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Immunosensor interface based on physical and chemical immunoglobulin G adsorption onto mixed self-assembled monolayers

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Abstract

An immunosensor interface based on mixed hydrophobic self-assembled monolayers (SAMs) of methyl and carboxylic acid terminated thiols with covalently attached human Immunoglobulin G (hIgG), is investigated. The densely packed and organised SAMs were characterised by contact angle measurements and cyclic voltammetry. The effect of the non-ionic surfactant, Tween 20, in preventing nonspecific adsorption is addressed by ellipsometry during physical and covalent hIgG immobilization on pure and mixed SAMs, respectively. It is clearly demonstrated that nonspecific adsorption due to hydrophobic interactions of hIgG on methyl ended groups is totally inhibited, whereas electrostatic/hydrogen bonding interactions with the exposed carboxylic groups prevail in the presence of surfactant. Results of ellipsometry and Atomic Force Microscopy, reveal that the surface concentration of covalently immobilized hIgG is determined by the ratio of COOH / CH_3-terminated thiols in SAM forming solution. Moreover, the ellipsometric data demonstrates that the ratio of bound anti-hIgG / hIgG depends on the density of hIgG on the surface and that the highest ratio is close to three. We also report the selectivity and high sensitivity achieved by chronoamperometry in the detection of adsorbed hIgG and the reaction with its antibody.

Keywords: Mixed self-assembled monolayers; Immunosensor; Human immunoglobulin G; Tween 20; Electrochemistry; Ellipsometry

1. Introduction

In the development of affinity biosensors, the fabrication of the interface plays a crucial role, since it determines the specificity, the reproducibility, and the stability of the entire sensor [1,2]. The nonspecific adsorption together with a proper bioreceptor orientation/distribution on the surface is an important factor that must be controlled in order to get the best biosensor performance. By the use of mixed SAMs, interfaces become tunable and the desired chemical properties [3] can be achieved.

Much work has been devoted [4–7] to the search of surfaces that minimize nonspecific adsorption of proteins, based on the understanding of protein adsorption mechanisms. It has been found that the major factors responsible for the interfacial activity and adsorption of proteins are the water structure-driven hydrophobic effect and the two types of interactions, electrostatic and strong hydrogen-bonding (often characterized by cooperative, multiple hydrogen bonds [8]). Surfaces with nonionic polyethylene oxide (PEO) grafts show greatly reduced protein adsorption [9], since they are able to minimize both electrostatic and hydrophobic interactions. The development of an interface of gold modified with tri(ethylene glycol)-terminated thiol has also been reported [10,11] as resistant to the nonspecific adsorption of some proteins; in this case, its laborious synthesis appears as a drawback in its use. In contrast, the addition of surfactants [12–14] during biocompounds immobilisation, such as sodium dodecyl sulphate (SDS) or polyethylene glycol sorbitan monolaurate (Tween 20) became common in biosensor preparation. However, most of the studies only refer to their blocking ability towards hydrophobic surfaces, during biomolecules covalent attachment [15].

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In the present work, the influence of the nonionic detergent, Tween 20, during the physical and covalent attachment of the bioreceptor, hIgG, in pure and mixed self-assembled monolayers containing methyl and carboxylic acid terminal groups, has been investigated by ellipsometry and Atomic Force Microscopy. The incorporation of both thiols enables a SAM interface with few linking groups for the covalent attachment [2,10,16] of hIgG, surrounded by hydrophobic moieties where physical adsorption is completely inhibited by the presence of Tween 20.

The adsorption of proteins and their biological performance at the electrode surfaces modified with self-assembled monolayers has been addressed by several techniques, such as chronoamperometry [17], ellipsometry [18], surface plasmon resonance [1,19,20], radiolabelling [21,22], quartz crystal microbalance [23,24], electrochemical impedance spectroscopy [25] and atomic force microscopy [15,26,27]. In the current paper, the biorecognition of both physically and covalently adsorbed hIgG towards its antibody in antiserum, over pure or mixed SAMs is evaluated by ellipsometry and chronoamperometry.

2. Experimental

2.1. Materials

hIgG, hIgG antiserum (anti-hIgG), human serum albumin antiserum (anti-HSA), Tween 20, 1-ethyl-3-(3-(dimethylamino)-propyl)carbodiimide (EDC), and *N*-hydroxysuccinimide (NHS) were obtained from Sigma. 11-Mercaptoundecanoic acid and 1-Hexanethiol were purchased from Aldrich.

Gold film (200 nm) on glass (1.1×1.1 cm^2) with a pre-layer of chromium (2–4 nm) purchased from Arrandee, was used as substrate for mixed SAMs preparation.

2.2. Buffer and solutions

Phosphate-buffered saline (PBS: (8.0 mM $Na_2PO_4 \cdot 1.14$ mM KH_2PO_4, 138 mM NaCl, and 2.7 mM KCl, pH 7.4) was prepared in Millipore water (18 MΩ cm at 25 °C). Solutions of NHS (0.05 M) and EDC (0.2 M) were prepared in Millipore water immediately before use. HIgG was diluted with PBS to obtain two different concentrations, 0.1 and 0.05 mg/ml, with and without the addition of 1% Tween 20 (v/v). Anti-hIgG serum and anti-HSA serum were diluted with PBS to 0.1 mg/ml with the addition of 1% Tween 20.

2.3. Gold surface modification

Prior to use, the gold slides were annealed in the cold part of a Bunsen flame and quenched in ultra pure water. This treatment produces a flat gold surface with predominant (111) orientation, as confirmed by STM. The average roughness factor of the substrates (1.2) was estimated by the iodine chemisorption method [28] and is in agreement with reported values for similar thin gold electrodes [29,30]. The mixed monolayers were prepared by substrate immersion in 1 : 1, 1 : 5, 1 : 10, and 1 : 20 11-Mercaptoundecanoic acid : 1-hexanethiol solutions in ethanol, in a total thiol concentration of 1 mM, for 24 h, and then removed, thoroughly rinsed with ethanol and pure water, and dried under a stream of nitrogen.

Mixed monolayers will be designated throughout the paper by the corresponding COOH percentage in the deposition solution: 50%, 20%, 10% and 5%.

2.4. Covalent immobilization of hIgG

The gold substrates modified with mixed SAMs were immersed into 0.05 M NHS and 0.2 M EDC solution for 15 min. After rinsing with water, the substrates were then immersed in 0.1 mg/ml hIgG solution containing 1% (v/v) Tween 20, for 30 min, followed by copious rinsing with water and drying with nitrogen.

2.5. Contact angle measurements

The measurements were conducted using the Sessile drop method. De-ionized water (4 μl) was gently dropped on the SAMs and the contact angle was read directly using a goniometer.

2.6. Electrochemical studies

All electrochemical measurements were performed using a PARSTAT 2263 electrochemical work station produced by PerkinElmer, and conducted in an one-compartment Teflon electrochemical cell, fitted with a Saturated Calomel Electrode (SCE) and a Platinum wire as reference and counter electrodes respectively. The gold substrates were mounted at the bottom of the electrochemical cell using an O-ring, which defines a geometric area of 0.57 cm^2. The electrolyte solution, NaOH 0.5 M or $K_4Fe(CN)_6$ 1 mM in KCl 1 M, was de-aerated with nitrogen (99.999%) for 30 min. All measurements were performed at room temperature (20±2 °C).

2.7. Ellipsometry

Ex situ ellipsometric data were obtained with a rotating analyzer type ellipsometer SE 400 (SENTECH Instruments GmbH, Berlin, Germany) fitted with a He–Ne laser (λ=632.8 nm). The measurements were carried out at an angle of incidence of 70°.

2.8. Atomic Force Microscopy

The measurements were performed in a Nanoscope IIIa Multimode AFM Microscope (Digital Instruments, Veeco) in *tapping* mode using etched silicon probes (oscillation frequency of about 300 kHz).

3. Results and discussions

3.1. Characterization of SAMs

3.1.1. Cyclic voltammetry

Electrochemical reductive desorption is one of the most used methodologies to characterize modified surfaces with self-