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Surface Enhanced Raman Scattering Spectroscopy of Flavodoxin on Graphite Electrode with Silver Colloids

Viktor BRABEC† and Katsumi NIKI*

†Institute of Biophysics, Czechoslovak Academy of Sciences, Brno 12, Czechoslovakia

Department of Physical Chemistry, Yokohama National University, Tokiwadai 156, Hodogaya-ku, Yokohama 240

Surface enhanced Raman scattering of flavin mononucleotide and flavodoxin adsorbed on the pyrolytic graphite electrode were recorded by depositing silver colloids onto the adsorbed layers. The SERS signals were sensitive to the electrode potential and accord very well with those obtained at silver electrode.

Although the mechanism of the surface enhanced Raman scattering (SERS) has not been completely explored, SERS is a powerful techinique for the *in situ* investigation of the interfacial and conformational properties of biological molecules at an electrolyte/electrode interface. The most intense SERS signals are usually found at an electrochemically pretreated silver electrode. Colloidal silver,[1-4] silver island films,[5] and silver films deposited on quartz or Teflon particles[6-9] have also been used as SERS active substrates. Among these substrates colloidal silver has been most widely used.

In this communication we report SERS of flavin mononucleotide (FMN) and flavodoxin (Fld) adsorbed on the basal plane of pyrolytic graphite (BPG) electrode by depositing silver colloids on the adsorbed layers of these substrates. The electrode reactions of the adsorbed species take place at the BPG electrode and the SERS signals of the reaction products are induced at the interface of the colloidal silver adsorbed on these biological molecules. The SERS activity of these systems are comparable to that at silver electrode surfaces.

FMN was purchased from Wako Pure Chemical and used without further purification. Fld was extracted from *D. vulgaris*, Hildenborough strain, and purified chromatographically. The impurities in Fld were not detectable by the polyacrylamide gel electrophoresis. The purified Fld was stored at -70 °C in 10 mM Tris-HCl buffer solution containing 0.005% sodium azide. Prior to each experiment, the electrolytes in Fld were removed by dialysis in dark so that the photoreduction of Fld by Tris-HCl was prevented. Then, Fld was dissolved in 30 mM phosphate buffer at pH about 7. The concentrations of FMN ($\varepsilon = 12500\ M^{-1}cm^{-1}$ at 445 nm) and Fld ($\varepsilon = 10600\ M^{-1}cm^{-1}$ at 456.5 nm) were determined spectrophotometrically. Both FMN and Fld were immobilized on the BPG electrode by the film transfer method.[10] Voltammetric and SERS experiments were carried out in 30 mM phosphate buffer solution at 25 °C. The electrode potentials were referred to silver/silver chloride in saturated potassium

chloride. The SERS spectra were excited by the 414.5-nm line of an argon laser and recorded with a Jasco R-800 Raman spectrophotometer. Silver colloids were prepared by mixing $1x10^{-3}$ M $AgNO_3$ and $1x10^{-3}$ M $NaBH_4$ (1:3) at 0 °C and were deposited on the BPG electrode pre-coated by FMN or Fld in the solution containing silver colloids. Then, these electrodes were subjected to SERS measurements after rinsing them thoroughly by 30 mM phosphate buffer solution. SERS measurements at ORC-treated silver electrodes were also made.

The cyclic voltammograms of FMN and Fld immobilized on the BPG electrodes are shown in Fig. 1. FMN exhibits well-defined redox peaks with the peak separation of 42 mV (sweep rate: 100 mV/s) and the electrode reaction is considered to be two consecutive one-electron reactions. The formal potential is estimated to be -0.445 V. On the other hand, Fld exhibits a voltammogram with the anodic at -0.365 V and cathodic peaks at -0.445 and -0.485 V (sweep rate: 100 mV/s). The two cathodic peaks become more evident at slower scan rates and merge into one peak at pH above 8.2. The electrode reaction of Fld is also considered to be two consecutive one-electron reactions, which are different from those determined by Dubourdieu and his coworkers,[11] and the formal potential is estimated to be -0.42 V at pH 7.3. The formal potentials of both FMN and Fld shifted toward negative with increase in pH. The formal potentials of FMN and Fld in the bulk are close to those of adsorbed species at the BPG electrode.

The SERS spectra of FMN in various oxidation states are shown in Fig. 2. The surface enhanced resonance Raman scattering (SERRS) is recorded at -0.40 V because the electronic absorption band of the oxidized form (quinone) coincides with 514.5 nm laser excitation line. At -0.50 V, however, weal SERS signals are recorded because FMN is in the fully reduced form (hydroquinone) so that no resonance effect is involved.

Figure 3 represents the SERS spectra of Fld at 0 V (quinone form) and at -0.55 V (hydroquinone). The SERS signals are recorded for the quinone form and no reso-

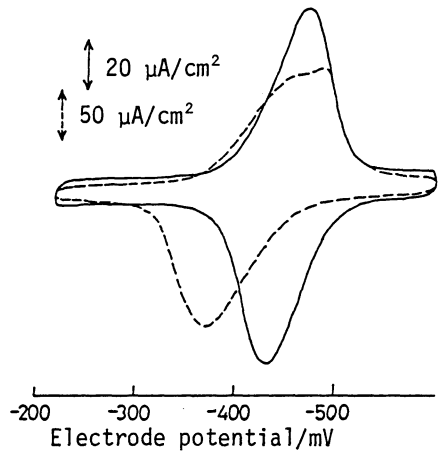

Fig. 1. Cyclic voltammograms of FMN (——) and flavodoxin (-----) adsorbed on BPG electrode in 30 mM phosphate buffer at pH 7.2. sweep rate: 100 mV/s.

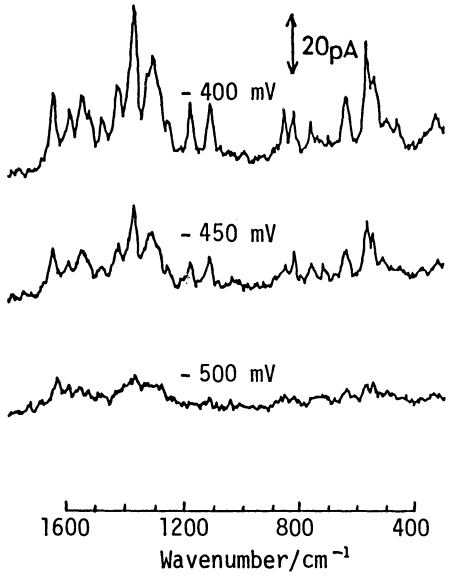

Fig. 2. SERS spectra of FMN adsorbed on silver electrode in 30 mM phosphate buffer at pH 7.1.

nance effect is observed for SERS of the hydroquinone form. The SERS bands of FMN in Fld are similar to those of FMN. However, the majority of the bands show significant differences in both wave numbers and intensities. The SERS intensities of Fld for the oxidized form are about one tenth of those of FMN. This is probably due to the fact that the prothetic group of Fld prevents a direct interaction between the chromophore and silver electrode so that the SERS signals of FMN in Fld are weaker. After two hours irradiation of the laser beam at 514.5-nm to Fld adsorbed on silver electrode, the SERS signals corresponding to the monomeric FMN start to appear, indicating that the slow dissociation of Fld takes place under the irradiation of the laser beam.

Our further experiments were aimed at obtaining the SERS spectra of FMN and Fld adsorbed on the BPG electrode by depositing silver colloids onto the adsorbed layers. The silver colloids were deposited on the BPG electrode coated with Fld by maintaining the electrode potential at -0.55 V for about two minutes so that good SERS signals could be obtained. As we expected, no SERS signals are observable at the BPG electrodes without silver colloids. After rinsing this electrode thoroughly by 30 mM phosphate buffer, it was subjected to the SERS measurements and the results are shown in Fig. 4. The SERS bands and their relative intensities at various electrode potentials are almost the same as those observed on the silver electrode shown in Fig. 3. However, the resolution and sensitivity of the SERS signals obtained on the Fld modified BPG electrode with silver col-

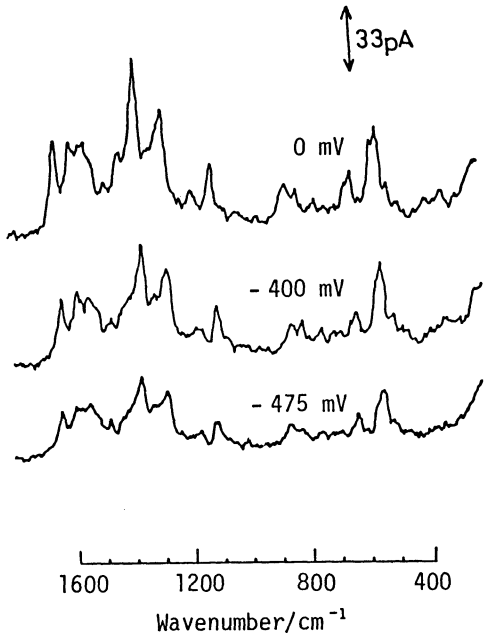

Fig. 4. SERS spectra of flavodoxin adsorbed on BPG electrode in the presence of silver colloids in 30 mM phosphate buffer at pH 7.1.

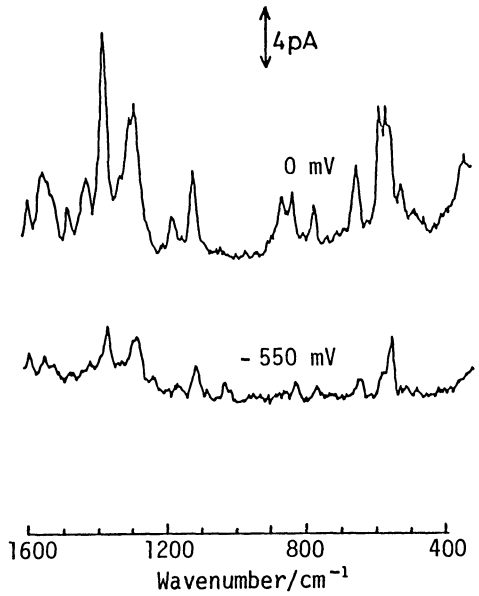

Fig. 3. SERS spectra of flavodoxin adsorbed on silver electrode in 30 mM phosphate buffer at pH 7.1.

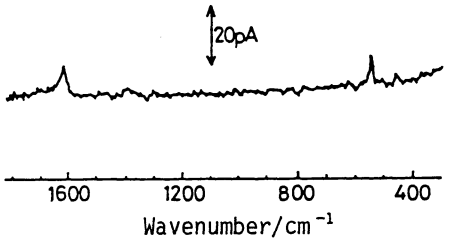

Fig. 5. SERS spectrum of FMN adsorbed on BPG electrode in the presence of silver colloids in 30 mM phosphate buffer at pH 7.1.

loids are better than those observed on silver electrode.

Figure 5 shows the SERS at the FMN modified electrode with silver colloids. The SERS signals were so weak that no signals were obtained under the same experimental conditions (single scan) as those in Fig. 4. The voltammetric measurement of this electrode, however, revealed that the electrode was fully covered with FMN and the same signals as shown in Fig. 2 were recorded after accumulating eight scans. The weak signals of FMN at the BPG electrode are probably due to the conformation of FMN at the electrode surface. FMN was shown to be adsorbed at a gold electrode with its isoalloxazine ring anchored onto the substrate with its ribitol and the phosphate moiety protruding into the solution phase.[12] It is reasonable to assume that the free FMN adopts the same interfacial configulation on the BPG electrode as that on gold electrode, and that the SERS spectrum of FMN at the BPG electrode could arise only due to the interaction of the isoalloxazine ring with silver colloid particles. The SERS inactivity of the free FMN adsorbed on the BPG electrode could be explained by assuming that the colloid particles are unable to enter the BPG electrode/electrolyte interface in such a way that they could contact with isoalloxazine ring of the adsorbed FMN. On the other hand, the FMN moiety in Fld adsorbed on the BPG electrode generates a good quality flavin SERS spectrum by the addition of silver colloids. This results suggests that the FMN moiety in the adsorbed Fld may adopt different configulation from the free FMN so that the colloid particles could contact with the FMN moiety in Fld for generating a good SERS spectrum.

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