Biosensors and Bioelectronics 52 (2014) 82–88

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Allergen immobilisation and signal amplification by quantum dots for use in a biosensor assay of IgE in serum

Geoffrey W. Platt [a], Francesco Damin [b], Marcus J. Swann [a], Isabelle Metton [c], Gilbert Skorski [c], Marina Cretich [b,*], Marcella Chiari [b]

[a] Farfield Group Ltd., 3000 Manchester Business Park, Aviator Way, Manchester M22 5GT, UK
[b] Consiglio Nazionale delle Ricerche, Istituto di Chimica del Riconoscimento Molecolare, Milano, Italy
[c] Phylogene S.A., 62 RN 113, 30620 Bernis, France

ARTICLE INFO

Article history:
Received 22 May 2013
Received in revised form
8 August 2013
Accepted 12 August 2013
Available online 27 August 2013

Keywords:
Biosensor
Polymer coating
Allergen
Quantum dots
Interferometry

ABSTRACT

The production of biosensors for point of care diagnostics usually requires the immobilisation and storage of protein (for example, antigen or antibody) on a sensor surface, in a manner that retains a high degree of activity and low levels of non-specific binding. These characteristics have been assessed for polymer immobilised antigens (allergens) using an IgG binding assay and demonstrated further by assay with serum containing reactive IgEs.

The activity of allergens immobilised on sensor chips using copoly(DMA–NAS–MAPS) and a spotting technique, as well as the specificity of their binding interactions with cognate immunoglobulins was assessed using Dual Polarisation Interferometry (DPI). The data obtained indicate that the allergens studied remain stable over long periods of time (at least 114 days). This performance compared favourably with other immobilisation methods. Allergen coated chips were tested in an anti-casein IgE assay using human serum from allergic and non-allergic donors. Detection of both total Ig and specific IgE was demonstrated using a secondary anti-IgE antibody. Furthermore, optical signal enhancement with streptavidin conjugated quantum dots was shown to yield responses for samples below 0.84 ng/mL (0.35 KU/L) of IgE, which overlap with the industrial quasi-standard ImmunoCAP® and is the clinically relevant threshold used to classify serum samples from allergic individuals.

1. Introduction

The study of molecular recognition events, such as antigen–antibody interactions, in both research and clinical settings can benefit from the development of peptide and protein microarrays that have the potential to allow such analyses to proceed in a time efficient and high throughput fashion with low reagent consumption. One of the key issues for the successful translation of protein microarray technology into diagnostics is the ability to control the quantity and the integrity of surface immobilised proteins. A suitable immobilisation strategy has to achieve the maximum binding capacity, keep the capture reagents in a functional state (even for long term storage) and prevent non-specific interactions in order to provide the high signal to noise ratio that is a prerequisite for high sensitivity (Hartmann et al., 2009).

Determining the optimal surface chemistry for each application is critical and influences the reliability of any protein microarray experiment (Rusmini et al., 2007). Previously, a functional polymer named copoly(DMA–NAS–MAPS), obtained by radical copolymerisation of *N*,*N* dimethylacrylamide, *N*-acryloyloxysuccinimide, and 3-(trimethoxysilyl)propyl methacrylate has been used to immobilise allergens on coated glass and silicon slides (Cretich et al., 2010). This allowed the efficient measurement of their interactions with allergen-specific Immunoglobulin E (IgEs) that are the universally accepted biomarkers for allergy testing in blood. The coating is prepared by a "dip and rinse" approach, where a silicon oxide substrate is immersed in an aqueous solution of the copolymer (10 mg/mL) at ambient temperature followed by washing with water. This procedure produces a thin film with an average thickness of a few nanometres (Cretich et al., 2009a) by a combination of physisorption and chemisorption. The coating is stable under aqueous conditions, where it swells up to 15 nm (Yalcin et al., 2009), and provides a generic covalent immobilisation strategy as the active esters present are able to react with surface exposed amine groups of proteins.

In many applications, such as point-of-care allergy tests, it is necessary to immobilise proteins onto a sensor surface and to store them for an extended period of time.

Covalent immobilisation is the preferred method for attaching proteins to sensor surface as this approach leads to formation of a strong and stable linkage. The most widely used method to

* Correspondence to: Consiglio Nazionale delle Ricerche, Istituto di Chimica del Riconoscimento Molecolare (ICRM), Via Mario Bianco, 9, 20131 Milano, Italy. Tel.: +39 02 2850 0042; fax: +39 02 2890 1239.
E-mail address: marina.cretich@icrm.cnr.it (M. Cretich).

0956-5663/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bios.2013.08.019

functionalize Si/SiO_2 sensors involves monodimensional silane coatings. However, the direct attachment of a protein to a surface without a spacer can cause steric constraint of the protein's reactivity or interaction capability compared to the protein in solution (Kusnezow and Hoheisel, 2003). Moreover, multiple direct contacts with the surface can induce denaturation. By introducing a spacer between the protein and the reactive group on the surface, these effects can be minimised. An effective way to space a protein from the surface is by the introduction of a polymeric coating (Milum and Sadana, 1997) which, besides reducing steric hindrance, increases the density of protein per unit area by providing a three dimensional binding scaffold.

The suitability of copoly(DMA–NAS–MAPS) for such a task, together with its stability and capacity to reduce the level of non-specific binding is studied here using Dual Polarisation Interferometry (DPI).

DPI is a surface-based optical technique that can measure both the amount of material deposited upon a SiO_xN_y chip surface and the average structural properties (thickness and density) of the layer formed (Swann et al., 2004). It is commonly used to study interactions between proteins and can give quantitative data including the binding stoichiometry and surface coverage. DPI is an evanescent waveguide technique in which light travels down adjacent sensing and reference waveguides and diffracts at the output to give a Young's interference pattern in the far field. The evanescent field is highly sensitive to changes in refractive index in the vicinity of the sensing surface, therefore, addition of layers to the sensing region of the chip will result in changes in the position (phase) of the fringes generated. The DPI supports both transverse magnetic (TM) and transverse electric (TE) polarisation modes, which generate different evanescent fields at the sensing surface. This means that the phase measurements can be used to provide sensitive thickness and density values of the layer, and allow for the further calculation of mass deposited. DPI has been used to study both interactions and resulting conformational changes of a range of biomaterials including proteins, lipids and DNA (Sanghera et al., 2009; Wang et al., 2010; Coan et al., 2012).

Here, a DPI assay was used to measure the interaction of copoly (DMA–NAS–MAPS) immobilised allergens with commercially available antibodies, which act as a mimic of the allergen-reactive IgEs present in human sera. These measurements allow analysis of the specificity of allergen–Immunoglobulin interactions and the effect of storage, over a range of time periods under various conditions, on the activity of the immobilised allergen. Furthermore, changes in the resistance of the polymer coated surface to fouling under various conditions over the range of storage periods is assessed.

The assay is then tested under quasi-clinical conditions with casein as the immobilised allergen against human serum samples containing controlled levels of IgE. The contribution from specific and non-specific binding is assessed from total serum protein containing IgG and IgE, using the primary binding response followed by a secondary antibody in a "label free" format. The secondary antibody response is then further tested using streptavidin conjugated quantum dots (QDs), which act as signal enhancement and improve the sensitivity in the low IgE concentration regime.

2. Experimental section

2.1. Materials

All chemicals and proteins were purchased from Sigma-Aldrich (Poole, UK) unless stated otherwise. DPI sensor chips (unmodified SiO_xN_y glass, amine functionalised or PEG-Biotin, FB80 *Ana*Chip plus) were obtained from Biolin Scientific SA (Gothenburg, Sweden). Quantum dots (Qdot 655) streptavidin conjugate was purchased from Invitrogen (Paisley, UK), BS^3 (Bis[sulfosuccinimidyl] suberate) was from ThermoScientific (Rockford, IL).

2.2. Sensor chip polymeric coating

Unmodified SiO_xN_y *Ana*chips (Biolin Scientific SA, Gothenburg Sweden) compatible with DPI experiments were used in this study. The planar *Ana*chips were coated with copoly(DMA–NAS–MAPS) by immersing the slides into a 1% (*w*/*v*) polymer solution in ammonium sulphate followed by rinsing with water and drying under vacuum at 80 °C.

2.3. Chip bio-functionalization

Allergen proteins, purchased from Sigma-Aldrich were spotted at 1 mg/mL in PBS (pH 7.4) using a FlexArrayer S5 piezo-electric spotter by Scienion, (Berlin, Germany). Two thousand protein spots (400 pL each) were deposited onto only one of the two active sensing channels present on the coated *Ana*chip and incubated overnight. The remaining active succinimide ester groups were then blocked by incubation of the spotted chips in 50 mM ethanolamine, 0.1 M Tris (HCl), pH 9, briefly rinsed with water and dried under a stream of Nitrogen. The functionalised chips were stored dry for various time periods either at room temperature or 4 °C before DPI data was acquired.

The same procedure was performed on amine functionalised chips using physisorption as the immobilisation mechanism, or on amine chips functionalised with BS^3 before blocking the unreactive sites with ethanolamine.

2.4. Casein specific IgE serum preparation

The concentration of IgE was determined in KU/L using ELISA, however for consistency between the different assays in this paper the ng/ml value is given as the concentration with KU/L values in brackets. Three sera to be used as negative samples were purchased from SeraCare Life Science and one serum reported as containing more than 240 ng/mL (100 KU/L) casein IgE, evaluated by Phadia ImmunoCAP technology, was purchased from Laboratoire Cerba. Specific IgE REAST kit, calibrators and biotinylated Casein (F78) allergen were purchased from Dr Fooke Laboratorien. Assay procedure as described by the kit provider was strictly followed, a cubic spline curve fitting was used to process data and estimate casein specific IgE content.

"Negative" sera were analysed without any dilution, sera with highly concentrated IgE were diluted 1:25 and 1:50 in negative serum.

A serum sample at high IgE level (120 ng/mL) (50 KU/L) was prepared by diluting the positive serum in a mix (1:1:1) of the three negative sera. Other levels (60 ng/mL, 24 ng/mL, and 2.4 ng/mL) (25 KU/L, 10 KU/L, and 1 KU/L) were prepared by serial dilution of the 120 ng/mL (50KU/L) sample in the negative serum mix. IgE concentrations of prepared samples were assessed by ELISA using the same method.

2.5. Dual Polarisation Interferometry (DPI)

All DPI measurements were acquired using an *Ana*light 4D (Farfield Group, Manchester, UK) running *Ana*light DAQ software. The experiments were performed at 20 °C and the running buffer used, unless stated otherwise, was 50 mM Tris (HCl) pH 7.6, 150 mM NaCl, 0.02% (*v*/*v*) Tween 20. After insertion of the functionalised chip containing immobilised allergen into the fluidic compartment of the *Ana*light 4D the proteins were hydrated by continuous flow of running buffer at 50 μL/min over