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Influence of the sulfation degree of glycosaminoglycans on their multilayer assembly with poly-L-lysine

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ABSTRACT

We report on the build-up and the intrinsic properties of polyelectrolyte multilayer films from poly-L-lysine and glycosaminoglycans (GAGs) with different sulfation degree, i.e. different charge. We used three complementary techniques, namely electrokinetic analysis (EKA), quartz-crystal microbalance with dissipation (QCM-D) and surface plasmon resonance (SPR), to characterize the assembly process and to assess the properties of the obtained films. EKA elucidated the contribution of the polymers charged groups to the net surface charge of the films and suggested that the assembly process is not solely driven by electrostatic interactions. The combined analysis of QCM-D and SPR data demonstrated that the mechanical properties of the films are dependent on the polymer charge: sulfated GAGs (heparin and chondroitin sulfate) form elastic films while hyaluronan (no sulfation) assembles into multilayer constructs with viscous behavior. The contribution of the water content to these distinct regimes is also discussed. Finally, we show that rather complete characterization of the film properties is possible by SPR employing the two-wavelength and two-media approach: thickness, adsorbed mass, refractive index, and interaction kinetics of the assembly process can be studied by SPR alone.

1. Introduction

During the last decades, layer-by-layer (LbL) assembly of oppositely charged polyelectrolytes (PEs) has attracted considerable attention in the biomedical field because of its simplicity, versatility and the possibilities that it offers for a good control of thickness, composition, viscoelasticity and surface properties of the obtained films [1,2]. The film properties can be tuned by the choice of PE pair (composition, charged groups type, density and molecular weight) and deposition conditions (pH, ionic strength, time and temperature) [3]. Among different polyanions, glycosaminoglycans (GAGs) have been widely employed to prepare bioactive LbL films with natural and synthetic polycations [4]. The GAGs are widespread in living organisms, where they are implicated in a number of vital functions via multivalent non-covalent interactions with other bioentities such as proteins [5]. Due to their high hydrophilicity and water retention ability, free GAGs and their conjugates (e.g. proteoglycans) appear as hydrogels, which are main building elements of the extracellular matrix. The roles they play in living organisms are quite diverse and intimately related to their peculiar chemistry including molecular weight, sulfation degree/position and the related degree of hydration.

In fact, the hydration is directly related with the affinity between the PEs and thus, it has an important role during biorecognition processes: ion pairing of oppositely charged groups (intrinsic compensation) leads to the release of counterions and water molecules from the hydration shells of the PE chains. The entropic gain due to this release is a major driving force of the multilayer assembly [6,7]. Moreover, the amount of water within the multilayers can be correlated with the number and type of cross-links between complementary groups of the PE pair in the film [6,8,9]. For instance, high intrinsic charge compensation or chemical cross-linking leads

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Abbreviations: EKA, electrokinetic analysis; QCM-D, quartz crystal microbalance with dissipation; SPR, surface plasmon resonance; LbL, layer-by-layer; PE, polyelectrolyte; GAG, glycosaminoglycan; PLL, poly-L-lysine; HA, hyaluronic acid; ChS, chondroitin sulfate; Hep, heparin; IEP, isoelectric point.

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to less hydrated films by contrast with high extrinsic compensation or electrostatically assembled networks, respectively. Hence, water content also determines the internal structure and the mechanical properties of the films.

While the importance of water in different interfacial biomolecular processes has been recognized, its quantification in nanostructures such as LbL films is not an easy and straightforward task [7,10–12]. Herein we employ the electrokinetic analysis (EKA) to follow the charge compensation between the PEs together with the "classical" combination of an acoustic (quartz-crystal microbalance with dissipation, QCM-D) and an optical (surface plasmon resonance, SPR) technique to follow the build-up and hydration of poly-L-lysine (PLL)/GAG films onto gold surfaces. Furthermore, we demonstrate the validity of the two-wavelength and two-media SPR approach to determine simultaneously the thickness and refractive index of multilayer films in situ [13–15]. This methodology has a main advantage over standard SPR and other optical techniques as it does not require determining or assuming any of the following parameters: thickness, refractive index, chromatic dispersion (dn/dλ) or refractive index increment (dn/dc). To the best of our knowledge, this approach has not been employed to characterize LbL-assembled films.

2. Materials and methods

2.1. Materials

We have used three GAGs that have different sulfation degree (Ds): the non-sulfated sodium hyaluronate (HA, 25 kDa, Lifecore Biomedical), chondroitin sulfate (ChS, 12 kDa, Ds = 1.6, Kraeber & Co GmbH) and sodium salt of heparin from intestinal mucosa (Hep, 30 kDa, Ds = 2.1, Sigma-Aldrich). Poly-L-lysine hydrobromide (15–30 kDa) and all the remaining chemicals were purchased from Sigma-Aldrich and used without further purification. Gold-coated AT-cut quartz crystals (QSX301, Q-Sense, Sweden) were used for the QCM-D experiments. The EKA measurements were performed using gold-coated glass slides (20 × 10 mm; 20 nm of e-beam deposited gold on top, with a titanium adhesion layer between gold and glass) as substrates. SPR gold-coated sensors (~50 nm) with a chromium adhesion layer (~2 nm) were obtained from BioNavis, Finland. All the substrates were cleaned by immersion in a hot (75 °C) solution of H_2O_2:NH_4OH:H_2O (1:1:5) for 15 min, followed by thorough washing with ultrapure water and absolute ethanol.

2.2. Assembly of polyelectrolyte multilayers

PEs were assembled onto gold surfaces by alternate deposition of 0.5 mg/mL (0.15 M NaCl) solutions of PLL or GAG (HA, ChS or Hep), prepared in Milli-Q water without any pH adjustment (pH ≈ 5.7). All build-ups started with a PLL layer. Each deposition step was followed by rinsing with an aqueous solution of 0.15 M NaCl to remove any unbound PE. In QCM-D experiments, the in-situ deposition of PEs and the rinsing step were both performed under a continuous flow of 100 μL/min for 15 min, whereas in SPR the flow rate was set to 50 μL/min and each step (deposition or rinsing) had a duration of 5 min. QCM-D and SPR measurements were carried out at 25 °C. The films for the EKA experiments were assembled at room temperature by alternately dipping the substrates into the respective PE or salt solutions for 15 min.

2.3. Surface zeta potential measurements

SurPASS electrokinetic analyzer (EKA, Anton Paar, Austria) [16] was used to determine the zeta potentials of the assembled films (up to the 6th layer). Two identical (dry) samples (20 × 10 mm^2) were mounted in the adjustable-gap cell and the gap was set to 100 ± 5 μm. The background electrolyte was 1 mM NaCl. Because the gold surfaces underneath the films are conductive, streaming currents (I_{str}) were measured instead of streaming potentials (U_{str}): I_{str} is not sensitive to surface conductivity effects, whereas U_{str} depends on the cell resistance [17,18]. I_{str} were determined at different pH values within the range 3–10 automatically adjusted with 0.05 M HCl and 0.05 M NaOH, respectively. Each zeta potential, ζ, was calculated and averaged over four measurements using Attract 2.0 software. This software calculates ζ from streaming current measurements employing the Smoluchowski equation Eq. (1):

$$\zeta = \frac{dI_{str}}{dp}\frac{\eta}{\varepsilon_0\varepsilon_r}\frac{L}{A} \quad (1)$$

where p is the pressure drop across the streaming channel, ε_0 is the vacuum permittivity ($8.854 \times 10^{-12}\,J^{-1}\,C^2\,m^{-1}$), ε_r and η are the dielectric constant and the viscosity of the electrolyte solution, respectively, and L and A are the length and the cross-sectional area of the streaming channel, respectively.

2.4. Quartz crystal microbalance with dissipation (QCM-D)

QCM-D measurements were performed in a QCM-D E4 instrument (Q-Sense, Sweden). The temperature was set to 25 °C and a stable baseline was acquired with 0.15 M NaCl before starting with the assembly of the PEs. Quartz crystal oscillation was driven at the fundamental frequency of 5 MHz (N = 1) and at its multiple overtones (15, 25, 35, 45, 55 and 65 MHz corresponding to N = 3, 5, 7, 9, 11 and 13, respectively). Changes in the frequency (Δf) caused by material deposition on the quartz crystal are directly related to the adsorbed mass (Δm) by the Sauerbrey's equation Eq. (2) for rigid films, uniformly distributed on the surface and with a small mass compared to that of the crystal

$$\Delta m = -C\Delta f_N/N \quad (2)$$

where C is the mass sensitivity constant that depends on the resonator (17.7 ng/cm^2 for the 5 MHz crystal) and Δf is the frequency shift caused by the variation of the adsorbed mass (Δm). The "acoustic thickness" (d_{ac}) is the Sauerbrey mass divided by the effective density (ρ_{eff}) of the film [19],

$$d_{ac} = \frac{-C\Delta f_N/N}{\rho_{eff}} \quad (3)$$

Beyond the Sauerbrey limit, the viscoelastic properties of the films can be accessed using an appropriate model (e.g. Maxwell or Voigt) [20]. The Maxwell model is usually applied to polymers with a liquid-like behavior, whereas the Voigt model is used for soft and dissipative films formed on the surface of QCM sensor. We have applied Voigt model to our data. Briefly, this model describes the propagation and damping of an acoustic wave in an oscillating piezoelectric crystal covered by a uniform viscoelastic film in contact with a semi-infinite Newtonian liquid, under no slip conditions [20–23]. The adsorbed film is treated as a viscoelastic solid with a frequency-dependent complex shear modulus given by Ref. [22]

$$G = G\prime + iG'' = \mu_f + i2\pi f\eta_f \quad (4)$$

where G' is the shear storage modulus, G'' is the shear loss modulus, μ_f is the elastic shear modulus, η_f the shear viscosity, and f the oscillation frequency. For adlayers within the boundary conditions of Voigt model, changes in frequency, Δf, and dissipation energy, ΔD, are given by Eqs. S1 and S2 in the Supporting information (ESI) [20]. A weighted least squares fit of the experimental data was performed using at least three harmonics and Q-tools software (v 3.0.6.213, Q-Sense AB). We assumed an effective multilayer density between 1150 and 1200 kg/m^3. The goodness of fits was evaluated by the χ^2 ($\leq 10^6$) and visual inspection.