Colloids and Surfaces B: Biointerfaces 110 (2013) 248–253

Contents lists available at SciVerse ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Electrophoretic interactions between nitrocellulose membranes and proteins: Biointerface analysis and protein adhesion properties

S.C. Low[a,*], R. Shaimi[a], Y. Thandaithabany[a], J.K. Lim[a], A.L. Ahmad[a], A. Ismail[b]

[a] School of Chemical Engineering, Engineering Campus, Universiti Sains Malaysia, Seri Ampangan, 14300 Nibong Tebal, S.P.S., Penang, Malaysia
[b] Institute for Research in Molecular Medicine, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

ARTICLE INFO

Article history:
Received 16 January 2013
Received in revised form 4 April 2013
Accepted 1 May 2013
Available online 9 May 2013

Keywords:
Streaming potential
Nitrocellulose membrane
Electrophoretic interaction
Protein adsorption
Immunodetection

ABSTRACT

Protein adsorption onto membrane surfaces is important in fields related to separation science and biomedical research. This study explored the molecular interactions between protein, bovine serum albumin (BSA), and nitrocellulose films (NC) using electrokinetic phenomena and the effects of these interactions on the streaming potential measurements for different membrane pore morphologies and pH conditions. The data were used to calculate the streaming ratios of membranes-to-proteins and to compare these values to the electrostatic or hydrophobic attachment of the protein molecules onto the NC membranes. The results showed that different pH and membrane pore morphologies contributes to different protein adsorption mechanisms. The protein adsorption was significantly reduced under conditions where the membrane and protein have like-charges due to electrostatic repulsion. At the isoelectric point (IEP) of the protein, the repulsion between the BSA and the NC membrane was at the lowest; thus, the BSA could be easily attached onto the membrane/solution interface. In this case, the protein was considered to be in a compact layer without intermolecular protein repulsions.

1. Introduction

The detection and identification of pathogen have become important primarily for health and safety reasons [1]. Biosensor technology, a relatively new technique compared to the conventional polymerase chain reaction (PCR) method, is one of the fastest growing pathogen detection technologies [2,3] due to its advantages, which include equally reliable results in shorter times, no skilled personnel required and advanced infrastructure. Biosensors can also be used to detect environmental pollutants and to measure the concentration of metal ions and the biochemical oxygen demand (BOD) levels in waste water treatment [4,5].

The principal analytical technique of biosensors is based on the specific interactions between a substance of interest (the target analyte) and the biological recognition elements (the capture analytes such as enzymes, antibodies, nucleic acids) that are immobilised onto the lateral flow membrane [6]. In a biosensor, the lateral flow membrane acts as the detector surface or carrier for the target analyte. In fact, the immobilisation of the biomolecule is critical in the development of the biosensor, as the immobilised biomolecules need to maintain its original functionality for the biosensor to work.

Proteins are the most common reagent applied onto the membrane surface, where the loading capacity of a protein depends on the compactness of its structure and the pH conditions [7,8]. In addition to the nature of the protein, the immobilisation of the biomolecule depends strongly on the membrane morphology and the available surface area for immobilisation. Different membrane-forming materials have different intrinsic affinity-binding capacities for different proteins [9,10]. The protein adsorption depends on various physiochemical interactions between the protein molecules and the membrane, including the electrostatic attraction, van der Waal interactions, hydrophobic interactions and hydrogen bonding [11–15]. Nitrocellulose (NC) is the most common membrane used in lateral flow biosensor. In terms of the physiochemical properties, the strong dipole of the nitrate group in the NC membrane would interact with the strong dipole of the peptide bonds in the protein, thus giving rise to an electrostatic affinity [16]. In this case, the electrostatic interaction between the membrane and protein is expected to be the major factor that dominates the adsorption behaviour. The polymer surface properties and the protein adsorption capacity onto the surfaces are important and may also influence the protein attachment [17].

Thus, there is a growing interest in quantitatively and qualitatively determining the membrane–protein interaction, which, in most cases, is closely associated with the materials' surface properties [18–22]. The streaming potential plays a major role in determining the electrophoretic interactions between the membrane and the protein. The streaming potential method is based

* Corresponding author. Tel.: +60 4 5996412; fax: +60 4 5941013.
E-mail addresses: chsclow@eng.usm.my, siewchun@gmail.com (S.C. Low).

0927-7765/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.colsurfb.2013.05.001

on the measurement of different electrostatic potentials when a hydrostatic pressure is applied over a charged membrane [23,24]. The index of the electrophoretic interactions can be evaluated by the product of the zeta potential of both the membrane and the protein molecules. However, protein adsorption onto biosensors remains a poorly understood phenomenon because the majority of biomolecule immobilisation studies have been conducted under physiological conditions for biomedical use. Separate in-depth studies of protein attachment onto membranes are essential as the differences among membranes, in terms of their materials and pore structures, result in different binding mechanisms that may require different measurements regarding the sensitivity of the binding line for a biosensor.

To accomplish this goal, we directly measured the intermolecular interactions between the membrane and protein using the static adsorption of bovine serum albumin (BSA) onto NC membranes at different pH values and with different membrane morphologies. The electrophoretic interactions between the membrane and protein were evaluated based on the streaming potential measurements to investigate how the physiochemical properties of the membrane and the pH affect the protein adsorption. This approach enabled us to better understand the two different protein adsorption mechanisms, hydrophobic and electrostatic interactions, at the membrane/solution interface.

2. Experimental

2.1. Materials

The NC membranes (HF-90, HF 135 and HF-180) were manufactured by Millipore Corporation (Bedford, MA). Bovine serum albumin (BSA, A1933) was used as the model protein and was supplied by Sigma (MO, USA) with a molecular weight of 66,430. The BSA solution was prepared using 0.05 M phosphate buffered saline solution (PBS). The electrolyte solution used for the streaming potential measurement was KCl of pure analytical grade. The solutions were prepared using ultrapure water with resistivity greater than 18 MΩ-cm, where the salt concentration was fixed at 10 mM but the pH ranged from 2.0 to 8.0. The pH was controlled by the addition of NaCl or HCl supplied by Merck (Darmstadt, Germany).

2.2. The properties of the membranes

Field emission scanning electron microscopy (FESEM SUPRA 35vp Zeiss, Germany) was used to visualise the surface morphologies of the membranes. The membrane samples were first coated with platinum (Pt) using a sputter coater to prevent the surface from becoming charged. The sizes of the membrane pores were measured from the FESEM micrograph, where the measured pore sizes were divided into groups and each group had a range of 2 μm. The pore size distribution was then determined based on the frequency count of the membrane pores that were distributed in each group. The porosity of the membrane (ε), based on the dry station of the membrane, was calculated according to the equation given by Yamane et al. [25] and Meier et al. [26].

$$\varepsilon = \frac{V_A - V_E}{V_A} \times 100\% \quad (1)$$

The flat sheet NC membrane was cut into 2 cm × 2 cm squares, and the membrane thickness was measured using a micro thickness gauge (Mitutoya 7301, Japan). The apparent volume of the membrane (V_A, cm^3) was calculated based on the film thickness and the surface area (2 cm × 2 cm). The membrane was then dried in an oven to eliminate any water vapour contained in the membrane. The existent volume of the membrane (V_E, cm^3) at the dried state condition was then determined using the polymer density (1.23 g/cm^3) and the membrane dry weight.

2.3. Protein properties

The electrophoretic mobility of the BSA was measured using a Zetasizer Nano (Malvern, UK) to evaluate the effective net surface charge of the BSA at different pH values. Prior to the analysis, the protein suspensions were diluted with de-ionised water to avoid multiple scattering effects, and 0.7 ml of the sample was then directly placed into the equipment (mean, $n = 3$).

2.4. Protein immobilisation

The adsorption capacity of the protein onto the membrane is dependent on the pH of the protein solution and the morphology of the membrane. The proteins were immobilised by immersing the flat sheet NC membranes into 10 ml of BSA solution (1 mg/ml) at specific pH values and then shaking the membranes for 1 hour at a constant temperature of 25 °C. The membrane sample was then washed three times with phosphate buffer to remove unbound proteins from the membrane surface. To quantify the protein bound onto the NC membrane and the binding interactions, photometric (Spectroquant Pharo 300, Germany) and streaming potential (Zetacad, France) analysis were performed on the samples.

2.5. Biointerface analysis: streaming potential and photometric measurements

The membrane's streaming potential is measured by imposing the movement of an electrolyte solution (10 mM KCl) through a streaming channel that is formed by two identical membrane samples that are placed facing each other. The potential difference, ΔE, across the membrane is recorded when the fluid is forced through the sample by applying a fluid pressure difference, ΔP. The streaming potential could be deduced from the slope of $\Delta E = f(\Delta P)$. In the present study, the membrane's electrokinetic potentials were studied at different pH values (from 2 to 8) and with different NC membrane morphologies (HF-90, HF-135 and HF-180), where the apparent streaming potential was estimated using the Helmholtz–Smoluchowski equation.

To quantify the amount of immobilised protein on the membrane, the sample replicate was transferred into a test tube. Subsequently, 2.0 ml of bicinchoninic acid working reagent (BCA) was added, and the test tubes were incubated at 37 °C for 30 min. The absorbance of the liquid content from the test tubes was photometrically measured at a wavelength of 562 nm. Using the preliminary standard curve plot, the absorbance reading for the sample was interpolated.

2.6. Immunoassay testing

A rabbit anti-goat IgG antibody solution prepared at a pH of 7.2 (IEP of IgG) was lined on the membrane surface using an automated Isoflow reagent dispenser (Kinematic M1600, USA) at a speed of 2 μl/cm. The membrane was then air dried, and the remaining active sites of the membrane were blocked and treated with the Western blocking reagent (Roche, Germany). After the blocking step was completed, the membrane was assembled as an immunoassay. The assembled membrane strip was mounted with 50 μl of running buffer (1 ml 10% BSA and 0.5 ml of Tween 20 in 98.5 ml of ICT PBS buffer, where the solution was adjusted to a pH of 7.2 and diluted to 1000 ml using deionised water) in the first well of a 96-well microplate. When the buffer solution reached the top and fully covered the membrane strip, the strip was placed into a second well containing 25 μl of buffer and 7 μl of a colloidal gold solution.