Modulation of Interprotein Electron Transfer Energetics by Heme-Ligand Variation

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The coordination environment of the metal ion in electron-transfer (ET) metalloproteins usually is fixed. In contrast, mixed-metal hemoglobin hybrids, [MP,Fe(L)P],[1] are ideal for studying the specific role of the metal ligands in interprotein long-range ET[2–4] because the exogenous ligand L coordinated to FeP is easily varied (neutral L ≡ L⁰ = H_2O, ImH; anionic L ≡ X⁻ = CN⁻, F⁻, N_3^-) with minimal structural perturbation.[5,6] We report here a dramatic dependence on ligand of both photoinitiated and thermally activated ET (eqs 1 and 2, respectively),

$$[^3(MP),Fe^{3+}(L)P] \xrightarrow{k_t} [(MP)^+,Fe^{2+}(L)P] \quad (1)$$
$$A^* \qquad\qquad\qquad I$$

$$[(MP)^+,Fe^{2+}(L)P] \xrightarrow{k_b} [(MP),Fe^{3+}(L)P] \quad (2)$$
$$I \qquad\qquad\qquad A$$

between the α_1 and β_2 subunits within these hybrids. Our kinetic

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(1) (a) The symbol in brackets for the hybrid represents an {α_2,β_2} T-state tetramer of hemoglobin (Hb), in which the irons in either the α or the β chains have been replaced with M = Mg or Zn. For L = H_2O, the data refer to [β(MP),α(Fe³⁺(L)P)]. For the remaining ligands, data were obtained for both [β(MP),α(Fe(L)P)] and [α(MP),β(Fe(L)P)] and were similar. (b) Other abbreviations: P = protoporphyrin IX; ImH = imidazole; Mb = myoglobin: k_t^M(L) and k_b^M(L) refer to the photoinitiated and thermal ET rate constants in [MP,Fe³⁺(L)P].

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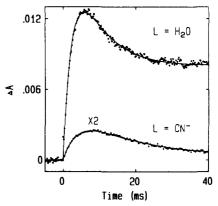(7) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* 1985, *811*, 265–322.

Figure 1. Kinetic progress curves monitored (λ = 435 nm, *T* = 278 K) for the electron-transfer intermediate (B) formed upon flash photolysis of [β(ZnP),α(Fe³⁺(L)P)]. Experimental points (•) and nonlinear least-squares fits (solid lines) for L = H_2O and L = CN⁻ are presented, with absorbance changes normalized to a zero-time triplet concentration (A_0^*) = 10⁻⁶ M. For [β(ZnP),α(Fe³⁺(H_2O)P)], k_b = 345 (30) s⁻¹, k_p = 134 (15) s⁻¹; for [β(ZnP),α(Fe³⁺(CN⁻)P)], k_b = 243 (25) s⁻¹, k_p = 65 (8) s⁻¹.

Table I. Ligand Dependence of k_b^M and k_t^M within [MP,Fe³⁺(L)P][a]

	k_t, s⁻¹		k_b, s⁻¹	
L	M = Zn	M = Mg	M = Zn	M = Mg
H_2O	80 (20)	30 (6)	325 (30)	155 (20)
Im[b]	75 (25)	35 (10)	300 (35)	150 (25)
CN⁻	6 (3)	5 (2)	243 (25)	135 (10)
F⁻	6 (4)	3 (2)	200 (35)	105 (10)
N_3^-	6 (4)	5 (3)	230 (40)	115 (15)

[a] Rate constants are the average of 5–10 measurements at 278 K. Rate constants for exogenous ligands were invariant over the concentration ranges: Im,[b] 200–500 mM; CN⁻ and N_3^-, 0.5–5 mM; F⁻, 0.8–1.2 M. [b] For M = Mg, Im = ImH; for M = Zn, Im = 1-methylimidazole.

results show that anionic ligands remain bound throughout the ET cycle, and electrochemical measurements indicate that anion binding modulates the rate constants k_t and k_b through major changes in energetics. The data are consistent with a reorganization energy of $\lambda \sim$ 1 eV for *both* reactions and suggest that the preexponential of k_b is enhanced over that of k_t.[7,8]

The rate constant for both the A* → I and the I → A processes can be determined after photoexcitation of A by analyzing the time course of the ET intermediate, I. This can only be done by monitoring the absorbance changes at an A*/A isosbestic point (λ = 435 nm for M = Zn; λ = 432 nm for M = Mg).[2a] At all other wavelengths, the transient absorbance changes are dominated by A*. In the kinetic traces of Figure 1,[9] the long-time exponential decays correspond to the net disappearance (rate constant k_p) of A*, which also can be measured by following A* directly. Deactivation of A* can occur by intrinsic triplet decay (rate constant k_D), by electron transfer (rate constant k_t), and possibly through other processes such as Forster energy transfer (rate constant k_o): $k_p = k_D + k_t + k_o$.[2b] Measurements of triplet quenching give the quenching rate constant, $k_q \equiv k_p - k_D = k_t + k_o$ which is therefore an upper bound to k_t. Recently, we noted that k_t can be determined directly by quantitation of the kinetic transient for B, whose magnitude is directly proportional to k_t.[2a] Analysis of the relatively large absorbance changes for [(ZnP)⁺,Fe²⁺P] (Figure 1) gives $k_t^{Zn}(H_2O)$ = 90 (30) s⁻¹, confirming, within error, the assignment[2b] of $k_t \approx k_p - k_D$ for L = H_2O. For [ZnP,Fe³⁺(CN⁻)P], both the long-time exponential fall of I and the triplet decay of A* give $k_t \leq k_q$ = 14 (4) s⁻¹; analysis of the smaller absorbance change associated with I (Figure 1) gives an even lower ET rate constant, $k_t^{Zn}(CN⁻)$ = 6 (3) s⁻¹. Thus,

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(9) Hybrid preparation and experimental protocols will be discussed elsewhere: Kuila, D.; Natan, M. J.; Baxter, W. W.; Gingrich, D. J.; Hoffman, B. M., manuscript in preparation.

replacement of H_2O by CN^- in the heme coordination sphere reduces k_t by over 1 order of magnitude. Quantitation of I for M = Mg and Zn and with all the ligands studied here yielded the values for $k_t^M(L)$ shown in Table I.

The rate constant $k_b^M(L)$ is determined from the short-time absorbance rise of I. The kinetic traces in Figure 1 give $k_b^{Zn}(H_2O)$ = 345 (30) s^{-1}, minimally larger than $k_b^{Zn}(CN^-)$ = 243 (25) s^{-1}. For L = H_2O, reduction of the aquo-bound heme most likely yields the five-coordinate ferroheme, $Fe^{2+}P$.[10] However, ligand dissociation from $Fe^{2+}(CN^-)P$ is slow,[11] and the I → A process involves reoxidation of the CN^--bound species, $Fe^{2+}(CN^-)P$. Indeed, the data in Table I indicate that all the anionic ligands remain associated with iron throughout the electron-transfer cycle of eqs 1 and 2.[12] First, if ET-induced ligand loss were rapid compared to the I → A electron-transfer process, then one would expect that $k_b^M(X^-)$ = $k_b^M(H_2O)$, contrary to observation. Second, for a given anion, $k_b^{Mg}(X^-)$ < $k_b^{Zn}(X^-)$; this metal dependence indicates that $k_b^M(X^-)$ cannot represent rate-limiting ligand dissociation from the $Fe^{2+}(X^-)P$ redox partner of state I, followed by fast ET (i.e., ligand "gating"[3a,13] of I → A).[14]

The data presented in Table I show that (i) the A* → I process is strongly reduced by anion binding to the heme, with $k_t^M(L^0)$ ≥ $10k_t^M(X^-)$, and (ii) in contrast, anion binding lowers the I → A rate constant k_b^M by less than 50%. These results can be interpreted as arising from changes in the activation free energy for ET, ΔG^*, that occur as the energetic consequence of persistent anion ligation on the ET time scale. Direct protein electrochemistry of $Mb^{3+}(CN^-)$ indicates that the $Fe^{3+}(X^-)P/Fe^{2+}(X^-)P$ redox potential is shifted negative by almost 500 mV relative to $Fe^{3+}(H_2O)P/Fe^{2+}P$;[15] this shift decreases the driving force for the A* → I ET process for a hybrid with either M^{2a} from $-\Delta G^°$ ≈ 1.0–1.1 eV (L = L^0) to $-\Delta G^°$ ≈ 0.5–0.6 eV (L = X^-), and increases that for the I → A reaction from $-\Delta G^°$ ≈ 0.7–0.8 eV (L = L^0) to $-\Delta G^°$ ≈ 1.2–1.3 eV (L = X^-).[16] With these changes in driving force, both the 10-fold-lower values of $k_t^M(X^-)$ as compared to $k_t^M(L^0)$ and the small reduction in $k_b^M(L)$ upon anion binding can be accommodated by the classical Marcus expression, ΔG^* = $(\lambda + \Delta G^°)^2/4\lambda$, with a single reorganization energy, λ ~ 1 eV, for both reactions of the protein complex.[17]

This value for λ places both the A* → I and I → A processes near the peak of the Marcus free energy curve for L = L^0 and attributes the reduction in k_b for L = X^- to an excursion into the inverted region of ET.[18] Within this context, the general result that $k_b^M(L)$ >> $k_t^M(L)$ appears to require that the preexponential factor for k_b^M is enhanced over that for k_t^M.[19] Measurement of

ET in hybrids prepared from Hb mutants is addressing this issue.

1,2-Dehydro-*o*-carborane[1]

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Though the similarity between benzene, the prototypal planar aromatic two-dimensional system of organic chemistry, and the icosahedral "super-aromatic" carboranes may at first seem remote, even forced, there is much the two have in common. Both owe their special stability to the presence of a fully filled system of bonding molecular orbitals, three for benzene, 13 for *o*-carborane.[2] Aromaticity is manifested in both cases by extraordinary thermal stability as well as unusual chemical reactivity. For instance, both species undergo aromatic substitution.[2c,3] There are many differences as well. Benzene requires ca. 1100 °C before two ring carbons move apart in a degenerate rearrangement,[4] but a mere 420 °C suffices to convert *o*-carborane to its meta isomer.[5] Connection between a benzene ring and an orbital on an α carbon dominates the chemistry of the benzyl system, yet the icosahedral frame and an α orbital are quite well insulated from each other.[6] We have been involved for some time in an exploration of the properties of carborane-based reactive intermediates.[7] Here we describe the first example of a dehydro-*o*-carborane (1) and make some comparisons to the related classically aromatic system, dehydrobenzene, or benzyne.[8]

The dots represent carbons - all other vertices are B–H.

1

In order to generate **1**, we have mimicked one of the earlier syntheses of benzyne in which an α-bromo anion is generated, only to suffer elimination of bromide.[9] After the failure of a number of direct routes from 1-bromo-*o*-carborane or 1,2-dibromo-*o*-carborane,[10] we proceeded by an indirect path in which the dianion **2** was treated with a single equivalent of bromine to generate the bromo anion, **3**. At 0 °C, this species is stable, unlike the corresponding α-bromo phenyl anion which requires temperatures as low as -100 °C to avoid rapid bromine loss.[11] Presumably,

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(14) Since $k_b^{Mg}(ImH)$ ≈ $k_b^{Mg}(H_2O)$ and $k_b^{Zn}(ImH)$ ≈ $k_b^{Zn}(H_2O)$, the fate of $Fe^{2+}(ImH)P$ is unclear.

(15) (a) We find that the reduction potential for the $Mb(Fe^{3+}L)/Mb$-$(Fe^{2+}L)$ couple is +0.05 V vs NHE for L = H_2O, and -0.43 V vs NHE for L = CN^- (to be submitted), as measured by cyclic voltammetry (ref 15b). (b) Nicholson, R. S.; Shain, I. *Anal. Chem.* **1964,** *36,* 706–723.

(16) Driving-force calculations utilize $E^°$′ = +0.95 vs NHE for the half-reaction $(ZnP)^+$ + e^- = ZnP (note the typographical error in ref 2a).

(17) We have taken the reorganization energies to be identical for reduction for all ligated hemes. This is likely a good approximation, even for L = H_2O, where ET is synchronous probably with ligand loss, because the inner-sphere contribution to the total reorganization energy is small.[4a] The λ reported here is slightly less than that reported in ref 4a. This is expected because that result includes a contribution from solvent reorganization about $[LRu(NH_3)_5]^{3+/2+}$.

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(19) This is true for both M considered separately. Despite very similar energetics, k_b and k_t for M = Zn are consistently larger than for M = Mg, indicative of a larger preexponential term in the former.

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