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Use of cellulose derivatives on gold surfaces for reduced nonspecific adsorption of immunoglobulin G

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ABSTRACT

This study addresses the design of protein-repellent gold surfaces using hydroxyethyl- and ethyl(hydroxyethyl) cellulose (HEC and EHEC) and hydrophobically modified analogues of these polymers (HM-HEC and HM-EHEC). Adsorption behavior of the protein immunoglobulin G (IgG) onto pure gold and gold surfaces coated with cellulose polymers was investigated and described by quartz crystal microbalance with dissipation monitoring (QCM-D), atomic force microscopy (AFM) and contact angle measurements (CAM). Surfaces coated with the hydrophobically modified cellulose derivatives were found to significantly outperform a reference poly(ethylene glycol) (PEG) coating, which in turn prevented 90% of non-specific protein adsorption as compared to adsorption onto pure gold. HEC and EHEC prevented around 30% and 60% of the IgG adsorption observed on pure gold, while HM-HEC and HM-EHEC were both found to completely hinder biofouling when deposited on the gold substrates. Adsorption behavior of IgG has been discussed in terms of polymer surface coverage and roughness of the applied surfaces, together with hydrophobic interactions between protein and gold, and also polymer-protein interactions.

1. Introduction

Adsorption of proteins onto solid surfaces has attracted a great deal of interest and is of relevance to a wide audience because of its many possible impacts on systems such as medical implants, biochips and biosensors, drug delivery, and within food- and biochemical processing [1,2]. Within the medical- and biological sciences, and regarding expression and treatment of various diseases, protein deposition and immunoresponses are often major contributors or malefactors. Triggering of an immune response involves binding of an antibody to a foreign substance/molecule (antigen). Antibodies constitute a class of proteins called immunoglobulins, which are produced by B-cells [3]. Immunoglobulins are composed of four polypeptide chains that are connected by disulphide bonds and noncovalent forces. These four polypeptide chains are grouped together in different fragments; two identical F_{ab} segments and one F_c segment, yielding a Y-shaped conformation of the protein. The antigen binding sites are located on the far ends of the F_{ab} segments. In turn, the F_{ab} segments are linked to the F_c segment by a hinge region, which varies in length and flexibility between the five antibody classes, namely IgM, IgA, IgD, IgE and IgG.

Perhaps the most common synthetic material that is used to resist nonspecific protein adsorption consists of polymers or oligomers based on ethylene glycol. The effectiveness of poly(ethylene glycol) (PEG) and other organic surface coating agents comparable to PEG in resisting protein adsorption, is typically related to a common set of properties [4,5], they should namely be (i) hydrophilic, (ii) electrically neutral, (iii) hydrogen bond acceptors and (iv) not hydrogen bond donors. Additionally, the packing density of the surface coating agent is of crucial importance as this determines whether the underlying surface is accessible and also dictates the extent of unfavorable steric interactions upon compression of the coating layer by incoming proteins [6]. Despite its ability to resist protein adsorption, PEG is susceptible to autoxidation in the presence of dioxygen and transition metal ions, typically found in biologically relevant solutions [7,8]. Additionally, the terminal hydroxyl group of PEG can be oxidized by alcohol dehydrogenase to an aldehyde, which in turn may react with proteins in vivo, or with other molecules having amine groups [9]. Thus, there is great interest in identifying alternatives to PEG as bio-inert surface coating materials.

Polysaccharide surface coatings should be similar to PEG coatings since polysaccharides can also provide neutral, low-surface-energy coatings with hydrated, randomly oriented chains. Recently, we reported the adsorption of hydroxyethyl cellulose (HEC) and ethyl(hydroxyethyl) cellulose (EHEC) and their respective hydrophobically modified counterparts HM-HEC and HM-EHEC

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onto planar gold and citrate-coated gold surfaces, as well as onto citrate-coated gold nanoparticles [10]. In this study, it was found that the hydrophobically modified cellulose derivatives displayed a higher propensity for adsorption than the unmodified counterparts did. In the case of the hydrophilic HEC dissolved in water, the good solvent conditions promote the chains to extend far into the bulk at high polymer concentrations.

Earlier studies have shown that surfaces coated with HEC [11] or EHEC [11,12] compare well with surfaces covered with PEG in preventing adsorption of fibrinogen [11] and lysozyme [12], respectively. In this investigation, we have studied the adsorption of immunoglobulin G (IgG) onto gold surfaces coated with HEC, EHEC and their hydrophobically modified counterparts, using the quartz crystal microbalance with dissipation monitoring (QCM-D) technique. To mimic physiological conditions, a phosphate buffered saline solution was employed as the bulk solvent since the ionic strength and pH of this buffer is close to what can be found in the living organism. Immunoglobulin G has a molecular weight of approximately 150,000 and a point of zero charge around pH 7.0, which renders the protein with a small negative charge under the conditions considered here. For comparison, adsorption of IgG to self-assembled monolayers of thiolated, short-chain PEG on planar gold surfaces was investigated. The hydrophobically modified polysaccharides HM-HEC and HM-EHEC were found to be superior to PEG in reducing adsorption of IgG. When ranking the efficiency of the materials in reducing protein adsorption of the surfaces studied here, the order was found to be: Au < HEC < EHEC < PEG < HM-HEC = HM-EHEC. The results are discussed in terms of hydrophobic interactions between gold and the coating polymers, inducing an oriented adsorption and varying surface coverage of the polymer layers, and then by correlating IgG adsorption to these findings.

2. Materials and methods

Immunoglobulin G and *O*-[2-(3-mercaptopropionylamino) ethyl]-*O*′-methylpolyethylene glycol 5′000 (PEG-thiol 5000) were both purchased from Sigma–Aldrich. A HEC sample (Natrosol 250 GR; Lot. No. A-0832) was obtained from Hercules, Aqualon Division, and this hydrophilic polymer served as the precursor for the synthesis of the hydrophobically modified analogue (HM-HEC). The degree of substitution of hydroxyethyl groups per repeating anhydroglucose unit for HEC is 2.5 (given by the manufacturer). The weight-average molecular weight (M_w = 400,000) of this sample in dilute aqueous solution was determined by intensity light scattering at 25 °C [13]. The HM-HEC sample was synthesized according to a procedure described elsewhere [14], and the details of the synthesis as well as the characterization of this sample have been reported previously [13]. The chemical structure and purity of the HM-HEC were ascertained by ^{1}H NMR and the degree of hydrophobic modification (glycidyl hexadecyl ether groups, n-$C_{16}H_{33}$) was determined from the peak ratios between the anisomeric protons and the methyl protons of the glycidyl hexadecyl chain [13]. The degree of substitution of the hydrophobic groups determined from NMR is 1 mol%.

Ethyl(hydroxyethyl) cellulose (EHEC) and a hydrophobically modified analogue (HM-EHEC) were both obtained from Akzo Nobel Surface Chemistry AB, Stenungsund, Sweden. Before use, the polymers were purified as described elsewhere [15]. Both EHEC and HM-EHEC have the same molecular weight ($M_w \approx 100{,}000$), and the degree of substitution of ethyl and hydroxyethyl groups are DS_{ethyl} = 0.8 and MS_{EO} = 1.8, respectively. The values of DS and MS correspond to the average number of ethyl and hydroxyethyl groups per anhydroglucose unit of the polymer, as they were given by the manufacturer. The hydrophobically modified polymer is equivalent to the EHEC sample, but with branched nonylphenol chains grafted onto the polymer backbone. The degree of substitution was determined to be 1.7 mol% (ca. 6.5 groups per molecule) relative to the repeating units of the polymer.

Dilute solutions of all the polymers were dialyzed against Millipore water for several days to remove low-molecular-weight impurities and were thereafter isolated by freeze-drying. Regenerated cellulose with a molecular-weight cutoff of approximately 8000 (Spectrum Medical Industries) was utilized as the dialyzing membrane. All the polymers have broad molecular weight distributions ($M_w/M_n > 2$), which is usually the case for most polysaccharides.

Phosphate buffered saline (PBS) was prepared by adjusting an aqueous solution of NaCl (137 mM), KCl (2.7 mM), Na_2HPO_4 (10 mM) and KH_2PO_4 (1.8 mM) to pH 7.4 using NaOH (1 M). All chemicals used for the PBS solution were acquired from Merck.

Gold coated quartz crystals were cleaned by immersion in a piranha solution (1:3, H_2O_2:H_2SO_4) for 20 min before rinsing with ultrapure water and drying under a stream of N_2-gas. Crystals were used immediately after preparation.

QCM-D measurements were conducted at 37 °C (to simulate physiological conditions for the protein) on a QCM-Z500 supplied by KSV Instruments Ltd. The principle, advantages and limitations of this technique have been extensively reviewed elsewhere [16,17]. Briefly, the instrument is based on the piezoelectric effect where a deposited mass is registered as changes in frequency of an oscillating quartz crystal. The adsorbed mass can then be calculated by using the Sauerbrey equation:

$$\Delta m = \frac{C\Delta f_n}{n} \quad (1)$$

In Eq. (1), Δm equals change in mass per unit surface area, C is the instrument sensitivity constant (17.7 ng cm^{-2} Hz^{-1}), Δf_n is the frequency change of the specific harmonic, where n denotes the number of the harmonic of oscillation. Moreover, the QCM-D also measures the ratio between the total dissipated energy during one oscillation cycle ($E_{dissipated}$) and the total energy stored in the oscillation (E_{stored}) (Eq. (2)). As mass is deposited onto the quartz crystal, this is considered to provide information about flexibility of the adhered layer, and is defined as the dissipation factor D:

$$D = \frac{E_{dissipated}}{2\pi E_{stored}} \quad (2)$$

By plotting changes in dissipation factor as a function of changes in frequency (ΔD vs. Δf plot), time as an explicit parameter is eliminated and this makes it possible to observe how the viscoelasticity of the adlayer changes as more molecules are deposited (or removed) [18]. Moreover, the dissipation factor can be used as a qualitative measure for the validity of the Sauerbrey equation, where a change in D less than 10^{-6} per 5 units change in frequency has shown a good correlation between calculated and actual adsorbed mass, as reported in previous studies [19–21]. The software provided by KSV Instruments Ltd. also offers the possibility to calculate the thickness of the adsorbed layers. By applying at least three of the measured harmonics (here all five were used), and through equivalent circuit modelling, the mechanical properties of the added layers on the quartz crystal can be assayed [22,23]. The remaining experimental data reported in this work refer to the 3rd (f_3 = 15 MHz) harmonic, as this showed the lowest signal-to-noise ratio, and have been normalized in all figures to represent correct adsorption values.

All experiments were conducted using PBS (pH 7.4) as baseline and solvent for both the polymer and the protein. In a typical adsorption experiment, the measurement chamber was first flushed with a 1.5 ml aliquot of the polymer solution (0.5 mg/ml), equilibrated and then flushed twice with the pure buffer before a 1.5 ml aliquot of the IgG solution (0.5 mg/ml) was added to the