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A study of glycoprotein–lectin interactions using quartz crystal microbalance

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ABSTRACT

A study of biospecific interactions between lectins and glycoproteins using a quartz crystal microbalance biosensor with dissipation monitoring (QCM-D) was reported. Four lectins were covalently immobilised on the thiol-modified gold electrode of the QCM chips in order to obtain sensing surfaces. The frequency shift served as analytical signal and the dissipation shift provided additional information about the viscoelastic properties of the glycoprotein–lectin complex formed on the surface of the QCM chip. The working conditions of the assay were optimised. The interaction between different lectins and glycoproteins was characterised by specific frequency shifts and each glycoprotein displayed its own unique lectin-binding pattern. This lectin pattern can serve as a finger print for the discrimination between various glycoproteins. The biosensor enabled quantitative determination of glycoproteins in the concentration range of 50 μg mL^{-1} to 1 mg mL^{-1} with good linearity and R.S.D. of less than 6.0%. An additional advantage of the proposed biosensor was the possibility to re-use the same lectin surfaces during a long period of time (2 month) without changes in analytical response. This was experimentally achieved by the application of a proper regeneration solution (10 mM glycine–HCl, pH 2.5). The lectin-based quartz crystal microbalance technique is suitable both for rapid screening and for quantitative assay of serum glycoproteins.

1. Introduction

Glycoproteins (GPs) are proteins that contain oligosaccharide chains (glycans) covalently attached to their polypeptide chains. Some of the GPs are integral membrane proteins and play key roles in a number of regulatory and neural functions, cell–cell interactions, cell communication, cell proliferation and death [1–3]. The GPs are used in medicine to recognise cancer cells, biomolecules and as markers for certain types of diseases based on changed carbohydrate interactions, e.g. infectious diseases, when the bacteria adhere to host tissues [4]. Glycans also have an important role in two distinct but related areas of drug development: either as part of glycoprotein therapeutics, modulating protein activity and stability, or alone as saccharide-based therapeutics. The majority of the proteins targeted by therapeutic compounds are GPs, including some pharmaceutical products for heart disease, cancer, neurodegenerative diseases and diabetes [5]. Therefore, the development of rapid and low-cost methodology for screening of GPs is highly important for diagnostics of diseases and for development of new, highly effective and specific therapeutics. Such approaches have been widely exploited, especially because GPs act as signalling molecules and changes in the glycoprotein structure are associated with disease progression. However, common methods for glycoprotein detection and analysis, such as staining with dye, fluorescent or radioactive reagents are time-consuming [6]. Mass-spectrometry [7,8], high-resolution NMR spectroscopy [9] and capillary electrophoresis [10] despite that they are capable to identify the structure of the glycans are complicated, expensive, and require skilled personal. Ultrasensitive masspectrometric methods can provide detailed carbohydrate sequence data for glycosylated proteins on low-femtomole samples, but nevertheless they are not suitable for routine assays.

A better approach to detect glycosylation changes for diagnostic purposes is to use bioaffinity probes in combination with label-free mass-sensitive detection. In this context the quartz crystal microbalance is a suitable instrument for the GP detection and identification being inexpensive, easy to operate and sufficiently sensitive. QCM can be used with label-free techniques and operates well in complex, optically opaque solution media [11]. The technology used in this study has a distinct advantage over our earlier reported SPR technique for screening serum GP [12] because this device is equipped with four channels which can be used in parallel. Thus, the time required for analysis of one sample is greatly reduced. The mass sensitivity of the QCM-D instruments is 5 pg mm^{-2} and for Biacore instruments it is ca. 0.5 pg mm^{-2}, but

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in practise this difference is not important, both techniques have adequate sensitivity and stability for the actual measurements.

The QCM technique is based on the change in the resonant frequency of a quartz crystal which in the case of rigid, uniformly distributed layers is linearly related to the mass accumulated on the surface (Sauerbrey, 1959) [13]:

$$\Delta f = \frac{-\Delta m}{nC},$$

where C is the mass sensitivity constant ($C = 17.7\ \text{ng} \times \text{cm}^{-2} \times \text{Hz}^{-1}$ at 5 MHz) and n is the overtone number ($n = 1, 3, 5, \ldots$). The crystal is excited to oscillation in the thickness shear mode at its fundamental (or an overtone) resonant frequency, f, by applying a suitable voltage across the electrodes deposited on two faces of the crystal. A mass, Δm, added to the electrodes, induces a decrease in the resonant frequency, which is proportional to Δm [13]. The direct, label-free QCM method makes it possible to measure very small mass changes and to follow binding interactions on the surface directly on-line. One advantage of the special QCM-D technique is that it allows simultaneous frequency and energy dissipation monitoring [14]. Dissipation measurements are done by periodically disconnecting the oscillating crystal from the driver circuit via a computer-controlled relay. Disconnection causes the crystal oscillation amplitude to decay following an exponentially damped sinusoidal function:

$$U(t) = U_0 \exp\left(\frac{-t}{\tau}\right) \sin(2\pi f t + \varphi), \quad t = 0,$$

where U is the voltage over the crystal, τ is the decay time constant and φ is the phase. The dissipation factor is inversely proportional to τ. The decay of the QCM oscillation is transferred to a computer and a numerical fit of equation is performed. Hence, both the resonance frequency and the dissipation factor can be measured simultaneously for the QCM oscillation [15]. Inclusion of dissipation measurements provides additional information about the structure and dynamic viscoelastic properties of surface-adsorbed protein layers.

Lectins are proteins or glycoproteins which can be found worldwide in plants, animals, fungi, bacteria and viruses. Lectins exhibit strong and specific binding to carbohydrates, carbohydrate moieties attached to glycoproteins and glycolipids through hydrogen bonds, metal coordination, van der Waals and hydrophobic interactions [16]. Lectins serve as excellent biorecognition elements due to high affinity for saccharide moieties via multivalent interactions arising from the spatial organisation of oligosaccharide ligands [17]. Therefore, combination of the QCM technique with lectins enables to develop approaches for detection of GPs.

The past decade has witnessed an explosive growth in the application of the QCM technique to the study of a wide range of molecular systems at the solution–surface interface, in particular, biopolymer and biochemical systems [11]. However, the application of the QCM instrument coupled with lectin panels as a biosensor device for the specific detection of GPs has not yet been described. Thus, the aim of the present study was to demonstrate possibility of using lectin panels in combination with the QCM technique for rapid qualitative screening and quantitative assay of serum GPs. Additionally, the goal of the study was to develop a proper regeneration technique enabling re-use of the same lectin-panel over many cycles.

2. Experimental

2.1. Chemicals

N-hydroxysuccinimide (NHS), 11-mercaptoundecanoic acid and 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), analytical grade, were purchased from Sigma (Stockholm, Sweden). Ethanolamine–HCl (1 M, pH 8.5), 10 mM glycine (pH 2.5) were supplied from BIAcore (GE Healthcare, Uppsala, Sweden). Phosphate buffered saline (PBS) (10 mM, pH 7.4) was prepared from the chemicals of analytical grade (Merck, Darmstadt, Germany) using deionised, highly purified water (18.2 $\text{M}\Omega\,\text{cm}^{-1}$) from an Elga Maxima water purification system (Elga Processed Water Ltd., Marlow, UK).

2.2. Lectins

The following freeze-dried native lectins: *Triticum vulgaris* (WGA), *Lens culinaris* (LCA), *Ulex europeus* (UEA) were purchased from Vector Laboratories (Burlingame, CA, USA). *Concanavalin A* (Con A) lectin was purchased from Sigma (Stockholm, Sweden). Lectins were dissolved in 10 mM PBS containing 1 mM Ca^{2+}, 1 mM Mg^{2+}, 1 mM Mn^{2+} salts at concentration 0.1 mg mL^{-1}.

2.3. Samples

The serum proteins transferrin, fetuin, asialofetuin, ribonuclease A (RNase), thyroglobulin and fibrinogen were purchased from Sigma (Stockholm, Sweden). Stock solutions of glycoproteins were prepared at a concentration 1 mg mL^{-1} in the PBS running buffer and stored at 4 °C when not used. Concentrations between 0.1 mg mL^{-1} and 1 mg mL^{-1} were prepared by dilution of stock solution with running buffer.

2.4. Quartz crystal microbalance measurements

The experiments were performed on the quartz crystal microbalance device Q-Sense E4 from Q-Sense (Göteborg, Sweden). The instrumentation comprising this QCM device has been elaborated elsewhere [18]. It consists of a measurement platform that carries four temperature controlled (set at 25 °C) flow-modules. Each module mounted with polished gold-coated quartz crystal (AT-cut, 14 mm diameter) with a fundamental resonant frequency 5 MHz (Q-Sense, Göteborg, Sweden). The running buffer was 10 mM PBS (pH 7.4). The surface of quartz crystals was modified according to the procedure described in Section 2.5. Samples were then introduced into the system with a peristaltic pump which was supplied with the device. To allow well-controlled flow measurements, the liquid enters from the side of the crystal. Injection time was 40 min (50 $\mu\text{L min}^{-1}$), followed by 30 min PBS rinse (50 $\mu\text{L min}^{-1}$). The system allows simultaneous measurements of changes in oscillation frequency (Δf) and energy dissipation (ΔD) with noise levels of 2 Hz and 0.2×10^{-6} U, respectively. The QCM-D instrument is integrated with a PC and the data obtained using the Q-Sense system can be visualised with QSoft 401 software and analyzed by Q-Tools (Q-Sense, Göteborg, Sweden). For clarity, changes in frequency and dissipation of the fifth overtone were presented. Bound GPs were released from the surface by injection of low pH glycine–HCl solution (pH 2.5). The sensor chips with immobilised lectins were stored at 4 °C.

2.5. Lectins immobilisation via thiol groups

Lectins were immobilised on the modified surface of gold-coated quartz crystal according to a simple amine coupling procedure. First, quartz crystals were cleaned in the boiling mixture of 35% of NH_3, 33% of H_2O_2 and ultrapure water in ratio 1:1:5 (v/v) for 10 min and then rinsed with ultrapure water and ethanol. Next, quartz crystals were immediately immersed into the alcohol solution of 1 mM 11-mercaptoundecanoic acid and kept overnight at ambient room temperature (20–25 °C) in order to obtain the

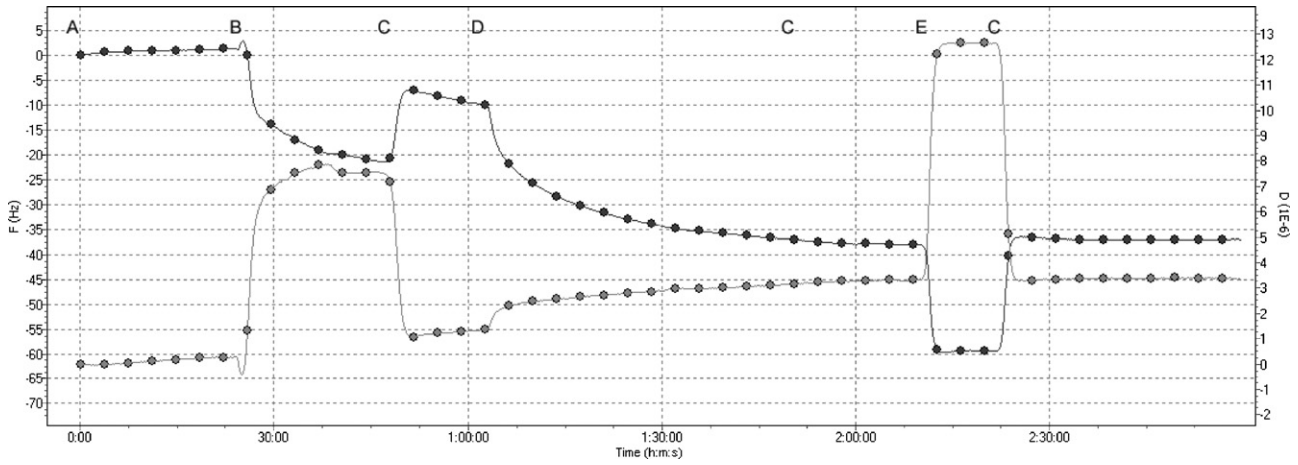

Fig. 1. The time-dependent frequency (upper curve) and dissipation shifts (lower curve) recorded during the immobilisation of *Concanavalin A* lectin on the surface of quartz crystal electrode: (A) thiol-modified electrode; (B) activation of the carboxyl groups with NHS:EDC mixture; (C) rinse with the running buffer; (D) immobilisation of the lectin (1 mg mL^{-1}, 60 min); (E) blocking of unspecific sites with ethanolamine–HCl (pH 8.5). PBS (10 mM, pH 7.4) containing 1 mM metal ions (Ca^{2+}, Mg^{2+} and Mn^{2+}) was pumped through the system at a flow rate 50 μL min^{-1}.

thiol self-assembled monolayers (SAM) on the gold surfaces. Afterwards unattached thiols were removed from the surface by rinsing with ethanol and ultrapure water followed by drying in a stream of nitrogen. The activation step was performed in flow-through mode. Crystals were mounted in the QCM devices and buffer was passed through the system until stable baselines of frequency and dissipation were achieved. Then freshly prepared aqueous mixture of 0.4 M EDC and 0.1 M NHS (in ratio 1:1) was used for activation of carboxyl groups on the gold surface. The mixture was injected into the units at a flow rate 50 μL min^{-1}. Lectin solutions in PBS (containing 1 mM Ca^{2+}, 1 mM Mg^{2+}, 1 mM Mn^{2+}) at concentration of 0.1 mg mL^{-1} were injected into each flow chamber for the duration of 1 h. Finally, nonspecific sites were blocked with 1 M ethanolamine–HCl, pH 8.5 (30 min). Each step was followed by 10 min buffer rinse. The system was allowed to equilibrate until a stable baseline was achieved.

3. Results and discussion

3.1. Optimisation of the assay conditions

As the gold surface is relatively chemically inert and unable to effectively retain adsorbed material, a modification of the quartz crystal surface was first conducted. This was conveniently performed by a three-step adsorption procedure. Firstly, crystals were dipped into a solution of 11-mercaptoundecanoic acid in order to form a well-ordered and crystalline self-assembled monolayer (SAM) that would incorporate different chemical groups. Although bonds between gold and sulphur form rapidly, typically within second to minutes, a few hours are required to introduce a significant amount of order into the assembly [19]. Therefore, SAM formation was performed in the off-line mode. Subsequently, the crystals were mounted in the QCM-D system and activation of surface was performed in on-line mode. All experiments were conducted in PBS buffer containing 1 mM Ca^{2+}, Mg^{2+} and Mn^{2+} since the presence of metal ions is essential for the binding activity of lectins [20]. The working flow rate of 50 μL min^{-1} was chosen in order to get the efficient mass transfer over the crystal surface and reduce the time for the assay. After a stable baseline was established the mixture of 0.1 M NHS and 0.4 M EDC was passed through the surface of the quartz crystals followed by PBS rinsing in order to exclude bulk effect which can appear in a microbalance system due to changes in viscosity, composition or temperature of injected solutions. Decrease in frequency was first observed, but after rinsing with PBS the resulting frequency shift was insignificantly small because of the small mass added to the surface.

Real-time frequency and dissipation changes associated with immobilisation of lectins are presented in sensorgram (Fig. 1). Lectin injection into the system was associated with quick frequency decrease followed by slower decrease when surface was saturated. Injection was conducted until no future changes in frequency and dissipation shifts were observed. After rinse with PBS the frequency remained unchanged, indicating that immobilised lectin molecules were firmly attached to the surface. Dissipation shifts were all positive. The values of frequency and dissipation shifts obtained during immobilisation procedure were as following: 27.0 Hz and 2.0×10^{-6} U for Con A; 15.0 Hz and 0.2×10^{-6} U for WGA; 29.0 Hz and 1.1×10^{-6} U for UEA; 30 Hz and 0.1×10^{-6} U for LCA. With this procedure maximum frequency shift was achieved for the UEA lectin.

The concentration of lectins remaining for efficient immobilisation procedure was optimised. If too low concentration of lectin is used the mass transfer might not be efficient which prolongs the procedure of the immobilisation. The usage of too high concentrations is not reasonable due to the high consumption of the reagents. Concentrations ranging from 0.01 mg mL^{-1} to 1 mg mL^{-1} were checked (data not shown) and a concentration of 0.1 mg mL^{-1} was chosen to provide fast and efficient saturation of the activated crystal surface. The lectin solution was injected until the frequency and dissipation stopped to change, which indicates that most active sites on the crystal had been occupied by the ligand. After that the remaining reactive sites on the surface of the crystal were blocked by an injection of 1 M ethanolamine–HCl (pH 8.5) to prevent nonspecific adsorption during the further experiments.

3.2. Screening of glycoproteins

The selection of lectins was based on their ability to interact with the main classes of the sugar structures. Two mannose binding lectins (Con A, LCA), one sialic acid binding lectin (WGA) and one fucose binding lectin (UEA) were chosen for the experiments. This selection of the lectin panel enabled identification and screening of the actual serum GPs.

In order to carry out an illustrative experiment the following serum glycoproteins were used: transferrin, fetuin, asialofetuin, ribonuclease A (RNase), thyroglobulin and fibrinogen. The GPs were injected into the sensor chamber until maximum changes in frequency and dissipation were achieved (Fig. 2). The shift in

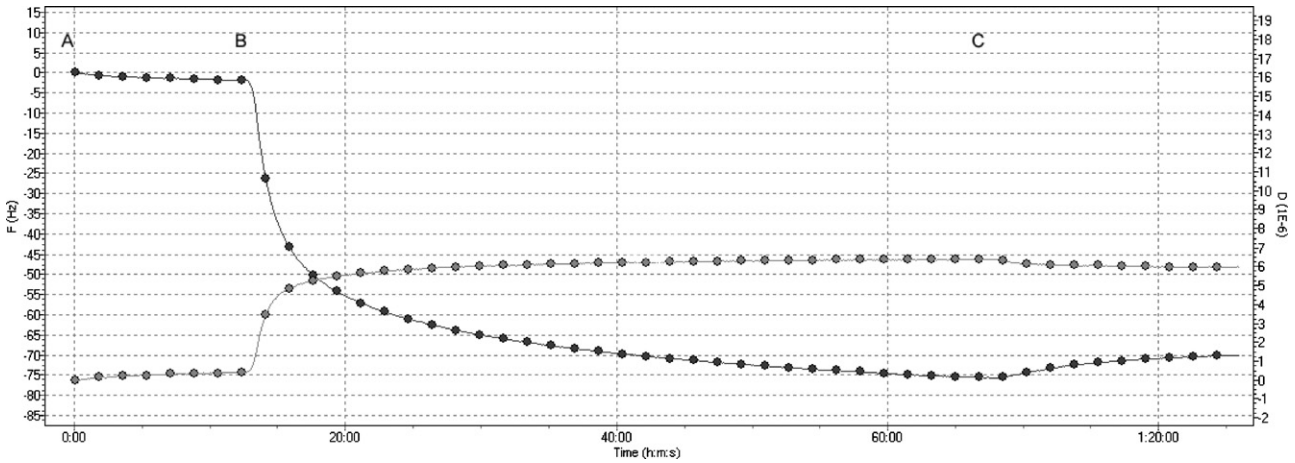

Fig. 2. Example of sensorgram for the interaction between asialofetuin and immobilised UEA lectin. Frequency (upper curve) and dissipation (lower curve) shifts as a function of time are presented: (A) WGA-modified surface; (B) injection of asialofetuin (1 mg mL^{-1}); (C) rinse with the running buffer; (D) regeneration with glycine–HCl (pH 2.5). PBS (10 mM, pH 7.4) containing 1 mM metal ions (Ca^{2+}, Mg^{2+} and Mn^{2+}) was pumped through the system at a flow rate 50 μL min^{-1}.

oscillation frequency served as analytical response and provided information about affinity properties of GPs. If no specific interaction occurs in a system two possible situations can take place. Firstly, no changes in oscillation due to glycoprotein addition will be observed. Secondly, frequency shift can be caused by the presence of bulk effects. In this case, after PBS rinse, oscillation frequency returns to the initial value corresponding to the baseline.

Specific interaction between lectins and GPs passed through the channel led to an increase in mass attached to the surface and, as a result, a decrease in oscillation frequency of the crystal. The amount of GP bound to the lectin surface was then expressed in Hz and obtained by taking the difference between the frequency signal 1 min prior to injection of GP and 1 min prior to injection of regeneration solution. The energy dissipation shift provided additional information about the viscoelastic properties of the formed layer. From the sensorgram (Fig. 2) the adsorption of GP to the lectin surface can be observed as a quick frequency drop (mass increase) followed by a slower frequency decrease as the adsorption saturates. After equilibrium in the system had been reached, the glycoprotein exposure was eventually interrupted by exchanging the protein solution in the QCM chamber for PBS solution and a slight increase in frequency could be observed due to bulk effect. Measurement of the dissipation shift indicated the formation of a soft layer of proteins on the lectin surface due to high changes in range of 1.0×10^{-6} U to 4.5×10^{-6} U. However, the softness of the formed layer induces changes only in dissipation response while the frequency is not affected by the type of GP and how it is bound to the lectin surface. Therefore, determination of a frequency shifts in combination with the lectin panel can serve as appropriate analytical tool for discrimination between various GPs by lectin-binding patterning.

GPs detection data is presented in Fig. 3. It can be seen from the diagram that the interaction between different lectins and GPs is characterised by a specific frequency shift and that each GP has its own unique lectin-binding pattern. This lectin pattern can serve as a finger print for the discrimination between various GPs. The

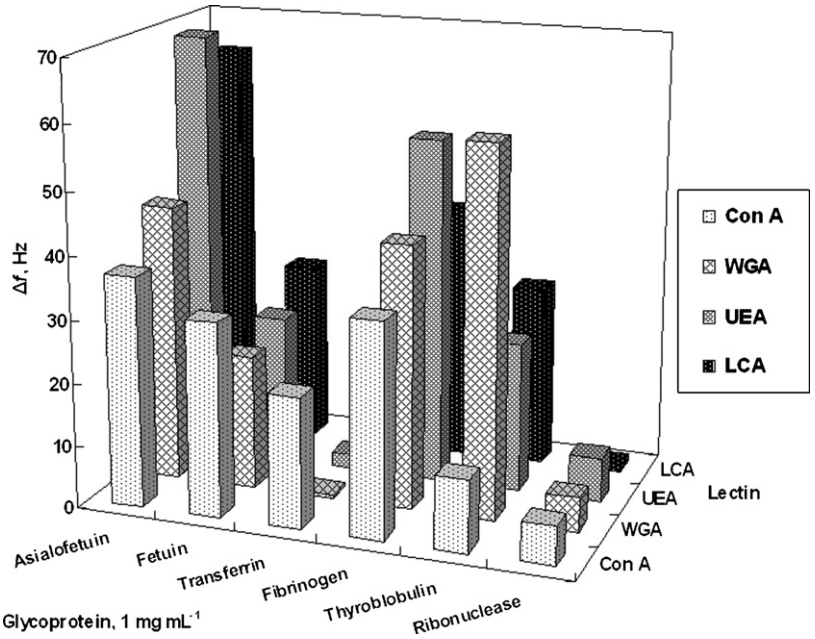

Fig. 3. Affinity of glycoproteins to different lectin surfaces (Con A, WGA, UEA, LCA). PBS (10 mM, pH 7.4) containing 1 mM metal ions (Ca^{2+}, Mg^{2+} and Mn^{2+}) was pumped through the system at a flow rate 50 μL min^{-1}. Glycoproteins (1 mg mL^{-1}) were injected for 40 min. Surfaces were regenerated with glycine–HCl (pH 2.5).

results obtained using immobilised lectins and QCM are in good correlation with the literature data. It was found that fetuin has a wider range of affinity as it can be bound by all the lectins tested. It can be explained by the fact that fetuin contains sialic acid, galactose, N-acetylglucosamine and mannose glycans attached through asparagine residues [21], and therefore all the lectins used could bind to fetuin with relatively strong affinity. Asialofetuin has a similar structure as fetuin [22]. Data obtained demonstrates that asialofetuin can be bound with the same lectins as fetuin but with a different binding pattern.

Consequently, the usage of various lectin recognition patterns makes it possible to distinguish and identify the GPs. The information about the lectin–glycoprotein interactions allows to determine not only the specificity of the lectin to a proper glycan, but also to estimate the affinity of this binding and thus the sensitivity of the detection. It has been reported that thyroglobulin contains fucose, sialic acid and mannose residues [23,24]. Present data demonstrates that this glycoprotein has affinity to mannose, fucose and sialic acid binding lectins, i.e. Con A, UEA, WGA, respectively. The primary glycan structure of bovine fibrinogen described in [25] was found by ^{1}H NMR. This glycoprotein comprises mannose and GlcNAc glycans in its structure that can be bound by mannose and sialic acid/N-acetylglucosamine binding lectins. As can be observed from Fig. 3, Con A, LCA, WGA showed strong affinity to those fibrinogen glycans.

3.3. Analytical merits of the proposed biosensor

Because GPs play a significant role in many regulation processes and are serving as signalling molecules for many pathogenic processes the quantitative analysis of GPs becomes extremely important for medical and biological purposes. The quartz crystal microbalance is known for being a technique sensitive to small mass changes on a crystal surface. Due to that ability quantitative assays of the GPs can be conducted. Typical calibration curves for QCM determination of GPs using immobilised lectins are represented in Fig. 4. All measurements were made in triplicates and R.S.D. of less than 6.0% was achieved. As seen in Fig. 4 the frequency shift after 40 min of GPs injection increased linearly from 7.8 Hz to 41.9 Hz for fibrinogen–WGA interaction; from 4.6 Hz to 40.8 Hz for fibrinogen–LCA interaction and from 9.0 Hz to 68.7 Hz for asialofetuin–UEA interaction when the concentration of each GP changes from 50 μg mL^{-1} to 1 mg mL^{-1}. Below a GP concentration of 50 μg mL^{-1} the frequency shift could not be distinguished from the background signal. The linear plot of the data provided

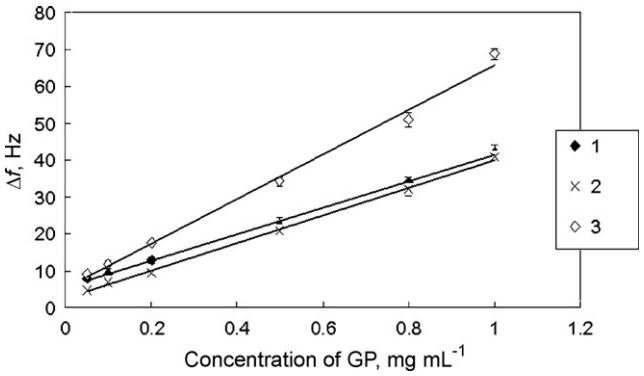

Fig. 4. Calibration curves for fibrinogen on surface modified with WGA (1), LCA (2) and calibration curve for asialofetuin on surface modified with UEA (3). PBS (10 mM, pH 7.4) containing 1 mM metal ions (Ca^{2+}, Mg^{2+} and Mn^{2+}) was pumped through the system at a flow rate 50 μL min^{-1}. Glycoproteins in concentration from 50 μg mL^{-1} to 1 mg mL^{-1} were injected for 40 min. Surfaces were regenerated with 10 mM glycine–HCl, pH 2.5.

the following correlation coefficients ($n = 3$, $R = 0.95$): $r^2 = 0.9977$, $r^2 = 0.9984$, $r^2 = 0.9935$ for fibrinogen–WGA, fibrinogen–LCA, and asialofetuin–UEA interactions, respectively. The obtained results demonstrate that using the assay based on the immobilised lectins and QCM the serum GPs can be determined within rather wide linear ranges and with reasonable sensitivity required for analysis of real samples.

3.4. Regeneration of the modified surface of the biosensor

The formation of the glycoprotein–lectin complexes on the working surface of the quartz crystal makes the further assay of glycan binding to that surface impossible. Application of an efficient regeneration procedure, however, enables reuse of the lectin surfaces thus reducing the total cost of the assay. The regeneration solution is required to remove GPs completely from the lectin surface without damaging it. Solutions with acidic or basic pH (of inorganic or organic nature), high ionic strength (inorganic salts) and some organic components (surfactants) are commonly used for the destruction of biospecific protein interactions. In this study we tested the following regeneration solutions: (1) 10 mM glycine–HCl (pH 2.5); (2) 25 mM glycine–HCl pH (2.5); (3) 100 mM glycine–HCl (pH 2.5); (4) 150 mM NaCl; (5) 1 M NaCl; (6) 0.05% sodium dodecyl sulfate (SDS); (7) 10 mM HCl; (8) 100 mM NaOH.

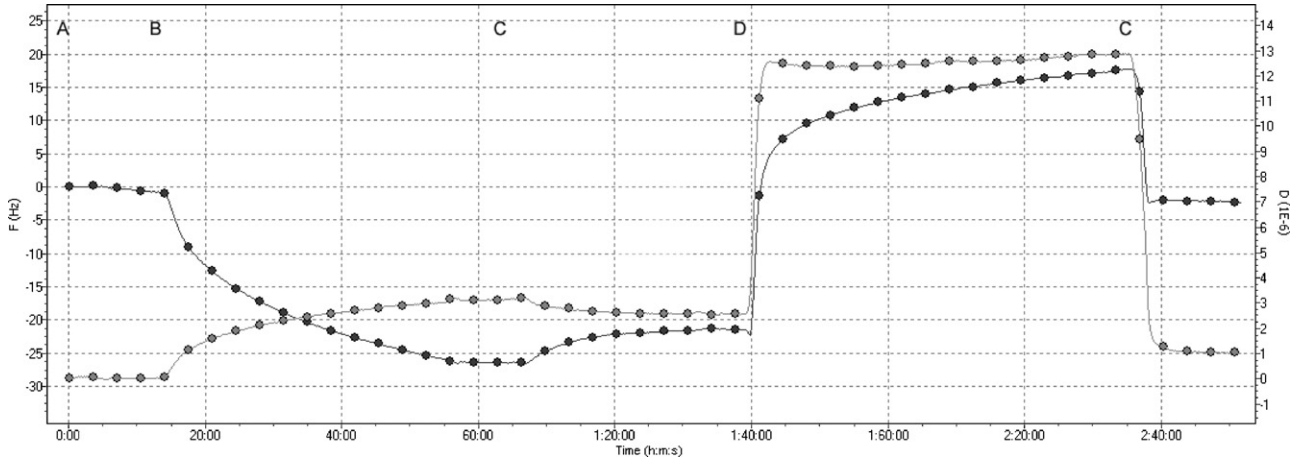

Fig. 5. Regeneration of modified surface. Frequency (upper curve) and dissipation (lower curve) shifts as a function of time are presented: (A) WGA-modified surface; (B) injection of fetuin (1 mg mL^{-1}); (C) rinse with the running buffer; (D) regeneration with glycine–HCl (pH 2.5). PBS (10 mM, pH 7.4) containing 1 mM metal ions (Ca^{2+}, Mg^{2+} and Mn^{2+}) was pumped through the system at a flow rate 50 μL min^{-1}.

It was established that the efficiency of the regeneration of the working surface depends on the affinity/strength of the GP–lectin interaction. The application of NaCl solutions as a regeneration solution was shown not to be efficient. This solution is not capable of removing the GPs from the crystal surface completely. The solution of 0.05% SDS was partly deactivating the lectins, probably due to its ability to denature the proteins. Examples of successful regenerations are shown in Fig. 5. Application of 10 mM glycine–HCl buffer (pH 2.5) followed by rinsing with PBS was the most effective procedure to remove the bound GPs. At first, it caused a disturbance of the equilibrium (increasing the oscillation frequency) that can be explained by changes of the density and viscosity of the solution used (shifting from the buffer media to aqueous solution). Then the glycine was rinsed away with continuous flow of the running buffer until a new stable baseline was obtained. Since the baseline came to its initial position (before GP addition) it was concluded that complete removal of GP from the surface had been achieved. All experiments were performed with the same panel of four lectins and no changes in analytical response was observed even after more than 100 injections onto the same surface indicating that the regeneration step does not damage the biosensing part of the system. Modified crystals were stored at 4 °C between measurements. No decrease in response was achieved during continuous use of the crystals for 2 months indicating excellent reproducibility and stability.

4. Conclusions

A preliminary study of lectin–glycoprotein interactions using different immobilised lectins and quartz crystal microbalance technique has been carried out. The frequency shift was used as analytical signal for qualitative and quantitative analysis of the glycoprotein adsorption to the various lectin surfaces. Dissipation shifts were measured and provided additional information about the viscoelastic properties of the formed layer. The lectin-based quartz crystal microbalance biosensor allows for a convenient, economical flow injection procedure for rapid screening and selective and specific assay of serum GPs based on lectin patterning. The possibilities for further developments and applications of the technique for monitoring and control of serum protein glycosylation status look very promising.

The results obtained indicate that analysis of the binding pattern already with the small number of lectins used here would have sufficient discriminatory power to detect changes in GP distribution and for monitoring of protein glycosylation processes. However, by investigating a larger variety of lectins and GPs at different glycosylation stages it will be possible to create libraries of lectin binding patterns for more selective and accurate analysis, which could serve as a good alternative to the time-consuming and complicated techniques generally involved in carbohydrate analysis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.aca.2009.12.004.

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