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Reduction of severe bovine serum associated matrix effects on carboxymethylated dextran coated biosensor surfaces

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ABSTRACT

Surface plasmon resonance (SPR) based biosensor technology has been widely used in life science research for many applications. While the advantages of speed, ruggedness, versatility, sensitivity and reproducibility are often quoted, many researchers have experienced severe problem of non-specific binding (NSB) to chip surfaces when performing analysis of biological samples such as bovine serum. Using the direct measurement of the bovine protein leptin, present in bovine serum samples as a model, a unique buffering system has been developed and optimised which was able to significantly reduce the non-specific interactions of bovine serum components with the carboxymethyl dextran chip (CM5) surface on a Biacore SPR system. The developed NSB buffering system comprised of HBS-EP buffer, containing 0.5 M NaCl, 0.005% CM-dextran, pH 9.0. An average NSB reduction ($n = 20$) of 85.9% and 87.3% was found on an unmodified CM5 surface and a CM5 with bovine leptin immobilised on the chip surface, respectively. A reduction in NSB of up to 94% was observed on both surfaces. The concentration of the constitutive components and pH of the buffer were crucial in achieving this outcome.

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1. Introduction

The need for more samples to be tested for an ever-increasing range of complex substances under more defined performance criteria has resulted in an increased demand for sensitive but robust analytical methods capable of high-throughput and with simple sample preparation procedure. Recent advances in bio-analytical sciences have greatly facilitated this need. One of the most frequently exploited techniques is real-time biospecific interaction analysis (BIA) utilising surface plasmon resonance (SPR) technology that is now widely used in many life science research applications [1–4]. The advantage of SPR biosensor method over other technologies is the ability to deliver rapid and reliable analyte detection in real-time, without the use of labels. The applicability of this technology in the fields of animal health monitoring and food analysis has made a valuable addition to the spectrum of analytical tools available.

A number of different commercial SPR platforms exist, but the technology provided by the GE Healthcare Company, Biacore (Biacore AB, Uppsala, Sweden) is a popular choice for method developers in food diagnostics [5,6]. Biosensor analysis based on carboxymethylated dextran (CM-dextran) surfaces has provided a reliable platform for the rapid determination of a wide range of compounds relevant to food safety and quality, and an equally wide variety of food and related sample types. But while the advantages of speed, ruggedness, versatility, sensitivity and reproducibility are often quoted, many researchers have experienced severe problem of non-specific binding (NSB) in biological samples. It is generally recognized that matrix interference, resulting in interaction between components of biological specimens and the sensor chip surface, is a major technical difficulty in assay development and has, more than any other single factor, limited the success of SPR biosensor system in the study of binding events in complex biological samples such as serum and blood due to an inability to control non-specific adsorption [7].

A number of strategies have been employed to resolve the problem of NSB in real-time BIA applications with various degrees of success. In some cases, worthwhile reductions in NSB have been achieved and workable assays have been developed. For example, Johnsson et al. introduced a simple precipitation step using saturated ammonium sulphate (SAS) to remove proteins of bovine serum and reported the development of a sensitive bioassay for the detection of drug residue (benzimidazole) in bovine serum [8]. However, this technique is not suitable for analysis of proteins since the precipitation step will remove all proteins from the serum sample. No apparent success has been reported in applications where the direct measurement of an analyte present in bovine serum on

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carboxymethylated dextran coated biosensor surfaces. Masson et al. claimed that better performance could be obtained for bovine serum assay by replacing the CM-dextran with other biocompatible polymers [9]. High-throughput analysis of serum samples is highly dependent on an ability to assay samples directly and without the need to perform time-consuming and expensive sample preparation prior to analysis.

The present study used the measurement of bovine leptin protein in bovine sera as a model to investigate the possibility of assembling a buffering system to minimise non-specific binding of bovine serum components to the surface of a CM-dextran coated chip (CM5). Leptin, a 146 amino acid peptide hormone (16 kDa) is produced and released from adipocytes [10], and is one of a group of potential protein biomarkers for the detection of anabolic steroid misuse in cattle.

2. Experimental

2.1. Instrument and reagents

The SPR biosensor instrument (Biacore® Q), carboxymethylated dextran coated sensor chips (CM5 research grade), HBS-EP buffer (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% P20, pH 7.4), the amine coupling kit containing *N*-hydroxysuccinimide (NHS), *N*-ethyl-*N*′-(3-diethyl-aminopropyl) carbodiimide (EDC), and ethanolamine hydrochloride (pH 8.5) were obtained from Biacore AB (Uppsala, Sweden). Biacore control software (version 3.0.1) was used for instrument operation and data handling. Recombinant bovine leptin (purity >95%) was purchased from Oxford Bio-Innovation Ltd (DSL-OBL, Oxon, UK). Synthetic leptin fragment ($_{77}$SNDLENLRDLLHLLAA$_{92}$) was supplied by AgriFood and Biosciences Institute (AFBI, Belfast, UK). Anti-leptin antibody was raised in guinea pigs against recombinant ovine leptin (a gift from Professor Arieh Gertler of the Hebrew University of Jerusalem, Israel) and has been used in radioimmunoassay (RIA) for the analysis of both bovine and ovine leptin. Carboxymethyl dextran sodium salts (MW: 75, 500, and 2000 kDa) were purchased from Ssens (Hengelo, The Netherlands). Sodium dihydrogen orthophosphate and di-sodium hydrogen orthophosphate were purchased from BDH Laboratory Supplies (Poole, UK). Carboxymethyl dextran sodium salt and all other chemicals were purchased from Sigma–Aldrich (Poole, UK). Deionised water was obtained using Millipore reverse osmosis and Milli-Q water polishing systems.

Tris–HCl buffer (pH 7.4) was prepared by mixing 44.2 ml 0.1 M solution of tris (hydroxymethyl aminomethane) and 50 ml of 0.1 M HCl, and then diluted to a total of 200 ml with deionised water. Phosphate buffer (pH 7.2) was prepared by mixing 28 ml of 0.2 M solution of sodium dihydrogen orthophosphate and 72 ml of 0.2 M solution of di-sodium hydrogen orthophosphate. Non-specific binding buffer (NSB buffer) was based on HBS-EP (10 mM HEPES, 3 mM EDTA and 0.005% Tween 20) contained 0.5 M NaCl and 0.005% CM-D500, and was adjusted to pH 9.0 using 1 M NaOH.

2.2. Methods

2.2.1. Preparation of the SPR sensor chip surface

Modified coupling procedures described previously by Johnsson et al. [11] were used for external immobilisation of recombinant bovine leptin or leptin peptide fragment onto the surface of a CM5 sensor chip. Briefly, the chip surface was activated with 50 μl of a mixture of 0.4 M (EDC) and 0.1 M (NHS) (1:1; v/v) for 20 min at ambient temperature on bench. The reactant was removed and 50 μl of leptin peptide fragment (1 mg ml^{-1} in 10 mM sodium acetate, pH 4.5) or whole bovine leptin (500 μg ml^{-1} in 10 mM sodium acetate, pH 4.5) was added and allowed to remain in contact with the chip surface for 2 h (peptide fragment) or overnight (whole leptin) at ambient temperature. Unreacted sites were blocked by the addition of 50 μl of 1 M ethanolamine (pH 8.5) for 20 min at room temperature. The reactant was removed and the chip surface was washed with deionised water and then dried under a stream of nitrogen gas. The prepared chip was stored at 4 °C in the present of desiccant.

2.2.2. Rmax determinations

Rmax is the maximum binding capacity of the surface ligand (peptide) for the anti-peptide antibody, as measured in resonance units (RUs; where 1000 RU ≈ 1 ng mm^2 for proteins). Rmax provides useful information about the ability of immobilised ligand and binding partner to interact. Rmax values were obtained by injecting high concentrations of antibody (e.g. ×10 dilution) over the chip surface at a low flow rate (5 μl min^{-1}) for up to 10 min (injection time). The increase in relative response units over this period is denoted as the Rmax value.

2.2.3. Preparation of bovine serum

Bovine serum was prepared by centrifugation (2500 rpm, 15 min) of clotted bovine blood obtained from local cattle. Serum samples were diluted 10-fold with appropriate buffer prior to analysis.

2.2.4. SPR biosensor assay

All experiments were performed on a Biacore® Q and Evaluation software 1.0 was used for data analysis. Freshly diluted bovine serum was mixed with the same volume of diluted antibody and injected over the sensor chip surface for 4 min at a flow of 20 μl min^{-1}. The surface was regenerated by a 1-min pulse of 50 mM sodium hydroxide at a flow rate of 20 μl min^{-1}.

The leptin assay was designed as a solution competition (inhibition) assay. The high molecular weight (HMW) interactant (anti-leptin antibody) was added to the bovine serum sample containing the analyte of interest. The analyte itself or an analogue (leptin or peptide fragment) was immobilised on the surface and used to determine the concentration of free HMW interactant in solution. Analyte concentration was measured in terms of inhibition of binding of the HMW interactant (antibody) to the surface. A low response indicated a high analyte concentration (the concentration of free interactant in solution was low due to their binding to the analyte in sample). When the concentration of analyte present was known, i.e. bovine serum spiked with a range of analyte concentrations, a calibration curve could be constructed and used to measure the analyte concentration in unknown samples.

For the study of the non-specific binding of serum components on the CM5 sensor chip surfaces, bovine serum diluted 10-fold with appropriate buffer was injected over the chip surfaces, i.e. blank and leptin immobilised surfaces, with or without the presence of anti-leptin antibody. Two sets of data (i.e. relative response units) were subjected to statistical analysis, as other assay parameters (buffer conditions etc.) remained the same. Preparation of calibration curves was not needed for this task.

3. Results and discussion

3.1. Preparation of SPR sensor chip surface

The sensor surface immobilised with recombinant bovine leptin displayed an Rmax of 12.6 kRU while the leptin fragment coated surface gave an Rmax of 17.4 kRU. For concentration measurements utilising small molecule coated surfaces (e.g. drug or peptides) to

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