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(28) The rate of epimerization in principle can be determined by the use of polarimetric techniques. Moreover, comparison of the rotation of the equilibrium mixture of diastereomers with that of freshly prepared solutions of the pure $(S)_C(S)_{Sn}$ diastereomer might offer insights concerning the relative contribution of the two chiral centers to the total optical rotation. However, the low solubility of the pure diastereomer at low temperatures precludes such measurements.
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(35) The actual distance is even smaller because in the conformer found in the solid the plane through the C_6H_4 group makes an angle of 12.5° with the mirror plane through the Ph_2SnBr part of II. Substitution of H_6 by a second intramolecular Me_2NCH_2 ligand results in an ionic triorganotin halide $[2,6-(Me_2NCH_2)_2C_6H_3]SnMeRBr$ (R = Me or Ph). The Sn–Br bond is dissociated (x-ray, R = Me; Sn···Br 5.2 Å) because the C_3Sn cationic state is now stabilized by Sn–N coordination of the two Me_2NCH_2 ligands resulting in a TBP configuration around tin.[26]

Electron Transfer Reactions of Trifluoroacetylated Horse Heart Cytochrome *c*

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Abstract: Rate parameters have been determined for the reactions of fully trifluoroacetylated (19 lysine residues) horse heart cytochrome *c* with the reductant $Fe(EDTA)^{2-}$ and the oxidants $Co(phen)_3^{3+}$ and $Fe(CN)_6^{3-}$. Respective second-order rate constants and activation parameters obtained for the reduction of trifluoroacetylated (TFA) ferricytochrome *c* by $Fe(EDTA)^{2-}$ and the oxidation of TFA ferrocytochrome *c* by $Co(phen)_3^{3+}$ are $(5.1 \pm 0.3) \times 10^3$ M^{-1} s^{-1}; $\Delta H^{\ddagger} = 3.9 \pm 0.5$ kcal/mol, $\Delta S^{\ddagger} = -28 \pm 3$ cal/mol·deg and $(1.67 \pm 0.05) \times 10^4$ M^{-1} s^{-1}; $\Delta H^{\ddagger} = 10.5 \pm 1.0$ kcal/mol, $\Delta S^{\ddagger} = -3.9 \pm 0.3$ cal/mol·deg (25.0 °C, μ = 0.1 M, pH 7.0 (phosphate)). Rate constants found for the oxidation of TFA and native cytochromes by $Fe(CN)_6^{3-}$ under the same conditions are $(1.20 \pm 0.06) \times 10^6$ M^{-1} s^{-1} (25.4 °C) and $(2.3 \pm 0.2) \times 10^7$ M^{-1} s^{-1} (25.4 °C), respectively. Ionic strength dependences of rate constants for TFA cytochrome *c* electron transfer reactions were fit using equations from transition state and Marcus theories, treating the protein charge as a variable. Electrostatics-corrected apparent TFA cytochrome *c* self-exchange electron transfer rate constants based on $Fe(EDTA)^{2-}$ and $Co(phen)_3^{3+}$ cross reactions are in good agreement with those obtained previously for the native protein.

Introduction

The study of the reactions of electron transfer proteins with inorganic reagents has recently been an area of active research. We have presented a method, based on the Marcus theory of outer sphere electron transfer, for quantitatively extracting several of the contributions to the energetics of these reactions from available kinetic data.[2-4] In view of the wealth of structural and rate data available for horse heart cytochrome *c*,[2] we have paid particular attention to its electron transfer reactions in our work. Compensation for electrostatic interactions between the reactants is an important consideration in the analysis of redox reactivity trends for cytochrome *c* within the framework of Marcus theory.[2,3] The availability of the fully trifluoroacetylated derivative of horse heart cytochrome *c* has now allowed us to test the analytical method developed to estimate Coulombic contributions to the activation free energies for reactions of the metalloprotein with oxidants and reductants. Rate parameters are reported in this

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paper for the reactions of trifluoroacetylated cytochrome *c* with the reductant $Fe(EDTA)^{2-}$ and the oxidants $Fe(CN)_6^{3-}$ and $Co(phen)_3^{3+}$; comparisons of these results with those for the analogous reactions with the native protein provide a basis for better understanding nonelectrostatic contributions to the activation process.

The reactivity of cytochrome *c* trifluoroacetylated at all 19 lysine residues (TFA cytochrome *c*) with low molecular weight redox agents is of particular interest, as the positively charged lysine residues are known to be important in the reaction of cytochrome *c* with its physiological partner, cytochrome *c* oxidase. It has been shown that all of the lysines surrounding the heme crevice, lysines 8, 13, 25, 27, 72, 79, and 87, are involved in binding to cytochrome *c* oxidase and cytochrome b_5.[5-9]

Experimental Section

Fully trifluoroacetylated (TFA) horse heart cytochrome *c* was prepared by the method of Fanger and Harbury,[10] using Sigma type VI protein as the starting material. The fluorodinitrobenzene method[10] indicated that 18.7 out of 19 lysines were trifluoroacetylated. The protein was passed through a BioGel P-60 column to remove any polymeric material and was never lyophilized, thus avoiding formation of polymeric material after the protein had been passed through the Bio-Gel P-60 column.[11] The absorbance band at 695 nm was identical with that of native cytochrome *c* to within experimental error (5%), indicating that the heme environment was essentially unmodified and no polymeric material was present. The protein in 0.01 M, pH 7 phosphate buffer was shell frozen and stored at −10 °C until use.

Sigma type VI cytochrome *c* was used as supplied for all experiments with the unmodified protein. Cytochrome *c* concentrations were determined by measuring the 550-nm absorbance change accompanying full reduction of the protein with ascorbate ($\Delta\epsilon_{550} = 18.5 \times 10^3$ M^{-1} cm^{-1}).[12] The visible absorption spectrum of fully trifluoroacetylated cytochrome *c* has been shown to be essentially identical with that of the native protein.[10]

Reagent grade chemicals were used without further purification, and triply distilled water was used in preparing solutions for kinetics measurements. Nitrogen gas used for deoxygenation of kinetics solutions was purified of oxidizing impurities by passage through two chromous scrubbing towers. Dialysis tubing was boiled extensively to remove sulfur-containing impurities. Serum caps were boiled in concentrated base before using.

Ferrous EDTA solutions were prepared from $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ and $Na_2H_2EDTA \cdot 2H_2O$ as previously described.[13] The concentration of EDTA was maintained in 20% excess over iron for all runs. Stock solutions of $Fe(EDTA)^{2-}$ were maintained under a nitrogen atmosphere, and dilutions were accomplished using Hamilton gas-tight syringes to transfer aliquots into serum-capped bottles containing deoxygenated phosphate buffer and ammonium sulfate. Reported concentrations are derived from the weight of ferrous ammonium sulfate used to prepare the stock solution.

Fisher potassium ferricyanide was used as supplied. Ferricyanide solutions were prepared by weight, and were protected from light in serum bottles wrapped in aluminum foil.

Tris(1,10-phenanthroline)cobalt(III) perchlorate dihydrate was prepared by the method of Schilt and Taylor.[14] The concentrations of $Co(phen)_3^{3+}$ solutions were determined spectrophotometrically in the region 380–320 nm (ϵ_{350} 3700 M^{-1} cm^{-1}; ϵ_{330} 4680 M^{-1} cm^{-1}).[15]

TFA ferricytochrome *c* solutions were prepared by dialyzing against triply distilled water and diluting in a volumetric flask containing weighed amounts of $NaH_2PO_4 \cdot H_2O$ and Na_2HPO_4 to give the desired pH and ionic strength. Solutions of the reduced TFA derivative were prepared by adding an equivalent amount of ascorbic acid to the deoxygenated ferric protein. Protein solutions were deoxygenated by slowly purging with N_2.

Native and TFA cytochrome *c* electron transfer reactions were followed at 550 nm on a Durrum D-110 stopped-flow spectrophotometer using standard techniques.[16] All kinetics measurements were performed with small molecule reagents in pseudo-first-order excess over the metalloproteins. Cytochrome *c* concentrations of ~2.5–5 μM were used in experiments with $Fe(EDTA)^{2-}$ and $Co(phen)_3^{3+}$; a value of ~1 μM was required for experiments with $Fe(CN)_6^{3-}$. Sodium phosphate buffers alone were used to maintain the ionic strength at the desired level in experiments with $Co(phen)_3^{3+}$ and $Fe(CN)_6^{3-}$. Ammonium sulfate and phosphate buffers were used for this purpose in the $Fe(EDTA)^{2-}$ studies. Kinetic data were collected as photographs of absorbance–time traces on a Tektronix Model 564B storage oscilloscope. Observed rate constants were obtained from the least-squares slopes of $\ln |A_t - A_\infty|$ vs. time plots; these plots invariably were found to be linear for at least 3 half-lives of the reactions.

The reduction potential of TFA cytochrome *c* in phosphate buffer (pH 7.0) was determined by titration with potassium ferrocyanide as described by Margalit and Schejter.[17]

All spectral data were acquired on a Cary 17 UV-visible spectrophotometer and an Ionalyzer Model 801 instrument was used to make pH measurements.

Results

I. Rate Laws for TFA Cytochrome *c* Electron Transfer Reactions with $Fe(EDTA)^{2-}$, $Co(phen)_3^{3+}$, and $Fe(CN)_6^{3-}$. Plots of k_{obsd} vs. reductant or oxidant concentration for the reduction of TFA ferricytochrome *c* by $Fe(EDTA)^{2-}$ and for the oxidation of TFA ferrocytochrome *c* by $Co(phen)_3^{3+}$ and $Fe(CN)_6^{3-}$ are given in Figures 1–3. Data obtained for the oxidation of native ferrocytochrome *c* by $Fe(CN)_6^{3-}$ are also included in Figure 3. The reagent concentration ranges used for $Fe(EDTA)^{2-}$, $Co(phen)_3^{3+}$, and $Fe(CN)_6^{3-}$ were 1.0×10^{-4} to 1.0×10^{-2}, 6.5×10^{-5} to 2.59×10^{-3}, and 1.04×10^{-5} to 2.09×10^{-4} M, respectively. In each case, the kinetics observed were first order in inorganic redox agent (and first order in metalloprotein). Respective second-order rate constants obtained for the reduction of TFA ferricytochrome *c* by $Fe(EDTA)^{2-}$, and for the oxidation of TFA ferrocytochrome *c* by $Co(phen)_3^{3+}$ and $Fe(CN)_6^{3-}$, are $(3.33 \pm 0.04) \times 10^3$ (25.0 °C), $(1.13 \pm 0.02) \times 10^4$ (24.9 °C), and $(1.96 \pm 0.04) \times 10^6$ M^{-1} s^{-1} (25.5 °C). All values pertain to ionic strength 0.5 M, pH 7.0 (phosphate). The rate constant obtained for the native ferrocytochrome *c*–$Fe(CN)_6^{3-}$ reaction under the same conditions is $(4.4 \pm 0.2) \times 10^6$ M^{-1} s^{-1} (25.6 °C).

Concentration dependence plots for the $Fe(CN)_6^{3-}$ oxidation of both native and TFA ferrocytochrome *c* (Figure 3) show small positive intercepts (2.6 and 2.9 s^{-1}, respectively), which may reflect slight deviations from a simple first-order $Fe(CN)_6^{3-}$ dependence. However, these intercepts fall within experimental error of zero and are therefore not considered significant.

II. Activation Parameters for the Reactions of TFA Cytochrome *c* with $Fe(EDTA)^{2-}$ and $Co(phen)_3^{3+}$. Observed rate constants were obtained as a function of temperature in the range 6–41 °C for the $Fe(EDTA)^{2-}$–TFA ferricytochrome *c* and TFA ferrocytochrome *c*–$Co(phen)_3^{3+}$ reactions at ionic strength 0.1 M, pH 7.0. Activation parameters derived from linear Eyring plots (Figures 4 and 5) are summarized in Table I along with parameters obtained previously for the analogous reactions with native cytochrome *c*. Also presented in Table I are parameters for the $Fe(CN)_6^{3-}$ oxidation of TFA ferrocytochrome *c* as measured by LeBon and Cassatt.[18] The room temperature rate constant for reduction of TFA ferricytochrome *c* by $Fe(EDTA)^{2-}$ is a factor of 5 smaller than that for the native protein; the activation parameters reveal that a decrease in $\Delta S^{\ddagger}$ of 10 cal/mol·deg offsets a small drop in $\Delta H^{\ddagger}$ of 2.1 kcal/mol. By contrast, the rate constant for $Co(phen)_3^{3+}$ oxidation of TFA ferrocytochrome *c* is a factor of 11 larger than that for the native protein at room temperature; an activation entropy more positive by 2.3 cal/mol·deg is predominantly responsible for the rate enhancement, as the $\Delta H^{\ddagger}$ value remains essentially unchanged.

III. Ionic Strength Dependences of Electron Transfer Rate Constants. Ionic strength dependences of second-order rate constants were evaluated over the interval 0.05–0.30 M for all of the reactions under consideration. In all cases the metallo-

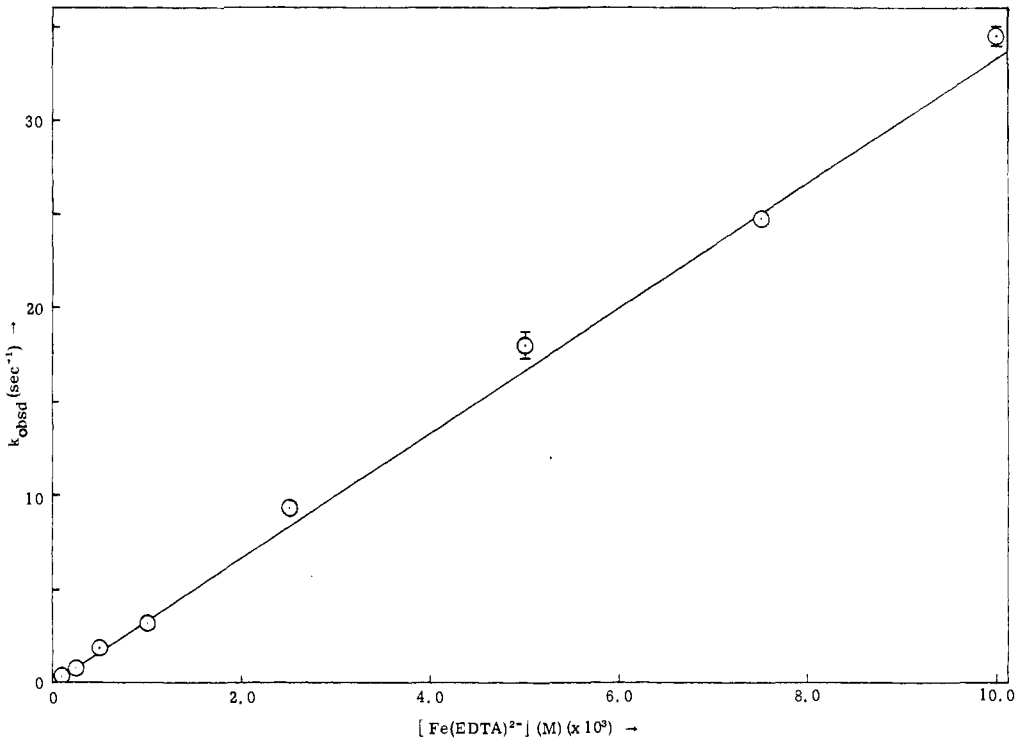

Figure 1. Plot of k_{obsd} vs. $[Fe(EDTA)^{2-}]$ for the reduction of TFA ferricytochrome *c* by $Fe(EDTA)^{2-}$ (25.0 °C, pH 7.0 (phosphate), μ = 0.5 M total, 0.4 M contributed by phosphate buffer). Data points plotted in all figures represent averages of at least two and in most cases three determinations.

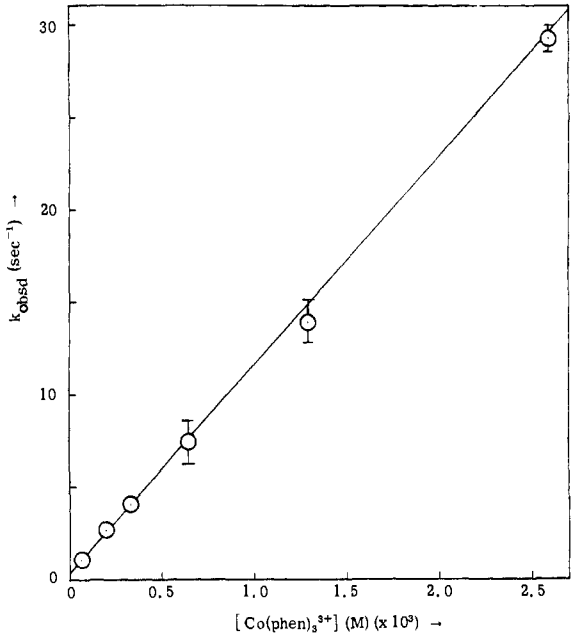

Figure 2. Plot of k_{obsd} vs. $[Co(phen)_3^{3+}]$ for the oxidation of TFA ferrocytochrome *c* by $Co(phen)_3^{3+}$ (24.9 °C, pH 7.0 (phosphate), μ = 0.5 M).

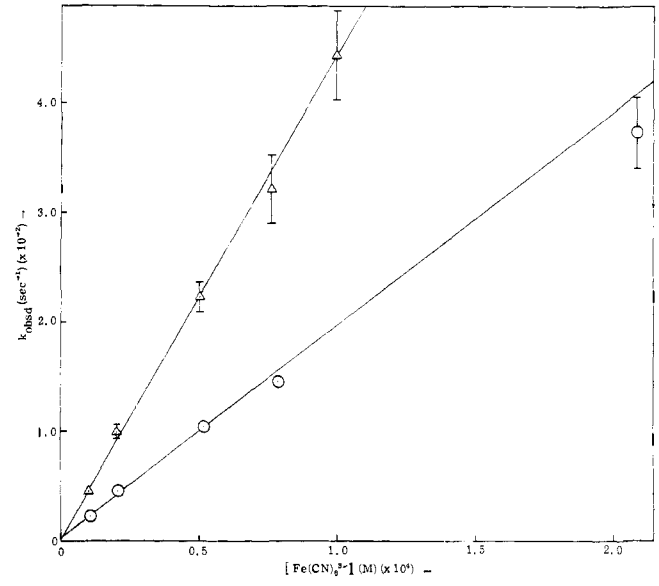

Figure 3. Plot of k_{obsd} vs. $[Fe(CN)_6^{3-}]$ for the oxidation of TFA (○), 25.5 °C, and native (△), 25.6 °C, ferrocytochrome *c* by $Fe(CN)_6^{3-}$ (pH 7.0 (phosphate), μ = 0.5 M).

protein initially in ionic strength 0.1 M, pH 7.0 buffer was mixed with equimolar solutions of the inorganic redox agent in phosphate buffers of varying concentration. Results for the $Fe(EDTA)^{2-}$-TFA ferricytochrome *c*, TFA ferrocytochrome *c*-$Co(phen)_3^{3+}$, TFA ferrocytochrome *c*-$Fe(CN)_6^{3-}$, and native ferrocytochrome *c*-$Fe(CN)_6^{3-}$ reactions are given in Table II. The rate of the first reaction undergoes a modest decrease of 30% as the ionic strength increases from 0.060 to 0.298 M; the rate constant for the second reaction is independent of ionic strength over the interval 0.050–0.254 M. The $Fe(CN)_6^{3-}$ oxidation rate of the TFA derivative varies little with ionic strength over the interval 0.050–0.281 M, whereas the reactivity of the native protein with $Fe(CN)_6^{3-}$ drops by a factor of 5 over the same range. Second-order rate constants for ionic strength 0.1 M interpolated from the data in Table II are included in Table I. At μ 0.1 M the native protein is oxidized nearly 20 times faster than the TFA derivative, whereas the reactivity advantage drops to a factor of ~5 at μ = 0.281 M. The rate constant reported here for the TFA ferrocytochrome *c*-$Fe(CN)_6^{3-}$ reaction is in fair agreement with that of 3.0×10^5 M^{-1} s^{-1} obtained recently by LeBon and Cassatt under slightly different conditions (25 °C, pH 7.0, 0.01 M phosphate buffer, μ = 0.1 M (KCl)).[18]

IV. Reduction Potentials of Native and TFA Cytochrome *c*. The reduction potentials ($E^{0\prime}$) of native and TFA cytochromes

Table I. Rate Parameters for Electron Transfer Reactions of TFA and Native Cytochrome *c*[a]

reagent	TFA cytochrome *c*			native cytochrome *c*		
	k, M^{-1} s^{-1}	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, cal/mol·deg	k, M^{-1} s^{-1}	$\Delta H^{\ddagger}$, kcal/mol	$\Delta S^{\ddagger}$, cal/mol·deg
$Fe(EDTA)^{2-}$	5.1×10^3	3.9 ± 0.5	-28 ± 3	2.57×10^4 [b]	6.0[b]	−18[b]
$Co(phen)_3^{3+}$	1.67×10^4	10.5 ± 1.0	-3.9 ± 0.3	1.50×10^3 [c]	11.3[c]	−6.2[c]
$Fe(CN)_6^{3-}$	1.2×10^6 [d]	0.4[e]	−33[e]	2.3×10^7 [d]	1.1[f]	−20[f]
	3.0×10^5 [e]			8.0×10^6 [f]		

[a] Values pertain to 25.0 °C, μ = 0.1 M, pH 7.0 (phosphate) unless otherwise specified. [b] H. L. Hodges, R. A. Holwerda, and H. B. Gray, *J. Am. Chem. Soc.*, **96**, 3132 (1974). [c] J. V. McArdle, H. B. Gray, C. Creutz, and N. Sutin, *J. Am. Chem. Soc.*, **96**, 5737 (1974). [d] 25.4 °C, pH 7 (phosphate), μ = 0.1 M; interpolated from Table II. [e] T. R. LeBon and J. C. Cassatt, *Biochem. Biophys. Res. Commun.*, **76**, 746 (1977); ionic strength maintained with KCl. [f] J. C. Cassatt and C. P. Marini, *Biochemistry*, **13**, 5323 (1974); ionic strength maintained with KCl.

Table II. Ionic Strength Dependences of Rate Parameters for the $Fe(EDTA)^{2-}$ Reduction, $Co(phen)_3^{3+}$ Oxidation, and $Fe(CN)_6^{3-}$ Oxidation of TFA Cytochrome *c* and $Fe(CN)_6^{3-}$ Oxidation of Native Cytochrome *c*[a]

reaction	μ, M	k_{obsd}, s^{-1}	k, M^{-1} s^{-1}
$Fe(EDTA)^{2-}$/TFA Cyt *c*[b]	0.060	5.69 ± 0.25	$(5.83 \pm 0.26) \times 10^3$
	0.084	5.30 ± 0.18	$(5.43 \pm 0.18) \times 10^3$
	0.154	4.39 ± 0.51	$(4.50 \pm 0.52) \times 10^3$
	0.203	3.96 ± 0.26	$(4.05 \pm 0.27) \times 10^3$
	0.255	3.87 ± 0.30	$(3.96 \pm 0.31) \times 10^3$
	0.298	4.00 ± 0.20	$(4.10 \pm 0.21) \times 10^3$
$Co(phen)_3^{3+}$/TFA Cyt3*c*[c]	0.050	4.94 ± 0.42	$(1.88 \pm 0.16) \times 10^4$
	0.070	4.38 ± 0.03	$(1.67 \pm 0.01) \times 10^4$
	0.127	4.59 ± 0.07	$(1.75 \pm 0.03) \times 10^4$
	0.168	4.43 ± 0.04	$(1.69 \pm 0.02) \times 10^4$
	0.210	4.52 ± 0.09	$(1.72 \pm 0.03) \times 10^4$
	0.254	4.72 ± 0.24	$(1.79 \pm 0.09) \times 10^4$
$Fe(CN)_6^{3-}$/TFA Cyt *c*[b]	0.050	20.6 ± 2.3	$(1.76 \pm 0.20) \times 10^6$
	0.073	23.6 ± 1.5	$(2.01 \pm 0.13) \times 10^6$
	0.137	23.4 ± 1.6	$(2.00 \pm 0.14) \times 10^6$
	0.184	23.3 ± 0.3	$(1.99 \pm 0.02) \times 10^6$
	0.231	14.9 ± 0.2	$(1.27 \pm 0.01) \times 10^6$
	0.281	15.8 ± 0.9	$(1.35 \pm 0.07) \pm 10^6$
$Fe(CN)_6^{3-}$/native Cyt *c*[d]	0.050	418 ± 21	$(3.58 \pm 0.18) \times 10^7$
	0.073	304 ± 21	$(2.60 \pm 0.18) \times 10^7$
	0.137	178 ± 21	$(1.52 \pm 0.18) \times 10^7$
	0.184	114 ± 3	$(9.77 \pm 0.25) \times 10^6$
	0.231	96.4 ± 2.9	$(8.24 \pm 0.25) \times 10^6$
	0.281	84.2 ± 9.8	$(7.20 \pm 0.84) \times 10^6$

[a] All values are the means of at least two separate determinations. [b] pH 7.1 (phosphate), 25.2 °C, $[Fe(EDTA)^{2-}] = 9.76 \times 10^{-4}$ M. [c] pH 7.1 (phosphate), 25.0 °C, $[Co(phen)_3^{3+}] = 2.63 \times 10^{-4}$ M. [d] pH 7.1 (phosphate), 25.4 °C, $[Fe(CN)_6^{3-}] = 1.17 \times 10^{-5}$ M.

c were found to be 260 ± 5 and 204 ± 5 mV, respectively, in potassium phosphate buffer, pH 7.0, 25 °C. These values are independent of ionic strength over the interval 0.04–0.20 M.

Discussion

Trifluoroacetylation of horse heart cytochrome *c* lysine residues causes essentially no change in the UV-visible spectrum of the metalloprotein,[5,10] and only very minor changes in the ORD spectrum.[19] The proton chemical shifts of the contact shifted methyl groups of TFA and native cytochrome *c* are identical except for a slight difference in the heme ring 1 methyl resonance.[5] Trifluoroacetylation does, however, shift the reduction potential by ~−50 mV. The bulk of the evidence indicates that trifluoroacetylation of all 19 lysine residues of cytochrome *c* leads to relatively minor changes in the coordination environment of the iron and the overall protein conformation. In view of this evidence, we conclude that differences in the redox reactivity of TFA and native cytochromes *c* may be interpreted (after corrections for differences in driving force) in terms of the steric and electrostatic consequences of derivatization, assuming that the structural differences between the native and modified proteins are not sufficient to bring about substantial changes in the electron transfer pathway preferred by each of the reagents.

Comparisons of activation parameters for the reactions of $Fe(EDTA)^{2-}$ and $Co(phen)_3^{3+}$ with native and TFA cytochromes *c* lend further support to the hypothesis that trifluoroacetylation of the metalloprotein does not change its inherent redox reactivity. Thus $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ values for the reaction of TFA ferricytochrome *c* with $Fe(EDTA)^{2-}$ are in reasonable agreement with those obtained for reduction of the native protein under conditions where some of the lysine residues are deprotonated ($\Delta H^{\ddagger}$ = 4.8 kcal/mol, $\Delta S^{\ddagger}$ = −24 cal/mol·deg, pH 9.0 (carbonate), μ = 0.1 M).[20] Similarly, differences between activation parameters for the oxidation of TFA and native cytochromes *c* by ferricyanide may be satisfactorily accounted for considering only electrostatic interactions involved in the formation of the activated complex.[18] That trifluoroacetylation does not substantially perturb the Franck-Condon activation barrier for oxidation of the Fe(II) center is indicated by the close agreement between $\Delta H^{\ddagger}$ values for the oxidation of TFA and native proteins by $Co(phen)_3^{3+}$.

Rate data for the reduction and oxidation of native cytochrome *c* have previously been analyzed[2,3] by relating cross reaction rate constants (k_{12}) to apparent protein self-exchange electron transfer rate constants (k_{11}) through the equation from relative Marcus theory for outer sphere electron transfer:

$$k_{11} = k_{12}^2/k_{22}K$$
$$= k_{12}^2/k_{22} \exp(38.94(\Delta E))$$

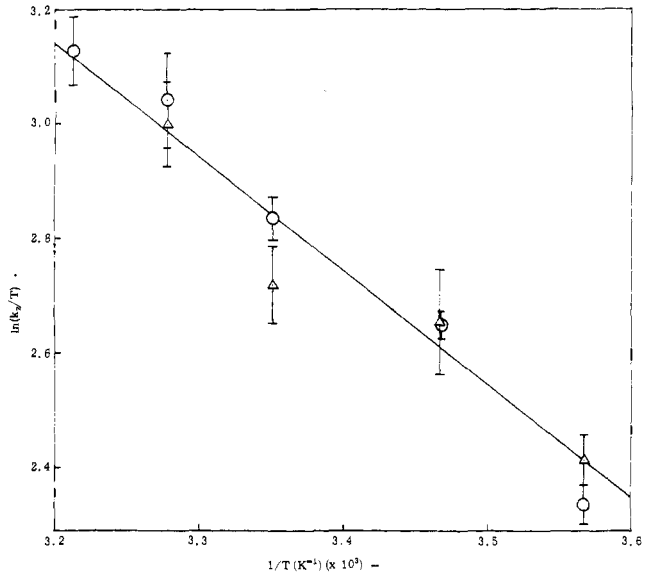

Figure 4. Eyring plot of rate data for the reduction of TFA ferricytochrome *c* by $Fe(EDTA)^{2-}$ (pH 7.0 (phosphate), μ = 0.1 M): (○), $[Fe(EDTA)^{2-}] = 1.00 \times 10^{-3}$ M; (△), $[Fe(EDTA)^{2-}] = 2.50 \times 10^{-3}$ M.

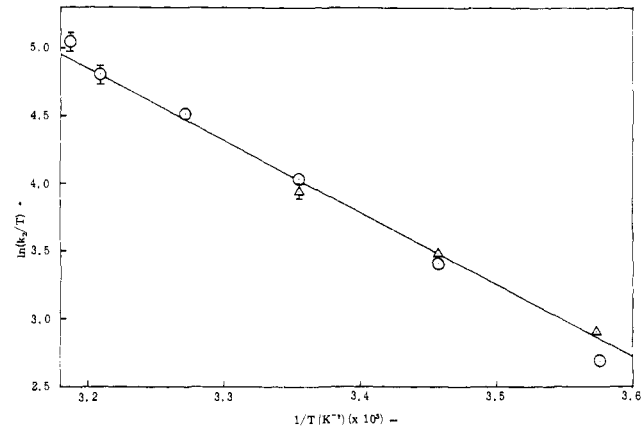

Figure 5. Eyring plot of rate data for the oxidation of TFA ferrocytochrome *c* by $Co(phen)_3^{3+}$ (pH 7.0 (phosphate), μ = 0.1 M): (○), $[Co(phen)_3^{3+}] = 1.284 \times 10^{-3}$ M; (△), $[Co(phen)_3^{3+}] = 3.21 \times 10^{-4}$ M.

where k_{22} refers to the self-exchange electron transfer rate constant for the small molecule reagent, K is the equilibrium constant for the reagent-protein cross reaction, and ΔE is the difference between the reduction potentials of the protein and the reagent in volts. Alternatively, this relationship may be expressed in terms of activation free energies and the standard free-energy change for the cross reaction, ΔG_{12}^0:

$$\Delta G_{11}^{*\text{uncor}} \equiv 2\Delta G_{12}^{*} - \Delta G_{22}^{*} - \Delta G_{12}^{0}$$

The analysis rests upon the principle that calculated k_{11} values should be invariant if the protein employs the same mechanism in cross reactions as it does in its self-exchange reaction. Deviations from constancy in calculated k_{11} values then may be understood in terms of (1) protein-reagent interactions that are not canceled by interactions in the reagent self-exchange; or (2) differences in the activation processes for the protein or reagent relative to those used in their respective self-exchange reactions.

The simplified Marcus theory equations given above ignore the electrostatic work required to bring the reactants together into the activated complex for electron transfer. Electrostatic work terms for bringing the reactants together in the cross reaction (w_{12}; for the products of the cross reaction $-w_{21}$) and in the protein (w_{11}) and reagent (w_{22}) self-exchange reactions

Table III. Native and TFA Cytochrome *c* Marcus Calculations (w/Ionic Strength Fit Charges)[a]

protein	reagent	k_{12}, M^{-1} s^{-1}	$\Delta E^{0\prime}$, V	w_{12}, kcal mol^{-1}	w_{21}, kcal mol^{-1}	w_{11}, kcal mol^{-1}	w_{22}, kcal mol^{-1}	ΔG_{11}^{*}uncor, kcal mol^{-1}	k_{11} uncor, M^{-1} s^{-1}	ΔG_{11}^{*}cor, kcal mol^{-1}	k_{11} cor, M^{-1} s^{-1}	ionic strength fits[b] Simp. T.S. theory Z_1	Simp. T.S. theory k_0	T.S. theory Z_1	T.S. theory k_0	Marcus theory[c] Z_1	Marcus theory[c] k_∞
TFA	$Fe(EDTA)^{2-}$	5.1 (10^3)	0.084	-0.249	-0.087	0.063	0.493	15.34	3.5 (10^1)	16.23	7.7 (10^0)	0.3	7.8 (10^3)	-0.3	7.3 (10^3)	3.3	3.3 (10^3)
native	$Fe(EDTA)^{2-}$	2.6 (10^4)	0.140	-0.612	-0.268	0.478	0.493	14.63	1.2 (10^3)	16.51	4.8 (10^0)	1.7	2.7 (10^5)	5.5	8.0 (10^6)	8.1	8.6 (10^3)
TFA	$Co(phen)_3^{3+}$	1.67 (10^4)	0.166	-0.151	0.040	-0.001	0.507	11.87	1.2 (10^4)	12.35	5.4 (10^3)	-0.01	1.8 (10^4)	2.2	6.1 (10^3)	-0.2	1.7 (10^4)
native	$Co(phen)_3^{3+}$	1.5 (10^3)	0.110	0.354	0.287	0.223	0.507	13.51	7.6 (10^2)	13.60	6.6 (10^2)	0.4	6.0 (10^2)	5.7	3.1 (10^1)	4.7	2.9 (10^3)
TFA	$Fe(CN)_6^{3-}$	1.2 (10^6)	0.229	-0.137	-0.324	0.025	2.408	11.71	1.6 (10^5)	14.59	1.2 (10^2)	0.2	2.6 (10^6)	-2.9	5.0 (10^5)	1.3	1.4 (10^6)
native	$Fe(CN)_6^{3-}$	2.3 (10^7)	0.173	-0.855	-1.281	0.613	2.408	7.00	4.5 (10^7)	12.14	7.7 (10^3)	0.8	1.1 (10^8)	2.6	4.6 (10^6)	8.1	4.1 (10^6)

[a] μ = 0.1 M, pH 7.0 (phosphate), 25 °C. [b] For description of parameters, see ref 2, 3. [c] The Z_1 *values* from Marcus theory ionic strength fits were used for k_{11} calculations.

may be included in the Marcus equation to correct ΔG_{11}^* (and k_{11}) for the effect of noncanceling Coulombic work:[2-4]

$$\Delta G_{11}^{*cor} = 2\Delta G_{12}^* - \Delta G_{22}^* - \Delta G_{12}^0 - w_{21} - w_{12} + w_{11} + w_{22}$$

Results of Marcus theory calculations for the reactions of both TFA and native cytochrome *c* with $Fe(EDTA)^{2-}$, $Co(phen)_3^{3+}$, and $Fe(CN)_6^{3-}$ are summarized in Table III. Work terms, ΔG_{11}^{*cor} values, and electrostatics-corrected cytochrome *c* self-exchange rate constants (k_{11}^{cor}) were evaluated using parameters for the reagents given previously.[2,3] The equation used for calculation of ΔG_{11}^{*cor} differs slightly from the above equation, because of the inclusion of the *f* factor.[2,3]

The choice of Z_1 (the electrostatic charge on the protein) is critical in the Marcus calculations. This choice must be compatible with the ionic strength dependences of the rate constants, as these measure the extent of electrostatic interactions between the reactants in the activated complex.[2-4] Ionic strength dependence results obtained for the reactions of TFA cytochrome *c* with $Fe(EDTA)^{2-}$ and $Co(phen)_3^{3+}$ and for the reactions of both TFA and native proteins with $Fe(CN)_6^{3-}$ have been analyzed in terms of equations given previously[2,3] based on transition state theory, simplified transition state theory, and Marcus theory treatments. Only Z_1 has been considered as a variable in these calculations; other parameters used are identical with those employed in previous calculations for the native protein.[2,3] Least squares fits of the ionic strength dependence data to each of these three equations were performed, and parameters derived from the fits for TFA and native cytochromes are compared in Table III. For the native protein, the best fit values for Z_1 based on the transition state and the Marcus theory equations are generally comparable with that estimated from the amino acid sequence. Such is not the case for the TFA derivative, as Z_1 values of $\sim 0 \pm 3$ are calculated from the ionic strength dependence data. The derived Z_1 values are not at all unreasonable, however, when it is recognized that the charge distribution on the surface of TFA cytochrome *c* is markedly asymmetric, owing to aspartic and glutamic acid residues that are clustered in a "negative patch" at the back of the molecule.[21] This negative patch is far removed from the heme *c* redox center, and apparently does not influence the electron transfer reactivity of the TFA protein with charged reagents.

An examination of the k_{11}^{cor} values obtained using ionic-strength-fit charges (Table III) shows that the basic reactivity pattern of the TFA cytochrome is similar to that of the native protein: $k_{11}^{cor}(Co(phen)_3^{3+}, Fe(CN)_6^{3-}) > k_{11}^{cor}(Fe(EDTA)^{2-})$. Previous calculations for cytochrome *c* and other metalloproteins have suggested that large variations in k_{11}^{cor} predominantly reflect differences in the adiabaticity (related to the extent of overlap between the reagent and protein redox orbitals) of the cross reactions and also in the extent of nonelectrostatic interaction between the protein and the reagents required to form the precursor complex for electron transfer.[2,3] In the case of $Co(phen)_3^{3+}$ and $Fe(EDTA)^{2-}$, our results support this conclusion, in that another potential source of variations in ΔG_{11}^{*cor} and k_{11}^{cor}, deviations from the model used for making the electrostatic work term calculations, is shown to be of minor importance by the reasonable agreement found between the values calculated for the native and modified proteins. The structural and electronic factors that contribute to the observed trend in k_{11}^{cor} values derived from $Fe(EDTA)^{2-}$ and $Co(phen)_3^{3+}$ cross reaction rate constants have been presented in detail elsewhere[2,3] and will not be discussed further here.

The k_{11}^{cor} value for $Fe(CN)_6^{3-}$ oxidation of cytochrome *c* in Table III indicates that $Fe(CN)_6^{3-}$ has lost some of its reactivity (compared to $Co(phen)_3^{3+}$) upon trifluoroacetylation of the protein. This finding may mean that a specific electrostatic interaction (with a positively charged lysine residue) allows a cyanide ligand on $Fe(CN)_6^{3-}$ to approach the heme *c* edge in the native protein. As all such residues are neutralized in the TFA derivative, such an interaction is not possible.

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Supplementary Material Available: A complete listing of observed rate constants (6 pages). Ordering information is given on any current masthead page.

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