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Adsorption effectiveness of β-lactoglobulin onto gold surface determined by quartz crystal microbalance

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ABSTRACT

Bovine β-lactoglobulin (LGB) is a transport protein that can bind to its structure hydrophobic bioactive molecules. Due to the lack of toxicity, high stability and pH-dependent molecular binding mechanism, lactoglobulin can be used as a carrier of sparingly soluble drugs. Dynamic light scattering has confirmed LGB's tendency to create oligomeric forms. The hydrodynamic diameter of LGB molecules varies from 4 nm to 6 nm in the pH range of 2–10 and ionic strength $I = 0.001–0.15$ M, which corresponds to the presence of mono or dimeric LGB forms. The LGB zeta potential varies from 26.5 mV to −33.3 mV for $I = 0.01$ M and from 13.3 mV to −16 mV for $I = 0.15$ M in the pH range of 2–10. The isoelectric point is at pH 4.8. As a result of strong surface charge compensation, the maximum effective ionization degree of the LGB molecule is 35% for ionic strength $I = 0.01$ M and 22% for $I = 0.15$ M. The effectiveness of adsorption is linked with the properties of the protein, as well as those of the adsorption surface. The functionalization of gold surfaces with β-lactoglobulin (LGB) was studied using a quartz crystal microbalance with energy dissipation monitoring (QCM-D). The effectiveness of LGB adsorption correlates strongly with a charge of gold surface and the zeta potential of the molecule. The greatest value of the adsorbed mass was observed in the pH range in which LGB has a positive zeta potential values, below pH 4.8. This observation shows that electrostatic interactions play a dominant role in LGB adsorption on gold surfaces. Based on the adsorbed mass, protein orientation on gold surfaces was determined. The preferential side-on orientation of LGB molecules observed in the adsorption layer is consistent with the direction of the molecule dipole momentum determined by molecular dynamics simulations of the protein (MD). The use of the QCM-D method also allowed us to determine the effectiveness of adsorption of LGB on gold surface. Knowing the mechanism of LGB adsorption is significant importance for determining the optimum conditions for immobilizing this protein on solid surfaces. As β-lactoglobulin is a protein that binds various ligands, the binding properties of immobilized β-lactoglobulin can be used to design controlled protein structures for biomedical applications.

1. Introduction

β-Lactoglobulin (LGB), which belongs to the lipocalin superfamily, is the major whey protein in cow's milk. Under physiological conditions (pH 7 and concentration > 50 μM) LGB is predominantly dimeric [1,2]. Self-association of LGB to form larger oligomers has been reported in the pH range 3.7–5.2 with a maximum at approximately pH 4.6, just below the isoelectric point [2,3]. The oligomerization is more pronounced for isoform A than for isoform B indicating the involvement of specific interactions in this process [3]. The biological function of LGB is still unclear. It can bind to physiologically relevant ligands such as steroids, fatty acids, retinoids, vitamin D, cholesterol and local anesthetics [4–7]. Despite of many investigations of LGB, adsorption on different surfaces, such as stainless steel [8], chromium [9,10], silicon [11], silica substances [12], polysulfone and polystyrene [13,14], the mode of the mechanism of its adsorption on a solid surface, especially on metals, remains to be clarified [15]. Understanding the adsorption behavior of LGB on metal surfaces is essential for the reduction of biofouling, a problem observed during food and drug production. Analysis of the experimental data shows that there are still inconsistent opinions about the mechanism of β-lactoglobulin protein adsorption concerning adsorption kinetics, structural reorientation or conformation, and protein aggregation whether in a solution or on the surface [16,17].

The properties of the adsorbed protein layer are highly dependent on the shape, effective charge and structure of the protein as these factors influence surface affinity, surface coverage, and hydration of the layer. The reversibility of the protein adsorption process depends on the polarity, hydrophobicity and surface roughness. Also, in the case of proteins, a preferential interaction with the surface is observed, a

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consequence of occurrence of the heterogeneous surface of the protein molecule. The development of new analytical techniques makes it possible to study protein adsorption with increasing accuracy [14,18–21]. Techniques such as quartz crystal microbalance (QCM), or surface plasmon resonance (SPR) enable a highly sensitive, qualitative, realtime, label-free, and noninvasive detection of adsorbed macromolecules.

In this work, we determined several basic physicochemical properties of LGB, including the diffusion coefficient (expressed as hydrodynamic diameter), electrophoretic mobility, which made it possible to determine the isoelectric point, and the non-compensated charge of the LGB molecule. As a substrate for adsorption, we chose gold owing to the fact that this metal is an attractive surface for many biological and medical applications, mostly due to its chemical stability and biocompatibility. Also, the number of investigations concerning the use of a gold surface in modern science is constantly increasing. In our previous work, by using a wide range of methods, we demonstrated that the conditions under which the adsorption occurred had a significant influence on the structure and properties of the adsorbed protein layer [22–24]. To qualitatively describe the LGB adsorption process on the surface of gold, we have determined the zeta potential of gold using the streaming potential method. The high sensitive technique of a quartz crystal microbalance with dissipation monitoring (QCM-D) allows us to determine the adsorbed amount and the visco-elastic properties of the adsorbed layer. Experimental data obtained from the QCM-D measurements were analyzed using the Sauerbrey based model to acquire quantitative information about viscoelastic properties of the protein layers formed on gold surfaces. These investigations lead to a more profound understanding of the self-assembling behavior of LGB layers, which are interesting candidates for drug delivery systems.

2. Materials and methods

2.1. Materials

A mixture of isoforms A and B of bovine β-lactoglobulin (≥90%, Sigma) was used in the study of adsorption. The protein was dissolved in a high purity NaCl solution with a controlled ionic strength ($I = 0.001$, 0.01, 0.15 M). The protein solutions were used without additional purification.

2.2. Chromatography of LGB

Fast Protein Liquid Chromatography (FPLC) was used to analyze a mixture of A and B isoforms of bovine LGB. Separation of the isoform mixture was carried out using the ion-exchange chromatography method with the FPLC ÄKTA purifier system (GE Healthcare).

Separation was performed on a MonoQ GL 5/50 anion exchange column (GE Healthcare) at a flow rate of 2 ml/min at room temperature. For the separation process, high purity deionized water and 0.7 M sodium acetate with pH 6.3 were used [25].

2.3. UV–vis measurements

The LGB adsorption spectrum was measured for protein solutions at concentrations of 1–1000 ppm at an ionic strength of $I = 0.001$ M NaCl, using a UV–vis Evolution 300 spectrophotometer (Thermo Scientific) at wavelengths from 190 to 1100 nm.

2.4. Density measurements

The density of LGB solutions was measured using a DMA 5000 M density meter (Anton Paar), which is based on the oscillating U-tube method. The measurements were conducted for a concentration range from 100 to 7000 ppm at pH 6.5 and ionic strength of $I = 0.001$ M NaCl at 25 °C.

2.5. Viscosity measurements

Viscosity was measured using a Lovis 2000 M/ME rolling ball microviscometer (Anton Paar). The apparatus measured viscosity in the range from 0.3 to 10,000 mPa·s with an accuracy of 0.05%. Measurements on LGB solutions were conducted for a concentration range from 100 to 7000 ppm at an ionic strength of $I = 0.001$ M NaCl at 25 °C.

2.6. Dynamic light scattering and electrophoretic mobility measurements

The size of LGB molecule was measured with the dynamic light scattering (DLS) method using Zetasizer Nano ZS (Malvern). This technique measures the time-dependent fluctuations in the intensity of scattered light that occur because particles undergo Brownian motion. The analysis of these intensity fluctuations enables the determination of the diffusion coefficients of particles, which are converted into a size distribution.

The electrophoretic mobility of LGB solutions was measured using the Doppler effect (LDV – Laser Doppler Velocimetry). The results were used to determine zeta potential and isoelectric point (i.e.p.) of the studied protein.

The hydrodynamic diameter and electrophoretic mobility of LGB at a concentration of 1000 ppm in a NaCl electrolyte solution were measured for three ionic strengths ($I = 0.001$ M, $I = 0.01$ M, $I = 0.15$ M) at pH ranging from 2 to 10.

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

Adsorption of the protein on a gold surface was monitored using a quartz crystal microbalance with energy dissipation monitoring (Q-Sense E1, Biolin Scientific). The flow rate in the measurement system was controlled via a peristaltic pump. The baseline was determined by a 10 min flow of NaCl solution with an appropriate ionic strength. Adsorption of the protein at a concentration of 5 ppm was conducted for $t = 90$ min. After adsorption, the system was rinsed with a NaCl solution of specified ionic strength for $t = 90$ min. Adsorption was conducted at a pH ranging from 3.5 to 9, the pH was adjusted by the addition of high purity HCl or NaOH. The measurements were conducted at 25 °C.

2.8. Surface zeta potential of gold QCM-D sensor

The surface zeta potential of macroscopic solids such as the gold sensor used in the QCM-D experiments is commonly determined from the streaming potential measurements using the classical Helmholtz-Smoluchowski equation

$$\zeta = \frac{dU_{str}}{d\Delta p} \times \frac{\eta}{\varepsilon \times \varepsilon_0} \times \kappa_B \tag{1}$$

where $dU_{str}/d\Delta p$ is the streaming potential coefficient, η and ε are the viscosity and dielectric coefficient of water, ε_0 is the vacuum permittivity, and κ_B is the electric conductivity of the aqueous solution. According to Eq. (1) the streaming potential, which is a d.c. voltage generated by the flow of an aqueous solution through a capillary surrounded by the sample surface gets compensated by the bulk conductivity of the electrolyte solution. The approximation of the conductance of the flow channel by the bulk conductivity is valid for the zeta potential analysis of non-conductive material surfaces. However, in the case of the gold sensor, the QCM-D quartz disk is covered by a conductive gold layer, which serves as the adsorbent surface but also as the electrode for activating the sensor oscillation in the QCM-D experiment. The additional conductance introduced by the gold surface and its effect on the streaming potential coefficient are not considered by Eq. (1) and commonly lead to an *apparent* zeta potential only. Therefore the alternative approach for surface zeta potential analysis, i.e., the measurement of