Carboxybetaine Modified Interface for Electrochemical Glycoprofiling of Antibodies Isolated from Human Serum

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Supporting Information

ABSTRACT: Impedimetric lectin biosensors capable of recognizing two different carbohydrates (galactose and sialic acid) in glycans attached to antibodies isolated from human serum were prepared. The first step entailed the modification of a gold surface by a self-assembled monolayer (SAM) deposited from a solution containing a carboxybetaine-terminated thiol applied to the subsequent covalent immobilization of lectins and to resist nonspecific protein adsorption. In the next step, Sambucus nigra agglutinin (SNA) or Ricinus communis agglutinin (RCA) was covalently attached to the SAM, and the whole process of building a bioreceptor layer was optimized and characterized using a diverse range of techniques including electrochemical impedance spectroscopy, cyclic voltammetry, quartz crystal microbalance, contact angle measurements, zeta-potential assays, X-ray photoelectron spectroscopy, and atomic force microscopy. In addition, the application of the SNA-based lectin biosensor in the glycoprofiling of antibodies isolated from the human sera of healthy individuals and of patients suffering from rheumatoid arthritis (RA) was successfully validated using an SNA-based lectin microarray. The results showed that the SNA lectin, in particular, is capable of discriminating between the antibodies isolated from healthy individuals and those from RA patients based on changes in the amount of sialic acid present in the antibodies. In addition, the results obtained by the application of RCA and SNA biosensors indicate that the abundance of galactose and sialic acid in antibodies isolated from healthy individuals is age-related.

1. INTRODUCTION

Glycosylation is the most common co- and post-translational modification of proteins; it may be estimated that approximately 70% of cytosolic and 80% of membrane-bound human proteins are glycosylated.1,2 Glycans play an important role in many different processes (e.g., viral infection, cancer development, cell-signaling and adhesion, proper functioning of an immune system), as they enhance the solubility and stability of many proteins but may also determine the function of proteins.3–10 The presence/absence of a single carbohydrate within a glycan structure can significantly influence the function of proteins. The addition of a single molecule of sialic acid (N-acetylneuraminic acid) to the glycan present in the Fc fragment of immunoglobulin (IgG), thereby changing the protein from being a pro-inflammatory to an anti-inflammatory agent, can serve as a good example.11,12 Although over 70% of all therapeutic proteins are glycoproteins,13 the first glyco-engineered therapeutic antibody was only launched as recently as in 2012;14 today significant focus is directed toward controlling the glycosylation of therapeutics.15

Received: March 13, 2015
Revised: May 22, 2015
Published: June 5, 2015
Changes in the glycan structure in selected glycoprotein biomarkers are often the result of a disease progression, but may also be associated with aging. In the case of autoimmune diseases (e.g., rheumatoid arthritis, RA), where the antibodies present in human plasma are produced against the host’s own tissues, their glycan composition is changed. Detailed analysis of glycans is only made possible by using a battery of instruments, but lectins (natural glycan decipherers) interacting with glycans attached to intact proteins can provide functional information about glycans. Lectins are (glyco-)proteins selectively binding some glycan residues or simple mono- or oligosaccharide structures present in more complex glycans. These molecules are potentially more useful in the search for new biomarkers in complex biological samples than the standard immunochemical protocols because, in lectin-based applications, prior knowledge of a biomarker’s identity or its structure is not required. For glycoocode-deciphering, lectins are often applied as an effective tool in many clinical diagnostic procedures, mostly in the microarray analysis, affording an extremely high throughput, simplicity, and reproducibility of assays.

Faradaic electrochemical impedance spectroscopy (EIS) is a powerful tool in bioanalysis, because it enables the determination of analytes down to the aM concentration range. The method is based on a small sinusoidal perturbation of a thin conductive or semiconductive surface layer using a low amplitude voltage; for a spectrum of different frequencies, it provides information about surface capacitance, solution, and charge-transfer resistance. Moreover, it represents a label-free detection method, hence reducing the negative effect of labeling on a biorecognition event and lectin structure. It has been successfully used for the detection of a variety of molecules, viruses, or even whole bacterial or eukaryotic cells in different configurations. Most recently, some new variations of this method have been presented, that is, impedance-derived electrochemical capacitance spectroscopy for the evaluation of lectin-glycoprotein binding affinity and immittance electroanalysis.

It is of the utmost importance to control nonspecific protein-binding, especially when using biosensor devices based on label-free detection platforms. While molecules bearing oligoethylene glycol (OEG) moieties have been successfully applied to resist nonspecific protein binding in the past, zwitterionic molecules that bind water molecules more strongly than OEG-based molecules, thus exhibiting greater repulsive force against protein adsorption, are becoming more widely used. Oligo- and poly-zwitterionic molecules attached to various interfaces can be used in a number of applications. In addition to the ability to resist nonspecific protein binding, zwitterionic polymers can provide nonbiofouling surfaces (resisting microbial adhesion), exhibiting bactericidal effects beneficial for wound-healing or the prolonged blood circulation of polymer-modified particles. Poly- and oligo-zwitterionic molecules are not ideal for the construction of impedimetric biosensors, because the interfacial layer of such devices has to be built up so as to produce a moderate initial charge-transfer resistance. Hence, in a previous study, thiolated sulphobetaine (SB) with only one zwitterionic unit was applied to the construction of an EIS lectin-based biosensor with the subsequent glycoprofiling of serum samples. Resistance to the adsorption of nonspecific proteins was guaranteed by the formation of a mixed SAM composed of 11-mercaptopentadecanoic acid (MUA, for covalent lectin immobilization) and SB (resisting nonspecific interactions and being a MUA diluent). The problematic mixing of these two thiols (SB dissolved in water and MUA in ethanol) resulted in the preparation of interfacial layers, which affected the performance of such a device. The present study’s first aim was to increase the reproducibility of the biosensor preparation by the application of a carboxybetaine-containing thiol (CB, applicable for covalent lectin immobilization and resisting nonspecific protein binding) to form a one-component SAM with high reproducibility. The second aim was to separate antibodies (i.e., IgG’s, Figure 1) from human serum from healthy individuals and RA patients to increase the sensitivity of glycoprofiling. It should be noted that a short aliphatic thiol bearing a carboxybetaine moiety was recently applied to gold patterning together with a boronate-containing thiol recognizing fructose for the label-free detection of this analyte.

2. MATERIALS AND METHODS

2.1. Chemicals. Potassium hexacyanoferrate(III) (ferrocyanide), potassium hexacyanoferrate(II) trihydrate (ferrocyanide), potassium chloride, phosphate buffer saline tablets, sulfuric acid, sodium hydroxide, N-hydroxyquinoline (NHS), N-([3-(dimethylamino)propyl])-N-ethylenediamine hydrochloride (EDC), Ricinus communis agglutinin (RCA, recognizing galactose, caution: handle with special care because it is a biological toxin), fetuin (FET, contains 8.7% of sialic acid), and asialofetuin (ASF, contains ≤0.5% of sialic acid) were purchased from Sigma-Aldrich (U.S.). Sambucus nigra agglutinin type I (SNA, 7148

DOI: 10.1021/acs.langmuir.3b00944
Langmuir 2015, 31, 7148–7157

Figure 1. Structure of human immunoglobulin G (IgG) (pdb code 1MCO) with (a) visible glycan moieties in the cavity created by two heavy chains in Fc fragment and (b) lateral view of the same molecule showing availability of two glycan chains for lectin biorecognition (red arrows).
Article

Scheme 1. Synthesis of Carboxybetaine-Containing Thiol Derivative 1

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recognizing sialic acid) lectin from \textit{Sambucus nigra} was purchased from EY Labs (U.S.). Ethanol for UV/vis spectroscopy (ultrapure) was purchased from Slavus (Slovakia). Biotinylated lectins and a carbo-free derivative of 100 mM NaOH under anaerobic conditions (100 scans from ~500 to ~1500 mV at a scan rate of 100 mV s\(^{-1}\)) and then the electrodes were polished mechanically using a polishing pad and alumina slurries (particle size 1 and 0.3 µm, each for 5 min) and left after short sonication in a hot piranha solution for 20 min (H\(_2\)O\(_2\) and H\(_2\)SO\(_4\) at 1:3 ratio, caution: handle with special care). The viscosity and permittivity of the electrolyte were calculated on the basis of the temperature using a calibration curve.

2.6. Electrochemical Impedance Spectroscopy (EIS) Measurements. On an electrode modified by a SAM, the biorecognition elements (SNA and RCA lectins) were immobilized using standard amine coupling with carboxylic groups of CB being activated by a 1 + 1 mixture of 0.2 M EDC in DW and 0.05 M NHS in DW for 10 min (Scheme 2). The lectins were covalently immobilized on the activated SAM layer from a 40 µL stock solution (1 mg mL\(^{-1}\) in PBS). All electrochemical impedance spectroscopy (EIS) measurements were performed in a filtered electrolyte containing 5 mM ferricyanide, 5 mM ferrocyanide, and 0.1 M KCl. The analysis was conducted at 50 different frequencies (ranging from 0.1 Hz to 100 kHz) using Nova Software 1.10 (Ecochemie, The Netherlands). The data acquired were evaluated by the same software using a Nyquist plot with a Randles–Elschner equivalent circuit \(R(C|RW)|\) employed. The change in charge-transfer resistance \(R_{\text{CT}}\) relative to a reference surface (a biosensor surface after the lectin immobilization and stabilization in a sterile 100 mM KCI solution for at least 15 min) expressed in % was used as a measurement signal. Each analyte/sample was measured in triplicate at least, using an independent biosensor device, and the results are shown with a standard deviation (±SD) calculated in Excel. Human samples were diluted in a sterile and filtered 10 mM PBS buffer, pH 7.4 containing chloride ions (tablets) to prevent any significant changes in ionic strength during the experiment. The analysis of each sample lasted for 40 min including incubation of the sample with the biosensor device for 20 min, and then the biosensor was incubated for 10 min in a sterile 100 mM KCI solution.
with a subsequent impedimetric analysis conducted for an additional 10 min. All stock solutions (lectins, standard glycoproteins, and human samples) were stored at −20 °C in aliquots for periods not exceeding 1 month.

### 2.7. Atomic Force Microscopy (AFM) Measurements

Peak force tapping mode atomic force microscopy (Scan Asyst, Bruker, U.S.) in air mode was carried out on a Bioscope Catalyst instrument and Olympus IX71 microscope in conjunction with NanoScope 8.15 software using ScanAsyst in air mode. Square-shaped gold chips (10 × 10 mm²), provided by the Institute of Electrical Engineering, Slovak Academy of Sciences; see section 2.3) modified as previously described for the planar gold electrodes were scanned using a SCANASYST-AIR silicon tip on a nitride lever (Bruker, U.S., with a contact angle of 63.5°, sharpened to a tip radius of 2 nm).

### 2.8. Quartz Crystal Microbalance (QCM) Measurements

All QCM measurements were performed using Autolab PGSTAT 128N (Ecochemie, The Netherlands) equipment using an EQCM module. The changes per mass were evaluated using Sauerbrey’s equation:

\[
\Delta f = -\frac{2f_0}{\sqrt{\rho q}} \Delta m
\]

where \(\Delta f\) is the frequency change (Hz), \(f_0\) is the nominal resonant frequency of the crystal (6 MHz), \(\Delta m\) is the change in mass (g cm⁻²) and \(\mu_s\) is the shear modulus of a quartz crystal (g cm⁻¹ s⁻²), \(A\) is the surface area, and \(\rho_q\) is the density of quartz in g mL⁻¹. For a 6 MHz crystal, the whole equation can be simplified to

\[
\Delta f = -C_i \Delta m
\]

where \(C_i\) is the frequency constant 0.0815 Hz ng⁻¹.

### 3. RESULTS AND DISCUSSION

#### 3.1. SAM Layer Formation and Characterization

**Contact Angle Measurements.** The bare Au surface (after the piranha cleaning procedure for a few minutes) exhibited a moderate level of hydrophobicity/hydrophilicity with a contact angle of 63.5°, a value comparable to the values of 70°⁴⁶ and 62°⁵⁴ obtained in previous studies. After formation of the CB monolayer on the Au surface, the hydrophilicity of an interface increased, as can be observed from the contact angle of 45° obtained on such a modified gold surface. This contact angle value is in agreement with the value of 34° obtained on a poly(carboxybetaine methacrylate)-modified surface⁶⁸ and within the contact angles of 33°–53° obtained on various poly(carboxybetaine methacrylate)-modified surfaces.⁷⁹

**AFM Measurements.** Monitoring of the differences in surface topology between the bare Au chips and the same chips modified with a CB monolayer using AFM exhibited no difference in surface roughness ((0.37 ± 0.18) nm for bare Au and (0.39 ± 0.12) nm for a CB SAM-modified surface, respectively; p > 0.05 for at least three different samples), suggesting that the topology of the SAM copied the surface topology of the bare gold surface, creating a uniform and dense layer.

**Zeta (ζ) Potential Measurements.** Zeta potential measurements at pH 7 revealed that, upon formation of CB SAM, the value of the ζ potential of the bare Au surface decreased from −80 to −120 mV. Note that the zeta potential values are very dependent on pH value and overall conductivity of the substrate surface and partially dependent on shape and structure of the substrate and zeta potential assay method. These values are comparable to the ζ potential of a similar system where the value for carboxylic acid-SAM was determined as −100 mV at pH 7.⁴⁰ Other studies refer to the ζ potential value of (−187 ± 7) mV for carboxylic acid-terminated SAMs.⁴¹,⁴² Moreover, mixed positive/negative charged SAMs have isoelectric point shifted to pH lower than η.⁵⁰ Even zwitterionic SAM on the gold solid surface might have significantly negative ζ potential value at pH 7.

All of these measurements together with XPS data on SAM (see Supporting Information) are consistent with the successful formation of CB SAM on a bare gold surface. Accordingly, the
CB-modified gold surface was ready for the subsequent activation of −COOH groups of CB for covalent immobilization of two different lectins, RCA and SNA.

### 3.2. Optimization of Activation Time Using EDC/NHS Chemistry

Because the CB SAM does not contain any diluting thiol, the density of the −COOH groups present in the SAM is too high, and the only way to control the density of the immobilized lectin is to optimize the time of activation by EDC/NHS. The optimal time of activation of the −COOH groups by EDC/NHS was followed by QCM (obtaining surface lectin density) and AFM (reading surface roughness). The results obtained from QCM and AFM consistently showed that the highest lectin density was obtained with an activation time of 10 min (Figure 2).

![Figure 2. Correlation between surface density of immobilized SNA lectin (black) and surface roughness of the surface with immobilized SNA expressed as Rq (red) obtained from QCM and AFM experiments, respectively. SNA lectin was immobilized from 1 mg mL⁻¹ stock solution in 10 mM PBS.](image)

The lectin surface coverage increased from a value of 4.2 pmol cm⁻² to a value of 6.2 pmol cm⁻² when the activation time was increased from 5 to 10 min. It is worth noting that the lectin surface coverage decreased to a value of 3.7 pmol cm⁻² when the surface confined −COOH groups were activated for 15 min. This low lectin surface coverage of 3.7 pmol cm⁻² can be explained by hydrolysis of the activated esters in an aqueous solution during incubation for 15 min, resulting in a decreased density of activated esters available for covalent lectin immobilization. The AFM experiments revealed a similar pattern, that is, an increased surface roughness Rq from a value of 0.39 nm (without activation, i.e., CB SAM) to a value of 0.96 nm (5 min activation) and further to a value of 1.4 nm (10 min activation) (Figure 3). Finally, a decrease to a value of 1.2 was observed when the surface was activated for 15 min (Figure 3).

On the basis of these results, a 10 min activation time was selected for the covalent immobilization of lectins in subsequent experiments.

### 3.3. EIS Characterization and Measurements

First, the charge-transfer resistance (R_CT) of the prepared surfaces, corresponding to a diameter in a semicircle region of a Nyquist plot, was investigated (Figure 4A). The bare Au electrodes exhibited an average initial R_CT value of 87 Ω. However, the R_CT value only shifted slightly to a value of (98 ± 4) Ω after incubation with a CB solution. After SNA lectin immobilization, R_CT increased to a value of (620 ± 10) Ω. A sharp increase in R_CT to a value of (2700 ± 640) Ω was observed upon incubation of the biosensor with IgG analyte (Figures 4A and 5). The cyclic voltammetry measurements (Figure 4B) are consistent with the EIS investigation, showing that immobilization of an SNA lectin on CB SAM-modified gold formed a quite effective barrier, resulting in a decreased reversibility of the ferriyanide electrochemistry with ΔE_p = 134 mV in comparison with ΔE_p = 94 mV observed on a CB SAM-modified Au surface.

### 3.4. Calibration of EIS Lectin Biosensor and Analysis of Real Biological Samples

Calibration of the EIS lectin biosensor was performed only with the SNA lectin immobilized, because the limit of detection and working concentration ranges for the SNA- and RCA-based lectin biosensors were found to be similar in a previous study. ASF containing a much lower amount of sialic acid than in FET can also be detected, but with a slightly lower sensitivity ((21.0 ± 1.5)% for ASF vs (30.8 ± 1.1)% for FET). For every calibration curve constructed (in Figure 6), an average SD was calculated. As noise for the measurement for a particular analyte, 3× average SD was calculated. In the subsequent step, the concentration of both analytes (FET and ASF) for both methods of analysis (EIS and MA) above this noise level was found, and this value is presented as the limit of detection (LOD). For EIS measurements, the LOD for FET was 3.5 × 10⁻⁶ mg mL⁻¹ (73 pM) and for ASF was 1.3 × 10⁻⁴ mg mL⁻¹ (2.7 nM). For the MA assays, the LOD for FET was 0.015 mg mL⁻¹ (310 nM) and for ASF was 0.020 mg mL⁻¹ (420 nM). The lectin-based microarray affords an LOD approximately 4 orders of magnitude higher than the EIS lectin-based biosensor for the main analyte FET (Figure 6). A much lower limit of detection for EIS than for MA was also observed in the previous study. It is worth noting that the SD in Figure 6 for EIS is not the SD of the assay but rather the SD of a biosensor preparation, because every calibration or sample measurement by EIS was performed with an independently prepared biosensor device. The LOD of the impedimetric SNA biosensor based on CB is 73 pM, a value much higher than the value of 24 fM (a value recalculated from ref 41) for the impedimetric SNA biosensor based on a mixed SAM composed of MUA and SB. This difference might be the result of a larger initial R_CT of the interface of the SNA biosensor previously published (32 kΩ) as compared to this study (620 Ω), indicating that a moderate initial R_CT is essential to obtain a high level of detection for impedimetric devices, as discussed previously. Moreover, the impedimetric SNA biosensor based on a mixed SAM composed of MUA and SB was more resistant to nonspecific protein binding (6.1% of the specific response), in comparison with the 30% of nonspecific response (using human serum albumin) observed for the current SNA biosensor (data not shown). The beneficial features of the current SNA biosensor over that previously published based on MUA and SB are the higher reproducibility of the biosensor construction (average RSD of 8.9% vs 16.1%), higher sensitivity of analysis (30.8% M⁻¹ vs 14.6% M⁻¹), and a more reliable calibration curve expressed as R² (0.993 vs 0.954). The increase in initial R_CT needed to render the EIS-based lectin biosensor more sensitive and robust can be achieved by blocking the biosensor surface with bovine serum albumin as previously proposed for suspended microchannel resonators; this blocking procedure made the analysis of analytes in serum reliable.

Suitable data for comparing the analytical performance of the proposed impedimetric biosensor with other glycoprofiling methods are not easy to find, but a lectin-based analogy of ELISA can offer an LOD down to the pM level with a linear range (LR) spanning 1 order of concentration magnitude; the lowest LOD for EIS-based biosensors is in aM–fM range with...
the LR spanning 3—9 orders of magnitude, and an LOD of 67 nM for SPR and QCM lectin-based methods with LR of 1—2 orders of magnitude was demonstrated. Label-based lectin biosensors offer an LOD down to 3 pM with the LR spanning 3 orders of magnitude. The main advantage of lectin-based glycoprofiling is the ability to analyze even intact cells (i.e., cancerous cells) without any treatment. Instrumental techniques make it possible to glycoprofile samples down to...
the attomolar–femtomolar level with a few microliters of the sample (\(\sim\) pM–nM level) with a linear range spanning 2–3 orders of magnitude.\(^{16,54,55}\)

Because the second aim of this study was to apply developed EIS-based lectin biosensors to the glycoprofiling of isolated IgG’s (antibodies), the analysis of which is more relevant to studying the progression of the RA disease than glycoprofiling a whole serum sample, affinity columns were applied to isolate IgG’s (antibodies) from the serum samples. A simple comparison of the relative output signal (i.e., \(\Delta R_{CT}\) divided by the total protein content) revealed an enhancement of the signal 65.6-fold (slope of 25.6 ± 1.2 vs 0.39 ± 0.01) (Figure 7), a feature important for the enhanced reliability of the analysis. In the subsequent experiment, the nonfouling properties of the CB SAM were tested. Figure 7 shows that the calibration curve for isolated IgG is affected by a large SD (average RSD of 22.6% as compared to average RSD of 8.9% for standard glycoprotein FET), which might be explained by the presence of small components within the serum depleted from highly abundant proteins. In forthcoming studies, other methods will be applied to purify the IgG fraction (i.e., using protein A/G columns). The LOD of 3.2 nM calculated for isolated IgG was quite high due to the large SD of the assays.

A gold surface patterned only by CB SAM was exposed to IgG’s isolated from human serum with different dilutions to probe nonspecific interactions. While only a negligible nonspecific signal was observed at dilutions 10 000x and 1000x the isolated IgG’s, when the same concentration of IgG’s was applied to the SNA-based biosensor, a good and reliable biospecific response was obtained (Figure 8). Incubation of the IgG’s with the CB SAM at a dilution of 100x resulted in a significant relative response of 10.6% as compared to a biospecific relative response of 63.8%. Accordingly, in subsequent experiments, isolated IgG’s diluted 1000x were applied to glycoprofiling.

The final part of this study is the application of RCA- and SNA-based biosensors to the glycoprofiling of IgG’s isolated from the serum of healthy individuals and of those suffering from the RA disease. In addition, a pool of samples from healthy individuals was divided into two batches by the age of these individuals. The results presented in Figure 9 show that the RCA lectin can hardly distinguish between the IgG’s isolated from healthy individuals and those suffering from RA (slope of 57.6 ± 2.0 vs 47.7 ± 1.6) at different dilutions of IgG’s. Interestingly, the glycoprofiling of IgG’s from young healthy individuals exhibited a larger signal...
The glycoprofiling of isolated IgG’s by the SNA-based biosensor revealed larger differences among all three types of samples in the present study as compared to the output obtained by the RCA-based biosensor. While a moderate difference was observed in the sialic acid content in the IgG’s isolated from the serum of healthy older individuals and RA patients (slope of $10.5 \pm 1.4$ vs $7.6 \pm 1.6$), the sialic acid on the IgG’s isolated from young healthy individuals was much higher (slope of $36.4 \pm 2.1$) than in the above two groups. In the most recent studies, it is suggested that altered glycosylation might be age-related, because glycan synthesis is influenced by the activity of two different types of enzymes, that is, anabolic (glycosyltransferases) and catabolic (exoglycosidases) enzymes. Age-driven changes in the enzymatic activities of these two types of glycan-processing enzymes thus result in an altered glycan synthesis. The present results indicate that age-related changes in the glycan profile should be taken into account to make glycoprofiling more reliable for future diagnostics of various diseases.

IgG’s from all three types of human serum samples were also glycoprofiled with SNA lectin in a microarray analysis. The results showed that the EIS-based biosensor was capable of providing reliable data, because validation of the biosensor by a widely applied lectin microarray method was in good agreement with $R^2 = 0.990$ (Figure 10). The large error bars shown in Figure 10 are due to the analysis of several samples with each of the three categories (RA, healthy older, healthy young) naturally differing in the level of IgG and glycosylation.

Extensive recent strategies aimed at discovering novel biomarkers have revealed that to have just a single biomarker for a particular disease is proving quite elusive and that, for the sensitive and specific diagnosis of different diseases, a panel of biomarkers is required. As well as the frequent application of several genetic and protein biomarkers, it is proposed that glycoprofiling will be an important biomarker for inclusion in the panel of biomarkers for future disease diagnostics.

4. CONCLUSIONS
The study investigated the preparation of a biointerface for an easy, reproducible, robust, and sensitive impedimetric detection of two carbohydrate residues (galactose and sialic acid) present on the IgG molecules isolated from the human sera of healthy individuals and patients with RA. Because the biological signal for the assay derives from an affinity biorecognition event, it is extremely important to adequately block nonspecific protein adsorption on the surface. For this purpose, a new CB derivative was synthesized, because zwitterionic materials are known to offer good properties for resisting nonspecific protein binding. By a simple chemical modification of gold electrode surfaces by SAMs composed of a newly synthesized thiol (CB), it was possible to prepare a highly sensitive impedimetric lectin-based electrochemical assay. In addition, the lectin-based biosensors were applied to the analysis of real human samples (IgG’s isolated from human sera with glycans still attached to the Fc fragment of IgG), and the SNA-based biosensor was able to distinguish between patients suffering from RA and healthy individuals. Furthermore, the study also revealed that the desialylation and degalactosylation of human IgG’s might be an age-related process, which should be taken into account for these kinds of analyses in future works. To enhance the robustness of glycoprofiling by the impedimetric lectin biosensor based on CB, a more efficient blocking of the interface is needed, which can result in a decreased LOD with increased specificity of analysis. The analysis of one sample can be made considerably less time-consuming by integration of the electrodes into an array format of analysis. Furthermore, improved purification of the antibodies from human serum is needed for overall enhancement of IgG glycoprofiling.
ASSOCIATED CONTENT

Supporting Information

Synthesis together with the characterization of CB and XPS spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.langmuir.5b00944.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Dr Sardar Ali, GPC Qatar University, for carrying out XPS analysis. Financial support received from the Slovak Scientific Grant Agency VEGA 2/0162/14 and from the Slovak Research and Development Agency APVV 0282-11 is acknowledged. The research leading to these results received funding from the European Research Council under the European Union’s Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement no. 311532. This publication was made possible by NPRP grant no. 6-381-1-078 from the Qatar National Research Fund (a member of the Qatar Foundation). The statements made herein are solely the responsibility of the authors.

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