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Calcium uptake by casein embedded in polyelectrolyte multilayer

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ABSTRACT

The aim of our work was to investigate formation of polyelectrolyte multilayer films containing α- and β-casein. Since in neutral pH casein is negatively charged (as verified by electrophoretic mobility measurements) it has been used as a polyanionic layer for the film build-up. Casein containing films were formed on Si/SiO_2 wafers. The formation of the film was investigated by liquid cell ellipsometry. After the multilayer films were formed they were contacted with solution containing calcium ions and changes in the film thickness were monitored. Additionally the surfaces of casein containing multilayers were analyzed with AFM for the structural changes within the films occurring after binding of calcium ions. Presence of calcium ions bound in the film was also monitored by XPS. We concluded that casein embedded in the polyelectrolyte multilayers preserves its ability to bind calcium ions.

1. Introduction

In recent years there has been an increased interest in intrinsically unstructured proteins (IUP), i.e., proteins that in their natural state do not adopt stable, folded structures [1–4]. Proteins of this type, abundant in nature, play an important role in living organisms. However, despite their function is well understood, our knowledge concerning adsorption and self-organisation is rather limited. A characteristic feature of IUP's is an open structure, which becomes preserved even after ligand binding. Casein is one of the most common IUP's. It is a phosphoprotein present in mammalian milk and its products, where it occurs in a micellar form made of four major types [5,6]: α_{s1}-, α_{s2}-, β-, κ-casein. α_{s1}- and α_{s2}-caseins comprise 40% and 10%, respectively, of the whole casein content of milk and are usually called α_s-caseins [7]. β-casein comprise 38% of total casein contents [8,9]. 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 [2,7,10,11].

In aqueous solution casein is surface active [11–14] and forms micellar aggregates. Single protein behaves as flexible, disordered, polyelectrolyte-like molecule [15], therefore, it should be easily integrated into polyelectrolyte films. Casein has ability to bind calcium ions and therefore, it can be used in biotechnology and in biomedical applications. Films containing caseins are used as paints, which may be diluted with water or adhesives for labeling of glass containers [16]. Materials covered with casein containing films can be also applied in diary industry for the prevention of calcium deposit formation.

Molecular structures of two types of caseins used in our investigations are illustrated in Fig. 1. α(s1)-casein has molecular weight (MW) 23,000. The chain is build from 199 amino acids with 17 proline residues. It has two hydrophobic regions, containing all the proline residues, separated by a polar region, which contains all but one of eight phosphate groups and is highly charged. Molecular diameter of α(s1)-casein is 9 nm. α(s2)-casein: MW 24,000; 207 residues, 10 prolines. Negative charges are concentrated near N-terminus and positive charges near C-terminus. Both caseins can be precipitated at very low levels of calcium. Molecular weight of β-casein is 24,000. It has 209 residues and 35 prolines. N-terminal region is highly charged, hydrophilic and C-terminal region is a hydrophobic. This amphiphilic protein acts like a detergent molecule. It is less sensitive to calcium precipitation. Its molecular diameter is 7.5 nm. MW of κ-casein is 19,000, 169 residues with 20 prolines. This casein is very resistant to calcium precipitation and it stabilizes other proteins and their micellar aggregates [20–22].

Proline acts as a structural disruptor in the middle of regular secondary structure elements such as alpha helices and beta sheets. However, proline is also commonly found as a first residues of an alpha helix and also in the edge strands of beta sheets. As proline lacks a hydrogen on the amino group, it cannot act as a hydrogen

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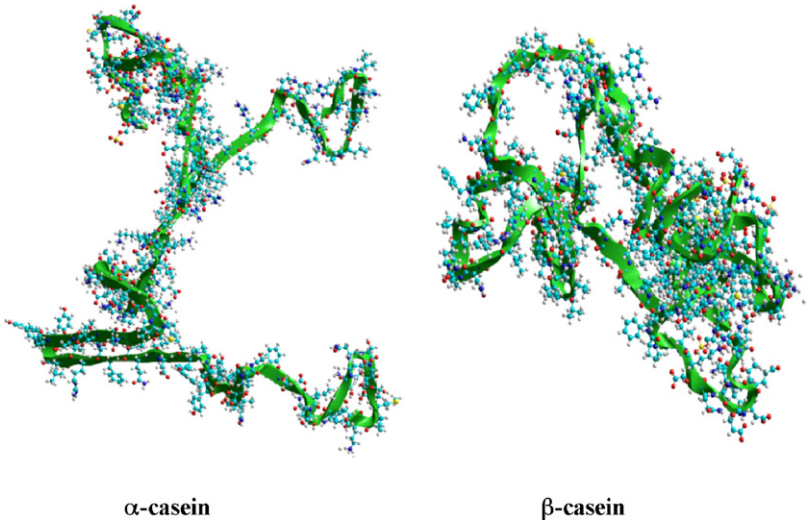

Fig. 1. Molecular models of α and β-casein derived by Thomas F. Kumosinski et al. [17,18] optimized using AMBER99 molecular mechanics force field [19].

bond donor, only as a hydrogen bond acceptor. The large number of proline residues in caseins causes particular bending of the protein chain and inhibits the formation of closely packed secondary structures [16]. Therefore, embedding of praline reach casein into a polyelectrolyte film should not effect to a large extend protein structure. Most of the casein proteins in solution form micelles, which are polydisperse, roughly spherical aggregates with diameter ranging between 150 and 300 nm [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, also referred to as layer-by-layer adsorption technique which was introduced by Decher et al. [24,25], has attracted in the recent years much attention. It has the advantage of producing multilayers films with well defined thickness and surface properties. The layer-by-layer technique can be useful in wide range of applications [26–28]. Embedding of proteins or other bio-active nanoparticles in polyelectrolyte multilayer films can contribute to formation of surface nanostructures [29], which can be used in a biomaterial area.

The aim of our present work was to investigate formation of polyelectrolyte multilayer films containing α- and β-casein and to verify if the casein embedded in the polyelectrolyte multilayers preserves its ability to bind calcium ions. The formation of the casein containing films was investigated by liquid cell ellipsometry. After the multilayer films were formed they were contacted with solution containing calcium ions and changes in the film thickness were monitored. Additionally the surfaces of casein containing multilayers were analyzed with AFM and XPS.

2. Materials and methods

2.1. Materials

α-casein (Cat. No. C6780-1G, min 70%) and β-casein (Cat. No. C6905-1G, min 90%) from bovine milk, poly-L-lysine hydrochloride (PLL), MW 30,000–70,000, (Cat. No. P-2636) and HEPES (N-2-Hydroxyethylpiperazine-N′-2-ethanesulfonic acid, Cat. No. H6147) 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).

2.2. Methods

Malvern NanoZeta dynamic light scattering (DLS) analyzer was used for zeta potential and size measurement of α- and β-casein. They were measured in dependence of pH of casein solution. pH was regulated by addition of HCl and NaOH. Zeta potential and size was measured also in presence of calcium ions. Concentration of Ca^{2+} was 2 and 50 mM.

Thickness of PLL/casein multilayer films were measured by imaging ellipsometer EP3 (Nanofilm). Ellipsometry is a sensitive optical technique that measures the change in the state of the polarization of light upon reflection from a planar interface in order to gain information about its structure and optical properties. In any ellipsometry experiment, upon reflection of the polarised light at a known angle of incidence and with a known wavelength λ, the relative change in the amplitude of the polarised light [expressed as $\tan(\psi)$] and the relative change in the phase difference between the s- and p-components of the light (expressed as Δ) are determined [30]. Fitting appropriate optical model to the measured values of ψ and Δ, thickness and optical properties (refractive index, absorbance) of the film adsorbed at surface are calculated [30].

We used ellipsometer equipped with liquid cell connected with system of syringes filled with appropriate solution needed for experiments (Fig. 2). In our case the wavelength of laser light was λ = 532 nm. The liquid cell was illuminated under the angle of incidence (AOI) 60°. For dry samples AOI = 75° was used.

To build-up multilayer films with casein and PLL we used layer-by-layer technique [24,25], schematically presented in Fig. 3.

The multilayer films were deposited on a silicon wafers Si/SiO_2. Before using as support for the films, wafers 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. To form polycation layer 100 ppm PLL solution in HEPES (10 mM, 0.15 M NaCl, pH 7) was used. PLL as a synthetic polypeptide, consists of the amino acid-lysine, which has an amine group at the end of the side-chain and therefore, it is positively-charged at neutral pH. As a polyanion α- or β-casein (0.1 g/l in HEPES) solution was used. Washing steps were realized by using HEPES buffer.

Multilayer films containing five layers—$(PLL/CAS)_2PLL$ and six layers $(PLL/CAS)_3$ were formed on surface of silicon wafers. Afterwards, wafers with multilayer films terminated with casein or with PLL were immersed in $CaCl_2$ solutions. Concentration of $CaCl_2$ was 2, 20 and 50 mM. Such prepared plates were dried and analyzed