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Factors impacting protein adsorption on layer-by-layer assembled stimuli-responsive thin films



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ABSTRACT

Studying the stimuli-response of polyelectrolyte multilayers (PEMs) towards protein adsorption requires coping with the multiplicity of factors influencing interfacial processes. In this study, we report the design of a nanostructured stimuli-responsive coating based on the sequential layer-by-layer assembly of poly(N-isopropylacrylamide) block copolymers with poly (allylamine hydrochloride) and scrutinize its ability to modulate protein adsorption. The results reveal that the stimuli-response of the film towards protein adsorption under T, pH and I changes is strongly influenced by the type of buffer. This latter parameter has indeed a significant impact on the adsorbed amount of proteins, while it has only slight effect on the stimuli-response of free block copolymer, on the mechanism of PEM growth and the properties of the obtained film. In conditions favorable to protein adsorption, the use of anionic buffer leads to the adsorption of an apparent monolayer of proteins, while in zwitterionic buffer, proteins adsorb in a significantly higher amount. This trend may be explained by a more efficient charge screening of macromolecules by zwitterions which reduce the repulsion between proteins, leading to their accumulation at the interface. Finally, the length of the poly(N-isopropylacrylamide) block is shown to be another key factor impacting the amount of adsorbed proteins. The findings reported in this paper provide relevant information and allow the evaluation of stimuli-response of PEM to be carried out under optimized conditions.

1. Introduction

Since the last decade, the use of block copolymers to build-up sequential layer-by-layer (LbL) assemblies has been increasingly recognized as a powerful method to enhance the functionalities of nanostructured films and to achieve specific properties, such as stimuli-responses [1–3]. In a pioneering work, Webber et al. have shown the ability of block copolymer micelles (BCM) to respond to pH stimulus when adsorbed on a solid surface [4]. Later, the incorporation of block copolymers in polyelectrolyte multilayers (PEMs) through a sequential LbL assembly has attracted a growing interest, showing the ability of these films to respond to multiple stimuli, including pH, temperature and ionic strength [2,5–7]. Poly(N-isopropylacrylamide) (PNIPAM) is one of the most employed polymer for the synthesis of stimuli-responsive block copolymers [8–10]. At temperatures lower than the lower critical solution temperature (LCST), PNIPAM adopts a random-coil conformation, which is maintained through hydrogen bonds between the exposed amide groups and water molecules. Upon a temperature increase above the LCST, a hydrophobic collapse of the chains occurs, leading to the

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formation of water insoluble globules. This property was extensively used for modulating the interaction between PNIPAM-modified surfaces and biomolecules [11,12,13]. The strategy of incorporating stimuli-responsive block copolymers in LbL assemblies is particularly relevant to control the adsorption of proteins. The main advantage lies in the high versatility of the LbL techniques and the widespread possibilities to elaborate nanostructured coatings with tuned properties on almost all types of surfaces. The successful incorporation of Poly(acrylic acid)-b-PNIPAM block copolymers in PEMs has been reported recently, showing their ability to respond to temperature and/or pH towards protein adsorption [14]. Though, the efforts dedicated to exploring PEM-containing block copolymers for the control of protein adsorption remain rather limited. The mechanism of interaction of proteins with PEMs is particularly complex, involving interfacial processes which are not encountered on solid surfaces, such as the formation of protein-polyelectrolyte complex [15] and the interpenetration of proteins within the film [16]. Moreover, this process is expected to be strongly influenced by parameters related to the properties of the block copolymers (block length) and parameters related to the medium (pH, temperature ionic strength and, type of buffer). Indeed, the construction of PEM and subsequent protein adsorption tests are most often performed in buffer solutions containing different ionic species which may be strongly involved at different levels: (i) build-up of PEM, (ii) stimuli-response of the block copolymer, (iii) interaction of proteins with PEM, etc.

In the present work, we investigate the build-up of PEM-containing PAA-b-PNIPAM block copolymers and focus on factors influencing their thermo-response towards protein adsorption. In addition to key parameters, namely temperature (T), pH and ionic strength (I), we study the effect of the type of buffer on the PEM stimuli-response. For this purpose, experiments were conducted in two buffer solutions broadly used in the context of protein adsorption but presenting different characteristics, namely acetate and 2-(4-morpholino)ethane sulfonic acid (MES) buffers. The influence of the block length of PAA-b-PNIPAM copolymer on the stimuli-responsive behavior of PEMs towards protein adsorption was also investigated and evaluated *in situ* by means of quartz crystal microbalance with dissipation monitoring (QCM-D).

2. Experimental section

2.1. Polyelectrolyte and protein solutions

Poly(allylamine hydrochloride) (PAH, Mw = 15 kDa) and poly(acrylic acid sodium salt) (PAA, Mw = 15 kDa) were purchased from Sigma-Aldrich (Belgium). PAA-b-PNIPAM block copolymers were synthesized following the procedure described in a previous report [14]. Their characteristics are given in Table S1 (Supplementary material). Sodium acetate, acetic acid and sodium hydroxide were purchased from Sigma-Aldrich (Belgium). 2-(4-morpholino)ethane sulfonic acid (MES) was purchased from Fisher Scientific (Belgium). Ultrapure MilliQ water (Millipore, France) was used for the preparation of all buffer solutions. Polyelectrolyte solutions were prepared in acetate or MES buffers (50 mmol L⁻¹, pH = 5.70 ± 0.1) at a concentration of 1 mg mL⁻¹. Albumin from chicken egg (ovalbumin, Mw = 46 kDa) was purchased as a powder from Sigma-Aldrich (Belgium). Ovalbumin solutions, with a concentration of 200 µg/mL, were prepared by dissolving the protein powder in a freshly prepared acetate or MES buffers (50 mmol L⁻¹, pH = 5.7 ± 0.1) at room temperature.

2.2. Dynamic light scattering (DLS)

DLS experiments were performed using a Malvern CGS-3 apparatus equipped with a He-Ne laser with a fixed wavelength (632.8 nm) and a thermostat. The device monitors the time-dependent fluctuation of intensity of scattered light from the particles at 90° angle. The raw data were processed using a CONTIN algorithm, a method based on a constrained inverse Laplace transformation of the data that gives access to a size distribution histogram and average hydrodynamic radius (R_H). The value of R_H was obtained from the Stokes-Einstein equation:

$$R_H = \frac{k_B T}{6\pi\eta D} \quad (1)$$

where k_B is the Boltzmann's constant, T is the absolute temperature (°K), η is the solution viscosity (mPa s), and D is the diffusion coefficient of the objects (m² s⁻¹).

PAA-b-PNIPAM was dissolved in acetate or MES buffer (50 mmol L⁻¹, pH = 5.7 ± 0.1) at a concentration of 1 mg mL⁻¹ and kept at room temperature for 1 h. Prior to the measurements, solutions were filtrated through PET membrane (0.2 µm). Heating was carried out by raising the temperature from 25 to 50 °C and the measurements were performed at least three times for each temperature.

2.3. Build-up of PEM

The build-up of (PAH/PAA-b-PNIPAM)_n or (PAH/PAA)_n multilayers was performed on silicon wafers (~1 cm², ACM, France), cleaned prior to use in a piranha solution H₂SO₄ (98%)/H₂O₂ (30%) 1/1 v/v (*Caution! Piranha solutions react violently with organic materials and should not be stored in closed containers*). The substrates were then extensively rinsed with MilliQ water and dried under a nitrogen gas flow. The build-up of PEM was performed by alternately dipping a cleaned silicon wafer in a positively charged PAH and negatively charged PAA or PAA-b-PNIPAM for 5 min. After each dipping step, the samples were rinsed in two different baths (2 min each) of the buffer solution used for LbL assembly.

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