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Adsorption Behavior of Serum Albumin on Electrode Surfaces and the Effects of Electrode Potential

Bei GUO, Jun-ichi ANZAI, and Tetsuo OSA*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980–77, Japan.

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The adsorption behavior of serum albumin onto the surface of platinum, gold, and glassy carbon electrodes was studied in relation to the electrode potential, by using cyclic voltammetry and a quartz-crystal microbalance. The kinetics of adsorption was significantly dependent on the electrode potential. The adsorption was highly accelerated by the application of positive potential to the electrode, suggesting an electrostatic interaction between the negatively charged albumin molecules and the positively polarized electrode as the origin of the accelerated adsorption. The adsorption of albumin on the electrodes was irreversible with respect to dilution of the albumin solution, while the albumin molecules were desorbed in part upon shifting the electrode potential in the negative direction. The quartz-crystal microbalance data showed that albumin forms a monomolecular layer on the electrode surface. Protein adsorption on electrode surfaces in serum was also examined.

Key words adsorption; serum albumin; electrode; cyclic voltammetry; quartz-crystal microbalance

It is known that the adsorption of proteins onto electrode surfaces disturbs electrochemical analysis of clinical samples.[1,2] Such electrode fouling induces changes in the availability of the electrode surface to analytes, resulting in modification of the electrochemical response. Therefore, the adsorption characteristics of proteins on electrode surfaces and its influence on the voltammetric behavior should be examined carefully to permit reliable electrochemical analysis of biological fluids. So far, few reports have appeared concerning protein adsorption on metal and carbon electrodes,[3,4] though the nonspecific adsorption of proteins on polymeric materials has been studied extensively in relation to their medical and clinical use.[5–8] We have already reported preliminary results on the adsorption of serum albumin on platinum and gold electrodes.[9] In the present paper, we show that serum albumin is adsorbed irreversibly on these electrode surfaces and modifies the voltammetric response of the electrodes, and that the adsorption is accelerated by keeping the electrode potential positive.

Experimental

Materials Human serum albumin (HSA) was purchased from Miles Lab. as a lyophilized powder. A control serum (Consera, Nissui Pharm.) was used after dilution with distilled and deionized water.

Measurements Cyclic voltammetry (CV) was carried out using a platinum (Pt), gold (Au), or glassy carbon (GC) disk electrode (3 mm diameter) as the working electrode, a Pt wire as the counter electrode, and a Ag/AgCl reference electrode. The polished disk electrode was exposed to HSA solution or serum and rinsed with buffer, then the cyclic voltammogram was recorded in phosphate-buffered saline (pH 7.4) in the presence of 2.5 mM $K_4Fe(CN)_6$ and 50 mM Na_2SO_4. The CV measurements were carried out at a scan rate of 0.1 V/s. In order to evaluate the effect of electrode potential on the adsorption behavior, the electrode potential was controlled *vs.* an Ag/AgCl electrode using a Potentiostat/Galvanostat HA-501 (Hokuto Denko) during the course of adsorption.

For the quartz-crystal microbalance (QCM) study, Pt and Au disk electrodes (5 mm diameter) sputtered on a 9 MHz AT-cut quartz crystal were used. The Pt or Au electrode was mounted in a cell and the resonant frequency of the quartz crystal was measured in the presence of HSA or serum using a Quartz Chemical Analyzer QCA-917 (Seiko EG & G). The electrode potential was controlled by the same potentiostat as used for the CV measurement. Phosphate-buffered saline (pH 7.4) was used throughout this study, unless otherwise noted. All measurements were carried out at *ca.* 20 °C.

* To whom correspondence should be addressed.

Results and Discussion

Adsorption of HSA Monitored by CV It has been reported that monolayer adsorption of amphiphile and proteins on an electrode surface can block the redox reaction of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions at the electrode surface and lower the peak current in CV depending on the surface coverage.[10,11] This is because $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions can not approach the electrode surface. In other words, one can monitor the adsorption behavior of protein on the electrode surface by measuring the CV in the presence of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions.

Figure 1A shows cyclic voltammograms of 2.5 mM $K_4Fe(CN)_6$ on Pt electrodes before and after treatment in 0.4% HSA solution (pH 7.4), which corresponds to the

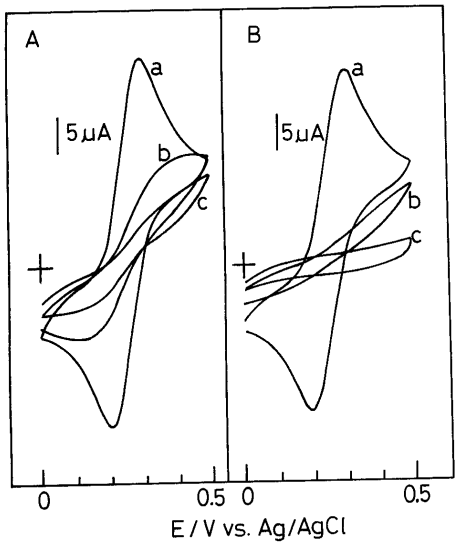

Fig. 1. Cyclic Voltammograms of 0.25 mM $F_4Fe(CN)_6$ on a Pt Eelectrode before (a) and after Treatment in 0.4% HSA Solution for 30 s (b) and 2 min (c)

(A) Electrode potential was not applied to the Pt electrode during the HSA treatment. (B) Electrode potential (0.5 V) was applied to the Pt electrode during the HSA treatment.

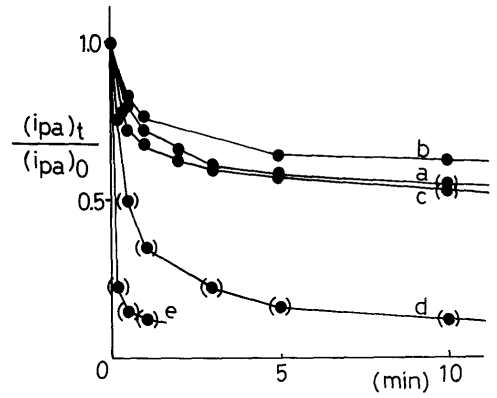

Fig. 2. The Time Course of HSA Adsorption on a Pt Electrode with and without Application of Electrode Potential

a, with no applied potential; b, −0.5 V; c, 0 V; d, 0.5 V; e, 1.0 V. The $(i_{pa})_t/(i_{pa})_0$ values for $K_4Fe(CN)_6$ were plotted against time, where $(i_{pa})_t$ and $(i_{pa})_0$ denote the anodic peak current at time t and time zero, respectively. The plots in parentheses were read from the current at 0.5 V in the CV due to disappearance of the anodic peak.

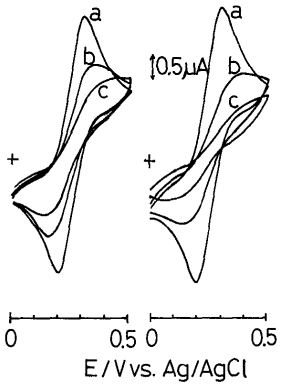

Fig. 3. Cyclic Voltammograms of 0.25 mM $K_4Fe(CN)_6$ on HSA-Adsorbed Pt Electrode before and after Rinsing

CVs were recorded on a bare Pt electrode (a) and an HSA-adsorbed Pt electrode before (b) and after rinsing for 12 h (c). HSA was adsorbed on the Pt electrode in open circuit (no applied potential) (left) and at 0.5 V (right).

HSA concentration in 10-fold diluted serum. The voltammogram exhibited clear redox peaks originating from the $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ couple before the treatment. The anodic peak current (i_{pa}) of the CV decreased to *ca.* 50% of the original value after 30 s exposure of the electrode to the HSA solution and continued to decrease during extended exposure, showing that the electrode surface became covered with HSA.

In the previous paper, we reported that the adsorption of bovine serum albumin (BSA) onto Pt and Au electrodes can be facilitated by the application of electrode potential to the electrodes.[9] Figure 1B depicts the cyclic voltammograms after 30 s and 2 min treatment of the electrode at 0.5 V *vs.* Ag/AgCl in 0.4% HSA solution. The redox peaks disappeared completely after 2 min and the voltammogram showed no further change thereafter, implying complete coverage of the electrode surface with HSA. It is clear that the adsorption of HSA is accelerated by the externally applied electrode potential.

We also checked the HSA adsorption in a slightly acidic medium (pH 5.0) and found that the adsorption is more significant in pH 5.0 than in pH 7.4 buffer. This tendency in pH dependence has also been reported previously for BSA adsorption.[9] The results were explained based on loss of the net charge of BSA at pH equal to its isoelectric point (pI), and, as a result, enhanced hydrophobicity. In the present study, our attention was focused exclusively on the adsorption behavior of HSA in pH 7.4 buffer, because the electrochemical analysis of biological fluids is usually carried out at neutral pH.

In order to evaluate the time course of the HSA adsorption, the Pt electrode was immersed in a diluted HSA solution (0.04%) with or without application of constant electrode potential for a specified time and the CV was measured in a solution of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions. The i_{pa} values in the CV were plotted as a function of the adsorption time (Fig. 2). The i_{pa} value decreased to about a half of the original value after 10 min adsorption without application of a constant potential (*i.e.*, open circuit). This means that HSA is significantly adsorbed onto the Pt electrode even from the diluted solution. On the other hand, the application of positive potential to the Pt electrode considerably accelerated the adsorption of HSA. The effect of applied potential was more significant at 1.0 V than at 0.5 V. In contrast to the effect of the positive potential, acceleration was not clearly observed at 0 or −0.5 V. These observations imply that electrostatic force of attraction is responsible for the accelerated adsorption of HSA, because the HSA molecule is negatively charged in the pH 7.4 buffer (the pI of HSA is *ca.* 4.8).[12] This does not necessarily mean that the main force of attraction between HSA and Pt electrode stems from the electrostatic interaction. It seems that the adsorption behavior of HSA is a complicated function of hydrophobicity, electric charges of both the surface and the protein, denaturation and conformation of proteins, co-existence of inorganic ions, *etc.*[3-8]

In order to evaluate the reversibility of the adsorption of HSA, the HSA-adsorbed Pt electrodes were rinsed by immersing them in an excess amount of buffer solution for 12 h and their cyclic voltammograms were measured again in the presence of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions (Fig. 3). The original forms of the voltammograms were not recovered after rinsing, but the i_{pa} values decreased further, suggesting that the adsorbed HSA molecules can not be desorbed from the electrode surface upon dilution of the HSA solution or rinsing (*i.e.*, irreversible adsorption). The fact that the i_{pa} values decreased substantially after rinsing suggests strongly that denaturation or conformational changes of the adsorbed HSA was induced by a strong interaction with the Pt surface. Surface-induced conformational changes of proteins have often been reported.[12-14] We hypothesize that the denaturation from native globular form to unfolded conformation enhances the surface area that is occupied by a single HSA molecule at the Pt surface and results in higher surface coverage in spite of the constant loading of HSA. Judging from the CV results, the denaturation is more significant for the HSA molecules adsorbed under the application of electrode potential (0.5 V) than for those adsorbed with no applied potential. The reason for this is not obvious.

Gold (Au) and GC electrodes are often used for electroanalysis, as well as Pt electrode. For this reason,

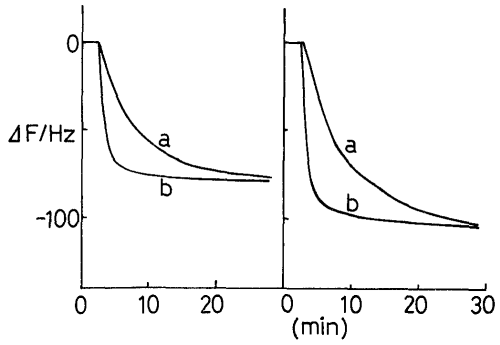

Fig. 4. Changes in the Resonant Frequency upon HSA Adsorption

The concentration of HSA was 10 μg/ml (left) or 100 μg/ml (right). The electrode potential was none (a) or 0.5 V (b).

we verified the adsorption behavior of HSA on these electrodes by means of CV. The adsorption of HSA on an Au electrode was significant and was accelerated by the application of positive potential, showing basically the same tendency as for the Pt electrode. In the case of the GC electrode, the effect of applied potential was less significant than for the metal electrodes. However, HSA adsorption to the GC surface was still not negligible.

Adsorption of HSA Monitored by QCM A QCM technique has been widely used to monitor surface phenomena which are accompanied with mass changes.[15,16] We employed QCM with a 9 MHz AT-cut quartz crystal to study the kinetics of HSA adsorption and the loading. Figure 4 depicts the frequency changes (ΔF) caused by the adsorption of HSA on the Pt-sputtered resonator. The resonant frequency (F) decreased upon addition of HSA in the buffer solution, which means that HSA molecules were adsorbed on the Pt electrode to increase the mass loading on the quartz crystal. The Sauerbrey equation (Eq. 1) can be used to calculate the mass change ($\Delta M/\mathrm{g\,cm^{-2}}$) from the frequency change (ΔF)

$$\Delta F = 2F_0^2 \Delta M / A(\rho\mu)^{1/2} \quad (1)$$

where frequency shift ΔF is a function of the initial frequency F_0, the mass loading ΔM, the active area of the electrode A (0.25 $\mathrm{cm^2}$), the density ρ (2.648 $\mathrm{g/cm^3}$) and the shear modulus μ (2.947×10^{11} $\mathrm{g/(cm\,s^2)}$) of the quartz.[17] Thus, for the 9 MHz AT-cut device, one nanogram of mass loading induces a frequency change of -0.91 Hz (Eq. 2).

$$\Delta F(\mathrm{Hz}) = -0.91 \Delta M(\mathrm{ng}) \quad (2)$$

Based on Eq. 2, the loading of HSA can be estimated to be *ca.* 330 $\mathrm{ng/cm^2}$ (in 10 μg/ml HSA solution) and *ca.* 420 $\mathrm{ng/cm^2}$ (in 100 μg/ml HSA solution). It should be noted here that accurate estimation of mass loading on the quartz resonator is very difficult because the resonant frequency has been found to be sensitive to not only mass loading, but also to viscosity, elasticity, surface roughness, *etc.*[18,19] This problem is serious, particularly in the case of organic macromolecules including proteins. Therefore, the calculated values of HSA loading based on Eq. 2 should be considered as rough estimates rather than accurate values. In practice, however, Eq. 2 has been widely used to estimate the mass loading of organic molecules, for convenience.[20-23] Assuming that HSA is an ellipsoidal

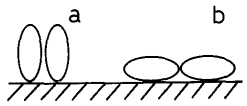

Fig. 5. Possible Orientations of HSA Molecules on the Electrode Surface

a, perpendicular (end-on type) orientation; b, parallel (side-on type) orientation.

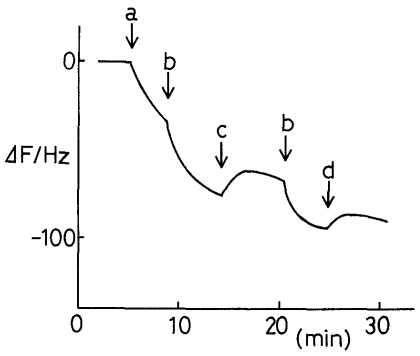

Fig. 6. Effects of *in-Situ* Stepping of Electrode Potential on the Adsorption Behavior of HSA on a Pt Electrode

a, 100 μg/ml HSA added; b, 1.0 V; c, −0.5 V; d, 0 V.

molecule[12] with dimensions of roughly $4 \times 4 \times 14\ \mathrm{nm^3}$, two different arrangements of HSA molecule on the electrode surface can be considered as extreme cases: a parallel (or side-on type) and a perpendicular (or end-on type) orientation against the electrode surface (Fig. 5). If the electrode surface is covered completely with a monomolecular layer of HSA which is adsorbed in the perpendicular orientation, the loading of HSA should be *ca.* 530 $\mathrm{ng/cm^2}$, calculated from the molecular mass of 66000. On the other hand, the parallel orientation would result in *ca.* 140 $\mathrm{ng/cm^2}$. The QCM data in Fig. 4 showed HSA loadings between these two extreme cases, which may suggest a random orientation of HSA on the electrode.

The decrease in resonant frequency was accelerated considerably under the influence of the applied electrode potential (0.5 V), showing facilitated adsorption of HSA. This tendency is in line with the CV results. It should be noted that the applied electrode potential did not alter the final ΔF values. In other words, it seems that the application of electrode potential affects the kinetics of HSA adsorption rather than the thermodynamics.

Figure 6 illustrates the effect of the electrode potential on the adsorption behavior; the electrode potential of the Pt electrode was regulated *in situ* during the adsorption. When positive potential (1.0 V) was applied to the electrode, the adsorption was accelerated, as expected. On the other hand, the resonant frequency increased slightly upon stepping the electrode potential *in situ* from 1.0 to 0 V or −0.5 V, implying a partial desorption of the albumin from the electrode surface. These results may be explained reasonably by assuming that the adsorbed albumin layer contains both tightly bound and loosely bound albumin molecules, depending on the orientation of the molecules on the electrode surface, as suggested by Kurrat *et al.*[4] It is plausible that tightly bound albumin is adsorbed on the surface irreversibly, while the loosely bound counterpart can be desorbed upon stepping the

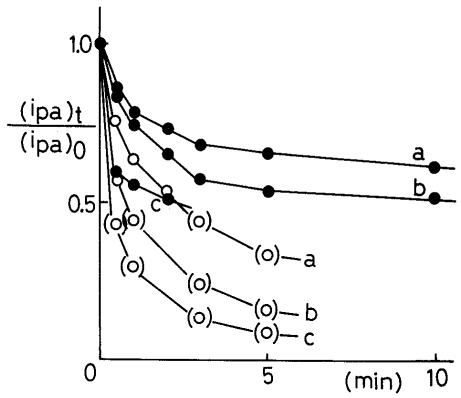

Fig. 7. Electrode Fouling in Diluted and Undiluted Sera with 0.5 V (—○—) and without (—●—) Application of Electrode Potential

Samples; 100-fold diluted (a), 10-fold diluted (b), and undiluted sera (c). The plots in parentheses were read from the current at 0.5 V in the CV because of disappearance of the anodic peaks.

electrode potential in the negative direction due to the increase of electrostatic repulsion or decrease of electrostatic attraction.

Electrode Fouling in Serum The electroanalysis of blood is usually carried out using serum, not whole blood, in order to circumvent possible interference arising from the coagulation of blood during the measurement. Judging from the fact that HSA is adsorbed onto the electrode surface significantly even from a dilute solution, the electrode surface is likely to be fouled in serum by adsorption of proteins and other constituents. Thus, we checked the electrode fouling in serum by means of CV, using the same procedure as in the estimation of HSA adsorption. Figure 7 shows the decrease of the i_{pa} values as a function of time. The i_{pa} values decreased gradually depending on the concentration of serum, but the final i_{pa} values for different concentrations of serum were not very different. This suggests that the electrode surface is essentially covered with serum proteins and that the electrode fouling involves irreversible adsorption. Another important point is that the application of potential to the Pt electrode (0.5 V) accelerated the electrode fouling. This is of practical importance, because, in the electroanalysis of biological fluids, the electrodes are often operated at a constant potential.

Conclusion

It has become apparent from the present study that (1) HSA binds to Pt, Au, and GC electrodes from dilute solutions to form an adsorption layer with random molecular orientation, (2) the adsorption of HSA is accelerated by the application of positive potential to the electrodes, probably due to enhanced electrostatic force of attraction, (3) the adsorption is virtually irreversible in relation to dilution of the HSA solution, (4) the adsorbed albumin can be desorbed in part by stepping the electrode potential in the negative direction, and (5) the electrode fouling is significant even in diluted serum. Consequently, we should take into consideration the occurrence of electrode fouling by the adsorption of albumin and other proteins in serum when electrodes are used for analytical purposes in contact with serum. A suitable modification of the electrode surface may be required to eliminate protein binding to the electrode.

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