Journal of Colloid and Interface Science 349 (2010) 265–274

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Protein interactions with bottle-brush polymer layers: Effect of side chain and charge density ratio probed by QCM-D and AFM

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ARTICLE INFO

Article history:
Received 4 March 2010
Accepted 15 May 2010
Available online 21 May 2010

Keywords:
Protein repellency
Protein adsorption
Surface forces
AFM
QCM-D
Reflectometry
BSA
Lysozyme
Bottle-brush polymer

ABSTRACT

Silica surfaces were coated with a range of cationic bottle-brush polymers with 45 units long poly(ethylene oxide) side chains, and their efficiency in reducing protein adsorption was probed by QCM-D, reflectometry and AFM. Preadsorbed layers formed by bottle-brush polymers with different side chain to charge ratio was exposed to two proteins with different net charge, lysozyme and BSA. The reduction in protein adsorption was found to depend on both the type of protein and on the nature of the polyelectrolyte layer. The most pronounced reduction in protein adsorption was achieved when the fraction of charged backbone segments was in the range 0.25–0.5 equivalent to a fraction of poly(ethylene oxide) side chains of 0.75–0.5. It was concluded that these polymers have enough electrostatic attachment points to ensure a strong binding to the surface, and at the same time a sufficient amount of poly(ethylene oxide) side chains to counteract protein adsorption. In contrast, a layer formed by a highly charged polyelectrolyte without side chains was unable to resists protein adsorption. On such a layer the adsorption of negatively charged BSA was strongly enhanced, and positively charged lysozyme adsorbed to a similar extent as to bare silica. AFM colloidal probe force measurement between silica surfaces with preadsorbed layers of bottle-brush polymers were conducted before and after exposure to BSA and lysozyme to gain insight into how proteins were incorporated in the bottle-brush polymer layers.

1. Introduction

Interactions between surfaces modified with polymers and biomolecules (e.g. proteins) are of great importance in numerous biomedical and food applications [1,2]. For controlling non-specific adsorption, biocompatibility and in some cases enhancement of protein adsorption at solid–liquid interfaces, understanding protein-surface interactions are essential. Typical coatings used for achieving low protein adsorption include adsorbed protein layers of BSA and casein, and coatings containing dextran or poly(ethylene oxide) [3–5]. Mucins, which are large glycoproteins, have also recently gained interest as a biomaterial coating due to low neutrophil activation [6,7]. It is well established that biofilms on artificial implants in humans tend to induce serious conditions such as thrombosis [8–10]. Unwanted protein adsorption can also trigger biological responses that can interfere with the functionality of contact lenses [9], blood contacting devices (e.g. catheters) [11] and surgical instruments [12]. On the other end of the spectrum, adsorbed proteins layers are also used as a conditioning layer to which for instance bacterial cells adhere and develop into mature biofilms [6,10,13].

Surfaces coated with polymers containing poly(ethylene oxide), PEO, have long been known as efficient for reducing protein adsorption, and in recent years proved to be one of the most attractive methods for controlling biological fouling of surfaces [14–18]. The molecular mechanism underlying the protein resistant properties of PEO has been suggested to be linked to the strong hydration and flexible nature of PEO that results in low van der Waals attraction and strong steric repulsion. Though in many cases the reduction in protein adsorption improve with increasing PEO grafting density [19–23], other factors like chain distribution, chain length and anchoring method are also of importance. PEO coatings where brush conformations are achieved has been shown to be very efficient in reducing protein adsorption [14,19,24–27]. However, a drawback with too high grafting density is dehydration of the chains and subsequent loss of chain flexibility that may lead to increased protein adsorption [28]. By using end grafted PEO, it has also been shown that the optimal chain density decreases with increasing PEO chain length [21].

In the present study we examine the ability of a group of cationic bottle-brush polymers, containing 45 unit long PEO side chains, to

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doi:10.1016/j.jcis.2010.05.061

reduce protein adsorption on hydrophilic silica surfaces. We particularly aim at clarifying how the structure of the bottle-brush polyelectrolyte affects the protein adsorption, and if adsorbed bottle-brush polymer layers can be used for reducing protein adsorption. The adsorption properties of these polymers have previously been determined on silica and mica [29–32], and also investigated theoretically using lattice mean-field calculations [33,34]. To assess the protein reducing ability of such surface layers we exploited mainly the QCM-D technique to analyse the protein adsorption, while the AFM colloidal probe technique was used to gain further insight into the layer structure.

2. Materials

The bottle-brush polymers (PEO_{45}MEMA:METAC-*X*) were synthesised by free-radical copolymerization as described elsewhere [32]. In this representation PEO_{45}MEMA stands for poly(ethylene oxide) methyl ethyl methacrylate, and METAC is abbreviation for methacryloxyethyl trimethylammonimum chloride. The quantity "*X*" denotes the molar percentage of METAC monomers in the backbone, which constitutes the permanently charged segments of the copolymer. The other backbone monomers contain one PEO side chain each. Some characteristics of the polymers used in this study are provided in Table 1.

The water was pre-treated with a Milli-RO Plus unit and then purified by a Milli-Q plus 185 system and filtered through a 0.2 μm Millipak filter. Lysozyme from chicken egg white and bovine serum albumin (BSA) were purchased from Sigma–Aldrich and obtained as lyophilized powders. Lysozyme with an isoelectric point at pH 11.1 [35], carries a net positive charge of about eight elementary charges at pH 7 as determined from hydrogen ion titration curves [36]. BSA, with the isoelectric point at pH 4.8, has a net charge of −11 at pH 6.8 as determined by titration. The effective charge of BSA at the same pH is slightly lower, about −8 as determined with electrophoresis NMR, due to counterion binding [37]. All protein stock solutions for adsorption and AFM experiments were prepared in 10 mM pH 7 phosphate buffer, by mixing K_2HPHO_4 and KH_2PO_4 (from Merck) in a 1:1 M ratio, 24 h before the measurements. The protein stock solutions were just prior to measurements diluted to a protein concentration of 100 ppm, using the same buffer.

3. Methods

3.1. Quartz crystal microbalance-dissipation (QCM–D)

QCM-D measurements were performed using a Q-sense E4 microbalance equipped with a measurement chamber specifically designed for controlled flow measurements. The solution under study was pumped through the measurement cells (four parallel cells operating concomitantly) with a peristaltic pump using a flow rate of 0.160 mL/min. The sensors were AT-cut quartz crystals with gold electrodes coated with silica by means of vapor deposition (Q-sense AB, Gothenburg, Sweden). The crystals are characterized by a fundamental resonance frequency of f_0 = 5 MHz and a sensitivity constant C = 0.177 mg (m^2 Hz)$^{-1}$.

Table 1
Data on the copolymers used in this study. The last column reports the molecular weight of the various copolymers as determined by static light scattering [31].

Brush polymers	METAC (mol%) in feed	METAC (mol%) in copolymer	M_w (kg mol^{-1})
PEO_{45}MEMA:METAC-10	10	10.6	760
PEO_{45}MEMA:METAC-25	25	25.8	660
PEO_{45}MEMA:METAC-50	50	51.3	680
PEO_{45}MEMA:METAC-75	75	75.0	520
Poly(METAC)	100	99.3	145

During measurement the Quartz crystal is allowed to oscillate at its resonance frequency in buffer solution. The resonance frequency (f) and the dissipation (D) of the crystal are accurately determined as described by Rodahl et al. [38]. The dissipation is measured by switching off the driving power and monitoring the amplitude decay profile. The amplitude decays as an exponentially damped sinusoidal function with a characteristic decay time (τ_d). The decay time is related to the dissipation factor (D) as:

$$D = \frac{2}{\omega \tau_d} \tag{1}$$

$$D = \frac{1}{Q} = \frac{E_L}{2\pi E_S} \tag{2}$$

where ω is the angular frequency, Q the quality factor, E_L the energy dissipated during one cycle, and E_S the energy stored in the oscillation.

The frequency decreases and the dissipation normally increases due to adsorption. The change in frequency (Δf) is for a thin and rigid layer related to the adsorbed mass by the Sauerbrey equation [39], but for the viscoelastic layers encountered in this study such a simple treatment is not appropriate [40,41]. Instead, we employed the Voigt model [40].

The sensed mass includes the mass of the adsorbing polymer as well as the mass of the water trapped within the layer, and it can be significantly larger than the adsorbed mass. The dissipation change (ΔD) due to the adsorption is related to the layer viscoelasticity [32,38], where formation of a highly viscoelastic layer results in a high dissipation change. The experiments were started by verifying the stability of both the resonance frequency and the dissipation of the bare substrate in the buffer solution (without polymer or protein present). Next, the bottle-brush polymer adsorption was followed until the frequency and dissipation stabilized at new values. The chambers were subsequently flushed with the same (polymer-free) buffer solution for a period of 10–30 min in order to remove non-adsorbed polymers from the measuring chamber. Next, the protein solution was injected and the frequency and dissipation changes were followed as a function of time. The experiments were concluded by a final rinse with (protein-free) buffer solution.

The silica crystals were cleaned with 2% Hellmanex (Hellma GmbH) for 30 min, followed by rinsing with copious amount of Milli-Q water. The surfaces were left overnight in Milli-Q water before the measurement. The silica surfaces used in all experiments (QCM-D, reflectometry and AFM) are completely wetted by water, suggesting that the surface is highly hydroxylated and thus similar to precipitated colloidal silica.

3.2. Reflectometry

A reflectometer as designed by Dijt et al. [42] was employed. In this instrument linearly polarized light is reflected from the Si/SiO_2/water interface at an angle close to the Brewster angle. The reflected light is split into its parallel and perpendicular components and the respective intensities, I_p and I_s, are recorded using photodiodes. The signal ratio ($S = I_p/I_s$) is continuously recorded during the experiment. The change in signal, ΔS, due to adsorption is related to the surface excess Γ via [42]:

$$\Gamma = \frac{1}{A_s}\frac{\Delta S}{S} \tag{3}$$

The sensitivity factor (relative change in S per unit surface excess), A_s, is determined from Fresnels reflectivity theory as outlined by Dijt et al. [43].