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Label-free method for anti-glucopeptide antibody detection in Multiple Sclerosis

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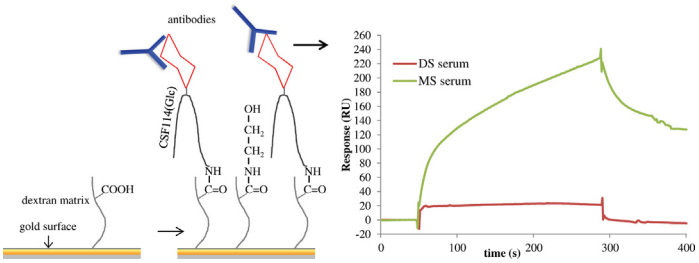
GRAPHICAL ABSTRACT



ABSTRACT

Surface plasmon resonance technique is particularly interesting in immunology because it has the potential to visualize label-free antigen-antibody interactions in real-time, thus enabling antibody detection and monitoring. Herein we release the guidelines for the correct use of a method to detect specific antibodies directly in Multiple Sclerosis patients' sera using a glucopeptide-based label-free biosensor. The protocol describes the strategy employed for the immobilization of glucopeptide antigen onto a gold sensor chip and the evaluation of the specific binding of serum antibodies to the immobilized antigen.

- Label-free method for the real time screening of disease-specific antibodies within a few minutes;
- The described protocol employs small quantities of glucopeptide antigen and blood serum samples saving method-cost;

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- Stability of the immobilized glucopeptide antigen guarantees the regeneration of the surface allowing re-use the immunosensor with high automated throughput.

The antibodies detected using the described methodology can be evaluated as biomarkers of Multiple Sclerosis. The SPR detection system is able to characterize antibodies significantly different from those evaluated in the classical enzyme-linked immunosorbent assays (ELISA).

ARTICLE INFO
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Method details

One of the major challenges in Multiple Sclerosis diagnosis is the set-up of simple immunodiagnostic methods. In fact, the gold standard for the diagnosis and prognosis of the disease is, up to now, the use of magnetic resonance imaging markers and cerebrospinal fluid analysis. Surface plasmon resonance (SPR) technique has been successfully used to measure the binding of a large number of biomolecular interactions including those of antibodies with cognate antigens [1]. The method for anti-glucopeptide antibody detection in Multiple Sclerosis described herein enables label-free specific antibody detection directly in patients' sera, using a previously described glucopeptide antigen, termed CSF114(Glc) [2]. A direct comparison of antibody profiles in Multiple Sclerosis patients' sera by means of enzyme-linked immunosorbent assay (ELISA) and SPR-based biosensor evidenced that, from a diagnostic point of view, results should be independently evaluated [3].

Glucopeptide antigen immobilization: selection of the immobilization buffer

The glucopeptide CSF114(Glc) was prepared by microwave-assisted solid phase peptide synthesis and further characterized by mass spectrometry and analytical HPLC as described elsewhere [4]. A stock solution of CSF114(Glc) was prepared in pure water (1 μg/μL) and stored at +4 °C. Immediately prior to immobilization procedure, peptide stock solution was diluted in the immobilization buffer to a final concentration of 10 μg/mL.

Sensor chip CM5 (GE Healthcare, Uppsala, Sweden) was inserted into the SPR detector (Biacore T100, GE Healthcare). The running buffer HBS-EP+ 10× (0.1 M HEPES, 1.5 M NaCl, 30 mM EDTA and 0.5% v/v Surfactant P20; yielded pH 7.4 when diluted) was diluted and flowed over the sensor chip channels. All experiments were conducted at +25 °C.

The immobilization buffer was previously selected using the pH scouting protocol, in which the peptide antigen, solved in different buffers, was flowed over the inactive sensor chip for 120 s at a flow rate of 10 μL/min. The regeneration of the chip surface was performed with a pulse of 0.1 M NaOH for 30 s at a flow rate of 10 μL/min after each solution injected. The immobilization buffers were used at pH between 3.5 and the isoelectric point of the antigen in order to achieve the electrostatic pre-concentration of glucopeptide in the dextran matrix of CM5 chip (pre-concentration is favored by low ionic strength in the buffer). The best immobilization buffer was selected injecting the glucopeptide in 10 mM carbonate buffer pH 9.6, PBS buffer pH 7.2, 10 mM, 1 mM and 0.1 mM acetate buffer at pH 4.5, 5.5 and 6.0. Buffers that give irregular sensorgrams or signals with irregular slopes, probably due to ligand aggregation/precipitation or chip saturation, were discarded. The buffer 0.1 mM sodium acetate pH 5.5 presented the highest sensorgram slope and for this reason was selected as the optimal immobilization buffer.

Glucopeptide antigen immobilization

The flow cell of the sensor chip surface was activated by injecting a 0.4 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and 0.1 M *N*-hydroxysuccinimide (NHS) mixture (50:50),