Electron Transfer in SAM/Cytochrome/Polyelectrolyte Hybrid Systems on Electrodes: A Time-Resolved Surface-Enhanced Resonance Raman Study

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Silver electrodes were covered with mixed self-assembled monolayers (SAMs) of 11-mercaptoundecanoic acid (MUA) and 11-mercaptoundecanol (MU) and subsequently coated with alternating layers of cytochrome *c* (Cyt) and poly(anilinesulfonic acid) (PASA). The immobilized protein is electroactive and retains its native structure. Compared to the case of systems on gold electrodes, the stability of the assembly was found to be decreased. The redox process of Cyt is accompanied by reversible oxidation−reduction of PASA as revealed by the comparative surface-enhanced resonance Raman (SERR) analysis of assemblies including Cyt and the redox-inactive apo-cytochrome *c*. Time-resolved SERR experiments show a fast electron exchange between the protein and the polyelectrolyte that may play a supporting role in the electric communication of thicker multilayer assemblies employed as sensors.

Introduction

The immobilization of redox proteins on biocompatible electrodes has received increasing attention over the last two decades from both fundamental and applied points of view. In the latter case, it is widely accepted that the unique properties of proteins and enzymes in terms of redox behavior, molecular recognition, and catalysis represent an important potential for the design of bioelectronic devices such as biosensors and biofuel cells.[1,2]

In this context, cytochrome *c* (Cyt) has been extensively used as a model system due to the stability and availability of this well characterized soluble heme protein.[3] Strategies have been developed for producing Cyt monolayers on coated electrodes with different properties.[4−7] In the simplest approach, Cyt is electrostatically adsorbed on Au or Ag electrodes coated with self-assembled monolayers (SAMs) of ω-carboxyl alkanethiols. Binding to these SAMs occurs via the positively charged ring of lysine residues surrounding the partially exposed heme, ensuring a largely uniform orientation and efficient electronic communication. Furthermore, it was shown that superoxide radicals are able to reduce the immobilized protein, which at an appropriate electrode potential can be reoxidized, producing a current that is proportional to the radical concentration.[8−12] The sensitivity of this simple sensor is correlated with the amount of immobilized protein.[13] Therefore, an improvement of the sensor performance could be achieved by constructing stable protein multilayers.

It was demonstrated that multilayer assemblies can be prepared by layer-by-layer deposition of heme proteins and polyelectrolytes.[14−19] However, inefficient electric communication of the proteins in the outer layers has restricted the applicability of the method to only two to five layers.[15,17,18] More recently, it was shown that alternating layer-by-layer deposition of Cyt and poly(anilinesulfonic acid) (PASA) leads to a nearly linear increase of the voltammetric current with the number of deposited bilayers up to 16.[20] The multilayer formation was further confirmed by electrochemical quartz crystal microbalance (QCM) studies.[21] The assembly displays a 4−6 times higher sensitivity toward

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superoxide than Cyt immobilized in a monolayer.[22] It was proposed that electron transfer in the multilayer arrays proceeds via protein−protein electron exchange between face-to-face oriented Cyt molecules.[20,22] This mechanism requires a certain degree of rotational flexibility of the Cyt molecules embedded in the PASA polyelectrolyte matrix.

In the present work, we have investigated SAM/Cyt/PASA hybrid systems on Ag electrodes by stationary and time-resolved surface-enhanced resonance Raman (SERR) spectroelectrochemistry. The unique advantage of this methodological approach is that it allows simultaneous monitoring of the two main components of the assemblies (Cyt and PASA) and, therefore, provides new insight into the structure and dynamics of the devices.

Experimental Section

Chemicals. Horse heart cytochrome *c* was provided by Sigma (Steinheim, Germany). 11-Mercapto-1-undecanoic acid (MUA) and 11-mercapto-1-undecanol (MU) were purchased from Aldrich (Taufkirchen, Germany). Sodium phosphate, sodium dihydrogen phosphate, sulfuric acid, silver sulfate, urea, and 2-mercaptoethanol were provided by Merck (Darmstadt, Germany). Acetone was purchased from Riedel de Haën (Seelze, Germany). Acetic acid was supplied by Fluka (Schnelldorf, Germany). All solutions were prepared with 18 MΩ Millipore water (Eschborn, Germany). Silver ring electrodes were machined from 99.99% Ag rods (GoodFellow, U.K.). Buffer A was a 5 mM sodium phosphate buffer (pH 7), and buffer B a 0.5 mM sodium phosphate buffer (pH 5).

Electrode Preparation. The working electrode (Ag ring, 8 mm in diameter, 2.5 mm high) was mechanically polished and electrochemically roughened to produce a SERR active surface following published procedures.[23] Subsequently, it was immersed in a 1:3 mixture of 5 mM MUA and 5 mM MU in ethanol for 2 days to form a stable SAM. After washing with ethanol and buffer A, the MUA/MU modified electrode was incubated for 2 h in a 20 μM Cyt solution (buffer A) and washed with buffer A. The system Ag-MUA/MU-Cyt was then used as a template for creating alternating multiple layers of anionic PASA and cationic Cyt. The multilayers were created using the layer-by-layer adsorption technique that consists of alternating immersion steps in PASA solution (0.2 mg·mL^{-1} in buffer B) and Cyt solution (20 μM in buffer B). For each step, the electrode was dipped for 10 min into the corresponding solution and washed afterward with buffer B. Some preparations were subjected to thermal treatment which was found to stabilize the multilayer assembly.[20,22] For this purpose, the electrodes were incubated in buffer B at 45 °C for 30 min. In some experiments, Cyt was replaced by the redox and SERR inactive apoprotein (Apo).

The coated Ag ring electrodes were mounted in an electrochemical cell containing buffer A. The cell was equipped with a Pt wire counter electrode, an Ag/AgCl (sat. KCl) reference electrode, and optical windows for spectroscopic measurements. Electrode potentials were controlled with an EG&G potentiostat (Princeton Applied Research). Cyclic voltammograms were acquired in the range −150 to +150 mV or −200 to +200 mV at a scan rate of 100 mV·s^{-1}. All potentials reported in this work refer to the Ag/AgCl (sat. KCl) electrode.

Apo-cytochrome *c* Preparation. Apo-cytochrome *c* was prepared by chemical removal of the heme group following the protocol of Stellwagen and Rysavy.[24] Cytochrome *c* (80 mg) was dissolved in 10 mL of Millipore water with subsequent addition of 2 mL of acetic acid and 15 mL of 0.8% silver sulfate. This mixture was kept at 44 °C in the dark for 4 h and dialyzed versus 0.2 M acetic acid overnight. For precipitation of the apoprotein, 10 volumes of cold (4 °C) acid acetone (100 mL of acetone and 1 mL of 5 N sulfuric acid) was added to the mixture. The precipitate was collected by centrifugation, washed by acetone, and put into 5 mL of 0.2 M acetic acid. Solid urea was added until the solution turned clear. After addition of 25 molar excess of 2-mercaptoethanol and 20 min incubation at room temperature, the solid silver was removed. Finally, the protein was dialyzed against 0.5 mM phophate buffer. The concentration of apo-Cyt was determined by UV−vis spectrophotometry.

Surface-Enhanced Resonance Raman (SERR) Spectroscopy. SERR measurements were performed with the 413 nm line of a Kr^+ laser (Innova 300, Coherent) using a confocal Raman microscope (LabRam HR-800, Jobin Yvon) equipped with a N_2(l)-cooled back-illuminated charge-coupled device (CCD) detector. The laser beam was focused on the surface of the working electrode by means of a long working distance objective (20×; numerical aperture 0.35). SERR spectra were acquired with laser powers of 2.5 or 1.4 mW, accumulation times of 4−8 s, a spectral resolution of 2 cm^{-1}, and an increment per data point of 0.57 cm^{-1}. All spectra shown in this paper are normalized to the power of 1 mW and accumulation time of 1 s.

For time-resolved (TR) SERR experiments, potential jumps of variable duration and size were applied to trigger the redox reaction.[5−7,25,26] The relaxation process was probed by measuring the SERR spectra at variable delay times δ after each jump. Synchronization of potential jumps and measuring laser pulses was achieved with a homemade four-channel pulse-delay generator. The measuring laser pulses were generated by passing the continuous wave laser beam through two consecutive laser intensity modulators (Linos) which gave a total extinction better than 1:25 000 and a time response of ∼20 ns. The real spectra acquisition time, corresponding to the product of the measuring laser pulse length Δ and the number of cycles, was 1−2 s. The time-dependent spectroscopic results are displayed as a function of the delay time δ defined as $\delta = \delta' + \Delta/2$. The working electrode was rotated at ∼5 Hz to avoid laser induced sample degradation. After polynomial baseline subtraction, the measured SERR spectra were treated with homemade component analysis software.

Results and Discussion

Structure and Redox Equilibria of Cyt in the Hybrid Systems. Cyt was electrostatically immobilized on Ag electrodes previously coated with mixed SAMs of MUA/MU and investigated by potential-dependent SERR. SERR spectra of the adsorbed protein recorded at potentials sufficiently negative or positive with respect to the formal potential are identical to the resonance Raman (RR) spectra of the ferrous and ferric Cyt in solution, respectively (see the Supporting Information). RR and SERR spectra of heme proteins measured under Soret band excitation (∼410 nm) are dominated by the totally symmetric modes of the porphyrin ring. The so-called marker band region (∼1300−1700 cm^{-1}) includes a number of bands that are particularly sensitive to the electronic and structural properties of the heme iron, such as ligation pattern, spin, and oxidation state.[3,5−7,27] Thus, it is concluded that the protein native structure is preserved in the electrostatic complex, at least at the level of the heme pocket. Accordingly, series of SERR spectra measured at various potentials from −0.2 to +0.14 V could be consistently described on the basis of only two spectral components, that is, the ferrous and ferric native Cyt, using only their relative contributions as adjustable parameters.[28] The results were

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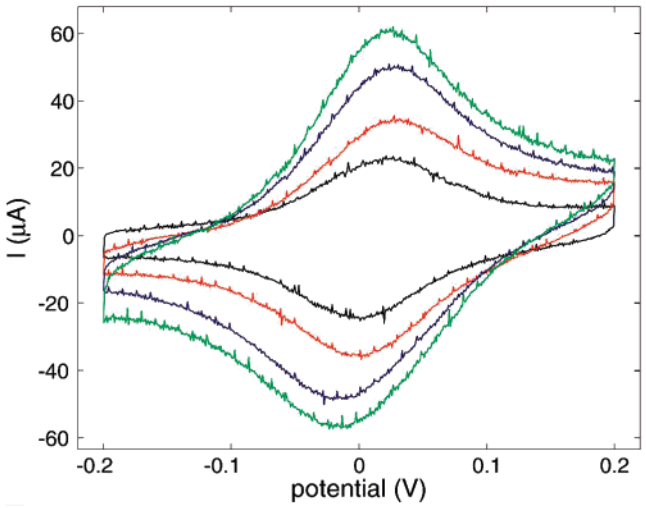

Figure 1. Cyclic voltammograms of Ag-MUA/MU-Cyt-(PASA-Cyt)$_n$ electrodes with n = (black) 1, (red) 3, (blue) 6, and (green) 10, measured immediately after preparation. Scan rate: 100 mV s^{-1}.

quantitatively treated in terms of the Nernst equation expressed as

$$E = E^0 - \frac{RT}{zF}\ln\frac{f_{Red}}{f_{Ox}} - \frac{RT}{zF}\ln\frac{I_{Red}}{I_{Ox}} \quad (1)$$

where I_i is the relative intensity of an individual spectral component and f_i is a factor proportional to the reciprocal SERR cross section of the species. The factors $f_{Red} = 0.28$ and $f_{Ox} = 1.00$ were estimated from the relative RR intensities of the fully reduced and fully oxidized Cyt, respectively, measured in solution under otherwise identical conditions.

This procedure yields nearly ideal Nernstian plots for a one-electron process with $E^0 = 20 \pm 10$ mV and $z = 0.9 \pm 0.1$. The formal potential obtained by SERR is in excellent agreement with the results of cyclic voltammetry (CV) measurements (see the Supporting Information) and with literature data for Cyt immobilized on SAM-coated electrodes.[4−10,12,29]

Using the Ag-MUA/MU-Cyt protein films as a template, multilayer assemblies of the type Ag-MUA/MU-Cyt-(PASA-Cyt)$_n$ were constructed by successive layer-by-layer deposition of PASA and Cyt and characterized by CV. As shown in Figure 1, the load of electrochemically addressable protein multiplies upon increasing n from 1 to 10, in agreement with previous results utilizing Au needle electrodes.[20,22] In sharp contrast, however, the upper layers are significantly less stable on roughened Ag electrodes as indicated by a steady decrease of the electrochemical signal during the first minutes after preparation. Probably, the different stabilities on the two metals are related to differences in the local electrostatics due to very different potentials of zero charge.[30,31] In addition, the surface roughness at the silver electrodes used within this study is much higher than at the gold electrodes used for sensor construction. Therefore, SERR investigations were restricted to the stable Ag/SAM/Cyt/PASA/Cyt assemblies ($n = 1$).

SERR spectra of these systems exhibit the typical bands of native reduced and oxidized Cyt. In addition, we observe a relatively strong band at ~1620 cm^{-1} (Figure 2), which is assigned to the C=C and C−C stretching vibrations of the quinoid and

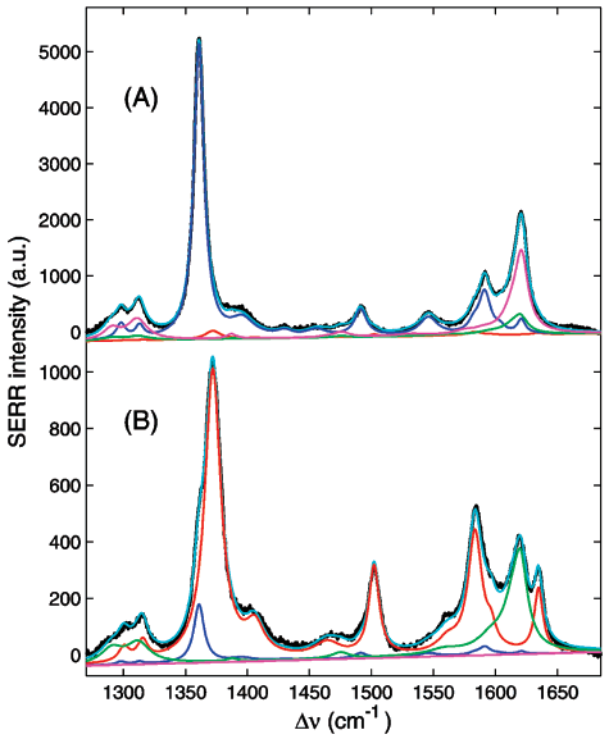

Figure 2. Measured SERR spectra and component analysis of the Ag-MUA/MU-Cyt-PASA-Cyt electrode at two different potentials: (A) −0.1 V and (B) +0.12 V. (black) Experimental spectra, (cyan) overall fit, (red) Cyt oxidized, (blue) Cyt reduced, (magenta) PASA1, and (green) PASA2.

benzenoic rings of the coadsorbed PASA polyelectrolyte.[32,33] Note that the spectral contribution of the polyelectrolyte in Figure 2 is divided into two components (PASA1 and PASA2, as described in the following section), while Cyt components are adopted from RR measurements in solution of the native ferrous and ferric species (see the Supporting Information).

Nernst analysis of potential-dependent SERR spectra of Cyt from several preparations yields an average value of $E^0 = 28 \pm 10$ mV for the formal potential of Cyt, which is in excellent agreement with the value determined by CV and very close to the value observed for Au-MUA/MU-Cyt preparations (*vide supra*). In addition, CV experiments show a linear relationship between the peak currents and scan rates, indicating that the electrochemically addressable protein is actually immobilized (see the Supporting Information).

Some of the Cyt/PASA preparations have been incubated at 45 °C prior to the measurements. Such a thermal treatment has been reported to substantially increase the stability of Cyt/PASA assemblies on gold.[20,22] For the hybrid systems on Ag electrodes after the treatment, we have found the same electrochemical and spectral properties except for a 2−4 fold increase of the SERR intensity of the polyelectrolyte modes. This finding indicates a rearrangement of the polyelectrolyte network enhancing the interaction of both components of the assembly. Even after thermal treatment, the immobilized protein retains its native structure in both the ferric and ferrous forms as judged from the careful analysis of the SERR spectra. The method, however, failed to stabilize the multilayer arrays on the rough silver, probably since the disassembling process occurs too fast.

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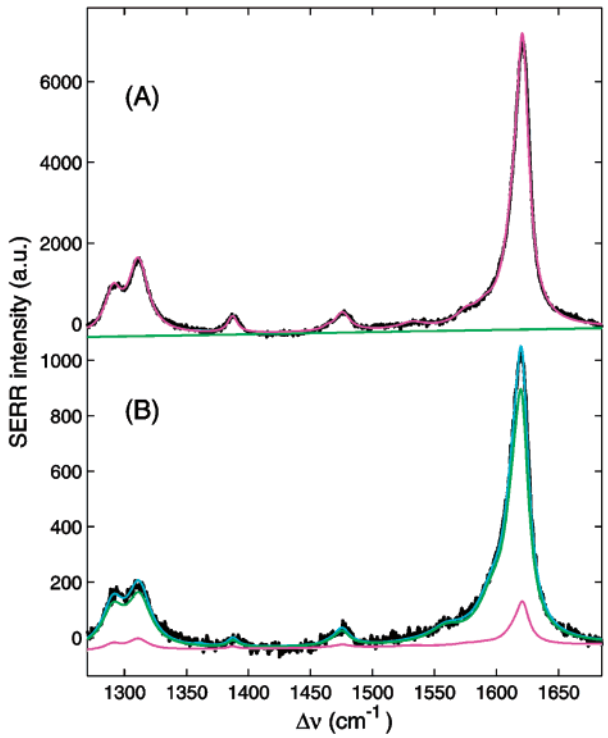

Figure 3. Measured SERR spectra and component analysis of the Ag-MUA/MU-Apo-PASA-Apo electrode at two different potentials: (A) −0.4 V and (B) +0.25 V. (black) Experimental spectra, (cyan) overall fit, (magenta) PASA1, and (green) PASA2.

Potential Dependence of the Polyelectrolyte Signals. Direct adsorption of PASA on the SAM-coated electrodes is very poor, as judged from the lack of a SERR signal. This can be understood, since both partners have the same charge. In contrast, assemblies of the type Ag/SAM/Apo/PASA/Apo, in which Cyt is replaced by the spectroscopically and electrochemically silent apoprotein (Apo), show strong characteristic PASA signals. Representative spectra recorded at two extreme potentials are shown in Figure 3. The affinity of PASA for the apoprotein reflects the opposite charge of the polyelectrolyte and the protein at pH 5 and is in agreement with independent surface plasmon resonance determinations (see the Supporting Information).

Notably, upon raising the electrode potential from −0.40 to 0.25 V, the SERR intensity drops by a factor of ∼7. This intensity change is fully reversible and, therefore, cannot be ascribed to a partial desorption of the polyelectrolyte.

Polyanilines are known to undergo redox transitions between the oxidized pernigraniline form through the emeraldine base to the reduced leucoemeraldine form.[34] In addition, protonation of the emeraldine base gives rise to the emeraldine salt, which can be conductive under certain conditions.[35] All these different polyaniline forms differ in color and thus in the absorption spectra.[36,37] The very strong PASA intensity at negative potentials can, therefore, be explained in terms of a strong resonance enhancement at the excitation wavelength of 413 nm. For different kinds of sulfonated polyanilines, an absorption band at ∼420 nm was assigned to the radical cation (emeraldine salt) form, which is responsible for the conducting properties of the polyelectrolyte.[38]

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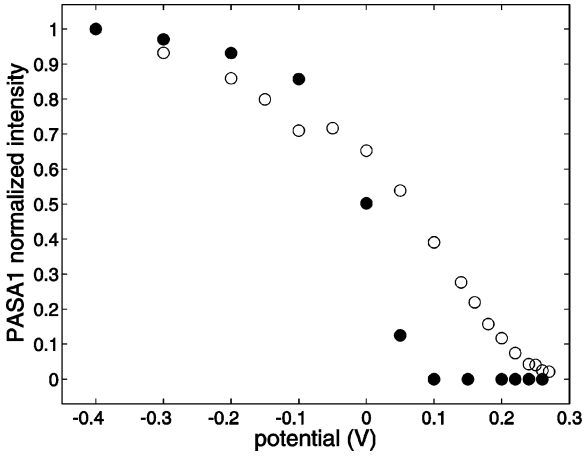

Figure 4. Normalized intensity of the PASA1 spectral component as a function of the electrode potential. (solid circles) Ag-MUA/MU-Cyt PASA-Cyt electrode and (hollow circles) Ag-MUA/MU-Apo-PASA-Apo electrode.

Table 1. Spectral Parameters of the Two PASA Components as Determined by SERR

PASA 1			PASA 2		
peak position (cm^{-1})	normalized intensity	fwhm (cm^{-1})	peak position (cm^{-1})	normalized intensity	fwhm (cm^{-1})
1290.6	0.16	16.3	1290.7	0.19	21.4
1311.1	0.30	19.3	1311.9	0.27	23.0
1387.1	0.07	9.6	1387.6	0.03	10.4
1475.6	0.08	14.8	1475.4	0.08	15.0
1575.1	0.05	20.1	1559.3	0.07	31.0
1590.0	0.05	20.0	1596.9	0.20	28.6
1614.3	0.33	19.7	1613.5	0.46	18.3
1621.2	1.00	12.0	1620.3	1.00	13.3

Thus, the SERR spectra recorded at the two extreme potentials most likely refer to different redox and protonation states of PASA. The states exhibit different absorption properties and SERR cross sections at the excitation wavelength; however, an unambiguous identification of these species is not possible on the basis of the present results. This interpretation is supported by the fact that the potential-dependent intensity drop is accompanied by small but distinct spectral changes that are indicative of chemically different species. Specifically, the most prominent PASA band at 1620 cm^{-1} is downshifted by ∼1 cm^{-1} at positive potentials. This shift is accompanied by a broadening and the appearance of a more pronounced shoulder on the low-frequency side. In addition, a potential-dependent change in the relative intensities of the two bands at 1290 and 1311 cm^{-1} is observed (Table 1).

The spectra obtained at −0.40 and 0.25 V, denoted as PASA1 and PASA2, were then used as component spectra for simulating the spectra recorded at intermediate potential values. A consistent description of the experimental spectra was obtained in the entire potential range by solely varying the relative contributions of the spectral components PASA1 and PASA2.

Unlike Cyt, the relative cross sections for the two PASA components are unknown, and therefore, their intensities cannot be converted to relative concentrations. Alternatively, we arbitrarily define the PASA1 intensity at the most negative electrode potential as equal to 1.0 to illustrate the potential-dependent variation of the relative contribution of PASA1.

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Figure 4 compares the potential dependence of the PASA1 component for Apo- and Cyt-containing electrodes.

Note that for Ag/SAM/Apo/PASA/Apo preparations the drop of the PASA1 contribution occurs over a very broad potential range. In contrast, Ag/SAM/Cyt/PASA/Cyt electrodes show a better defined (reversible) transition at ~15 mV, which is very close to the formal potential of the immobilized Cyt. This observation suggests, but does not prove, an electron exchange between Cyt and PASA.

Electron Transfer Dynamics. The time response of Ag/SAM/Cyt/PASA/Cyt electrodes to changes of the poised potential was investigated by time-resolved (TR) SERR. Spectra were measured at variable delay times δ after a potential jump from an initial value E_i to a final potential E_f. TR-SERR spectra were submitted to the same kind of component analysis as used for stationary measurements. For all TR-SERR experiments, we selected $E_i = -80$ mV. At this potential, Cyt is nearly fully reduced and the polyelectrolyte signal is largely dominated by the intense PASA1 component, such that the spectral changes resulting from potential jumps in the positive direction can be easily followed (Figure 5).

Assuming a one step relaxation process, the time evolution of the relative concentrations for ferric and ferrous Cyt can be described according to eq 2

$$\frac{\Delta c_\delta}{\Delta c_0} = \frac{c_{red}(t=\delta) - c_{red}(t=\infty)}{c_{red}(t=0) - c_{red}(t=\infty)} = \exp[-(k_{ox} + k_{red})t] \quad (2)$$

where $c_{red}(t = \delta)$ denotes the concentration of reduced Cyt at delay time δ and $c_{red}(t = \infty)$ and $c_{red}(t = 0)$ refer to the equilibrium concentrations at the final potential E_f and at the initial potential E_i, respectively. The oxidation and reduction rate constants k_{ox} and k_{red}, respectively, are linked through the equilibrium constant $K = k_{ox}/k_{red}$, which is determined from the stationary potential-dependent SERR experiments. The one step relaxation model appears to be justified, since logarithmic plots according to eq 2 yield straight lines with intercept values very close to zero.

For potential jumps to the redox potential of Cyt, that is, $E_f = 25$ mV, the average value for k^0_{ox} ($= k^0_{red}$), obtained for several Ag/SAM/Cyt/PASA/Cyt preparations, was found to be 17 ± 7 s^{-1}, which is very close to the value determined for Ag/SAM/Cyt systems, that is, in the absence of PASA ($k^0_{ox} = k^0_{red} = 22 \pm 8$ s^{-1}; see the Supporting Information).

As shown in Figure 6, the polyelectrolyte undergoes spectral changes on a similar time scale. The intensity decay of the PASA1 component, however, cannot be satisfactorily treated as a single exponential, and instead a minimum of two exponentials is required. This, however, does not rule out an even more complex kinetics, as one might expect from the structural complexity of the devices.

The average values of the two decay constants for the PASA1 component of several Ag/SAM/Cyt/PASA/Cyt preparations are $k_1^{app} = 35 \pm 15$ s^{-1} and $k_2^{app} = 1 \pm 1$ s^{-1} (Figure 6). Within the experimental error, the value of the first decay constant is similar to the electron transfer rate constant of Cyt.

Potential jumps to $E_f = 140$ mV, which correspond to an overpotential of ~115 mV for Cyt oxidation, produce an acceleration of the apparent rate constant of oxidation of Cyt ($k_{ox} = 120 \pm 30$ s^{-1}) as well as of the first PASA decay constant ($k_1^{app} = 135 \pm 30$ s^{-1}), while the second PASA decay constant remains unaltered within the experimental error ($k_2^{app} = 3 \pm 2$ s^{-1}). Control experiments with Ag/SAM/Apo/PASA/Apo preparations yield $k_1^{app} = 2 \pm 1$ s^{-1} and $k_2^{app} = 0.01 \pm 0.01$ s^{-1} for jumps to $E_f = 25$ mV, and nearly no acceleration is observed for $E_f = 140$ mV. Consistently, the apparent formal potential of PASA immobilized with the help of Apo on a MU/MUA-coated electrode is about +140 mV (the PASA signal can be only observed at small scan rates). SPR measurements indicate that Apo possesses a stronger binding affinity to the surface than Cyt. Therefore, the presence of Apo strongly bound to the MU/MUA-SAM is expected to slow down the direct electron transfer from PASA to the electrode.

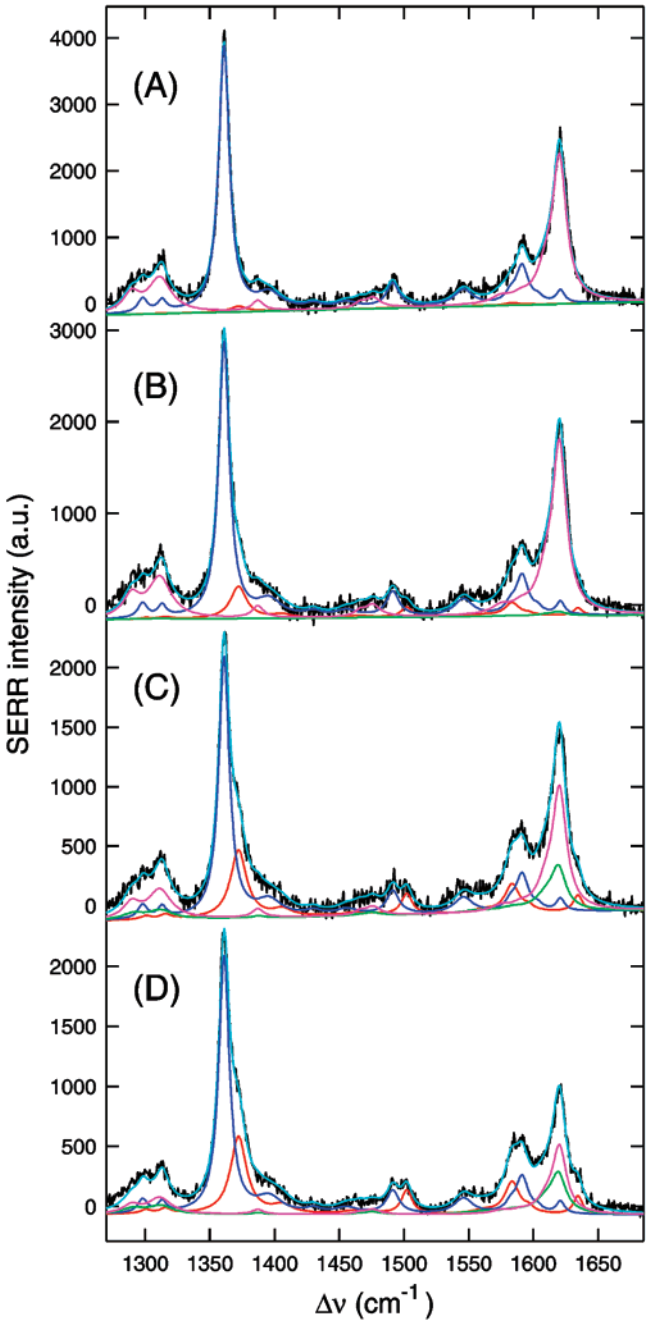

Figure 5. TR-SERR spectra of a Ag-MUA/MU-Cyt PASA-Cyt electrode at different delay times after a potential jump from $E_i = -80$ mV to $E_f = 25$ mV: (A) $\delta = 0$ ms; (B) $\delta = 21$ ms; (C) $\delta = 210$ ms, and (D) $\delta = \infty$. (black) Experimental spectra, (cyan) overall fit, (red) Cyt oxidized, (blue) Cyt reduced, (magenta) PASA1, and (green) PASA2.

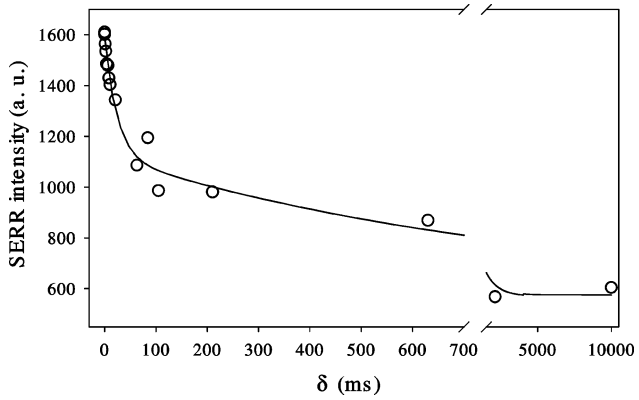

Figure 6. Absolute SERR intensity of the PASA1 spectral contribution as a function of the delay time δ after a potential jump from −80 to 25 mV for a Ag-MUA/MU-Cyt-PASA-Cyt electrode.

(k_1^{app}). This observation is consistent with a mechanism in which the heterogeneous electron transfer is limited by electron tunneling between the electrode and the first Cyt layer electrostatically adsorbed to the SAM. These Cyt molecules then undergo a rapid electron exchange with the nearby redox centers of the PASA molecules. The remaining fraction of PASA redox sites may be oxidized/reduced via direct electron transfer to/from the electrode (k_2^{app}).

Such a mechanism may also be relevant in thicker multilayer Au/SAM/Cyt/(PASA/Cyt)$_n$ systems, for which the electron transfer between the different protein layers was found to be slower than the electrode−Cyt electron exchange, and face-to-face interprotein electron transfer has been proposed as the main reaction pathway.[20,22]

Conclusions

(1) Cytochrome *c* immobilized in Ag/SAM/Cyt/PASA/Cyt assemblies by layer-by-layer deposition retains its native structure even after thermal treatment. The protein is electroactive and exhibits a nearly ideal Nernst behavior with $E^0 = 25$ mV. On rough Ag electrodes, additional PASA/Cyt adlayers are unstable and tend to desorb within a time scale of minutes.

(2) The polyelectrolyte undergoes reversible potential-dependent changes as judged by SERR spectroscopy. Two spectrally different forms were identified, which differ in relative intensities, peak positions, and absolute intensities.

(3) Comparison of the redox equilibria and dynamics of Cyt and PASA in Ag/SAM/Cyt-PASA/Cyt and Ag/SAM/Apo/PASA/Apo assemblies provides evidence for efficient electron exchange between the protein and the polyelectrolyte. Thus, we conclude that polyelectrolyte−protein electron transfer may play an active role in supporting long-range electron transport within metal-MUA/MU-Cyt-(PASA-Cyt)$_n$ multilayer assemblies. The results obtained here for a two-protein layer system, however, do not exclude the involvement of interprotein electron transfer as previously proposed.[20,22]

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Supporting Information Available: RR, SERR, electrochemical, and SPR data. This material is available free of charge via the Internet at http://pubs.acs.org.

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