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Glycoprofiling as a novel tool in serological assays of systemic sclerosis: A comparative study with three bioanalytical methods

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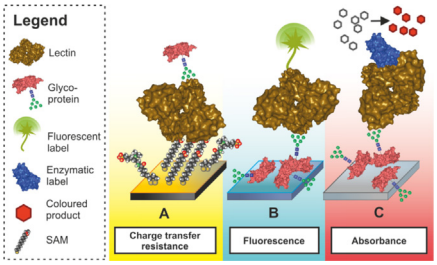
HIGHLIGHTS

- Assays of systemic sclerosis (SSc) in human serum by three bioanalytical methods.
- Lectin-based glycoprofiling using electrochemical impedance spectroscopy (EIS), microarrays and ELISA-like approach.
- Ultrasensitive glycoprofiling by EIS with LOD down to 10 aM.
- Increased level of sialic acid-containing glycoproteins in control sample compared to SSc samples.
- The first direct glycoprofiling assays of SSc in human serum.

GRAPHICAL ABSTRACT

Glycoprofiling of human serum by: (A) lectin-based electrochemical impedance spectroscopy, (B) microarray with fluorescently labeled lectin and (C) enzyme-linked lectin assay (ELLA) with enzyme-labeled lectin.



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ABSTRACT

Systemic sclerosis (SSc) is an autoimmune disease seriously affecting patient's quality of life. The heterogeneity of the disease also means that identification and subsequent validation of biomarkers of the disease is quite challenging. A fully validated single biomarker for diagnosis, prognosis, disease activity and assessment of response to therapy is not yet available. The main aim of this study was to apply an alternative assay protocol to the immunoassay-based analysis of this disease by employment of sialic acid recognizing lectin *Sambucus nigra* agglutinin (SNA) to glycoprofile serum samples. To our best knowledge this is the first study describing direct lectin-based glycoprofiling of serum SSc samples. Three different analytical methods for glycoprofiling of serum samples relying on application of lectins are compared here from a bioanalytical point of view including traditional ELISA-like lectin-based method (ELLA), novel fluorescent lectin microarrays and ultrasensitive impedimetric lectin biosensors. Results obtained by all three bioanalytical methods consistently showed differences in the level of sialic acid present on glycoproteins, when serum from healthy people was compared to the one from patients

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having SSc. Thus, analysis of sialic acid content in human serum could be of a diagnostic value for future detection of SSc, but further work is needed to enhance selectivity of assays for example by glycoprofiling of a fraction of human serum enriched in antibodies for individual diagnostics.

1. Introduction

Systemic sclerosis (SSc) is a chronic and progressive autoimmune disease of a connective tissue and can affect virtually every organ in the body [1–4]. Total survival time of patients is shortened with a mortality rate of 15% after 10 years due to heart failure and renal insufficiency. Initial classification of patients was done using American College of Rheumatology preliminary criteria developed in 1980 [5], but over time it was obvious that such classification was not well-suited for identification of early stage of the disease in SSc patients. Analysis of serum autoantibodies might identify the early stage of the disease, but the level of autoantibodies in individual patients can vary significantly [6]. The heterogeneity of the disease also means that identification and subsequent validation of biomarkers of the disease is quite challenging and the situation might be even more complicated since SSc patients might have other autoimmune diseases [7].

Despite significant progress in searching for prospective disease biomarkers by an increase in the number of published SSc biomarker studies, a fully validated single biomarker for diagnosis, prognosis, disease activity and assessment of response to therapy is not yet available [8]. Different techniques such as protein microarrays [9], peptide aptamer microarrays [10], optical biosensor [11] and ELISA [12] proved to be promising approaches for finding/analysis of protein(autoantibody)-based biomarkers of SSc. Recent studies suggest that microRNAs [13] and glycans might play an important role in the disease as judged from presence of anti-glycan antibodies [14] or other glycan-recognizing proteins (Siglecs) [15] in samples from SSc patients. Other autoimmune diseases are emerging at an increasing speed due to combination of genetic and environmental factors. At the same time all these factors together result in the dysregulation of synthesis of glycans stimulating immune system [16]. An increasing number of studies prove that protein glycosylation plays an important role in regulation of immune responses and in the development of autoimmune diseases, as well [17].

Recent advances in material chemistry, especially with introduction of various nanomaterials, together with launching of novel transducing protocols and biorecognition elements are behind a huge development in ultrasensitive and multiplexed analysis of a wide range of disease biomarkers [18–23]. It is well-known that presence of labels can negatively affect binding [24,25] and thus label-free methods of analysis are preferential option for diagnostic purposes. One of the most sensitive biosensing detection platform is electrochemical impedance spectroscopy (EIS), offering robust, extremely sensitive method for monitoring of biorecognition events in a label-free mode of operation [19,26–28]. This method is based on the application of small amplitude alternating voltage (perturbation) to the interfacial layer on the electrode, measuring the double layer capacitance (C_{DL}) and charge transfer resistance (R_{CT}), respectively [29–31]. Moreover, recently we developed reliable EIS assay for detection of glycoproteins directly in human serum with low level of non-specific binding [28].

In this study, we present a novel approach for analysis of a systemic sclerosis disease by detection of glycan moieties on proteins in diluted sera without any further pre-treatment using SNA lectin recognizing terminal sialic acid of a glycan moiety on glycoproteins. Moreover, in this study three different bioanalytical approaches for glycoprofiling of human sera are described, including traditional ELISA-like method enzyme-linked lectin assay (ELLA), novel multiplexed lectin microarray format of analysis and ultrasensitive EIS-based biosensing with immobilized lectins. Since glycosylation is the most common posttranslational modification of proteins (≥50% of all eukaryotic proteins and ≥70% of all therapeutic proteins) [19,32] a huge scientific effort is devoted to better understanding of their role in cell processes or to apply such knowledge to design better therapeutics or diagnostic tools [33–40]. Thus, glycoprofiling of samples from SSc patients can be an alternative way for future early stage diagnostics of the SSc disease and other diseases including various types of cancer associated with aberrant glycosylation.

2. Experimental

2.1. Materials

11-Mercaptoundecanoic acid (MUA), potassium hexacyanoferrate(III), potassium hexacyanoferrate(II) trihydrate, sodium chloride, potassium chloride, potassium phosphate monobasic, potassium phosphate dibasic, 1,3-propanesultone, *N*-hydroxysuccinimide (NHS), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), sodium sulfate, *N*,*N*-dimethylethylene diamine, dicyclohexylcarbodiimide (DCC), ethanolamine hydrochloride, dichloromethane, tetrahydrofuran (THF), Tween 20, *Ricinus communis* agglutinin (RCA recognizing galactose, caution: it is a biological toxin), concanavalin A (Con A recognizing mannose and glucose), anti-BSA (bovine serum albumin) antibody, polyvinylalcohol (PVA) and protein A from *Staphylococcus aureus* were purchased from Sigma–Aldrich (USA). (R)-Lipoic acid was purchased from TCI Europe. *Sambucus nigra* agglutinin (SNA recognizing sialic acid) lectin from *S. nigra* was purchased from EYLabs (USA). Ethanol for UV/Vis spectroscopy (ultrapure) was purchased from Slavus (Slovakia). Phosphate buffer saline tablets (PBST) were from Merck (Slovakia). Eight biotinylated lectins *Aleuria aurantia* lectin (AAL), *Lens culinaris* agglutinin (LCA), *Maackia amurensis* lectin (MAL), *Phaseolus vulgaris* agglutinin (PHAE), *R. communis* agglutinin and *S. nigra* agglutinin, concanavalin A and wheat-germ agglutinin (WGA); and avidin-peroxidase (AV-HRP) were purchased from Vector Laboratories (USA). CF555-streptavidin fluorescent label was purchased from Biotium (USA). Sulfobetaine ((R)-3-((2-(5-(1,2-dithiolan-3-yl)-pentanamido) ethyl) dimethylammonio)-propane-1-sulfonate (DPS) was synthesized according to a previously published protocol [28].

2.2. Apparatus

Electrochemical investigation was performed on a potentiostat PGSTAT 128N (Ecochemie, Netherlands) run under Nova Software 1.10 (Ecochemie, Netherlands) in a three electrode cell system, using modified gold electrode, auxiliary Pt and reference Ag/AgCl electrode.

Lectin microarray was prepared by spotting samples using SpotBot3 Microarray Protein edition (Arrayit, USA) on epoxide-coated slides Nexterion E (Schott, Germany), which were scanned after experiment using InnoScan710 scanner (Innopsys, France) at a wavelength of 532 nm. The slide image was evaluated using the software Mapix 5.5.0 by evaluation of the intensity of fluorescence and intensity of all independent array spots on the array.