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Adsorption characteristics of various proteins on a metal surface in the presence of an external electric potential

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ABSTRACT

The effect of the properties of a protein on its adsorption to a metal surface in the presence of external electric potential was investigated. Protein adsorption processes at different surface potentials were measured for fifteen types of proteins using an *in-situ* ellipsometry. The tested proteins were classified into three groups, based on the amount of protein that was adsorbed as a function of the surface potential: In First group of proteins, an increasing trend for the amount adsorbed with a more positive surface potential was found; The amount adsorbed of α-chymotrypsinogen A and ribonuclease A (Second group) were roughly constant and independent of the applied surface electric potentials; In Third group, the amount adsorbed decreased with increasing surface potential. This protein classification was correlated with the isoelectric points of the proteins (First group: ≤9.3; Second group: 9.3–10; Third group: >10). Increasing the pH positively and negatively shifted the surface potentials, allowing ß-lactoglobulin (First group) and lysozyme (Third) to become adsorbed, respectively. The surface potential range for protein adsorption was also markedly shifted depending on the metal substrate type. These findings were interpreted based on the electrostatic interactions among the protein, surface hydroxyl groups, and the applied external electric field.

1. Introduction

The adsorption of proteins onto solid surfaces is a topic that has extensively and continuously intrigued researchers in the fields of surface science and bioengineering [1]. In the adsorption of protein onto a solid surface, various conditions including the pH [2,3], the surface material being investigated [4–8], temperature [9,10], protein type [11–14], type and concentration (ionic strength) of electrolyte [15,16] and related factors [17] have been examined. Regarding the mechanism responsible for the adsorption of a protein to a metal surface, including Cr, Ni, and stainless steel, the electrostatic interactions of the protein molecule with the oxidized surface of the surface passive layer have been reported to be essential [18,19]. Since the net charge of an adsorbed protein molecule and the charge state of the metal (oxide) surface can vary markedly, depending on the pH, the pH dependence of protein adsorption may be explained based on the net charge of the protein and the nature of the solid surface.

Controlling the adsorption of proteins to solid surfaces is a subject of considerable interest in a variety of fields [20]. For example, reducing the amount of adsorbed protein onto the wall in a manufacturing facility in the food and drug industry would facilitate the cleaning of reaction vessels, which involve the use of considerable amounts of water, detergent, energy and are time-consuming [1]. On the other hand, the intentional immobilization of proteins on a solid surface is also a key technology for developing and improving nano-devices such as protein chips [1,20].

Based on the findings that protein-surface electrostatic interactions are crucial for such an adsorption, as described above, it is possible that protein adsorption might be controlled by electrochemically controlling the electric potential of the adsorbent surface. Previous studies have reported on attempts to control (reduce or enhance) protein adsorption by applying an electric potential to the adsorbent metal surface [21–25]. In our previous study [26], we also reported in the influence of an external electric field on the adsorption of a protein to a base metal oxide surface, using lysozyme (LSZ) and six types of base metals (St, Ti, Ta, Zr,

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Cr, and Ni) as model substrates. The results demonstrated the feasibility of the electrochemical control of protein adsorption: The negative polarization of the metal (oxide) surface tended to suppress protein adsorption, whereas it was strongly enhanced when the surface potential range was above a certain value. However, only a limited number of protein types that have been examined studies of adsorption behaviour under an external electric field.

In this study, the adsorption of ß-lactoglobulin (ß-Lg), one of the constituents involved in milk fouling [27,28], to a base metal (oxide) surface was investigated as a function of applied surface applied potential (−1.5∼+0.4 V vs. Ag/AgCl). The amount of protein adsorbed was monitored by an *in-situ* ellipsometry technique. The observed trend for the absorption of ß-Lg to a metal surface was found to be opposite that for the adsorption of LSZ [26]. This strongly suggests that the protein structural characteristics can have a significant effect on its adsorption to a metal surface when a potential is applied to its surface. Hence, the impact of protein type on its adsorption to a base metal (oxide) surface in the presence of an external electric field was examined in this study. The dependence of protein adsorption on the applied surface potential was investigated, using a total of fifteen types of proteins. Relationships between protein characteristics with adsorption to a metal surface as a function of surface potential were investigated.

2. Materials and methods

2.1. Materials

Fifteen types of proteins, model adsorbates, were purchased from Sigma-Aldrich Co. (St. Louis, MO). All of the proteins were of the highest purity (>99%) and were used without further purification. Information on the proteins is listed in Table 1. Stainless steel SUS316L plate (St) (30 × 50 × 2 mm) having mechanically polished mirror surface was normally used in this study. The auger electron spectroscopy analysis of the St plate [44] indicated that the chemical composition on the St surface was [6.9% C, 55.4% O, 5.2% Cr, 29.4% Fe, 3.0% Ni], while [0.0% C, 1.0% O, 17.5% Cr, 68.1% Fe, 12.3% Ni] in the bulk, and the thickness of the passive layer thickness was 3.2 nm of SiO_2 basis. The St plate surface was analyzed by a contact-mode scanning probe microscopy (SPM) using Shimadzu SPM-9500J microscope system (Shimadzu Co., Kyoto, Japan) with a triangular silicon nitride cantilever (spring constant: 0.57 N/m). From the SPM image [Fig. S1], the average surface roughness (R_a) and maximum height (R_z) of the St plate surface were determined to be 0.34 nm and 4.15 nm, respectively. Alternatively, the plates (30 × 50 × 1 mm) of titanium (Ti), chromium (Cr), Tantalum (Ta), Nickel (Ni), and zirconium (Zr) were also used in this study. These base metal plate surfaces had previously been characterized in terms of refractive index, extinction coefficient and hydrophilicities [45,46]. All these metal plates were used in this study and were obtained from the Furuchi Chemical Co. (Tokyo, Japan). Potassium chloride powder and hydrogen peroxide solution (ca. 12 M) were obtained from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). The sample plates that had been used for the adsorption experiment were regenerated by an H_2O_2-electrolysis treatment [47] and were repeatedly used in this study.

2.2. Methods

2.2.1. Protein adsorption onto a metal surface in presence of an external electric field

The adsorption of the protein onto the sample metal surface with an applied electric potential was conducted using the same experimental setup as was used in our previous study [26]. In brief, a sample metal plate was immersed in 20 mM KCl, and a prescribed electric potential was applied to the sample plate, using a potentiostat. Protein adsorption was initiated by adding the protein stock solution so as to produce a final protein concentration of 10 μg/mL. The thickness of the layer of the adsorbed protein was monitored by means of an ellipsometer, in which the sample plate was irradiated with a He-Ne laser (633 nm, wavelength), and the glass cell solution was purged with nitrogen gas at the flow rate of 35 mL/min to prevent gas dissolution and to constantly stir the solution. The refractive indexes (RI) and extinction coefficients of the tested metal surfaces as well as the RI value of the adsorbed protein, used for the calculation of the adsorption layer thickness, had been determined in our previous studies [45,46].

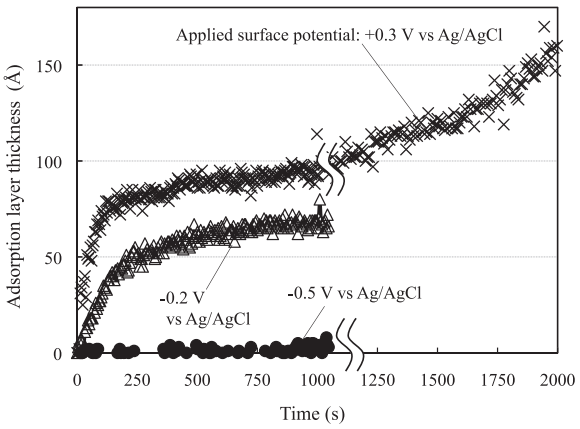

Fig. 1. Courses for the thickness of the adsorbed ß-lactoglobulin (ß-Lg) layer on the surface of a polarized stainless steel plate in a 20 mM KCl solution containing 10 μg/mL ß-Lg at 25 ± 2° C. The pH was 5.8 ± 0.1.

2.3. Fourier transform infrared spectroscopy of protein adsorbed on a metal surface

The relationship between the measured thickness of the protein adsorption layer and the amount of adsorbed protein were determined for all the tested proteins following the same way as was used in our previous studies [26,45,48]. Namely, known amounts (mg) of protein were dry-fixed on the sample plates and analysed for the IR absorption peaks due to protein amide I bands (∼1650 cm^{-1}) by Fourier transform infrared reflection-absorption (FTIR-RA) spectroscopy. The amide I band area of the fixed protein was plotted against the surface protein density (mg-protein/m^2-surface). Next, the correlation between the protein adsorption layer thickness and the amide I band area was determined as follow: After the protein adsorption experiment (Section 2.2.1), the adsorption solution containing protein was replaced with the protein-free one. The thickness of the protein adsorption layer promptly decreased to a constant value (usually within 20 min) and then was measured by the ellipsometer. Hereafter, the sample plate was withdrawn from the protein-free solution and dried by flushing dry nitrogen gas. The IR spectrum of adsorbed protein on the sample plate was measured by an FTIR-RA measurement, from which the area of the amide I band was determined. The amide I band area was converted to the amount adsorbed using the preliminarily obtained calibration line between the amide I band area and the amount of the fixed protein. Finally, the conversion factors from the protein adsorption layer thickness to the amount of adsorbed protein were determined for all the tested proteins and metal plates.

3. Results and discussion

Fig. 1 shows representative time courses for the thickness of adsorbed ß-Lg on a stainless steel surface. As exemplified by the