Rate of Conformational Change of Cytochrome c during Electron Transfer Determined by Double Potential Step Chronoelliptometry

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The role of the conformational changes of cytochrome *c* that occur during electron transfer with its in vivo reaction partners has attracted attention for many years.[1-14] These studies have largely used stopped-flow methods coupled with various spectroscopic probes. We report here results from double potential step electrochemical experiments that used circular dichroism (CD) and magnetically induced (or enhanced) circular dichroism (MCD) spectroscopy to monitor rates of conformational change attendant to both reductive and oxidative electron-transfer reactions. Large overpotential double potential steps were used to reduce and oxidize solution-resident cytochrome *c* at a diffusion-controlled rate.[15] Under these conditions the rates of heterogeneous electron-transfer reactions are known and controlled. During double potential step experiments the CD or MCD change of cytochrome *c* was recorded versus time, monitoring the Soret band at 417 nm using indium oxide transparent electrodes in a transmission optical arrangement.[15] The CD and MCD signals of cytochrome *c* in the Soret region are sensitive to structural differences between the oxidized and reduced forms.[16] The results presented here embody the rates of conformational change that are induced by direct electron transfer reactions.

Figure 1a shows the CD change as function of time when 0.5-s potential steps, from +0.60 to −0.25 V vs NHE and then back to +0.60 V, were applied to the working electrode. The sample in the electrochemical cell is ferricytochrome *c* (the formal potential of cytochrome *c* is approximately +0.26 V vs NHE[17]). The reactions occurring during the forward (+0.60 to −0.25 V) and reverse (−0.25 to +0.60 V) steps can be modelled by the simple reaction sequences 1 and 2.

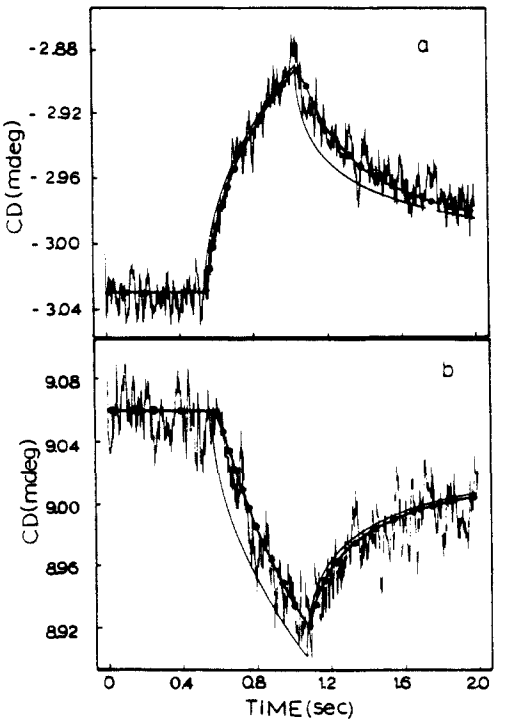

Figure 1. Double potential step chronoelliptometry (DPS/CD) experiments of cytochrome *c*. Results shown were signal averaged 1000 times, wavelength 417 nm: (a) ferricytochrome *c* 275 μM in pH 7 Tris/cacodylic acid buffer (μ = 0.2); (b) ferrocytochrome *c* 310 μM in pH 7 Tris/cacodylic acid buffer (μ = 0.2).

$$\mathrm{Cyt(III)} + \mathrm{e} \xrightarrow{k_f} \mathrm{Cyt(II)}^* \xrightarrow{k_1} \mathrm{Cyt(II)} \qquad (1)$$

$$\mathrm{Cyt(II)} - \mathrm{e} \xrightarrow{k_b} \mathrm{Cyt(III)}^* \xrightarrow{k_2} \mathrm{Cyt(II)} \qquad (2)$$

The model assumes that both electron-transfer reactions occur at a diffusion-controlled rate to produce intermediates Cyt(II)* and Cyt(III)*, which then relax to the equilibrium conformation of the reduced (eq 1) or oxidized (eq 2) cytochrome *c*.

A simulated CD response for conformational changes that occur at a diffusion-controlled rate (i.e., at the same rate as electron transfer) is shown by a smooth solid line in Figure 1a. During the forward potential step (reduction) the simulated CD response is not distinguishable from the experimental data, while during the back potential step (oxidation) the experimental CD response differs from the simulated CD response. The conformational change being monitored by the CD during the back potential step (oxidation) occurs after, and more slowly than, oxidative electron transfer. The model described above was used to fit simulated CD responses to experimental data (the solid line with dots in Figure 1a was simulated) with $k_1 \geq 50\ s^{-1}$ (simulated CD responses for values of k_1 greater than 50 s^{-1} are the same) and $k_2 = 10\ s^{-1}$ producing the best fit. Concerns that this effect might be due to an experimental artifact led to doing the same experiment on solutions of ferrocytochrome *c* (Figure 1b). The conformational change monitored by CD on oxidation, now occurring during the forward potential step, is again shown to occur at a rate slower than the rate of electron transfer (identical simulation parameters were used to fit data of Figure 1b; $k_1 \geq 50\ s^{-1}$ and $k_2 = 10\ s^{-1}$). Voltammetry data, not shown here, also establish that these samples contain only native, monomeric cytochrome *c*, under these experimental conditions.

The conformation of native ferrocytochrome *c* is more stable than that of ferricytochrome *c*.[18] This is consistent with this

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(1) Ferguson-Miller, S.; Brautigan, D. L.; Margoliash, E. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. VII, Chapter 4 and reference therein.
(2) Greenwood, C.; Palmer, G. *J. Biol. Chem.* **1965**, *240*, 3660-3663.
(3) Wilson, M. T.; Greenwood, C. *Eur. J. Biochem.* **1971**, *22*, 11-18.
(4) Lambeth, D. O.; Campbell, R. Z.; Palmer, G. *J. Biol. Chem.* **1973**, *248*, 8130-8136.
(5) Davis, L. A.; Schejter, A.; Hess, G. P. *J. Biol. Chem.* **1974**, *249*, 2624-2632.
(6) Land, E. J.; Swallow, A. J. *Arch. Biochem. Biophys.* **1971**, *145*, 365-372.
(7) Pecht, I.; Faraggi, M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 902-906.
(8) Lichtin, N. N.; Shafferman, A.; Stein, G. *Biochim. Biophys. Acta* **1973**, *314*, 117-135.
(9) Wilting, J.; Van Buuren, K. J. H.; Braams, R.; Van Gelder, B. F. *Biochim. Biophys. Acta* **1975**, *376*, 285-297.
(10) Tabushi, I.; Yamamura, K.; Nishiya, T. *J. Am. Chem. Soc.* **1979**, *101*, 2785-2787.
(11) Forster, M.; Hester, R. E.; Cartling, B.; Wilbrandt, R. *Biophys. J.* **1982**, *38*, 111-116.
(12) Carling, B.; Spiro, T. G. *J. Chem. Phys.* **1985**, *83*, 3894-3905.
(13) Liang, N.; Kang, C. H.; Ho, P. S.; Margoliash, E.; Hoffman, B. M. *J. Am. Chem. Soc.* **1986**, *108*, 4665-4666.
(14) Peerey, L. M.; Kostic, N. M. *Biochemistry* **1989**, *28*, 1861-1868.
(15) Heineman, W. R.; Hawkridge, F. M.; Blount, H. N. In *Electroanalytical Chemistry*; Bard, A. J., Ed.; Marcel Dekker: New York, 1984; Vol. 13, pp 1-113.
(16) Myer, Y. P.; Pande, A. In *The Porphyrins*; Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. III, p 297.
(17) Cusanovich, M. A. In *Bioorganic Chemistry*; van Tamelen, E. E., Ed.; Academic: New York, 1978; Vol. 4, pp 117-145.
(18) Moore, G. R.; Williams, R. J. P. *Eur. J. Biochem.* **1980**, *103*, 513-521.

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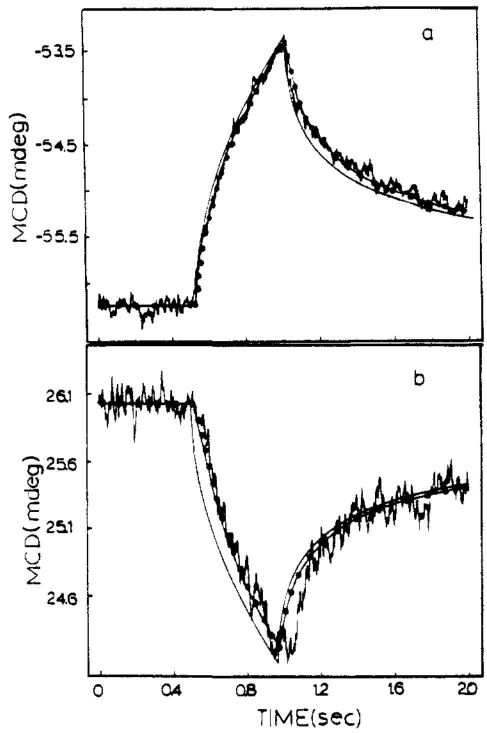

Figure 2. Double potential step MCD (DPS/MCD) experiments of cytochrome *c*. Results shown were signal averaged 100 times, wavelength 417 nm: (a) ferricytochrome *c* 355 μM in pH 7 Tris/cacodylic acid buffer (μ = 0.2); (b) ferrocytochrome *c* 250 μM in pH 7 Tris/cacodylic acid buffer (μ = 0.2). Magnetic field: 15 kg.

finding that k_2 is less than k_1, i.e., the rate of conformational change following reduction is faster than the rate following oxidation.

MCD experiments were conducted as were the CD experiments described above. Figure 2a shows the experimental and simulated results with the bulk solution species being ferricytochrome *c*. Figure 2b shows the analogous results for a sample of ferrocytochrome *c*. Again, when simulated responses (smooth solid line with dots) were compared with experimental data, best fits were found for the values $k_1 \geq 50$ s^{-1} and $k_2 = 20$ s^{-1}. The rate constant for the Cyt(III)* $\rightarrow$ Cyt(III) conformational change is found to be larger when monitored by MCD ($k_2 = 20$ s^{-1}) versus CD ($k_2 = 10$ s^{-1}). This is not surprising. The CD of the Soret band reflects the changes in the chiral environment of the heme moiety while the MCD is more sensitive to changes occurring in the coordination sphere of the metal ion.[19] Both CD and MCD are sensitive to the conformational changes of cytochrome *c* upon reduction and oxidation. However, MCD is more sensitive than CD to localized structural changes such as those that would be associated with changes in the iron–sulfur bond occurring upon electron transfer. Other conformational changes may then be propagated through the protein from the immediate environment about the heme following electron transfer. The differences in the rates of conformational change as observed here by CD and MCD are consistent with this view.

In conclusion, the experiments described here provide a means of studying the kinetics of conformational change of heme proteins during both reductive and oxidative electron-transfer reactions. Detailed studies of binding effects and pH effects on the conformational changes of cytochrome *c* during electron-transfer reactions are being pursued.

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(19) Holmquist, B. In *The Porphyrins*, Dolphin, D., Ed.; Academic Press: New York, 1978; Vol. III, Chapter 5.

Hexakis(trimethylphosphine)tungsten(0): Synthesis, Structure, and Reactivity

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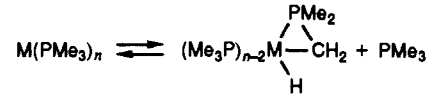
Zerovalent homoleptic trimethylphosphine complexes, M(PMe_3)$_n$, have been isolated for several of the transition metals, including Mo, Fe, Os, Co, Ni, Pd, and Pt.[1] Interest in these complexes arises from the strong σ-donor coupled with weak π-acceptor properties of the trimethylphosphine ligand, a combination that generates metal centers in which the valence electrons are of high energy. Such metal complexes are often termed "electron rich" and show great promise for activating normally unreactive substances by oxidative addition, e.g. alkane C–H bonds.[1b,2] A consequence of the high reactivity of the metal centers in M(PMe_3)$_n$ complexes is the facile formation of cyclometalated divalent derivatives accompanied by elimination of PMe_3 (eq 1).[1,3] For example, Mo(PMe_3)$_6$ has been shown to be in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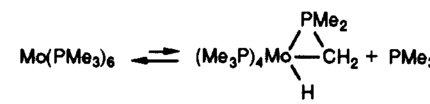
equilibrium with low concentrations of the complex Mo(PMe_3)$_4$(η^2-CH_2PMe_2)H and PMe_3 (eq 2).[1a,b] However, the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analogous tungsten complex, W(PMe_3)$_6$, has so far remained elusive. Attempts to prepare W(PMe_3)$_6$ by both the co-condensation of tungsten atoms with PMe_3 and the reduction of WCl_6 with alkali metal reducing agents with PMe_3 as a reactive solvent resulted in the isolation of the cyclometalated product W(PMe_3)$_4$(η^2-CH_2PMe_2)H in each case.[3a–c] Here we report that W(PMe_3)$_6$ can indeed be isolated, and we describe the synthesis, structure, and kinetics and thermodynamics of its conversion to W(PMe_3)$_4$(η^2-CH_2PMe_2)H.

W(PMe_3)$_6$ is isolated as a yellow crystalline solid in good yield (>50%) by the reduction of WCl_6 with Na(K) alloy with PMe_3 as a reactive solvent (eq 3),[4] using a similar procedure to that

(1) (a) Brookhart, M.; Cox, K.; Cloke, F. G. N.; Green, J. C.; Green, M. L. H.; Hare, P. M.; Bashkin, J.; Derome, A. E.; Grebenik, P. D. *J. Chem. Soc., Dalton Trans.* **1985**, 423–433. (b) Cloke, F. G. N.; Cox, K. P.; Green, M. L. H.; Bashkin, J.; Prout, K. *J. Chem. Soc., Chem. Commun.* **1982**, 393–394. (c) Ermer, S. P.; Shinomoto, R. S.; Deming, M. A.; Flood, T. C. *Organometallics* **1989**, *8*, 1377–1378. (d) Klein, H.-F.; Karsch, H. H. *Chem. Ber.* **1975**, *108*, 944–955. (e) Klein, H.-F. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 362–375. (f) Karsch, H. H.; Klein, H.-F.; Schmidbaur, H. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 637–638. (g) Karsch, H. H.; Klein, H.-F.; Schmidbaur, H. *Chem. Ber.* **1977**, *110*, 2200–2212. (h) Timms, P. L. *Angew. Chem., Int. Ed. Engl.* **1975**, *14*, 273–277. (i) Rathke, J. W.; Muetterties, E. L. *J. Am. Chem. Soc.* **1975**, *97*, 3272–3273. (j) Ugo, R. *Coord. Chem. Rev.* **1968**, *3*, 319–344. (k) Malatesta, L.; Cenini, S. *Zerovalent Compounds of Metals*; Academic Press: New York, 1974.

(2) (a) Green, M. L. H.; O'Hare, D. *Pure Appl. Chem.* **1985**, *57*, 1897–1910. (b) Cloke, F. G. N.; Fyne, P. J.; Gibson, V. C.; Green, M. L. H.; Ledoux, M. J.; Perutz, R. N.; Dix, A.; Gourdon, A.; Prout, K. *J. Organomet. Chem.* **1984**, *277*, 61–73. (c) Shinomoto, R. S.; Desrosiers, P. J.; Harper, G. P.; Flood, T. C. *J. Am. Chem. Soc.* **1990**, *112*, 704–713.

(3) Metalated derivatives of the constitution M(PMe_3)$_n$(η^2-CH_2PMe_2)H have been reported for Mo, W, Fe, Ru, and Os. (a) Gibson, V. C.; Grebenik, P. D.; Green, M. L. H. *J. Chem. Soc., Chem. Commun.* **1983**, 1101–1102. (b) Gibson, V. C.; Graimann, C. E.; Hare, P. M.; Green, M. L. H.; Bandy, J. A.; Grebenik, P. D.; Prout, K. *J. Chem. Soc., Dalton Trans.* **1985**, 2025–2035. (c) Green, M. L. H.; Parkin, G.; Chen, M.; Prout, K. *J. Chem. Soc., Dalton Trans.* **1986**, 2227–2236. (d) Werner, H.; Gotzig, J. *Organometallics* **1983**, *2*, 547–549. (e) Gotzig, J.; Werner, R.; Werner, H. *J. Organomet. Chem.* **1985**, *290*, 99–114. (f) Werner, H.; Werner, R. *J. Organomet. Chem.* **1981**, *209*, C60–C64. (g) Graimann, C. E.; Green, M. L. H. *J. Organomet. Chem.* **1984**, *275*, C12–C14.

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