Sensors and Actuators B 194 (2014) 127–133

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

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

Label free biosensor for sensitive human influenza virus hemagglutinin specific antibody detection using coiled-coil peptide modified microelectrode array based platform

Sunil K. Arya*, Patthara Kongsuphol, Chee Chung Wong, Lee Joseph Polla, Mi Kyoung Park

Institute of Microelectronics, 11 Science Park Rd., Singapore Science Park II, 117685 Singapore, Singapore

ARTICLE INFO

Article history:
Received 17 October 2013
Received in revised form 7 December 2013
Accepted 16 December 2013
Available online 24 December 2013

Keywords:
Coiled-coil peptide
HA-antibody
Biosensor
Electrochemical impedance
Self-assembled monolayer

ABSTRACT

Coiled-coil peptide (CCP) modified microelectrode array with comb structure (MACS) has been utilized to fabricate an electrochemical biosensor for antibody detection. Thiol terminated CCP has been employed for self-assembling on MACS and peptide chains connecting amino acid sequence 98-106 (YPYDVPDYA) of human Influenza virus hemagglutinin (HA) has been used as a molecular receptor for HA-antibody. Electrochemical impedance (EIS) studies, cyclic voltammetry studies, atomic force microscopic imaging and contact angle measurements were utilized to characterize self-assembly of CCP on MACS surface. Further, EIS studies were used to investigate interaction between CCP and HA-antibody at different concentrations. EIS measurements revealed that the specific interaction of HA-antibody with CCP gives rise to a clear increase in the value of interfacial charge transfer resistance (R_{ct}). A linear relationship between R_{ct} and the logarithm of HA-antibody concentration was found for the concentration range of 1 pg ml^{-1} to 100 ng ml^{-1}, with a detection limit of 1 pg ml^{-1}. Negligible change of R_{ct} was observed by incubating the sensor in solutions of other proteins such DO1, anti-GFAP, anti-IL6 and BSA, suggesting high selectivity of descried immunosensor for HA-antibody. These results demonstrated that the CCP modified MACS based platform can provide a versatile matrix for sensitive detection of desired target.

1. Introduction

Electrochemical immunosensors have recently aroused much interest for the detection of desired proteins, immunoglobin, biomarkers and biological toxins in various food, environment, and clinical diagnostics situations [1–4]. Among electrochemical immunosensor, electrochemical impedance spectroscopy (EIS) based label free sensing has gained much attention [5–8]. EIS is a very powerful non-destructive method and can be employed to measure biological interactions and analyze interfacial properties related to bio-recognition events occurring at the modified surfaces. In EIS, data points are generated using a small perturbation in signal that reduces the matrix interference. The main drawback of EIS is lower sensitivity and lower detection limits compared to labeled electrochemical techniques; however, this can be overcome through the use of microelectrodes. Microelectrodes offer several benefits over larger area macro-electrodes, such as higher sensitivity with minimum variability due to radial diffusion profile as opposed to a planar diffusion profile in macroelectrodes [8–11].

In immunosensor fabrication, the selection of a proper biorecognition element and its binding strategy is critical to realize immunosensors with improved selectivity and stability [12]. Further, oriented immobilization of biorecognition molecule is desired to promote an ideal sensitivity enhancement via optimized presentation of the immobilized molecules that produces unobstructed recognition sites for the analyte. Studies have indicated that use of the self-assembled monolayer (SAM) and covalent binding provides options for strong and stable immobilization of a biomolecule in the very near vicinity of the sensor surface [9,13]. However, binding of biorecognition element on SAM usually requires surface modification and activation, which makes system cumbersome and increases the cost of fabrication. Also most of time it does not guaranties the proper orientation of bound recognition element. For specific recognition immunosensor platforms usually employ specific antibodies (Abs) as recognition elements [4,5]. Production of specific Abs, however, is difficult, expensive and very time-consuming. Also, antibodies easily lose their activity when subjected to changing environmental conditions [14,15]. To overcome antibody associated shortcomings, DNA aptamers have been explored as an alternative. However, aptamers are not available naturally for most of the targets and their synthesis requires extensive procedure to search for specific DNA sequence to match the specific target. Therefore, there is a need to explore alternative

* Corresponding author. Tel.: +65 67705621; fax: +65 64640517.
E-mail address: sunilarya333@gmail.com (S.K. Arya).

0925-4005/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.snb.2013.12.066

and more robust specific receptor ligands for the development of immunosensors.

Recently, it has been proposed that two-stranded α-helical coiled-coil peptides (CCP), one of nature's favorite ways of creating a dimerization motif, can provide improved matrix and adaptable interface for next generation biosensors [16,17]. Coiled-coil unit consist of several key structural features that makes it of great potential value in designing molecular interface for many applications including biosensors, protein expression and affinity purification tag [18]. Using knowledge of protein folding studies and design model systems, a novel heterodimeric coiled-coil protein can be synthesized [19]. Studies have shown that CCPs are both structurally and kinetically stable in solution at neutral pH [17]. Most importantly, it contains two non-covalently assembled helical peptide building blocks in which one of the coil can be modified with thiol group at its terminal for easy surface functionalization and the other coil can be fused with the first coil via receptor a protein. This configuration facilitates easy immobilization and enables proper orientation of the recognition element on the sensor surface for wide variety of applications. Coiled-coil protein can be synthesized in vitro [19]; thus, allowing production of large quantities at relatively low cost with long storage life. Furthermore, CCP has advantage over antibodies in term of lower production cost and better stability. Thus, use of CCP has great potential for providing function-adaptable biosensor interfaces [16]. Altogether, the unique structure, the chemical resistance properties, and the lower production cost of CCP make it a powerful tool for development of biosensors.

Influenza, commonly known as "the flu" is an infectious disease of birds and mammals caused by the influenza virus. The influenza viruses are known for their high mutation rate, thus necessitating the frequent updates in composition of influenza virus vaccines to match their antigenicity [20]. Effective vaccines which can induce protective immunity can be correlated with the presence of virus-specific antibodies (Abs) in serum that are directed against the external coat proteins of the virion, hemagglutinin (HA). HA-specific antibodies that interfere with influenza virus can be used to correlate with the vaccine protective efficacy [20,21]. Thus, accurate monitoring of the antibody specific for influenza in blood is necessary for early detection of influenza. The hemagglutination inhibition (HI) test is the most widely used serological test for the detection of anti-influenza virus antibodies and is used routinely to determine the serological outcome of vaccinations [20]. The basis of the HI assay is that the antibodies to influenza virus will prevent attachment of the virus to red blood cells. Therefore, hemagglutination is inhibited when antibodies are present. It is an indirect assay in which the highest dilution of serum that prevents hemagglutination is called the HI titer of the serum. It is reported that serum HI antibody titers of 40 i.e. dilution of 1:40 are associated with at least a 50% reduction in risk for influenza infection or disease in populations" [22]. The assay itself is technically simple but difficult to automate and standardize.

In this paper, we describe a label-free EIS based biosensor for the detection and estimation of HA specific antibodies. Coiled-coil peptide (CCP) with integrated HA-antibody specific peptide (YPYDVPDYA) was employed as a molecular recognition element. CCP was immobilized onto the microelectrode array with comb structure (MACS) by self-assembly technique, which results in simpler modification protocol with desired orientation. The formation of self-assembled CCP layer on MACS electrode surface was confirmed by contact angle, atomic force microscopic (AFM) image, cyclic voltammetry (CV) and EIS. The concentration studies using EIS suggest that sensor can be employed for sensitive detection of HA antibodies up to 1 pg ml^{-1}. To our best knowledge, it is first example of CCP modified microelectrode array based electrochemical biosensor for the determination of HA-antibody, which can be extended for detection of any desired target.

2. Materials and methods

2.1. Chemicals and reagents

Coiled-coil peptide with N-terminus to C-terminus sequence (EKKLAQLEWENQALEKELAQG-YPYDVPDYA-AQLKKKLQANKKELA-QLKWK-CH2-CH2-SH) was procured from Biosynthesis, Inc. HA (F7) antibody (200 μg ml^{-1}) from mouse was procured from Santa Cruz biotechnology, dimethylsulfoxide (DMSO) was purchased from Sigma-Aldrich. Phosphate buffer solution (PBS, pH = 7.4) was bought from Invitrogen, USA. All other chemicals were of analytical grade and used without purification. Working solutions of HA-antibody were prepared by dilution in PBS. Stock solution of CCP (1 mg ml^{-1}) was prepared in DMSO and stored at −20 °C till use.

2.2. Measurement and apparatus

Electrochemical impedance (EIS) and cyclic voltammetry (CV) were utilized to characterize the CCP/MACS electrodes and to estimate HA-antibody concentration. EIS measurements were carried out at equilibrium potential called open circuit potential (OCP) without bias voltage in the frequency range of 0.5 Hz–50 KHz with a 25 mV amplitude using Autolab Potentiostat/Galvanostat (Eco Chemie, the Netherlands). Three-electrode cell configuration with gold as a pseudo reference and counter electrode was used for measurements. EIS measurements were carried out using 60 μl PBS solution (10 mM, pH 7.4) containing a mixture of 5 mM $Fe(CN)_6^{4-}$ (Ferrocyanide) and 5 mM of $Fe(CN)_6^{3-}$ (Ferricyanide) i.e. 5 mM $Fe(CN)_6^{3-/4-}$ as a redox probe. Using the redox probe (5 mM $Fe(CN)_6^{3-/4-}$), change in the charge transfer resistance at the electrode/electrolyte interface has been investigated in electrochemical impedance studies. Experiments were carried out in triplicate and Autolab Frequency Response Analyzer version 4.9.006 was used to assess charge transfer resistance values. Contact angle measurements were conducted to check the hydrophilic/hydrophobic nature of the surface before and after CCP modification by the Sessile drop method using a drop shape analyzer (DSA 100) from Kruss Gmbh Hamburg. AFM studies were carried out using Digital Instruments NanoScope AFM in tapping mode. The change in the surface morphology was related to CCP SAM layer formation on the gold surface.

2.3. Experimental conditions optimization

Peptide density on MACS, antibody incubation time, and buffer pH conditions were optimized and used in the present study. For CCP SAM formation, incubation time of 1 min, 2 h, 5 h and overnight was investigated (Supplementary data 1). It is observed that CCP SAM formed with 2 h incubation gives the optimum response, thus utilized in present work. All experiments were studied at room temperature to maintain uniformity and simplicity. HA-antibody incubation time for concentration studies were investigated at 10, 20, 30 and 40 min. Better results were observed at 30 or 40 min, however the good signal resolution and sensitivity was observed even at 20 min, thus 20 min incubation time was chosen for shorter analysis time. The pH studies were performed to investigate its effect on HA-antibody binding on CCP. Experiments were carried out for CCP SAM formation and HA-antibody binding in range of pH 6.0 to 8.0 (Supplementary data 2). It was observed that the binding is most effective in pH range of 7.0 to 7.5, thus all experiments were carried out at physiological pH of 7.4.