Back-bonding Manifestation on the Stability of a Pyridinethiolate Surface Modifier at a Gold Electrode

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Heterogeneous electron transfer (ET) reactions of horse heart cytochrome *c* (cyt *c*) have been an intense subject of study in the last two decades.[1] It is well documented that the strong adsorption of this species at naked electrodes leads to protein unfolding, making difficult the assessment of the ET rate.[2] Conversely, rapid electron transfer of cyt *c* has been observed at gold-modified electrodes with 4-mercaptopyridine (pyS) and 4,4′-dipyridyl disulfide (pySS) with no evidence of protein conformation loss.[2b] Despite the successful use of these electrodes, there is a remarkable instability of the adlayers formed from pyS and pySS at gold, yielding adsorbed atomic and oligomeric sulfur species due to the C−S bond cleavage[3].

We undertook the $[Ru(CN)_5]^{3-}$ metal center as a π-donor system coordinated to the pyS ligand to design the first inorganic complex[4] applicable to the assessment of the inherent rate of the cyt *c* ET reaction. A key point is that the coordination of a π-donor transition metal center to a nitrogen on a pyridine ring of the pyS would affect its electronic density,[5] enhancing the C−S bond strength with consequent stability gain of the adlayer.

The modified electrode[6] was characterized by infrared reflection spectroscopy.[7] Figure 1 shows the spectra of a $[Ru(CN)_5(pyS)]$−Au adlayer (RupySAu), $K_4[Ru(CN)_5(pyS)]\cdot 3H_2O$ (RupyS), and pyS dispersed in KBr with a comparative purpose. The cyanide stretching frequency at 2055 cm^{-1} in the spectra of RupySAu and RupyS not only is consistent with the presence of the reduced Ru(II) form[8] but also suggests no cyanide adsorption at the gold surface.[9] The band at 1115 cm^{-1} assigned to the C═S stretching[10] in the spectra of pyS (Figure 1c) and RupyS (Figure 1d) is not present in the spectrum of RupySAu. These observations strongly suggest that the complex binds the gold surface through sulfur atoms.

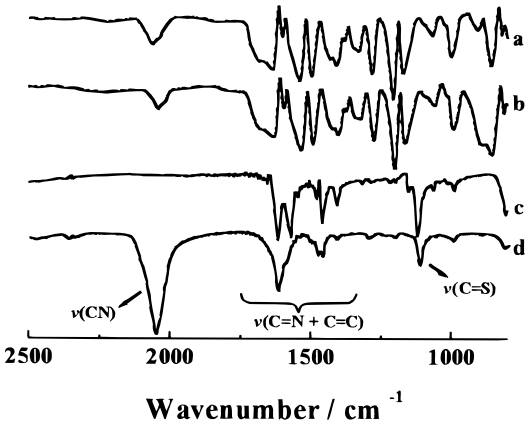

Figure 1. Infrared reflection spectra for adlayers immersed in 20 mM aqueous solution of RupyS for 24 h (a) and 5 min (b). Infrared transmission spectra of pyS (c) and RupyS (d).

The bands present between 1600 and 1400 cm^{-1} are currently assigned to the coupled in-plane stretching modes (ν(C═C) + ν(C═N)) of the pyridyl ring.[3,10,11] The C−H bends in plane $\delta(CH)_{ip}$ and out of plane $\delta(CH)_{op}$ are also evident at 1210 and 800 cm^{-1}, respectively.[3,11] All of these spectral data qualitatively confirm the chemical composition of the $[Ru(CN)_5(pyS)]$-derived adlayer at the gold surface. Contrary to that observed for the pySAu adlayer,[3] the spectra obtained for freshly prepared RupySAu (Figure 1b) and more extended immersion time in the precursor solution (Figure 1a) have not shown any significant change indicative of the adlayer degradation.

The following discusses the finding and implications of different sets of electrochemical studies taking the RupySAu as the working electrode to assess the ET of cyt *c* probe redox protein. The first set of studies achieved the experimental

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(4) RupyS was synthesized acording to the procedure for RuPySS preparation (Moreira, I. S.; Franco, D. W. *Inorg. Chem*. **1994**, *33*, 1607−1613). Previous study of $[Ru(CN)_5(pySS)]$−Au film formation led us to select the $[Ru(CN)_5]^{3-}$ moiety (Diógenes, I. C. N.; Nart, F. C.; Tanaka, A. A.; Moreira, I. S. Manuscript in preparation). Also, the cyanide environment of this class of complexes is known to interact electrostatically with the lysine groups of cyt *c* (Moreira, I. S.; Sun, J.; Cho, M. O. K.; Wishart, J. F.; Isied, S. S. *J. Am. Chem. Soc*. **1994**, *116*, 8396−8397).

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(6) The adlayers were prepared by changing the immersion time of the polycrystalline gold electrodes (areas of 0.0314 and 2.0 cm^2) in a 20 mM aqueous solutions of the promoters. The gold electrodes were polished as described by Qu et al.: Qu, X.; Lu, T.; Dong, S. *J. Mol. Catal.* **1995**, *102*, 111−116.

(7) Reflection spectra were obtained using p-polarized light incident on the samples at 80° with respect to the surface normal. All spectra are presented as % R/Ro, where R and R_0 represent the reflectances of the adlayers and naked gold, respectively.

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conditions for best and reproducible performance of the RupySAu toward the efficiency of cyt c redox transformation. Accounting for the time required to reach the saturation coverage of the RupyS self-assembled monolayer at gold, the best cyt *c* electrochemical response was achieved for gold electrode immersed in RupyS solution for 5 min. From this period of immersion to 60 min, we did not detect any reasonable difference in the current values or in the redox wave separations of the cyt *c* redox process. This suggests that a complete formation of the monolayer occurs within 5 min under the experimental conditions.[12] Further increases in the immersion time did not affect the electrochemical performance of the gold-modified surface to facilitate the ET of cyt *c*.

We have taken two of the best promoters reported in the literature[1−3] (pyS and pySS) along with RupyS in order to modify the gold surface and perform cyt *c* electrochemistry. The current response obtained by using RupySAu clearly confirms its best performance to facilitate the ET of cyt *c*. Cyclic voltammograms are shown in the Supporting Information for this report.

A decrease in the performance of pySAu and pySSAu adlayers toward the electrolysis of cyt *c* with increasing immersion time of the gold electrode was observed as reported in the literature.[3] The voltammetric curves of cyt *c* for gold immersed overnight in these promoter solutions were barely distinguishable from the background current. Conversely, the curve obtained for RupySAu showed a performance similar to that shown by curves obtained for shorter immersion times. Furthermore, the cyt *c* electrochemical response for RupySAu prepared within 5 min immersion and kept out of the promoter solution overnight was about the same as for freshly prepared electrode.

Figure 2 presents the reductive linear sweep voltammetric curves of the adlayers as a function of the immersion time of gold in pyS and RupyS solutions. As reported in the literature,[3] the voltammetric curve (Figure 2a) of the pyS adlayer for 5 min immersion time is dominated by a cathodic wave with an E_{rd} of − 0.56 V assigned to the reduction desorption (AuSpy + e^- → Au + pyS^-). As the immersion time increases, a wave at − 0.95 V is observed and grows at the expense of the − 0.56 V wave (Figure 2d). This wave has been assigned to an adsorption-induced activation of the oxidative cleavage of the C−S bond and the resulting formation of an adlayer composed of atomic and oligomeric forms of sulfur.[3] The E_{rd} observed for freshly prepared RupySAu (Figure 2b) is significantly more negative (−0.73 V) than pySAu, suggesting that the complex is more strongly bound to gold than the free ligand. This is consistent with the anticipated back-bonding effect, (Ru^{II}) $d\pi \rightarrow p\pi^*$ (pyS), that increases the electronic density of pyS improving the chemisorption process.

The wave assigned to desorption of sulfur forms appears after 30 min immersion time (Figure 2c) with a very small charge consumed (17 μC cm^{-2}). Therefore, the formation of this undesirable adlayer is minor compared to a similar process[3] for pySAu (ca. 180 μC cm^{-2}). Accounting for the rate of the RupyS aquation, $t_{1/2}$ = 96 min^{-1}, the competitive adsorption of the dissociated free ligand (pyS) must be considered for large immersion time modifications. Consequently, it would be reasonable to assume that the wave at −0.95 V (Figure 2c) derives from pySAu decomposition instead of RupySAu.

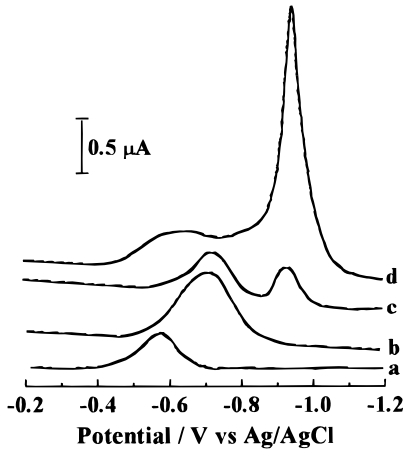

Figure 2. Voltammetric desorption curves for adlayers immersed in 20 mM aqueous solutions of pyS for 5 min (a) and 30 min (d) and RupyS for 5 min (b) and 30 min (c): electrolyte, 0.5 M KOH(aq); ν = 100 mV s^{-1}.

The data collected on the system described here strongly demonstrated that the π back-bonding effect, so well established by Taube's research group,[5,13] from the $[Ru(CN)_5]^{3-}$ metal center[4,14] plays an important role in the stability of the RupySAu improving the assessment of the ET of cyt *c*. To extend such study to other π-donor systems, experiments with $[Fe(CN)_5]^{3-}$, $[Ru(NH_3)_5]^{2+}$, and $[Os(NH_3)_5]^{3+}$ metal centers are currently under investigation at our laboratory. The results will be reported in a forthcoming paper.[15] The efficiency of this metal-centered back-bonding toward the sulfur−sulfur bridge stability of the pySS molecule has been clearly demonstrated in the study of mixed-valence complexes.[4,8e,16,17]

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Supporting Information Available: Reproductions of cyclic voltammetry of cyt *c* at gold electrode immersed for 5 min in 20 mM aqueous solution of RupyS, pyS, pySS, and naked electrode. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) A 20 mM aqueous solution of RupyS; 100 μM cyt *c* in 100 mM phosphate buffer, pH = 7.00, ν = 100 mV s^{-1}. The protein was previously purified according to Brautigan et al.: Brautigan, D. L.; Fergusson-Miller, S.; Margoliash, E. In *Methods in Enzymology*; Fleischer, S., Pucker, L., Eds.; Academic Press: New York, 1978; Vol. LIII.

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