the two exchange integrals of similar origine, J_{14} and J_{23}. A difference of about 65 cm^{-1} is observed, which is in good agreement with what might be expected from the literature.[23] Due to the symmetry of the cluster, the values of the two exchange integrals deduced from the calculation cannot be unambiguously attributed to a specific pair of copper atoms. The higher value is probably associated with the pair that has the most acute bridge angle Cu1-Cu4 (Cu1-O5-Cu4 = 119.80° vs. Cu2-O6-Cu3 = 121.68°).[23,24] No definite correlation of the magnetic and structural properties has been found for monohydroxy-bridged dicopper complexes. For tetragonal complexes exchange interactions span the range -32 to -500 cm^{-1} while the corresponding CuO(H)Cu angles vary from 110 to 147°.[33] In the present study, intermediate values are obtained for both parameters. It is interesting to note that all but one[33c] data conform to the general trend that the more obtuse the CuO(H)Cu angle, the stronger the antiferromagnetic coupling.[23,24]

The weak interaction of Cu5 with its four neighbors is probably the most surprising result. Consideration of the orientation of the magnetic orbitals ($d_{x^2-y^2}$ for all coppers) would predict that some overalap is possible on the catechols oxygens. Moreover, the magnitude of the Cu5-O-Cu angles seems to warrant that a significant interaction may be operative. We think that the nonplanarity of the system is responsible for the reduced coupling of Cu5. This is better illustrated in Figure 3, which presents only the five copper atoms with their immediate O and N environment. It clearly appears that the coordination plane of Cu5 is not coplanar with the one of any other copper atom. Actually, the dihedral angles between these planes average to 129°. This situation is analogous to the one observed very recently in a tetranuclear copper catecholate.[15] In the latter case, no coupling was present inside a pair of copper atoms despite the fact that a significant overlap of the magnetic orbitals was expected on a bridging oxygen. The absence of coupling was attributed to the fact that the axis of the magnetic orbital (d_{z^2}) of one copper made an angle of 124° with the basal plane of the other. This situation is reminiscent of the folding observed in some dihydroxy-bridged dicopper complexes,[34a] which has been shown theoretically to produce smaller singlet-triplet splittings as a result of decreasing both the ferromagnetic and the antiferromagnetic contributions.[34b]

Conclusions

This publication reports the structural and magnetic characterization of a novel pentacopper(II) cluster built around two molecules of a trinucleating catecholate ligand. To our knowledge this is the second example of a catecholate bonding three metal atoms. We recently found the first one in a compound that resulted from the dimerization of a dicopper complex of a binucleating catecholate.[15] The trinucleating character of the present ligand is obviously the driving force of the molecular assembly and it can probably be used with other metals to obtain clusters of higher nuclearity. Work is currently in progress along these lines.

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Supplementary Material Available: Listings of general temperature factors (Table III) and calculated hydrogen atom positions (Table IV) (3 pages); a listing of structure factor amplitudes (Table V) (24 pages). Ordering information is given on any current masthead page.

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Electron Transfer from Cytochrome *c* to Tris(1,10-phenanthroline)cobalt(III) and Its Electrically Neutral Sulfonated Analogue as a Probe for Direct, Image, and Nonlocal Electrostatic Interactions at the Protein Surface

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We have investigated the kinetics of electron transfer between cytochrome c^{II} and the triply charged and electrically neutral $[Co(phen)_3]^{3+}$ and $[Co(phen\text{-}SO_3^-)_3]$ complexes in aqueous Tris, sodium *p*-toluenesulfonate (NaTS), and chloride media at pH 7.00 (phen = 1,10-phenanthroline). The rate constant, activation enthalpy ΔH_A, and apparent activation entropy ΔS_A^{app} of the former in 0.05 mol dm^{-3} Tris/NaTS are 2.92×10^3 dm^3 mol^{-1} s^{-1}, 49 ± 2 kJ, and -15 ± 5 J K^{-1}, respectively, and of the latter are 2.35×10^5 dm^3 mol^{-1} s^{-1}, 30 ± 2 kJ, and -40 ± 5 J K^{-1}, respectively. The rate constants are smaller in media containing protein-binding ions (chloride or phosphate), while ΔH_A is little affected. The reaction of $[Co(phen\text{-}SO_3^-)_3]$ is independent of the ionic strength μ, and thus this species behaves as a neutral molecule, while the reaction of $[Co(phen)_3]^{3+}$ follows a Debye-Hückel law in the μ range 0.1-0.2 mol dm^{-3}. The charge product is 3.2 for nonbinding ions and is lower for binding anions. The data have been analyzed by means of a model in which Co(III) is represented as a conducting sphere embedded in a dielectric and cyt *c* is another dielectric of low dielectric constant. The rate parameters can be understood in terms of reorganization in both dielectric regions and of image force and nonlocal electrostatic work terms. Small electronic transmission coefficients, around 10^{-5}, emerge when the activation entropies are calculated on the basis of the model.

Introduction

We have recently reported a search for electron-transfer systems that might both belong to the diabatic limit and be well-represented by the simplest conceivable models for the reactant ions and solvent[1] resting on a conducting-sphere approximation for the ions interacting through coulombic terms that are screened by a ma-

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croscopic structureless solvent dielectric.[2]

The reactions

$$[Co(terpy)_2]^{2+} + [Co(phen)_3]^{3+} \rightarrow [Co(terpy)_2]^{3+} + [Co(phen)_3]^{2+} \quad (1)$$

$$[Co(terpy)_2]^{2+} + [Co(phen\text{-}SO_3^-)_3] \rightarrow [Co(terpy)_2]^{3+} + [Co(phen\text{-}SO_3^-)_3]^- \quad (2)$$

(terpy = terpyridine, phen = 1,10-phenanthroline, and phen-SO_3^- = 5-sulfonated phen) in aqueous sodium *p*-toluenesulfonate (NaTS) solutions were found to conform with these conditions. Both rate constants and activation parameters are consistent with single values of the reorganization free energies, with electronic transmission coefficients in the range (0.5–2) × 10^{-5}, and with solvent reorganization free energies and work terms in accordance with calculated values for spherical ions and the bulk dielectric constant and its temperature coefficient. The last item is notable since the temperature variation of the dielectric constant is the only significant source of activation entropy in this model.

Neither the analogous bpy complexes (bpy = 2,2′-bipyridine) nor any of these reactions in ethylene glycol/water or glycerol/water solutions correspond to the simplest solute and solvent representations. These reactions, however, do conform with a more detailed representation of the medium, resting on nonlocal dielectric theory. The crucial feature of this approach is that it incorporates, within the continuum formalism, solvent structural elements in the form of a correlation of the solvent dipolar reorientation within a spatial range corresponding to the local structure of the solvent[3] (1 or 2 molecular diameters).

The adequacy of the simplest representation for the aqueous phen/phen-SO_3^- system suggests that this might be a suitable "probe system" for diabatic electron transfer in other contexts. We report here data for electron transfer from cytochrome *c* (cyt *c*) to the two Co(III) complexes. Both these processes and electron transfer involving several other small metalloproteins[4-6] exhibit substantial activation energies. This observation, as well as the protein nature, is incompatible with the conducting-sphere and structureless solvent models. We shall consider the data in terms of another model that incorporates the protein structure of cyt *c* as an infinitely large sphere of low dielectric constant close to a conducting sphere that represents the Co(III) complex. Within this model the reorganization free energy of both aqueous and protein regions and the work terms can be calculated and provide a consistent frame for all the data. However, this can only be achieved by invoking both image[7] and nonlocal[3] work terms. Since the model provides the activation entropy, the electronic transmission coefficient can also be obtained. Very notably, it turns out to be small and close to the value for the reactions of the two Co(III) complexes with $[Co(terpy)_2]^{2+}$.

Experimental Section

Materials. AnalaR grade reagents and Millipore water (four-housing Milli-Q system) were used throughout. $[Co(phen)_3](ClO_4)_3$ was prepared as in ref 8 and $[Co(phen\text{-}SO_3^-)_3]\cdot 10H_2O$ as in ref 1. The products were checked spectrophotometrically, and the sulfonated complex was also examined by elemental analysis for C, N, H, and S.[1]

Cytochrome *c* was from horse heart, Sigma type VI. New samples were used, as supplied. Even the best commercial products are, however, reported to contain deamidated forms.[9] The data were therefore checked by samples that had been purified on a Whatman CM52 cellulose coloumn as in ref 9. Within experimental accuracy both rate constants and activation energies and their ionic strength dependence for the purified samples were found to coincide with those of unpurified, but freshly

Table I. Examples of Rate Constants for the Reaction of $[Co(phen)_3]^{3+}$ with cyt *c* at Different Temperatures[a]

T, K	$10^{-3}k$, dm³ mol⁻¹ s⁻¹	*T*, K	$10^{-3}k$, dm³ mol⁻¹ s⁻¹
291.7	1.95 ± 0.01	306.7	5.45 ± 0.04
298.2	2.74 ± 0.02	311.2	7.07 ± 0.04
303.2	3.95 ± 0.03		

[a] Conditions: ionic strength 0.102 mol dm⁻³ (0.05 mol dm⁻³ Tris + HCl (pH 7.00) + 0.05 mol dm⁻³ NaTS); [Co(III)] = 1.5 × 10^{-3} mol dm⁻³.

Table II. Rate Constants at 25 °C (dm³ mol⁻¹ s⁻¹), Activation Enthalpies (kJ), and Apparent Activation Entropies (J K⁻¹) for Reactions of $[Co(phen)_3]^{3+}$ with cyt *c* in Different Ionic Media[a]

medium	ionic strength	$10^{-3}k$	ΔH_A	ΔS_A^{app}
0.05 M Tris[b]	0.093	2.92 ± 0.02	49 ± 2	−15 ± 5
+HCl	0.140	5.06 ± 0.04	46 ± 2	−20 ± 5
+NaTS	0.188	6.63 ± 0.04	49 ± 2	−8 ± 5
0.05 M Tris[c]	0.053	0.96 ± 0.01	48 ± 2	−25 ± 5
+HCl	0.149	1.88 ± 0.02		
+NaCl				
0.2 M Tris[d]	0.143	1.75 ± 0.02		
+HCl				
0.05 M phosphate[e]	0.084	1.53 ± 0.02		
+NaCl	0.124	1.83 ± 0.02		
	0.211	2.37 ± 0.02		

[a] pH 7.00 with HCl; $[[Co(phen)_3](ClO_4)_3]$ = 1.5 × 10^{-3} mol dm⁻³. [b] 0.05 M Tris, 1 M HCl for pH adjustment, and ionic strength variation with NaTS. [c] As for *b*, but ionic strength variation with NaCl. [d] 0.2 M Tris, 1 M HCl for pH adjustment. [e] 9.3 × 10^{-3} M KH_2PO_4, 1.36 × 10^{-2} M Na_2HPO_4 at pH 7.00, and variable concentrations of NaCl.

Table III. Examples of Rate Constants for the Reaction of $[Co(phen\text{-}SO_3^-)_3]$ with cyt *c* at Different Temperatures[a]

T, K	$10^{-5}k$, dm³ mol⁻¹ s⁻¹	*T*, K	$10^{-5}k$, dm³ mol⁻¹ s⁻¹
294.4	2.05 ± 0.03	307.0	3.49 ± 0.04
299.2	2.39 ± 0.03	310.6	4.06 ± 0.05
303.2	2.93 ± 0.04		

[a] Conditions: ionic strength 0.0927 mol dm⁻³ (0.05 mol dm⁻³ Tris + HCl + 0.05 mol dm⁻³ NaTS); [Co(III)] = 5.0 × 10^{-4} mol dm⁻³.

Table IV. Rate Constants at 25 °C (dm³ mol⁻¹ s⁻¹), Activation Enthalpies (kJ), and Apparent Activation Entropies (J K⁻¹) for Reactions of $[Co(phen\text{-}SO_3^-)_3]$ with cyt *c* in Different Ionic Media[a]

medium[b]	ionic strength	$10^{-5}k$	ΔH_A	ΔS_A^{app}
0.05 M Tris	0.093	2.35 ± 0.03	30 ± 2	−40 ± 5
+HCl	0.140	2.22 ± 0.03	29 ± 2	−45 ± 5
+NaTS	0.188	2.48 ± 0.03	30 ± 2	−40 ± 5
0.05 M Tris	0.047	1.93 ± 0.03	27 ± 2	−55 ± 5
+HCl	0.156	1.79 ± 0.02	30 ± 2	−40 ± 5
0.05 M phosphate	0.081	1.82 ± 0.03	31 ± 2	−40 ± 5
+NaCl	0.199	1.76 ± 0.02	32 ± 2	−35 ± 5

[a] Conditions: pH 7.00; [Co(III)] = 5.0 × 10^{-4} mol dm⁻³. [b] Compositions as in Table II.

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Table V. Rate Constants at 25 °C (dm^3 mol^{-1} s^{-1}), Activation Enthalpies (kJ), and Apparent Activation Entropies (J K^{-1}) for Oxidation of $[Co(terpy)_2]^{2+}$ by $[Co(phen)_3]^{3+}$ and $[Co(phen\text{-}SO_3^-)_3]$[a]

complex	k	ΔH_A	ΔS_A^{app}
phen	1.14×10^2	27 ± 2	−115 ± 10
phen-SO_3^-	1.20×10^4	20 ± 2	−100 ± 10

[a] No electrolyte was added. Values are from ref 1.

Table VI. Standard Redox Potentials (V) and Their Formal Entropies (J K^{-1}) for the Half-Reactions in Eq 5 (0.05 mol dm^{-3} NaTS) and for cyt c

complex	$E_{1/2}$	$S°_{1/2}$
phen[a]	0.399	103 ± 5
phen-SO_3^-[a]	0.498	63 ± 5
cyt c[b]	0.261	−150 (−126)

[a] From ref 1. [b] From ref 11. The value in parentheses is for 0.05 mol dm^{-3} phosphate.[12]

supplied, samples. Aged samples gave different values of the rate parameters, but values coinciding with those of the fresh or purified samples could be obtained after purifying the aged samples by the method of ref 9.

Kinetics. The reactions were followed by stopped-flow spectrophotometry using a Durrum–Gibson instrument fitted with Teflon drive syringes, a Beckman DU monochromator, and data collecting equipment for direct data processing. The processes were monitored at the wavelength 550 nm, where the absorption is dominated by cyt c^{II} and $\Delta\epsilon$ reported to be 18 700 dm^3 mol^{-1} cm^{-1}.[10] Solutions of cyt c^{III} were prepared by weight, the appropriate buffer and salt were added, and cyt c was reduced by addition of slightly less than the equivalent amount of ascorbic acid. cyt c^{II} concentrations were about 2×10^{-5} mol dm^{-3} and those of the Co(III) complexes in the range $(1–2) \times 10^{-3}$ mol dm^{-3}. The absorbance–time data could be fitted to the pseudo-first-order equation

$$\ln [(D - D_\infty)/(D_0 - D_\infty)] = k[\text{Co(III)}]_t \qquad (3)$$

where D, D_0, and D_∞ are the absorbances at time t, zero, and infinity, respectively, [Co(III)] is the concentration of the Co(III) complex, and k is the second-order rate constant. Reported rate constants are the average of three determinations, and accurate fits of this equation were always obtained for over 90% of the reaction.

Results

Tables I–IV summarize rate constants and activation parameters for the reactions of $[Co(phen)_3]^{3+}$ and $[Co(phen\text{-}SO_3^-)_3]$ with cyt c in different ionic media, for pH 7.00. The activation parameters normally refer to the temperature range 20–40 °C and were calculated from the equation

$$k = (k_B T/h) \exp(\Delta S_A^{app}/k_B) \exp(-\Delta H_A/k_B T) \qquad (4)$$

where k_B is Boltzmann's constant, T is the temperature, h is Planck's constant, and ΔH_A and ΔS_A^{app} are the activation enthalpy and apparent entropy, respectively. For comparison, the rate parameters for the reactions of $[Co(terpy)_2]^{2+}$ are shown in Table V.[1] Table VI summarizes the standard redox potentials $E_{1/2}$ (V) and $\Delta S°_{1/2}$ for the half-reaction Co(III) + $e^- \rightleftarrows$ Co(II), reported previously.[1]

The redox potential for the cyt $c^{III/II}$ couple has been obtained by potentiometry,[11] by spectrophotometric investigation of the cyt $c^{II}/[Co(phen)_3]^{3+}$ equilibrium, and by cyclic voltammetry.[13] The standard redox potential is 0.261 V,[11] but this value varies with added ion concentration and in opposite directions for binding and nonbinding ions. The effects on the activation parameters are insignificant, even though the overall charges on cyt c are quite different in the two cases,[11,14] but the rate constants are smaller

Table VII. Reaction Free Energies (kJ mol^{-1}), Enthalpies (kJ mol^{-1}), and Entropies (J K^{-1}) for the Oxidation of cyt c^{II} and $[Co(terpy)_2]^{2+}$

complex	ΔG_0	ΔH_0	ΔS_0
phen[a]	−13.3	60 (55)	250 ± 10 (225)
phen-SO_3^-[a]	−22.8	40 (35)	210 ± 10 (190)
phen[b]	−10.9	−5	19 ± 6
phen-SO_3^-[b]	−20.4	−26	−21 ± 5

[a] Oxidation of cyt c^{II}, from the present work. [b] Oxidation of $[Co(terpy)_2]^{2+}$, from ref 1. Values in parentheses are for 0.05 mol dm^{-3} phosphate.[12]

for the binding ions chloride and phosphate than for the (presumably nonbinding) TS^- and protonated Tris ions. This difference is larger for the charged $[Co(phen)_3]^{3+}$ than for the neutral $[Co(phen\text{-}SO_3^-)_3]$ complex. In the following we shall therefore use the value 0.261 V and compare rate constants extrapolated to zero ionic strength for the Tris/NaTS solutions by the observed Debye–Hückel dependence (cf. below). These values are 3.7×10^2 dm^3 mol^{-1} s^{-1} for $[Co(phen)_3]^{3+}$, for both Tris and NaTS solutions, and 2.35×10^5 dm^3 mol^{-1} s^{-1} for $[Co(phen\text{-}SO_3^-)_3]$.

The formal entropy of the cyt $c^{III/II}$ half-reaction has been determined by the T dependence of the redox potential[11] and by calorimetry of the $[Co(phen)_3]^{3+}$/cyt c^{II} reaction.[12] At low ionic strength $\Delta S°_{1/2}$ is −150 ± 10 J K^{-1} but is less negative at larger ionic strengths.[11,12] Table VII finally summarizes the reaction free energies, enthalpies, and entropies obtained by combining values for the separate redox couples.

The most important results to be noted from tables I–VII are as follows:

(A) The ratio between the rate constants of the substituted and unsubstituted complexes for reactions with cyt c is about 600, which is larger by a factor of 6 than for the reactions with $[Co(terpy)_2]^{2+}$. The higher rates for the sulfonated complex are due to the higher standard redox potentials of this couple and the absence of long-range electrostatic work terms. The rate constants and activation enthalpy for the $[Co(phen)_3]^{3+}$/cyt c^{II} system agrees well with those of previous reports.

(B) The ionic strength dependence for the unsubstituted Co(III) complex follows a Debye–Hückel law with an apparent charge product of 3.2. Ionic media with binding anions give lower Debye–Hückel slopes. There is no ionic strength dependence for the sulfonated complex, which in this respect behaves as a neutral molecule with no localized sulfonate group charges. All these features are closely similar to the behavior of the reactions with $[Co(terpy)_2]^{2+}$.

(C) The activation parameters are quite different for the processes involving $[Co(terpy)_2]^{2+}$ and cyt c, ΔH_A being much larger and ΔS_A^{app} much less negative in the latter case. A similar pattern is seen for oxidations of other cytochromes,[4b,6a] for the blue copper proteins azurin and plastocyanin[5] (while stellacyanin follows a different pattern of reaction[5]), and for iron–sulfur proteins.[5] In contrast, reduction of corresponding oxidizing forms of small metalloproteins has commonly small activation enthalpies and large negative apparent activation entropies.[15–19]

What is also notable from the data in the tables is that activation enthalpies of both the charged and neutral Co(III) complexes are large and that the difference between them is about 3 times the difference for the $[Co(terpy)_2]^{2+}$ reactions. An expected "simple" electron-transfer mechanism for the cyt c reactions should leave

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the same or smaller differences. Oxidant-induced conformational changes or work terms and reorganization energies that incorporate the macromolecular nature of the heme surroundings are possible reasons for the different reaction patterns, and we shall investigate the latter possibility in the following section.

Reorganization and Work Terms Involving Dielectric Image Forces

The notable differences between the reactions of cyt c and those of $[Co(terpy)_2]^{2+}$ are (a) the much larger activation enthalpies for both Co(III) complexes, (b) the much less negative apparent activation entropies, and (c) the much larger activation enthalpy difference between the charged and the neutral Co(III) complex. The last observation suggests that the electrostatic work terms are of a different nature in the $[Co(terpy)_2]^{2+}/[Co(phen)_3]^{3+}$ and cyt $c^{II}/[Co(phen)_3]^{3+}$ reactions.

We approach these points by means of recent reformulations of electron-transfer theory as have been reported in ref 20 in particular and by invoking a specific model for the solvent and protein reorganization contributions to the activation free energy. The elements of electron-transfer theory needed in our analysis are summarized in part A of the Appendix.[20-23] This formalism is rather general and rests only on linear molecular and environmental responses to the field changes associated with electron transfer (the harmonic approximation).

In designing a suitable simple specific model, we note first that conducting-sphere reactants and macroscopically screened work terms, found to be satisfactory for the reactions with $[Co(terpy)_2]^{2+}$, are obviously inadequate for cyt c, in view of both the size and protein structure of this molecule and the numerical implications of such a model. We shall use another representation that, in the simplest possible fashion, incorporates both the protein and the aqueous environments, namely a charged conducting sphere close to a dielectric that represents cyt c. Onto the dielectric surface are stuck a number of fixed surface charges, simulating charged amino acids. In order to preserve "transparency" of the various free energy terms, we shall regard the radius of cyt c as much larger than that of the Co(III) complexes, so that the dielectric surface is essentially planar. In reality the cyt c radius is only 2 or 3 times that of the Co(III) complexes, but this will not affect our conclusions. Also, for the charged Co(III) complex it appears that the dielectric regions must be regarded as nonlocal,[3] this being at the expense of an increased number of little-known parameters. Rather than being an accurate reproduction of reality, this crude model is to be regarded as the simplest possible explicit incorporation of the protein part. The parameters of the model, extracted from the data, are then indicative as to real nuclear rearrangements in the protein and aqueous parts.

We consider first the overall electrically neutral phen-SO_3^- complex. The negatively charged sulfonate groups are localized on the surface of the Co(III) complex and in principle are available for "specific" interactions with positively charged surface groups on cyt c. However, if this would lead to a rigidly oriented location of Co(III), one of the $-SO_3^-$ groups would point away from the cyt c heme group. Each positive cyt c surface group would furthermore be exposed predominantly to a single negative $-SO_3^-$ charge and the triply charged Co(III) "background". This would almost certainly lead to detectable ionic strength variation of the rate constants. Since this is not observed, we shall assume that the interaction between $[Co(phen\text{-}SO_3^-)_3]$ and cyt c can be ignored and the work terms in eq A2–A6 disregarded. In view of the crudeness of the model, it is also legitimate and greatly facilitates the estimates to regard the heme group and Co(III) complex as located symmetrically with respect to the phase boundary and being represented by spheres of equal radius. The overall environmental protein and outer solvent reorganization free energy and entropy are then given by eq B1 and B2 in part B of the Appendix,[24] by means of which we can estimate the activation parameters.

We can put $l = r_0 = 7$ Å in eq B1 and B2.[1,20] Further, the bulk dielectric constants are $\epsilon_0^w \approx \epsilon_0^p \approx 1.8$, $\epsilon_s^w = 78$, and $\epsilon_s^p \approx 4$.[25,26] Finally, a value of the Co(III) contribution to E_r of about 40 kJ emerges from the analysis in ref 1 and 20. The contribution to E_r from cyt c is small. An upper limit of 27 kJ for the cyt $c^{III/II}$ exchange reaction has been calculated on the basis of the structural differences between crystalline cyt c^{II} and cyt c^{III},[27] but this included all the protein motion, which is represented by the low-permittivity part in our model. A value of less than 5 kJ corresponded to the local modes around the heme group, and we can ignore this part. Inserting the dielectric constants into eq B1 gives $E_s = 42$ kJ, and together with the reaction free energy in Table VII, θ becomes 0.36.

Equation B2 takes the numerical form $-\partial E_s/\partial T \approx (1.2 \times 10^4)\gamma_s^p$ J K^{-1} (γ_s^p in K^{-1}). Insertion of $E_s + E_r$, ΔH_0, and $\partial E_s/\partial T$ into eq A6 gives ΔH_A (kJ) $\approx 30 - 12T\gamma_s^p$, to be compared with the experimental value of 30 kJ. This leaves little room for the γ_s^p-dependent term. Bulk values of γ_s^p for solid polypeptide films are about (2–3) $\times 10^{-3}$ K^{-1},[25,26] i.e. positive (while γ_s^w is negative[28]). This would give $\Delta H_A = 20$–25 kJ, which can perhaps be considered as satisfactory in view of the simplicity of the model.

ΔH_A would also be smaller if the discrete nature of the $-SO_3^-$ groups would lead to an overall repulsive work term. However, this would be at variance with the negligible ionic strength variation of the observed rate constants.

The activation entropy (eq B2) becomes ΔS_A (J K^{-1}) $= 70 - (1.2 \times 10^4)\gamma_s^p$. This should be corrected by the steric "accessibility" of the heme group, which is about 1–3 %,[29] or −30 to −40 J K^{-1}, i.e. $\Delta S_A \approx 35$–45 J K^{-1}. In comparison with the experimental value of $\Delta S_A^{app} = -40$ J K^{-1}, this leaves −75 to −85 J K^{-1} for the quantity $k_B \ln [\kappa_e(\hbar\omega_{eff}/k_BT)]$. By ref 1 and references there, $\hbar\omega_{eff}/k_BT$ can be estimated to about 0.5. The notable result then emerges that $\kappa_e \approx (0.6\text{–}1) \times 10^{-4}$; i.e., with the dielectric model used, the reaction turns out to be strongly diabatic. If the γ_s^p term is ignored, κ_e is lower by 1 order of magnitude. Diabaticity is perhaps to be anticipated in view of the electronic structure of the Co(III)/Co(II) system, the diabatic nature of the reactions with $[Co(terpy)_2]^{2+}$ that emerges on the basis of conducting-sphere and bulk electrostatic models, and the partly buried position of the heme group in cyt c.

The diabaticity conclusion can be checked by comparison with the reaction of the charged $[Co(phen)_3]^{3+}$ complex, which reacts with $[Co(terpy)_2]^{2+}$ by a pattern similar to that of $[Co(phen\text{-}SO_3^-)_3]$. We shall then need a representation for the electrostatic work terms.

The large activation enthalpy difference between the phen and phen-SO_3^- complexes in their reactions with cyt c, compared with that for their reactions with $[Co(terpy)_2]^{2+}$, reflects the larger positive ΔH_0 and the repulsive work term. Combination of the E_s and E_r values already obtained with ΔG_0 (Table VII) gives $\theta = 0.42$ for the phen complex. By the same procedure as above, we obtain ΔH_A (kJ) $\approx (42\text{–}46) - 12T\gamma_s^p$, or if bulk values of γ_s^p for solid polypeptide films are inserted. $\Delta H_A = 35$–40 kJ, i.e. quite close to the experimental value, which includes work terms. However, the work terms are free energy terms, and their form

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must be examined in order to obtain their enthalpy and entropy components.

For details of this investigation we refer to part C of the Appendix and proceed to extraction of the experimental work term information by means of the formalism in this part. Values of the symmetry factors estimated above for the two reactions, ΔG_0 in Table VII, and the assumption that the transmission coefficient ratio coincides approximately with that for the reactions with $[Co(terpy)_2]^{2+}$ (this ratio is not far from unity), first give the work term for the unsubstituted Co(III) complex, $U_r^{us} = 16.8$ kJ. For $r_0 = 7$ Å, this implies that about one-third of the work term (5.8 kJ) originates from the repulsion of $[Co(phen)_3]^{3+}$ by its own image charge and the rest from interaction with protein surface charges and their images. If the distances of these charges from the phase boundary can be regarded as small, the last two groups of terms furthermore approximately coincide, leaving 5.5 kJ to the work term from either group.

From the observed activation enthalpy difference we can next calculate the enthalpy part of the work term, ΔH_A^w, and the parameter combination $\gamma_s^p(\epsilon_s^p/\epsilon_s^w)$. The result is $\Delta H_A^w = 12$ kJ and $\gamma_s^p(\epsilon_s^p/\epsilon_s^w) = 2.7 \times 10^{-3}$ K^{-1}. However, if this is combined with the bulk values of ϵ_s^p and ϵ_s^w, then $\gamma_s^p = 5 \times 10^{-2}$ K^{-1}, i.e. about 1 order of magnitude larger than bulk values for polypeptides[25,26] and with what is compatible with the reorganization free energies. It is not excluded that γ_s^p could be different for the phen and phen-SO_3^- complexes, for example if the charged reactant induces conformational transitions in the protein. $\gamma_s^p = 0.05$ K^{-1} is, on the other hand, far too high and would give ΔH_A more than twice the experimental value when combined with eq B1 and B2.

If we wish to maintain γ_s^p and ϵ_s^p to be consistent with the other data, we are then forced to use γ_s^w and ϵ_s^w different from bulk values. This would be consistent with the concepts of nonlocal dielectric theory[3] as applied for example in our previous papers.[1,30,31] It is possible to extract from the data corresponding nonlocal values of γ_s^w and ϵ_s^w, while γ_s^w and ϵ_s^w cannot be obtained separately. The correlation between γ_s^w and ϵ_s^w consistent with the experimental ΔH_A and rate difference, and for bulk γ_s^p and ϵ_s^p, is

$$\gamma_s^w\ (K^{-1}) \approx 1.0 \times 10^{-3} - 0.85/(\epsilon_s^w)^2 \qquad (5)$$

The value $\epsilon_s^w = 26$ corresponds to the $[Co(phen)_3]^{3+}$ image term reaching the value of U_r^{us} estimated above and is therefore a lower limit. The two limits $\epsilon_s^w = 26$ and 78 give $\gamma_s^w = -2.3 \times 10^{-3}$ and -1.2×10^{-3} K^{-1}, respectively, i.e. larger than the bulk value of -4.6×10^{-3} K^{-1}.[28] Physically this would be caused by finite extension of the solvent structure characterized by the polarization correlation length λ, its temperature derivative (negative), and the short-range dielectric constant ϵ_i and by erosion of this structure with increasing temperature. The nonlocal dielectric permittivity function[3]

$$\epsilon_s^w(r) = \epsilon_s/[1 + (\epsilon_s/\epsilon_i - 1)\exp(-r/\lambda)] \qquad (6)$$

(ϵ_s being the bulk dielectric constant), and the two limits of ϵ_s^w and γ_s^w (for $r = r_0 = 7$ Å) give $\lambda = 3.5$ Å and $d\lambda/dT \approx -0.01$ Å K^{-1} for $\epsilon_s^w = 26$ and $d\lambda/dT \approx -0.1$ Å K^{-1} and $\lambda \to 0$ for $\epsilon_s^w = 78$, i.e. values in line with observations in other contexts.[1,3,31]

This can be compared with estimates from the activation entropy difference (Tables III and IV and eq C5). By insertion of bulk values, the difference is -44 J K^{-1}, while it is about zero and 15 J K^{-1} for $\gamma_s^w = -2.3 \times 10^{-3}$ and -1.2×10^{-3} K^{-1}, respectively. The experimental difference is 20–30 J K^{-1}. The remaining difference is thus quite small when nonlocal parameters are used and consistent with the ratio $\kappa_e^{us}/\kappa_e^{sub} \approx 4$ used above to obtain U_r^{us}, but far too large when bulk dielectric parameters are used.

Inspection shows that $\epsilon_s^w = 50$–60 and $\gamma_s^w = -1.2 \times 10^{-3}$ to -1.3×10^{-3} K^{-1} are the best values consistent with both the experimental activation enthalpy and entropy differences and with the transmission coefficient ratio from ref 1.

We notice finally that interaction solely with surface charges, screened by the bulk dielectric constant of water,[32] is incompatible with the experimental activation and reaction enthalpies. Insertion of these into eq C3 and omission of the image terms leave a positive value of $U_r^{us}(1 + \gamma_s^w T)$ of about 12 kJ, whereas the bulk value of this quantity is negative. This discrepancy can only disappear if image forces and/or nonlocal dielectric effects are incorporated.

Discussion

The kinetic pattern for the reactions of $[Co(phen)_3]^{3+}$ and $[Co(phen\text{-}SO_3^-)_3]$ with cyt *c* differs from that of the reactions with $[Co(terpy)_2]^{2+}$ by significantly larger activation enthalpies, a much larger activation enthalpy difference for the charged and neutral complexes, and much less negative activation entropies. The simple conducting-sphere model and bulk dielectric screening found suitable for the reactions between these two Co(III) complexes and $[Co(terpy)_2]^{2+}$ cannot account for the cyt *c* data. The simplest continuum representation of the protein reactions involves a dielectric interphase, reorganization of both dielectric spatial parts, image forces,[7] and nonlocal dielectric effects.[3]

The primary cause for these large activation enthalpies, compared to the only slightly less exoergic reactions with $[Co(terpy)_2]^{2+}$, is the substantial positive reaction enthalpies, the origin of which is the large negative entropy associated with cyt c^{II} oxidation.[11,12] This feature is also encountered in oxidation of other small metalloproteins,[4-6] whereas metalloprotein reduction[15-19] commonly exhibits small activation enthalpies. Vanishing activation enthalpy or approximate coincidence between the activation enthalpy and reorganization free energy does not, however, imply that the reactions belong to the activationless and barrierless regions,[21-23] respectively. These regions are not determined by reaction enthalpies but by Gibbs free energies, which are here numerically much smaller, and the apparently "abnormal" activation enthalpies for metalloprotein oxidation and reduction reflect temperature dependence of the reorganization free energies and work terms rather than critical locations of the appropriate reactant and product potential surfaces.

The specific, but crude, dielectric interface representation provides a frame for the activation parameters. In combination with bulk dielectric constants it is quite good for the phen-SO_3^- complex, for which work terms are absent. The calculated ΔH_A is too low by 6–10 kJ (20–30%), but this might be improved if the finite size of cyt *c* were taken into account. In terms of the model the larger activation enthalpy compared with that of the $[Co(terpy)_2]^{2+}$ reaction then arises from the reorganization terms in both the aqueous and protein phases and from a larger reaction enthalpy. The latter originates from a positive reaction entropy contribution, i.e. a positive γ_s^p, which reflects increasing protein mobility with increasing temperature. In comparison, the entropy contribution from the aqueous phase is of minor importance for the phen-SO_3^- complex. These features could be reformulated in terms of conformational relaxation in the protein, provided that specific conformational modes could be identified.[33]

The activation enthalpy difference between the reactions of $[Co(phen)_3]^{3+}$ and $[Co(phen\text{-}SO_3^-)_3]$ leaves an enthalpic work term contribution of 12 kJ. This cannot originate from normal screened electrostatic repulsion from the protein surface charges, which would give a negative value. The data indicate two possible origins of this. First, repulsion from image charges is numerically equally important as direct repulsion and has a quite different temperature coefficient, quantitatively in line with the observed

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activation enthalpy difference. Its numerical value, expressed by the quantity γ_s^P, is, however, far higher than reported bulk values[25,26] and also too high to be compatible with the individual activation enthalpies for both the phen and phen-SO_3^- complexes. This appears to be tractable only by the second possible origin of the positive enthalpic work term component, namely nonlocal dielectric effects[3] in the aqueous phase, such effects being reflected more strongly in the work terms than in reorganization terms.

The small negative activation entropies can be satisfactorily attributed to the positive γ_s^P and the nonlocal work terms. This is in striking contrast to the case for the reactions with $[Co(terpy)_2]^{2+}$ in water, for which the sole origin of the large negative activation entropy is the temperature derivative of the work terms. With the consistently calculated activation entropies in hand, the electronic transmission coefficients can be determined from the preexponential factor. Notably, the transmission coefficients are small, i.e. about 10^{-5}, and quite close to the values for the reactions with $[Co(terpy)_2]^{2+}$ obtained by the conducting-sphere model and bulk dielectric screening. This conclusion would remain valid if the finite size of cyt *c* were incorporated into the continuum model.

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Appendix

A. Elements of Diabatic Electron-Transfer Rate Theory Recast into the Form of Absolute Rate Theory. The bimolecular electron-transfer rate constant, k, can be given the form[20]

$$k = \kappa_e \frac{\omega_{eff}}{2\pi} \Delta V \exp(-\Delta G_A/k_BT)$$
$$\Delta G_A = \Delta \tilde{G}_A - k_BT \ln\left(\frac{\hbar\omega_{eff}}{k_BT}\kappa_e \Delta V\right) \quad \text{(A1)}$$

where $\hbar = h/2\pi$, ω_{eff} is the "effective" vibrational frequency representing a weighted average of all the classical nuclear modes shifted during the reaction, and ΔV is the "reaction zone", i.e. the volume of the region within which electron transfer occurs. ΔV is close to 1 cm^3 mol^{-1} for small molecules.[1,20] We shall use this value but subsequently introduce a "steric" factor to account for the limited "accessibility" to the heme group in cyt *c*. Finally, κ_e is the electronic transmission coefficient, ΔG_A the activation free energy obtained from absolute rate theory (eq 4), and $\Delta \tilde{G}_A$ the "real" activation free energy.

Several terms contribute to ΔG_A

$$\Delta G_A \approx \theta\, \Delta G_0 + U_r + \theta(U_p - U_r) + \Delta G_A^{out} + \Delta G_A^{in} - k_BT \ln\left(\frac{\hbar\omega_{eff}}{k_BT}\kappa_e\right) \quad \text{(A2)}$$

where ΔG_0 is the reaction free energy, U_r and U_p are the reactants' and products' work terms, and ΔG_A^{out} and ΔG_A^{in} are the contributions from reorganization of the solvent, including the protein, and the molecular structure, respectively. Finally, θ is the symmetry factor, i.e. $\theta \approx (-k_BT)\mathrm{d}\ln k/\mathrm{d}\Delta G_0$, in principle obtained by minimzing ΔG_A with respect to θ.[20] ΔG_A^{out} and ΔG_A^{in} have the general form

$$\Delta G_A^{out} = \theta(1-\theta)E_s \qquad \Delta G_A^{in} = \theta(1-\theta)R_r \quad \text{(A3)}$$

where E_s and E_r are the environmental and molecular reorganization free energies, respectively. We can here and in the following neglect nuclear tunneling.[1,20]

The apparent activation entropy is formally given by the temperature derivative of ΔG_A and is related to the real activation entropy, $\Delta \tilde{S}_A$, by

$$\Delta S_A^{app} = -\partial \Delta G_A/\partial T = \Delta \tilde{S}_A + k_B \ln\left(\kappa_e \frac{\hbar\omega_{eff}}{k_BT}\right) \quad \text{(A4)}$$
$$\Delta \tilde{S}_A = -\partial \Delta \tilde{G}_A/\partial T$$

By disregarding the quantity $U_p - U_r$ in eq A2, we can write eq A4 as

$$\Delta S_A^{app} \approx \theta\, \Delta S_0 - \partial U_r/\partial T - \theta(1-\theta)\, \partial E_s/\partial T - k_B \ln\left(\kappa_e \frac{\hbar\omega_{eff}}{k_BT}\right) \quad \text{(A5)}$$

ΔG_A^{in} being largely independent of T. ΔS_0 is the reaction entropy.

Finally, ΔH_A is obtained from the relation $\Delta H_A = \Delta G_A + T\Delta S_A$

$$\Delta H_A \approx \theta\, \Delta H_0 + U_r - T\, \partial U_r/\partial T + \theta(1-\theta)(E_s + E_r) - \theta(1-\theta)T\, \partial E_s/\partial T \quad \text{(A6)}$$

where ΔH_0 is the reaction enthalpy.

B. Environmental Reorganization Free Energy and Entropy for Symmetric Charge Transfer across a Planar Boundary between Two Dielectrics. We represent the electron-transfer process from the heme group in cyt *c* to the Co(III) complexes as electron transfer between spheres of equal radii r_0, located symmetrically with respect to a planar phase boundary that separates two dielectric media of low (protein) and high (water) dielectric constant. The quantity E_s is then[24]

$$E_s = e^2\left[\left(\frac{1}{\epsilon_0^w} - \frac{1}{\epsilon_s^w}\right) + \left(\frac{1}{\epsilon_0^p} - \frac{1}{\epsilon_s^p}\right)\right]\left(\frac{1}{2r_0} + \frac{1}{l} - \frac{r_0^3}{6(2l)^4}\right) \quad \text{(B1)}$$

where ϵ_0 and ϵ_s are the optical and static dielectric constants, the superscripts referring to the aqueous (w) and protein (p) parts, e is the elementary charge, and l is the distance between the charge centers and the phase boundary.

Only the protein part in eq B1 has a significant entropy component

$$-\partial E_s/\partial T \approx -\frac{1}{\epsilon_s^p}\gamma_s^p e^2\left(\frac{1}{2r_0} - \frac{1}{4l} - \frac{r_0^3}{6(2l)^4}\right) \quad \text{(B2)}$$

where $\gamma_s^p = \mathrm{d}\ln\epsilon_s^p/\mathrm{d}T$.

C. Work Terms Including Dielectric Image Forces. The free energy, entropy, and enthalpy parts of the work terms can be obtained from the relations

$$k^{sub}/k^{us} = \exp\{-[(\theta^{sub}\Delta G_0^{sub} - \theta^{us}\Delta G_0^{us})/k_BT] + (U_r^{us}/k_BT) + \ln(\kappa_e^{sub}/\kappa_e^{us})\} \quad \text{(C1)}$$

$$\Delta S_A^{app,sub} - \Delta S_A^{app,us} \approx \theta^{sub}\Delta S_0^{sub} - \theta^{us}\Delta S_0^{us} + k_B \ln(\kappa_e^{sub}/\kappa_e^{us}) \quad \text{(C2)}$$

$$\Delta H_A^{sub} - \Delta H_A^{us} \approx \theta^{sub}\Delta H_0^{sub} - \theta^{us}\Delta H_0^{us} - U_r^{us} - T\alpha_s \quad \text{(C3)}$$

where the superscripts "sub" and "us" refer to the phen-SO_3^- and phen complexes, respectively, and we have denoted $-\partial U_r^{us}/\partial T$ by α_s.

The specific form of the work term U_r^{us} for the planar two-dielectric model is[7]

$$U_r^{us} = \frac{1}{4}\frac{z^2e^2}{\epsilon_s^w r_0}f + \frac{ze^2}{\epsilon_s^w}\sum_i\left(\frac{z_i}{r_i} + \frac{z_i}{r_i}f\right) = \left\{\frac{1}{4}\frac{z^2e^2}{\epsilon_s^w r_0} + \frac{ze^2}{\epsilon_s^w}\sum_i\frac{z_i}{r_i}\right\}f + \frac{ze^2}{\epsilon_s^w}\sum_i\frac{z_i}{r_i} \quad \text{(C4)}$$

$$f = \frac{\epsilon_s^w - \epsilon_s^p}{\epsilon_s^w + \epsilon_s^+}$$

where ze is the charge of $[Co(phen)_3]^{3+}$, r_0 its radius, r_i the distance between the center of $[Co(phen)_3]^{3+}$ and the ith (positive) surface charge, and r_i the distance to the corresponding image charge on the protein side. The first term on the right-hand side of eq C4 is the interaction between $[Co(phen)_3]^{3+}$ and its own image, the second group of terms the direct interaction with the surface charges, and the third group the interaction with the images of the protein surface charges. Since $\epsilon_s^w > \epsilon_s^p$, all the terms are repulsive. Further, even if $\epsilon_s^w \gg \epsilon_s^p$, we cannot a priori disregard

ϵ_s^p in comparison with ϵ_s^w in eq C4, because the corresponding enthalpy and entropy contributions are in a crucial way determined by the temperature coefficients of both these quantities.

The entropy associated with eq C4 is

$$\Delta S_A^w = -\partial U_r^{us}/\partial T \approx \frac{1}{\epsilon_s^w}\left(\gamma_s^w + 2\frac{\epsilon_s^p}{\epsilon_s^w}\gamma_s^p\right)\left[\frac{1}{4}\frac{z^2e^2}{r_0} + \sum_i \frac{z_i}{r_i}\right] + \gamma_s^w \frac{1}{\epsilon_s^w} ze^2 \sum_i \frac{z_i}{r_i} \approx \gamma_s^w U_r^{us} + 2\frac{1}{\epsilon_s^w}\frac{\epsilon_s^p}{\epsilon_s^w}\gamma_s^p\left(\frac{1}{4}\frac{z^2e^2}{r_0} + \sum_i \frac{z_i}{r_i}\right) \quad (C5)$$

if $\epsilon_s^w \gg \epsilon_s^p$. In eq C5 $\gamma_s^w = d \ln \epsilon_s^w/dT$ and $\gamma_s^p = d \ln \epsilon_s^p/dT$. The enthalpy is

$$\Delta H_A^w = U_r^{us} + T\Delta S_A^w = (1 + T\gamma_s^w)\frac{ze^2}{\epsilon_s^w}\sum_i \frac{z_i}{r_i} + \frac{1}{\epsilon_s^w}\left(1 + T\gamma_s^w + 2\gamma_s^p T\frac{\epsilon_s^p}{\epsilon_s^w}\right)\left[\frac{1}{4}z^2e^2\frac{1}{r_0} + ze^2\sum_i \frac{z_i}{r_i}\right] = U_r^{us}(1 + T\gamma_s^p) + 2\frac{1}{\epsilon_s^w}T\gamma_s^p\frac{\epsilon_s^p}{\epsilon_s^w}\left[\frac{1}{4}z^2e^2\frac{1}{r_0} + ze^2\sum_i \frac{z_i}{r_i}\right] \quad (C6)$$

We notice that since $|T\gamma_s^w| > 1$ and $\gamma_s^w < 0$, the activation enthalpy contribution from the direct interaction with the protein surface charges is negative. The contribution from the second group of terms, i.e. the image terms, must therefore be positive and significant.

Registry No. cyt *c*, 9007-43-6; $[Co(phen)_3]^{3+}$, 18581-79-8; $[Co(phen\text{-}SO_3^-)_3]$, 99747-69-0; Cl^-, 16887-00-6; PO_4^{3-}, 14265-44-2.

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Kinetics of the Oxidation of High-Potential Iron-Sulfur Protein from *Chromatium* by Ferrocenium Derivatives

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Kinetic studies for the oxidation of high-potential iron-sulfur protein from *Chromatium vinosum* by five ferrocenium derivatives are reported. Second-order rate constants for protein oxidation by ferrocenium and its 1,1′-dimethyl, hydroxymethyl, chloromercurio, and phenyl derivatives are $(2.2 \pm 0.1) \times 10^6$ M^{-1} s^{-1}, $(0.35 \pm 0.03) \times 10^6$ M^{-1} s^{-1}, $(14 \pm 1) \times 10^6$ M^{-1} s^{-1}, $(5.1 \pm 0.8) \times 10^6$ M^{-1} s^{-1}, and $(13 \pm 1) \times 10^6$ M^{-1} s^{-1}, respectively, at pH 7.0 (phosphate), $\mu = 0.13$ (phosphate/NaCl), and 25 °C. The protein oxidation by ferrocenium was found to depend upon pH ($pK_a = 6.90 \pm 0.12$) with the protonated protein being about half as reactive as its conjugate base. No rate saturation is observed at high ferrocenium derivative concentration nor is inhibition by either highly positively or negatively charged redox-inactive complexes observed. Three of the reactions have been studied as a function of temperature, and the temperature dependence of the equilibrium constant has been obtained for two of these. These results are used to discuss the site of electron transfer on the protein's surface and to estimate the protein's self-exchange electron-transfer rate constant and activation enthalpy.

High-potential iron-sulfur protein (HiPIP) (*Chromatium vinosum*) is a small (M_R 9300) protein with an Fe_4S_4 active core capable of undergoing one-electron oxidation-reduction with a potential of 0.350 V at pH 7.[1] Its relatively high stability in both oxidation states, a redox potential close to that of many available redox agents, and the availability of the X-ray crystal structure[2] of both the oxidized ($HiPIP_o$) and reduced ($HiPIP_r$) protein have made it an attractive molecule for numerous physical and chemical studies. These studies have included the determination of its redox kinetics with small inorganic reagents[3-7] and with other metalloproteins.[7-10]

This paper reports our studies of the oxidation of $HiPIP_r$ by five ferrocenium ion derivatives. Of particular interest are the nature and location of the reactive site on $HiPIP_r$ for ferrocenium electron transfer, the effect that electrostatic and hydrophobic interactions have on the reaction rate, and the influence of $HiPIP_r$ protonation on its redox activity. In addition, these studies are used to estimate the self-exchange rate constant for HiPIP and its self-exchange enthalpy of activation.

Experimental Section

Laboratory distilled water was further purified by reverse osmosis and ion exchange (Sybron/Barnstead Nanopure). All chemicals were reagent grade or of the highest purity available. Argon gas, passed through two chromous or vanadous scrubbing towers to remove traces of molecular oxygen, was used for preparing anaerobic solutions.

Ferrocene, ferrocenium, their derivatives, and their solutions were prepared as described previously.[11,12] The ferrocenium ion solutions vary greatly in their stability at pH 7.0,[11,12] with (chloromercurio)ferrocenium and 1,1′-dimethylferrocenium solutions being stable for hours while phenylferrocenium undergoes significant decomposition in minutes. Experiments with phenylferrocenium were performed by preparing solutions of its hexafluorophosphate salt in dilute acid, in which phenylferrocenium is much more stable, and bringing these solutions to the desired pH at the time of mixing in the stopped-flow. Phenylferrocenium

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