Turning now to the CD spectrum, we come to the major objective of this work. It is clear from Figure 4 that there is a mirror-image relationship between the spectra of the enantiomorphic molecules, due allowance being made for a slightly sloping base line. The instrument used to measure these spectra is insensitive beyond about 620 nm, but it is clear that in the range 590-620 nm a CD band lying further to the red is being approached and that this is opposite in sign to the one at 490 nm. This relationship of opposite signs for the $\delta \rightarrow \delta^*$ and $\delta_{xy} \rightarrow \delta_{x^2-y^2}$ CD bands has been observed consistently in the past[5] and is to be expected theoretically.

All previous theorizing about the relationship between the absolute chirality of a twisted $Mo_2L_nL'_{8-n}$ molecule and the sign of the CD band for the $\delta \rightarrow \delta^*$ transition can be summarized in the sector diagram at the top of Figure 8. To apply this diagram, we consider only the two sets of four-coordinated atoms, one set on each metal atom. The diagram shows the upper metal atom and its four ligands. If the lower ML_4 group is rotated so that the M-L bonds are no longer directly under those shown for the upper ML_4 group, the sign of the $\delta \rightarrow \delta^*$ CD band is given by the signs of the sectors in which they fall. Two important points are (1) the presence of chains of atoms connecting upper and lower L atoms and the positions of those chains are irrelevant, since the chirality at the chromophore is dominated by the eight coordinated atoms, and (2) a twist angle of χ and one of $\chi - 90°$ have the same effect on the sign of the CD.

The $Mo_2Cl_4(DIOP)_2$ molecules studied here are the first ones of the general class represented in Figure 1b to have χ between 45° and 90°. Figure 8 also shows axial views of the two isomers of $Mo_2Cl_4[(R,R)\text{-DIOP}]_2$. According to the sector diagram the Δ and Λ forms of such a molecule should show - and + CD bands, respectively, for the $\delta \rightarrow \delta^*$ transition. Since a solution of $Mo_2Cl_4[(R,R)\text{-DIOP}]_2$ contains 1.7 times as many P molecules as S molecules, the rule predicts that the solution should show a net positive CD band for the $\delta \rightarrow \delta^*$ transition and, for the reason mentioned earlier, a negative CD band for the transition at 490 nm. This is what is observed, as Figure 4 shows.

Concluding Remarks. The work reported here has benefited from two pleasant surprises, namely that the compound (which contains two fused eight-membered rings) could actually be prepared and that it displayed a mean torsion angle in the 45° $< \chi <$ 90° range. Both of these features may be attributed to the conformational restraints imposed by shape and rigidity of the incorporated five-membered dioxo ring. This helps to overcome the entropic factor disfavoring the closure of such a large ring and also imposes restraints that lead to the large torsion angles about the Mo-Mo bond. Still another surprise was that this molecule was not only able to exist in two conformationally isomeric forms in solution, but that in the crystal they were able to coexist and share the same set of sites, just as had much simpler $\beta\text{-}M_2X_4(LL)_2$ molecules where there was no chirality inherent in the ligands. All of these features taken together have enabled us to acquire information about $\beta\text{-}M_2X_4(LL)_2$ molecules that had heretofore been inaccessible.

The results obtained here also suggest some further studies that we shall attempt to carry out. One of the most interesting will be to examine the NMR spectra of other $\beta\text{-}M_2X_4(diphos)_2$ in solution to see if they, too, exist as mixtures of two isomers. In all previous discussions it has been tacitly assumed that for a compound like $Mo_2Cl_4[(S,S)\text{-dppb}]_2$ where only one isomer occurred in the crystal,[4] only this one isomer would be present in solution. It is now clear that this need not be true. From what we have learned in the present study, it seems certain that should a second isomer be present, we shall be able to detect it by NMR. It is also now of interest, and clearly feasible, to see if other $\beta\text{-}Mo_2X_4(diphos)_2$ molecules that do show two isomers in the crystal will continue to do so in solution, and also to determine the ratio. Once ratios of isomers for the resolved chiral compounds are known, it may be possible to obtain quantitative relationships between the rotational strengths of CD bands and the internal twist angles.

Acknowledgment. We thank the National Science Foundation for support.

Supplementary Material Available: Complete tables of anisotropic thermal parameters, bond distances, bond angles, torsional angles for the central portion of the molecule, and distances and angles of selected protons of $\Delta\text{-}Mo_2Cl_4[(R,R)\text{-DIOP}]_2$ and $\Lambda\text{-}Mo_2Cl_4[(R,R)\text{-DIOP}]_2$ (16 pages); listings of observed and calculated structure factors (32 pages). Ordering information is given on any current masthead page.

Electron-Transfer Self-Exchange Kinetics of Cytochrome b_5

Dabney White Dixon,*,† Xiaole Hong,† Scott E. Woehler,† A. Grant Mauk,*,‡ and Bhavini P. Sishta‡

Contribution from the Department of Chemistry and Laboratory for Microbial and Biochemical Sciences, Georgia State University, Atlanta, Georgia 30303, and the Department of Biochemistry, University of British Columbia, Vancouver, British Columbia V6T 1W5, Canada.
Received December 12, 1988. Revised Manuscript Received September 13, 1989

Abstract: The electron-transfer self-exchange rate constant of trypsin-solubilized bovine liver microsomal cytochrome b_5 has been measured as a function of temperature and ionic strength. Calculations based on ^1H NMR spectra and using the inversion-recovery method determined this rate constant to be 2.6×10^3 M^{-1} s^{-1} [pH 7.0, μ = 0.1 M (sodium phosphate), 25 °C] with $\Delta H^* = 5.5$ kcal mol^{-1} and $\Delta S^* = -23$ eu (μ = 0.1-0.3 M). This rate constant increases with ionic strength, reaching a value of 4.5×10^4 M^{-1} s^{-1} at μ = 1.5 M. Analysis of the data in terms of Marcus theory gives a reorganization energy, λ, for self-exchange in the range 0.9-1.3 eV mol^{-1}. The components of the dipole moments through the exposed heme edge of the reduced and oxidized protein are estimated to be -280 and -250 D, respectively.

Metalloprotein electron-transfer reactions are fundamental phenomena that are characteristic of biological processes such as photosynthesis, oxidative phosphorylation, xenobiotic detoxification, and the catalytic cycles of several enzymes.[1] The rate of electron transfer between metalloproteins is a function of several factors including the thermodynamic driving force, distance between the donor and acceptor centers, reorganization energy of

†Georgia State University.
‡University of British Columbia.

(1) (a) Michel-Beyerle, M. E., Ed. *Antennas and Reactions Centers of Photosynthetic Bacteria*; Springer-Verlag: New York, 1985. (b) Hatefi, Y. *Annu. Rev. Biochem.* **1985**, *54*, 1015. (c) Blumberg, W. E. *Q. Rev. Biophys.* **1978**, *4*, 481. (d) Dreyer, J. L. *Experientia* **1984**, *40*, 653. (e) Pettigrew, G. W.; Moore, G. R. *Cytochromes c. Biological Aspects*; Springer-Verlag: New York, 1987; p 29.

0002-7863/90/1512-1082$02.50/0

the system, and the nature of medium intervening between the donor and acceptor centers.[2] Recently, conformational change has also been emphasized as a possible controlling factor for biological electron transfer.[3]

Historically, the study of electron-transfer self-exchange kinetics has played a seminal role in the understanding of reaction mechanisms of both inorganic complexes and metalloproteins.[4] Following the pioneering work of Kowalsky, Redfield, and Gupta on cytochrome *c*,[5] the electron-transfer self-exchange kinetics of several metalloproteins have been analyzed.[6] The current report addresses this issue through analysis of the ionic strength and temperature dependence of the electron-transfer self-exchange kinetics of the tryptic fragment of bovine liver microsomal cytochrome b_5.

Several considerations make cytochrome b_5 an excellent candidate for such analysis. For example, cytochrome b_5 fulfills a pivotal electron-transfer role in stearyl-CoA desaturation,[7] the cytochrome P-450 catalytic cycle,[8] and maintenance of hemoglobin[9] and hemerythrin[10] in the functional, reduced (Fe(II)) state. In addition, the three-dimensional structures of the lipase-solubilized, bovine liver microsomal form of both ferri- and ferrocytochrome b_5 have been determined.[11] Finally, synthetic genes coding for the erythrocytic forms of rat[12] and bovine[13] cytochrome b_5 have recently been prepared and expressed efficiently in *E. coli*. These latter efforts allow the possibility of producing specifically mutated forms of this protein for the purpose of characterizing those structural elements that regulate the rate of electron transfer demonstrated by cytochrome b_5 in various situations. The eventual combination of crystallographic, molecular genetics, and NMR techniques in the study of the cytochrome b_5 electron-transfer self-exchange reaction should prove to be critical for understanding the potentially more complex processes in which cytochrome b_5 transfers electrons to other metalloproteins[2b,d,14] or inorganic complexes.[15]

Table I. Kinetic Parameters for Electron Self-Exchange of Cytochrome b_5

	ionic strength, M; [cytochrome b_5], mM		
	0.3; 3.1	0.3; 1.0	0.1; 1.0
	Self-Exchange Rate Constants, $\times 10^{-3}$ M^{-1} s^{-1}		
temp, °C			
10.0	3.1		
20.0	4.0	3.7	
25.0	4.5	4.6	2.6
30.0	6.6	5.0	3.4
35.0		6.2	3.9
40.0	7.9	7.6	4.4
	Activation Parameters		
$\Delta H^{\ddagger}$, kcal mol^{-1}	5.2 ± 1.0	5.8 ± 0.5	5.6 ± 0.9
$\Delta S^{\ddagger}$, eu	−24.0 ± 3.0	−22.4 ± 1.5	−23.3 ± 1.1
$\Delta G^{\ddagger}$,[a] kcal mol^{-1}	12.4 ± 1.4	12.5 ± 1.3	12.5 ± 1.4

[a] 25 °C.

Experimental Section

Protein Purification and Sample Preparation. Cytochrome b_5 was isolated from bovine liver microsomes following tryptic solubilization to a purity ratio ($A_{412.5}/A_{280}$) of ≥5.9.[15a,16] After kinetic measurements, the protein was re-cycled (4 °C) by loading the protein solution (ca. 0.5 mL of 3 mM protein) onto a DEAE-cellulose anion exchange column (DE-52, Whatman BioSystems Ltd.) (5 mm × 65 mm) that had been equilibrated with 0.02 M pH 7.2 (4 °C) sodium phosphate buffer. The column was then washed with 0.02 M pH 7.2 sodium phosphate buffer (ca. one column volume of buffer), and the cytochrome was eluted from the column with 0.15 M pH 7.2 sodium phosphate buffer in a volume of 6–8 mL. Ferricytochrome b_5 concentration was determined by elec-

(2) (a) Scott, R. A.; Mauk, A. G.; Gray, H. B. *J. Chem. Educ.* **1985**, *62*, 932. (b) Mayo, S. L.; Ellis, W. R., Jr.; Crutchley, R. J.; Gray, H. B. *Science* **1986**, *233*, 948. (c) McLendon, G. *Acc. Chem. Res.* **1988**, *21*, 160. (d) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265. (e) Dixon, D. W. In *Molecular Structure and Energetics: Principles of Enzyme Activity*; Liebman, J. F., Greenberg, A., Eds.; Elsevier: New York, 1988; p 169.

(3) Hoffman, B. M.; Ratner, M. A. *J. Am. Chem. Soc.* **1987**, *109*, 6237.

(4) (a) Sutin, N. *Inorganic Biochemistry*; Eichhorn, G. l., Ed.; Elsevier: New York, 1973; p 651. (b) Sutin, N. *Bioinorganic Chemistry II*; Raymond, K. N.; American Chemical Society: Washington, DC, 1977; ACS Adv. Chem. Ser. 162, p 156. (c) Wherland, S.; Gray, H. B. In *Biological Aspects Inorganic Chemistry*; Addison, A. W., Cullen, W. R., Dolphin, D., James, B. R., Eds.; Wiley: New York, 1978; p 289.

(5) (a) Kowalsky, A. *Biochemistry* **1965**, *4*, 2382. (b) Gupta, R. K.; Redfield, A. G. *Science* **1970**, *169*, 1204. (c) Gupta, R. K.; Koenig, S. H.; Redfield, A. G. *J. Magn. Reson.* **1972**, *7*, 66. (d) Oldfield, E.; Allerhand, A. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 3531. (e) Gupta, R. K. *Biochim. Biophys. Acta* **1973**, *292*, 291.

(6) (a) Phillips, W. D.; McDonald, C. C.; Stombaugh, N. A.; Orme-Johnson, W. H. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 140. (b) Keller, R. M.; Wüthrich, K.; Pecht, I. *FEBS Lett.* **1976**, *70*, 180. (c) Beattie, J. K.; Fensom, D. J.; Freeman, H. C.; Woodcock, E.; Hill, H. A. O.; Stokes, A. M. *Biochim. Biophys. Acta* **1975**, *405*, 109. (d) Dahlin, S.; Reinhammar, B.; Wilson, M. T. *Inorg. Chim. Acta* **1983**, *79*, 126. (e) Dahlin, S.; Reinhammar; Wilson, M. T. *Biochem. J.* **1984**, *218*, 609. (f) Timkovich, R.; Cork, M. S. *Biochemistry* **1984**, *23*, 851. (g) Timkovich, R.; Cork, M. S.; Taylor, P. V. *Biochemistry* **1984**, *23*, 3536. (h) Groeneveld, C. M.; Canters, G. W. *Eur. J. Biochem.* **1985**, *153*, 559. (i) Armstrong, F. A.; Driskcoll, P. C.; Hill, H. A. O. *FEBS Lett.* **1985**, *190*, 242. (j) Ûgürbil, K.; Mitra, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 2039. (k) Moore, G. R.; Williams, R. J. P.; Peterson, J.; Thomson, A. J.; Mathews, F. S. *Biochim. Biophys. Acta* **1985**, *829*, 83. (l) Concar, D. W.; Hill, H. A. O.; Moore, G. R.; Whitford, D.; Williams, R. J. P. *FEBS Lett.* **1986**, *206*, 15. (m) Groeneveld, C. M.; Dahlin, S.; Reinhammar, B.; Canters, G. W. *J. Am. Chem. Soc.* **1987**, *109*, 3247. (n) Groeneveld, C. M.; Canters, G. W. *J. Biol. Chem.* **1988**, *263*, 167. (o) Groeneveld, C. M.; Ouwerling, M. C.; Erkelens, C.; Canters, G. W. *J. Mol. Biol.* **1988**, *200*, 189. (p) Timkovich, R.; Cai, M. L.; Dixon, D. W. *Biochem. Biophys. Res. Commun.* **1988**, *150*, 1044. (q) Lommen, A.; Canters, G. W.; Van Beeumen, J. *Eur. J. Biochem.* **1988**, *176* 213. (r) See also references cited in: Shirazi, A.; Barbush, M.; Ghosh, S.; Dixon, D. W. *Inorg. Chem.* **1985**, *24*, 2495.

(7) Strittmatter, P.; Spatz, L.; Corcoran, D.; Rogers, M. J.; Setlow, B.; Redline, R. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4565.

(8) (a) Estabrook, R. W.; Hildebrandt, A. G.; Baron, J.; Nettor, K. J.; Liebman, K. *Biochem. Biophys. Res. Commun.* **1971**, *42*, 132. (b) Peterson, J. A.; Prough, R. A. *Cytochrome P-450. Struture, Mechanism, and Biochemistry*; Ortiz de Montellano, P. R., Ed.; Plenum Press: New York, 1986; p 89.

(9) (a) Hultquist, D. E.; Passon, P. G. *Nature (London), New Biol.* **1971**, *229*, 1252. (b) Sannes, L. G.; Hultquist, D. E. *Biochim. Biophys. Acta* **1978**, *544*, 547. (c) Hegesh, E.; Hegesh, J.; Kaftory, A. N. *Engl. J. Med.* **1986**, *314*, 757.

(10) Utecht, R. E.; Kurtz, D. M., Jr. *Biochim. Biophys. Acta* **1988**, *953*, 164.

(11) (a) Mathews, F. S.; Levine, M.; Argos, P. *J. Mol. Biol.* **1972**, *64*, 449. (b) Argos, P.; Mathews, F. S. *J. Biol. Chem.* **1975**, *250*, 747. (c) Mathews, F. S.; Czerwinski, E. W. *The Enzymes of Biological Membranes*; Martonosi, A. E., Ed.; Plenum Press: New York, 1976; Vol. 4, p 143. (d) Mathews, F. S.; Czerwinski, E. W., Argos, P. *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. VII, p 107. (e) Mathews, F. S. *Biochim. Biophys. Acta* **1980**, *622*, 375. (f) Mathews, F. S.; Czerwinski, E. W. In *Enzymes of Biological Membranes* 2nd ed.; Martonosi, A. N., Ed.; Plenum: New York, 1986; Vol. 4, p 235.

(12) von Bodman, S. B.; Schuler, M. A.; Jollie, D. R.; Sligar, S. G. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 9443.

(13) Funk, W. D.; Mauk, M. R.; Mauk, A. G.; MacGillivray, R. T. A. M. Unpublished material.

(14) Eltis, L.; Mauk, A. G.; Hazzard, J. T.; Cusanovich, M. A.; Tollin, G. *Biochemistry* **1988**, *27*, 5455.

(15) (a) Reid, L. S.; Mauk, A. G. *J. Am. Chem. Soc.* **1982**, *104*, 841. (b) Chapman, S. K.; Davies, D. M.; Vuik, C. P. J.; Sykes, A. G. *J. Am. Chem. Soc.* **1984**, *106*, 2692. (c) Reid, L. S.; Mauk, M. R.; Mauk, A. G. *J. Am. Chem. Soc.* **1985**, *106*, 2182. (d) Reid, L. S.; Lim, A. R.; Mauk, A. G. *J. Am. Chem. Soc.* **1987**, *108*, 8197. (e) Davies, D. M.; Lawther, J. M. *J. Chem. Soc., Chem. Commun.* **1986**, 385.

(16) This form of the protein consists of 82 amino acid residues and differs from the lipase-solubilized form that has been characterized crystallographically[11] by removal of two residues from the amino terminus and 9 residues from the carboxyl terminus. The electrostatics calculations performed in this work are based on the corrected sequence for the trypsin-solubilized form as revealed by the sequence of a bovine liver cytochrome b_5 cDNA clone (Christiano, A. B.; Steggles, A. W. *Nucl. Acids Res.* **1989**, *17*, 799) and by gas-phase sequence analysis of tryptic peptides obtained from a tryptic hydrolysate of apocytochrome b_5.[13] The corrected sequence differs from the reported sequence (Ozols, J.; Strittmatter, P. *J. Biol. Chem.* **1969**, 244, 6617) in that residue 57 is now known to be Asn and residues 11 and 13 are known to be Glu and Gln, respectively (based on the sequence numbering convention of Mathews and co-workers[11]).

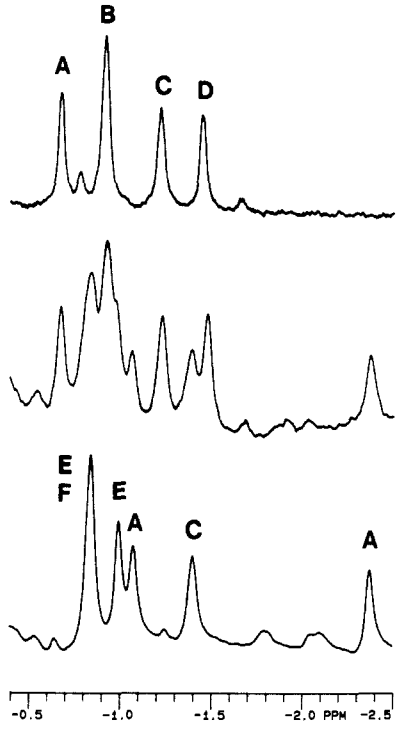


Figure 1. Upfield region of the NMR spectrum of cytochrome b_5: top, Fe(II); middle, Fe(II) and Fe(III) mixture; bottom, Fe(III). Assignments of the methyl group resonances: A, Leu-25; B, Leu-46; C, Ile-76; D, Val-61; E, Leu-23; F, Leu-32.[18a]

tronic spectroscopy with a Bausch & Lomb 2000 spectrophotometer based on $\epsilon_{412.5} = 117\,000$.[17]

The purified cytochrome (ca. 2 mL) was exchanged into D_2O following concentration (to ca. 0.15 mL) by centrifugation (1 h at 6000 g; Sorvall RC2B centrifuge (4 °C)) in a Centricon-10 microconcentrator (Amicon). After being diluted with approximately 1 mL of $\mu = 0.1$ M sodium phosphate, pH 7.0 D_2O (99.8% D) buffer, the sample was reconcentrated with the Centricon. This procedure was repeated at least 3 times. Residual [HOD] after this treatment was <0.5%. The resulting solution was transferred into an NMR tube, diluted to about 0.5 mL with sodium phosphate buffer ($\mu = 0.1$ M, pH 7.0) (99.96% D), and capped with a serum stopper (Wilmad No. 526-PP) or septum (Wilmad No. 528-TR). The solution was bubbled with nitrogen for 8–10 min in the NMR tube prior to data collection. Samples with ionic strength higher than 0.1 M were prepared by direct addition of NaCl to the NMR tube. Ferricytochrome b_5 was reduced by the anaerobic addition of solid $Na_2S_2O_4$ (Fischer) directly into the NMR tube in a nitrogen-filled glovebox.

Kinetic Measurements. NMR measurements were performed 20–120 min after preparation of the ferri-/ferrocytochrome b_5 samples. The ratio of oxidized to reduced protein was determined from the integrated areas of the two forms of the protein in the NMR spectrum. Measurements of a single rate constant required about 6 h, during which time the ratio of Fe(II)/Fe(III) cytochrome b_5, as calculated from the peaks in the region just upfield of 0 ppm, was stable to better than ±10%. In this region of the spectrum, peaks from the oxidized and reduced protein are in slow exchange at 400 MHz.

Trypsin-solubilized cytochrome b_5 is a mixture of two isomers that differ in the orientation of heme binding to the apoprotein through 180° rotation around the α–γ meso-carbon axis.[18] At equilibrium, the two forms are present in about a 10:1 ratio. The rate of isomer interconversion is very slow on the time scale of electron exchange ($t_{1/2} = 30$ h at pH 7.0 and 25 °C for ferricytochrome b_5[18c]). The rate constants reported here were measured on the major isomer and assume that the rates exhibited by the major and minor isomers are identical. The minor isomer was not investigated because the Fe(II)/Fe(III) cytochrome b_5 ratio did not remain constant over the prolonged data acquisition times required to measure rate constants on the low concentration of the minor component. The rate constants determined in this study were found to be independent of protein concentration within experimental error.

(17) Ozols, J.; Strittmatter, P. *J. Biol. Chem.* **1964**, *239*, 1018.

(18) (a) Keller, R. M.; Wüthrich, K. *Biochim. Biophys. Acta* **1980**, *621*, 204. (b) La Mar, G. N.; Burns, P. D.; Jackson, J. T.; Smith, K. M.; Langry, K. C.; Strittmatter, P. *J. Biol. Chem.* **1981**, *256*, 6075. (c) McLachlan, S. J.; La Mar, G. N.; Burns, P. D.; Smith, K. M.; Langry, K. C. *Biochim. Biophys. Acta* **1986**, *874*, 274.

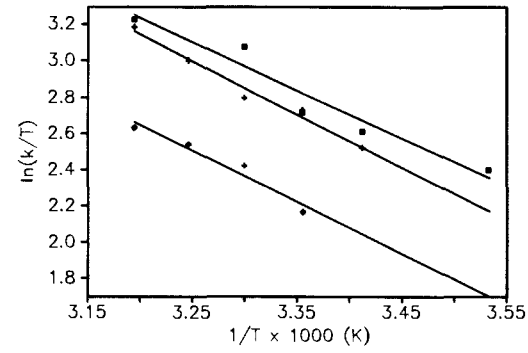


Figure 2. Eyring plots of the cytochrome b_5 electron-transfer self-exchange rate constants at various protein concentrations and ionic strengths: (■) $\mu = 0.3$ M, [cytochrome b_5] = 3.1 mM; (+) $\mu = 0.3$ M, [cytochrome b_5] = 1.0 mM; (♦) $\mu = 0.1$ M, [cytochrome b_5] = 1.0 M.

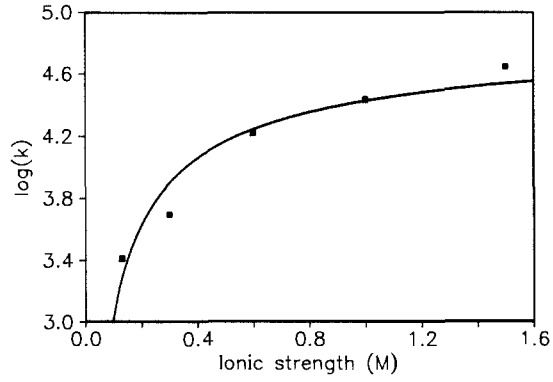


Figure 3. The ionic strength dependence of the cytochrome b_5 electron-transfer self-exchange rate constant (pH 7.0 (phosphate), 25 °C). The solid line is a best fit to the data for $Z_{ox} = -7.5$ and $Z_{red} = -8.5$. The fitted values are $D'_{ox} = -250$, $D'_{red} = -280$, and $k_{inf} = 3.7 \times 10^5$ M^{-1} s^{-1} where the dipole moments are the component of the dipole moment through the exposed heme edge.

Proton NMR spectra were recorded on a Varian VXR-400 spectrometer operating at a magnetic field of 399.954 MHz. The NMR probe temperature was calibrated to ±0.4 °C with use of a methanol thermometer[19] and regulated to ±0.1 °C in the range 5.0–60.0 °C. Rate constants were measured with use of an inversion-recovery pulse sequence in which a 180° selective pulse was applied through the decoupler. The strength of the 3-ms pulse was 122 Hz, calibrated via the Bloch-Siegert shift. The details of the method are described elsewhere.[20]

Results

Relaxation Measurements and Kinetics. A mixture of oxidized and reduced cytochrome b_5 undergoes electron transfer in the slow exchange time limit, as shown in Figure 1, where resonances from both the oxidized and reduced protein are seen clearly in a mixture of the two. In particular, both of the Leu-25 methyl resonances from the oxidized protein and one of the resonances from the reduced protein are seen in a 2-ppm range in the mixture of the two oxidation states. Selective inversion recovery techniques were used to measure the rate constant for electron self-exchange.[20] Measurement of the recovery after inversion of the heme ring methyl groups of the ferric form of the protein allowed calculation of the rate constant, which at 25 °C and 0.1 M ionic strength is 2.6×10^3 M^{-1} s^{-1}. Cytochrome b_5 self-exchange rate constants obtained for a variety of other solution conditions are set out in Table I.

Thermodynamic Studies. The temperature dependence of the cytochrome b_5 electron self-exchange rate constants was studied at three combinations of protein concentration and ionic strength (Figure 2). The activation parameters derived from linear

(19) van Geet, A. L. *Anal. Chem.* **1968**, *40*, 2227.

(20) (a) Dixon, D. W.; Hong, X.; Woehler, S. E. *Biophys. J.* **1989**, *56*, 339–351. (b) Dixon, D. W.; Hong, X. *Adv. Chem. Ser.*, in press.

least-squares fits to these data are set out in Table I. The values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ at 0.1 M ionic strength and 1.0 mM protein are 5.6 ± 0.9 kcal mol^{-1} and −23.3 ± 1.1 eu, respectively. The activation parameters measured at somewhat different concentrations of protein or ionic strength were very similar. Data were collected over the temperature range 10–45 °C. The upper limit was restricted by the formation of a high-spin form of cytochrome b_5 above 45 °C.[21,22] The lower limit was dictated by the magnitude of the rate constants. The inversion-recovery technique requires that the rate of the reaction is comparable to the T_1 of the peak of interest; at temperatures lower than 10 °C, electron exchange contributes too little to the relaxation to satisfy this requirement.

Electrostatic Studies. The ionic strength dependence of the cytochrome b_5 self-exchange rate is shown in Figure 3. As seen from this figure, the rate constants for cytochrome b_5 electron self-exchange increase monotonically with ionic strength, as expected for a reaction between two similarly charged proteins. Several proposals have been advanced for treating the ionic strength dependence of electron-transfer reactions between two proteins. van Leeuwen's approach[23] has proven to be effective at the high ionic strengths needed for NMR studies and to be readily implemented.[24,25] This method treats the electrostatics in terms of monopole–monopole, monopole–dipole, and dipole–dipole interactions and assumes that electron transfer occurs at the partially exposed heme edge. Consequently, the component of the dipole through the heme edge is required. This value can be obtained through application of the following relationships:

$$\ln (k_1/k_{inf}) = -\{Z_{ox}Z_{red} + (ZD)(1 + \kappa r) + (DD)(1 + \kappa r)^2\}(q^2/4\pi\epsilon_0\epsilon kTr)f(\kappa) \quad (1)$$

$$ZD = (Z_{ox}D'_{red} + Z_{red}D'_{ox})/qr \quad (1a)$$

$$DD = D'_{ox}D'_{red}/(qr)^2 \quad (1b)$$

$$f(\kappa) = (1 - \exp(-\kappa r))/\kappa r(1 + \kappa r/2) \quad (1c)$$

Z_{ox} and Z_{red} are the net charges of the oxidized and reduced protein, D'_{ox} and D'_{red} are the components of the dipole moments through the exposed heme edge, r is the sum of the radii of the two electron transfer partners, κ is 0.329 $\mu^{1/2}$, k_1 is the rate constant at a given ionic strength, and k_{inf} is the rate constant at infinite ionic strength. A fit of the current data to these equations gives $D'_{ox} = -250$, $D'_{red} = -280$ D, and $k_{inf} = 3.7 \times 10^5$ M^{-1} s^{-1} when the ionic strength is calculated on the basis of small ion concentrations plus the charge on the protein multiplied by the protein concentration. The van Leeuwen formalism also allows calculation of an interaction energy, w_r, of 3.1 kcal mol^{-1} [$w_r = -RT \ln (k_1/k_{inf})$] for the two proteins in a heme edge-to-heme edge geometry, presumed to be the favored geometry for electron transfer.

Discussion

Eyring Parameters. The insensitivity of the activation parameters to protein concentration establishes that over the range of protein concentration studied, the contribution of the protein to the effective ionic strength is not a kinetically significant factor. While the activation parameters might be expected to vary as a function of ionic strength, the values determined in the current study for μ = 0.1 and 0.3 M (a relatively narrow range) are within experimental error of each other. We note that the values of $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ observed here are remarkably similar to those reported for several other electron-transfer reactions of cytochrome b_5 (Table II), though the origin of this similarity is not apparent at present.

Table II. Thermodynamic Parameters for Cytochrome b_5 Electron-Transfer Reactions[a]

reaction	$\Delta H^{\ddagger}$	$\Delta S^{\ddagger}$	$\Delta G^{\ddagger}$
self-exchange	5.5	−23	12.4
DME-cytochrome b_5/Fe^{II}(EDTA)[b]	4.5	−30	13.4
deuterocytochrome b_5/Fe^{II}(EDTA)[c]	6.8	−26.2	14.6
cytochrome b_5/Fe^{II}(EDTA)[d]	5.4	−29.2	14.1
cytochrome b_5/Fe^{III}(NTA)[e]	6.7	−21.7	13.2
cytochrome b_5/Co^{III}(EDTA)[f]	8.6	−24.4	15.9

[a] All values listed have the units kcal/mol (free energies and enthalpies) or eu (entropies). The $\Delta G^{\ddagger}$ values are calculated at 25 °C. [b] Reid, L. S.; Mauk, M. R.; Mauk, A. G. *J. Am. Chem. Soc.* **1984**, *106*, 2182. [c] Reid, L. S.; Lim, A. R.; Mauk, A. G. *J. Am. Chem. Soc.* **1986**, *108*, 8197. [d] Reid, L. S.; Mauk, A. G. *J. Am. Chem. Soc.* **1982**, *104*, 841. [e] Reid, L. S.; Gray, H. B.; Dalvit, C.; Wright, P. E.; Saltman, P. *Biochemistry* **1987**, *26*, 7102. [f] Chapman, S. K.; Davies, D. M.; Vuik, C. P. J.; Sykes, A. G. *J. Am. Chem. Soc.* **1984**, *106*, 2692.

The electron-transfer self-exchange rate constant for the form of cytochrome b_5 studied here is remarkably similar to that previously reported for horse heart cytochrome *c* by others.[5,20] Assuming that the self-exchange reaction for both cytochromes occurs at the partially exposed heme edge, it is unexpected that the rates exhibited by the two proteins are so similar because the heme group in cytochrome b_5 is significantly more exposed to solvent than is the heme group in cytochrome *c*.[2,20,26] Possible reasons for the similarity in rate constants are discussed below.

Cytochrome b_5 Reorganization Energy. The rate of electron transfer is determined in part by the reorganization energy of the reaction, e.g., the energy required for the oxidized partner to assume the geometry of the reduced partner and vice versa. The reorganization energy can be calculated from the dependence of the rate constant on temperature, from the dependence of the rate constant on driving force, or from the crystal structures of the oxidized and reduced protein. Given an expression for the factors that control the electron self-exchange rate constant, the reorganization energy can also be calculated from the rate constant at a given ionic strength and temperature. We have used this last approach in calculating the reorganization energy for the cytochrome b_5 self-exchange reaction.

The cytochrome b_5 self-exchange rate constant can be expressed as[2d]

$$k_{et} = SK_a\nu_n\kappa_{el}\exp(-\Delta G_r^{*}/RT) \quad (2)$$

where S is the steric factor, which reflects the hypothesis that electron transfer occurs primarily at the exposed heme edge, K_a is the association constant for formation of the precursor state from the two separated electron-transfer partners, ν_n is the nuclear frequency factor, κ_{el} is the probability of electron tunneling once the nuclear transition state has been formed, and ΔG_r^{*} is the free energy of activation (the sum of both inner-sphere and outer-sphere reorganization energies).

A value for K_a, the association constant of the two cytochromes, can be estimated by calculating the effective volume over which the reaction occurs along the reaction coordinate multiplied by an electrostatic work term [$\exp(-w_r/RT)$]

$$K_a = 4\pi Nr^2\delta(r)\exp(-w_r/RT) \quad (3)$$

where N is Avogadro's number, r is the sum of the radii of the two proteins, and $\delta(r)$ is the range of internuclear separations that contribute significantly to the reaction rate. The value for $\delta(r)$ is usually taken as β^{-1}, the distance at which the electron-transfer rate constant decreases to 1/e of its value in the heme edge-to-heme edge complex. With the use of the van Leeuwen formalism described above, the work necessary to bring the two species to an encounter complex at an ionic strength of 0.1 M is 3.1 kcal mol^{-1} for the heme edge-to-heme edge cytochrome b_5/cytochrome b_5 complex. Therefore, for r = 31.8 Å and $\delta(r)$ = 1.11 Å, we calculate K_a = 0.045 M^{-1} (Table III).[27]

(21) Sugiyama, T.; Miki, N.; Miura, R.; Miyake, Y.; Yamano, T. *Biochim. Biophys. Acta* **1982**, *706*, 42.
(22) Bendzko, P.; Pfeil, W. *Biochim. Biophys. Acta* **1983**, *742*, 669.
(23) van Leeuwen, J. W. *Biochim. Biophys. Acta* **1983**, *743*, 408.
(24) (a) Rush, J. D.; Lan, J.; Koppenol, W. H. *J. Am. Chem. Soc.* **1987**, *109*, 2679. (b) Rush, J. D.; Koppenol, W. H. *Biochim. Biophys. Acta* **1988**, *936*, 187.
(25) Dixon, D. W.; Barbush, M. In preparation.
(26) Stellwagen, E. *Nature* **1978**, *275*, 73.

Table III. Calculation of Reorganization Energies from $k_{et} = SK_a\nu_n\kappa_{el}\exp(-G_r^*/RT)$[a]

	cytochrome *c*	cytochrome b_5
$\text{heme}_{\text{surface area}}/\text{protein}_{\text{surface area}}$	0.007	0.038
steric factor, *S*	0.0012	0.036
radius, Å	16.6	15.9
$4\pi r^2 dr$	9.27	8.50
work, kcal/mol	2.7	3.1
K_a	0.097	0.045
heme–heme distance	8.9	7.5
$\kappa_{el} = \exp(-\beta(d - d_0))$	4.9×10^{-3}	1.7×10^{-2}
$SK_a\nu_n\kappa_{el}$	5.9×10^{6}	2.9×10^{8}
k_{et}(exptl)[b]	5.1×10^{3}	2.7×10^{3}
ΔG_r^*, kcal/mol	4.2	6.9
λ, eV	0.72	1.2

[a] μ = 0.1 M, 25 °C. [b] Cytochrome *c* data from ref 20.

Given K_a, the quantity SK_a is the equilibrium constant for formation of the electron-transfer complex in which the electron-transfer sites of the partners are in contact. The steric factor *S* accounts for the fact that electron transfer to the cytochrome has a strong angular dependence, occurring largely at the exposed heme edge. If we use the approximation that electron transfer occur only through the exposed heme edge, and if electron transfer is slow relative to the diffusion of the two partners, then in the simplest picture the cytochromes will react only when the two exposed hemes are in contact and *S* can be set equal to the square of the fraction of the protein surface area that is heme.[2d] For cytochrome b_5, the fraction of the protein surface area that is heme is 0.038, calculated from the crystal structure using a probe sphere of 1.5 Å and the algorithms in the BIOGRAF software.[28c] However, as Marcus and Sutin have pointed out, electron transfer can take place from a variety of distances and orientations and it is necessary to integrate over all of these. To account for this, we have multiplied the exposure of each heme by a factor of 5.[29] These assumptions give a value for *S* of 0.036. Cytochrome *c*, which has a much less exposed heme edge,[20,26] has an *S* value of 0.0012.

When the reaction is adiabatic, κ_{el} is 1. However, self-exchange reactions of cytochromes need not be adiabatic because local protein structure may prevent close approach of the two heme edges.[2d] Electron transfer is thought to fall off exponentially with distance. In this case, $\nu_n\kappa_{el}$ can be expressed as

$$\nu_n\kappa_{el} = 10^{13}\exp[-\beta(d - d_0)] \quad (4)$$

where *d* is the closest heme–heme distance, d_0 is the value of *d* at which $\kappa_{el} = 1$, and β varies with the system but is close to 0.9 $Å^{-1}$ for cytochrome *c*.[30]

(27) The association constant can, in some instances, be measured directly. The ^1H NMR resonances of cytochrome b_5 show small changes in chemical shift and line width as a function of concentration.[20a] If these changes are due to dimerization, then one can estimate an association constant of 25–30 M^{-1}. The value of 25–30 M^{-1} is not the K_a of eq 3 in the present analysis, because the latter includes the term $\exp(-w_r/RT)$ which in the van Leeuwen formalism is a work term for formation of the heme edge-to-heme edge complex. The value of 25–30 M^{-1} is best compared with the value of $4\pi r^2\delta r$ of 8.5 in eq 3; these are in good agreement. It also must be remembered the NMR changes as a function of concentration may not be due to dimerization. At the high end of NMR concentrations the solution is as much as 4% by volume protein; small spectral changes may be due to factors other than specific protein–protein interactions at these high protein concentrations.

(28) (a) Connolly, M. E. *J. Appl. Crystallogr.* **1983**, *16*, 548. (b) Connolly, M. E. *Science* **1983**, *221*, 709. (c) BIOGRAF Version 1.40; BioDesign, 199 S. Los Robles Ave., Suite 270, Pasadena, CA 91101.

(29) Marcus and Sutin have estimated the value of *S* as 0.01 for cytochrome *c*.[2d] Because the surface of cytochrome *c* is 0.7% heme, this value of *S* translates to enhancement of electron transfer by an additional factor of 15. We have looked in detail at the steric effects on electron self-exchange in cytochromes *c*, c_{551}, and b_5.[20a] The model assumed that electron transfer falls off exponentially with distance, that the orientation of the two hemes and the nature of the intervening residues had no effect on the electron-transfer rate constant, and that electron transfer occurred only at the surface of the protein. Given these assumptions, electron transfer at the heme edge accounts for 40% (cytochrome *c*) to 80% (cytochrome b_5) of the total electron transfer. These values correspond to enhancements of 2.4 for cytochrome *c* and 1.3 for cytochrome b_5. In the present work we have chosen an enhancement factor of 5, between the upper and lower estimates described above.

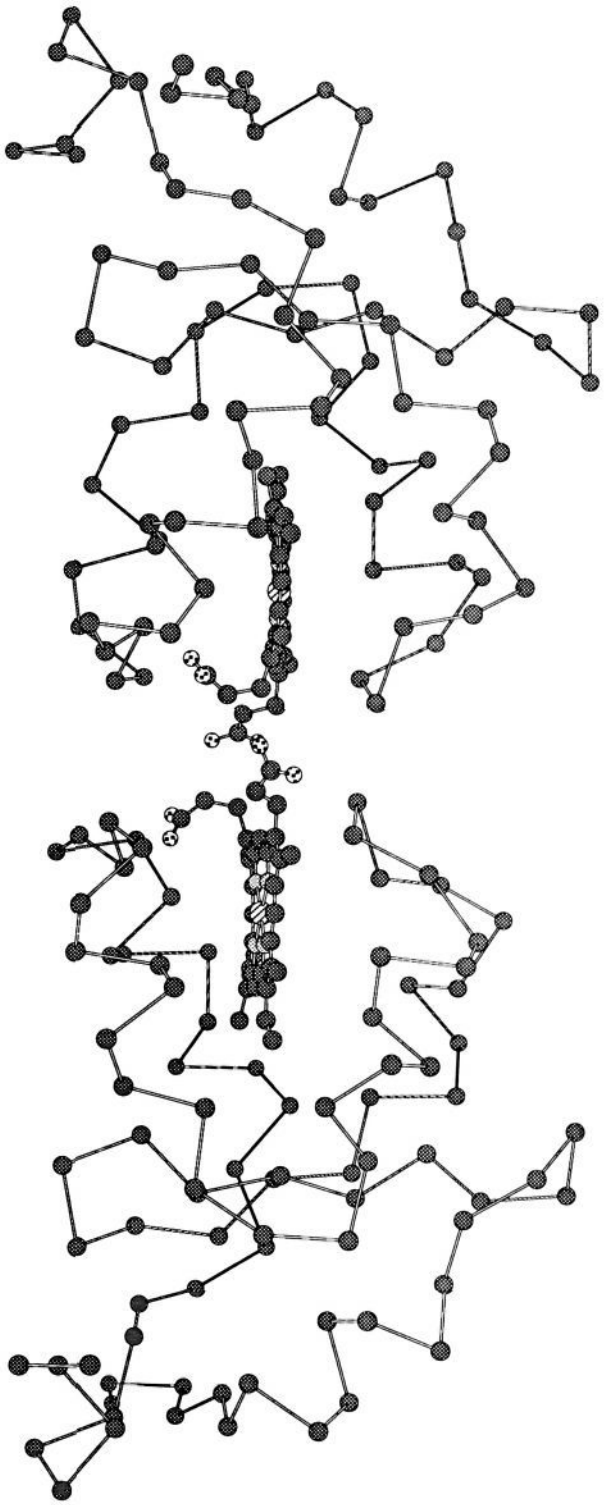


Figure 4. Heme edge-to-heme edge geometry of two cytochromes b_5.

Figure 4 shows a model for the cytochrome b_5–cytochrome b_5 self-exchange complex. We have obtained an estimate for d in this complex by docking the two proteins in the heme edge-to-heme edge geometry and minimizing to RMS = 0.6 (DREIDING force field in the BIOGRAF software).[28c] The closest approach (carbon-to-carbon distance) is 7.5 Å. Using the standard value for d_0 of 3 Å,[2d,30] and a β of 0.9 $Å^{-1}$,[30] we calculate a value for $\nu_n\kappa_{el}$ of 1.74×10^{11} s^{-1}.

(30) Gray, H. B.; Malmström, B. G. Submitted for publication.

We have also performed similar calculations for cytochrome *c*. In this case, the heme is less exposed; the fraction of the surface of the protein that is heme is only 0.007. In addition, computer modeling of the heme-edge to heme-edge complex shows that the two hemes are slightly farther away in the cytochrome *c*/cytochrome *c* complex than in the cytochrome b_5/cytochrome b_5 complex, being 8.9 Å for cytochrome *c* and 7.5 Å for cytochrome b_5. Our cytochrome *c*/cytochrome *c* model is in very good agreement with that of Weber, in which a heme–heme distance of 9.4 Å was calculated with no minimization.[2d]

Having made estimates for S, K_a, ν_n, and κ_{el} we can now calculate ΔG_r^* from the rate constant at a given ionic strength and temperature. At 25 °C and μ = 0.1 M, the values of ΔG_r^* for cytochrome b_5 and cytochrome *c* are 6.9 and 4.2 kcal mol^{-1}, respectively (Table III).

Classical Marcus theory predicts that the rate constant for electron transfer will depend on the driving force for the reaction ($\Delta G°'$) and the reorganization energy of the reaction, λ, as

$$\Delta G_r^* = (\lambda/4)[1 + \Delta G°'/\lambda]^2 \quad (5)$$

where $\Delta G°'$ is given by

$$\Delta G°' = \Delta G° + w_p - w_r \quad (6)$$

Here, $\Delta G°$ is the free energy change of the reaction, and w_p and w_r represent the work required to bring the reactants and products to separation achieved in the electron-transfer complex. For self-exchange reactions, the terms w_p and w_r are equal to each other. When the thermodynamic driving force for the reaction, $\Delta G°$, is zero (as in the self-exchange reaction), $\lambda = 4\Delta G_r^*$. Therefore, the reorganization energies, λ, for the self-exchange reactions of cytochromes b_5 and *c* are 1.2 and 0.7 eV, respectively.

What is the numerical basis of the difference in reorganization energies between cytochromes *c* and b_5? Cytochromes *c* and b_5 are proteins of similar size and shape. In addition, the absolute values of the net charge and dipole moment are very similar for the two proteins, leading to very similar work terms.[20] However, the heme of cytochrome b_5 is far more exposed to solvent than that of cytochrome *c* and the closest approach between the two proteins is shorter for cytochrome b_5 than for cytochrome *c*. Both of these differences are numerically significant and lead to the prediction that the cytochrome b_5 electron self-exchange rate constant should be substantially greater than that of cytochrome *c*. However, the measured rate constants for the two proteins are very similar at a given ionic strength and temperature. The necessary consequence of the electron-transfer model given in eq 2 is that the reorganization energy is larger for cytochrome b_5 than for cytochrome *c*. As pointed out above, small changes in the fraction of the surface area of the protein that is heme are numerically significant because this parameter is squared in the calculation of S. For cytochrome b_5, a static model that assumes electron transfer only through the exposed heme edge leads to a calculated λ of 0.9 eV. On the other hand, fluctuations that would increase the heme exposure would result in a higher value for λ. In the present analysis, an increase in the heme exposure by a factor of 10 would lead to a λ of 1.3 eV. It is also possible that the two hemes can approach more closely in solution than is indicated by the docking of the crystal structures. Wendoloski et al. have run picosecond dynamics of the interaction between cytochrome *c* and cytochrome b_5 and found that the inter-iron distances in two simulations were 1.1 to 2.1 Å smaller than the 17.8-Å distance in the static model.[31] For the cytochrome b_5 self-exchange reaction, a decrease in the heme edge-to-heme edge of 2 Å would give a reorganization energy of 1.4 eV.

The chemical basis of the greater reorganization energy for cytochrome b_5 may be due to the different orientation of the heme prosthetic group in the two proteins. In cytochrome *c*, both heme propionate groups are located internally,[32] while in cytochrome b_5, the heme propionate groups are located on the protein surface at the presumed site of electron exchange, with one of the groups fully extended into solution and completely exposed to solvent.[11] Consequently, these propionate groups provide a significant electrostatic barrier to formation of the precursor complex that can offset the rate advantage provided by the greater heme exposure. In the terms of eq 2, this could be reflected in the work term (which would be larger in a more detailed model than it is in the van Leeuwen model) and in an increased reorganization energy. Similar conclusions have been reached previously on the basis of studies performed on a derivative of cytochrome b_5 in which the heme propionate groups have been converted to the corresponding methyl esters.[15c] In addition, calculations of λ for the cytochrome *c* self-exchange reaction based on the crystal structures of the oxidized and reduced protein gave a λ of 0.3 eV in the cytochrome environment but a much larger 1.4 eV for the two hemes in water (8 Å heme edge-to-heme edge distance).[33]

Table IV. Reorganization Energies for Heme Protein Electron-Transfer Reactions

reaction	dependence	λ, eV
Ru-cytochrome *c*	*T*	1.2[a,b]
Ru-(Fe/Zn)cytochrome *c*	*E*	1.5[c]
cytochrome *c*/cytochrome b_5	*E*	0.8[d]
cytochrome *c*/CCP	*E*	1.5[e]
	T	1.3[e]
Zn^{II}/Fe^{III} Hb	*T*	2.1[f]
Ru-Mb	*E*	1.3[g]
Ru + (Fe/Zn)cytochrome *c*	*E*	1.2[c]
porphyrins + cytochrome *c*	*E*	1.0[h]
cytochrome b_5 + cytochrome b_5	*T*	1.2
cytochrome *c* + cytochrome *c*	*T*	0.7

[a] Nocera, D. G.; Winkler, J. R.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 5145. Meade, T. J.; Gray, H. B. *J. Am. Chem. Soc.* In press. [b] Isied, S. S.; Kuehn, C.; Worosila, G. *J. Am. Chem. Soc.* **1984**, *106*, 1722. [c] Elias, H.; Chou, M. H.; Winkler, J. R. *J. Am. Chem. Soc.* **1988**, *110*, 429. [d] Reference 29. [e] Conklin, K. C.; McLendon, G. *J. Am. Chem. Soc.* **1988**, *110*, 3345. [f] Peterson-Kennedy, S. E.; McGourty, J. L.; Kalweit, J. A.; Hoffman, B. M. *J. Am. Chem. Soc.* **1986**, *108*, 1739. [g] Karas, J. L.; Lieber, C. M.; Gray, H. B. *J. Am. Chem. Soc.* **1988**, *110*, 559. Cowan, J. A.; Upmacis, R. K.; Beratan, D. N.; Onuchic, J. N.; Gray, H. B. *Ann. N.Y. Acad. Sci.* In press. [h] Cho, K. C.; Che, C. M.; Ng, K. M.; Choy, C. L. *J. Am. Chem. Soc.* **1986**, *108*, 2814.

The Reorganization Energy of the Cytochrome *c*/Cytochrome b_5 System. The only other study to date concerning the reorganization energy of electron transfer involving cytochrome b_5 is that of McLendon and Miller,[34] who measured electron-transfer rate constants in four cytochrome *c*/cytochrome b_5 complexes as a function of driving force. In a series of electron-transfer reactions with different driving forces, the rate constant should exhibit a maximum when the reorganization energy equals the free energy of the reaction (i.e., $\Delta G° = -\lambda$) (eq 5). From their data on intracomplex electron transfer in four cytochrome *c*–cytochrome b_5 pairs, McLendon and Miller[34] calculated a λ of approximately 0.8 eV for the sum of the reorganization energies of cytochromes *c* and b_5. The geometry of the cytochrome *c*/cytochrome b_5 electron-transfer complex is thought to be heme-edge to heme-edge as proposed first by Salemme[35] and detailed more recently by Wendoloski et al.[31] NMR evidence, however, allows but does not require alternative geometries.[36]

The reorganization energy, λ, for the cytochrome *c*/cytochrome b_5 system can also be calculated from the self-exchange data

$$\lambda = (\Delta G_{b5}^* + \Delta G_c^*)/2 \quad (7)$$

This value of 0.95 eV is in very good agreement with Miller and McLendon's value[34] of approximately 0.8 eV. Although this

(31) Wendoloski, J. J.; Matthew, J. B.; Weber, P. C.; Salemme, F. R. *Science* **1987**, *238*, 794.
(32) (a) Takano, T.; Dickerson, R. E. *J. Mol. Biol.* **1981**, *153*, 79. (b) Takano, T.; Dickerson, R. E. *J. Mol. Biol.* **1981**, *153*, 95.
(33) Churg, A. K.; Weiss, R. M.; Warshel, A.; Takano, T. *J. Phys. Chem.* **1983**, *87*, 1683.
(34) McLendon, G.; Miller, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 7811.
(35) Salemme, F. R. *J. Mol. Biol.* **1976**, *102*, 563.
(36) Hartshorn, R. T.; Mauk, A. G.; Mauk, M. R.; Moore, G. R. *FEBS Lett.* **1987**, *213*, 391.

agreement may be in part fortuitous, it is gratifying that a series of experiments on intracomplex electron transfer as a function of driving force and a series on intermolecular electron transfer as a function of temperature and ionic strength lead to the same conclusion.

Heme Protein Reorganization Energies. Our reorganization energies for cytochrome *c* and cytochrome b_5 can be compared with those for electron transfer in a number of heme proteins given in Table IV. The reorganization energies have been calculated from the dependence of the electron-transfer rate constants on temperature (T) or on the driving force (E) of the reaction. Both protein-protein and protein-small molecule reagent pairs have been investigated; reactions studied include those with intramolecular, intracomplex, and bimolecular electron transfer. The reorganization energies are between 0.8 and 2 eV. Our values for cytochromes *c* and cytochrome b_5 are in the range of other heme protein electron-transfer reactions, indicating that comparisons of intramolecular and intermolecular electron transfer as well as calculations via the dependence of the rate constants on temperature or energy all give similar values for the reorganization energy.

Acknowledgment. We thank the National Institutes of Health (Grant DK 38826 to D.W.D.) and the Medical Research Council of Canada (Grant MT-7182 to A.G.M) for support of this work. D.W.D. thanks the National Science Foundation for a Career Advancement Award (NSF CHE 8707447). The Varian VXR400 NMR spectrometer at G.S.U. was purchased with funds from the NSF Instrumentation Program (CHEM-809599).

Registry No. Cytochrome b_5, 9035-39-6.

(37) Sutin, N.; Creutz, C.; Linck, R. G. In *Inorganic Reactions and Methods*; Zuckerman, J. J., Ed.; VCH Publishers: Deerfield Beach, FL, 1986; Vol. 15, p 3.

Periodic Trends in Charge Distribution for Transition-Metal Complexes Containing Catecholate and Semiquinone Ligands. Synthetic, Physical, and Stereodynamic Properties of the Tris(3,5-di-*tert*-butylquinone) Complexes of Ruthenium and Osmium

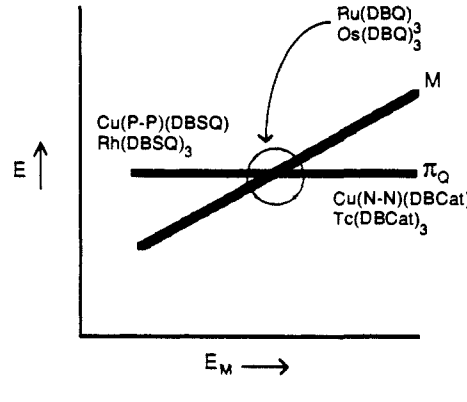
Samaresh Bhattacharya, Steven R. Boone, Glenn A. Fox, and Cortlandt G. Pierpont*

Contribution from the Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309. *Received March 27, 1989*

Abstract: Tris(3,5-di-*tert*-butylbenzoquinone) complexes of Ru and Os have been synthesized in the interest of investigating periodic trends in charge distribution for tris(quinone) complexes of second- and third-row transition metals. Infrared spectra of the two complexes are similar but differ from spectra obtained on related semiquinone [$Fe(DBSQ)_3$] and catecholate [$Re(DBCat)_3$] complexes. Both complexes undergo two oxidation and two reduction reactions at similar potentials. Crystallographic characterization on the cis and trans isomers of $Ru(DBQ)_3$ and on *trans*-$Os(DBQ)_3$ at −60 °C shows short M–O bond lengths, typical of complexes containing high oxidation state forms of Ru and Os. Ligand C–O bond lengths are found to be intermediate between semiquinone and catecholate values, with lengths that are more semiquinone-like for $Ru(DBQ)_3$ and more catecholate-like for $Os(DBQ)_3$. This subtle difference in charge distribution between the second- and third-row metals appears to contribute to marked differences in the stereodynamic properties of the two complexes. Both are diamagnetic and show sharp NMR spectra at room temperature. Eight *tert*-butyl and eight ring proton resonances are observed for $Ru(DBQ)_3$ at room temperature, indicating the presence of stereochemically rigid cis and trans isomers. Two *tert*-butyl and two ring proton resonances are observed for $Os(DBQ)_3$ at room temperature. At −85 °C eight *tert*-butyl and eight ring proton resonances appear as molecular rearrangement rates decrease on the NMR time scale. Analysis of the temperature dependence of the spectrum of $Os(DBQ)_3$ has indicated racemization by a trigonal twist mechanism at lower temperatures, with structural isomerization and racemization by a rhombic twist mechanism at higher temperatures.

Localized quinone and metal electronic levels in complexes containing semiquinonate and catecholate ligands are close in energy. The resulting ambiguity in charge distribution has become a unique property of complexes containing chelated quinone ligands. Studies have shown that, for a particular ligand, effects that change the order of metal orbital energy relative to the energy of the quinone π orbital can result in a change in the electron distribution within the complex. The most widely studied quinone ligand in this regard is 3,5-di-*tert*-butylbenzoquinone (DBBQ), coordinated in its reduced catecholate (DBCat) and semiquinonate (DBSQ) forms. Charge distribution in the $L_2Cu^{II}(DBCat)/L_2Cu^{I}(DBSQ)$ unit has been shown to depend upon the donor nature of the counter ligand L.[1] Hard nitrogen donors favor Cu(II); soft phosphine donors result in the Cu(I) charge distribution (Chart I). A change in the net charge of a complex may result in a change in electron distribution. This property has been studied for the $Mn^{II}(DBSQ)_2/Mn^{III}(DBCat)_2^-$ and $V^{III}(DBSQ)_3/V^{V}(DBCat)_3^-$ couples where, ironically, reduction of the complex leads to oxidation of the metal.[2,3] Thermal changes

Chart I



(I)

(1) Buchanan, R. M.; Wilson-Blumenberg, C.; Trapp, C.; Larsen, S. K.; Greene, D. L.; Pierpont, C. G. *Inorg. Chem.* **1986**, *25*, 3070.

0002-7863/90/1512-1088$02.50/0