with acyl chlorides (RCOCl) to form the corresponding esters. It is to be noted that elemental analyses of **4** and **5** give satisfactory agreement[11] with the compositions we have assigned.

The studies have been extended to other nucleophiles, and the results are summarized in Scheme I. Conversion to products as indicated is essentially quantitative, except for CN^-, where 25% of either **2** or **3** is found to be reduced to hydroxyruthenocene. The reactions are slower for the anionic nucleophiles than they are for the phosphines and, in the case of the former, may be governed by the rate of dissolution of the corresponding alkali-metal salts. The products were characterized by their ^1H NMR spectra.[12]

The activation for substitution on η^5-$C_5H_5^-$ by cyclopentadienone as coligand raises questions about the reaction mechanism. Attempts to do kinetic studies in the case of the homogeneous systems, by using ^1H NMR to follow the course of the reaction, failed because of the rapidity of the reactions.

Of particular interest is the role of coordinated nucleophile (CH_3CN in the case of **2**) in affecting the course and the rates of the reactions.

Acknowledgment. Support by the National Science Founation Grant CHE8816422 is gratefully acknowledged.

(12) ^1H NMR spectra of the reaction products of the reaction of **3** with SCH_3^-, $SC_6H_5^-$, and CN^-, i.e. attack on the Cp ring, and **2** with P (C_6H_5)$_3$ and $SC_6H_5^-$, i.e. attack on the C_5H_4O ring (δ, ppm, nitromethane-d_3, 20 °C): 4.84 (b, 1 H), 4.72 (t, 2 H), 4.66 (t, 2 H), 4.57 (s, 2 H), 4.27 (t, 2 H), 2.27 (s, 3 H); 7.2–7.0 (m, 5 H), 4.70 (t, 2 H), 4.69 (t, 2 H), 4.62 (t, 2 H), 4.22 (t, 2 H); 5.38 (b, 1 H), 5.09 (t, 2 H), 4.79 (t, 2 H), 4.78 (t, 2 H), 4.38 (t, 2 H); 8.00–7.78 (m, 15 H), 5.21 (m, 1 H), 4.76 (m, 1 H), 4.57 (s, 5 H), 4.16 (m, 1 H); 7.35–7.20 (m, 5 H), 4.99 (b, 1 H), 4.91 (2d, 1 H), 4.64 (2d, 1 H), 4.59 (s, 5 H), 4.46 (2d, 1 H).

Reactions between Cytochrome *c* and Plastocyanin Indicate That Choice of Docking Sites on Protein Surfaces May Depend on Thermodynamic Driving Force for Electron Transfer

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Various aspects of electron-transfer reactions can be examined with metalloproteins.[1] A pair of them can form multiple complexes,[2-7] and this phenomenon requires kinetic investigation. This study indicates that a protein (plastocyanin, pc) can form structurally different precursor complexes with virtually identical proteins differing in reduction potential (native and zinc-reconstituted cytochrome *c*, cyt and Zncyt).

Plastocyanin ($E°$ = 0.36 V vs NHE) has a negative patch remote (14–19 Å) from the copper atom and an electroneutral patch proximate (3–9 Å) to it.[8] Electron transfer to copper should be much more efficient from the latter than from the former;[9] the choice between the patches is often attributed simply to the charge of the other reactant.[8] Cytochrome *c* ($E°$ = 0.26 V) has a positive patch near the exposed heme edge.[10,11] In the electrostatic cyt/pc complex the heme patch abuts the remote patch,[12-22] but analysis[23-26] of dependence on ionic strength of the bimolecular rate constant k_1 excludes this as the reactive configuration.[27] The electron-transfer rate constant k_2 is large (1300 ± 200 s^{-1}) for the electrostatic complex, but undetectably small

$$\text{cyt(II)} + \text{pc(II)} \xrightarrow{k_1} \text{cyt(III)} + \text{pc(I)} \quad (1)$$

$$\text{cyt(II)/pc(II)} \xrightarrow{k_2} \text{cyt(III)/pc(I)} \quad (2)$$

(less than 0.2 s^{-1}) for the complex reinforced by noninvasive covalent cross-links between the heme patch and the remote patch,[28,29] which impede protein rearrangement.[30,31]

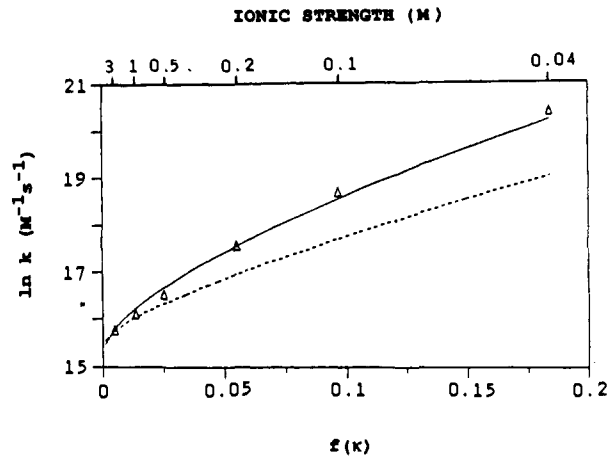

Figure 1. Dependence of k_3 on ionic strength at pH 7.0 and 25 °C. The protein parameters, function f(κ) of ionic strength, and the configuration-defining angle are explained elsewhere,[27] and k_∞ = 1.5 × 10^6 M^{-1} s^{-1}. The fitting (—) of experimental results (Δ) yields the angle of 36°; the other curve (– –) corresponds to the angle of 86°, characteristic of cytochrome *c* binding at the proximate patch (His 87) of plastocyanin.

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(28) Some evidence for this cross-linking is given in refs 14–16, 19, and 20. Our UV–vis, CD, and MCD spectra show that the covalent and electrostatic cyt/pc complexes have very similar structures,[29] and the protein orientation in the latter is known.[12-22] Moreover, plastocyanin whose carboxylate groups in the remote patch are blocked[16] cannot be cross-linked with cytochrome *c*.[29]

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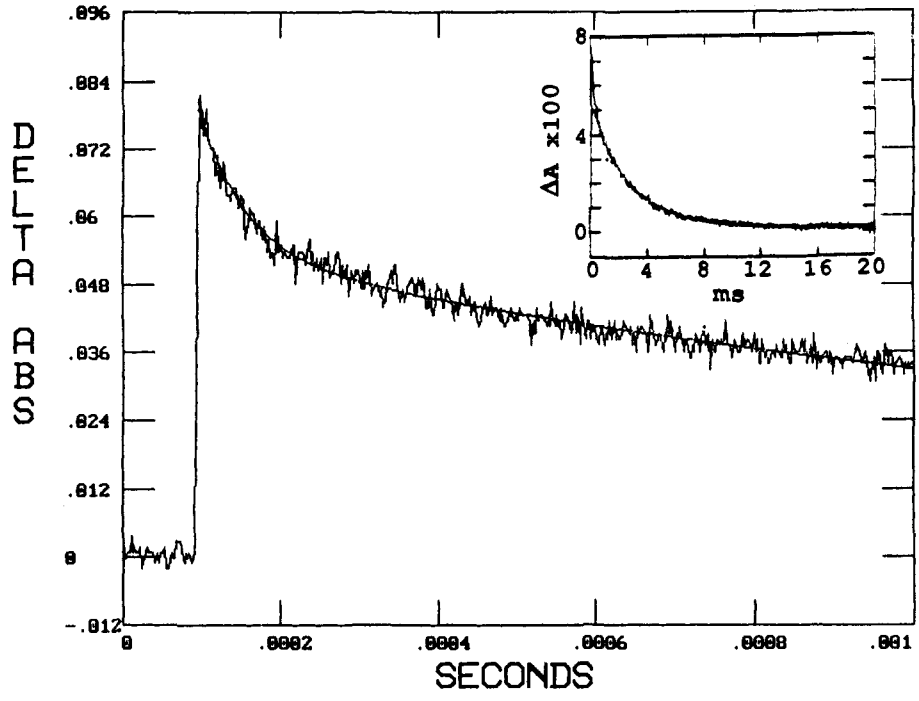

Figure 2. Transient absorbance at 460 nm in a solution containing 10 μM covalent complex Zncyt/pc (derivative 5 from a column of CM52) in phosphate buffer of ionic strength 10 mM, pH 7.0, at 25 °C. The solid line is a biexponential fit. Inset: The same, over 0.02 s, to show complete recovery of the ground state.

This study concerns "forward" (k_3 and k_4) and "back" (k_5) electron-transfer reactions of zinc cytochrome *c* and plastocyanin. Replacement of iron(II) by zinc(II) does not perturb the conformation[32] of cytochrome *c* and its association with other proteins.[33,34] The triplet state of zinc cytochrome *c* is a strong donor ($E°$ = −0.88 V).[2,35-38] Because heme excitation should not appreciably change the dipole moment of cytochrome *c*,[39] the protein configuration for the reaction k_3 was calculated with the parameters used[27] for the reaction k_1. As Figure 1 shows, the

$$^3\text{Zncyt} + \text{pc(II)} \xrightarrow{k_3} \text{Zncyt}^+ + \text{pc(I)} \quad (3)$$

$$^3\text{Zncyt/pc(II)} \xrightarrow{k_4} \text{Zncyt}^+\text{/pc(I)} \quad (4)$$

$$\text{Zncyt}^+\text{/pc(I)} \xrightarrow{k_5} \text{Zncyt/pc(II)} \quad (5)$$

defining angle[27] is 36 ± 10°, inconsistent with docking of zinc cytochrome *c* at the proximate patch (ca. 90°) and consistent with its docking at the remote patch (30–34°) in plastocyanin.

The covalent complex Zncyt/pc was prepared like the covalent complex cyt/pc[19,30] and characterized[29,30] and kinetically studied[40] by known methods. Besides efficiently and noninvasively cross-linking the proteins, carbodiimide converts certain carboxylate groups into neutral *N*-acylurea groups.[41] Indeed, cation-exchange chromatography[42] yielded eight derivatives of the complex Zncyt/pc, which differ in location and number of these neutralized groups, but not in configuration; in all of them the cross-links join the heme patch and the remote patch.[28-30]

The eight derivatives of the covalent complex Zncyt/pc(I) at various concentrations (5.0–30 μM) and ionic strengths (10 mM–1.00 M) and the electrostatic complexes Zncyt/pc(I) and Zncyt/apopc at low ionic strength (10 mM) all show exponential decay of the triplet state with the rate constant of 190 ± 10 s^{-1}. The decay is not complicated by electron transfer (because the copper atom is reduced or absent) or by energy transfer (because the triplet emission spectrum and the cuproplastocyanin absorption spectrum do not overlap). Since the decay rate depends on the heme environment,[33,34] these covalent and electrostatic complexes evidently have similar configurations.

The eight derivatives of the covalent complex Zncyt/pc containing both pc(I) and pc(II) show biexponential decay of the triplet state; the two exponentials are completely separable. The rate constant of the faster process does not, whereas that of the slower process does, depend on the complex concentration and ionic strength over the aforementioned ranges. These quenching processes, respectively, are the unimolecular (k_4) and bimolecular (k_6) redox reactions. Indeed, the relative amplitudes of the two

$$^3\text{Zncyt/pc(I)} + \text{Zncyt/pc(II)} \xrightarrow{k_6} \text{Zncyt}^+\text{/pc(I)} + \text{Zncyt/pc(I)} \quad (6)$$

exponentials match the proportion of cupriplastocyanin and cuproplastocyanin in the covalent complex. The reaction k_6 was not investigated further.

The rate constant k_4 is (2.5 ± 0.4) × 10^5 s^{-1} for the electrostatic complex[40] and (2.2 ± 0.5) × 10^4 s^{-1} for all the eight covalent derivatives; evidently, these eight have similar configurations, and the reaction within the covalent complex is unaffected by variation in surface charge (*N*-acylurea groups). This latter value, determined accurately by monitoring the disappearance of ^{3}Zncyt (Figure 2), and the value of 1.3 × 10^4 s^{-1}, determined less accurately by monitoring Zncyt$^+$ at 675 nm, are consistent with each other. The rate constant k_5, determined by monitoring Zncyt$^+$, is (1.1 ± 0.5) × 10^6 s^{-1} for the electrostatic complex[40] and 7 ×

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10^4 s^{-1} for the covalent complex. Similar relative decreases in both k_4 and k_5 from the electrostatic to the covalent complex probably reflect some small difference in protein orientation or in conformational fluctuation[43] between these complexes. Remarkably, both reactions k_4 and k_5 within the covalent complex remain fast. The same increase in the driving force, from 0.10 to 1.2 eV, is accompanied by different increases in the rate constant from k_2 to k_4, from 1300 to 2.5×10^5 s^{-1} in the electrostatic diprotein complex, but from virtually 0 to 2.2×10^4 s^{-1} in the covalent diprotein complex. The last two numbers indicate that the electron-transfer pathway via the remote patch in plastocyanin, to which each donor is cross-linked, is unfavorable for the reaction k_2 and favorable for the reaction k_4.

Comparison of electrostatic precursor complexes (k_1 vs k_3) and comparison of reactions in covalent complexes (k_2 vs k_4) consistently indicate that ferrocytochrome *c* does not, whereas zinc cytochrome *c* does, reduce cupriplastocyanin when bound at the remote patch. Although the two reductants have the same topography and electrostatic properties, perhaps the stronger one (the latter) can overcome the long distance and small electronic coupling,[44] whereas the weaker one (the former) must seek more favorable conditions, presumably at the proximate patch. Perhaps thermodynamic driving force should be considered when analyzing pathways for electron transfer in proteins.

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Registry No. Cytochrome *c*, 9007-43-6.

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Fiber Optic Attenuated Reflection Spectroscopy (FO-ATR) for Investigation of Organometallic Polymeric Films

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The use of optical fibers in spectroscopy has been demonstrated for both optical[1] and fluorescence[2] spectra. Infrared has received considerably less attention.[3] Attenuated reflection spectroscopy (ATR) has provided a very sensitive spectroscopic method[4] and in fact FT-IR-ATR has been used in the investigation of organic monolayers and assemblies on substrates such as glass Ge, ZnSe, and Si.[5] In this communication we report a novel method for performing sensitive infrared spectroscopic measurements on organometallic thin films. This method combines the use of optical chalcogenide IR glass fibers and ATR (FO-ATR). The characterization of thin films of this type is difficult, and the reports that do exist[6] include a need for new and improved techniques.

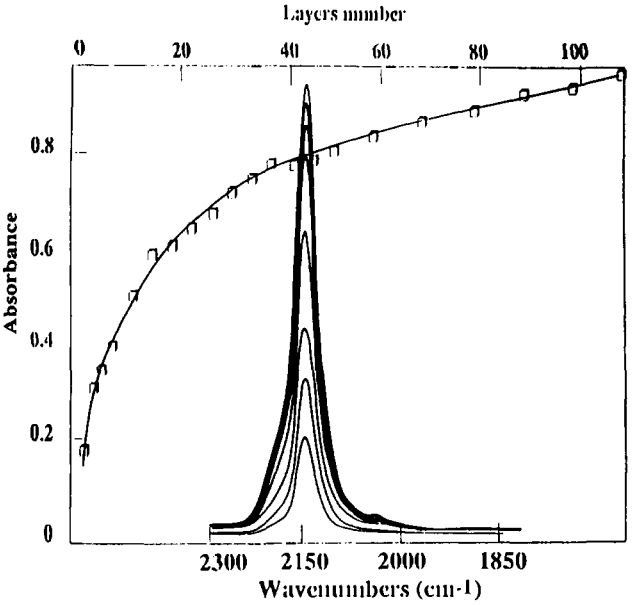

Figure 1. Absorbance bands $\nu(C{\equiv}N)$ as increasing number of layers are casted. In ascending order, 2, 4, 8, 20, 40, 60, 80, 100 layers (lower *x* axis). Upper axis: graphic representation of above bands as function of number of casted layers.

The advantages in using chalcogenide IR glass optical fibers in this way are numerous. They are cheap, compact, and easily implemented. The fibers provide an inert surface for deposition of nonfree-standing films. In addition to providing a proper deposition media the fiber then is easily able to efficiently guide the "signal" to and from the test sample. Changing the fiber diameter per length unit allows one to obtain different sensitivities.[7] Experiments are able to be monitored on line as we demonstrate here.[8] Therefore the combination of ATR spectroscopy[4,9] with its significant sensitivity and the use of chalcogenide optical fibers considering their technological implications may serve to make this method a powerful tool for both basic and applied research in this area.

This short article attempts to exemplify just how this FO-ATR method may serve in the characterization of thin organometallic films. For this purpose two types of experiments are described. The first deals with casting of a thin organometallic film onto a chalcogenide IR glass fiber from a ready solution of the polymer. This serves to test the relationship between the sensor response and number of polymer layers. Provided that the index of refraction of these films are known, this experiment enables one to calculate film thickness. The second deals with preparation of

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