SERS Studies of Cytochrome c Immobilized on Modified Silver Electrode

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1 INTRODUCTION

Surface-enhanced Raman scattering (SERS) and Surface-enhanced resonance Raman scattering (SERRS) are powerful methods in the studies of biomolecules. Hildebrandt and Stockburger¹⁾ have shown that cytochrome c directly adsorbed on the silver electrode at a negative potential (-0.4 V vs. SCE) exhibits the six-coordinated low spin (6cLS) state which is the same as that in the solution. On the other hand, the adsorption at a positive potential (+0.1 V) gives rise to a mixture of five coordinated high spin (5cHS) and 6cLS configurations. Niki et al.²⁾ have shown that cytochrome c adsorbed on a roughened silver electrode is displaced by bis(4-pyridyl) disulfide (4-PyS) on which cytochrome c is immobilized. SERS signals of this electrode excited at 514.4 nm are almost identical to those of the 4-PyS modified electrode and no response from cytochrome c is obtained.

In the present work, we have characterized the oxidation, coordination and spin states of cytochrome c immobilized on the 4-PyS modified gold and silver electrodes.

2 EXPERIMENTAL

Horse heart cytochrome c (Type VI from Sigma) was purified chromatographically. 4-PyS (>98% from Aldrich) was used

without further purification. The gold and silver electrodes were mechanically polished by 0.05 μ m alumina powder followed by an ultrasonic cleaning in purified water. The electrodes were roughened by oxidation-reduction cycle (ORC) in 0.1M KCl aqueous solution. The adsorption of both 4-PyS and cytochrome c on gold and silver electrodes were made by the film transfer technique. The samples were excited by the Kr⁺ laser beam (Coherent Innova 100). Raman spectra were obtained by OMA III Raman system (EG&G model 1421, model 1460). The potential measurements were carried out in a quartz cell filled with 30 mM phosphate buffer solution of pH 7.0. All potentials were referred to SCE.

3 RESULTS AND DISCUSSION

Figure 1 represents the SERS spectrum (excitation wavelength is 647.1 nm) of the 4-PyS modified gold electrode on which cytochrome c is immobilized. The SERS signals are almost identical to those of the 4-PyS modified gold electrode. Whereas, the SERRS spectrum of the silver electrode excited at 413.1 nm gives rise to the signals of cytochrome c, which is immobilized on the 4-PyS modified silver electrode, and no SERS signals of 4-PyS are observable (Fig.2). Since the SERS is limited to molecules or functional groups located at distances ranging up to 0.5 nm from the electrode surface, the Raman signals of cytochrome c are probably due to the resonance Raman effect with an electronic transition of cytochrome c at the γ -peak.

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Fig. 1 SERS spectrum of cytochrome c adsorbed on 4-PyS modified gold electrode at open circuit potential (excitation wavelength : 647.1 nm).

Fig. 2 SERRS spectrum of cytochrome c adsorbed on 4-PyS modified silver electrode at -100 mV (excitation wavelength : 413.1 nm).

Fig. 3 SERRS spectrum of cytochrome c adsorbed on a bare silver electrode at -400 mV (excitation wavelength : 413.1 nm).

Fig. 4 Potential dependence of the marker band (ν_4 mode) of cytochrome c adsorbed on 4-PyS modified silver electrode. (○ : forward potential scan, ● : reverse potential scan)

The conformation of the cytochrome c adsorbed directly to silver electrode is monitored by the shift of the marker band, ν_3 , and is a mixture of 6cLS (1493 cm⁻¹) and 5cHS (1469 cm⁻¹) states as shown in Fig.3. However, the spectrum represented in Fig.2 reveals that cytochrome c immobilized on the 4-PyS adsorbed layer is in the 6cLS (1495 cm⁻¹), which is the same as the native form in the solution. The formal potential of cytochrome c immobilized on the 4-PyS modified silver electrode can be estimated from the midpoint of the shift of the marker band, ν_4 , at 1372 cm⁻¹ for the oxidized form and 1363 cm⁻¹ for the reduced form (Fig.4). The estimated formal potential agrees quite well with the formal potential determined by the UV-vis electroreflectance method³⁾. In both cases, the hysteresis was always observed and this is probably due to the slow equilibrium of the conformational change of the protein structure.

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