Metalloprotein–Cobalt Cage Electron Transfer and the Stereoselective Reduction of Spinach Plastocyanin by Λ- and Δ-[Co((N(CH$_3$)$_3$)$_2$-sar)]$^{4+}$

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Electron transfer kinetics for the reduction of spinach plastocyanin and horse heart cytochrome *c* by several cobalt-cage complexes have been determined. The reduction of plastocyanin by [Co((N(CH$_3$)$_3$)$_2$-sar)]$^{4+}$ shows saturation kinetics with $K_{Co(II)} = (1.5 \pm 0.2) \times 10^3$ M^{-1}, and $k'_{et} = 4.0 \pm 0.2$ s^{-1}. This reaction is stereoselective (k_Λ/k_Δ = 1.7) and the stereoselectivity is independent of ionic strength. The reaction is observed to depend upon pH, pK_a = 5.3 ± 0.1, with the protonated protein being much less reactive than its conjugate base. The reaction is inhibited by added [Co((N(CH$_3$)$_3$)$_2$-sar)]$^{5+}$, $K_{Co(III)} = (3.9 \pm 0.1) \times 10^4$ M^{-1} and the protein-inhibitor complex has about 7% of the redox reactivity of the free protein, $k''_{et} = (4.4 \pm 0.2) \times 10^2$ M^{-1} s^{-1}. The reduction of plastocyanin by [Co((NH$_2$)$_2$-sar)]$^{2+}$ occurs with a second-order rate constant of $(1.13 \pm 0.06) \times 10^6$ M^{-1} s^{-1} and without stereoselectivity. Both reactions were studied at pH 7.5, I = 0.050 M, and 25 °C. Reduction of horse cytochrome *c* by [Co((NH$_2$)$_2$-sar)]$^{2+}$ and [Co(NO$_2$-capten)]$^{2+}$ are observed to occur with simple second-order kinetics with rate constants of $(4.06 \pm 0.05) \times 10^4$ M^{-1} s^{-1} and $(2.02 \pm 0.06) \times 10^5$ M^{-1} s^{-1}, respectively, at pH 7.0, I = 0.050 M, and 25 °C. The relevance of these results to the mechanisms of metalloprotein electron transfer and the suitability of cage complexes as metalloprotein redox mediators is discussed.

The use of metal complexes that are stable to ligand exchange over a wide range of pH to derivatize proteins[1,2] and electrodes[3] for the study of metalloprotein reactivity and to construct useful bioelectrical devices[2,3] is a developing research area. The metal cage complexes of sarcophagine and sepulchrate and related molecules, Figure 1, are especially suitable for this work since they are robust[4,5] over a wide range of pH and some work has already been done to utilize these complexes in biological studies. For example, Sykes and co-workers[6] have studied the oxidation of parsley ferredoxin by [Co(sepulchrate)]$^{2+}$, Toma and Murakami[7] have examined the reduction of horse cytochrome *c* by [Co(sepulchrate)]$^{2+}$ and Scott et al.[1c] have covalently attached [Co((NH$_2$)$_2$-sar)] to several surface sites on cytochrome *c* and measured the rate constants for intramolecular electron transfer from cobalt to iron. This paper reports kinetic studies of the reactions of three cobalt cage complexes with plastocyanin and cytochrome *c* and provides information on the intrinsic reactivity of these complexes with proteins that are relevant to the use of these exceptionally stable molecules as potential protein redox titrants, electrochemical mediators, and electrode modifiers. The stability of these complexes to ligand exchange and subsequent racemization also allowed the observation of stereoselectivity in one of these reactions.

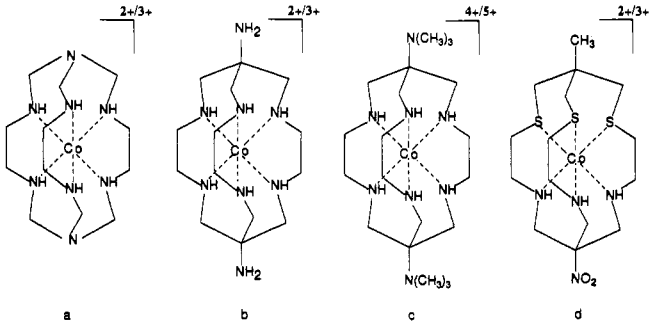

Figure 1. Structures of (a) [Co(sepulchrate)]$^{3+/2+}$, (b) [Co((NH$_2$)$_2$-sar)]$^{3+/2+}$, (c) [Co((N(CH$_3$)$_3$)$_2$-sar)]$^{5+/4+}$, and (d) [Co(NO$_2$-capten)]$^{3+/2+}$.

Stereoselectivity in the electron transfer reactions between metalloproteins and chiral coordination compounds has only recently been reported.[8-10] Bernauer and Sauvain[8a] have made direct kinetic observation of the stereoselective reduction (k_Δ/k_Λ = 1.6) of spinach plastocyanin by labile, chiral, iron complexes and more recently[8b] have reported comparable selectivity for the

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oxidation of spinach ferredoxin by related cobalt(III) complexes. Temperature studies for these systems reveal counterbalancing enthalpic and entropic contributions to the overall selectivity.[8c] Sakaki and co-workers[9] have observed relatively smaller chiral induction by measuring the circular dichroism spectrum of the product mixtures for the oxidation of horse cytochrome *c* by [Co(2,4-pentanedionate)$_3$] and [Co(2,2′-bipyridine)$_3$]$^{3+}$. In both reactions the magnitude of the selectivity depends on the solvent composition (ethanol/water mixtures) and pH. The larger rate constants and stereoselectivity for [Co(2,2′-bipyridine)$_3$]$^{3+}$ as compared to [Co(2,4-pentanedionate)$_3$] are interpreted as indicative of the 2,2′-bipyridine complex gaining better access to the heme edge via the crevice on the surface of cytochrome *c*. Ficke et al.[10] have also observed stereoselectivity in the oxidation of horse cytochrome *c* by [Co(oxalate)$_3$]$^{3-}$, employing both direct kinetic measurements and circular dichroism spectra of products to establish stereoselectivity in the electron transfer. For this reaction the stereoselectivity is nearly independent of pH, ionic strength, and buffer identity, and the substantial attractive electrostatic forces involved allow the identification of the likely protein surface site at which the observed stereoselectivity is expressed.

An important issue in these studies is the origin of the stereoselectivity since it can arise from differential ion pairing constants for the protein and the individual enantiomers, from differences in the electron transfer rate constants within the ion pair, or from some combination of the two effects.[11] If the stereoselectivity arises from differences in the intramolecular rate constants for electron transfer within ion pairs of approximately equal stability, then subtle orientational effects that modify the intramolecular electron transfer pathway or that modify the effectiveness of a single path must be the origin of the selectivity. Detailed information regarding the relationship between donor–acceptor distance, orientation, electron pathway, and electron transfer rate is being actively sought.[1,11,12]

Studies on stereoselective electron transfer appear to show the importance of close association of complex and metalloprotein in chiral discrimination. The two proteins chosen for study differ substantially in charge at pH 7, with copper (I) spinach plastocyanin bearing a net charge of −9 and iron (II) horse cytochrome *c* a charge of +7. Since the three cage complexes used are positively charged, it was anticipated that the extent of association of cage complex with protein prior to electron transfer would be greatly different for the two proteins and that these differences may substantially influence the electron transfer kinetics and the extent of stereoselectivity. Moreover, the detailed interpretation of stereoselectivity is aided by knowledge of the protein's crystal structure and surface site(s) for complex association and electron transfer. In addition to the crystal structure,[13] information is available for the association of positively-charged, hydrophilic complexes on the surface of plant plastocyanins,[12a,14-18] and these proteins are especially attractive for examination of stereoselective effects in metalloprotein electron transfer reactions of cationic partners. This paper focuses on the mechanisms of electron transfer reactions between proteins and complex ions and on the stereoselective reduction of copper (II) spinach plastocyanin by Λ- and Δ-[Co((N(CH$_3$)$_3$)$_2$-sar)]$^{4+}$ ((1,8-bis(trimethylammonio)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]-icosane)cobalt(II)).[19] This cobalt complex, Figure 1c, was chosen because of its relatively high positive charge, relatively small self-exchange electron transfer rate constant and inertness to racemization or decomposition in both oxidation states over a wide range of pH.

Recent work on plant plastocyanins has also focused on the relative electron transfer efficiencies[18] of what are believed to be the two principal electron transfer sites on the surface of the protein and the factors that favor electron transfer at each site or migration[20] of redox partners between initial binding and eventual redox sites. This work provides additional information on factors that favor electron transfer at the remote, acidic site.

Experimental Details

The cobalt complexes used in these studies were prepared and characterized by published methods: [Co((NH$_2$)$_2$-sar)]Cl$_3$·2HCl·H$_2$O[4] ((NH$_2$)$_2$-sar = 1,8-diamino-3,6,10,13,16,19-hexaazabicyclo[6.6.6]icosane); [Co(NO$_2$-capten)](ClO$_4$)$_3$·H$_2$O[21,22] (NO$_2$-capten = 1-methyl-8-nitro-3,13,16-trithia-6,10,19-triazabicyclo[6.6.6]icosane); [Co((N(CH$_3$)$_3$)$_2$-sar)]Cl$_5$·6H$_2$O[19] (((N(CH$_3$)$_3$)$_2$-sar) = 1,8-bis(trimethylammonium)-3,6,10,13,16,19-hexaazabicyclo[6.6.6]icosane). The cobalt(II) forms of the complexes were usually prepared by Zn·Hg amalgam reduction of the cobalt(III) salts under an inert atmosphere. In particular, for the reduction of [Co((N(CH$_3$)$_3$)$_2$-sar)]$^{5+}$, Zn·Hg reductions were continued for more than 12 h to ensure complete reduction and avoid contamination of reactant solutions with oxidized complex. In some experiments a single solution of relatively concentrated [Co((N(CH$_3$)$_3$)$_2$-sar)]$^{5+}$ was reduced and diluted to varying extents with deoxygenated buffer immediately prior to reaction. In other experiments a [Co((N(CH$_3$)$_3$)$_2$-sar)]$^{5+}$ solution was diluted prior to reduction, and each diluted solution was reduced exhaustively and used directly without further dilution. It was reasoned that if either incomplete reduction of the Co(III) or oxidation by trace amounts of oxygen was responsible for significant amounts of contaminating Co(III) in the reaction mixtures that it was unlikely that these two approaches would lead to the same pattern of Co(III) contamination in the two sets of experiments. Equivalent results were obtained by both methods, suggesting that systematic errors in measured rates were not

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accumulating because of Co(III) contamination. As will be described later, the protein reduction was sensitive to trace amounts of $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ in the reaction mixture, and these experiments were designed both to minimize this effect and to determine the extent to which the method of solution preparation might lead to systematic errors in reaction rates. Solutions of $[Co(NO_2\text{-capten})]^{2+}$ were prepared by the reduction of the cobalt(III) salt for 3 h with a platinum catalyst under an atmosphere of molecular hydrogen. Since it is possible to reduce the nitro group on this complex as well as the metal center, solutions of $[Co(NO_2\text{-capten})]^{2+}$ were air oxidized evaporated to dryness and the IR spectrum was obtained. This spectrum was observed to be identical to the IR spectrum of the complex before reduction with platinum/hydrogen. Consequently, we conclude that the nitro group remained intact following reduction by this method. All solutions were transferred under an atmosphere of molecular nitrogen or argon using gastight syringes. The concentrations of the cobalt(II) solutions were determined from visible spectra of the original cobalt(III) solutions and by two spectrophotometric assays. One of these assays utilized the reduction of horse cytochrome *c*; the other, the reduction of the 1,10-phenanthroline complex of iron(III). In the cytochrome assay, an aliquot of the cobalt(II) solution was added to an excess of cytochrome *c* in pH 7.5 HEPES (*N*-[2-hydroxyethyl]-piperazine-*N'*-ethanesulfonic acid) buffer and the concentration of the cobalt(II) was then calculated from the absorbance change at 550 nm.[23] In the 1,10-phenanthroline assay, an aliquot of the Co(II) solution was transferred to a small volume, ca. 0.20 mL, of a solution that contained 5×10^{-3} M iron(III) nitrate and 5×10^{-2} M 1,10-phenanthroline maintained at pH 5.8 with acetate buffer. Following mixing and reaction, this solution was diluted to 5.00 mL and its absorbance change measured at 508 nm ($\Delta\epsilon = 1.10 \times 10^4$ M^{-1} cm^{-1}). These spectrophotometric assays and the determination of the Co(II) concentrations from the spectrum of the starting Co(III) solutions usually agreed within the uncertainty of the assay, typically 1–3%.

Horse cytochrome *c* (Sigma Type VI) was used without further purification. Plastocyanin was prepared from fresh spinach leaves by a modification[24] of the method of Yocum et al.[25] and purified to an absorbance (*A*) ratio A_{278}/A_{597} of 1.4 or less using ion-exchange and gel chromatography and FPLC. Concentrations of the oxidized protein were determined from the absorbance of the solutions at 597 nm ($\epsilon = 4.9 \times 10^3$ M^{-1} cm^{-1}).[26] Buffered solutions of plastocyanin were stored frozen at 77 K and were stable for several months under these conditions.

Kinetic measurements were made by observing the decrease in absorbance at 597 nm as plastocyanin was reduced or the increase in absorbance at 550 nm as cytochrome *c* was reduced by the cobalt(II) complexes. All experiments were done under concentration conditions of pseudo-first-order excess of the cobalt(II) complex over the protein and the absorbance changes recorded with an Applied Photophysics stopped-flow spectrophotometer, Model SF.17 MV, coupled to an Archimedes 410/1 computer. The mixing block was enclosed in a disposable glovebag and purged with molecular nitrogen throughout the experiment. Data were treated using standard algorithms provided with this instrument and all data reported gave good fits to a single exponential decay. No systematic deviations from the first-order fits were observed. Typically, measurements were repeated four times with each pair of reactant solutions and the resulting rate constants averaged. These average rate constants are reported and used in functional fits described subsequently. Kinetic measurements at pH's other than 7.5 were done by mixing a solution containing the cobalt complex in a solution of high concentration of desired buffer with a solution of protein in dilute pH 7.5 buffer. The reported pH was attained on mixing the two solutions in the stopped-flow. This method minimizes exposure of the protein to pH conditions under which the protein is not stable over longer time periods, in particular low pH.

Results

Reduction of Plastocyanin by Racemic $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$. The reduction of plastocyanin was studied with the Co(II) complex, $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$, in excess of the oxidized protein, pc(II), and the progress of the pseudo-first-order reaction was observed by following the absorbance decrease at 597 nm due to protein reduction. The reaction was studied at 25 °C and as a function of pH and ionic strength. Observed rate constants at pH 7.5 (0.050 HEPES) and $I = 0.050$ M (HEPES, NaCl) are collected in Table 1 of the supplementary material and displayed in Figure 2. These data display saturation kinetics consistent with the following kinetic scheme:

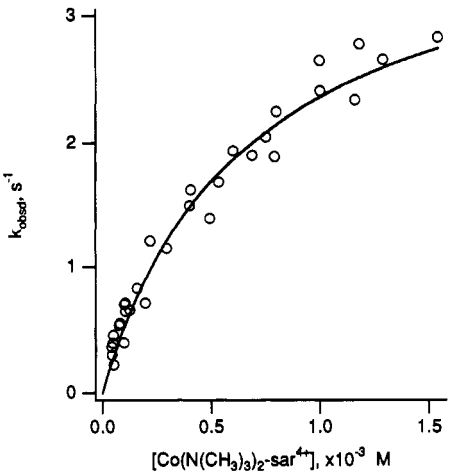

Figure 2. Observed first-order rate constants for the reduction of pc(II) by $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ versus the concentration of reducing agent at 25 °C, pH 7.5 (HEPES), and $I = 0.050$ M.

$$Co(II) + pc(II) \leftrightarrow Co(II),pc(II) \quad K_{Co(II)} \qquad (1)$$

$$Co(II),pc(II) \rightarrow Co(III) + pc(I) \quad k'_{et} \qquad (2)$$

where pc(II) and pc(I) are the oxidized and reduced forms of plastocyanin respectively, $K_{Co(II)}$ is the equilibrium constant for association of Co(II) with the oxidized protein, and k'_{et} is the rate constant for electron transfer from within the ion pair. When the association process is rapid relative to electron transfer, this scheme leads to the following rate law:

$$-d[pc(II)]/dt = k'_{et}K_{Co(II)}[Co(II)][pc(II)]/(1 + K_{Co(II)}[Co(II)]) \qquad (3)$$

which, under conditions of pseudo-first-order excess of Co(II), leads to an observed first-order rate constant

$$k_{obsd} = k'_{et}K_{Co(II)}[Co(II)]/(1 + K_{Co(II)}[Co(II)]) \qquad (4)$$

The solid trace in Figure 2 is obtained from a nonlinear least-squares fit of the observed pseudo-first-order rate constants and initial Co(II) concentrations to this expression which yields $k'_{et} = 4.0 \pm 0.2$ s^{-1} and $K_{Co(II)} = (1.5 \pm 0.2) \times 10^3$ M^{-1}.

Many experiments were used to establish this trace because uncertainty in each measurement is relatively high. The reason for the uncertainty is believed to be related to the effective inhibition of the reaction by trace amounts of the Co(III) form of the complex, $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$, present in the reaction mixture. While the amount of Co(III) in the reducing mixture was believed to be very small and detectable neither spectrally nor by redox analysis, some is surely present and even amounts of ca. 1% can influence the reaction rate, as will be shown subsequently. This effect was minimized by reducing the cobalt complex over long times with Zn·Hg amalgam under molecular nitrogen and taking care to exclude oxygen while handling the solutions. Furthermore, air oxidation of this complex was slow. In addition, spectrophotometric redox analysis of the Co(II) reactant solutions at the time of mixing was used to assure total reduction within the sensitivity of the analysis. Stock solutions for reaction were prepared by two different methods with the hope of detecting any errors due to a systematic variation in the amount of contaminating Co(III)) in the reaction mixtures. In some experiments a concentrated stock solution of Co(II) was

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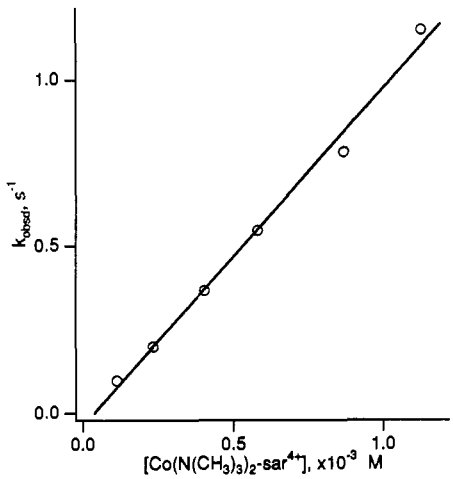

Figure 3. Observed first-order rate constants for the reduction of pc(II) by $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ versus the concentration of reducing agent at 25 °C, pH 7.5 (HEPES), and I = 0.50 M.

prepared and diluted to produce individual reaction solutions, while in others Co(II) reaction solutions were prepared directly by exhaustive reduction of a diluted Co(III) stock solution. Since no differences were observed in the results from these two types of experiments, we conclude that the curvature apparent in Figure 2 arises from the behavior depicted by eqs 1–2 and not from systematic inhibition of the reaction rate by trace amounts of Co(III) in starting reaction mixtures.

In addition, since Co(III) is a product of the electron transfer, small amounts of Co(III) are produced as the reaction proceeds. While it is impossible to state exactly how much inhibition is produced from the Co(III) product because its association constant with pc(I) is not known, one expects that this association constant is probably similar in magnitued to, if not larger than, that for pc(II) which is known to be 3.9×10^4 M^{-1}. Consequently, the Co(III) product probably is divided nearly equally between association with pc(I) and pc(II) following the reaction. Since the largest pc(II) starting concentration used was 4×10^{-6} M, the highest possible Co(III) available for inhibition is approximately 2×10^{-6} M and is available at this level only at the end of the reaction. The average Co(III) available for inhibition of pc(II) reduction over the course of the reaction would therefore be only 1×10^{-6} M. This results in a 3% contribution to the denominator of eq 8 in the worst case and much less than this in most experiments. Therefore, it is unlikely that product inhibition makes a significant contribution to the curvature observed in Figure 2. In support of this contention is the observation that k_{obsd} was not dependent on variations in the starting concentration of pc(II) which would have been expected if Co(III) product inhibition of the reaction was significant.

This reaction was also studied at an I = 0.50 M (HEPES, NaCl). Under these conditions no rate saturation was detected, Table 1 of the supplementary material, and the observed rate constants were linearly dependent on the Co(II) concentration, Figure 3. The second-order electron transfer rate constant obtained from a least-squares fit to these data, solid line in Figure 3, is $(1.01 \pm 0.05) \times 10^3$ M^{-1} s^{-1}.

Stereoselectivity in the Electron Transfer between pc(II) and $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$. The rates of reduction of pc(II) by optically pure Δ- and Λ-$[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ were measured at pH 7.5 (0.050 M HEPES) and at three ionic strengths: 0.050, 0.10, and 0.50 M (HEPES, NaCl) at 25 °C. Observed rate constants are collected in Table I. At $I = 0.050$ M, the average ratio of rate constants, k_Λ/k_Δ, is 1.70 and this is insensitive to ionic strength.

Inhibition of Electron Transfer by $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$. The reduction of pc(II) by $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ was substantially inhibited by the addition of Co(III) complex, $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$. Data displayed in Figure 4 illustrate this inhibition. This type of behavior is well-known[12a,14,15] for plastocyanin redox reactions that involve cationic redox partners and is indicative of competition between the inhibitor and the redox partner for a redox-active site on the protein's surface. This competition is represented by the following reaction scheme:

Table I. Observed Rate Constants, k_{obsd}, for the Reduction of Plastocyanin[a] by Λ- and Δ-$[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ at 25 °C and pH 7.5 (HEPES)

$10^4[[Co((N(CH_3)_3)_2\text{-sar})]^{4+}]$, M	isomer	ionic strength, M	k_{obsd}, s^{-1}
0.75	Λ	0.050	0.638
0.75	Λ	0.050	0.405
0.75	Λ	0.050	0.683
0.75	Δ	0.050	0.425
0.75	Λ	0.050	0.679
0.75	Δ	0.050	0.386
0.75	Λ	0.050	0.692
0.75	Δ	0.050	0.389
0.50	Λ	0.050	0.619
0.50	Δ	0.050	0.419
0.50	Λ	0.050	0.638
0.50	Δ	0.050	0.429
0.50	Λ	0.10	0.254
0.50	Δ	0.10	0.155
0.55	Λ	0.10	0.257
0.50	Δ	0.10	0.154
0.50	Λ	0.50	0.0887
0.50	Δ	0.50	0.0508
0.50	Λ	0.50	0.0920
0.50	Δ	0.50	0.0530

[a] Plastocyanin = $(2–4) \times 10^{-6}$ M.

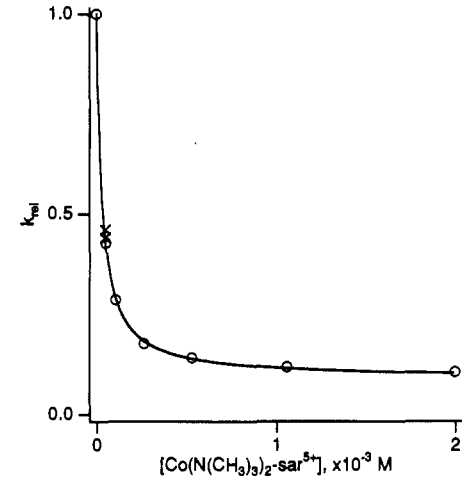

Figure 4. Ratio of the observed rate constants in the presence of inhibitor, $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$, to the observed rate constants in the absence of this inhibitor, k_{rel}, versus the concentration of inhibitor for the reduction of pc(II) by $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ at 25 °C, pH 7.5 (HEPES), and $I = 0.050$ M. Key: circles, racemic inhibitor; crosses, resolved inhibitor.

$$Co(III) + pc(II) \leftrightarrow Co(III),pc(II) \quad K_{Co(III)} \qquad (5)$$

$$Co(III),pc(II) + Co(II) \rightarrow 2Co(III) + pc(I) \quad k''_{et} \qquad (6)$$

$$Co(II) + pc(II) \rightarrow Co(III) + pc(I) \quad k'_{et}K_{Co(II)} \qquad (7)$$

where $K_{Co(III)}$ is the equilibrium constant for association of the Co(III) inhibitor, $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$, with the oxidized protein, k''_{et} is the second-order rate constant for electron transfer to the protein-inhibitor complex from $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$, and $k'_{et}K_{Co(II)}$ is the association and electron transfer between protein without bound inhibitor and $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$, the process already described in eqs 1–2.

The following relationship for the observed rate constant in the presence of inhibitor, k_{obsd}, can be derived from this scheme

Table II. Observed Rate Constants, k_{obsd}, for the Inhibition of the $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ Reduction of Plastocyanin by $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ at 25 °C, pH 7.5 (HEPES), and I = 0.050 M (NaCl)[a]

$10^4[[Co((N(CH_3)_3)_2\text{-sar})]^{5+}]$, M	isomer	k_{obsd}, s^{-1}
0.00	racenmic	0.659
0.528	racemic	0.281
1.06	racemic	0.188
2.64	racemic	0.115
5.30	racemic	0.092
10.6	racemic	0.076
20.0	racemic	0.069
0.50	Λ	0.303
0.50	Δ	0.303
0.50	Λ	0.303
0.50	Δ	0.310

[a] Experiments done with $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ at an initial concentration of 1.26×10^{-4} M and in first-order excess of pc(II) (~(2–4) × 10^{-6} M).

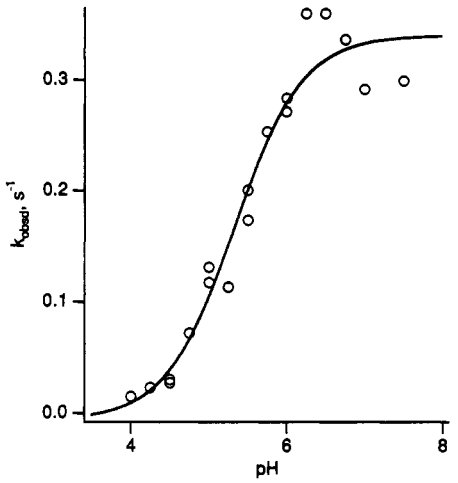

Figure 5. Observed first-order rate constants for the reduction of pc(II) by $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ versus pH at 25 °C and I = 0.050 M.

when Co(II) is in pseudo-first-order excess of pc(II).

$$k_{obsd} = \{(k'_{et}K_{Co(II)} + k''_{et}K_{Co(III)}[Co(III)])/(1 + K_{Co(II)}[Co(II)] + K_{Co(III)}[Co(III)])\}[Co(II)] \quad (8)$$

A series of experiments, Table II, were done at a single Co(II) concentration with varying amounts of inhibitor added and the observed rate constants fitted to eq 8 holding k'_{et} and $K_{Co(II)}$ at their previously determined values. The values obtained from this procedure are k''_{et} = (4.4 ± 0.2) × 10^2 M^{-1} s^{-1} and $K_{Co(III)}$ = (3.9 ± 0.1) × 10^4 M^{-1}. What is plotted in Figure 4 is k_{rel}, which is the ratio of the observed rate constants in the presence of inhibitor to those in the absence of inhibitor at the Co(II) concentration used for these studies. The solid trace in Figure 4 is obtained from the fitted parameters and eqs 4 and 8. Several inhibition experiments were also done with chiral inhibitor and these data are also recorded in Table II and displayed in Figure 4. No significant differences in inhibitory effect were observed between Δ-$[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ and Λ-$[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$.

pH Dependence of the Reduction of pc(II) by $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$. The reduction of pc(II) by $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ was observed to depend on pH as displayed in Figure 5. This pH dependence is consistent with the following reaction scheme:

$$H^+pc(II) \rightarrow H^+ + pc(II) \quad K_a \quad (9)$$

$$Co(II) + H^+pc(II) \rightarrow Co(III) + H^+pc(I) \quad k_H \quad (10)$$

$$Co(II) + pc(II) \rightarrow Co(III) + pc(I) \quad k \quad (11)$$

For this reaction k_H makes no detectable contribution to the observed rate, k represents the process already described, eqs 1–2, and the data in Table 2 of the supplementary material fit the expression, eq 12, which yields a pK_a of 5.3 ± 0.1.

$$k_{obsd} = kK_a[Co(II)]/(K_a + [H^+]) \quad (12)$$

Equilibrium Dialysis. In an attempt to determine if the association of $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ with pc(II) is stereoselective, racemic complex (1.0 mL, 3.78×10^{-3} M) was placed in one side of a dialysis cell and pc(II) (1.0 mL, 1.0×10^{-3} M) was placed on the other side of the membrane and the solution allowed to attain equilibrium. The optical rotation of the solution from the complex side of the membrane was then measured in a 1.0-dm pathlength cell. The solution had a very small negative rotation (–0.020°) and large absorbance (1.4 au) at 468 nm indicating that the dextrorotatory (Λ) enantiomer was preferentially associated with the protein. However, this signal is only slightly above the background signal under these conditions and represents 1.5% excess of Δ isomer in the dialysate and 4.2% stereoselectively in the binding of complex to protein.

Table III. Observed Rate Constants, k_{obsd}, for the Reduction of Plastocyanin[a] by $[Co((NH_2)_2\text{-sar})]^{2+}$ at 25 °C, pH 7.5 (HEPES), and I = 0.050 M (NaCl)

$10^5[[Co((NH_2)_2\text{-sar})]^{2+}]$, M	isomer	k_{obsd}, s^{-1}
9.90	racemic	113
5.60	racemic	62.2
2.40	racemic	27.0
9.90	Δ	104
5.49	Δ	64.0
2.72	Δ	30.0

[a] [Plastocyanin] = (2–4) × 10^{-6} M.

Reduction of Plastocyanin by $[Co((NH_2)_2\text{-sar})]^{2+}$. The reduction of plastocyanin by the Co(II) complex, $[Co((NH_2)_2\text{-sar})]^{2+}$, with complex in pseudo-first-order excess of the oxidized protein, pc(II), was observed by following the absorbance decrease at 597 nm due to protein reduction. The reaction was studied at 25 °C, pH 7.5 (0.050 HEPES), and I = 0.050 M (HEPES, NaCl) and found to be first order in each reactant with no rate saturation apparent over the concentration range studied. Work at this pH assures nearly complete deprotonation of the amine groups (pK_1 = 3.31 ± 0.02, pK_2 = 2.37 ± 0.02 for $[Co((NH_3)_2\text{-sar})]Cl_5\cdot 2H_2O$ and pK_1 = 6.29 ± 0.01, pK_2 = 5.40 ± 0.01 for $[Co((NH_3)_2\text{-sar})]Cl_4\cdot 5H_2O$ at 25 °C, I = 0.1 M).[5] The reduction was studied with racemic complex as well as with optically pure dextrorotatory isomer and no stereoselectivity outside the error of the rate measurements was observed. The observed rate constants are collected in Table III and a least-squares fit of these data yields a second-order rate constant of (1.13 ± 0.06) × 10^6 M^{-1} s^{-1}.

Reduction of Cytochrome c by $[Co((NH_2)_2\text{-sar})]^{2+}$. The reduction of cytochrome c by the Co(II) complex, $[Co((NH_2)_2\text{-sar})]^{2+}$, with complex in pseudo-first-order excess of the oxidized protein, was observed by following the absorbance increase at 550 nm due to protein reduction. The reaction was studied at 25 °C, pH 7.0 (0.050 HEPES), and I = 0.050 M (HEPES, NaCl) and found to be first order in each reactant with no rate saturation apparent. The reduction was studied with racemic complex as well as with optically pure dextrorotatory isomer and no stereoselectivity was observed. The observed rate constants are collected in Table IV, and a least-squares fit of these data yields a second-order rate constant of (4.06 ± 0.05) × 10^4 M^{-1} s^{-1}.

Reduction of Cytochrome c by $[Co(NO_2\text{-capten})]^{2+}$. The reduction of cytochrome c by the Co(II) complex, $[Co(NO_2\text{-capten})]^{2+}$, with complex in pseudo-first-order excess of the oxidized protein was observed by following the absorbance increase at 550 nm due to protein reduction. The reaction was studied at 25 °C, pH 7.0 (0.050 HEPES), and I = 0.050 M (HEPES, NaCl) and found to be first order in each reactant with no rate saturation apparent over the concentration range studied. The

Table IV. Observed Rate Constants, k_{obsd}, for the Reduction of Cytochrome c^a by $[Co((NH_2)_2\text{-sar})]^{2+}$ at 25 °C, pH 7.5 (HEPES), and I = 0.050 M (NaCl)

$10^4[[Co((NH_2)_2\text{-sar})]^{2+}]$, M	isomer	k_{obsd}, s^{-1}
8.53	racemic	34.6
6.35	racemic	25.1
2.86	racemic	11.4
1.34	racemic	5.16
0.49	racemic	1.85
6.85	Δ	27.6
3.17	Δ	12.0
1.67	Δ	6.51

[a] [Cytochrome c] = (2–3) × 10^{-6} M.

Table V. Observed Rate Constants, k_{obsd}, for the Reduction of Cytochrome c^a by $[Co(NO_2\text{-capten})]^{2+}$ at 25 °C, pH 7.0 (HEPES), and I = 0.050 M (NaCl)

$10^4[[Co(NO_2\text{-capten})]^{2+}]$, M	k_{obsd}, s^{-1}	$10^4[[Co(NO_2\text{-capten})]^{2+}]$, M	k_{obsd}, s^{-1}
16.2	326	3.38	71.0
5.93	113	1.07	21.9

[a] [Cytochrome c] = (2–3) × 10^{-6} M.

Table VI. Comparison of Experimental and Calculated Electron Transfer Rate Constants for Metalloprotein-Cobalt Cage Reactions at 25 °C

reaction	k_{exptl}, M^{-1} s^{-1}	k_{calcd}, M^{-1} s^{-1} [a]
cytochrome c/$[Co(sep)]^{2+}$	2.7×10^5 [b]	9.4×10^5
cytochrome c/$[Co((NH_2)_2\text{-sar})]^{2+}$	4.06×10^4 [c]	4.2×10^5
cytochrome c/ $[Co(NO_2\text{-capten})]^{2+}$ [e]	2.02×10^5 [c]	5.0×10^4
pc(II)/$[Co(NH_2)_2\text{-sar})]^{2+}$	1.13×10^6 [c]	5.6×10^6
pc(II)/$[Co(N(CH_3)_3)_2\text{-sar})]^{4+}$	6.0×10^3 [c,d]	6.8×10^3

[a] Calculations were done using the Marcus cross relationship.[12b,27] $E^{o\prime}$ values and self-exchange rate constants used are as follows: 0.370 V and 1×10^4 M^{-1} s^{-1} for plastocyanin,[12a] 0.26 V and 1×10^3 M^{-1} s^{-1} for cytochrome c,[28] –0.30 V and 5.1 M^{-1} s^{-1} for $[Co(sep)]^{2+}$,[5] –0.32 V and 0.5 M^{-1} s^{-1} for $[Co((NH_2)_2\text{-sar})]^{2+/3+}$,[45] 0.15 V and 4×10^4 M^{-1} s^{-1} for $[Co(NO_2\text{-capten})]^{2+/3+}$,[22,29] and 0.01 V and 1.1×10^{-2} M^{-1} s^{-1} for $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$;[19] a value of 1×10^{11} M^{-1} s^{-1} was used for the collision frequency Z, and the calculations are not corrected for work terms. The self-exchange value used for plastocyanin is an estimate based on the upper limit of 2×10^4 M^{-1} s^{-1},[12a] and the value used for $[Co(NO_2\text{-capten})]^{2+/3+}$ is an estimate based on the self-exchange rate constants for the related azacapten and NH_2-capten compounds.[22] [b] Reference 7. [c] This work. [d] This value represents the product $k'_{et}K_{Co(II)}$. [e] Low-spin cobalt(II).

observed rate constants are collected in Table V and a least-squares fit of these data yields a second-order rate constant of $(2.02 \pm 0.06) \times 10^5$ M^{-1} s^{-1}.

Discussion

Because of their exceptional inertness[4,5] to hydrolysis and to loss of ligand as redox cycling occurs, metal complexes of the encapsulating ligands based on sarcophagine and sepulchrate are potential metalloprotein titrants, biological electrochemical mediators, and reagents for derivatizing electrodes. While the cage complexes are known to behave predictably in their electron transfer reactions with themselves and other coordination compounds,[5] limited information is available regarding their intrinsic reactivity with metalloproteins.[1c,6,7] Table VI compares experimental and calculated[12b,27,28] second-order electron transfer rate constants for the reactions of cytochrome c and plastocyanin with several cage complexes. What can be seen from these results is that the cage complexes thus far studied behave in a consistent manner, considering the limitations of the calculations for metalloprotein cross-reactions. It follows that useful estimates of metalloprotein–cage complex electron transfer rate constants for similar systems should be possible. All of the calculated rate constants are within a factor of 10 of the experimental values, and considering the accumulation of error inherent in such calculations, the uncertainty of the applicability of the protein self-exchange rate constants, and that no attempt was made to account for work associated with bringing the charged reactants together, the agreement is satisfactory. It is useful to know that the agreement extends across a range of nearly 0.5 V in complex redox potentials and more than a factor of 10^6 in complex self-exchange rate constants. Moreover, the large number of stable cage derivatives, variety of metal complexes formed, range of potentials, and self-exchange rates accessible make these complexes attractive as redox mediators and reagents for electrode derivatization in bioelectronic devices. In particular, the sulfur-containing cages with low-spin Co(II) and Co(III) have large self-exchange rate constants[22] and undergo facile metalloprotein redox reactions even at modest driving forces.

It is interesting to compare the measured second-order rate constant from this study ($(4.06 \pm 0.05) \times 10^4$ M^{-1} s^{-1}) with the first-order rate constants measured by Scott et al.[1c] for intramolecular electron transfer to cytochrome c iron(III) from the same cobalt cage, $[Co((NH_2)_2\text{-sar})]^{2+}$, which has been covalently attached to the protein via the -NH_2 cap. Seven such intramolecular electron transfer rate constants have been measured for seven isomers with cage-to-heme distances that vary from about 10 to 19 Å with the unexpected result that the rate constants are all between 1 and 3 s^{-1} and are more or less insensitive to the intermetal distance. If one assumes for our bimolecular study that electron transfer follows cytochrome-cage association and that the association constant is less than 1 M^{-1} (probably much less than this given the high positive charge on the protein and the +2 charge on the complex), an intramolecular rate contant from within the weakly formed complex of $\geq 4 \times 10^4$ s^{-1} is obtained. While no unequivocal explanation is available for the distance independence of the intramolecular rate constants, it is clear for the molecules with covalently attached $[Co((NH_2)_2\text{-sar})]^{2+}$ that neither the small rate constants nor their invariance with distance can be attributed to the intrinsic reactivity of the cage complex. Apparently none of the covalent attachments places a $[Co((NH_2)_2\text{-sar})]^{2+}$ in effective proximity to the heme edge or to a superexchange pathway competitive with the more direct route available to the free complex.

The absence of detectable stereoselectivity in the two reactions with cytochrome c is not surprising considering the high positive charge on the protein, the positive charge on the complexes, and the likely result that the ion-pairing association constants will be small. One intuitively expects stereoselectivity to be more pronounced when these association constants are large because the subtle structural differences possessed by enantiomers can be expressed as the reaction partners make more intimate contact. The marked dependence of stereoselectivity on solvent[9,11] for several systems supports this idea as does the fact that the majority of reactions of coordination compounds for which stereoselective electron transfer has been detected involve partners of opposite charge.[11] While less data is available for metalloprotein-small molecule reactions, this appears to be true for metalloprotein reactions as well.[8-10] For the eight metalloprotein reactions displaying stereoselective electron transfer with small complexes reported to date, five have oppositely charged reactants, two involve a neutral complex, and one involves reactants of like charge. Of course, for metalloprotein reactions, to the extent that charge is important, it is reactive site ("local") charge rather than net charge that needs to be considered. However, several reactions have been examined where stereoselectivity is detected for partners

(27) Marcus, R. A. *Discuss. Faraday Soc.* **1960**, *29*, 21. Marcus, R. A. *Annu. Rev. Phys. Chem.* **1964**, *15*, 155.
(28) Wherland, S.; Gray, H. B. *Biological Aspects of Inorganic Chemistry*; Addison, A. W., Cullen, W. R., Dolphin, D., James, B. R., Eds.; Wiley: New York, 1977; p 289.
(29) Osvath, P.; Sargeson, A. M. Unpublished results.

of like charge or for one uncharged partner. For these systems hydrogen bonding and nonelectrostatic forces (hydrophobic interactions) may well play a crucial role in bestowing selectivity on the favored enantiomer in spite of the fact that the association of partners may be too weak to detect kinetically.

There is a large body of experimental work[1d,12a,e,14-18] which supports the view that electron transfer occurs primarily at two sites on the surface of plant plastocyanins. Electron transfer at the adjacent hydrophobic site ("north site") near His-87 where the copper atom is closest to solvent (ca. 6 Å) is argued to be favored for neutral, anionic, and hydrophobic partners and is generally viewed as being a more favored, and more direct pathway for electron transfer than the remote site ("acidic patch" or "east site"). The remote site, while involving electron transfer over a much greater distance, ca. 10–15 Å, is competitive with adjacent electron transfer when cationic, hydrophilic reagents are used which can make use of specific, efficient, superexchange ("through bond") pathways that involve association of the redox partner at the negatively charged surface focused around Tyr-83. Recent calculations compare the efficiency of electron transfer from these two sites and via various competing paths.[18] The saturation kinetics illustrated by Figure 2, inhibition of this redox by $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$, Figure 4, and the inhibition of the reaction by protein protonation, Figure 5, are evidence of the association of pc(II) with $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ prior to electron transfer and clearly indicative of a reaction that is occurring primarily at the remote site. Given the high charge and hydrophilic nature of the $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ reductant, this is not surprising. For all of the small complexes used to reduce pc(II) this equilibrium constant for prior association of reactants ($K_{Co(II)}$ = 1.5×10^3 M^{-1}) is the largest reported to date and gives nearly total ($k'_{et}K_{Co(II)}/k''_{et} \sim 0.93$) dominance of the remote site over the adjacent site in electron transfer. Electron transfer from this remote site would occur with weak overlap and be expected to be very sensitive to the orientation of the electron donor and the immediate acceptor along the through-bond pathway. The inhibition by $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ is striking ($K_I = 3.9 \times 10^4$ M^{-1}) and further illustrates the effective match of complementary forces that bring protein and complex together, both in the reactant ion pair and in the protein–inhibitor ion pair. The pH-dependence ($k_H \sim 0$) also supports a nearly complete remote site redox mechanism for this complex as described below. The observed saturation kinetics, nearly total inhibition by cationic inhibitor, and pH dependence also resemble the reactivity profile of pc(II)'s physiological partner, cytochrome *f*, more closely than other small redox partners which are often significantly partitioned between the remote and adjacent sites, display only weak or no association with pc(II), or display significant reactivity with protonated pc(II). These observations lead us to conclude that $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ substantially mimics cytochrome *f* in several key features of its reactivity and accomplishes redox almost exclusively via intramolecular electron transfer from the remote site from within a relatively tightly formed protein–complex ion pair. This conclusion is important to the analysis of the observed stereoselectivity which follows.

Figure 6 depicts in a simple way the complementary structural features of a possible plastocyanin–$[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ adduct. No attempt was made at an electrostatic minimization of this structure. The scaled figure simply shows the approximate relative geometric and electrostatic features of the two molecules that may account for the relatively strong association at the remote site responsible for the kinetic features reported here.

The dependence of this reaction on pH is striking. It is well-known[12a] that pc(I) undergoes both active site protonation and remote site protonation and that active site protonation results in a coordination change[13] that renders the protein redox inactive. However, pc(II) protonation neither alters the coordination geometry nor renders the protein redox inactive in general. For

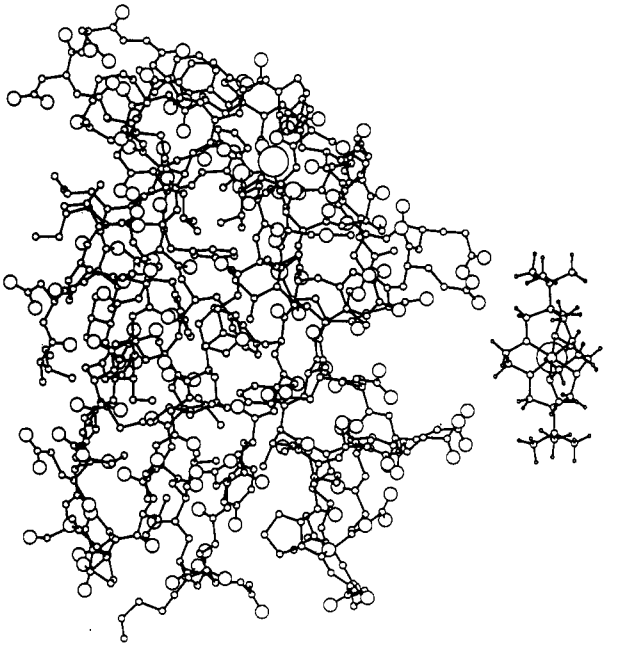

Figure 6. Computer-generated view of a possible complex formed between plastocyanin and manually "docked" $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$.

example, the $[Fe(CN)_6]^{4-}$ reduction of pc(II) is insensitive[12a] to changes in pH from 4.5 to 7.5 and the reduction by $[Ru(NH_3)_5(py)]^{2+}$ is pH dependent ($pK_a = 5.05$) with the protonated protein being about half as reactive as its conjugate base,[30] while reduction of plastocyanin by its natural partner, cytochrome *f*, is very sensitive to pH ($pK_a = 5.07$) with protonated protein having 10% or less of the reactivity of the conjugate base.[14h] These observations are reconciled by the conclusion that the reducing agents are being partitioned differently between the adjacent and remote redox sites on pc(II) and that only remote site redox is pH dependent, with $[Fe(CN)_6]^{4-}$ utilizing exclusively the adjacent site, $[Ru(NH_3)_5(py)]^{2+}$ utilizing both sites, and cytochrome *f* utilizing the remote site almost exclusively. Consistent with this, is our conclusion that $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ is using the remote site nearly exclusively for redox since we observe a pH-dependent reduction ($pK_a = 5.3$) with pc(II) reacting at a rate at pH 4.0 that is only 5% of the rate at neutral pH. The protein denatures at low pH, and it was not possible to get data at a pH lower than 4.0. Therefore it is difficult to know whether this represents residual reactivity of the protonated protein or not. However, it does not change our conclusion that the electron transfer in this system is occuring almost exclusively at the remote site. It is intriguing that remote site protonation can have such a dramatic effect. One might expect that protonation would decrease binding of cationic partners at the remote site but also might expect that redox agents such as the complex studied here or cytochrome *f* would still be able to undergo electron transfer at the remote site, albeit with somewhat lower efficiency. It would be interesting to know if the dramatic decrease in redox reactivity that accompanies remote site protonation results from destabilizing of the pc(II)–complex or pc(II)–cytochrome *f* ion pairs or by making inaccessible a crucial surface residue at the remote site that prevents any significant superexchange electron transfer from occurring. At the adjacent site the relative ineffectiveness of these two reagents may be because neither can gain reasonable access to the His-87 which is in a hydrophobic pocket. The energy costs of shedding solvent by the hydrophilic complex and the highly charged active site of the cytochrome in gaining such access may be too high.

In spite of efforts to observe chiral discrimination by a metalloprotein for enantiomeric coordination compounds, un-

(30) McGinnis, J.; Sinclair, J. D.; Sykes, A. G. *J. Chem. Soc., Dalton Trans.* **1986**, 2011.

equivocal demonstration of this effect has only recently been obtained and has thus far been observed for only a few reactions.[8-11] Moreover, the extent of the selectivity has been relatively small, typically about 10% or less preference of protein for one enantiomer over the other. The largest stereoselectivity reported prior to this work has been that of Bernauer and co-workers for the reduction of spinach plastocyanin by the iron(II) complex Fe(alamp) (alamp^{2-} is pyridine-2,6-diyl-bis(4-(3-aza-2-methylbutanoate))) for which they observe $k_\Delta/k_\Lambda = 1.6$ at 25 °C.[8a] They conclude that this uncharged iron complex is reacting at the adjacent site on plastocyanin. However, this iron complex is labile, and no firm conclusion can be drawn whether the origin of this stereoselectivity is an outer-sphere or an inner-sphere reaction. Our results show a stereoselectivity of similar magnitude ($k_\Lambda/k_\Delta = 1.7$ at 25°) and this applies to electron transfer at the remote site as already discussed and must originate from an outer-sphere process. However no meaning can be attached to the Λ/Δ ratio in terms of specific binding since the reagents are stereochemically so different in the two examples. While it is interesting that this protein can apparently express selectivity of a similar magnitude at two different sites on its surface, at this time we regard this as coincidental and now turn to a discussion of the remote site stereoselectivity of our reaction.

The ability to isolate the remote site as the location of redox in this system is important in considering the possible origin of the observed effects. Given the strong association of the complex with protein, one might anticipate that the overall selectivity arises from a preferential association of the Λ isomer during ion pair formation. Indeed, in numerous systems it has been concluded that the origin of the selectivity is preferential ion pairing of one isomer.[11] If preferential ion pairing is the origin of the stereoselectivity, one might also expect to see stereoselective inhibition of the reaction by the $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ product since it presumably is binding at the same protein surface site and by a similar mechanism as the reductant $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$. While we do observe dramatic inhibition by $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ as already described, the inhibition is not stereoselective. Another means of detecting stereoselectivity in the ion pairing process is by equilibrium dialysis. In experiments to detect stereoselective association of $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ with pc(II), an excess of racemic $[Co((N(CH_3)_3)_2\text{-sar})]^{5+}$ was allowed to equilibrate across a dialysis membrane and the dialyzing solution was analyzed for optical activity following equilibration. In these experiments, the expected activity can be calculated if the origin of the observed selectivity is the ion pairing process. What we observe is a very small (slightly above the background noise level) activity in the dialyzed solution which at most could account for a small (ca. 20% or less) part of the observed overall stereoselectivity. Moreover, the rate ratio of k_Λ/k_Δ does not change appreciably as the ionic strength is varied from 0.05 to 0.50 M. In particular, at $I = 0.50$ M the overall rate constant for electron transfer decreases by about a factor of 6 from that observed at $I = 0.050$ M, and the rate saturation observed at the lower strength is no longer detectable, suggesting that the association of complex with protein is much weaker at the higher ionic strength as would be expected. Collectively these observations bring us to conclude that the ion pairing process, while very strong, is likely to originate primarily from nonstereospecific electrostatic forces and is not the source of the overall stereoselectivity in the redox process. This behavior is analogous to what has been described by McLendon[31] as the "velcro" motif for protein binding. In this picture the binding patch on the protein's surface is rather large and the association of a partner can occur with nearly equal effectiveness in several orientations and "roll" around on the surface. In this sense the electrostatic forces guide the long-range recognition of partners, but other more subtle structural and chemical features control reactivity within the ion-pair.

Given the lack of stereoselective association for this reaction, we conclude that the electron transfer stereoselectivity is due to different rate constants for intramolecular electron transfer from within the two chiral ions pairs. Given the large electrostatic forces that apparently bring the reacting partners together, it does not seem likely that the donor–acceptor distance in the two ion pairs is greatly different. Moreover, current data indicate that complex association with plastocyanin does not significantly modify its redox potential.[12a,e] Consequently, we believe the most likely origin of the stereoselectivity is a significant difference in orientation of Λ- vs Δ-$[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ within the protein–complex ion pair. The conclusion that almost a factor of 2 in rate contants is attainable for the subtle orientational differences from within apparently equally stable ion pairs at probably nearly identical distances from the protein copper reinforces the emerging picture that the orientation of redox partners is crucial in long range intramolecular electron transfer in metalloproteins.[12h,18,21,31] These orientational effects may be especially important when electrons are being transferred over long distances by superexchange mechanisms because the orbital overlap integrals can be very sensitive to orientation, especially if changes in orientation result in a requirement of even small "through space" electron transfer.

While tight association of the reaction partner in this reaction is not the source of the stereoselectivity, it is important to the effectiveness of the remote site redox reactivity. For this site to be competitive with adjacent site redox it is necessary to minimize the involvement of solvent and initiate favorable overlap of the donor with atoms along the superexchange pathway.[18] Tight association might do this by "squeezing" solvent out of a critical interfacial region.

It is difficult to make a detailed interpretation of the role that subtle structural differences in the cage complexes have on the stereoselectivity of electron transfer with plastocyanin. The absence of detectable stereoselectivity of the $[Co((NH_2)_2\text{-sar})]^{2+}$ reduction of plastocyanin as compared to the selectivity observed in the $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ reaction may be due to the more intimate association between the protein and the latter complex that results from the higher complex charge, the steric bulk of the methyl groups versus hydrogen atoms on the caps, or some combination of these differences. Moreover, $[Co((N(CH_3)_3)_2\text{-sar})]^{4+}$ is known[19] to undergo a conformational change on electron transfer in solution from lel_3 in the Co(II) complex to ob_3 in the Co(III) complex while $[Co((NH_2)_2\text{-sar})]^{3+/2+}$ remains lel_3 in both oxidation states in solution (*lel* and *ob* refer to the orientation of the C–C axes of the 1,2-ethanediamine fragments relative to the C_3 axis of the complex ion, *i.e.*, parallel or oblique). Sufficient information is not available at this time to draw detailed conclusions about the influence of these features on electron transfer stereoselectivity other than to say that a conformational change accompanying the electron transfer must be somewhat more inhibiting than the situation where no such change occurs.

A number of studies have been directed at assessing the rate of intramolecular electron transfer from the remote site to the copper center in plastocyanins. Jackman et al.[32] covalently attached $Ru(NH_3)_5$– to histidine residues near this site on two species of plastocyanins and found the intramolecular rate of electron transfer from within these molecules to be immeasurably small (for *Scenedesmus obliquus* 0.04 ± 0.22 s^{-1}) in contrast to the result that electron transfer from the remote site from within the electrostatic ion pair for the related imidazole complex of ruthenium ($[Ru(NH_3)_5IM]^{2+}$) was deduced to occur with a relatively large rate constant ($>5 \times 10^3$ s^{-1}). Kostic and coworkers[20] have recently examined electron transfer between

(31) McLendon, G. Electron-Transfer Reactions in Metalloproteins *Met. Ions Biol. Syst.* **1991**, *27*, 183.

(32) Jackman, M. P.; McGinnis, J.; Powls, R.; Salmon, G. A.; Sykes, A. G. *J. Am. Chem. Soc.* **1988**, *110*, 5880.

plastocyanin and cytochrome *c* and Zn-substituted cytochrome *c* by observing electron transfer for both systems from within the electrostatic ion pairs and from within the covalently attached plastocyanin–cytochrome *c* adducts. In these ion pairs, the high negative charge near the remote site on plastocyanin is apparently aligned with the highly positive charged region around the presumed redox active site of the cytochrome and the covalent adducts are locked in this approximate arrangement by covalent attachment. Since they observe much larger rate constants for electron transfer from within the electrostatic complexes relative to the covalently attached molecules, they conclude that the cytochrome in the electrostatic adducts must migrate along plastocyanin's surface to the adjacent site for more rapid electron transfer after initial formation of the ion pair at the remote site. Given our results, which show that significant differences in rate can result from subtle changes in donor–acceptor orientation, and the results of Jackman et al.,[32] which show that great differences in rate can result in remote site reactivity when the electron donor is covalently attached, we believe that another explanation for the plastocyanin–cytochrome *c* results is worth considering. It seems possible that the much greater rates of electron transfer for the electrostatic complexes of plastocyanin and cytochrome *c* might arise from a more subtle rearrangement from within the ion pair at the remote site rather than from the relatively longer-distance migration (against a large electrostatic gradient) of the cytochrome in the electrostatic adducts to the adjacent site on plastocyanin as suggested. Such a subtle rearrangement may not be available to the more rigidly held cytochrome in the covalent adducts. Or the covalent adduct may perturb the protein structure enough to inhibit electron transfer. Furthermore, if cytochrome *c* in the electrostatic complex reaction finishes by transferring an electron at the adjacent site, it is hard to understand how protein protonation at the remote site can effectively turn off electron transfer since the adjacent site remains redox active over a wide pH range as discussed above.

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Supplementary Material Available: Lists of observed rate contsants, Tables 1 and 2 (3 pages). Ordering information is given on any current masthead page.