the sample and buffer is designed the way that the injected sample can pass through all four channels with different immobilisations (Fig. 1). Sensorgrams showing the modification of the sensor chip with LCA and SNA lectins in on-line mode via amine coupling are presented in Fig. 2 (green dotted and blue dashed lines). A continuous flow of a degassed running buffer at a flow rate of 5 μL min^{-1} was used throughout the procedure. Before, the running buffer was injected into each flow cell sequentially in order to obtain the stable baseline (Fig. 2, part 1). The running buffer contained 1 mM Ca^{2+}, Mg^{2+} and Mn^{2+}, as the presence of metal ions is essential for the binding activity of lectins [32]. Before the immobilisation the sensor chip surface was activated by injecting an aqueous mixture of EDC and NHS into each of four flow channels at a flow rate of 5 μL min^{-1} (Fig. 1, part 2). The activation step was terminated by rinsing of the flow channels with running buffer to minimise the bulk effect, which may occur due to the changing of the solutions composition (Fig. 2, part 3). After that, different lectins were sequentially immobilised onto individual channels of the sensor chip. The ligands bind to the activated surfaces containing the highly reactive succinimide ester through their amine and other nucleophilic groups. The immobilisation step was carried out at a flow rate of 5 μL min^{-1} providing efficient mass transfer over the flow channel. The binding of the lectins to the activated surface results in an increase of the SPR response. The concentration of the injected ligands was optimised in order to achieve a good coverage of the surface but at the same time avoiding unnecessarily high consumption of lectins. A concentration of 0.1 mg mL^{-1} provided good immobilisation yield resulting in a high response of 9000–14,000 RU (Fig. 2, part 4) and was therefore chosen for further modification of the sensor chip. The lectins were injected until the surface of each flow cell got saturated and the response remained unchanged. That indicates that the most of the activated sites were occupied by the immobilised lectins. The modification process was completed by injection of 1 M ethanolamine hydrochloride (pH 8.5) in order to block the unoccupied activated sites (Fig. 2, part 5). Unaltered responses after rinsing of the flow channels with the running

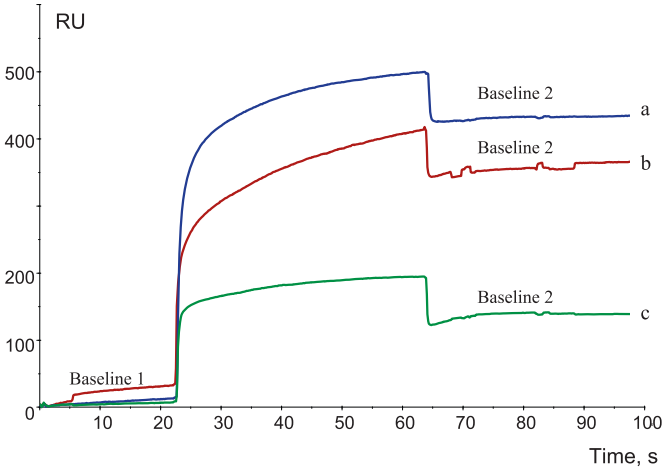

Fig. 3. Sensorgrams illustrating the binding of fibrinogen to the immobilised lectins. (a) Fibrinogen–MAL (431 RU), (b) fibrinogen–ConA (323 RU), and (c) fibrinogen–PNA (120 RU).

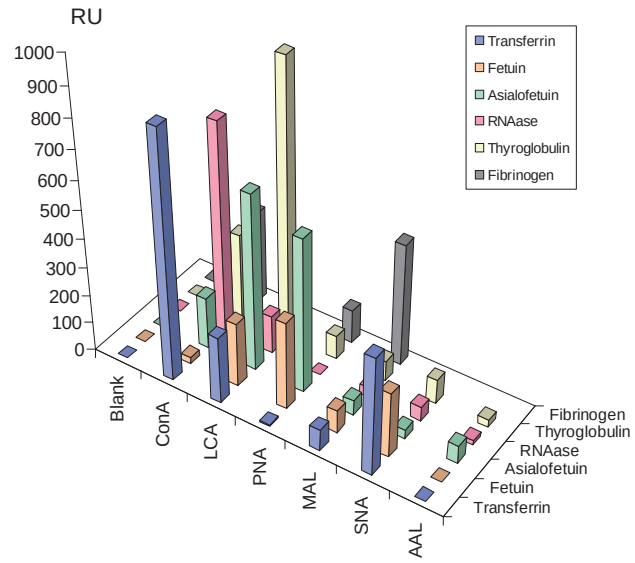

Fig. 4. Affinity of the tested glycoproteins to the immobilised lectins (ConA, LCA, PNA, MAL, SNA and AAL). GPs at concentration 1 mg mL^{-1} were injected at a flow rate of 5 μL min^{-1}. Running buffer: 10 mM HEPES (pH 7.4) containing 1 mM of Ca^{2+}, Mg^{2+} and Mn^{2+}.

buffer (Fig. 2, part 6) indicate that the lectins are firmly attached to the surface. One flow cell in each sensor chip contained no lectin and served as a reference channel (blank) in order to control any possible non-specific adsorption. Its surface was only activated with the mixture of EDC and NHS followed by blocking with 1 M ethanolamine hydrochloride (pH 8.5) (Fig. 2, red solid line).

The modified sensor chip can be stored at 4 °C for at least 2 months with no significant loss of the activity of the immobilised lectins.

3.2. Qualitative analysis of GPs using lectin recognition patterns and SPR

As each sensor chip contains four flow channels, up to four different lectins can be immobilised individually on its surfaces and the binding of the injected glycoprotein to all four surfaces can be monitored simultaneously in a single injection. The lectins were selected according to their affinity to the main classes of the sugar structures. Such variability of lectins provides the pattern recognition for specific detection of selected glycoproteins. Two mannose binding lectins (ConA and LCA), one galactose binding lectin (PNA), 2 sialic acid binding lectins (MAL and SNA) and one fucose binding lectins (AAL) were used as specific receptors. In this work three lectins with different affinities were immobilised on the flow channels of each chip and one channel was used as a reference (blank).

In order to carry out the experiment the following serum glycoproteins were used: transferrin, fetuin, asialofetuin, RNAase, thyroglobulin and fibrinogen. Before the GP injection the stable constant signal value corresponding to the initial baseline (baseline 1, Fig. 3) was achieved. Then, the GP was injected into the flow channels containing the immobilised lectins and the shift of refractive index (in RU) was monitored until it reached the maximum constant value or plateau. Specific interaction between the injected glycoprotein and the immobilised lectin causes the increase in the response due to the increasing of the mass concentration on the channel surface. The signal growth is proportional to the concentration of the analyte injected. If the modified surface has a high binding capacity it binds more molecules of the analyte. From one hand, it results in a larger response and makes it possible to achieve greater sensitivity of the analysis. From another hand, injection of the larger volume of the analyte at a slow flow rate leads to the prolongation of the analysis. That is not desirable for the routine screening. Thus, in this case the reasonable compromise should be made between the sensitivity of the detection and its rapidity. The injection of 30 μL of GPs at a flow rate of 5 μL min^{-1} provided both the sufficient analytical response and the rapidity of the assay and, thus, was used to carry out further measurements. The slight decrease of the signal (Fig. 3) after binding may be explained by the bulk effect due to the temperature differences between the analyte solution and the running buffer. Then running buffer was allowed to pass through the flow channel in order to remove unbound analyte and a new baseline was reached (baseline 2, Fig. 3). If the level of baseline 2 is higher than that of baseline 1 after rinsing the flow channel with buffer, it indicates firm binding between the ligand and the analyte. The analytical signal is calculated as a difference between two baselines. If no specific binding occurs between the ligand and the analyte, in most of the cases the analytical response remains unchanged. Sometimes it may increase because of the bulk effect if the analyte is dissolved in the solvent other than running buffer or due to their temperature differences. However, in this case the signal quickly returns to the level of baseline 1 after rinsing the channel with running buffer.

Fig. 4 illustrates the affinity of the immobilised lectins to the tested glycoproteins. No response was observed when the GPs were injected into the blank (reference) flow cell. Injection of the GPs into other flow cells containing the immobilised lectins resulted in different affinities and gave different analytical responses. The obtained results are in a good agreement with the literature data. It is established that mannose binding lectins – ConA and LCA – had the broader range of affinity and bound to all tested glycoproteins. That can be explained by the fact that those glycoproteins carry mannose residues in their carbohydrate structures, e.g. transferrin and thyroglobulin [33–35]. The highest analytical signals with ConA were observed for transferrin and RNAase (864 and 740 RU, respectively); asialofetuin and thyroglobulin showed the high response to LCA (618 and 961 RU, respectively). As galactose residues are found in fetuin and asialofetuin [36,37], those GPs bind to PNA and give a rather high response of 310 and 543 RU, respectively. The primary glycan structure of bovine fibrinogen has been determined by ^{1}H NMR [19]. Fibrinogen contains mannose and GlcNAc that can be recognised by the appropriate lectins ConA and MAL (323, 431 RU, respectively). Asialofetuin has very similar structure as fetuin, but does not contain sialic acid (or Neu5Ac) [37]. The obtained data showed that both GPs can be recognised by sialic acid binding SNA

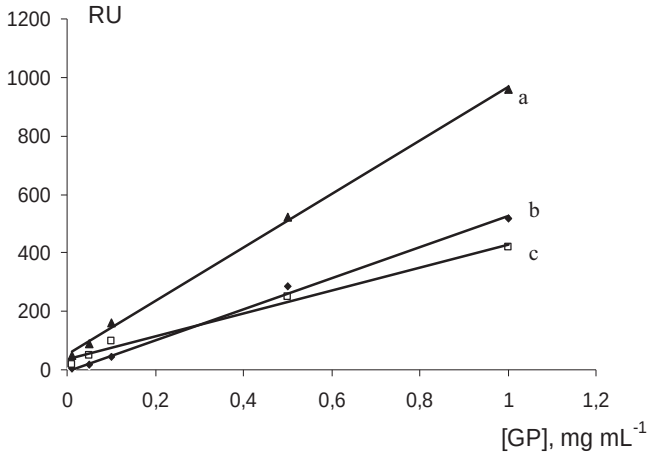

Fig. 5. The calibration curves corresponding to the interactions (a) LCA–thyroglobulin, (b) LCA–fetuin, and (c) SNA–transferrin. GPs at concentration range of 0.01 mg mL^{-1} to 1 mg mL^{-1} were injected at a flow rate of 5 μL min^{-1}. Running buffer: 10 mM HEPES (pH 7.4) containing 1 mM of Ca^{2+}, Mg^{2+} and Mn^{2+}.

but the response to fetuin was much higher than to asialofetuin (230 RU vs. 33 RU). It should be mentioned that the obtained results are in good agreement with our previous study on a similar theme using quartz crystal microbalance technique [28].

Thus, the selected lectin recognition panel combined with SPR detection is highly specific and makes it possible to clearly distinguish and identify even the glycoproteins with similar glycan structure by simple and quick screening. If the affinity of the immobilised lectin to certain glycoprotein is high, the GP binding is relatively strong. This binding can be destroyed using special regeneration solutions (described in Section 3.4) and not just by rinse with the running buffer. When any interference as non-specific binding occurs, the bound molecule can be removed by simple buffer rinse. Blocking the unoccupied ester active binding sites during the surface modification step helps to reduce or even to minimise the non-specific binding and, thus, to increase the selectivity of the GP assay. If still some non-specific binding is observed, it can be controlled using the subtraction of data from the reference cell (containing no immobilised lectin).

3.3. Quantitative analysis of GPs using lectin recognition patterns and SPR

The key role of many glycoproteins in biological processes has an importance to detect them also quantitatively. Such highly sensitive technique as surface plasmon resonance makes it possible to measure GPs at low concentrations. The calibration curves corresponding to the interactions fetuin–LCA, thyroglobulin–LCA and transferrin–SNA are presented in Fig. 5. The obtained data showed good linearity for all three GPs in the concentration range of 0.01–1.0 mg mL^{-1}, which corresponds to the micro- and nanomolar level. The correlation coefficients were $r^2 = 0.9967$ for fetuin, $r^2 = 0.9988$ for thyroglobulin and $r^2 = 0.9867$ for transferrin. Although the GPs at concentrations below 0.01 mg mL^{-1} can be distinguished from the background signal, they may not be detectable with any higher level of accuracy. The data obtained were highly reproducible. All measurements were performed in triplicates and a RSD of 1.8–2.8% was achieved.

The proposed method was tested on human glycosylated serum as an example of a real sample. The experimental principle was as described above. The diluted sample was injected into the flow channels at a flow rate of 5 μL min^{-1}. Significant responses were obtained from LCA and SNA surfaces corresponding to 143.43 ± 2.5 and 176.8 ± 3.1 RU, respectively ($n = 3$). The interaction was much weaker with ConA surface (28.6 ± 1.3 RU $n = 3$) and almost negligible with AAL surface (11 ± 0.2 RU, $n = 3$). PNA did not show any binding to the carbohydrates present in the serum sample.

At this stage using the data set we have obtained, we can only assume that mannose and sialic acid residues and non-significant amount of galactose might be present in the analysed serum. However, in order to screen the real samples and obtain more reliable results further investigations of the larger group of GPs and creation of a broader lectin panel need to be done. The expanded lectin recognition panel will help to predict the structure of the analysed GPs more precisely. Nevertheless, even at this stage it is clear that the lectin-based SPR has a promising future to become a suitable method both for rapid screening and for quantitative measurement of serum glycoproteins in real samples. The assay is simple, does not require sample preparation except dilution of serum in order to prevent clogging of the microfluidic paths. In order to control the possible interferences it is recommended to use one or even two flow cells as a blank to perform the reference subtraction of the obtained data if needed. It should be mentioned that combination of SPR and mass spectrometry (SPR–MS) broadens the perspectives and increase the power and potential of SPR technique for real sample analysis. The tandem technique provides both a means of selective binding and a structural information which facilitates the identification and characterisation of specific molecule in a complex biological mixture [38].

3.4. Regeneration of the sensor chip surface

Specific complex formed due to the binding of GP to the immobilised lectin occupies the binding sites on the chip surface. Thus, the further accurate measurements with the same surface become impossible. As the main expenses of the assay come from the cost of the sensor chip, the efficient regeneration procedure makes it possible to re-use the same chip and, thus, reduce the total cost of the analysis. The successful regeneration must destroy the specific complex and remove the bound glycoprotein from the lectin surface to make it available for the next binding. However, the lectin layer is required to remain undamaged and active. The optimised regeneration helps to shorten the whole assay procedure as the same lectin surface can be used for several measurements without any immobilisation step before each experiment. In order to destroy the biospecific complex the solutions with acidic or basic pH (inorganic or organic), the concentrated solutions of inorganic salts with high ionic strength ($MgCl_2$, NaCl) and surfactants (SDS) are commonly used.

Different combinations of the solutions were tested in order to find the most suitable one and optimise the regeneration step. 10 μL of each solution were injected into the flow channel at a flow rate of 5 μL min^{-1} after the glycoprotein–lectin binding.

The following combinations were tested:

(a) 1 M NaCl, 10 mM HCl;
(b) 1 M NaCl, 10 mM EDTA, 10 mM glycine–HCl (pH 2.5);
(c) 10 mM glycine–HCl (pH 2.5);
(d) 10 mM HCl, 10 mM glycine–HCl (pH 2.5).

The fact that the baseline after regeneration (2, Fig. 6) gives the same signal as the initial baseline (1, Fig. 6) obtained before the experiment indicates the complete successful removal of the bound GP from the chip surface.

Combinations (a) and (c) were not efficient enough to remove the bound GPs completely. EDTA in combination (b) was used to remove the bivalent metal ions Ca^{2+}, Mg^{2+} and Mn^{2+} essential for the binding activity of lectins. However, this combination did not provide the complete surface regeneration and partially inactivated

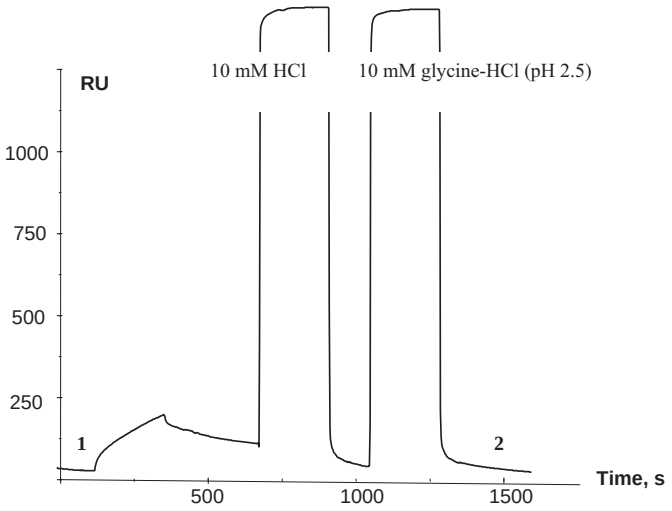

Fig. 6. Successful regeneration of the sensor chip surface after injection of 10 mM HCl followed by the injection of 10 mM glycine–HCl (pH 2.5). 1 – Incident baseline obtained before the experiment. 2 – Baseline obtained after the regeneration.

the immobilised ligands (data not shown). The combination (d) enabled to remove the bound glycoproteins completely from all lectin surfaces (Fig. 6). Also it was established that no alteration in analytical response was observed after multiple injections of the same GP. It means that this combination does not inactivate the surface and does not deteriorate the binding activity of the immobilised lectins. Increase of the response after each injection of the regenerating solution can be referred to the bulk effect due to the changing of the solvent composition. Thus, 10 mM HCl in combination with the injection of 10 mM glycine–HCl (pH 2.5) was selected for the further regenerations of the sensor chip surfaces. The total time for simultaneous analysis of glycoprotein in a single injection onto four different surfaces is 15–20 min including the regeneration step.

3.5. Determination of the kinetic parameters of GP–lectin interaction

Rate constant and association and dissociation constants (K_A and K_D) are the fundamental parameters of the specific interaction. The rate constant indicates what fraction of the molecules in the initial compartment is traveling through the pathway per unit time. The association and dissociation constants represent the affinity between the ligand and the analyte. The smaller the dissociation constant is, the tighter the ligand binds to the analyte. Studying the affinity and kinetic parameters helps to select the ligand suitable for the immobilisation, estimate the level of active immobilised ligand in order to improve the selectivity of binding and thus, the sensitivity of the analysis.

The kinetic parameters of the lectin–glycoprotein interactions were evaluated using the immobilised lectin and injecting different concentrations of the same glycoprotein. The obtained sensorgrams were fitted, normalised and the kinetic parameters were calculated by the BiaEvaluation software included in the Biacore software package 3.2 R1, which contains all kinetic models. In general, lectins have two or more binding sites. They have the potential to bind two or more molecules. However, if low amount of lectin is immobilised on the chip surface and glycoprotein is injected at low concentration the mass transport effect is negligible. Then it can be assumed that all binding events are independent and simple monomolecular interaction model can be used [39].

It has been found that the immobilised lectins varied greatly by the binding rate and affinity to the tested GPs. For instance, the binding LCA–thyroglobulin was characterised by relatively high (under the studied conditions) rate constant of $(1.0 \pm 0.1) \times 10^{-3}\ s^{-1}$ and high affinity. The association constant for this interaction was calculated as $K_A = (1.7 \pm 0.2) \times 10^7\ M^{-1}$ ($K_D = 59 \pm 2$ nM). The interaction SNA–thyroglobulin was slightly faster but weaker. The calculated reaction rate for this binding was $(1.4 \pm 0.2) \times 10^{-3}\ s^{-1}$ $K_A = (4.5 \pm 0.03) \times 10^6\ M^{-1}$ ($K_D = 0.22 \pm 0.01$ μM). The binding LCA–fetuin occurred slower and it was one order of magnitude weaker, than SNA–thyroglobulin. The rate constant for this interaction was $(0.23 \pm 0.02) \times 10^{-3}\ s^{-1}$, $K_A = (4.5 \pm 0.03) \times 10^5\ M^{-1}$ ($K_D = 2.2 \pm 0.1$ μM)

4. Conclusion

A preliminary study of biospecific interactions between the immobilised lectins and glycoproteins using surface plasmon resonance in flow-injection mode has been carried out. The increase of refractive index due to the binding of GPs to various lectins surfaces was used as an analytical signal. All tested glycoproteins showed their own unique lectin-binding pattern. Combination of the lectin panel and surface plasmon resonance makes it possible to carry out both qualitative screening and quantitative detection of serum glycoproteins. Although, in order to obtain more reliable results, further investigations with larger group of GPs and the creation of broader lectin library need to be done, this method looks promising and has a potential to be applied for monitoring of protein glycosylation process. It may be concluded that the unique selectivity, high sensitivity, excellent reproducibility, simplicity, absence of the labelling agents, rapidity, automation and small consumption of reagents make the reported method a good alternative to the more complicated and time-consuming glycoprotein analysis techniques based on structural investigations and quantitative determination of the carbohydrate composition.

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