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Characterization of casein and poly-L-arginine multilayer films

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ABSTRACT

Thin films containing casein appear to be a promising material for coatings used in the medical area to promote biomineralization. α- and β-casein and poly-L-arginine multilayer films were formed by the layer-by layer technique and their thickness and mass were analyzed by ellipsometry and quartz crystal microbalance with dissipation monitoring (QCM-D). (PLArg/casein) films deposited in 0.15 M NaCl exhibit fast (exponential-like) growth of the film thickness with the number of layers. The resulting films were c.a. 10 times thicker than obtained for poly-L-arginine and natural polyanions. We investigated the effect of the type of casein used for the film formation, finding that films with α-casein were slightly thicker than ones with β-casein. The effect of polyethylene imine anchoring layer on the thickness and mass of adsorbed films was similar as for linear polyelectrolyte pairs. Thickness of "wet" films was c.a. two times larger than measured after drying that suggests their large hydration. The analysis of the mass of films during their post-treatment with the solutions of various ionic strength and pH provided the information concerning films stability. Films remain stable in the neutral and weakly basic conditions that includes HEPES buffer, which is widely used in cell culture and biomedical experiments. At the conditions of high ionic strength films swell but their swelling is reversible. Films containing caseins as polyanion appear to be more elastic and the same time more viscous than one formed with polyelectrolyte pairs. XPS elemental analysis confirmed binding of calcium ions by the casein embedded in the multilayers.

1. Introduction

Casein belongs to the heterogeneous group of phosphoproteins precipitated from raw skimmed milk. It is known as one of the most common intrinsically unstructured proteins, IUP's. In mammalian milk and its products it occurs as micelles made up of four major components [1,2] α_{s1}-, α_{s2}-, β- and κ-casein. The function of caseins is to store and transport bio-available metal ions (especially, Ca(II) and Mg(II)) by sequestering and transporting them from mother to the neonates [3–6].

Caseins possess a number of favorable characteristics suitable for the development of hydrogel biomaterials, such as high hydrophilicity, good biocompatibility particularly in oral delivery applications, lack of toxicity and availability of reactive sites for chemical modification. In aqueous solution single protein behaves as flexible, disordered, polyelectrolyte-like molecule [7], therefore, it is easily integrated into polyelectrolyte films [8,9]. Since one of the main features of casein is its ability to bind calcium ions, therefore, it can be used in biotechnology and in biomedical applications to promote biomineralization. A good example are materials for dental implants, which surface modified by films containing calcium crystallites provides better osteointegration [10]. Due to the unique physicochemical properties as natural polymeric surfactant caseins are good candidates for the preparation of conventional and novel drug delivery systems [11]. They can also serve as shield against radiation, particularly UV light, utilizing its strong absorbance around 200–300 nm [12,13]. Casein films have been shown to exhibit high tensile strength making them suitable as coatings for tablets [13–15]. Films containing caseins are used as water based paints or adhesives for labeling of glass containers [16]. Materials covered with casein containing films can be applied in diary industry for the prevention of calcium deposit formation. Caseins can be also used in biosorbents for removal of multivalent metal ions (zinc, cadmium, mercury, chromium) [17,18]. Poly-L-arginine hydrochloride (PLArg) (Fig. 1) is the positively charged, synthetic polyamino acid having one HCl per arginine unit. It is a biocompatible crystalline solid soluble in water. Poly-L-arginine was used as a component of microcapsules for protein or anticancer drug carriers [19]. It can serve as the immunostimulant in the anticancer vaccine [20]. Poly-L-arginine hydrochloride applications additionally include the polyelectrolyte film formation by the

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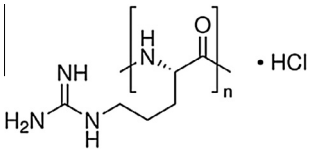

Fig. 1. Poly L-arginine hydrochloride.

layer-by-layer (LbL) deposition technique [21] and the complexation with nucleic acids as sensor components [22] or for gene expression [23].

Sequential adsorption of charged nanoobjects at interfaces is a very versatile technique to form nanostructured thin films. In particular the sequential adsorption of polyelectrolytes (frequently referred to as the layer-by-layer (LbL) technique), which was introduced by Decher et al. [24,25], has attracted much attention in the recent years. Due to the ability of producing multilayers films with well defined thickness and surface properties, the LbL technique can be useful in a wide range of applications [26–29]. Embedding of proteins or other bio-active nanoparticles in polyelectrolyte multilayer films can contribute to the formation of surface nanostructures, which can be used in the biomaterial area [10,29,30].

In our previous work we demonstrated that using poly-L-lysine (PLL) as polycation the polyelectrolyte films containing casein can be formed at surface of silicon wafers [8]. We found that the films were stable in neutral and weakly basic conditions and in the NaCl concentration range from 0.015 to 0.15 M. Casein embedded in such films preserved its ability to bind calcium ions when exposed to calcium chloride solutions [8]. In the present paper we concentrated on formation of polyelectrolyte multilayer films containing α- and β-casein with another biocompatible cationic poly amino acid – poly-L-arginine. Films were formed at the surface of silicon wafers and quartz crystal plates covered with gold (QCM sensors) and their thickness/mass were analyzed in both dry (ellipsometry) and wet (QCM-D) conditions. They were subjected to the post-treatment with the solutions of various composition to determine their stability. Changes of the viscoelastic properties of casein containing films were investigated during their formation and post-treatment. The elastic shear modulus and viscosity of the films were compared with the same parameters for ones formed with polyelectrolyte pairs. Films were also exposed to solutions containing calcium ions to evaluate their stability and the ability of casein embedded in the multilayers to bind calcium.

2. Experimental setup

The α-casein (Cat. No. C6780-1G, min 70%, α-cas) and β-casein (Cat. No. C6905-1G, min 90%, β-cas) from bovine milk, poly-L-arginine hydrochloride (PLArg), MW 15–70 kDa, (Cat. No. P7762), polyethyleneimine (PEI) MW 750 kDa, HEPES (N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid, Cat. No. H6147) and calcium chloride were obtained from Sigma. Sodium chloride pure p.a., hydrochloric acid, sodium hydroxide, sulfuric acid and hydrogen peroxide were obtained from POCh, Poland. Polished silicon wafers were purchased from On Semiconductor Czech Republic, a.s. (Cz/100T-0.5 mm/(100)/P Type). Gold covered quartz crystals QCX301 for QCM-D experiments were obtained from Q-Sense, Sweden.

The substrates for the multilayer deposition, namely silicon wafers and QCM sensors were washed in piranha solution (H_2SO_4:H_2O_2, 1:1), boiled three times in distilled water and rinsed with excess of distilled water. In such a way on the top of silicon wafers a layer of silica was formed. Its thickness was determined by ellipsometry multi-angle analysis and was equal to 3 ± 1 nm. To form the polycation layer 0.1 g/dm^3 PLArg solution in HEPES (10 mM, 0.15 M NaCl, pH7.4) was used. Negatively charged α- or β-casein layers were adsorbed from 0.1 g/dm^3 solution of HEPES buffer (10 mM, 0.15 M NaCl, pH = 7.4). If not noted otherwise the washing steps were performed using HEPES buffer. Further details of the multilayer film formation method can be found elsewhere [8,9]. Before the ellipsometric measurements films were carefully dried in the stream of argon.

The NanoZS Malvern dynamic light scattering (DLS) analyzer was used for zeta potential determination of α- and β-casein and poly-L-arginine. They were measured in 0.15 M NaCl and in dependence of pH of the solution, which was regulated by addition of HCl and NaOH. The Smoluchowski formula was used to calculate zeta potential from the electrophoretic mobility data.

The thickness and optical properties (refractive index, absorbance) of (PLArg/casein)$_n$ multilayer films adsorbed on silicon wafers Si/SiO_2 were determined by the single wavelength (λ = 632 nm) imaging ellipsometer EP^3(Nanofilm) by fitting the "constant $n{\cdot}k$" optical model for film consisting of two layers on solid support – (Si/SiO_2/PE film) to the measured values of the ellipsometric angles Ψ and Δ [31]. The refractive index of PLArg/casein film was found by the multiangle analysis as n = 1.6, while its absorption coefficient k was negligible.

QCM-D (Q-Sense AB Gothenburg, Sweden) technique was used to analyze the mass, thickness and viscoelastic property of polyelectrolyte-casein films adsorbed on AT-cut piezoelectric quartz, gold coated sensors. During the build-up of the alternating layers of PLArg and casein one observed the decrease in frequency of crystal resonance and increase of dissipation of the energy of oscillations, respectively. Changes in oscillating resonance frequency, Δf, are related to mass changes; a negative frequency shift is induced by the mass increase due to polyelectrolyte adsorption. The energy dissipation, ΔD, is related to the viscous losses in the film. A high value of ΔD and its spread for the oscillation overtones indicates a soft film structure. The experimental Δf and ΔD values for the multilayer films during their build-up and conditioning were interpreted basing on the viscoelastic Voigt model [32] and the thickness of the "wet" film, its elastic shear modulus and viscosity were calculated as the best fit parameters.

The X-ray Photoelectron Spectroscopy was utilized for the characterization of surface composition of silicon plates covered with multilayer (PLArg/cas)$_n$ films. XPS/ESCA measurements were carried out using a X-ray photoelectron spectrometer equipped with a semispherical analyzer SES R4000 (Gammadata Scienta) and monochromatic Mg Kα source (1253.6 eV), 400 W power. The extraction angle of a photoelectron was fixed at 90° and the base pressure in the vacuum chamber during measurements was around 8×10^{-10} mbar. Spectra were collected from area of the 3 mm^2 and were worked out by CasaXPS 2.3.12 software. Background was approximated by Shirleys' algorithm. Lines of the spectra were deconvoluted (decomposed) and fitted by Voigt's function. For XPS experiments the multilayer films were prepared on silicon wafers using the same conditions as for ellipsometry. Further details of the procedure of measurements are given elsewhere [8].

3. Experimental results

In our previous work [9] we demonstrated that the optimal conditions for the formation of the multilayer PLL/casein films at pH 7 concerning electrolyte concentration was 0.15 M NaCl i.e. close to the physiological conditions. As it is illustrated in Fig. 2 in those conditions zeta potential of caseins is negative between −40 and −60 mV, while the zeta potential for poly-L-arginine is