Evaluation of adsorption properties of Bovine Serum Albumin on TiO₂ membrane by quartz-crystal microbalance

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Properties of protein solutes adsorbed on ceramic membrane by using a quartz-crystal microbalance (QCM) were evaluated in situ. This frequency of piezoelectric AT-cut quartz resonator changed according to the amount of solute adsorbed on the quartz-crystal surface. Ceramic thin membrane, which was mainly titanium oxide (TiO₂), was coated on the QCM surface using sol-gel method. An adsorption behavior of bovine serum albumin (BSA) on the TiO₂-QCM was investigated.

The amount of BSA adsorption increased with increasing BSA concentration, and reached a maximum value near the isoelectric point of BSA solution. The cohesion force acting among BSA molecules was considered to become strongest because the electrostatic repulsion force was weakened, and then the BSA molecules were accumulated by adsorption onto the membrane surface. A higher ionic strength gave a lower BSA adsorption at a pH near the isoelectric point. The pH at maximum adsorption of BSA in a high ionic strength shifted to a more acidic pH than that in a low ionic strength. The adsorption amount of BSA increased by hydrophobilization of TiO₂ surface. The equilibrium amount of BSA adsorbed at pH4.8 could be expressed by a Freundlich type’s adsorption isotherm.

Key words : quartz-Crystal microbalance/ceramic membrane/adsorption/protein/bovine serum albumin/ionic strength
1. Introduction

In the membrane filtration, an interaction between a membrane surface and a protein solute on the adsorption is complicated. This is because the chemical characteristic of a membrane surface and a protein solute changes in a progress of a filtration. The study of adsorption behavior of solutes onto a membrane is also very important to the development of membrane separation techniques, although the membrane fouling is much discussed with plugging of pores and/or forming of gel layer.

When a protein solution is filtrated by using a ceramic membrane, the adsorption of a solute is also dependent on the electrochemical property of the membrane surface. Moreover, the influence of the adsorption of a solute on the filtrate resistance becomes more significant in the case of the low feed concentration.

The electric charge of ceramic membrane is affected by the solution pH. Protein solutes are also an amphoteric electrolyte and their electric charge changes with the pH except for the isoelectric point. Ceramic membranes and protein solutes have a positive charge in the pH range under the isoelectric point, and a negative charge in the pH range over the isoelectric point. Therefore, the interaction of membrane and protein solute is very important to understand the filtrate property. Although, no direct measurement of the amount of solute adsorbed onto the membrane had been reported because the adsorption delicately changes with filtrate conditions.

Recently, the amount of the solute adsorbed on a thin membrane has been analyzed in situ by using a quartz-crystal microbalance (QCM) method. The resonant frequency of QCM decreases in proportion to the adsorption amount of the solute. Moreover, quantitative microanalysis of the solute can be estimated by nanogram level.

The objective of this study is the coating of TiO₂ ceramic skin layer onto the QCM with sol-gel method, and the evaluation of adsorption properties of protein solutes on the TiO₂-QCM. The influences of protein solute concentration, solution pH, ionic strength and hydrophilic-hydrophobic characteristics of the membrane on the adsorption were studied.

2. Experimental

2.1 Fundamental of QCM

The resonant frequency of QCM decreases in proportion to the amount of solute adsorbed on the electrode. The relationship of the resonant frequency shift of QCM and the amount of adsorption is given by Sauerbrey’s relationship:

\[ \Delta f = -2f_0 \Delta m/A (\rho_0 \cdot \mu_0)^{1/2} \]

Where, \( \Delta f \) denotes the amount of resonant frequency shift and is determined from the difference between the initial frequency and equilibrium one of QCM. The basic resonant frequency \( f_0 \) is 5.946 MHz. The effective surface area of QCM electrode \( A \) is 1.327 \text{ nm}^2. The density of AT-cut quartz \( \rho_0 \) is 2.648 \times 10^3 \text{ kg} \cdot \text{m}^{-3}, the shear modulus \( \mu_0 \) is 2.947 \times 10^{10} \text{ N} \cdot \text{m}^{-2}, \Delta m \) is the mass change on the QCM. Therefore, the adsorption amount per unit frequency change can be calculated to be 16.6 ng/Hz. When the BSA molecule of 4 \times 14 \text{ nm}^3 in size absorbs in mono-molecule layer with “side-on” mode, the adsorption amount of mono-molecule

layer is described as

\[ q = \frac{M_w \cdot A}{S \cdot A_0} \]  

(2)

where, \( q \) denotes the adsorption amount of mono-molecule layer of BSA of which molecular weight \( M_w \) is 67,000. The area of BSA mono-molecule \( S \) (BSA molecule form is assumed as rectangle, and is projected vertically on the QCM) is \( 4 \times 10^{-18} \text{ m}^2 \), \( A_0 \) is the Avogadro’s number. Therefore, \( M_w / (S \cdot A_0) \) shown in Eq. (2) denotes BSA mono-molecule adsorption per unit area, and the value is about 2 mg m\(^{-2}\). The BSA mono-layer amount adsorbed onto QCM is calculated to be 264 ng. This is equivalent to the resonant frequency shift of about 15.9 Hz.

2.2 Experimental apparatus and detail of QCM structure

The AT-cut QCM of the basic resonant frequency of 6 MHz was supplied by Hokuto Denko Corporation. Its size is 25 mm in diameter and 0.27 mm in thickness. A thin Au electrode circuit has been vapor-deposited on both crystal surfaces. Figure 1 shows the structure of QCM and an experimental apparatus. A ceramic skin layer of a micron or less in thickness was coated on the QCM electrode surface by dip coating using sol-gel method. Its effective area is \( 1.327 \times 10^{-4} \text{ m}^2 \). This coated QCM was attached on a testing batch cell, and immersed in a protein solution maintained at 298 K. The resonant frequency shift of QCM on the adsorption of protein was measured with stirring slowly by a magnetic bar. The resonant frequency data was stored in a personal computer together with once a second. The amount of frequency change is equal to the amount of protein adsorbed. The protein solute mainly used is bovine serum albumin (BSA, Sigma Chemical Co.). Its molecular weight is 67,000 and the isoelectric point is pH 4.8. This protein solute was dissolved in a distilled and deionized water at various concentrations, pHs and ionic strengths. The ionic strength of the solution was adjusted with NaCl. The pH of the solution was adjusted with HCl or NaOH solution.
2.3 TiO$_2$ coating on the QCM by sol-gel method and adsorption of protein solute

TiO$_2$ skin layer was coated on QCM by a sol-gel process as shown in Figure 2. Titanium tetraisopropoxide of metal alkoxide was prepared and to be followed by dissolution in absolute ethanol, and titania sol was obtained hydrolysis under acidic condition. The optimum mol ratio of sol-gel reagents adopted Ti [OCH (CH$_3$)$_2$]$_4$: C$_2$H$_5$OH: H$_2$O: HNO$_3$=1: 100 : 10 : 0.04. This sol solution was dip-coated on the QCM dried after defatting by acetone, and was gelled with drying at 333 K. Next, TiO$_2$ ceramic skin layer was fixed on the QCM by sintering at 623 K. The sintering temperature was increased with 2°C/min, stored for 10 minutes at 623 K, and decreased with 2°C/min until a room temperature. This TiO$_2$-QCM was attached on the module, the adsorption of the protein solution was examined in a beaker kept at 298 K. This measurement temperature was necessary to control as precisely as possible using a thermostat, because the QCM frequency per unit degree increases about 20 Hz/°C.

3. Results and discussion

3.1 Effect of protein adsorption on coating conditions of ceramic skin layer by sol-gel method

The skin layer was observed to be homogeneous about 1 µm or less in thickness as shown in Figure 3. A higher thickness of a membrane led to a lower sensitivity of resonant frequency of QCM. The rugged surface view of TiO$_2$ membrane might be formed with thermal contraction when the gel was heat-dried or sintered. The layer was obtained with two times coating by dip-coat-
Fig. 5 Change in frequency with adsorbing of BSA

The skin layer could be sufficiently formed with about two or three times coating. The sintering temperature of QCM for fixing ceramic skin layer was needed to maintain within 673 K. An unreasonable increase of the sintering temperature changed a crystal structure of QCM and gave an unstable frequency response.

3.2 Effect of solution pH and ionic strength on protein adsorption

The resonant frequency changed by adsorbing protein onto the QCM coated with ceramic skin layer. Figure 5 shows a time course of the resonant frequency shift ($-\Delta f$) with adsorbing of BSA solute. The BSA solute was added after 20 min from the frequency counts started. The adsorptions of BSA reached equilibrium value after about 20 min from the addition of BSA solute with repeating adsorption and desorption. The $-\Delta f$ value was calculated with subtracting the initial resonant frequency value after 20 min from the final experimental value. An adsorption time of 20 min was mainly used in all further experiments. As shown in Fig. 5, the BSA molecule adsorbed onto the TiO$_2$-QCM with mono-layer immediately after the addition of the solute, and further a second-adsorption occurred from after about 30 min. The cohesion force of BSA molecules is considered to become strong near the isoelectric point with no salt addition. The adsorption amount per frequency change is calculated to be 16.6 ng/Hz according to Eq. (1). Figure 6 shows the pH dependence on the equilibrium adsorption of BSA and Myoglobin solutes. The isoelectric point of these proteins is pH 4.8 and 6.8, respectively. These solutes showed the highest adsorption near their isoelectric point because of the higher cohesion force. The protein molecules are easy to flocculate due to the neutralization of the electric charge near the isoelectric point. When the BSA molecules adsorb on mono-molecule layer with “side-on” mode, the resonant frequency shift has about 15.9 Hz. This value is converted to 2 mg·m$^{-2}$ as amount of BSA adsorbed. The protein solute was found to adsorb in a multi-molecule layer near the isoelectric point. Figure 7 shows time courses of the resonant frequency shift on BSA adsorption with var-
ious ionic strengths. Figure 8 shows relationships of the amount of BSA adsorbed in steady-state and the ionic strength. These results showed that the adsorbed amount of BSA decreased with increasing ionic strength. The increment of the ionic strength results in a decrease in the thickness of the electrical double layer, which operates on the electrostatic attraction of molecule themselves, and thus the coagulation of BSA molecules became weakly by the addition of NaCl\(^{13}\). Figure 9 shows the change of BSA adsorbed at various pHs with changing ionic strength. When the ionic strength of the solution increased, the pH at maximum adsorption of BSA shifted to a more acidic pH region, compared with that of low ionic strength. This is due to the fact that the apparent isoelectric point of BSA might shift to a more acidic pH region because the chloride ions of hydrochloric acid added for pH adjustment adsorbed preferentially onto BSA molecules. Shirahama et al.\(^{14,15}\) had also recognize the phenomenon in the adsorption of BSA onto hydrophobic polystyrene latex. They reported that the isoelectric point of BSA and bovine hemoglobin shifted when the coexisted electrolyte anions such as chloride ion existed.

Figure 10 shows an adsorption condition
of electrolyte ions when the NaCl was added stepwise. The increase in the ionic strength enhanced the adsorption of electrolyte ions onto the TiO₂-QCM. Both BSA molecules and TiO₂ membrane are positively charged in the range under pH 4.8. The affinity between these molecules and the membrane might be caused by the attraction of the chloride ions adsorbed on the BSA or the membrane.

3.3 Effect of BSA concentration and hydrophobilization of TiO₂-QCM surface on BSA adsorption

Figure 11 shows the changes in adsorption amount with time at various BSA concentrations at pH 4.8. BSA concentration was increased with every 20 minutes step until 1 kg·m⁻³. The result of hydrophobilized TiO₂-QCM is also shown in this figure. This hydrophobilization was carried out with rinsing by siliconized reagent (Siliconize : L-25) supplied by Fuji Systems Company. The methyl group was introduced on the TiO₂ membrane surface in this treatment. The equilibrium adsorption amounts increased with increasing of BSA concentration, furthermore, increased greatly by hydrophobilization of the membrane surface. This is probably due to the increases in the hydrophobic affinity and van der Waals force between BSA molecules and the TiO₂ membrane. Figure 12 shows the adsorption isotherms of the equilibrium adsorption amount versus BSA concentrations. The isotherm was expressed by a Freundlich’s relationship as follows.

\[ q = k \cdot c^{1/n} \]  (3)

These lines changed near the BSA concentration of 0.1 kg·m⁻³. A higher amount of BSA adsorbed was shown regardless of a lower concentration because the BSA molecules adsorbed preferentially onto the membrane surface. The 1/n value of hydrophilic and hydrophobic TiO₂ membrane was obtained from logarithm linear correlation of q vs. c up to 0.1 kg·m⁻³. These values resulted similar 0.25 m⁻³, although the strong interaction was shown between the hydrophobic TiO₂ membrane and BSA molecules.

Conclusion

The adsorption of BSA solute on ceramic
coated-film of TiO₂ was studied using a quartz crystal microbalance (QCM). This ceramic membrane could be homogeneously coated on the QCM at about 1 μm thickness by sol-gel method. The adsorption amount was affected by solution pH, ionic strength, BSA concentration and hydrophobilization of TiO₂ membrane. The adsorption amount of BSA was maximized at pH 4.8 near the isoelectric point of BSA, because BSA molecules adsorbed in multi-layer onto TiO₂-QCM at pH 4.8. The pH at maximum adsorption of BSA in a high ionic strength shifted to a more acidic pH region. The adsorbing amount increased with a higher BSA concentration and a greater hydropho-

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bilization of TiO₂ membrane. The equilibrium amount of BSA adsorbed near the isoelectric point could be explained using Freundlich type’s adsorption isotherm.

References

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