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LONG-RANGE ELECTRON TRANSFER AT FIXED AND KNOWN DISTANCE WITHIN PROTEIN COMPLEXES

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ABSTRACT

We have used zinc-substituted hemoproteins to study long-range electron transfer between redox centers at fixed and known distances. The photo-excited zinc triplet state in one subunit of the α_1-β_2 electron transfer complex of [Zn,Fe] hybrid hemoglobin transfers an electron to its partner aquoferriheme subunit at a rate, k_t=100 s^{-1}. The temperature dependence of this electron transfer from 77K to 313K is indicative of non-adiabatic electron tunnelling in which the accompanying nuclear rearrangements proceed by nuclear tunnelling.

For the complex between zinc-substituted yeast cytochrome c peroxidase (CCP) and native yeast cytochrome c, electron transfer occurs at a rate, k_t=138 s^{-1}, compared to k_t = 17 s^{-1} in the complex between the yeast enzyme and horse cyt c. The difference demonstrates the species specificity involved in physiological electron transfer. Oxidation of ferroporphyrin by the zinc porphyrin radical is more rapid, and, for the yeast cytochrome, occurs with a rate, $k_h \sim 10^4$ s^{-1}.

INTRODUCTION

Recently, we have discovered (ref.1-3) that long-range electron transfer (ref.4,5) between centers held at fixed and known distances can be studied by substituting zinc protoporphyrin for heme in one of the partners of a protein electron transfer complex. A significant feature of the approach is that it permits us to measure transfer rates over a wide range of temperatures because the electron transfer process is photoinitiated and occurs unimolecularly, within the previously formed complex.

Mixed-metal, [M,Fe], hybrid hemoglobins represent the best characterized system employed. Chains of one type (α or β) are substituted with closed shell zinc protoporphyrin (ZnP) and chains of the opposite type are oxidized to the aquo-ferriheme ($Fe^{III}P$) state (ref.1,2). Electron transfer occurs within the α_1-β_2 electron transfer complex between chromophores that are separated by protein residues and at a metal-metal distance of 25 Å (ref.6) (Fig. 1). The ZnP

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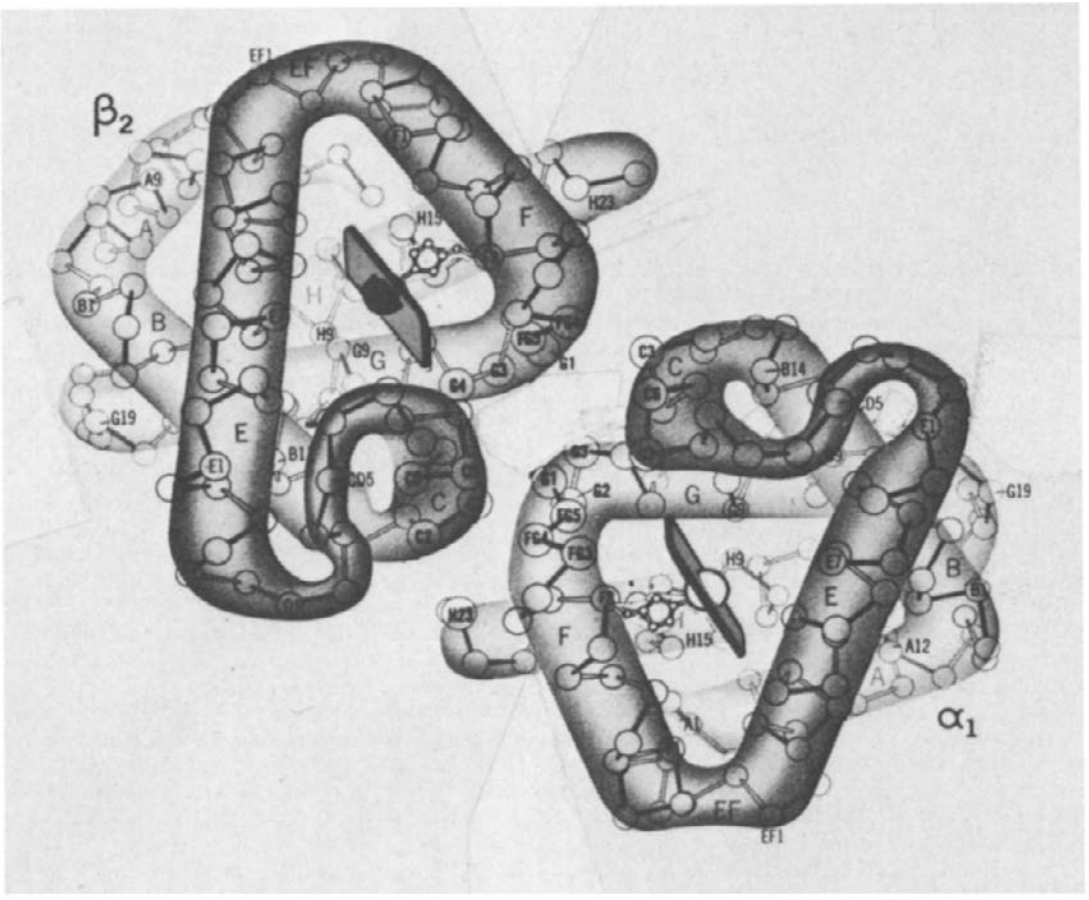

Fig. 1. Representation of the (α_1^{Fe},β_2^{Zn}) subunit pair, electron transfer complex, within [α^{Fe},β^{Zn}] hybrid hemoglobin in the deoxy-Hb (T-state) quaternary structure. The distance between Fe(o) and Zn(o) atoms is 24.7 Å (ref.6).

and FeP planes are roughly parallel, and the shortest distance between atoms of the rings is about 20 Å.

This approach also has been applied to an archetypical physiological electron transfer reaction, that between yeast cytochrome c peroxidase (CCP) and cytochrome c (cyt c) (ref.7), by employing the complex between zinc-substituted CCP (ZnCCP) and native cyt c (ref.3). Modeling studies of this complex by Poulos and Kraut (ref.8) led them to propose a structure in which the two heme planes are nearly parallel, at a metal-metal distance of 25 Å and an edge-to-edge distance of 17-18 Å. In this report we first describe the basis of these measurements, then summarize the results to date.

ELECTRON TRANSFER PROCESSES

Reversible electron transfer within a zinc-substituted, protein electron transfer complex is initiated by flash photoproduction of the slowly decaying

zinc protoporphyrin triplet state (^{3}ZnP). The ^{3}ZnP is a good reductant and can decay back to the ground state or can reduce its ferriheme partner by long-range electron transfer

$$^3ZnP + Fe^{III}P \xrightarrow{k_t} (ZnP)^+ + Fe^{II}P \qquad (a)$$

As a consequence, ^{3}ZnP within the complex decays with a rate constant, k_P, which is the sum of the intrinsic triplet decay rate constant, k_D, and of that for electron transfer: $k_P = k_D + k_t$. The redox potential for this reaction varies with the identity of the partners within a protein electron-transfer complex, but falls within a range, $\Delta E_o' \sim 0.8$-0.9 V (ref.2,3).

The intermediates formed by (a) regenerate the ground state in a reaction directly analogous to a physiologically important process, namely electron transfer from $Fe^{II}P$ to thermalized porphyrin cation radical, ZnP^+

$$(ZnP^+) + Fe^{II}P \xrightarrow{k_h} ZnP + Fe^{III}P \qquad (b)$$

The potential of this reaction again varies with the system, but in general it is comparably exoergic with (a): $\Delta E_o' \sim 0.85$-1.0 V.

ELECTRON TRANSFER WITHIN THE MIXED-METAL Hb HYBRIDS

Figure 2 presents the progress curve for the decay of the ^{3}ZnP produced by flash excitation of the mixed-metal hybrid in which electron transfer is blocked because the heme is initially in the high-spin, Fe^{II} state, $[\alpha^{Fe^{II}}, \beta^{Zn}]$. The triplet decay rate, $k_D = 50 \pm 5\ s^{-1}$, is the same as that for ^{3}ZnP in an individual subunit of fully substituted ZnHb (ref.9) and thus long-range energy transfer and paramagnetic quenching of ^{3}ZnP are negligible. However, when the partner subunit is initially in the aquoferriheme, Fe^{III}, state (Fig 2), the triplet decay rate is almost tripled, $k_P = 150 \pm 10\ s^{-1}$. The triplet decay enhancement is found to be independent of protein concentration, and, as confirmed by observations of ferriheme reduction (see below), represents the contribution from intramolecular electron transfer within the α_1-β_2 complex. Thus, the rate constant for electron transfer at room temperature between a ^{3}ZnP and aquoferriheme rigidly held 25 Å apart within the α_1-β_2 electron transfer complex is $k_t = k_P - k_D = 100 \pm 10\ s^{-1}$.

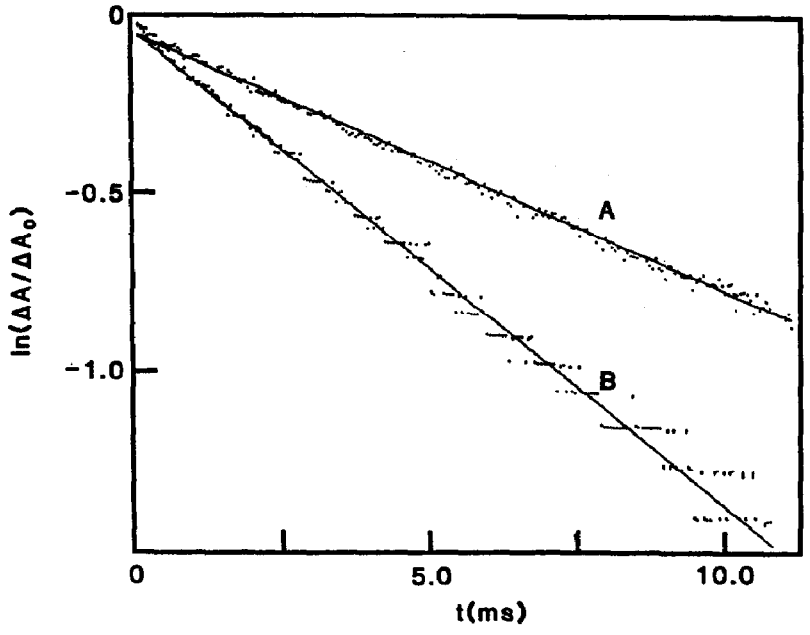

Figure 2. Progress curve of the [^{3}ZnP-ZnP] difference spectrum subsequent to flash photolytic excitation of [α^{Fe},β^{Zn}] hybrid hemoglobins, as monitored at 415 nm, the isosbestic of the [$Fe^{III}P$-$Fe^{II}P$] difference spectrum (ref.1). A) [$\alpha^{Fe^{II}},\beta^{Zn}$]; decay rate, 50(±3) s^{-1}. B) [$\alpha^{Fe^{III}},\beta^{Zn}$]; decay rate, 155(±10) s^{-1}.

The $(ZnP)^+$ π-cation radical product of electron transfer oxidizes its ferroporphyrin partner according to reaction (b). The process is rapid, and because $k_h >> k_t$, the reversibly formed (ZnP^+, $Fe^{II}P$) intermediate is in low steady-state concentration and has not been observed directly in the hybrids. However, when following the $Fe^{II}P$ concentration subsequent to a flash excitation, one observes a small net heme reduction corresponding to ca. 15% of the ^{3}ZnP formed. This implies that the intermediate not only reverts to the initial state by (b), but that $(ZnP)^+$ in the heme pocket of Hb in small part also undergoes a competing spontaneous reduction (rate constant, k_m) without accompanying ferroheme oxidation, by as yet unidentified protein residues. Analysis of these observations with kinetic equation for the full reaction scheme indicates that at room temperature, $k_m + k_h >> k_t$ and $k_h/k_m \sim 3$.

The electron transfer rate, k_t, for the [$\alpha(Fe^{III}H_2O)$; β(Zn)] hybrid falls smoothly from the room temperature value to a non-zero value, $k_t = 9 \pm 4$ s^{-1}, which is effectively invariant from 170 K down to 77 K (ref.2) (Fig. 3). Data

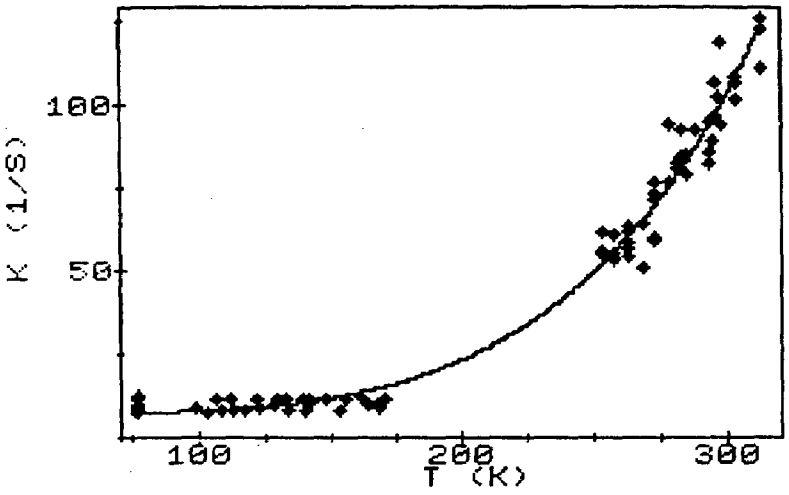

Fig. 3. Temperature dependence of the electron transfer rate, k_t, in the [$\alpha(Fe^{III}H_2O)$; $\beta(Zn)$] hybrid hemoglobin. The solid line is a plot of eq. 1, employing the parameters given in the text (ref.2).

in the temperature dependent region (T > 253 K) can be fit to the Arrhenius expression ($k = A \exp(-E_a/kT)$), to give $E_a \approx 2$ kcal/mole. The low temperature rate constant is comparable to that reported to occur over comparable distance in frozen glasses (ref.10). The temperature response of k_t is similar to that in the classic case of nonadiabatic electron transfer from cytochrome to chlorophyll in C. vinosum (ref.11). Only in these two systems have the full temperature response, including observation of a low-temperature plateau, been observed, and only with the [Zn,Fe] hybrid is the molecular architecture known.

The regime of temperature independence indicates that the transfer process involves nonadiabatic electron tunnelling in which the accompanying nuclear rearrangement proceeds by nuclear tunnelling; the temperature-dependence indicates coupling of the transfer process to thermal vibrations and/or fluctuations (ref.4). For purposes of comparison to C. vinosum, the temperature-dependence of k_t (Fig. 3) can be described by the simplest form of the semiclassical (ref.11) treatment of thermally assisted, nonadiabatic electron transfer. It incorporates vibronic coupling to oscillators of a single frequency, ω, and may be written in terms of three parameters (ref.4)

$$k_t = \alpha \sqrt{\tanh(T_c/T)} \quad \exp(-\beta \tanh(T_c/T)) \qquad (1)$$

Here, $T_c \equiv \hbar\omega/2k_B$; and the other parameters are interpreted as follows: $\beta \equiv E^\dagger/k_B T_c \equiv (\Delta E_o' - \lambda)^2/2k\omega\lambda$, where the activation energy in the high temperature, classical limit[4] is $E^\dagger = (\Delta E_o' - \lambda)^2/4\lambda$, and λ is the reorganization energy; $\alpha = (2\pi/\hbar)\,|H|^2/\sqrt{2\pi\hbar\omega\lambda}$; H is the electron tunnelling matrix element. The solid curve in Figure 3 represents the result of a nonlinear least squares fit of the data to eq 1 and employs the parameters, T_c = 275K, $\alpha = 2.14 \times 10^5\ s^{-1}$, β = 10.3 K. Calculations with β and T_c give $E^\dagger$ = 0.244 V and employing $\Delta E_o'$ = 0.8 V[2] one obtains λ = 2.3 (0.27) eV, with the alternate values arising from the quadratic nature of β. For comparison, the values for C. vinosum are 2.24 (0.090) eV. Taking either value of λ, one obtains from α the tunnelling matrix element for the Hb hybrid, $|H| \simeq 3\text{-}4\times10^{-6}$ eV, which is comparable to values expected on the basis of simple parameterization schemes (ref.4).

ELECTRON TRANSFER WITHIN THE ZnCCP/cyt C COMPLEX

The triplet decay curves of ^{3}ZnCCP and of a [^{3}ZnCCP/cyt c] complex, in which electron transfer has been blocked by prior reduction of horse cytochrome c heme, are exponential, with the same decay rate, $k_D = 126 \pm 2\ s^{-1}$, in each case. Upon titration of ZnCCP with horse cyt c^+, the ^{3}ZnCCP decay remains first order, but the rate increases from k_D, linearly with horse cytochrome c, until a 1:1 ratio is reached, and then remains constant at a plateau value characteristic of the complex itself: $k_P = 143 \pm 3\ s^{-1}$ (ref. 3) (Fig. 4). Because k_D and k_P are similar in magnitude, the ^{3}ZnP progress curves for solutions with substoichiometric cyt c^+ exhibit a composite rate, $k_{obs} = (1\text{-}f)k_D + f\,k_P$, where f is the fraction of [ZnCCP/cyt c^+] complex; Figure 4 in fact presents $\Delta k_{obs} = k_{obs} - k_D = (k_P - k_D)f$. The shape of the titration indicates that ZnCCP and the cytochrome c form a strong 1:1 complex ($K_A > 10^8\ M^{-1}$), consistent with previous studies (ref.7). The enhanced triplet decay within the complex represents long-range electron transfer according to eq (a), with the plateau rate being $k_P = k_D + k_t$. From the measured k_P and k_D, one obtains the electron transfer rate to the low-spin ferriheme within the [ZnCCP/horse cyt c^+] complex: $k_t = 17 \pm 3\ s^{-1}$ at 20°C.

First order analysis of the ^{3}ZnP decay curves upon addition of yeast cyt c^+ to ZnCCP gives a titration curve like that of Figure 4. However, the plateau rate characteristic of the complex itself is much greater for the yeast cyt c^+: $k_P = 264 \pm 11\ s^{-1}$. Careful examination of the substoichiometric progress curves indicates that they can be analyzed as the sum of a slow phase, free ZnCCP, and a rapid phase, the complex. Fixing the rate constants for slow and rapid

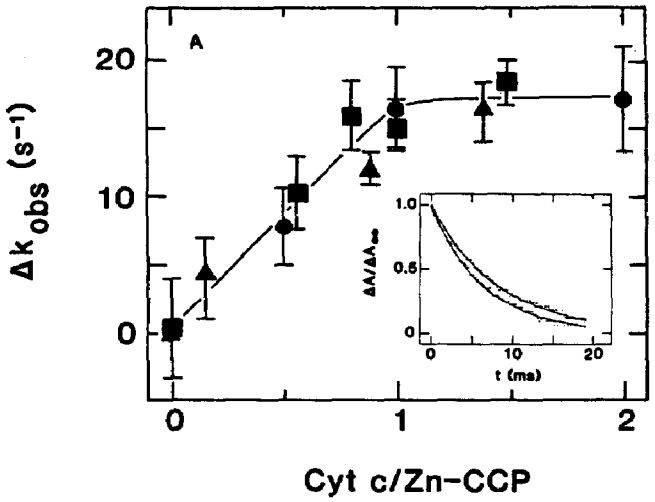

Fig. 4. Change (Δk_{obs}) in the observed rate constant (k_{obs}) for the first order decay of ^{3}ZnP upon titration of ZnCCP with horse cyt c^+. The curve represents protein binding assuming a composite rate as described in text and a single cyt c^+ binding site having $K_A = 10^8$ M^{-1}. Inset: The ^{3}ZnP decay traces for the uncomplexed ZnCCP and fully complexed ZnCCP; solid lines are theoretical, employing $k_{obs} = 126$ s^{-1} and 144 s^{-1}, respectively (ref.3).

components at $k_D = 126$ s^{-1} and $k_P = 264$ s^{-1}, the fraction of peroxidase exhibiting the fast phase grows linearly with the addition of cyt c^+, reaching unity at equimolar concentrations (Fig. 5). The break at 1:1 stoichiometry again corresponds to complex formation with $K_A > 10^8$ M^{-1}. From k_P and k_D one obtains a much higher rate for electron transfer within the [ZnCCP/yeast cyt c^+] complex: $k_t = 138 \pm 12$ s^{-1} at 20°C. Thus, although yeast and horse cytochromes c are highly similar in all ways (ref.7), electron transfer from the ^{3}ZnP of the peroxidase to the ferriheme of the cytochrome occurs roughly 10-fold faster with the evolutionarily homologous yeast cytochrome.

The kinetic difference spectra obtained from the complex between the two yeast proteins disclose a small absorbance that decays with rate k_P and matches that of the electron transfer intermediate, [$(ZnCCP)^+$/cyt c]. Preliminary steady-state kinetic analysis of this transient at wavelengths corresponding to the (^{3}ZnCCP/ZnCCP) isosbestics, indicates that $k_t/k_h \sim 1/100$, and thus, $k_h \sim 10^4$. This represents the first direct measurement of the rate for the thermal process, (h), an exact analogue of the physiological electron transfer

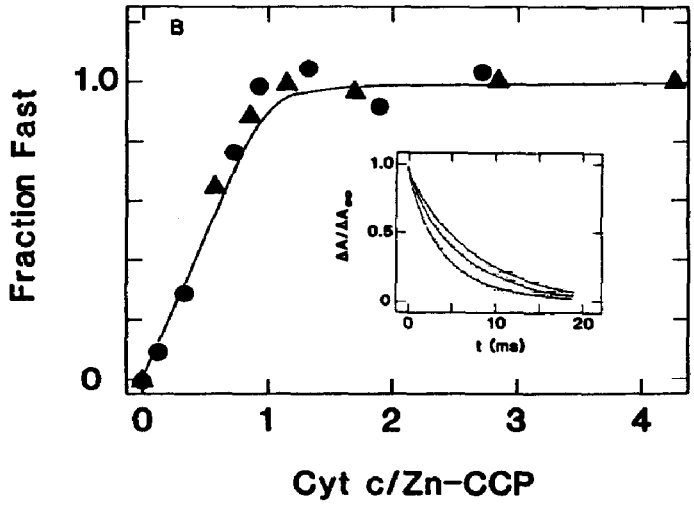

Fig. 5. Proportion of the rapid component in a description of the ^{3}ZnCCP decay curve by a two exponential function (free ^{3}ZnCCP decay, k_D = 126 s^{-1}; complex, k_P = 264 s^{-1}; see text) during titration of ZnCCP with yeast cyt $\underline{c}^+$. The curve represents protein binding assuming a single cyt $\underline{c}$ binding site having K_A = 10^8 M^{-1}. Inset: Experimental and theoretical progress curves for ^{3}ZnP decay as the fraction of [yeast cyt $\underline{c}^+$/ZnCCP] complex assumes the values, 0, 0.32, and 1.0 (ref.3).

reaction. Note particularly the large difference in 20°C rates for two processes, (a) and (b), which are comparably exoergic.

CONCLUSIONS

Our results to date, summarized in Table I, represent a first step in measuring electron transfer between redox centers in structurally well-characterized protein complexes. The observation of electron tunnelling within the crystallographically known environment of the Hb hybrids will permit chemical modulation of the energetic and vibronic aspects of the process through variation of the macrocycles, metals and ligands of donor and acceptor chromophores.

The difference in electron transfer rates between complexes of yeast ZnCCP with yeast and horse cytochromes $\underline{c}$ demonstrates the fine degree of species specificity involved in physiological electron transfer, and must reflect subtle structural differences between the two complexes. Studies of a wide variety of natural and chemically modified cytochromes should permit a correlation of electron transfer rate with protein structure.

TABLE 1.

Summary of Rates and Distances of Electron Transfer

	k_D[a] [s^{-1}]	k_t[a] [s^{-1}]	R[b] [Å]
[α[$Fe^{III}H_2O$);β(Zn)] hemoglobin hybrid	55±3	100±10	25
[α(Zn);(β$Fe^{III}H_2O$)] hemoglobin hybrid	55±3	100±10	25
ZnCCP·cyt c^+ (from horse heart)	126±2	17±3	~25
ZnCCP·cyt c^+ (from yeast isozyme)	126±2	138±12	~25

[a] k_D and k_t are the rate constants at room temperature
[b] R is the metal-to-metal distance.

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