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A New Ferrocenophane Surface Derivatizing Reagent for the Preparation of Nearly Reversible Electrodes for Horse Heart Ferri-/Ferrocytochrome *c*: 2,3,4,5-Tetramethyl-1-((dichlorosilyl)methyl)[2]-ferrocenophane

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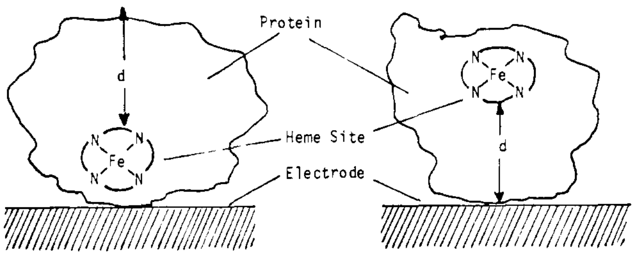
Abstract: Pt gauze electrodes functionalized with hydrolytically unstable 2,3,4,5-tetramethyl-1-((dichlorosilyl)methyl)[2]-ferrocenophane (I) exhibit persistent cyclic voltammetry waves consistent with a surface-confined redox couple, $\text{PMFc}^+/\text{PMFc}^0$, having an $E^{\circ\prime} = +0.04$ V vs. SCE in H_2O/1.0 M $NaClO_4$ at pH 7.0. Such electrodes will effect the one-electron reduction of horse heart ferricytochrome *c* (cyt c_{ox}) to ferrocytochrome *c* (cyt c_{red}) as well as the oxidation of cyt c_{red} to cyt c_{ox}, under conditions where the naked electrode does not effect such redox processes. n-type Si photoanodes functionalized with I will effect the uphill oxidation of cyt c_{red} upon $\geq E_g$ illumination and will effect cyt c_{ox} reduction in the dark. Derivatized electrodes are durable and do not significantly lose activity with use; a 4.4 mM cyt *c* solution can be alternately exhaustively oxidized and reduced at +0.3 or −0.3 V vs. SCE in controlled-potential electrolyses for >10 cycles without electrode deterioration. Electrodes derivatized with I come into charge-transfer equilibrium with cyt c_{ox}/cyt c_{red}, $E^{\circ\prime} = +0.02$ V vs. SCE, via heterogeneous electron-exchange processes involving the $\text{PMFc}^+/\text{PMFc}^0$ redox centers; such electrodes can be used as potentiometric sensors for cyt *c*.

Modification of electrode surfaces may serve to overcome two interrelated problems, adsorption and sluggish kinetics, that plague studies of the redox behavior of large biological reagents at electrodes.[1,2] One problem is electrode fouling resulting from the strong adsorption of biological reagents to electrode surfaces. The adsorption may block access to the electrode by solution species. Modified electrodes can have different adsorption properties that depend on the properties of the surface reagent. Since it is well established that many surfaces can be modified with the same reagent,[3] a surface reagent that reduces the problem of adsorption would allow the use of a variety of electrode materials that could be chosen on the basis of practical factors such as durability, surface area, and cost. Further, it is often of interest to be able to study "as is" biological samples that could be impure with respect to materials that adsorb and block electrode access. Surface modification could prove useful in overcoming this problem as well.

Scheme I



A second problem in the study of biological redox reagents would appear to be the low magnitude of the heterogeneous electron-transfer rate constant, k_{het}, owing to structural constraints that can prevent a close approach of the buried redox center to the electrode surface. In fact, surface modification to prevent adsorption could exacerbate this problem by holding the redox site further away from the electrode. But the problem of adsorption and a small k_{het} may be related as shown in Scheme I. Functionality of the biomolecule that brings about adsorption could be such that the redox center is held away from the electrode surface (left) and the redox system is a distance *d* away from the surface, which is sufficiently great that the electron exchange rate with the electrode is low. Alternatively, the adsorption may be such that the redox center closely approaches the electrode surface but an incoming reagent's redox center is >*d* away from that of the adsorbed species. In either case, the orientation of the adsorbed species can reduce the rate of mediated reaction that could proceed

$$[\text{cyt } c^*_{\text{ox}}]_{\text{ads}} + [\text{cyt } c_{\text{red}}]_{\text{soln}} \rightleftarrows [\text{cyt } c^*_{\text{red}}]_{\text{ads}} + [\text{cyt } c_{\text{ox}}]_{\text{soln}} \qquad (1)$$

without desorption of the adsorbed species, eq 1. Thus the actual value of k_{het} may be obscured by the adsorption problem. The observed rate of redox product formation can be controlled by either the slow electron-exchange process represented by eq 1 or by slow physical-exchange processes represented by eq 2 and 3

$$[\text{cyt } c_{\text{ox}}]_{\text{ads}} + [\text{cyt } c^*_{\text{red}}]_{\text{soln}} \rightarrow [\text{cyt } c^*_{\text{ox}}]_{\text{ads}} + [\text{cyt } c_{\text{ox}}]_{\text{soln}} + \text{e} \qquad (2)$$

$$[\text{cyt } c_{\text{red}}]_{\text{ads}} + [\text{cyt } c^*_{\text{ox}}]_{\text{soln}} + \text{e} \rightarrow [\text{cyt } c^*_{\text{red}}]_{\text{ads}} + [\text{cyt } c_{\text{red}}]_{\text{soln}} \qquad (3)$$

for oxidation of cyt c_{red} and reduction of cyt c_{ox}, respectively. In eq 1–3, the asterisk indicates labeled cyt *c* to emphasize in eq 1 that the cyt *c* is immobilized and in eq 2 and 3 that physical exchange is occurring.

In many cases it has been found that solution redox mediators can give good kinetics for reaction with biological redox reagents,[2] and in principle, a surface-confined reagent of this kind can retain good kinetics. Thus, when k_{het} is small, molecular derivatization of the surface could improve the observed heterogeneous kinetics. The mediator could penetrate the biological redox reagent to access the buried redox site, and retention of this property upon attachment of the mediator to a surface, while preserving a structure that will allow the mediator to rapidly exchange electrons with the electrode, is desirable. Equations 4 and 5 represent the

$$[\text{M}^+]_{\text{surf}} + \text{cyt } c_{\text{red}} \xrightarrow{k_{\text{ox}}} [\text{M}^0]_{\text{surf}} + \text{cyt } c_{\text{ox}} \qquad (4)$$

$$[\text{M}^0]_{\text{surf}} + \text{cyt } c_{\text{ox}} \xrightarrow{k_{\text{red}}} [\text{M}^+]_{\text{surf}} + \text{cyt } c_{\text{red}} \qquad (5)$$

mechanism that could obtain for a surface-confined mediator. The system is the heterogeneous analogue of the classic solution

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(1) (a) Margoliash, E.; Schejter, A. *Adv. Protein Chem.* **1966**, *21*, 113. (b) Eddowes, M. J.; Hill, H. A. O. *Biosci. Rep.* **1981**, *1*, 521.

(2) (a) Szentrimay, R.; Yeh, P.; Kuwana, T. *ACS Symp. Ser.* **1977**, *38*, 143. (b) Heineman, W. R.; Meckstroth, M. L.; Norris, B. J.; Su, C.-H. *Bioelectrochem. Bioenerg.* **1979**, *6*, 577. (c) Kuwana, T.; Heineman, W. R. *Acc. Chem. Res.* **1976**, *9*, 241. (d) Prince, R. C.; Linkletter, S. J. G.; Dutton, P. L. *Biochim. Biophys. Acta* **1981**, *635*, 132.

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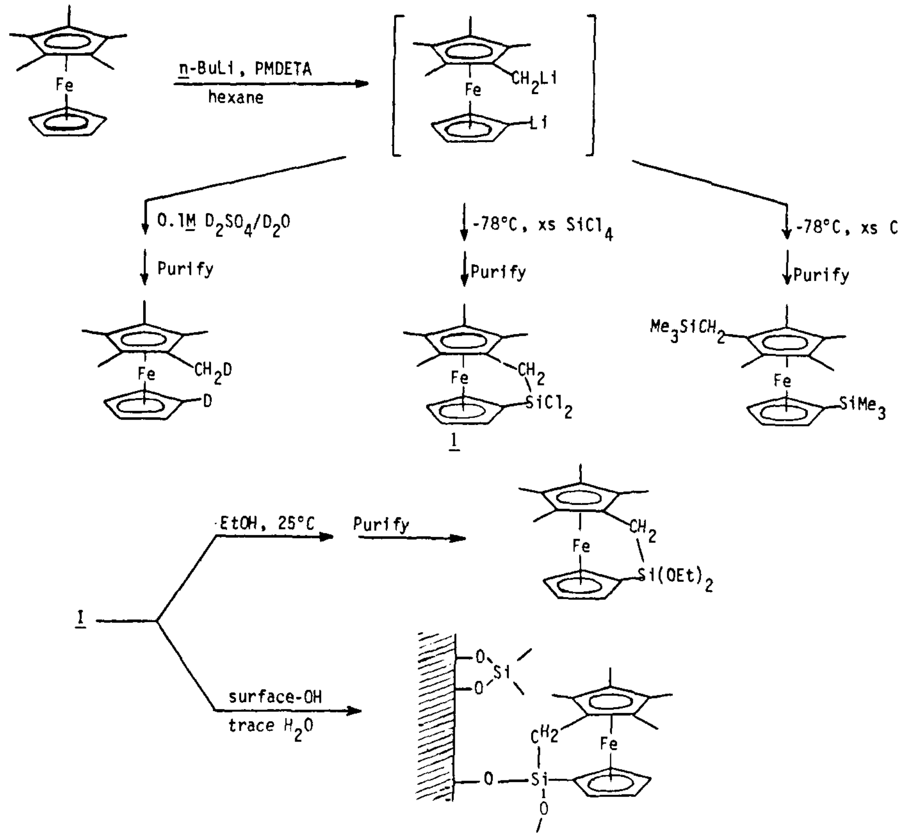
Scheme II. Representation of the Synthetic Procedures used To Prepare Reagent I



mediators.[2] The surface-confined mediator system has the advantage that the redox levels of the biomolecule can be manipulated on a synthetic scale without the complication of introducing undesirable solution species. Further, when a biomolecule does foul an electrode, access to the electrode by the solution mediator itself is blocked.

Studies of both solution mediators[2] and surface-bound redox reagents[3] provide at least three guidelines for obtaining desirable properties for surface mediators: (i) the solution analogue of the surface reagent should function as a mediator; (ii) the formal potential, $E°'$, of the $[M^{+/0}]_{surf}$ system should be close to that of the biomolecule in order to effect both oxidation and reduction with good kinetics; and (iii) the $[M^{+/0}]_{surf}$ must be durable in all redox levels involved.

A recent report from this laboratory[4] establishes that a surface-confined mediator can work. Electrodes of Pt, Au, or p-type Si can be derivatized with an *N,N'*-dialkyl-4,4'-bipyridinium, PQ^{2+}, reagent[5] that is an analogue of the well-known viologen mediator *N,N'*-dimethyl-4,4'-bipyridinium, MV^{2+}.[6] The Pt, Au, or p-type Si electrodes bearing $[(PQ^{2+})_n]_{surf}$ are useful in effecting the reduction of cyt c_{ox} to cyt c_{red} with good kinetics but only at electrode potentials where the $[(PQ^{2+})_n]_{surf} \rightarrow [(PQ^{+})_n]_{surf}$ reduction could be effected. The $E°'[(PQ^{2+/+})_n]_{surf} = -0.55 \pm 0.05$ V vs. SCE,[4,5] whereas $E°'(\text{cyt } c_{ox}/\text{cyt } c_{red}) = +0.02$ V vs. SCE,[7,8] and, accordingly, the oxidation of cyt c_{red} to cyt c_{ox} could not be effected with electrodes bearing $[(PQ^{2+})_n]$. Further, owing to the differences in $E°'$'s, the reduction of cyt c_{ox} requires considerable overvoltage with the $[(PQ^{2+/+})_n]_{surf}$ system. Nonetheless, the knowledge that MV^{+} will rapidly reduce cyt c_{ox}[9] led to the demonstration that electrodes bearing $[(PQ^{2+})_n]_{surf}$ could be useful in synthetic scale reductions of cyt c_{ox} without the use of solution mediators.[4] It is particularly noteworthy that good kinetics could be obtained even at high concentrations of cyt c_{ox} (up to 17 mM), where effects from adsorption should be more severe.

In our hands, cyt c_{ox} is not rapidly reducible, at any potential, at naked Si, Pt, or Au surfaces. Generally, the cyt c_{ox}/cyt c_{red} is not well-behaved at electrode surfaces.[10] However, at indium tin oxide electrodes, the cyt c_{ox}/cyt c_{red} system gives a nearly ideal response,[11] and a very recent report suggests[12] that electrode fouling is due to removable[13] impurities in the purest form of cyt *c* commercially available (Sigma Type VI)[14] and routinely used in this laboratory.[5,6a] However, Hill et al.[15] use the same purification procedure[13] and find that the cyt c_{ox}/cyt c_{red} only responds well at Pt or Au if 4,4'-bipyridine (or a structurally related molecule) is added to the solution to facilitate the charge transfer. Presumably, the added 4,4'-bipyridine blocks cyt *c* adsorption, while serving as a bridge between the electrode and the cyt *c*. Electropolymerization of MV^{2+} onto Au grid electrodes leads to good response to the cyt c_{ox}/cyt c_{red} couple, but the surface polymer is not completely characterized and is not a redox active MV^{2+} derivative.[16] At least it is not redox active near the $E°'$ of the

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(4) Lewis, N. S.; Wrighton, M. S. *Science (Washington, D.C.)* **1981**, *211*, 944.

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(14) Sigma Type VI is nominally 98% pure as supplied.

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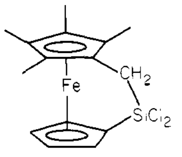
cyt c_{ox}/cyt c_{red} or $MV^{2+/+}$ couples. Thus, it would seem that the surface polymer from MV^{2+} functions in a manner similar to solution 4,4′-bipyridine except that it is permanently attached to the electrode and does not contaminate the solution.

We now report the synthesis, characterization, and use of a surface derivatizing reagent, I (Scheme II), that gives a surface redox system [$PMFc^{+/0}$] having an $E^{\circ\prime}$ on the surface of Pt electrodes of +0.04 V vs. SCE. n-type Si or Pt electrodes functionalized with I show much improved response to cyt c_{ox}/cyt c_{red} compared to naked electrodes, and it is noteworthy that electrodes functionalized with I can be used to effect both reduction of cyt c_{ox} to cyt c_{red} and oxidation of cyt c_{red} to cyt c_{ox} at high concentrations of cyt c. Thus, it appears that the [$PMFc^{+/0}$]$_{surf}$ is a durable surface mediator that operates as represented in eq 4 and 5.



I

Experimental Section

Chemicals. 1,1,4,7,7-Pentamethyldiethylenetriamine (PMDETA, Eastman Organic Chemicals) was distilled from Na under Ar before use. Silicon tetrachloride and chlorotrimethylsilane (Petrarch Research Systems, Inc.) were freshly distilled from Mg, and reagent grade hexanes and isooctane were distilled from CaH_2 under dry N_2 before use. *n*-BuLi (1.4 M in *n*-hexane, Alfa Products), D_2O, and D_2SO_4 (Aldrich) were used as received. Polarographic grade [n-Bu_4N]ClO_4 (Southwestern Analytical Chemicals) was dried under vacuum at 80 °C for 24 h and stored in a desiccator until used. Cytochrome *c* from horse heart was Type VI from Sigma Chemical Co. Anhydrous $NaClO_4$ (G. Frederick Smith Chemical Co.), $NaH_2PO_4{\cdot}H_2O$, $HClO_4$ (J. T. Baker Chemical Co.), absolute EtOH, spectroquality isooctane, and acetonitrile were used as received. Distilled H_2O was deionized before use as solvent for aqueous electrolyte solutions.

Synthesis of I. Scheme II represents the synthetic route used to prepare I. The ($Fe(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CH_3)_5)$) is a known compound[17] that was prepared by reaction of $FeCl_2$ in THF with 1 equiv each of Na-[C_5H_5]·1,2-dimethoxyethane[18] and Na[$C_5(CH_3)_5$].[19] The reaction yields $Fe(\eta^5\text{-}C_5H_5)_2$, $Fe(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CH_3)_5)$, and $Fe(\eta^5\text{-}C_5(CH_3)_5)_2$, and the desired complex can be purified by fractional sublimation to give ~60% yields of $Fe(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CH_3)_5)$: UV-vis λ_{max} (isooctane) 430 (ϵ 120), 325 nm (sh); ^1H NMR (C_6D_6) τ 8.13 (s, 15), 6.35 (s, 5); mass spectrum, *m/z* (rel intensity) 256 (100), 257 (22), 258 (2.8).

The dilithio species shown in Scheme II was prepared according to the following typical procedure: 0.5 g of $Fe(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CH_3)_5)$ is stirred in 10 mL of hexane, and 0.5 mL of 1,1,4,7,7-pentamethyldiethylenetriamine (PMDETA) is added. The *n*-BuLi (7.5 mmol) is then added to yield a brick-red precipitate after ~1 h, which is the dilithio reagent. The precipitate is collected by filtration, washed with hexane, and dried under vacuum. The dilithio reagent was characterized by reaction with 0.1 M D_2SO_4/D_2O and with $ClSiMe_3$.

Addition of 0.1 M D_2SO_4/D_2O to a suspension of the precipitate in hexane yields $Fe(\eta^5\text{-}C_5H_4D)(\eta^5\text{-}C_5(CH_3)_4CH_2D)$ after workup and purification by sublimation as determined by ^1H NMR and MS: ^1H NMR ($CDCl_3$) τ 8.13 (s, 14), 6.30 (s, 4); mass spectrum, *m/z* (rel intensity) 258 (100), 259 (53), 260 (12). Addition of an excess of $ClSiMe_3$ at -78 °C to a hexane suspension of the dilithio reagent yields $Fe(\eta^5\text{-}C_5H_4SiMe_3)(\eta^5\text{-}C_5(CH_3)_4CH_2SiMe_3)$ after warm-up and sublimation: ^1H NMR (CCl_4) τ 10.10 (s, 9), 9.83 (s, 9), 8.27 (s, 2), 8.13 (d, 12), 6.62 (t, 2), 6.40 (t, 2); mass spectrum, *m/z* (rel intensity) 400 (100), 401 (28).

Reagent I is synthesized by the addition of excess $SiCl_4$ at -78 °C to a hexane solution of the dilithio reagent. Warm-up and sublimation yield I: ^1H NMR (C_6D_6) τ 8.40 (s, 6), 8.05 (s, 6), 7.33 (s, 2), 6.20 (t, 2), 5.80 (t, 2); mass spectrum, *m/z* (rel intensity) 352 (100), 353 (28), 354 (65), 355 (16), 356 (13).

I was also characterized by reaction of I with excess EtOH in hexane at 25 °C to yield (after sublimation) the diethoxy analogue (cf. Scheme II): ^1H NMR (C_6D_6) τ 8.75 (t, 6), 8.20 (s, 6), 7.88 (s, 6), 7.73 (s, 2), 6.13 (m, 4), 6.04 (t, 2), 5.85 (t, 2); mass spectrum, *m/z* (rel intensity) 372 (100), 373 (28), 374 (8).

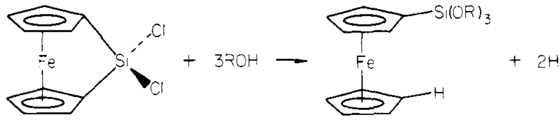
Electrodes. Most of the Pt working electrodes were prepared in the following way. Pt gauze (80 mesh, 0.003 in. diameter wire) was cut in 2.5 × 1.0 cm rectangular pieces and rolled into open-ended baskets ca. 0.8 cm in diameter. The Pt pieces were pierced, and a copper wire as a lead was attached and made secure with conducting Ag epoxy. The connection and copper wire were insulated from the solution by covering with ordinary epoxy and encasement in a 3-mm i.d. glass tube. The electrode was then cleaned in concentrated HNO_3 for 5 min and used after rinsing in distilled H_2O. Just prior to derivatization with reagent I, the Pt electrode surfaces were anodized at +1.9 V vs. SCE for 5 min in 0.5 M H_2SO_4 and then cycled between the O_2 and H_2 evolution potentials (-0.1 to 1.1 V vs. SCE, linear sweep, 100 mV/s), in 0.5 M H_2SO_4 until the cyclic voltammograms were constant (~2 h).[20] The electrode was then held at +1.1 V vs. SCE in that medium until the current declined to <1 $\mu A/cm^2$ (~5 min), washed with distilled H_2O and acetone, and allowed to dry in air. Smooth Pt wire electrodes (0.6-cm long, 0.5-mm diameter) or smooth Pt disks (4 mm in diameter) were petreated similarly. Derivatizaiton was carried out in the dark in a Vacuum Atmospheres drybox by dipping the pretreated Pt electrode into an isooctane solution of 0.1 M I at 25 °C for times varying from 4 to 24 h. After derivatization, the electrode was washed with isooctane followed by absolute EtOH.

Single-crystal, P-doped, n-type Si (111 face exposed) was obtained from Monsanto Co., St. Louis, MO. The polished wafers were 0.40-mm thick with resistivities between 3 and 7 Ω cm. Electrodes were made by cutting the Si into pieces ca. 0.2 cm^2 and mounting as previously reported.[21] The back surface was scratched with a diamond-tip scriber and rubbed with Ga-In eutectic and attached with conducting Ag epoxy to a coiled Cu wire. The Cu wire was passed through a glass tube. The electrode surface was defined by insulating all other surfaces with ordinary epoxy. An electrode area of ca. 0.1 cm^2 was generally used. Immediately preceeding derivatization with reagent I, the n-Si photoelectrode surfaces were etched in concentrated HF (15-60 s) and rinsed in distilled H_2O. After the HF etch process, electrodes were treated in 10 M NaOH for 60 s, then washed with distilled H_2O, followed by acetone and air-dried. Derivatization was carried out in the dark in a Vacuum Atmospheres drybox by employing essentially the same procedure and conditions adopted for the Pt electrodes.

Equipment and Procedures. Electrochemical experiments were carried out with a PAR 173 potentiostat equipped with a PAR 179 coulometer and a PAR 175 universal programmer. Traces were recorded on a Houston Instruments X-Y recorder. A two-compartment cell was employed with the saturated calomel (SCE) reference electrode and the working electrode separated from the Pt gauze counterelectrode by a fine-grade glass frit. Irradiation of the n-Si photoelectrode was supplied by a He-Ne laser emitting ca. 5.6 mW at 632.8 nm. Stirring of the cyt *c* solution was provided by a magnetic stirring bar. All aqueous electrolyte solutions were purged with Ar for 20 min prior to addition of cyt *c*, and a constant stream of Ar swept above the cyt *c* solution during the electrochemical studies. Visible absorption spectra were recorded on a Cary 17 spectrometer. The mass spectra were obtained with a Varian MAT 44 spectrometer, and ^1H NMR spectra were obtained with a Bruker 270 MHz Fourier transform spectrometer.

Results and Discussion

Behavior of Pt and n-Type Si Electrodes Functionalized with I. As indicated in Scheme II, the polyalkylated ferrocene derivative I is expected to be reactive with -OH functionality. The reaction of I with EtOH at 25 °C to form the diethoxy analogue is an expected result, owing to the reactive Si-Cl bonds. The reaction of I with -OH thus contrasts with the reactivity of the previously studied reagent II (eq 6), which suffers cleavage of the strained



(6)

II

Si-C bond in addition to known reaction of Si-Cl bonds.[22] For

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I, the two-atom bridge connecting the two cyclopentadienyl rings is not strained, and reaction with –OH is restricted to that of the two Si–Cl bonds.

Pretreated Pt (gauze, 80 mesh, 0.003 in. diameter wire, or smooth surfaces) or polished, single-crystal n-type Si can be functionalized by using reagent I. The reaction presumably involves both covalent attachment and polymerization via reaction of the two Si–Cl bonds with surface–OH or surface–O^-Na^+ and trace H_2O. Surface-confined, electroactive material can be detected by cyclic voltammetry scans in aqueous or nonaqueous electrolyte solutions. Integration of the cyclic voltammetry waves shows that >monolayer coverage of electroactive material is obtained. In CH_3CN/0.1 M [n-Bu_4N]ClO_4, the cyclic voltammetry waves are centered at +0.28 V vs. SCE, close to the $E^{\circ\prime}$ = +0.25 V vs. SCE for the diethoxy analogue of I dissolved in the same solvent/electrolyte solution. The $E^{\circ\prime}$ of the diethoxy analogue of I was determined by cyclic voltammetry at ~1 mM concentration. Thus, we attribute the cyclic voltammetry waves at +0.28 V vs. SCE *in* CH_3CN/0.1 M [n-Bu_4N]ClO_4 *for Pt derivatized* with I to be due to the intact ferrocene system illustrated in Scheme II that we represent as $[PMFc^{+/0}]_{surf}$. The $E^{\circ\prime}$ for a solution species and the surface-confined analogue would be expected to be within 100 mV.[23]

The behavior of n-type Si electrodes functionalized with I in CH_3CN/0.1 M [n-Bu_4N]ClO_4 is as expected for a photoanode in contact with a redox system having an $E^{\circ\prime}$ situated between the top of the valence band, $E_{VB} \approx +0.8$ V vs. SCE, and bottom of the conduction band, $E_{CB} \approx -0.3$ V vs. SCE.[21] The oxidation of $[PMFc^0]_{surf}$ to $[PMFc^+]_{surf}$ does not occur in the dark, even for electrode potentials significantly more positive than $E^{\circ\prime}$-$[PMFc^{+/0}]_{surf}$. However, illumination of the derivatized electrode with >band gap (E_g = 1.1 eV) light results in the $[PMFc^0]_{surf} \rightarrow [PMFc^+]_{surf}$ process with a peak current at a potential as negative as ~–0.3 V vs. SCE for 632.8-nm (~50 mW/cm^2) irradiation. The extent to which this photoanodic current peak is more negative than the anodic $[PMFc^0]_{surf} \rightarrow [PMFc^+]_{surf}$ current peak on Pt, ~580 mV, is a measure of the extent to which the light can be used to drive the oxidation of the $[PMFc^0]_{surf}$ in an uphill sense. The light is required to effect oxidation on n-type Si because no minority carrier holes (h^+) are available in the dark. Reduction of $[PMFc^+]_{surf}$ involves the majority charge carriers, electrons (e), that are available in the dark. However, the reduction of $[PMFc^+]_{surf}$ to $[PMFc^0]_{surf}$ occurs significantly negative of $E^{\circ\prime}$ owing to the barrier to the reduction for potentials positive of E_{CB}.

The important results pertaining to the use of electrodes derivatized with I concern aqueous electrolytes. While electrodes derivatized with II do show persistently attached, electroactive material, the simple ferrocene systems in the oxidized form are not rugged in H_2O, particularly in nonacidic media.[24] Electrodes derivatized with I should be more durable for several reasons: (i) the polyalkylation makes the surface more hydrophobic; (ii) the polyalkylation makes the oxidized form a less potent oxidant, with each alkyl substituent lowering the oxidizing power by ~50 mV; and (iii) the bridge connecting the two cyclopentadienyl rings should tend to lessen problems from loss of the rings. Compared to surfaces derivatized with II, electrodes derivatized with I are generally far more rugged in aqueous solution at pH 7.0.

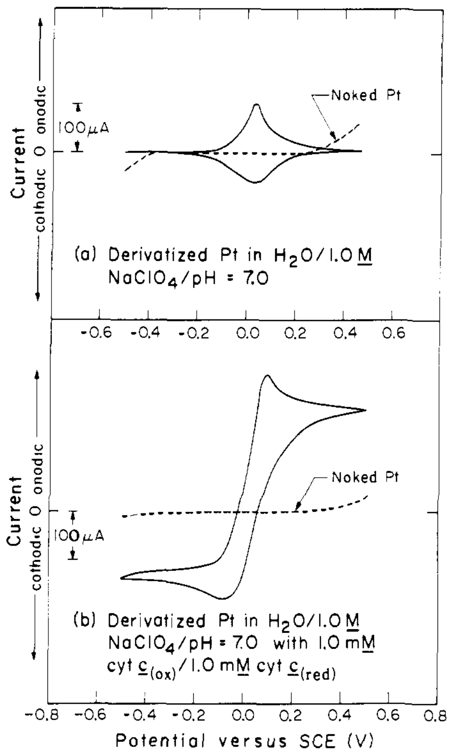

Figure 1. (a) Pt gauze electrode functionalized with I scanned at 5 mV/s under the conditions indicated (—) compared to an identical naked electrode (---). (b) Current–potential scan at 5 mV/s for the electrodes in (a) after addition of cyt c as indicated. Scans are carried out under Ar in stirred solutions. Coverage of the derivatized electrode is ~1 × 10^{-8} mol/cm^2 of actual surface area.

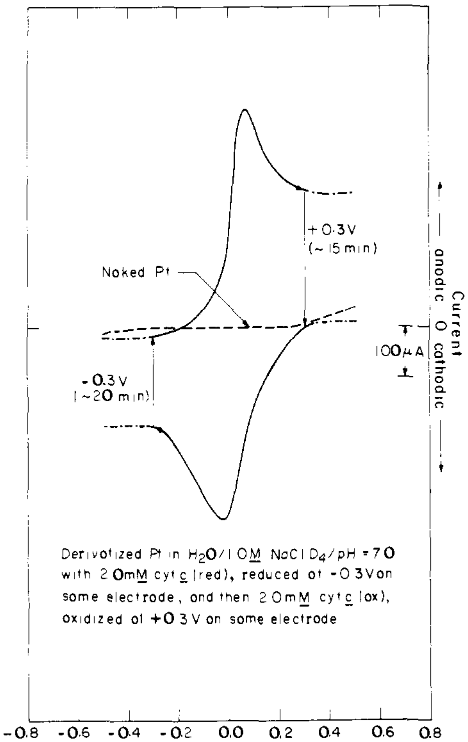

Figure 2. Pt gauze electrode functionalized with I (~1 × 10^{-8} mol/cm^2, total actual area = 3.5 cm^2) scanned at 5 mV/s in a stirred 1.50-mL phosphate buffer solution containing 2.0 mM cyt c_{red} or 2.0 mM cyt c_{ox}. The desired cyt c protein solutions were freshly synthesized with the same electrode by exhaustive electrolysis at applied potentials of –0.3 or +0.3 V vs. SCE, respectively, for the time indicated.

Figure 1a shows the cyclic voltammetry of a derivatized Pt gauze electrode compared to an otherwise identical Pt gauze electrode in aqueous/1.0 M $NaClO_4$ at pH 7.0. The coverage of electroactive material from integration of either the anodic or cathodic wave is 1 × 10^{-8} mol/cm^2. The $E^{\circ\prime}[PMFc^{+/0}]_{surf}$ = +0.04 V vs. SCE in aqueous solution from averaging the $E^{\circ\prime}$'s from over 50 independently prepared Pt electrodes. The $E^{\circ\prime}$ for a given electrode is the average position of anodic and cathodic current peaks. It appears that the $E^{\circ\prime}$ in H_2O is ~240 mV more negative than that in CH_3CN, a result that we found to be

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[23] Lenhard, J. R.; Rocklin, R.; Abruna, H.; Willman, K.; Kuo, K.; Nowak, R.; Murray, R. W. *J. Am. Chem. Soc.* **1978**, *100*, 5213.

[24] (a) Wrighton, M. S.; Palazzotto, M. C.; Bocarsly, A. B.; Bolts, J. M.; Fischer, A. B.; Nadjo, L. *J. Am. Chem. Soc.* **1978**, *100*, 7264. (b) Lenhard, J. R.; Murray, R. W. *Ibid.* **1978**, *100*, 7870. Generally, we have had much better durability for surface-bound ferricenium in low pH media than in pH 7.0 media for electrodes functionalized with reagents described in (a).

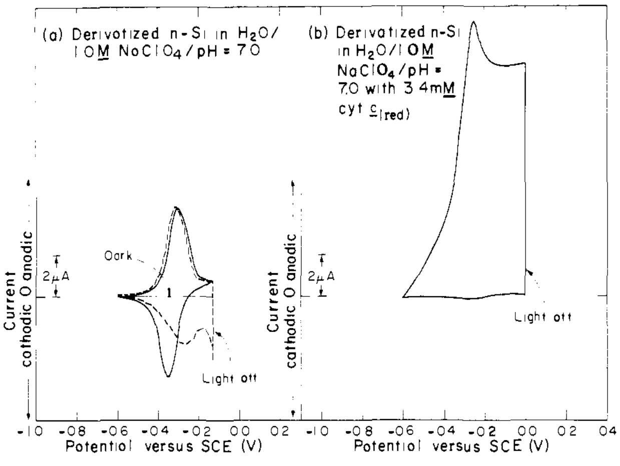

Figure 3. Cyclic voltammetry of n-type Si electrode derivatized with 1 ($\sim 1 \times 10^{-8}$ mol/cm^2, actual surface area = 0.1 cm^2) scanned at 5 mV/s in a stirred phosphate buffer solution. (a) Electrode scanned under illumination (—) and with the light blocked at the anodic limit with a mechanical shutter (---). (b) Same electrode and solution as in (a) except 3.4 mM cyt c_{red} was added. Scan with light blocked at the anodic limit shows much larger anodic current and little cathodic current compared to (a) due to mediated cyt c_{red} oxidation by surface-confined PMFc$^+$. Light source was a 5-mW He-Ne laser, at 632.8 nm, beam expanded to provide ~50 mW/cm^2 over the entire electrode surface.

paralleled in studies of electrodes derivatized with II that give $E^{\circ\prime} \approx +0.5$ V vs. SCE in CH_3CN/electrolyte and $E^{\circ\prime} \approx +0.3$ V vs. SCE in H_2O/electrolyte. Further, the cyclic voltammetry waves for Pt/$[PMFc^{+/0}]_{surf}$ in H_2O/electrolyte illustrated in Figure 1a are clearly nonideal. Such is likely due to a combination of two factors: (i) the large coverage of $[PMFc^{+/0}]_{surf}$ and (ii) the hydrophobic character of $[PMFc^0]_{surf}$. In CH_3CN/electrolyte, the waves are symmetrical, even at high coverage of $[PMFc^{+/0}]_{surf}$. Solvent effects like those found here for electrodes derivatized with I are quite similar to effects from solvent on electrodes derivatized with polyvinylferrocene.[25]

n-type Si electrodes derivatized with I are quite durable in aqueous media and can be characterized by cyclic voltammetry as shown in Figures 3 and 4. The position of the photoanodic peak from I is routinely more negative than for n-type Si derivatized with II, and the n-type Si electrodes derivatized with I are more durable in aqueous solution (particularly nonacidic solutions) than are those derivatized with II.[26] The more negative current peak of the $[PMFc^{+/0}]_{surf}$ is attributable to its more negative $E^{\circ\prime}$. The improvement in durability could be due to the greater durability of $[PMFc^{+/0}]_{surf}$ for the reasons outlined above, but the growth of insulating SiO_x is less severe at the less positive potentials necessary to completely oxidize $[PMFc^0]_{surf}$ compared to the potential generally required to oxidize the ferrocene derivative from II.

As for redox material from derivatization with II, we find that the $[PMFc^{+/0}]_{surf}$ redox system on Pt gives a linear increase of peak current upon linear increase in scan rate in the cyclic voltammograms for scan rates up to 0.2 V/s in CH_3CN or H_2O. Even beyond this, low coverage ($\sim 5 \times 10^{-9}$ mol/cm^2) electrodes can give a linear increase in peak current with increasing scan rate, with a peak position that is independent of scan rate. These findings indicate that the $[PMFc^{+/0}]_{surf}$ exhibits good kinetics on Pt. Generally, similar results obtain for n-type Si/$[PMFc^{+/0}]_{surf}$ except that the illumination intensity must be great enough to provide the h$^+$'s at the needed rate. In the experiments to be described below concerning cyt *c*, the rate of oxidizing and reducing the $[PMFc^{+/0}]_{surf}$ system has been determined to *not* be limiting the observed currents. This has been established by determining the peak current associated with the redox processes $[PMFc^+]_{surf} \rightleftarrows [PMFc^0]_{surf}$ as a function of scan rate under the conditions used to study cyt *c* to show that higher current density can obtain than is observed in the steady-state oxidation of cyt c_{red} or steady-state reduction of cyt c_{ox}.

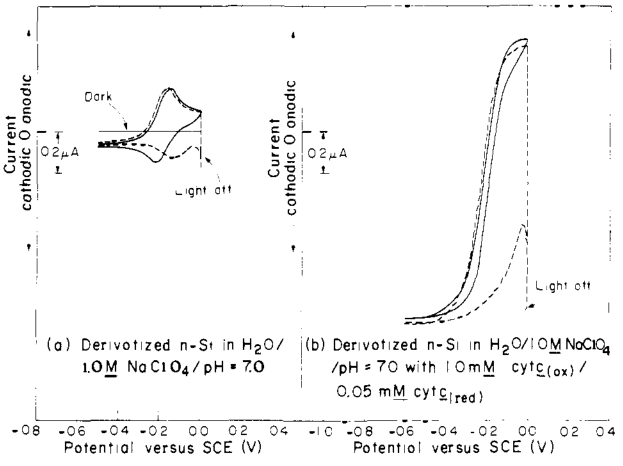

Figure 4. Cyclic voltammetry of an n-type Si electrode derivatized with 1 ($\sim 6 \times 10^{-10}$ mol/cm^2, actual surface area = 0.1 cm^2) scanned at 5 mV/s in a stirred phosphate buffer solution. (a) Electrode scanned under illumination (—) and with the light blocked at the anodic limit by a mechanical shutter (---). (b) Same electrode and solution as in (a) except 1.0 mM cyt c_{ox} and 0.05 m*M* cyt c_{red} freshly prepared from the solid protein. Scan with light blocked at the anodic limit shows marked increase in the cathodic current compared to (a) due to mediated cyt c_{ox} reduction by surface-confined PMFc^0. Light source was a 5-mW He–Ne laser, at 632.8 nm, beam expanded to provide ~50 mW/cm^2 over the entire electrode surface.

Table I. Controlled-Potential Electrolyses of Cyt *c* with Derivatized Pt[a]

substrate[b]	potential, V vs. SCE	coulombs passed (time, min)	% convn	absorbance at 550 nm[c] obsd (#)[d]	calcd
cyt c_{ox}	−0.3	0.00 (0.00)	0.0	0.35 (1)	0.35
		0.20 (2.93)	30	0.55 (2)	0.56
		0.40 (8.22)	63	0.78 (3)	0.79
		0.62 (22.30)	100	1.03 (4)	1.04
cyt c_{red}	+0.3	0.00 (0.00)	0.0	1.03 (4)	1.04
		0.22 (2.58)	38	0.77 (5)	0.79
		0.42 (7.03)	70	0.55 (6)	0.55
		0.59 (17.46)	100	0.34 (7)	0.34

[a] These data are culled from the spectral data given in Figure 5; cf. Figure 5 caption. [b] 4.4 mM cyt *c* in H_2O/1.0 M $NaClO_4$ (pH 7.0). [c] Observed absorbance at 550 nm and the calculated absorbance at 550 nm assuming 100% current efficiency for cyt *c* reduction and oxidation for solutions analyzed; cf. Figure 5 caption. [d] These numbers refer to the spectra in Figure 5.

Redox Behavior of Cyt *c* at Pt Derivatized with I. Pt and n-type Si electrodes bearing $[PMFc^{+/0}]_{surf}$ can be used to oxidize cyt c_{red} and to reduce cyt c_{ox}, Figures 1–5. Generally, there is no observable current for cyt $c_{ox} \rightleftarrows$ cyt c_{red} at electrodes that are not derivatized with I but are otherwise treated in the same manner. It is quite evident from the data in Figure 1 that naked Pt gauze does not exhibit as much current as the derivatized electrode in the presence of a 1/1 ratio of cyt c_{ox}/cyt c_{red}. Further, the current for the oxidation of pure cyt c_{red} or the reduction of pure cyt c_{ox} (Figure 2) is significant for the derivatized electrode whereas the naked Pt gauze electrodes do not give detectable current attributable to cyt $c_{ox} \rightleftarrows$ cyt c_{red}.

The oxidation and reduction currents in the presence of cyt c_{red} and cyt c_{ox}, respectively, for the Pt/$[PMFc^{+/0}]_{surf}$ electrodes are steady-state currents that can be maintained at the high concentrations of cyt *c* used to collect the data for Figures 1 and 2. The derivatized Pt gauze electrodes can be used to exhaustively

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(25) (a) Daum, P.; Murray, R. W. *J. Electroanal. Chem. Interfacial Electrochem.* **1979**, *103*, 289; *J. Phys. Chem.* **1981**, *85*, 389. (b) Flanagan, J. B.; Margel, S.; Bard, A. J.; Anson, F. C. *J. Am. Chem. Soc.* **1978**, *100*, 42–48.

(26) (a) Bocarsly, A. B.; Walton, E. G.; Wrighton, M. S. *J. Am. Chem. Soc.* **1980**, *102*, 3390. (b) Bolts, J. M.; Bocarsly, A. B.; Palazzotto, M. C.; Walton, E. G.; Lewis, N. S.; Wrighton, M. S. *Ibid.* **1979**, *101*, 1378.

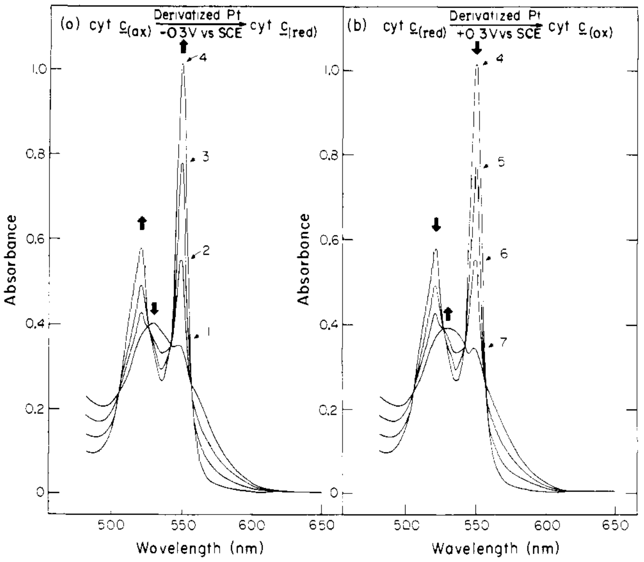

Figure 5. Spectrophotometric analyses accompanying controlled-potential reduction and oxidation of cyt c by using a Pt gauze electrode functionalized with I, ~1 × 10^{-8} mol/cm^2. The solution electrolyzed was a 1.50-mL solution of H_2O/1.0 M $NaClO_4$ buffered to pH 7.0 with phosphate containing initially 4.4 mM cyt c_{ox}. The analyses were performed by removing 25.0 μL and diluting to 3.00 mL in an optical-quality cuvette to give the spectra shown. Part a shows quantitative reduction of cyt c_{ox} to cyt c_{red}, charge passed was 1.00 ± 0.05 equiv; part b shows the regeneration of cyt c_{ox}, charge passed was 1.00 ± 0.05 equiv. All manipulations and electrolyses were carried out under Ar. The spectral properties for cyt c_{ox} and cyt c_{red} agree with the literature.[11,27] Data in Table I detail the electrolysis times associated with these optical changes.

reduce high concentrations of cyt c_{ox} to cyt c_{red} or to oxidize high concentrations of cyt c_{red} to cyt c_{ox} in electrolysis times that are very short compared to times required with naked Pt gauze electrodes. Figure 5 gives some quantitative data in this regard, and Table I summarizes the essentials. Controlled-potential electrolyses (−0.3 V vs. SCE for reduction and +0.3 V vs. SCE for oxidation) of a 1.50-mL 4.4 mM cyt c solution have been monitored spectrophotometrically by withdrawing 25.0-μL samples and diluting to 3.00 mL. As shown by the spectral changes in Figure 5, the current observed at −0.3 V corresponds to the reduction of cyt c_{ox} with 100 ± 5% coulombic efficiency, and the current at +0.3 V vs. SCE corresponds to the oxidation of cyt c_{red} with 100 ± 5% coulombic efficiency. Note that reduction of cyt c_{ox} to cyt c_{red} is >95% complete in <30 min and the oxidation of cyt c_{red} to cyt c_{ox} is also >95% complete in <30 min. During the electrolyses the observed current is directly proportional to the concentration of the species oxidized or reduced. Electrolyses under the same conditions except with naked Pt gauze electrodes give barely detectable optical spectral changes after ~24 h of electrolysis time. The spectra for cyt c_{red} and cyt c_{ox} are those expected for these species.[11,27]

The Pt/$[PMFc^{+/0}]_{surf}$ system is durable. For example, the 1.50-mL, 4.4 mM cyt c solution was alternately exhaustively reduced, cyt c_{ox} → cyt c_{red}, and oxidized, cyt c_{red} → cyt c_{ox}, more than ten times without any detectable changes in the electrode (constant coverage of $[PMFc^{+/0}]_{surf}$) or its performance (measured by the time required to effect the electrolysis, <30 min). Assuming that the $[PMFc^{+/0}]_{surf}$ is a mediator as represented in eq 4 and 5, the turnover number, the number of cyt c molecules oxidized or reduced per PMFc center, exceeds 2000 without any detectable loss of redox activity.

Consistent with the conclusion that Pt gauze electrodes bearing the $[PMFc^{+/0}]_{surf}$ system will come into charge-transfer equilibrium with the cyt c system, we have shown that the derivatized Pt electrodes will bring the cyt c_{ox}/cyt c_{red} ratio to the value predicted from the Nernst equation and the known $E°'$ of +0.02 V vs. SCE. Thus, independent of whether we start with 2.0 mM cyt c_{ox} or 2.0 mM cyt c_{red}, potentiostatting the derivatized Pt electrode to +0.02 V vs. SCE brings the solution to approximately a 1/1 ratio cyt c_{ox}/cyt c_{red}. The cyt c ratio can be determined ±1% spectrophotometrically; cf. Figure 5. The equilibration does take long periods of time (~6 h for 2.0-mL solutions of 2 mM cyt c), but naked Pt electrodes cannot be used at this concentration of cyt c since no detectable changes in composition are detected in >24 h. Further, potentiostatting at +0.08 or −0.04 V vs. SCE brings the cyt c_{ox}/cyt c_{red} ratio to approximately 10/1 and 1/10, respectively. These results establish that the derivatized electrode could be used to establish the $E°'$ of cyt c.

Smooth Pt wires or Pt disks functionalized with I also show improved response to cyt c, but owing to the smaller surface areas observed, currents are smaller and do not allow electrolyses of the cyt c on a convenient time scale. Electroanalytical experiments with smooth n-type Si derivatized with I (vide infra) have been used successfully to establish a lower limit for the values of k_{ox} and k_{red}.

The small-area, smooth Pt electrodes have allowed the establishment that the $[PMFc^{+/0}]_{surf}$ minimizes problems from adsorption of cyt c. The cyclic voltammetry (100 mV/s) of 0.5 mM $Fe(CN)_6^{3-}$/0.5 mM $Fe(CN)_6^{4-}$ having an $E°'$ of +0.20 V vs. SCE at a Pt wire electrode is unaffected by modification with I for coverages of <1 × 10^{-10} mol/cm^2. When 1.0 mM cyt c_{ox} is added to a pH 7.0, 0.1 M $NaClO_4$ solution containing 1.0 mM $Fe(CN)_6^{3-/4-}$, the cyclic voltammetry waves for the $Fe(CN)_6^{3-/4-}$ are rapidly attenuated at the naked Pt wire by the adsorption of the cyt c or impurities contained in the sample. The wave attributable to the $Fe(CN)_6^{3-/4-}$ disappears in <10 min after the addition of cyt c. In a parallel experiment with a Pt wire derivatized with I, the $Fe(CN)_6^{3-/4-}$ wave is not attenuated by the addition of the cyt c even after 12 h. Thus, the $[PMFc^{+/0}]$surf significantly reduces the problem of adsorption of the cyt c. It is noteworthy that the $Fe(CN)_6^{3-}$ is a commonly used[28] molecular oxidant for cyt c_{red}; the adsorption of the cyt c onto Pt blocks the mediator from the Pt and precludes its use as a good mediator at 1 mM concentration. The $[PMFc^{+/0}]_{surf}$ minimizes adsorption and is itself a mediator that allows either oxidation or reduction of the cyt c.

The experiments with $Fe(CN)_6^{3-/4-}$ raise the possibility that the cyt c could respond well at the electrodes derivatized with I without actually exchanging electrons with the $[PMFc^{+/0}]_{surf}$ system. That is, the hydrophobic character of the surface alone might be sufficient to bring about a good response to cyt c. Additional experiments with derivatized Pt, however, point to an important role for similar $E°'$'s for the surface redox reagent and cyt c. First, Pt electrodes functionalized with II can be used to oxidize cyt c_{red} to cyt c_{ox}, but significant current is not observed until the surface species is oxidized (onset ~+0.2 V vs. SCE). Further, Pt electrodes derivatized with II cannot be used to reduce cyt c_{ox}, presumably because the reduced form of the species derived from II is not a sufficiently strong reductant that there can be a significant rate. This result follows from an $E°'$ of ~+0.3 V vs. SCE for the surface–ferrocene system derived from II. The results for the Pt electrodes derivatized with II parallel the findings[5] for Pt electrodes derivatized with the $[(PQ^{2+})_n]_{surf}$ system that has an $E°'$ far away from the $E°'$ of cyt c; for the $[(PQ^{2+})_n]_{surf}$ system, only the reduction of cyt c is possible. These results, taken together, show that surface mediated oxidation and reduction of cyt c, if it occurs at all, will occur near the $E°'$ of the surface mediator. It follows that both oxidation and reduction are possible when the $E°'$'s of the surface mediator and the biological system are similar.

The data from electrodes derivatized with I, II, and $[(PQ^{2+})_n]_{surf}$ for cyt c appear to show that $E°'$'s for the surface mediator and the biological reagent must be close in order to observe electrocatalysis for both the oxidation and the reduction. Thus, minimizing adsorption is necessary, but not sufficient, to observe the

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(27) Margoliash, E.; Frohwirt, N. *Biochem. J.* **1959**, *71*, 570.

(28) The use of $Fe(CN)_6^{3-}$ to oxidize cyt c_{red} is well-documented; cf. for example: Sutin, N. *Adv. Chem. Ser.* **1977**, *162*, 156. Morton, R. A.; Overnell, J.; Harbury, H. A. *J. Biol. Chem.* **1970**, *245*, 4653.

Table II. Minimum Rate Constants for Oxidation and Reduction of Cyt *c* at Derivatized Electrodes

electrode[a]	area, cm^2	E_{PA}[b]	E_{PC}[b]	coverage,[c] mol/cm^2	[cyt c_{ox}],[d] $\mu mol/cm^3$	[cyt c_{red}],[d] $\mu mol/cm^3$	i, μA[e]	$k_{het} \times 10^4$,[f] cm/s	k_{ox} or $k_{red} \times 10^{-4}$,[g] $cm^3/(mol\ s)$
Pt-PMFc	3.5	+0.04	+0.02	1×10^{-8}	1.0		140	4.2	4.2
					2.0		200	3.0	3.0
						1.0	200	6.0	6.0
						2.0	300	4.4	4.4
Pt-PMFc	3.0	+0.08	0.00	2×10^{-9}	1.4		100	2.5	13
					2.5		200	2.8	14
						1.4	125	3.0	15
						2.5	175	2.4	12
Pt-PMFc	3.5	+0.07	−0.02	1.1×10^{-8}	0.8		120	4.4	4.0
						0.7	160	6.8	6.2
Pt-PMFc	3.5	+0.10	+0.02	6×10^{-9}	1.0		100	3.0	4.9
						0.5	100	5.9	9.9
Pt-PMFc	3.5	+0.10	+0.02	1×10^{-8}	0.6		80	4.0	4.0
					1.0		150	4.4	4.4
						0.5	140	8.3	8.3
						1.3	380	8.7	8.7
Pt-Fc	3.0	+0.40	+0.24	4×10^{-8}		1.5	1700	39	9.8
Pt-Fc	3.0	+0.38	+0.24	3.3×10^{-8}		1.5	1750	40	12
n-Si-PMFc[h]	0.1	−0.14	−0.22	5×10^{-11}	0.6		1	1.8	360
						0.9	3	3.5	700
n-Si-PMFc[h]	0.1	−0.28	−0.34	1×10^{-8}		3.4	10	3.0	3.0
n-Si-PMFc[h]	0.1	−0.15	−0.26	5×10^{-9}		0.9	3	3.5	7.0
n-Si-PMFc[h]	0.1	−0.15	−0.22	6×10^{-10}	1.0		1	1.0	17

[a] Pt or n-Si derivatized with I denoted PMFc or with II denoted Fc. [b] Anodic and cathodic current peak in a linear sweep voltammogram of the derivatized electrode in H_2O/1.0 M $NaClO_4$ (pH 7.0) in volts vs. SCE. [c] Coverage of electroactive material from I or II determined by integration of cyclic voltammogram. [d] Concentration of the redox active form of cyt *c*. [e] Steady-state current observed for oxidation of cyt c_{red} or reduction of cyt c_{ox} at a potential of +0.3 V vs. SCE or −0.3 V vs. SCE, respectively, for Pt-PMFc electrodes; at +0.5 V vs. SCE for Pt-Fc electrodes for cyt c_{red} oxidation; and at 0.00 V vs. SCE and −0.45 V vs. SCE, respectively, for illuminated, 632.8 nm (50 mW/cm^2), n-Si-PMFc electrodes. [f] Minimum heterogeneous electron-transfer rate constant calculated using eq 10. [g] Electron-transfer rate constant calculated by dividing k_{het} by coverage of electroactive material from I or II; see text. [h] Data are for illuminated n-Si electrodes; 632.8 nm (50 mW/cm^2).

electrocatalysis with the surface mediators described here. In principle, it should be possible to effect redox reaction even when the $E°'$'s are unfavorable, as has been pointed out by others.[26a,29] However, the question is how far can the $E°'$'s differ and still yield a significant rate? When the mechanism for the electron exchange is dominated by outer-sphere considerations, the self-exchange rates of the surface mediator and biological reagent will largely control the observed rate at a given set of potentials. The self-exchange rates for large biological reagents are generally small, presumably owing to geometrical constraints precluding close approach. This suggests that the $E°'$ for the surface mediator must always be quite close to that for the biological reagent. However, when the mechanism for the reaction between mediator and biological reagent takes on inner-sphere character the requirement to have nearly the same $E°'$ may be relaxed. In the case at hand, the $E°'[PMFc^{+/0}]_{surf}$ is ~20 mV more positive than $E°'$(cyt c_{ox}/cyt c_{red}), and we generally find a somewhat larger current for oxidation of cyt c_{red} than for reduction of an equal concentration of cyt c_{ox} at the same driving force for the two processes, 0.3 V beyond the $E°'$ of cyt *c*, cf. the steady-state oxidation and reduction currents in Figure 1. Additional data developed below further substantiate this finding.

Behavior of Cyt *c* at n-Type Si Derivatized with I. As shown in Figures 3 and 4, the $[PMFc^0]_{surf} \rightarrow [PMFc^+]_{surf}$ oxidation occurs on illuminated n-type Si with an onset of ~−0.4 V vs. SCE, and in the presence of cyt c_{red}, additional current is observed that we attribute to the mediated oxidation of cyt c_{red}. When the solution initially contains only cyt c_{red} (Figure 3), the onset of photocurrent corresponding to cyt $c_{red} \rightarrow$ cyt c_{ox} occurs at about the same potential as does the $[PMFc^0]_{surf} \rightarrow [PMFc^+]_{surf}$ process. These data establish that visible light can be used to drive the oxidation of cyt c_{red} to cyt c_{ox} in an uphill sense. Further, the ability to generate $[PMFc^+]_{surf}$ only when the light is on, while $[PMFc^+]_{surf}$ can be reduced in the dark at negative potentials, allows us to directly establish that the oxidation can proceed via eq 7. The

$$[PMFc^+]_{surf} + \text{cyt } c_{red} \xrightarrow{k_{ox}} [PMFc^0]_{surf} + \text{cyt } c_{ox} \qquad (7)$$

point is that at the positive limit of the potential sweep the illumination can be terminated and the photogenerated $[PMFc^+]_{surf}$ is detectable in the dark by scanning to a negative potential while monitoring current for the process represented by eq 8. In Figure

$$[PMFc^+]_{surf} + e \rightarrow [PMFc^0]_{surf} \qquad (8)$$

3a, the dashed curve is a negative sweep after illumination is terminated at the positive limit, and a well-defined cathodic current peak for the process represented by eq 8 is found. However, as Figure 3b shows, the cathodic current peak is absent in the presence of a stirred cyt c_{red} solution. This shows that the process represented by eq 7 leads to nearly complete consumption of the $[PMFc^+]_{surf}$ on the time scale of the negative sweep from 0.00 V vs. SCE. Experiments of this kind have been reported previously for n-type Si functionalized with II to determine values of electron-transfer rate constants.[30] The data establish eq 7 as representing a significant mechanistic path for the oxidation of cyt c_{red} using electrodes derivatized with I, and in the future our studies will include a quantitative evaluation of k_{ox} by measuring the $[PMFc^+]_{surf}$ as a funciton of time in the presence of variable cyt c_{red} concentration.

Data in Figure 4 establish that the n-type Si electrodes functionalized with I are able to effect the reduction of cyt c_{ox} at sufficiently negative potentials. For n-type Si, the reduction of $[PMFc^+]_{surf}$ to $[PMFc^0]_{surf}$ occurs with good kinetics only for potentials that are more negative than $E°'$, since $E°'$ is between E_{VB} and E_{CB} and there is a barrier to the flow of cathodic current

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(29) (a) Anson, F. C. *J. Phys. Chem.* **1980**, *84*, 3336. (b) Murray, R. W. *Philos. Trans. Roy. Soc. London, Ser. B*, to be published, and private communication. (c) Andrieux, C. P.; Dumas-Bouchiat, J. M.; Saveant, J. M. *J. Electroanal. Chem. Interfacial Electrochem.* **1980**, *114*, 159. (d) Oyama, N.; Anson, F. C. *Anal. Chem.* **1980**, *52*, 1192.

(30) (a) Lewis, N. S.; Bocarsly, A. B.; Wrighton, M. S. *J. Phys. Chem.* **1980**, *84*, 2033. (b) Lewis, N. S.; Wrighton, M. S. *ACS Symp. Ser.* **1981**, *146*, 37.

for any potential positive of the so-called flat-band potential that is ~0.1 V more positive than E_{CB}. However, when $[PMFc^0]_{surf}$ is produced, the reduction of cyt c_{ox} can occur at the n-type Si. Note that the n-type Si electrodes used are polished surfaces functionalized with I and give excellent response to the cyt *c*. Naked Si electrodes do not give current that can be attributed to a response to cyt *c*. Indeed, the naked n-type Si undergoes photoanodic corrosion in aqueous electrolytes that precludes any reproducible studies whatsoever.[26]

Rate of Oxidation and Reduction of Cyt *c* at Electrodes Derivatized with I. Table II summarizes some data from experiments relating to the observed rates for the oxidation and reduction of cyt *c* at electrodes modified with I. Two entries concern data for Pt electrodes modified with II. The data are obtained from steady-state current–voltage curves like those shown in Figures 1–4 for stirred solutions of cyt *c*. The current at Pt electrodes derivatized with I is that found at −0.3 V vs. SCE for the reduction of cyt c_{ox} or at +0.3 V for the oxidation of cyt c_{red}. These potentials are chosen because the current is not dependent on potential at these values, since the $[PMFc^{+/0}]_{surf}$ is fully oxidized at +0.3 V or fully reduced at −0.3 V vs. SCE. For Pt electrodes derivatized with II, the current is for the oxidation of cyt c_{red} at +0.5 V vs. SCE where the surface ferrocene is fully oxidized and the current is independent of potential. For n-type Si derivatized with I, the oxidation current is for an illuminated (632.8 nm, ~50 mW/cm^2) surface at 0.0 V vs. SCE where $[PMFc^{+/0}]_{surf}$ is fully oxidized (cf. Figures 3 and 4). At n-type Si, the reduction current in the dark is for an electrode at −0.45 V vs. SCE where $[PMFc^{+/0}]_{surf}$ is fully reduced. The data from these experiments reveal some important qualitative results concerning the rate constants, k_{ox}, for oxidation of cyt c_{red} (eq 7) and k_{red}, for reduction of cyt c_{ox}, (eq 9).

$$[PMFc^0]_{surf} + \text{cyt } c_{ox} \xrightarrow{k_{red}} [PMFc^+]_{surf} + \text{cyt } c_{red} \qquad (9)$$

Consider first the observed current densities. Assuming that the heterogeneous electron-transfer rate constant, k_{het}, is given by eq 10, we see that k_{het} for the oxidation of cyt c_{red} is in the range

$$k_{het} = \text{current density}/(F[\text{cyt } c]) \qquad (10)$$

$$(F = \text{Faraday constant})$$

2.4–8.7 × 10^{-4} cm/s (or 1.0–4.4 × 10^{-4} cm/s for reduction of cyt c_{ox}) for a wide variety of independently prepared electrodes having different coverages and for a variety of cyt *c* solutions of variable concentration prepared from different lots of cyt *c*. For electrocatalysis of cyt *c* by added 4,4′-bipyridine, k_{het} of ~10^{-2} cm/s has been obtained,[15] showing that these rates are about 1–2 orders of magnitude lower. For Pt electrodes derivatized with II, the driving force for the oxidation of cyt c_{red} is about 0.3 V, and we observe about an order of magnitude increase in k_{het} to 4 × 10^{-3}. This increase is perhaps lower than would be expected on the basis of the Marcus theory,[31] but we do not know the self-exchange rates of either the material derived from I or from II, and, moreover, the stirring may be sufficiently poor that the current is mass-transport limited. For this reason, we regard all of the values of k_{het} in Table II as lower limits.

Consistent with the larger value of k_{het} for electrodes derivatized with II compared to those of I, we also find the value of k_{het} for the oxidation of cyt c_{red} by $[PMFc^+]_{surf}$ to be larger than k_{het} for reduction of cyt c_{ox} by $[PMFc^0]_{surf}$. The consistency of these results follows from the fact that $E°′[PMFc^{+/0}]_{surf}$ is ~20 mV more positive than $E°′(\text{cyt } c_{ox}/\text{cyt } c_{red})$. Thus, for a given electrode, the k_{het} for oxidation of cyt c_{red} is 1.5–3.5 times larger than k_{het} for reduction of cyt c_{ox}.

The expression given in eq 10 is justifiable for a given solution owing to the observation that the current for cyt *c* oxidation or reducton is directly proportional to the concentration of the redox-active form in a controlled-potential electrolysis. However, as can be seen in Table II, the observed current does not always vary linearly with changes in total cyt *c* concentration. The departures from linearity are not severe but do suggest that impurities could be a variable that block redox activity. This point requires further investigation.

In the discussion above concerning the values of k_{het} for material from I or II, we have assumed that the mechanism for redox reaction of cyt *c* is via processes like those represented by eq 7 or 9. The coverage of $[PMFc^{+/0}]_{surf}$ is variable, but in most cases, exceeds monolayer. Since the cyt *c* is a large molecule, it likely does not penetrate the polymer from I or II. Thus, only the outermost layer of redox sites are likely to be accessible to cyt *c*. The values of k_{ox} or k_{red} in Table II have been calculated by dividing the value of k_{het} by the coverage of the surface-confined mediator. Notice that while there are only modest variations in k_{het}, the value of k_{ox} varies from 2.0 to 700 × 10^4 cm^3/(mol s). This large variation in calculated k_{ox} or k_{red} is consistent with accessibility only to the outermost layer of the surface polymer from I or II. Thus, the values of k_{ox} and k_{red} for the n-Si electrode having a coverage of $[PMFc^{+/0}]_{surf}$ of 5 × 10^{-11} mol/cm^2, likely most accurately reflects the true rate constants for oxidation and reduction of cyt *c* by $[PMFc^+]_{surf}$ and $[PMFc^0]_{surf}$, respectively. Thus, the value for k_{ox} is at least 7 × 10^6 cm^3/(mol s), and the value for k_{red} is at least 3.6 × 10^6 cm^3/(mol s). Assuming the self-exchange rate of cyt *c* to be 10^6–10^7 cm^3/(mol s)[28] and the self-exchange rate of the $[PMFc^{+/0}]_{surf}$ to be ~10^9 cm^3/(mol s), the approximate value of $Fe(\eta^5\text{-}C_5H_5)_2{}^{+/0}$,[32] the values of k_{ox} and k_{red} are lower than would be predicted from the Marcus relationship,[31] which would be approximately 10$^{7.5}$–10^8 cm^3/(mol s). It is possible that the effective self-exchange rate for the $[PMFc^{+/0}]_{surf}$ system is considerably smaller, but a quantitative determination remains to be made. It is noteworthy that surface species from II were found to give a k_{ox} a factor of 10 lower than for a solution species for the oxidation of I^-.[30] A detailed study of the interfacial electron-transfer kinetics of cyt *c* at derivatized photoelectrodes will be reported elsewhere.

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Registry No. I, 83928-46-5; II, 66083-73-6; Pt, 7440-06-4; Si, 7440-21-3; $SiCl_4$, 10026-04-7; $Fe(\eta^5\text{-}C_5H_5)(\eta^5\text{-}C_5(CH_3)_5)$, 83928-47-6; $Fe(\eta^5\text{-}C_5H_4Li)(\eta^5\text{-}C_5(CH_3)_4CH_2Li)$, 83928-48-7; $Fe(\eta^5\text{-}C_5H_4D)(\eta^5\text{-}C_5(CH_3)_4CH_2D)$, 83928-49-8; $Fe(\eta^5\text{-}C_5H_4SiMe_3)(\eta^5\text{-}C_5(CH_3)_4CH_2SiMe_3)$, 83928-50-1; 2,3,4,5-tetramethyl-1[(diethoxysilyl)methyl][2]-ferrocenophane, 83928-51-2; cytochrome *c*, 9007-43-6.

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