Long-Range Electron Transfer from Iron(II)-Cytochrome *c* to (Zinc-Cytochrome *c* Peroxidase)(+) within the 1:1 Complex

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Long-range electron transfer[1] within crystallographically defined protein complexes can be studied by substituting zinc protoporphyrin IX for heme in one partner.[2-5] Electron transfer within such a complex is initiated by flash photoproduction of the zinc protoporphyrin triplet state (^{3}ZnP),[6] which either can decay back to the ground state or can reduce a ferriheme partner,

$$\underset{A}{[ZnP,Fe^{III}P]} \underset{k_D}{\overset{h\nu}{\rightleftharpoons}} \underset{A^*}{[^3ZnP,Fe^{III}P]} \xrightarrow{k_t} \underset{B}{[ZnP^{\bullet+},Fe^{II}P]} \quad (1)$$

The redox intermediate B returns to the ground state by thermal electron transfer from $Fe^{II}P$ to the cation radical $ZnP^{\bullet+}$,

$$\underset{B}{[ZnP^{\bullet+},Fe^{II}P]} \xrightarrow{k_b} \underset{A}{[ZnP,Fe^{III}P]} \quad (2)$$

Until now, reaction 2 has not been observed directly. This report demonstrates the process in the archetypical 1:1 protein electron transfer complex between yeast cytochrome *c* peroxidase (C*c*P) and cytochrome *c* (C*c*)[7,8] and shows that it exhibits a much more pronounced dependence on the cytochrome *c* employed than does the photostimulated, forward reaction.[4] It is noteworthy that in this case the thermal reaction (2) is equivalent to the physiological oxidation of Fe^{II}C*c* by the H_2O_2-oxidized enzyme.

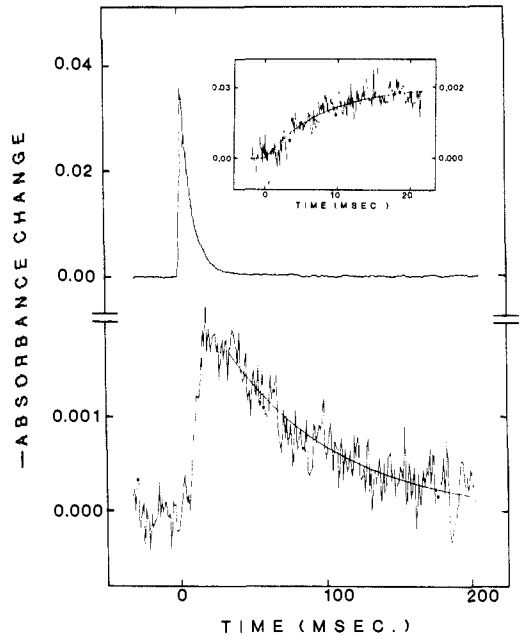

Figure 1. (Upper) Time dependence of ^{3}ZnP absorbance in [ZnC*c*P,-Fe^{III}C*c*(tuna)] complex: λ = 434 nm. (Inset) Instantaneous rise and subsequent decay of absorbance of ^{3}ZnP (left ordinate); growth of absorbance of intermediate B and least-squares fit to eq 3 (right ordinate). Both decay of ^{3}ZnP and growth of B are exponential, with rate constants $k_p = 140 \pm 4\ s^{-1}$. (Lower) Time dependence of absorbance of the electron-transfer transient B = $[(ZnP)^+,Fe^{II}P]$, for the complex between ZnC*c*P and tuna C*c*; λ = 444.5 nm. The solid line is a fit to eq 3 of the data for $(k_p t) > 5$. Conditions: 1.0 mM potassium phosphate buffer pH 7.0 at 25 °C.

By the kinetic scheme, eq 1 and 2, decay of the ^{3}ZnC*c*P excited state within the [ZnC*c*P,Fe^{III}C*c*] complex[9] is first order, with decay rate $k_p = k_D + k_t$. The ^{3}ZnP decay rate is $k_p = 140 \pm 4\ s^{-1}$ for the complex with tuna Fe^{III}C*c* and $k_p = 381 \pm 10\ s^{-1}$ for yeast iso-2Fe^{III}C*c*. Since $k_D = 115 \pm 8\ s^{-1}$, the rate of electron transfer from ^{3}ZnP to Fe^{III}P within the heterologous complex with tuna C*c* is $k_t = 25 \pm 9\ s^{-1}$, whereas $k_t = 266 \pm 13\ s^{-1}$ for the homologous complex with yeast C*c* complex.[10] By eq 1 and 2, the time course of intermediate B is

$$B(t) = A^*_0\left(\frac{k_t}{k_> - k_<}\right)e^{-k_< t}(1-e^{-(k_> - k_<)t}) \quad (3)$$

where A^*_0 is the initial concentration of ^{3}ZnP and $k_>$ and $k_<$ refer to the larger and smaller of k_b and k_p. Equation 3 corresponds to an exponential rise and fall with the maximal concentration at time τ: $B(\tau)/A^*_0 = (k_t/k_>)\exp[-k_<\tau]$; $\tau = \ln(k_>/k_<)/(k_> - k_<)$. For all conditions of interest, $k_t/(k_> - k_<) << 1$, and $B(\tau)/A^*_0 \lesssim (k_t/k_>) << 1$. Therefore, we initiated the search for spectroscopic evidence of B at the ^{3}ZnC*c*P/ZnC*c*P isosbestic point, λ = 444.5 nm, by comparing the kinetic traces taken at this wavelength with the time course of ^{3}ZnC*c*P measured at the Fe^{III}C*c*/Fe^{II}C*c* isosbestic point, λ = 434 nm. Conveniently, this

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(6) Abbreviations: C*c*, cytochrome *c*; C*c*P, cytochrome *c* peroxidase; ZnP, zinc protoporphyrin IX; FeP; iron protoporphyrin IX.

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(9) ZnC*c*P was prepared by a procedure modified from that reported for the preparation and heme reconstitution of the apoperoxidase (Yonetani, T. *J. Biol. Chem.* **1967**, *242*, 5008–5013) and this preparation will be discussed in detail elsewhere. Yeast iso-2 C*c* was employed. All experiments reported here were done at 25 °C using 1 mM potassium phosphate buffer, pH 7.0. Flash photolysis experiments were performed on a computer-interfaced apparatus (Stanford, M. A.; Hoffman, B. M. *J. Am. Chem. Soc.* **1981**, *103*, 4104–4114. Signal-to-noise ratios of weak, short-time absorbance transients were enhanced by signal averaging. However, full power excitation by the flash-lamp pumped dye laser (R6G; ~0.5 J) causes a small net photoreduction. Thus, data acquisition was limited to ≲30 transients, which produced insignificant (≲5%) net reduction.

(10) In the present experiments, k_t was measured at 25 °C with samples prepared in 1 mM pH 7.0 potassium phosphate buffers. Our earlier study[4] employed 10 mM buffer and 20 °C; k_t reported here for yeast C*c* is greater because of these differences.

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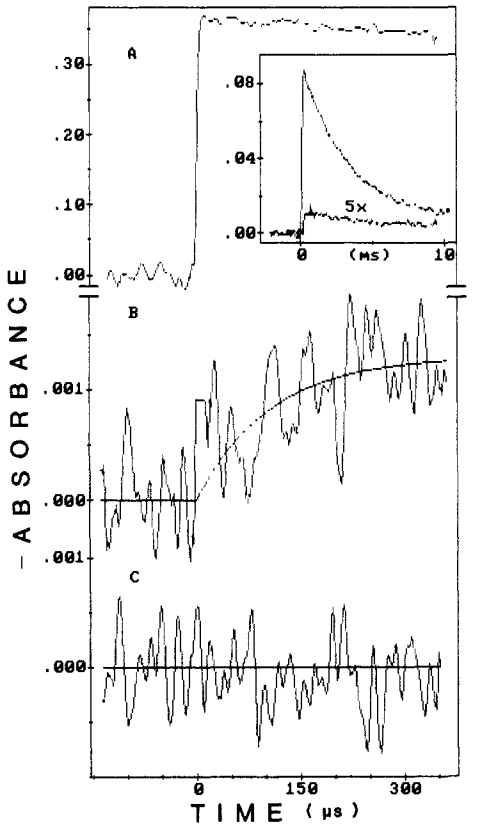

Figure 2. (A) Short-time absorbance change for ^{3}ZnP in [ZnC*c*P,-Fe^{III}C*c*(yeast-isozyme-2)], monitored at 434 nm. (Inset) Long-time decay of absorbance of ^{3}ZnP (low photolysis power) and of intermediate B (x5). (B) Growth of intermediate B, after photolysis of [ZnC*c*P,-Fe^{III}C*c*(yeast-isozyme-2)]; λ = 444.5 nm. Solid line is a fit to eq 3. Because of a signal from scattered light, five channels after t = 0 have been suppressed; these were not included in the fitting procedure. (C) Control: [ZnC*c*P,Fe^{II}C*c*] flashed under the same experimental condition as in (B). Conditions: 1.0 mM potassium phosphate buffer pH 7.0 at 25 °C. The signal in panel A represents the accumulation of 10 transients; the inset to (A) involved 1 transient for ^{3}ZnP and 16 for B; signals in (B) and (C) each represent 32 transients.

wavelength also is the maximum of the ^{3}ZnC*c*P/ZnC*c*P difference spectrum.

Flash excitation of the [ZnC*c*P,Fe^{III}C*c*(tuna)] complex gives a small but well-defined transient absorbance at 444.5 nm, whereas ZnC*c*P and [ZnC*c*P,Fe^{II}C*c*(tuna)] show a clean isosbestic point at 444.5 nm, and tuna Fe^{III}C*c* alone gives no observable transient. During the lifetime of the ^{3}ZnP, the transient absorbance grows with a rate constant equal to k_p (Figure 1, Inset), as predicted by eq 3 under the condition $k_b << k_p$. In this case B should persist after ^{3}ZnP has disappeared and decrease slowly, with rate constant k_b, precisely as seen in Figure 1. A fit to the long-time decay of the transient signal gives $k_b = 12 \pm 4$ s^{-1}. Because B persists, the transient also could be detected at long time after the complete decay of the ^{3}ZnP, using other wavelengths. The rate constant, k_b, is invariant with λ and the extrapolated zero-time absorbance change, $\Delta A'_0 = [\epsilon_B - \epsilon_A]A^*_0 k_t/(k_p - k_b)$ (eq 3), agrees in sign and magnitude with that expected from measured rate constants and static absorbance spectra.

A slow transient is not observed at any wavelength for the homologous complex[11] [ZnC*c*P, yeast Fe^{III}C*c*]. However, a rapid transient is detectable. At short times (Figure 2), the ^{3}ZnP signal, monitored at 434 nm, appears with the instrumental time constant and then remains essentially invariant. In contrast, at 444.5 nm a weak absorbance associated with intermediate B is seen to rise with a high, but finite, rate (Figure 2B) and then decay in parallel with ^{3}ZnP (inset, Figure 2A). As with the tuna C*c*, no signal is observed at 444.5 nm with the reference compounds Fe^{III}C*c*, ZnC*c*P, and [ZnC*c*P,Fe^{II}C*c*] (Figure 2C). This behavior is consistent with eq 3 in the limit $k_b >> k_p$, in which case k_b governs the increase of the absorbance of B at 444.5 nm; analysis gives $k_b = (1.1 \pm 0.5) \times 10^4$ s^{-1}.[11] As with tuna Fe^{III}C*c*, the sign and magnitude of the absorbance change in Figure 2B are consistent with the small amount of B predicted by the measured rates $(B(\tau)/A^*_0 \lesssim 10^{-2})$ and static absorbance spectra.

The large difference in rate constants for the thermal reaction (2) in complexes with two highly similar[12] C*c*, k_b(yeast)/k_b(tuna) $\sim 10^3$, is a striking display of influence of the protein on electron transfer in the physiological direction. This difference undoubtedly reflects different C*c*P–C*c* docking in the homologous complex, consistent with the higher affinity of C*c*P for yeast C*c* than for the horse or tuna proteins.[7e] If this charge-transfer process involves superexchange contributions[13] from intervening residues, as can be inferred from the modeling studies[8] and the evolutionary conservation of phenylalanine 82 of C*c*,[12] then it could be especially sensitive to subtle conformational alterations of the protein–protein interface.[14] In addition, application of the principle of microscopic reversibility to the protein dependence of the k_b/k_t ratio, $k_b/k_t \sim 0.5$ for tuna C*c*, but $k_b/k_t \sim 500$ for yeast C*c*, indicates a conformational rearrangement within the complex following the ^{3}ZnP → Fe^{III}P electron transfer.[15] This, of course, is as expected:[14] Solution[12] and X-ray diffraction structural studies[16] show that C*c* undergoes a conformational change upon reduction and changes in C*c*P also may occur. Clearly, a more precise understanding of the structures of the complexes between oxidized and reduced forms of C*c*P and of C*c* will be required in parallel with electron-transfer measurements.

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(11) Results are similar for yeast iso-2 C*c*, yeast iso-1 C*c*, and yeast iso-1 C*c* that has been carboxymethylated at cysteine 103.

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Synthesis and Crystal and Molecular Structure of In(C_5Me_5): An Apparent Octahedral Cluster

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The synthesis and structural characterization of group 13 compounds can play an important role in the development of main-group organometallic chemistry. A topic of current interest centers on compounds with the metal in positive oxidation states which are less than 3. In group 13 chemistry the fully characterized compounds in the +1 oxidation state include In(C_5H_5)[1,2] and Tl(C_5H_5).[3] The indium(I) compound is most readily prepared from InCl and LiC_5H_5 in diethyl ether.[2] An X-ray structural

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