ELECTROCHEMICALLY ACCELERATED ADSORPTION OF SERUM ALBUMIN ON THE SURFACE OF PLATINUM AND GOLD ELECTRODES

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Adsorption of serum albumin on the surface of platinum and gold electrodes was highly accelerated by the application of a constant potential to the electrodes. The accelerated adsorption was significant at the electrode potential of 0.5 – 1.0 V vs. Ag/AgCl, even in the diluted solution of albumin (0.01%).
KEYWORDS serum albumin;metal electrode;accelerated adsorption

Nonspecific adsorption of albumin proteins on the surface of polymeric materials has been studied extensively in relation to their medical and clinical use.[1] On the contrary, the systematic study on the adsorption of albumin on the electrode surface is still limitted in spite of a wide usage of electrode systems as a tool for the analysis of biological fluids[2]. The present communication reports the preliminary finding that the adsorption of serum albumin on platinum (Pt) and gold (Au) electrodes from its dilute solution is highly accelerated by the application of a constant potential to the electrodes. The present results are of practical importance because, in the analysis of biological fluids, the electrodes are often operated at a constant potential.

Pt and Au disk electrodes (diameter: Pt, 3.0 mm and Au, 1.6 mm) were immersed in bovine serum albumin solutions at pH 3.2, 5.0, and 7.4. The pH of the solutions was adjusted using a 0.1 M acetate buffer (for pH 3.2 and 5.0) and a phosphate-buffered saline (for pH 7.4). A constant potential was applied to the electrodes through a potentiostat vs. a Ag/AgCl reference electrode. The electrodes thus treated were rinsed in the working buffer and the adsorption of albumin was evaluated by cyclic voltammetry (CV) using a conventional three-electrode system. The electrolyte solution for the CV measurements was a phosphate-buffered saline containing 10 mM Na_2SO_4 and 1.5 mM $K_4Fe(CN)_6$ (pH 7.4). All measurements were carried out at room temperature (ca. 20°C).

Figure 1 shows CV curves of the Pt electrode before and after the treatment with a 0.01% albumin solution at pH 5.0. The CV curve exhibited clear redox peaks originating from the $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ redox couple in the solution before the electrode was treated with the albumin solution (curve **A**). After the immersion of the Pt electrode in the albumin solution for 3 min with no applied potential to the electrode, the redox current for $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ was decreased to some extent (curve **B**). The electrode surface is considered to be occupied and blocked in part by albumin molecules which were adsorbed spontaneously from the solution, because the peak current of this system should depend on the effective surface area of the electrode. This view is supported by the fact that monolayer immobilization of amphiphiles[3] and proteins[4] on an electrode surface can block the redox reaction of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ at the electrode surface. On the other hand, the redox peaks disappeared completely after the treatment of the electrode in the same solution for 3 min with the application of a constant electrode potential (1.0 V) (curve **C**). This shows that albumin molecules covered the electrode surface completely to insulate it toward electrical

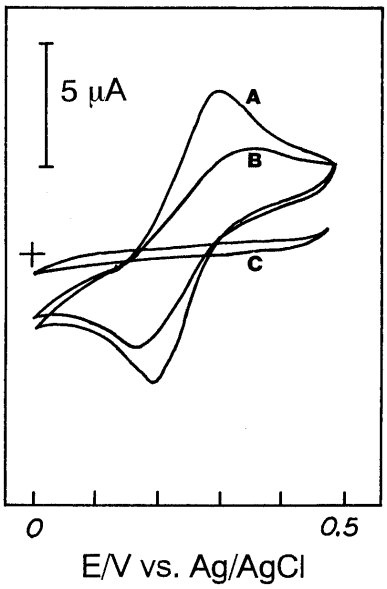

Fig. 1. Cyclic Voltammograms of Pt Electrodes in the Presence of 1.5 mM $K_4Fe(CN)_6$ at pH 7.4 before and after the Treatment with Albumin Solution
(A)Bare Pt electrode, (B)after the treatment with 0.01% albumin solution (pH 5.0) for 3 min (no electrode potential applied), and (C)after similar treatment to that in (B)with the applied potential to 1 V to the electrode.

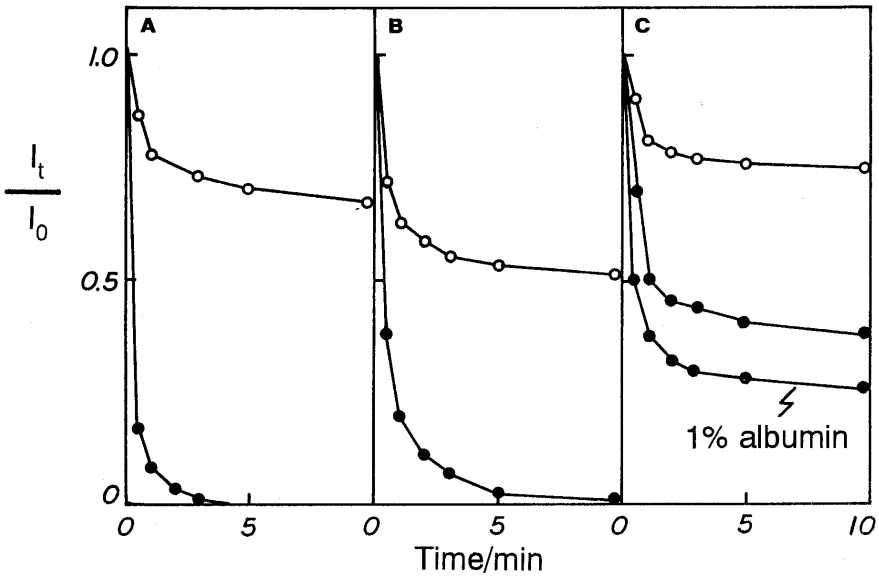

Fig. 2. Time Course of the Adsorption of Albumin on the Pt Electrodes in pH 3.2 (A), 5.0 (B), and 7.4 Media (C) with (-●-) and without (-○-)the Application of the Constant Potential of 1.0 V to the Electrode
The 0.01% albumin solution was used for all cases except for one case (1% albumin) in C.

connection with $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions in the solution. Thus it is demonstrated clearly that the adsorption of albumin molecules on Pt electrode is highly accelerated under the influence of the applied electrode potential. The acceleration of the adsorption rate was much more significant at 0.5 – 1.0 V than at 0 – 0.5 V.

In order to check the reversibility of the adsorption of albumin, the albumin–adsorbed electrodes were rinsed in a large quantity of buffer solution for 3 days, and then the CV measurements were performed again. No appreciable recovery of the redox current was observed for any electrodes, confirming that the adsorption of albumin is practically irreversible. The irreversible adsorption of albumin has also been reported for polymeric

materials.[5] Consequently, the electrode surface has to be polished mechanically to remove the adsorbed albumin molecules. We have observed nearly the same behavior in albumin adsorption, using Au electrode instead of the Pt electrode.

Figure 2 illustrates the time course of the adsorption of albumin on the Pt electrode from the 0.01% solutions at pH 3.2, 5.0, and 7.4. The relative values of the anodic current in CV for the oxidation of $Fe(CN)_6^{4-}$ ion (I_t/I_0, where I_0 and I_t are current at 0.28 V at the time 0 and t) were plotted as a function of time, although the I_t/I_0 values at this potential are a rough estimate of the progress of BSA adsorption rather than a quantitative evaluation of the surface coverage. It should be noted that, in all solutions tested, the adsorption of albumin was highly accelerated by the application of electrode potential. At pH 5.0, for example, 10 min suffices the complete coverage of the electrode surface by albumin at the electrode potential of 1.0 V, while more than 3 days are required by simple adsorption without applied potential in the same solution. The acceleration of adsorption was remarkable in pH 3.2 and 5.0 media. From the viewpoint of the practical use of the electrodes for the analysis of biological fluids including blood, it is important to evaluate the adsorption behavior of albumin at physiological conditions. For this purpose we used a phosphate-buffered saline at pH 7.4. In this medium, the rate of simple adsorption was not very fast. However, the application of electrode potential of 1.0 V accelerated the adsorption considerably. As expected, the adsorption was faster in 1% albumin solution than in 0.01% solution. Therefore, biochemical and/or clinical analysts should take these effects into consideration when the electrode is exposed to samples containing albumin or to blood itself, in view of the fact that the albumin content in human sera is roughly 4–5 g/100 ml.

The mechanisms by which the adsorption of albumin is accelerated by the application of the constant electrode potential are not unequivocal at the present stage, because many factors seem to be involved in determining the adsorption behavior of albumin, including hydrophobicity[6] and electric charges[7] of both the surface and protein, denaturation and conformational changes of the protein,[8] and even co-existence of small ions[9]. In any case, it should be emphasized that the application of a constant potential to the electrode accelerates the adsorption of albumin on the electrode even from the dilute solution (0.01%) and that, in some cases, electrochemistry on the electrode surface is blocked significantly.

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