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Adsorption of lysozyme on base metal surfaces in the presence of an external electric potential

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ABSTRACT

The impact of external electric potential on the adsorption of a protein to base metal surfaces was examined. Hen egg white lysozyme (LSZ) and six types of base metal plates (stainless steel SUS316L (St), Ti, Ta, Zr, Cr, or Ni) were used as the protein and adsorption surface, respectively. LSZ was allowed to adsorb on the surface under different conditions (surface potential, pH, electrolyte type and concentration, surface material), which was monitored using an ellipsometer. LSZ adsorption was minimized in the potential range above a certain threshold and, in the surface potential range below the threshold, decreasing the surface potential increased the amount of protein adsorbed. The threshold potential for LSZ adsorption was shifted toward a positive value with increasing pH and was lower for Ta and Zr than for the others. A divalent anion salt (K_2SO_4) as an electrolyte exhibited the adsorption of LSZ in the positive potential range while a monovalent salt (KCl) did not. A comprehensive consideration of the obtained results suggests that two modes of interactions, namely the electric force by an external electric field and electrostatic interactions with ionized surface hydroxyl groups, act on the LSZ molecules and determine the extent of suppression of LSZ adsorption. All these findings appear to support the view that a base metal surface can be controlled for the affinity to a protein by manipulating the surface electric potential as has been reported on some electrode materials.

1. Introduction

The adsorption of protein to a solid surface often plays a vital role in various industrial fields, such as the construction of biosensors and biochips [1,2], immobilized enzyme preparations [3–5] manifestation of the biocompatibility of artificial implants [6,7], and proteinaceous fouling on manufacturing equipment surfaces in industrial settings [8–11]. Hence protein adsorption is a topic that has been extensively and continuously investigated for more than 50 years [8,9,11].

The factors that determine the protein adsorption behavior are generally classified into three, namely, the nature of the protein, surface characteristics, and the type of medium. Structural flexibility (or fragility) [12,13], isoelectric point (or net charge) [13], and molecular size [14] of proteins directly affect the extent and strength of the adsorptive interactions between a protein molecule and a solid surface as well as the surface area occupied by the adsorbed protein molecule; Metal [15], semiconductor [16,17], glass [18,19], and plastic materials [20–22] all have different adsorption characteristics and presumably different adsorption mechanisms are operative for these materials; The pH of the adsorption medium as well as the electrolyte type and concentration naturally have effects on the ionization state of a protein and the nature of the solid surface [17,23–25], which can have a significant impact on protein adsorption behavior [17,25].

On the other hand, a solid surface always has some electric potential that varies depending on the material type and the contacting conditions. The surface electric potential is naturally considered to be one of the factors that affect protein adsorption behavior. In actual fact, protein adsorption can be altered by applying an external potential to the adsorptive surface, in which several electrode surfaces as adsorption surface have been used [2,26–32]. The adsorption of albumin, cytochrome c, and soybean peroxidase to a Au surface was reported to increase as the result of imposing

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both negative and positive external potentials [28–30], and a similar tendency was observed for the adsorption of fibrinogen to a platinum surface [26]. In the case for a carbon-based electrode as an adsorptive surface, increasing the surface potential from negative to positive resulted in a monotoneous decrease in the surface coverage by the adsorbed protein (bovine serum albumin and fibrinogen) while the adsorption rate showed a complicated dependence on the surface potential [26,31]. It was also reported that the rate and amount of protein adsorption on an optically transparent carbon electrode (OTCE) were increased when a positive external potential was imposed [26,31], which was more significant for a hard protein rather than for a soft one [32]. All these reports demonstrate that it is possible to control protein adsorption by adjusting the external electric potential [2,30].

On the other hand, base metals, such as stainless steel and titanium, are important materials that are used in various industrial processes. The surface of a base metal is usually covered with a thin oxide film, which also has a strong affinity for protein molecules [33–36], resulting in the formation of proteinaceous soiling [8,9,11]. For this reason, the necessity of controlling protein adsorption on base metal (oxide) surfaces has been a subject of intense interest for a long time [3,37]. However, only a few studies have been conducted on how and why the external electric potential affects the adsorption of a protein on the base metal (oxide) surface [27].

The aim of this study was to understand the impact of an external electric potential on the adsorption of a protein to base metal (oxide) surfaces. Six types of base metal substrates and hen egg-white lysozyme were used as an adsorptive surface and a protein, respectively. Protein adsorption in the presence of different external potentials were measured in situ under different conditions, including pH, electrolyte type and concentration, and substrate material, by means of ellipsometry. The attained thickness (amount) of the adsorbed protein and the initial adsorption rate were investigated for the surface potential dependencies. Based on the experimental results, a proposed mechanism for the impact of an external electric potential on the adsorption of a protein on a base metal (oxide) surface is discussed.

2. Materials and methods

2.1. Materials

Six types of base metal plates (Table 1) ($30 \times 50 \times 1$ or 2 mm) that had been mechanically polished to a mirror sheen were purchased from Fruuchi Chemical Co. (Tokyo, Japan). Lysozyme from hen egg white (LSZ) (L-6876) was obtained from Sigma-Aldrich Co. (St. Louis, MO). Potassium chloride, magnesium chloride, and potassium sulfate as an electrolyte and ca. 12 M hydrogen peroxide solution, for the regeneration of the protein-adsorbed base metal surfaces, were the products of Wako Pure Chemicals Industries, Ltd. (Osaka, Japan).

Table 1
Refractive indexes, extinction coefficients, and water contact angles of base metal surfaces used in this study as well as those of the adsorbed lysozyme layer. Standard deviations ($N \geq 10$) for each value are also shown.

Material	Refractive index (−)	Extinction coefficient (−)	Water contact angle (°)
SUS316L (St)	2.6 ± 0.2	4.3 ± 0.2	44 ± 3
Titanium (Ti)	2.5 ± 0.1	3.1 ± 0.2	9 ± 1
Zirconium (Zr)	2.2 ± 0.1	2.7 ± 0.05	66 ± 3
Tantalum (Ta)	2.16 ± 0.05	2.36 ± 0.05	40 ± 7
Chromium (Cr)	4.43 ± 0.02	4.51 ± 0.02	28 ± 4
Nickel (Ni)	2.08 ± 0.05	4.03 ± 0.05	46 ± 6
Lysozyme	1.38 [27]	0	–

2.2. Methods

2.2.1. Protein adsorption onto base metal surfaces with applied electric potential

The adsorption of protein (LSZ) on the base metal surface in the presence of an external electric potential was conducted using the same experimental setup as was used in our previous studies [27,28]. The base metal plate was immersed in 20 mM electrolyte solution (pH 5.8, 550 mL) in a glass cell, and a prescribed electric potential was applied to the plate, using a potentiostat (HSV-100, Hokuto Denko Co., Tokyo, Japan). An Ag/AgCl electrode (immersed in saturated KCl solution) and a platinum sheet ($5 \times 5 \times 0.1$ mm) were used as reference and counter electrodes, respectively. Nitrogen gas was purged into the glass cell solution at a flow rate of 35 mL/min (throughout the adsorption experiment) to minimize gas dissolution and provide constant stirring. Several milliliters of the electrolyte solution in the glass cell was then replaced with the same volume of the LSZ stock solution (~10 mg/mL, in 20 mM KCl solution) so as to give a final protein concentration of 10 μg/mL. Immediately thereafter, the solution in the glass cell was vigorously stirred using a pencil-type mixer (GL. Sciences Co., Tokyo, Japan) with a hand-made rotating tip (8 mm width, 50 mm length) for 10 s, which initiated the adsorption of the LSZ on the sample plate surface. On the other hand, as described later, this study revealed that the application of certain range of potential to base metal surfaces except for Ta and Ti mostly avoided the adsorption of LSZ. Hence, alternatively, the LSZ adsorption was initiated by switching from the non-adsorptive surface potential to a prescribed adsorptive one after the sample plate was sufficiently (~1 h) immersed in the LSZ adsorption with applying the non-adsorptive potential. Both the procedures for the LSZ adsorption exhibited the same adsorption processes, which demonstrated that the 10-s agitation sufficiently homogenized the LSZ solution in the glass cell. The amount of the LSZ adsorbed on the sample plate surface during the LSZ adsorption was measured as the thickness of the layer of LSZ that was adsorbed using an ellipsometer (Mizojiri Optical Co. Ltd., Tokyo, Japan). In the ellipsometric measurement, the sample plate surface was irradiated with a He-Ne laser (633 nm, wavelength) through an optical glass window of the glass adsorption cell. The reflected ray was collected at 1.68-s intervals with a rotating light detector and then converted into the LSZ adsorption layer thickness. In the calculation of the adsorption thickness, the refractive index (RI) of the adsorbed LSZ layer was fixed to be 1.38 on the basis of our previous analyses [38]: Although this constant RI assumption overrode the information on the density of the adsorbed LSZ layer and converted the calculated thicknesses into the apparent ones, it provided a sufficient correlation between the adsorption layer thickness and the amount adsorbed [38]. The RI values and extinction coefficients for the bare base metal surfaces were measured in the LSZ-free aqueous solution using the ellipsometer before the initiation of the LSZ adsorption (Table 1) and used for the calculation of the adsorption layer thickness.

Alternatively, the adsorption experiments were conducted at pHs of 4 and 7 where the solution pH was adjusted by adding small amounts of HCl or KOH, respectively. The pH changes during the adsorption experiments were at most ±0.3.

2.2.2. Fourier transform infrared spectroscopy of adsorbed LSZ on a base metal surface

The relationship between the measured thickness of LSZ adsorption layer and the amount of adsorbed protein were determined following procedures that were used previously [38,39]: (i) The calibration line between the amount of LSZ on the sample plate and infrared (IR) absorption intensity was determined for each sample plate material. Namely, several μL of a LSZ solution (~0.1 mg/mL, in electrolyte free water) were placed on the center of the sample

plate and the sample was then dried to fix LSZ on the sample plate. The sample plate with a known amount of LSZ was then set on the sample stage of a reflection-absorption (RA) accessory (FT-80; SpectraTech, Shelton, CT) inserted in a Fourier transform spectrometer (Magna 560; Nicolet, Madison, WI), so that entire fixed LSZ stain was included inside the sample stage window (13 mm in diameter) to be irradiated by IR light. The IR spectra of different amounts of LSZ on the sample plates were obtained at a resolution of 8 cm^{-1} with 64 scans. The area of the IR band at around 1650 cm^{-1} due to carbonyl stretching vibration (amide I band) of the fixed LSZ was determined from the obtained IR spectrum and plotted against the surface LSZ density (mg-LSZ/m^2-surface), derived from the amount of fixed LSZ divided by the area of the sample stage window; (ii) IR spectra of the sample plates, that were subjected to the LSZ adsorption (and for which the thickness of the LSZ adsorption layer was measured), were obtained from time to time using the same procedure as described above (i). The area of the IR absorption due to amide I band of the adsorbed LSZ was determined from the IR spectrum and converted into the amount of adsorbed LSZ (mg/m^2) by using a calibration line obtained by procedure (i). Based on the data sets for the LSZ adsorption layer thickness and the amount of adsorbed LSZ for each sample plate material, and by assuming that the thickness of the adsorbed layer is proportional to the amount adsorbed [38,39], the thickness of the LSZ adsorption layer was converted into the amount of adsorbed protein.

2.2.3. Regeneration of sample plates

All the sample plates were used repeatedly in the LSZ adsorption experiment. For the regeneration of a sample plate that had been used for LSZ adsorption as well as the pretreatment for the LSZ adsorption, the sample plate was subjected to a H_2O_2-electrolysis cleaning procedure [38,40] to completely remove the adsorbed LSZ. Namely, after the LSZ adsorption experiment, the LSZ solution in the glass adsorption cell was replaced with a protein-free 20 mM KCl solution containing 10 mM H_2O_2. A negative electric potential ($-0.4 \sim -1.6$ V vs Ag/AgCl) was then applied to the sample plate with the adsorbed LSZ using the same potentiostat system as was used in the LSZ adsorption experiment. The adsorbed LSZ on the sample plate was effectively removed from the sample plate by hydroxyl radicals generated due to the electrolysis of H_2O_2 ($H_2O_2 + e^- \rightarrow {}^{\bullet}OH + OH^-$) [40], which was monitored by an ellipsometer. The H_2O_2-electrolysis treatment was continued for more than 30 min until a further decrease in the adsorption layer thickness was negligible.

All of the above experiments were done at $25 \pm 2\,^{\circ}C$ and repeated at least in duplicate for each condition.

2.2.4. Contact angle measurement

The contact angles of a sessile water droplet on the base metal substrates used in this study were measured by means of a goniometer at $25 \pm 2^{\circ}$ C to evaluate the hydrophobicity/hydrophilicity of the substrates. More than ten independent measurements were conducted at different locations of the surface for each base metal substrate.

3. Results and discussion

Fig. 1 shows the representative processes for the adsorption of LSZ on a base metal surface (St) at different applied electric potentials. Typically, the increase in the thickness of the LSZ layer proceeded via three steps, as shown in Fig. 1. Namely, (1) the LSZ adsorption layer thickness rapidly increased immediately after the start of the adsorption; (2) The increase in the adsorption layer thickness then slowed down, (3) followed by reaching a maximum value within ca. 3000 s, although the LSZ adsorption layer thickness continued to increase throughout the experimental period ($\sim$7000 s) under certain conditions (curve for -0.3 V vs Ag/AgCl, in Fig. 1). Hence, the LSZ adsorption processes under different conditions were analyzed from the maximum amount of adsorbed LSZ and the initial adsorption rate at a given LSZ concentration.

In Fig. 2(a), the amount of LSZ adsorbed on a St surface at pHs 4.0, 5.8, and 7.0 are shown as a function of the applied surface potential. In the cases of the consecutive adsorption, the amounts of LSZ adsorbed at a time point of 7000 s were retrieved and are also plotted in Fig. 2(a) as closed keys. As shown in Fig. 2(a), increasing the surface potential decreases the amount of LSZ that is adsorbed, and the relationship between the amount of LSZ adsorption and the surface potential appears to be shifted toward a more positive potential with increasing pH. It should be noted that the adsorption of LSZ is minimized in potential ranges above certain thresholds.

In our previous study [27], the adsorption of ß-lactoglobulin, which is acidic (pI 5.1 [41]), on a St surface was found to be largely prevented when a negative potential was applied to the St surface.

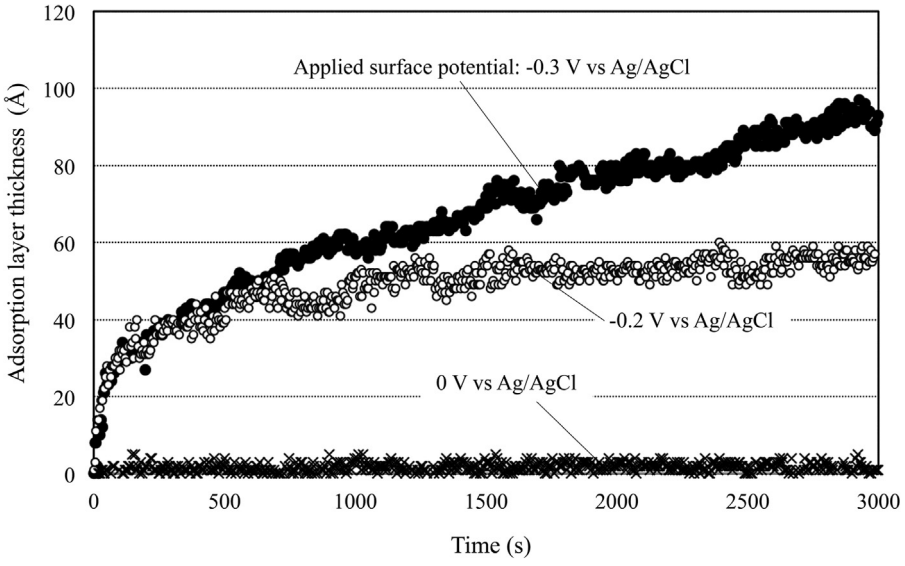

Fig. 1. Courses for the thickness of the adsorbed LSZ layer on externally polarized stainless steel plate surface in a 20 mM KCl solution containing 10 μg/mL LSZ at $25 \pm 2^{\circ}$ C.

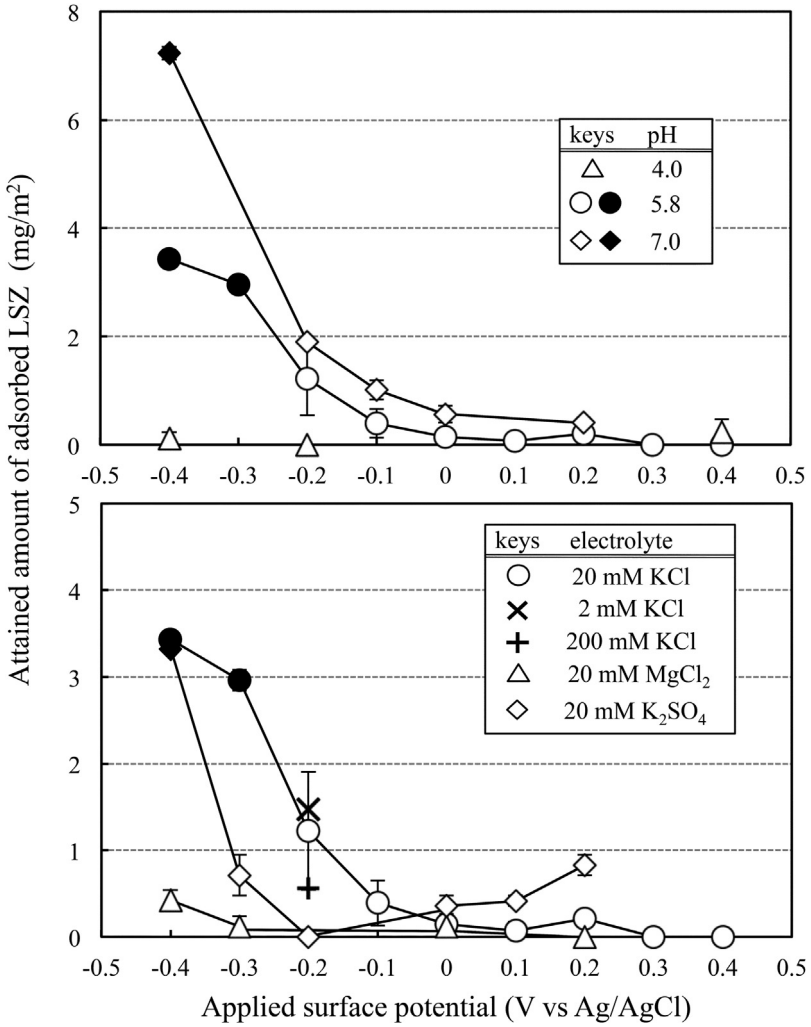

Fig. 2. Amounts of LSZ adsorbed on St as a function of applied surface potential under different conditions, including (a) pH and (b) type of electrolyte (pH 5.8). The electrolyte concentration was 20 mM (a) or varied from 2 mM to 200 mM (b). LSZ concentration and temperature were 0.01 mg/mL and 25 ± 2° C, respectively. Closed keys denote the amount adsorbed at 7000 s for the cases of consecutive adsorption. Error bars represent the highest and lowest values obtained for each condition.

On the other hand, as shown in Fig. 2(a), the adsorption of the basic LSZ was minimal in the positive potential ranges. These suggest that the protein adsorption onto a base metal surface also can be controlled by controlling the surface electric potential, as reported for those onto the electrode materials [2,26–32], and that the surface potential range for the protein adsorption control is varied depending on the net charge of the protein.

In an aqueous solution, the oxide surface on a base metal is covered with hydroxyl groups, and the surface −OH groups can acquire various charges, i.e., cationized ($-OH_2^+$) or anionized ($-O^-$) at pHs below or above the isoelectric point of the surface, respectively [23,24]. Our previous studies [13,35,42] revealed that these ionized surface hydroxyl groups serve as sites for electrostatic interactions with acidic and basic amino acid residues of a protein, which are largely responsible for protein adsorption on a base metal surface (without applying any electric potential). Acidic and basic proteins thus tend to adsorb on a base metal surface under acidic and basic conditions, respectively, where surface hydroxyl groups largely exist as $-OH_2^+$ and $-O^-$, respectively [23,24]. On the other hand, an electrically polarized surface would naturally attract or repel protein molecules. Such an electric field-based interaction was actually proposed as one of the main mechanisms for the adsorption of a protein on electrode surfaces in previous studies [2,26–32].

Based on these considerations and according to the pI value of St surface (5.8 [43]), the dependences of the amount of LSZ that is adsorbed on pH and applied surface potential (Fig. 2(a)) can be explained as follows: In the case of a pH below the surface pI value, electrostatic repulsions likely occur between LSZ molecules and surface $-OH_2^+$ groups (upper cases in Fig. 3), and the range of negative applied potential used may not induce a sufficiently strong an electric field-based attraction to overcome the repulsion against surface $-OH_2^+$ groups at pH 4 (upper left in Fig. 3). As the pH becomes higher, the electrostatic repulsion between LSZ and surface hydroxyl groups is weakened by the conversion of surface $-OH_2^+$ to −OH or $-O^-$ (middle cases in Fig. 3), and it would follow that the adsorption of LSZ would occur due to an electric field-based attraction in the negative potential range (middle left in Fig. 3), as observed at pH 5.8 (Fig. 2(a)). When the pH increases above the surface pI value (bottom cases in Fig. 3), the electrostatic repulsion between LSZ and surface hydroxyl groups then turns into attractive forces. Therefore, a more positive surface potential (and thus a greater electric repulsion for LSZ) may be required to minimize LSZ adsorption by the increased electrostatic interactions between LSZ and surface $-O^-$ groups (bottom right in Fig. 3), as observed in Fig. 2(a).

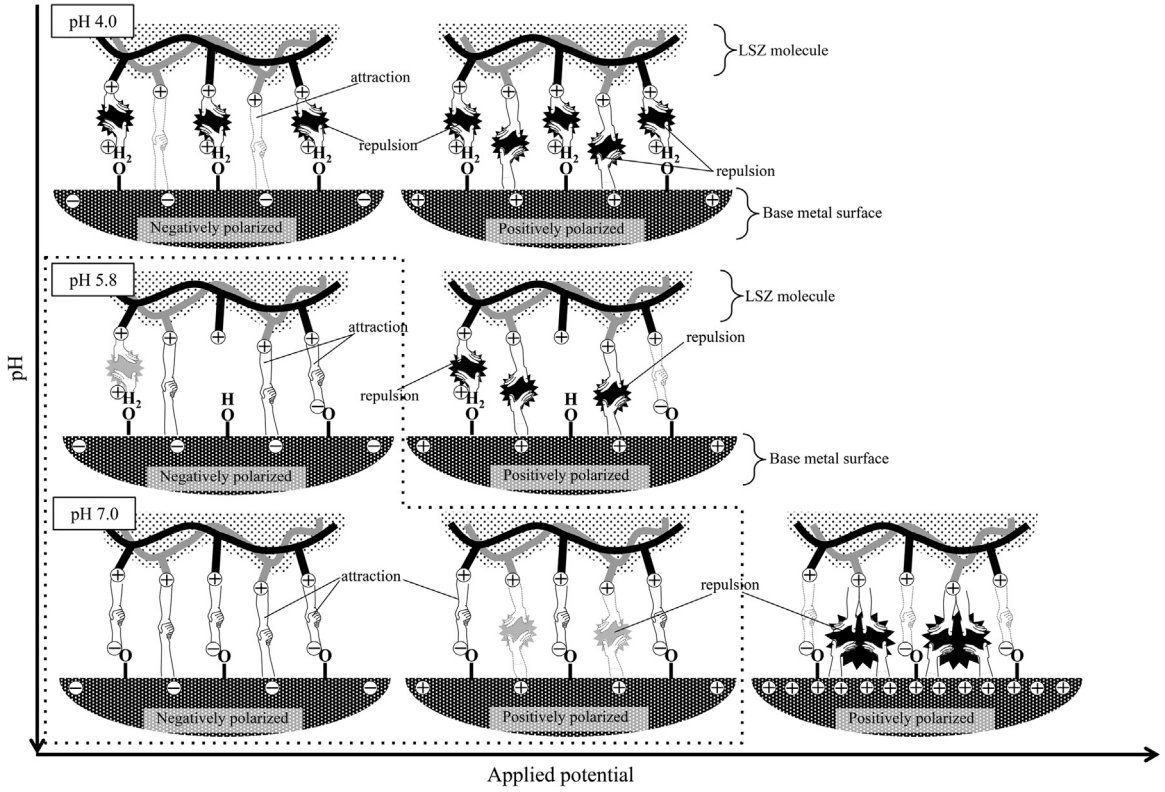

Fig. 3. Possible mechanism of the dependences of LSZ adsorption on pH and applied surface potential. The cases surrounded by dotted lines allow the LSZ adsorption on a base metal surface.

As shown in Figs. 1 and 2(a), when the applied surface potential is lowered below a certain value, the adsorbed layer of LSZ continues to increase in thickness throughout the experiment (~7000 s). A similar phenomenon has been observed in previous studies [31,32]. Benavidez and Garcia [31] proposed that an external electric potential may induce polarization of the protein; One end of the polarized protein binds to the surface (or adsorption layer) and the other end serves as an adsorption site(s) for consecutive protein lamination (adsorption). However, from the fact that the LSZ adsorption does not occur on the positively polarized surface, it is considered that a LSZ molecule may not be polarized so as to provide the binding (negative) sites for the positively polarized surface. This conflicts with the above mechanism based on the intramolecular polarization of the adsorbed protein [31]. The other possible explanation is as follows: When the sample plate surface is extensively covered with the adsorbed LSZ, the screening of the surface potential by the adsorbed LSZ molecules would become significant. By the potential drop due to the electric resistance of the adsorbed LSZ layer, an additional potential may be applied to the sample plate, which would induce further LSZ adsorption via electric field-based attraction. Consequently, the adsorption of LSZ and amplification of the applied surface potential may continue to occur, resulting in the continuous adsorption of LSZ, as shown in Fig. 1.

The amounts of LSZ that were ultimately adsorbed (on a St surface at pH 5.8) were measured in the presence of different types of electrolytes (KCl, $MgCl_2$, K_2SO_4) as well as at different KCl concentrations (Fig. 2(b)). In the negative surface potential range, an increase in KCl concentration and the presence of 20 mM $MgCl_2$ or K_2SO_4 instead of KCl resulted in a decrease in the final amount of adsorbed LSZ, as shown in Fig. 2(b). This can be attributed to the increase in ionic strength, which is consistent with the contribution of an external surface electric field on LSZ adsorption. On the other hand, a slight increase in the amount of LSZ that was adsorbed was detected at the positive surface potentials in the presence of 20 mM K_2SO_4. This can be attributed to the surface-bound divalent anion (SO_4^{2-}) functioning as a bridge between the positively charged LSZ molecules and the positively polarized surface [44,45].

The LSZ adsorption processes were measured for different five base metals in the applied potential ranges where the generated electric currents were ignorable (<several $\mu A/cm^2$). In Fig. 4, the amount of adsorbed LSZ on different base metal surfaces are plotted as a function of the applied surface potential, as well as the amount of LSZ adsorbed at 7000 s for the case of the consecutive adsorption (closed keys in Fig. 4). As shown in Fig. 4, increasing the surface potential generally tends to decrease the LSZ adsorption, which is similar to the tendency observed for the St substrate (Fig. 2). Interestingly, the Ta surface shows a noticeable increase in the amount adsorbed at around +0.2 V vs Ag/AgCl, and a possible mechanism for this is discussed below.

The tested base metals except Cr show the consecutive LSZ adsorption when the amount of adsorbed LSZ exceeds ca. 3 mg/m^2. The threshold for the amount adsorbed coincides with the adsorption of a monolayer of protein [3]. This suggests that the surface area occupied by the adsorbed LSZ molecule may be the same for the tested base metal surfaces and, furthermore, supports the above interpretation that origin of the consecutive adsorption may be due to the screening of the surface potential as the result of the adsorbed LSZ.

It is naturally expected that the surface roughness of sample plate could affect the adsorbed amount of a LSZ monolayer. However, the adsorbed amount thresholds for the consecutive adsorption for different base metal surfaces appear within the same range (Fig. 4), indicating that the base metal plates used in this study did not have any significant difference in the surface roughness.

As shown in Fig. 4, the potential ranges where the LSZ adsorption is minimal are different among the examined base metals. Namely, (when compared at pH 5.8,) the adsorption of LSZ on St and Ti surfaces is minimized at potentials at around and above 0 V

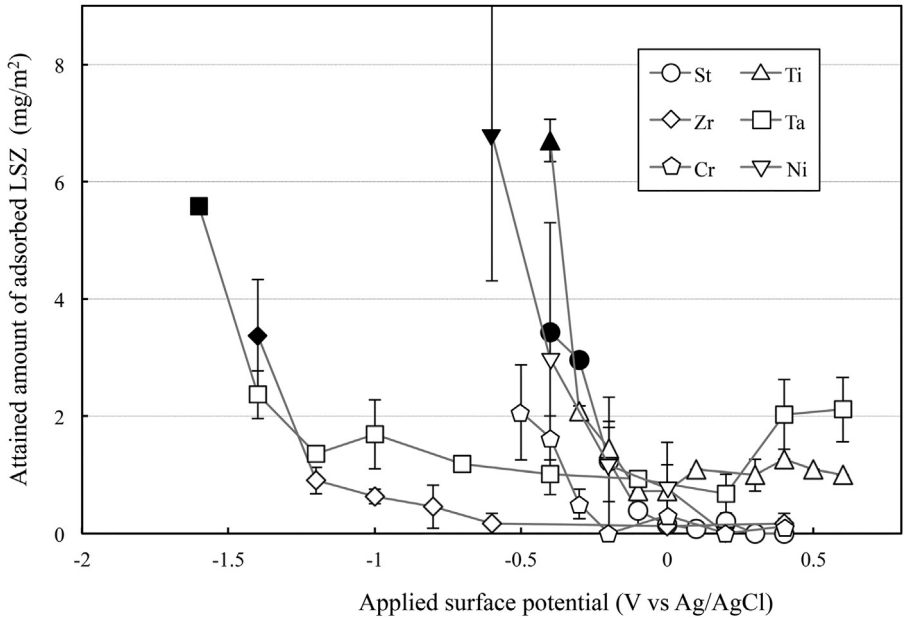

Fig. 4. Amounts of LSZ adsorbed on different base metal surfaces as a function of applied surface potential. The electrolyte concentration was 20 mM (pH 5.8). LSZ concentration and temperature were 0.01 mg/mL and 25 ± 2° C, respectively. Closed keys denote the amount adsorbed at 7000 s for the cases of consecutive adsorption. Error bars represent the highest and lowest values obtained for each condition.

vs Ag/AgCl, and, for Ta and Zr, LSZ adsorption is minimized in the potential ranges >−0.4 and >−0.6 V vs Ag/AgCl, respectively. The threshold potentials for Cr and Ni appear slightly (±0.2 V) more negative and positive than that for St and Ti, respectively. Regarding the minimum values of the amount of LSZ adsorbed, the extent of absorption was approximately 1 mg/m^2 for Ta and Ti, even in the potential range where LSZ adsorption is minimized. The amounts of LSZ adsorbed on the other surfaces are largely decreased below 0.3 mg/m^2 in the potential ranges for the minimal LSZ adsorption (Fig. 4).

Based on the above interpretation of the dependences of LSZ adsorption on pH and applied surface potential (Fig. 3), the potential range for allowing LSZ adsorption (Fig. 4) may indicate the magnitude of the electrostatic repulsion between LSZ and surface cationized hydroxyl groups: In the cases of Ta and Zr, a more negative surface potential is required to overcome the repulsion between LSZ and surface $-OH_2^+$ groups and to allow the LSZ adsorption to proceed than in the cases for the others. However, there found no correlation of the adsorption threshold potential with the isoelectric point of base metal [24,43,46] although the ionization state of surface hydroxyl groups is determined by the surface isoelectric point. Such a conflict may occur because the surface treatment history can alter the isoelectric point [47,48] while further investigations would be needed to confirm this.

As shown in Figs. 2 and 4, the adsorption of LSZ on St, Ti, Zr, Cr, and Ni surfaces occurs only in potential ranges below certain values. Similarly, a carbon-based electrode was also reported to support protein adsorption only in the positive or negative potential range [28,30]. On the other hand, previous studies indicated that the adsorption of a protein on Au [28,30] and Pt [26] was significant both in the negative and positive potential ranges. Accordingly, the electroconductive surfaces can be categorized into two types, according to the dependence of protein adsorption on the surface potential. Considering the contribution of a divalent anion (SO_4^{2-}) on the adsorption of LSZ on the St surface at positive potentials (Fig. 2(b)), the difference in the surface potential dependences could have originated from the binding of counterions on the surfaces. Namely, counterions, including monovalent ones, could be bound on a noble metal surface so tightly as to bridge protein molecules with the repulsive charges to the surface. This bridging effect of surface-bound counterions may also be responsible for the adsorption of LSZ on the Ta surface at potentials >0.2 V vs Ag/AgCl (Fig. 4). In contrast, the high hydrophobicity of the carbon-based electrode surface and the hydroxyl groups on the base metal surface may preclude any tight surface binding of monovalent counterions, resulting in minimizing protein adsorption at potentials that are repulsive to protein molecules.

Our previous studies demonstrated that electrostatic interactions between ionized groups of a protein and surface hydroxyl groups largely contribute to the adsorption of a protein on base metal surfaces [13,35,42,49]. On the other hand, it has also been suggested that hydrophobic interactions [50] and complexation with oxide species [51,52] could be related to the adsorption of a protein on a solid surface. These, as well as counterion-bridging, might explain the persistence of the LSZ adsorption on Ti and Ta surfaces in the positive potential range (Fig. 4). However, the contact angles of the base metal surfaces that were examined (Table 1) indicate that the base metal surfaces are generally hydrophilic, and the contribution of hydrophobic interactions may thus be quite limited for the adsorption of LSZ to the base metal surfaces, including the persistent ones to the positively polarized Ta and Ti surfaces (Fig. 4).

The first order adsorption rate constants were determined from the adsorption process by dividing the initial increasing slope by the thickness of the attained adsorption layer (Fig. 5). As shown in Fig. 5, the rate constants for the adsorption of LSZ are mostly in the same order of ~0.01 s^{-1} and appear to be independent of the applied surface potential and the surface material as well as the type of or the concentration of the electrolyte used. On the other hand, previous studies dealing with the adsorption of protein on electrode surfaces [2,26,28–32] indicated that the initial adsorption rate was increased in the presence of an external electric field. In this study, the protein concentration used (10 μg/mL) was one tenth or lower than those in the reported literature (100–500 μg/mL) [2,26,28–32], and thus the diffusion of LSZ molecules on the sample plate surface can be assumed to be the rate-limiting step in the

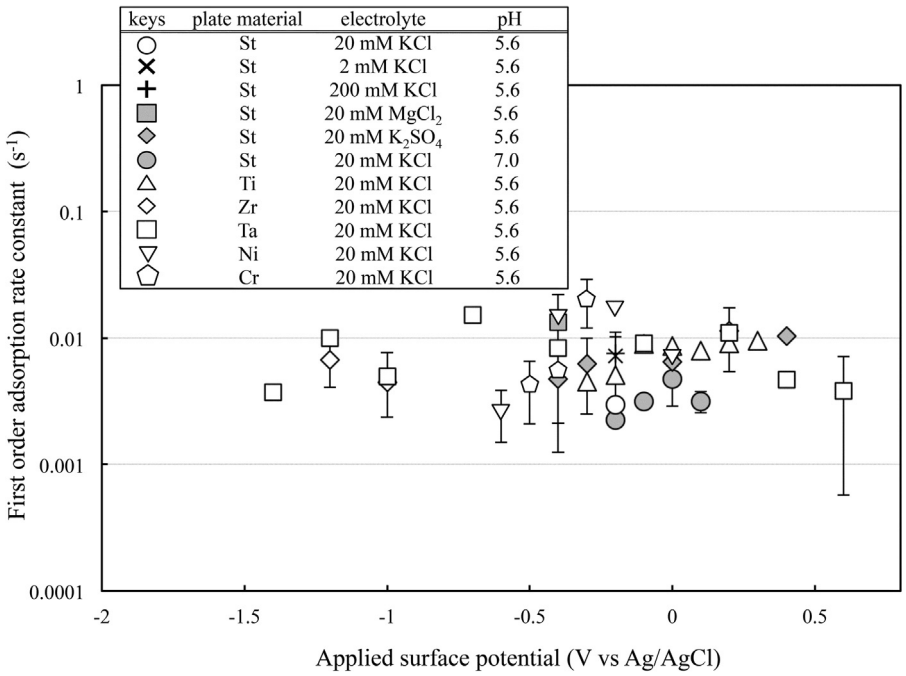

Fig. 5. Initial LSZ adsorption rate under different conditions as a function of applied surface potential. The LSZ concentration and temperature were 0.01 mg/mL and 25 ± 2° C, respectively. The adsorption rate constants were determined by dividing the initial slopes of the LSZ adsorption process by the amount of adsorbed LSZ. The adsorption rate constants for consecutive adsorption were not determined because of the absence of the amount adsorbed. Error bars represent the highest and lowest values obtained for each condition.

adsorption in the testing conditions. Accordingly, the initial rate of adsorption may not be increased, even in the case of an additional mechanism (electric field-based one) for LSZ adsorption, as shown in Fig. 5.

According to these findings, the adsorption of protein on an electrically-polarized base metal surface can be considered to be closely related to the two modes of interactions, that is, electrostatic interactions between the protein and ionized surface hydroxyl groups and electric field-based interactions. This study suggests that the nature of the ionization of surface hydroxyl groups may vary with the type of surface material, and the external electric potential clearly determines the direction (attraction or repulsion) and strength of the electric field-based interaction. Considering these factors, it may be possible to control the adsorption of a protein on a base metal surface appropriately adjusting the applied surface potential, as has been indicated for the protein adsorption on the electrode materials [2,30]. However, while lysozyme is a small rigid molecule, a large and/or structurally flexible protein would undergo a significant conformational change upon its adsorption [3,11,12], which may affect adsorption behavior in the presence of an external electric field. Hence, further studies using different types of proteins are planned as the next step of this study to examine the general validity of the methodology for controlling protein adsorption on a base metal surface by applying an electric potential to the surface.

4. Conclusions

The adsorption of hen egg white lysozyme (LSZ) on base metal surfaces (stainless steel SUS316L (St), Ti, Ta, Zr, Cr, or Ni) in the presence of an external electric field was investigated, focusing on the impacts of surface electric potential, pH, electrolyte type and concentration, and base metal type. The adsorption of LSZ for different conditions was characterized by the amount of LSZ that was adsorbed and the initial adsorption rate at a given LSZ concentration (10 μg/mL). The amount of adsorbed LSZ was increased with more negative potentials in the potential range below a certain threshold and was minimized at potentials above the threshold. The threshold potential for the adsorption of LSZ was higher at higher pH, and when the amount of LSZ adsorbed increased above that of a monolayer, the adsorption turned from a finite mode into a consecutive one. Considering these results and the knowledge of the ionization states of hydroxyl groups on a base metal surface, the adsorption of LSZ was deduced to depend on (i) the electric force by an external electric field and (ii) electrostatic interactions between the positively charged LSZ and ionized surface hydroxyl groups. Namely, the adsorption of LSZ on a base metal surface may occur when the (i) electric field-based interaction surpasses the (ii) electrostatic repulsion between positively charged LSZ and surface $-OH_2^+$ groups (or when LSZ molecules form stronger (ii) electrostatic interactions with surface $-O^-$ groups than (i) electric repulsion to the surface). The presence of a divalent anion (SO_4^{2-}) instead of a monovalent one (Cl^-) resulted in an increase in LSZ adsorption in the positive potential range, possibly because the surface-bound divalent anion (SO_4^{2-}) serves as a connection between both the positively charged (polarized) LSZ and the surface. Among the different base metal types, the Ta and Zr surfaces exhibited a markedly lower threshold potential for LSZ adsorption than the others, implying the existence of more cationized surface hydroxyl groups at the tested pH (5.8). All these findings support the conclusion that the adsorption of protein on a base metal surface can be controlled via the use of an external electric potential, which has been suggested also for the protein adsorption on the electrode materials. On the other hand, protein adsorption often causes a conformational change, which is more significant for larger and/or softer proteins and would likely have an effect on the adsorption of a protein to an electrically polarized surface. The surface electric field-based influence on the adsorption of a protein to a base metal surface should therefore investigated using different types of proteins and findings in the area will be reported in the near future.

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