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The adsorption process of bovine serum albumin (BSA) onto a stainless steel surface was investigated using the quartz crystal microbalance based on admittance analysis. The adhered mass change Δm increased with time as a result of contacting the BSA solution, and considerably long period (>2 h) was required for the attainment of the asymptotic values of Δm as well as dissipation factor ΔD. The relation between ΔD and Δm suggested that the layer of adsorbed BSA molecules became stiffer with increasing time at higher BSA concentration. The relation between Δm after 2 h and the final BSA concentration was described well by the Langmuir adsorption isotherm. However, the time course of Δm clearly deviated from the Langmuir adsorption model. The stretched exponential function model described the time course of Δm well although it was an empirical one.

Key words: QCM-A; protein adsorption; bovine serum albumin; stretched exponential function; dissipation factor

Adsorption of proteins onto solid surfaces is an important issue in food industry. During food manufacturing processes, it has been reported that proteinaceous deposits are often formed on equipment surfaces.[1] Deposits like this potentially act as a nutrient for product spoilage micro-organisms and pathogens. They also can cause damage and impaired performance of equipment. Therefore, the equipment surface must be cleaned regularly to remove them. Cleaning of food manufacturing equipment requires much water, chemicals, energy, and time. To raise cleaning efficiency of food manufacturing equipment and food processing line, understanding the behavior of food protein adsorption on the food-contact surface is important.

There have been many studies about food protein adsorption onto a solid surface.[2–5] Because stainless steel has been used extensively as a material for food production equipment, adsorption behavior of food proteins onto a stainless steel surface was investigated for various food proteins such as bovine β-lactoglobulin,[6]

lysozyme,[7] ovalbumin,[7] ovomucoid,[7] bovine serum albumin,[8–11] and gelatin.[8]

The quartz crystal microbalance (QCM) has seen an impressive broadening of uses for study of biomolecule adsorption onto a solid surface.[12,13] It is a very sensitive mass measuring device and has been used as a real-time and non-labeling mass sensor in aqueous solution. When elastic substances adhere on the QCM plate, the resonance frequency (F_r) decreases linearly with increasing mass Δm on the QCM plate. The change in resonance frequency ΔF_r is described by the Sauerbrey equation as shown below.[14]

$$\Delta F_r = -\frac{2F_0^2}{A\sqrt{\mu_q \rho_q}}\Delta m \qquad (1)$$

where F_0, A, μ_q, and ρ_q are the change in F_r, the fundamental resonance frequency of the quartz crystal, the electrode area, the shear modulus of quartz, and the density of quartz, respectively.

However, it has been recently pointed out that the Sauerbrey equation is not applicable when viscoelastic substances such as most of hydrated biomolecules because not only mass change but also change of viscosity which will come from both of bulk solution and adhered viscoelastic substances affected the change of resonance frequency.[12,13,15,16] Therefore, the quantitative QCM measurement of adsorption of biomolecules such as protein sometimes becomes difficult. To overcome or complement this drawback of QCM, the new type of QCM has been developed recently such as QCM-D[16–18] and QCM-A.[13,19,20] Both methods can estimate the viscosity contribution separately by obtaining the energy dissipation (D factor) that indicates energy loss from the viscous components of both solution and adhered substances. The QCM-D (quartz crystal microbalance with dissipation) has become a popular method to investigate adsorption and the following structural changes in biomolecules on the QCM plate.[21–24] However, the QCM-D still cannot estimate the amount of adhered substance without effect of change of viscosity from both solution and adhered substance. On the contrary, the QCM-A (quartz crystal microbalance based on admittance analysis)[13,19,25] can

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separately estimate the amount of adhered substance as well as *D*-factor. The purpose of this study is to observe the adsorption process of model food protein (BSA) onto a stainless steel surface using the technique of QCM-A in order to understand (1) effect of concentration of BSA on adsorption mode and (2) adsorption kinetics of BSA adsorption onto a stainless steel surface.

Materials and methods

Materials. Bovine serum albumin was used as a protein sample for three reasons. Firstly, it has been used as a model protein for various research fields including food science. Secondly, it is inexpensive since large quantities of it can be readily purified from bovine blood. Finally, it has similar properties to other food proteins such as β-lactoglobulin (major milk whey protein) and ovalbumin (major egg white protein); they are all acidic proteins, readily soluble in water and form aggregates or gels upon heating their solutions. The powder sample of bovine serum albumin was purchased from Sigma–Aldrich (St. Louis, USA). All other chemicals used were of reagent grade.

BSA solution. The powder of BSA was dissolved in 50-mM HEPES buffer (pH 7.0). The concentration of BSA was 1, 5, and 10%, respectively.

QCM-A. AFFINIX QN pro was used as a QCM-A instrument (Ulvac Inc. Chigasaki, Japan). It had one 500-μL cell that was equipped with a 27 MHz quartz crystal plate (8.7 mm diameter of a AT-cut quartz plate having an effective area of 0.049 cm^2 of a stainless steel (SUS304) electrode; Ulvac Inc. Chigasaki, Japan) at the bottom of the cell having a stirring device (Fig. 1). The detection range of adhered mass on the plate was from 10 pg to 10 μg; this corresponded to $2 \times 10^{-5} - 2$ g/m^2. Before adsorption experiment, the surface of stainless steel electrode was cleaned with piranha solution (3:1 mixture of sulfonic acid and hydrogen peroxide) based on the makers manual,[13,26] then rinsed by Milli-Q water and blown dry with air.

In the QCM-A,[13,19,20] a vector network analyzer measures the current at a certain applied alternating voltage over a specific range of frequencies (Fig. 2). Then the conductance (*G*) is plotted at the function of the swept frequency and three important frequencies are obtained, F_s, F_1, and F_2 (Fig. 2(a)). F_s is the frequency where *G* shows the maximum value,
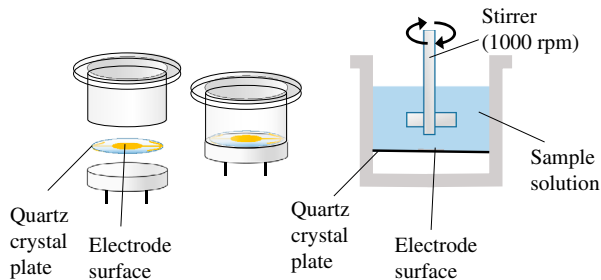
corresponds to the resonance frequency of the quartz crystal plate F_r., F_1, and F_2 are the frequencies at the half-height of the curve. When the mass is loaded on the QCM plate without viscosity change, the curve shifts to a lower frequency without altering the shape of curve (Fig. 2(b)). In this case, the change in F_s is directly related to the mass difference as Saurbrey equation (Equation (1)).

When the viscoelastic substance is added to the cell and adhesion of the substance occurred on the QCM plate, the curve shifts to a lower frequency and the bandwidth ($F_2 - F_1$) becomes broadened. In this case, F_s is effected by both the mass adhered on the plate, viscosity of the substances and viscosity change of bulk solution in the cell. Therefore, the change in mass cannot be determined by the change of F_s. On the contrary, F_2 is not affected by the viscosity of the substances and viscosity change of bulk solution in the cell.[19,25] By entering ΔF_2 instead of ΔF_S to the Saurbrey equation, the mass change Δm is obtained.

$$\Delta F_2 = - \frac{2F_0^2}{A\sqrt{\mu_q \rho_q}} \Delta m \qquad (2)$$

In addition, the energy dissipation (*D*) is also obtained by

$$D = \frac{F_2 - F_1}{(F_1 + F_2)/2} \qquad (3)$$

The change in dissipation ΔD is relative to the viscosity change of bulk sample solution in cell and adhered substances. If the viscosity change of bulk sample solution in cell is ignorable, the change of dissipation is a reflection of some change in the layer of adhered substances, such as reorganization or swelling.[12,13,16–18,21–23]

The QCM-A procedure used in this study is as follows: first, 400 μl of 50 mM HEPES buffer (pH 7.0) was put in the cell and stabilized for 3 h with vigorous stirring (1000 rpm). Then 2 to 30 μL of the BSA solution (10 to 100 mg/mL; the final BSA concentration in the cell was from 0.0498 to 6.98 mg/mL) was injected to the cell, and the ΔF_2 and ΔD values were obtained per every 2 s for 2 h. The temperature of sample solution was controlled to 25 °C during the experiments.

All experiments were done in triplicate or quadruple.

Results

Figure 3 shows the example of time course of ΔF_2. Upon injection of BSA solution to the cell (*t* = 0) F_2 decreased, indicating that the mass on the QCM electrode increase by adsorption of BSA molecules. Using the Equation (2), the mass change Δm on the QCM electrode was calculated. The time course of Δm for all BSA concentration examined was shown in the Fig. 4 as well as those of ΔD. It should be noted that even 2 h after the injection, the values of Δm and ΔD did not saturate.

Figure 5 shows the plot of mass change at *t* = 7200 s (Δm_t = 7200 s) as a function of final BSA concentration. The plot was similar to the Langmuir type isotherm and actually can be apparently fitted well by the equation of Langmuir isotherm as

Fig. 1. Schematic illustration of QCM-A sample cell.

(a) 3 important frequencies F_s, F_1, F_2
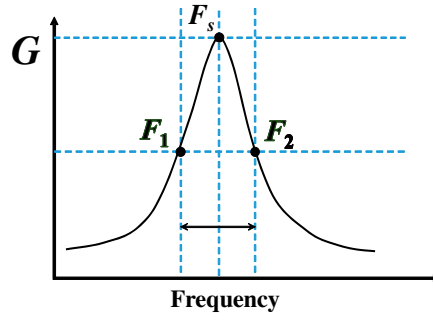
(b) Mass increase
No viscosity changes
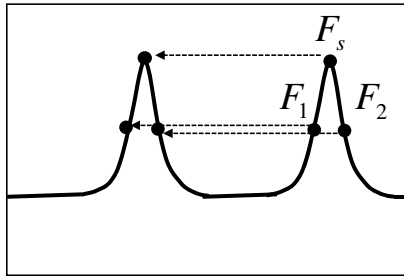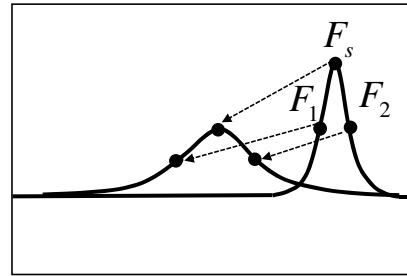
(c) Viscosity also increases

Fig. 2. Principle of QCM-A measurement. (a) The definition of F_s, F_1, F_2. (b) Changes of conductance curve, F_s, F_1, and F_2 when mass increases without viscosity change. (c) Change of conductance curve, F_s, F_1, and F_2 when both mass and viscosity increases.
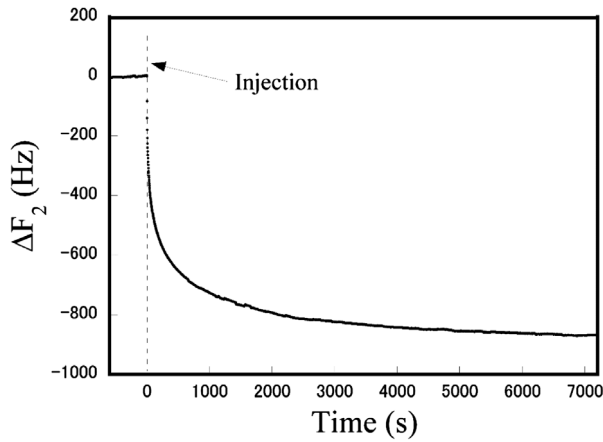

Fig. 3. Typical time course of ΔF_2. Final BSA concentration: 2.44 mg/mL.

$$\Delta m_{t=7200} = \frac{\Delta m_{c=\infty} KC}{1 + KC} \quad (4)$$

where K, C, and $\Delta m_{C=\infty}$ are the adsorption equilibrium constant, the final concentration of BSA in the cell, and the Δm at $C = \infty$, respectively.

To obtain further insight into the adsorption of BSA onto the stainless steel surface, ΔD was plotted as the function of Δm as similar way in previous researches[16,17,21,23,24] (Fig. 6). The values of ΔD reflect both the change of bulk solution viscosity and change of viscosity component of the layer of BSA molecules on the electrode surface. At the final BSA concentration of 0.0492 mg/mL, the plot shows linear;
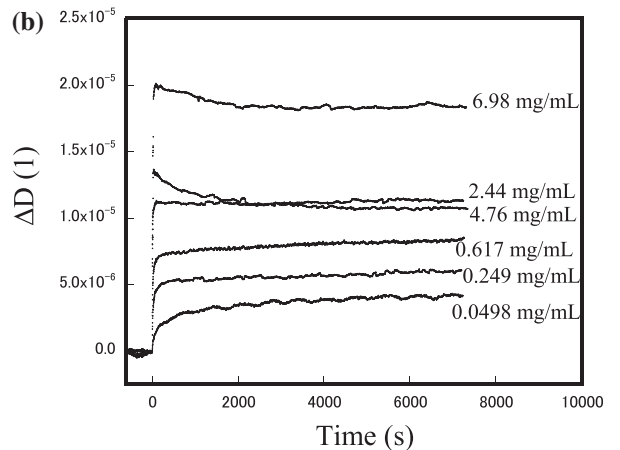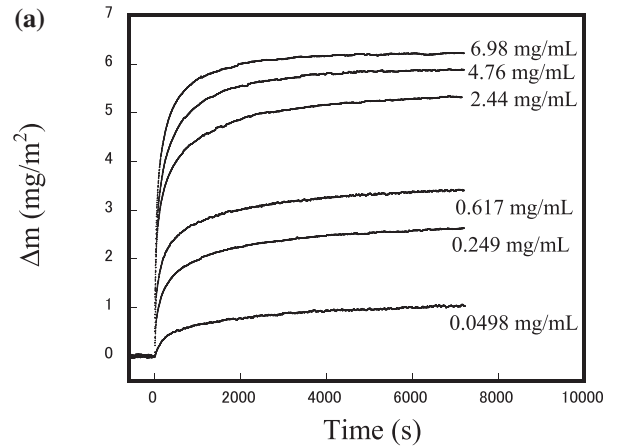

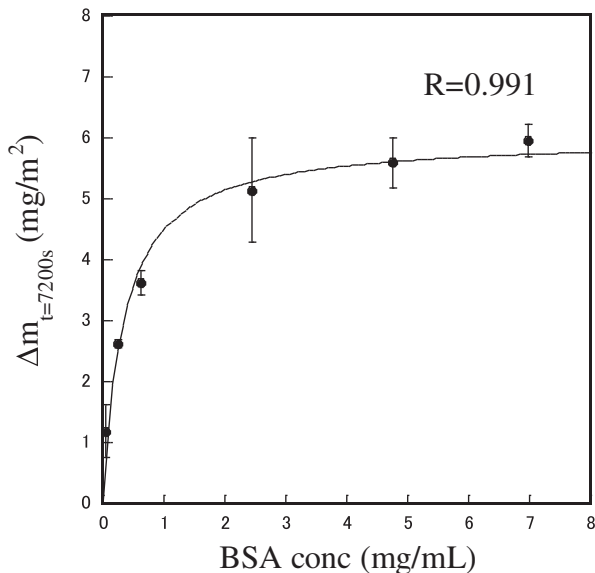Fig. 4. Typical time course of Δm (a) and ΔD (b).

Fig. 5. Plot of Δm at 7200 s as a function of final BSA concentration. Error bars represent the standard deviation of three or four replicas. The solid line in the plot is the result of fitting by the Langmuir adsorption isotherm equation.
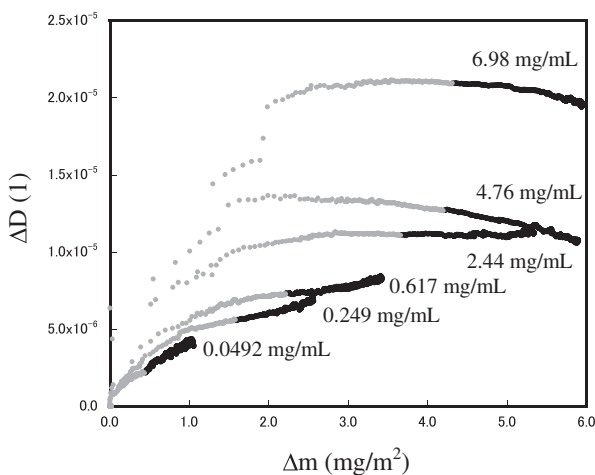

Fig. 6. Plots of ΔD as a function of Δm. Grey symbols (○); the data obtained within 5 min after injection. Black symbols (●); the data obtained 5 min after injection.

with increasing mass change, the ΔD also increase linearly. This can be interpreted as the result of increase of amount of adhered BSA molecules having a viscoelastic property on the electrode surface. However, increasing the final BSA concentration the slope of the plot tended to decrease with increase in Δm; at 4.76 and 6.98 mg/mL, the slopes took negative values. This indicates that the layer of BSA on the electrode became *less viscous* with increase in Δm.

Discussion

Understanding of protein adsorption onto a stainless steel surface is important for cleaning efficiency of food manufacturing equipment and food processing line. Many researches investigating adsorption amount of food protein and effect of external conditions such as pH, temperature, and salt concentration. However,

there were few researches that observed the kinetical process of protein adsorption onto a stainless steel surface. The QCM-A-based technique may enable a real-time and non-labeling monitoring of a protein adsorption process.

Other researchers estimated the saturation amount of BSA adsorbed onto a stainless steel surface by the depletion method such as ~2.3 mg/m²[10] (40 °C, pH 7.0, solvent: 10^{-3} M KNO_3), 3 – 4.2 mg/m²[11] (25 °C, solvent; Hank's balances salt solution), ~1 mg/m²[29] (30 °C, pH 7.0, solvent: 10^{-1} M KNO_3). In general, adsorption amount of protein is dependent on temperature and solvent condition.[6,7] Furthermore, in this study, Δm did not reach the saturated value even 120 min after the injection. Therefore, direct comparison of Δm to those reported values is difficult. However, it was true that the maximum Δm (5.95 ± 0.27 mg/m²; 6.98 mg/mL BSA at 120 min) was larger than those saturated values. This may be from the water molecules coupled with BSA. In the QCM-A, it was reported that mass of protein in hydrated state is detected.[16,19] So the mass detected by QCM-A is from both protein molecules and water molecules coupled with protein. Ozeki et al.[16] reported that the weight ratio of water molecules coupled with one BSA molecule in TE buffer (pH 7.8, 10 mM Tris–HCl, 200 mM NaCl) was about 1.5. That is to say, the mass of BSA is detected 2.5 times larger than its dried mass by QCM-A. Therefore, it may be said that the maximum value of Δm obtained in this study was consistent with the previous reported results.

The change in ΔD indicates the energy loss from the viscous components of bulk solution and adhered substances.[13,16–24] Immediately after injection of BSA solution, rapid increase in ΔD was observed. This was due to both the increase in bulk solution viscosity in the cell by addition of BSA solution and adsorption of BSA molecule having a viscoelasticity. Because the sample in the cell was vigorously stirred (1000 rpm), the effect of increase in the bulk solution viscosity by the injection of BSA solution was only appeared at early stage of experiment. The fact that the overshoot was only observed in the case when the BSA concentration was higher than a certain value (2.44 mg/mL) may also coincide this. If the stirring speed was not enough, the concentration distribution of BSA in the sample cell would not become even quickly and result in the overshoot of ΔD at all BSA concentration, which was not different from the results. In addition, the sample temperature was controlled to 25 ± 0.1 °C which is lower enough than denaturation temperature of BSA at pH 7.0 (61.2 °C[27]). So change in viscosity by denaturation of BSA did not occur. From these aspects, it was indicated that the change in ΔD in this study came from viscosity component of the layer of adhered BSA molecule except for the early stage of experiment. From here, we focus only the change in ΔD 5 min after injection of BSA solution, where the concentration distribution of BSA in the sample would be even enough. For 0.0492 mg/mL, ΔD increase is approximately linearwith Δm. This increase in behavior can be interpreted as the result of increasing adhered amount of BSA molecule that is viscoelastic. However, higher BSA concentration resulted in lower increase in ΔD
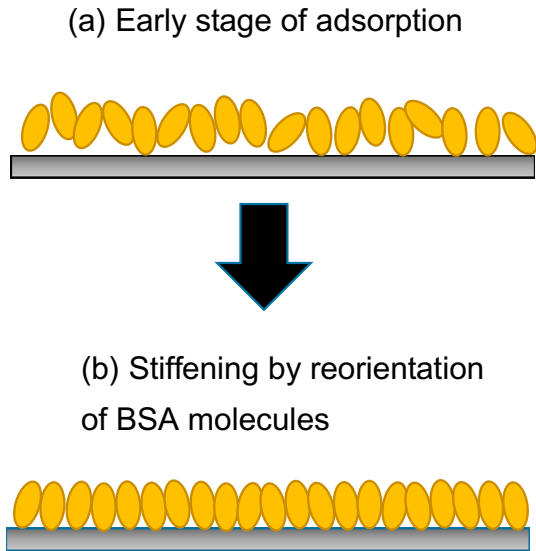
(a) Early stage of adsorption

(b) Stiffening by reorientation of BSA molecules

Fig. 7. Schematic illustration of the mechanism for stiffening of BSA layer.

with Δm. Especially at 4.76 mg/mL and 6.98 mg/mL, ΔD decreases with increase in Δm, suggesting an increase in the elasticity of the adhered BSA layer. Similar behavior was observed in the process of BSA adsorption onto a platinum surface[28] and lipid vesicles adsorption onto an oxidized gold surface.[16] It has been previously shown that these behaviors of decrease in ΔD against mass change increase occur as the layer of adhered substance becomes stiffer.[16,21,29,30] Considering these previous researches, the mechanism of decrease in ΔD against mass change at higher BSA concentration may be possible as follows (Fig. 7). Upon injection of BSA solution, BSA molecules adhered onto the stainless surface. Since the concentration of BSA is relatively higher, the surface is almost covered by BSA molecules. This initial adsorption reaction occurred so rapidly that BSA molecules have little time to take energetically preferred orientation. As a result, packing efficiency of BSA molecule in the layer lowered at the initial adsorption. As the time passes, the BSA molecules start to take energetically favored orientation and packed together gradually. This packing also allows more species to adsorb on the surface. As a result, the layer of adhered BSA becomes stiffer.

The concentration dependence of $\Delta m_t = 7200$ was fitted well by the equation of Langmuir isotherm model. Since isotherm data from protein adsorption studies often appear to be fitted well by the Langmuir isotherm model[10,31,32] as same as in this study, estimates of protein binding affinity have often been carried out from its use, although none of the conditions required for a Langmuir adsorption process may be satisfied for this type of application.[33] In this study, protein molecule reorientation in the adsorption layer was suggested, which is not considered in the Langmuir adsorption model. Using the time course of Δm, deviation of Langmuir adsorption model would also be evident. According to the Langmuir adsorption model, adsorption amount Δm is described by the following equation[33]
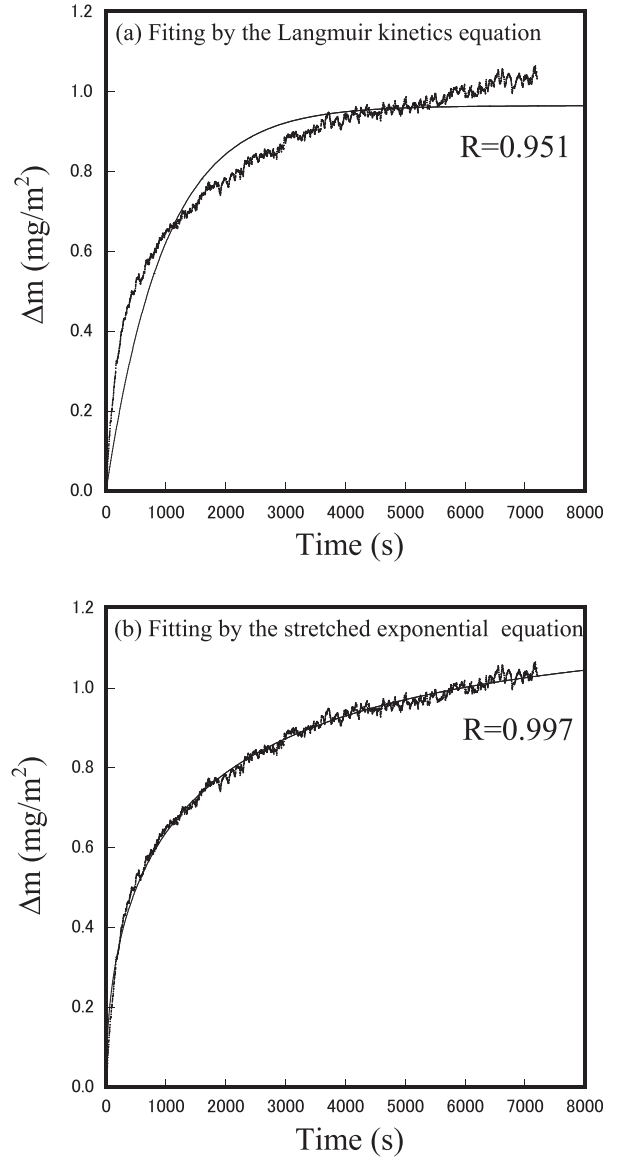

Fig. 8. Typical fitting results of time course of Δm by (a) the Langmuir kinetic equation and (b) the stretched exponential equation, respectively.

$$\Delta m(t) = \Delta m_\infty [1 - \exp(-kt)] \quad (5)$$

where Δm_∞ and k are the saturated adsorption amount and adsorption rate constant, respectively.

Figure 8(a) shows the example of results of fitting by the Equation (5) for 0.0492 mg/mL BSA. It is clear that the equation could not be fitted well although the isotherm was fitted well by the Langmuir adsorption model. At all other BSA concentrations, the Equation (5) was unable to fit the time course of Δm, neither (data is not shown). These results clearly indicate the deviation of the Langmuir adsorption model. Because QCM-A can monitor the amount of adherence on the surface, checking the Langmuir adsorption model is possible by one experiment without adsorption isotherm. This is one of the merits of QCM-A.

From the facts mentioned above, the question arises as to what the equation capable to approximate the time course of Δm. The following empirical equation was used to fit the data on time course of Δm.

Stretched exponential function model[34,35]

Table 1. Summary of the parameters obtained using the stretched exponential function equation for the fitting of time course of Dm.

BSA conc. (mg/mL)	k (s^{-1})		Δm_∞ (mg/m^2)		β		R	
	Average	SD	Average	SD	Average	SD	Average	SD
0.0498	7.32×10^{-4}	3.13×10^{-4}	1.30	0.42	0.522	0.021	0.994	0.001
0.249	1.98×10^{-3}	5.0×10^{-4}	2.74	0.15	0.436	0.026	0.994	0.007
0.617	5.17×10^{-3}	4.22×10^{-3}	3.73	0.11	0.381	0.023	0.997	0.002
2.44	5.86×10^{-3}	3.14×10^{-3}	5.29	0.86	0.359	0.017	0.998	0.001
4.76	4.84×10^{-3}	7.6×10^{-4}	5.67	0.38	0.404	0.019	0.999	0.001
6.98	6.92×10^{-3}	1.12×10^{-3}	5.98	0.26	0.453	0.016	0.998	0.001

$$\Delta m = \Delta m_\infty \left[1 - \exp(-(kt)^\beta) \right] \qquad (6)$$

where Δm_∞: saturated adsorption amount; k: adsorption rate constant; β: fitting parameter, representing the deviation from the Langmuir adsorption model.

Figure 8(b) shows the example of results of fitting by Equation (6). Table 1 summarizes parameters obtained by the fitting. The time courses of Δm for all initial BSA concentration examined in this study were described well by the stretched exponential function model function model. In spite of lack of physical meaning, this empirical approach can evaluate apparent adsorption rate constant, which may be useful for practical purpose, such as quantitative comparison of adsorption rate among different solid surfaces.

Author contributions

Tomoaki Hagiwara and Takaharu Sakiyama designed the research. Phosri Nattawut performed the experiments. Phosri Nattawut and Tomoaki Hagiwara analyzed the data. Mario Shibata participated in the review of the analysis results. Tomoaki Hagiwara wrote the manuscript. Mario Shibata also participated in the review of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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