Modification of a Glassy Carbon Electrode with Diols for the Suppression of Electrode Fouling in Biological Fluids

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The surface of a glassy carbon (GC) electrode was modified covalently with ethyleneglycol, diethyleneglycol, 1,2-propanediol, and 1,3-propanediol by electrochemical oxidation in order to suppress the electrode fouling originating from non-specific adsorption of serum proteins. Human serum albumin (HSA) was adsorbed significantly on the surface of a bare GC electrode, which was monitored by cyclic voltammetry in the presence of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions. In contrast, the diol-modified GC electrodes were scarcely fouled in HSA solution and even in human serum. The results were explained reasonably based on the hydrophilic nature of the diol-modified GC surface.

Key words protein adsorption; glassy carbon electrode; diols; anodic oxidation; chemical modification; serum albumin

Serum proteins are often adsorbed non-specifically onto the surface of electrodes and thus disturb the electrochemical reaction in biological fluids. We have reported that serum albumin is adsorbed onto the surface of gold (Au), platinum (Pt), and glassy carbon (GC) electrodes, and the electrochemical redox reaction of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions on the electrodes is blocked by the albumin layer.[1-3] Therefore, the non-specific adsorption of albumin should be eliminated for a reliable electroanalysis of biological samples. In the case of Au and Pt electrodes, the adsorption of serum albumin can be eliminated by modifying the electrode surface with a monomolecular layer of 2-mercaptoethanesulfonate (2-MS).[4] This is based on the strong affinity of thiol groups to Au and Pt surfaces.[5] The 2-MS-modified electrodes provide a highly hydrophilic surface due to the sulfonate residue of 2-MS. Unfortunately, this technique cannot be applied to the GC electrode because there is no affinity of thiols to GC. For this reason, we have treated the GC electrode with diol compounds in order to enhance the hydrophilicity of the electrode surface and to suppress the protein adsorption on the GC electrode. The diol-modified GC electrodes were prepared by electrochemical oxidation in the presence of diol compounds. In this context, Downnard and Roddick reported that protein adsorption on GC electrodes can be suppressed by modifying the surface covalently with aromatic diazonium salts through electrochemical reduction.[6] The present study reports the preparation of diol-modified GC electrodes and the adsorption property of human serum proteins on the electrodes.

Experimental

Materials A GC electrode was prepared by mounting a GC rod (3 mm diameter, from Tokai Carbon Co., Tokyo) in a Teflon support, and was used after polishing the surface thoroughly with alumina powder. Human serum albumin (HSA) and liophylized human serum were purchased from Miles Lab., Inc. and Nissui Pharmaceutical Co., respectively. Ethyleneglycol, diethyleneglycol, 1,2-propanediol (a mixture of *R*- and *S*-forms), 1,3-propanediol, ethanol, and 2-methoxyethanol were of reagent grade.

Modification of GC Electrode In order to modify the GC surface, the GC electrode was immersed in each solution of diols or alcohols (5 ml) containing 0.27 ml of 1 M H_2SO_4, and the electrode potential was repeatedly swept five times between 0 and 2 V *vs.* Ag/AgCl at a scan rate of 10 mV/s according to the reported procedure.[7] The modified electrode was washed with methanol and water before use.

Estimation of Protein Adsorption The modified or unmodified GC electrode was immersed in a HSA solution (0.4%) or in human serum for an appropriate time, and after rinsing the electrode with a phosphate-buffered saline (PBS), the cyclic voltammogram (CV) was recorded in PBS (pH 7.4) containing 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 using a Potentiostat/Galvanostat HA-501 (Hokuto Denko Co.). All measurements were carried out at *ca.* 20 °C.

Results and Discussion

It has been demonstrated that the adsorption of serum albumin on the electrode surface interferes with the redox reaction of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions at the electrode surface, resulting in the suppression of a peak current in the CV depending on the surface coverage.[1-3,8] It is plausible that the $Fe(CN)_6^{4-}/Fe(CN)_3^{3-}$ ions can not diffuse into the electrode surface due to blocking by the albumin molecules. This indicates that the adsorption

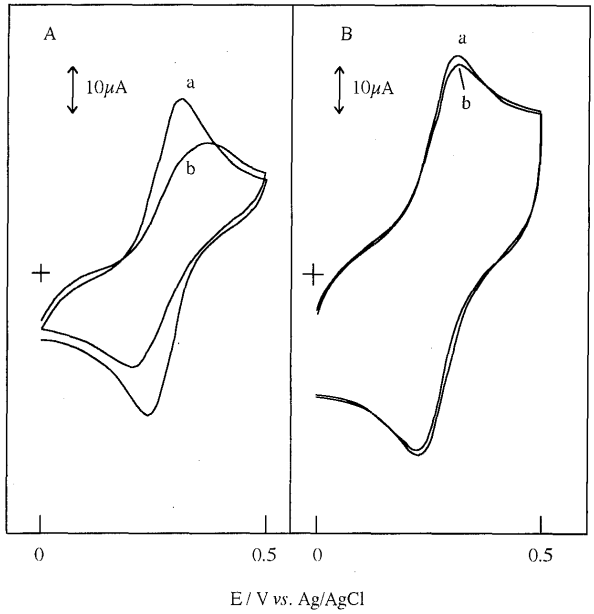

Fig. 1. CV of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ Ions on a Bare GC (A) and Ethyleneglycol-Modified GC (B) Electrodes before and after Adsorption of HSA

(a): CV before HSA adsorption and (b): CV after the electrode was treated with 0.4% HSA solution for 1 h.

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behavior of proteins on the electrode surface can be monitored by means of cyclic voltammetry in the presence of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions.

Figure 1 shows the CVs of 2.5 mM $K_4Fe(CN)_6$ on a bare GC electrode before and after treatment in 0.4% HSA solution (the albumin level in human serum is *ca.* 4%). When no HSA was adsorbed on the electrode surface before the HSA treatment, the CV exhibited a couple of reversible redox peaks which were centered at 0.27 V *vs.* Ag/AgCl with 60 mV separation (*i.e.*, $\Delta E_p = 60$ mV), showing a smooth electron transfer of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions to/from the GC electrode. On the other hand, after exposure of the electrode to the HSA solution, the peak current (i_p) in the CV decreased gradually and was attenuated down to *ca.* 65% of the original value, suggesting that the electrode surface was covered in part with the HSA molecules. The decrease in i_p is less significant than those observed for Pt and Au electrodes under the same experimental conditions.[1,2] However, the HSA adsorption to the GC electrode is still not negligible and probably induces some problems in using the electrode for the analysis of biological fluids.

It has been well established that the adsorption of serum albumin on the hydrophilic surface is lower than that on hydrophobic surfaces.[9-11] In order to make the GC surface more hydrophilic, we have tried to modify the electrode surface with diol compounds. It has been reported that the GC surface can be modified covalently with alcohols by sweeping the electrode potential between 0—2 V in alcohol containing a small amount of H_2SO_4.[7] Thus, it may be possible to introduce –OH groups at the GC surface through the electrochemical oxidation in diols (Chart 1). Figure 1B illustrates a cyclic voltammetric behavior of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions on the ethyleneglycol-modified GC electrode before and after treatment of the electrode with 0.4% HSA solution for 1 h. The CVs recorded before and after the HSA treatment were nearly identical to each other (the decrease in peak current was only 4% after the HSA treatment). This means that the redox reactions of $Fe(CN)_6^{4-}/Fe(CN)_6^{3-}$ ions occur smoothly in both cases, which, in turn, suggests that the surface of the ethyleneglycol-modified GC electrode is not fouled by HSA. In other words, the adsorption of HSA was suppressed almost completely by modifying the GC surface with ethyleneglycol due to the hydrophilic nature of the terminal –OH group.

It should be noted that the i_p value of the ethyleneglycol-modified electrode was enhanced slightly after the electrochemical oxidation as compared with the bare GC electrode (*cf.*, compare curve **a** in Fig. 1A with curve **a** in Fig. 1B). This probably originates from the formation of surface oxides through the electrochemical oxidation.[12] To verify the effect of the oxidation of the GC surface on protein binding, the GC electrode was oxidized in an aqueous 1 M H_2SO_4 in the absence of ethyleneglycol. As expected, the adsorption of HSA was reduced to some extent; the i_p value after HSA treatment was about 80% of the original i_p value in the CV, under the same experimental conditions as in Fig. 1. We also checked the possibility of irreversible adsorption of ethyleneglycol to the GC surface, by immersing the GC electrode in ethyleneglycol containing a small amount of 1 M H_2SO_4 for 1 h without any electrochemical treatment. After rinsing the electrode with methanol and water, the electrode was treated with the HSA solution for 1 h and the CV was measured similarly. The CV was almost the same shape and intensity as that measured on the bare GC electode, confirming no adsorption of ethyleneglycol on the GC surface. These observations suggest that electrochemical oxidation is essentially required for modifying the GC surface covalently with ethyleneglycol.

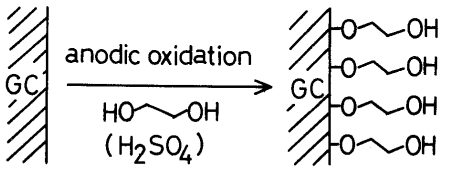

Chart 1

Table 1. Cyclic Voltammetric Behavior of Modified and Unmodified GC Electrodes before and after Treatment with HSA and Serum

Modifier	$i_{p,a}$ [a] (μA)	$i_{p,a}^{HSA}/i_{p,a}$ [b]	$i_{p,a}^{serum}/i_{p,a}$ [c]
None	37	0.65	0.84
Ethyleneglycol	47	0.96	0.97
Diethyleneglycol	50	0.96	0.96
1,2-Propanediol	45	0.96	0.93
1,3-Propanediol	42	0.93	0.97
Ethanol	55	0.79	0.89
2-Methoxyethanol	45	—[d]	—[d]

a) Anodic peak current for 2.5 mM $K_4Fe(CN)_4$ on the modified and unmodified electrodes before the treatment with HSA or serum. *b*) $i_{p,a}^{HSA}$ denotes the peak current in CV after the electrodes were treated with 0.4% HSA solution for 1 h. *c*) $i_{p,a}^{serum}$ denotes the peak current in CV after the electrodes were treated with serum for 1 h. *d*) The anodic peak disappeared completely in CV after the HSA and serum treatments because of full coverage of the electrode surface with serum proteins.

Table 1 lists the adsorption behavior of HSA and serum proteins on the diol- and alcohol-modified GC electrodes. Ethyleneglycol, diethyleneglycol, 1,2-propanediol, and 1,3-propanediol were effective as surface modifiers to prevent the GC surface from being fouled. In contrast to the diol compounds, the GC electrodes modified with ethanol and 2-methoxyethanol were fouled in the HSA solution and in serum. The protein adsorption on the 2-methoxyethanol-modified electrode was more significant than on the bare GC electrode. These results provide us with general descriptions that 1) the GC surface can be modified with all alcoholic compounds used, 2) diol compounds are effective as surface modifiers to suppress the HSA adsorption, and 3) alcohols with hydrophobic terminal groups (*i.e.*, ethanol and 2-methoxyethanol) are not effective modifiers, or rather, the HSA adsorption is accelerated in the case of 2-methoxyethanol.

Thus, it is concluded that the adsorption of serum proteins on the GC electrode can be suppressed considerably by modifying the electrode surface with diols by the electrochemical oxidation reaction. The suppressed binding of proteins is considered to originate from the enhanced hydrophilicity of the GC surface. Diol-modified GC electrodes would be useful for the electroanalysis of biological samples.

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