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Faster Interprotein Electron Transfer in a [Myoglobin, b₅] Complex with a Redesigned Interface

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Abstract

Direct measurements of electron transfer (ET) within a protein-protein complex with a redesigned interface formed by physiological partner proteins myoglobin (Mb) and cytochrome b₅ (b₅) reveal interprotein ET rates comparable to those observed within the photosynthetic reaction center. Brownian dynamics simulations show that Mb in which three surface acid residues are mutated to lysine binds b₅ in an ensemble of configurations distributed around a reactive most-probable structure. Correspondingly, charge-separation ET from a photoexcited singlet zinc porphyrin incorporated within Mb to the heme of b₅ and the follow-up charge-recombination exhibit distributed kinetics, with median rate constants, $k_f^s = 2.1 \times 10^9 \text{ second}^{-1}$ and $k_b^s = 4.3 \times 10^{10} \text{ second}^{-1}$, respectively. The latter approaches that for the initial step in photosynthetic charge separation, $k = 3.3 \times 10^{11} \text{ second}^{-1}$.

Ultrafast electron transfer (ET) has evolved within photosystems I and II (1) to occur on time scales of 10^{-11} to 10^{-12} s. Most biological redox processes involve long-range transfer of an electron or hole between redox centers separated by a noncovalent, dynamically fluctuating, protein-protein interface. These processes are many orders of magnitude slower, typically on time scales longer than 10^{-6} s (2–5). Here we report that redesigning the interface between the physiological ET partners, myoglobin (Mb) and cytochrome b₅ (b₅), leads to the fastest inter-protein ET rate constants yet observed, with values that approach those occurring within the photosynthetic reaction centers. This ultrafast ET occurs within

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Materials and Methods

SOM Text

Fig. S1

References

an interprotein ET photocycle (Fig. 1A) in which both charge separation and recombination exhibit distributed kinetics, in keeping with Brownian dynamics (BD) simulations (Fig. 1, B to D), which show that the complex exists as an ensemble of bound configurations. Relatively small overall differences among similar configurations within the ensemble lead to a broad distribution in their ET rate constants.

We recently described a strategy for redesigning the interface between weakly bound (dynamically docked) ET partner proteins, thereby enhancing and coupling both their binding and reactivity, by optimizing the placement of surface charges within the putative docking interface (6). We applied this strategy to the [Mb, b₅] complex and obtained a triple mutant, in which three acidic surface residues surrounding the Mb (horse) heme (Asp⁴⁴, Asp⁶⁰, and Glu⁸⁵) are replaced by Lys [Mb(+6)], that exhibits both strongly enhanced binding to b₅ (bovine) and intracomplex ET from the triplet state of Zn-deuteroporphyrin (ZnD)–substituted Mb to the Fe³⁺ b₅ heme (Fe³⁺P). Intriguingly, variations in the apparent efficiency of intersystem crossing from the photoexcited ZnD singlet state (¹ZnD) to the ZnD triplet state (³ZnD) during ET quenching titrations of ZnDMb(+6) by Fe³⁺ b₅ were interpreted as providing indirect evidence for extremely rapid intracomplex ET from ¹ZnD to the Fe³⁺P of Fe³⁺ b₅, $k \sim 10^9 \text{ s}^{-1}$. Here we report direct measurements of ultrafast interprotein ET across the noncovalent [Mb, b₅] interface through femtosecond transient absorption (fs-TA) measurements of the intracomplex photo-cycle (Fig. 1A) that begins when laser excitation generates the ¹ZnD state, which reacts by ¹ZnD → Fe³⁺P ET to form the charge-separated intermediate, **I** = [ZnD⁺Mb, Fe²⁺b₅], and concludes with Fe²⁺ → ZnD⁺ charge recombination. The photocycle energetics are given in the supporting online materials (SOM) (7).

The measurements use Mb(+6) reconstituted with Zn-deuteroheme dimethyl ester, with the heme neutralization having increased the Mb charge by an additional +2 [denoted ZnMb(+8)]. fs-TA experiments were performed with ZnMb(+8) by itself (fig. S1) (7) and with a sample in which more than 95% of the Mb is in the [ZnMb, Fe³⁺b₅] complex (Fig. 2). The photoexcited isolated ¹ZnMb(+8) converts almost quantitatively (inter-system crossing yield ~ 98%) to ³ZnMb(+8), with a time course (Fig. 3, left) that is well described at all wavelengths by the exponential decay of ¹ZnMb(+8) and the concomitant appearance of ³ZnMb(+8) (Eq. 1),

$$\Delta A = A \exp(-^1k_D t) + B [1 - \exp(-^1k_D t)] = [A - B] \exp(-^1k_D t) + B \quad (1)$$

where ¹k_D = 2.5 × 10⁸ s⁻¹. The wavelength-dependent amplitude factor, *A*, corresponds to the zero-time excited singlet-ground state difference spectrum (fig. S1), *B* to the persistent triplet-ground difference spectrum, and thus [*A* − *B*] to the ¹ZnMb-³ZnMb difference (8).

Figure 2 displays representative transient absorption-difference spectra of the [ZnMb(+8), Fe³⁺b₅] complex taken from 425 to 725 nm at delay times ranging from ~1 ps to ~4 ns. Focusing first on wavelengths λ < 525 nm, the initial difference spectrum corresponds to the ¹ZnMb-ground state difference, its most prominent feature being a maximum at 452 nm. With time, this maximum decreases in intensity and gradually blue-shifts toward 445 nm, the maximum of the ³ZnMb-ground state difference (8, 9). The decay of ¹ZnMb complexed

by Fe^{3+}b_5 is like that for $^1\text{ZnMb}$ alone, in being homogeneous for $\lambda < 530$ nm. This similarity is seen more clearly in the inset, where the longest-time (~ 4 ns) absorbance difference has been subtracted from each trace, generating a $^1\text{ZnMb}$ - $^3\text{ZnMb}$ difference spectrum that homogeneously decays to zero at the longest time. The decay after photoexcitation of $^1\text{ZnMb}$ in the complex is much faster than for $^1\text{ZnMb}$ alone, and it cannot be described by a single exponential. It can be fit by a biexponential (Fig. 3, blue) in which both decay constants are sharply increased by intracomplex ET ($k_{1f} = 1.1 \times 10^{10} \text{ s}^{-1}$, $k_{2f} = 1.2 \times 10^9 \text{ s}^{-1}$; $f_1/f_2 = 3/4$). However, such a fit implies the presence of two distinct (classes of) configurations for the complex, whereas the BD simulations (Fig. 1) do not show such a bimodal clustering of “hits.” Rather, the simulation yields an ensemble of BD hits that is smoothly distributed over a single region of the Mb surface in the vicinity of the heme (Fig. 1B). This is emphasized by a plot of these hits as a function of their Boltzmann-weighted calculated electrostatic binding energy (V_B) relative to the dissociated complex, and a parameter that reflects the b_5 location, the center-of-mass distance (d_{COM}) (Fig. 1C). This plot shows that the ensemble of hits is rather tightly distributed around a single most-probable maximum at an electrostatic energy of $V_B \sim -10$ kcal/mol, and center-of-mass distance of $d_{\text{COM}} \sim 26 \text{ \AA}$. Thus, although the biexponential function provides a satisfactory description of the decay of $^1\text{ZnMb}$ in complex with Fe^{3+}b_5 , its use is not physically justified.

Careful consideration of the structures associated with the BD hits shows that even though the hits exhibit a narrow range of distances, there is a considerable distribution in the positioning of b_5 relative to Mb, and this should cause a distribution in ET rate constants. Figure 1D superimposes onto the coordinates of the “target” Mb the b_5 coordinates of four representative configurations from among 224 hits within $\pm 0.25 \text{ \AA}$ and ± 0.25 kcal/mol of the most-probable configuration. Focusing on the b_5 hemes (black) shows a substantial variation among their positions and orientations, even among these similarly bound configurations. The driving force for ET should vary little, if at all, among the different configurations, but the structural variations within the ensemble will produce wide variation in the ET pathways that cross the protein-protein interface, and the correspondingly large variations in the preexponential term will cause large variations in the ET rate constant (10). These variations will not be time-averaged during the decay because dynamic interconversion among the configurations of the complex must be slower than the ET time scale set by the ultrafast interprotein ET that we observe. As a result, the ET process involves an ensemble of static configurations, each with its own structure and its own ET rate constant, and one expects to observe distributed kinetics. ET will occur first in the complexes with the largest rate constant. As the more ET-active configurations progressively become depleted, the remaining complexes with a ^1ZnD will progressively have smaller ET rate constants. As a result, the effective rate constant will decrease in time as the reaction progresses.

Prompted by these considerations, the singlet decay traces from the short wavelength range also were fit with a distributed decay—a version of Eq. 1 with a “stretched exponential” (11)

$$\Delta A = [A - B] \exp[-(^1k_D t) - (k_f^s t)^n] + B \quad (2)$$

This function corresponds to a distribution in ET rate constants whose width varies inversely with n , but can equivalently be thought of in terms of an ET rate constant that decreases in time; the constant, k_f^s , which fixes the “1/e” time for ET, corresponds to the median ET rate constant for the distribution (11). The fits to Eq. 2 (Fig. 3A) give $k_f^s = 2.1(5) \times 10^9 \text{ s}^{-1}$ and a distribution parameter, $n = 0.57(5)$ (Table 1).

The homogeneous decay of the difference spectrum for wavelengths $\lambda < 525 \text{ nm}$ indicates that the charge-separated ET intermediate **I** either is not spectroscopically distinct in this wavelength range or does not accumulate to a level detectable at these wavelengths because the system displays “inverted kinetics.” However, the results for $\lambda > 525 \text{ nm}$ confirm the formation of **I** and can be used to monitor the charge-recombination ET return to the ground state. The difference spectra in this range do not vary homogeneously in time (Fig. 2). This is best seen at $\sim 560 \text{ nm}$, a ^1ZnD -ground isosbestic and a wavelength range where the difference spectrum is governed by absorbance changes associated with the $\text{Fe}^{2+}\text{b}_5/\text{Fe}^{3+}\text{b}_5$ redox change (8). One observes a difference maximum that grows with time and then decays (Fig. 2); the corresponding kinetic trace at this wavelength (Fig. 3B) clearly shows the appearance and loss of the $[\text{ZnD}^+\text{Mb}, \text{Fe}^{2+}\text{b}_5]$ charge-separated intermediate as it is formed by excitation of ZnD to the ^1ZnD excited state, followed by $^1\text{ZnD} \rightarrow \text{Fe}^{3+}\text{P}$ charge separation, and disappears by $\text{Fe}^{2+}\text{P} \rightarrow \text{ZnD}^+$ charge recombination.

Following the above discussion, the time course for **I** is appropriately described by assuming a stretched exponential function for both the charge-separation and charge-recombination ET processes (Eq. 3) (12, 13). Numerical solution to the differential equations for this model in Mathcad gives a progress curve equivalent to that of the heuristic distributed function

$$\Delta A = I \{ \exp[-(^1k_D t) - (k_f^s t)^n] - \exp(-k_b^s t)^m \} \quad (3)$$

The fit to the progress curve for **I** is excellent (Fig. 3B); fixing k_f^s and n as the values determined for the singlet ET progress curves gives $k_b^s = 4.3 \times 10^{10} \text{ s}^{-1}$ ($m = 0.49$) as the median rate constant for charge recombination, roughly 20 times the k_f^s for charge separation (Table 1). The similarity of the distribution parameters for charge separation and recombination ET reactions ($n \approx m = 1/2$) is as expected because the ET reactions occur on a time scale that is short compared to the time scale for structural rearrangement of the individual reactive complexes. However, fits to the full photocycle with stretched exponentials would predict a maximum concentration of **I** roughly twice that determined from the observed maximum absorbance change and reported difference spectrum (8), an indication that the distribution in rate constants associated with the stretched exponentials (11) represents the ensemble of structures only imperfectly.

The striking nature of these results is highlighted by comparison to representative rate constants previously reported for ET across a protein interface, as well as to those for ET between redox sites within the reaction center of cyanobacteria, algae, and plants. The median rate constant for photoinitiated $^1\text{ZnDMb} \rightarrow \text{Fe}^{3+}\text{b}_5$ charge separation, k_f^s , is more than three times the rate constant for the fastest interprotein ET process previously

reported, ^1Zn -cytochrome c (Cc) $\rightarrow$ cytochrome c oxidase (CcO) singlet-state ET, and the charge-recombination rate constant, $k_b^s = 4.3 \times 10^{10} \text{ s}^{-1}$, is more than 60 times as large. In fact, this median interprotein rate constant is only roughly one-eighth the rate constant for the initial charge separation within the *Blastochloris viridis* photosynthetic reaction center (Table 1).

We examined features of the distribution in rate constants, $H(k_b^s)$, associated with the observed distribution parameter, $m = 1/2$ (11). The most-probable value for k_b^s is about one-fifth of the median value, yet almost one-fifth of the configurations have rate constants that match (or exceed) that for photosynthetic charge separation. Clearly, a substantial fraction of the ensemble of [Mb(+8), b₅] structures have extremely efficient ET pathways, although the pathways for many are poor.

The ^3ZnD that remains after the ^1ZnD photo-cycle is complete undergoes $^3\text{ZnD} \rightarrow \text{Fe}^{3+}\text{P}$ ET within the complex (6). This charge separation is three orders of magnitude slower than for ^1ZnD , but still slightly faster than the corresponding process in other Zn-substituted protein ET pairs (Table 1). The driving force for $^3\text{ZnD} \rightarrow \text{Fe}^{3+}\text{P}$ ET is less than for $^1\text{ZnD} \rightarrow \text{Fe}^{3+}\text{P}$, but considerations of the ET energetics suggest that this difference should make the ^3ZnD reaction faster, not slower (inverted region) (7). We suggest that the ^3ZnD reaction is so much slower because highly reactive configurations of the complex preferentially undergo ^1ZnD ET, and intersystem crossing to the triplet occurs only in configurations with much poorer ET pathways. As a result, ET from the triplet porphyrin presumably is gated by the dynamics of conformational inter-conversion to reactive conformations, and not controlled by the actual charge-separation process. Thus, the interface redesign strategy has created a complex that not only has unprecedented rate constants for interprotein ET, but also reveals details of the conformational distributions and dynamics that underlie protein-protein binding and reaction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

ET photocycle and corresponding BD simulation for the docking of $\text{Fe}^{3+}b_5$ (red) onto Mb(+6) (cyan) (**A**) The vertical spacing between states corresponds roughly to their energetic difference (see SI). The labels correspond with “1/e” times for ET. Spatial distribution (**B**), contour plot (**C**), and four representative configurations (**D**) in the vicinity of the most-probable configuration as defined by Boltzmann weighting the electrostatic potential energies (V_B). Details: Center-of-mass (COM) reaction criterion ($d = 39$ Å) as described in (6); 10^4 trajectories; 293 K; $\mu = 18$ mM; pH 7.0; mutation sites in yellow; the representative configurations are within ± 0.25 kcal/mol and ± 0.25 Å of the most-probable configuration.

Fig. 2. fs-TA absorbance-difference measurements subsequent to photoexcitation of [ZnMb(+8), Fe³⁺b₅]. Representative time-resolved spectra at delay times of 1.5 ps (black), 107 ps (red), 527 ps (purple), 1027 ps (blue), and $A_{\infty} = 3827$ ps (green). (**Inset**) Redisplay of selected spectra after subtraction of the residual difference at $t = 3.8$ ns. Arrows refer to representative time slices shown in Fig. 3. Conditions: 0.1 mM ZnMb(+8), 0.2 mM Fe³⁺b₅, 95% of Mb bound to b₅ ($K_a = 8.0 \times 10^5 \text{ M}^{-1}$); 10 mM potassium phosphate; pH 7.0; ambient temperature; 2 mm pathlength; fresh sample was used for each time point.

Fig. 3.

Representative kinetic progress curves. (A) Traces at 475 nm for decay of $^1\text{ZnMb}(+8)$ (gray) and its complex with $\text{Fe}^{3+}b_5$ (red). $\text{ZnMb}(+8)$ trace was fit with Eq. 1; trace for complex was fit with a biexponential function (blue) and Eq. 2 (black). (B) Progress curve for **I** obtained by averaging time slices between 556 and 563 nm. Trace was fit with Eq. 3 (black). $[\text{I}]_{\text{max}} \sim 0.2 \mu\text{M}$, on the basis of the maximum absorbance change and reported difference spectrum (8). Conditions: as in Fig. 2.

Table 1

Rate constants for interprotein and photosynthetic electron transfer. Cc, cytochrome c; CcO, cytochrome c oxidase; Pc, plastocyanin; P, bacteriochlorophyll special pair; (P)^{*}, photoexcited P; B, bacteriochlorin; H, bacteriopheophytin; Q, quinone.

ET	[Donor, acceptor]	ET rate constant (s ⁻¹)		Reference
		Charge separation	Charge recombination	
Protein-protein	[¹ ZnMb(+8), Fe ³⁺ b ₅]	2.1×10^9 [*]	4.3×10^{10} [†]	This work
	[¹ ZnCc, Cu _A ²⁺ CcO]	5.7×10^8	–	(14)
	[³ ZnMb(+6), Fe ³⁺ b ₅]	1.0×10^6	–	(6)
	[³ ZnCc, Cu _A ²⁺ CcO]	4.5×10^5	–	(14)
	[³ ZnCc, Cu ²⁺ Pc]	2.3×10^5	9.6×10^5	(15)
	[Fe ²⁺ Cc(Ru39), Cu _A ²⁺ CcO]		6.0×10^4	(16)
	[Reaction center (P ⁺), Fe ²⁺ Cc ₂]		1.0×10^6	(17)
Photosynthetic	[(P) [*] , B]		3.3×10^{11}	(18)
reaction center [‡]	[B ⁻ , H]		1.1×10^{12}	
(<i>Bl. viridis</i>)	[H ⁻ , Q]		5.0×10^9	

^{*} ET rate constant from stretched-exponential fit (Eq. 2, see text); biexponential fit, $k_{1f} = 1.1 \times 10^{10} \text{ s}^{-1}$, $k_{2f} = 1.2 \times 10^9 \text{ s}^{-1}$.

[†] ET rate constant from stretched-exponential fit (Eq. 3; see text).