tyrosine and tryptophan. No heme vibrations are observed even though the heme absorption at 230 nm is comparable to that of the three tyrosines and two tryptophans present in the protein.[11] Indeed, the intense heme resonance Raman intensities which occur with visible-wavelength excitation have prevented normal Raman studies of the globin protein.

It appears possible to selectively excite particular aromatic amino residues within a protein. Our recent pH study[12] of the UVRR spectra of myoglobin was able to detect the deprotonation of the single tyrosine in myoglobin which has a pK = 10.5; we were able to selectively enhance this tyrosinate residue. The other two tyrosines in myoglobin have higher pK values.[11]

These results indicate that UVRR spectroscopy shows promise as a new technique for the study of aromatic amino acid residues in proteins. The aromatic residues may be examined free of interference from Raman bands of other residues; histidine and N-methylacetamide (a model for a peptide bond), for example, are not sufficiently enhanced with 225-nm excitation to be detected at concentrations even 100 times greater than that of the aromatic amino acids.[12]

Acknowledgment. We gratefully acknowledge support for this study from NSF Instrumentation Grant PCM-8115738 and NIH Grant 1 R01 GM 30741-02.

Note Added in Proof. While this paper was in press another UV Raman study of aromatic amino acids appeared.[13] Although shorter excitation wavelengths were used, the conclusions are similar to those reported here.

Registry No. Phe, 63-91-2; Tyr, 60-18-4; Trp, 73-22-3.

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Temperature Dependence of Long-Range Electron Transfer in [Zn,Fe^{III}] Hybrid Hemoglobin

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We have shown recently[1] that mixed-metal, [M,Fe], hybrid hemoglobins[2] can be used to study long-range electron transfer[3] between chromophores that are rigidly held at fixed *and* crystallographically known distance and orientation.[4] In these experiments hemoglobin 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 aquoferriheme (Fe^{III}P) state. Electron transfer occurs within the α_1–β_2 electron-transfer complex between chromophores that are separated by two heme pocket walls and are at a metal–metal distance of 25 Å. Flash photoexcitation of ZnP to its triplet state initiates the primary process,[5]

$$^3\text{ZnP} + \text{Fe}^{III}\text{P} \xrightarrow{k_t} (\text{ZnP})^+ + \text{Fe}^{II}\text{P}\ \Delta E_0' \approx 0.8\ \text{V} \quad \text{(a)}$$

thereby forming an intermediate that returns to the ground state by back electron transfer from Fe^{II}P to the thermalized cation radical, ZnP$^+$,

$$(\text{ZnP}^+) + \text{Fe}^{II}\text{P} \xrightarrow{k_b} \text{ZnP} + \text{FeP}\ \Delta E_0' \approx 1.0\ \text{V} \quad \text{(b)}$$

We now report the temperature dependence of k_t, which indicates that reaction a involves electron and nuclear tunnelling.[3]

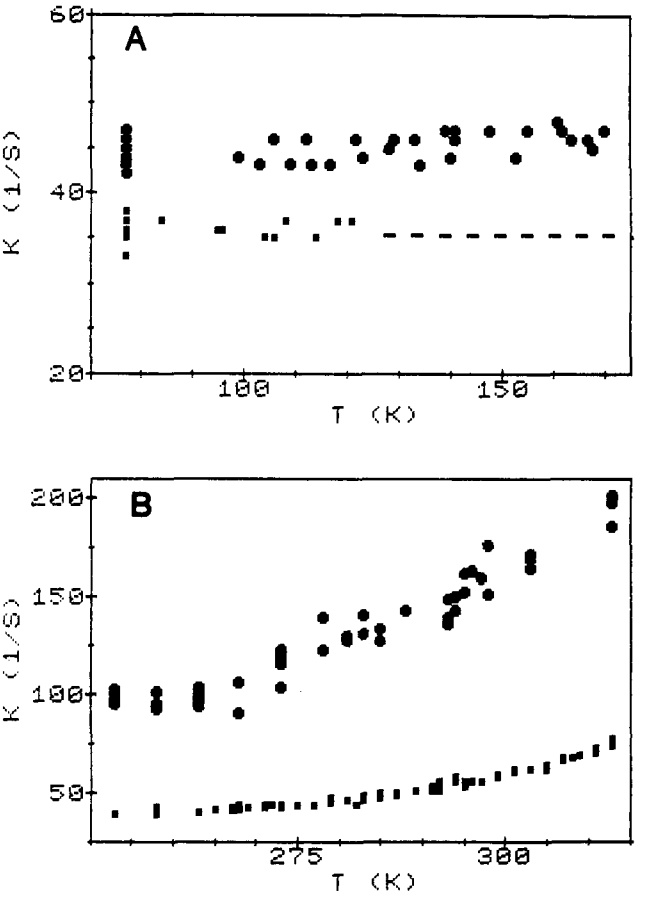

Figure 1. Temperature dependence of the ^{3}ZnP decay rate constants for the oxidized [α(Fe^{III}H$_2$O);β(Zn)] (•) and reduced [α(Fe^{II});β(Zn)] (■) hemoglobin hybrids. (A) Data between 77 and 170 K. The dotted line indicates that the rate constant for the reduced species is invariant in this range, being the same at 250 K as at 120 K. (B) Data between 250 and 315 K. Data for the reduced and oxidized hybrids represent 5 and 16 independently prepared samples, respectively. See ref 6 for details of experimental procedures and conditions.

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(4) Other recent work in this area includes the following: (a) Winkler, J. R.; Nocera, D. G.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1982**, *104*, 5798–5800. (b) Isied, S. S.; Worosila, G.; Atherton, S. J. *Ibid.* **1982**, *104*, 7659–7661. (c) Calcaterra, L. T.; Closs, G. L.; Miller, J. R. *ibid.* **1983**, *105*, 670–671. (d) Kostic, N. M.; Margalit, R.; Che, C.-M.; Gray, H. B. *Ibid* 1983, *105*, 7765–7767.

(5) (a) The concentration of ^{3}ZnP subsequent to flash excitation was monitored, as described in ref 1. Improvements in technique have led to some differences between room-temperature rates reported here and in ref 1. (b) The redox potentials for processes a and b differ substantially from those in ref 1, to the extent that $\Delta E_0'$ (b) > $\Delta E_0'$ (*a*), not the reverse. The original values employed the reduction potential for (ZnP)$^+$ incorporated into horseradish peroxidase. A more appropriate value is that for (ZnP)$^+$ incorporated into hemoglobin, and to our surprise, work in progress indicates that a much larger value than that for peroxidase, namely $E_0'((\text{ZnP})^+) \approx +1.2$ V (NHE) for $e^- + (\text{ZnHb})^+ \rightleftharpoons \text{ZnHb}$. In addition we take $\Delta E_T = E(^3\text{ZnP}) - E(\text{ZnP}) = 1.84$ V (ref 5c) and $E_0'(\text{Hb}^+) = 0.15$ V (NHE, ref 5d) in the equations— $\Delta E_0'(a) = \Delta E_T - E_0'((\text{ZnP})^+) + E_0'(\text{Hb}^+)$; $\Delta E_0'(b) = \Delta E_t - \Delta E_0'(a)$. (c) Stanford, M. A.; Hoffman, B. M. *J. Am. Soc.* **1981**, *103*, 4104–4114. (d) For example: Bull, C.; Hoffman, B. M. Proc. *Natl. Acad. Sci. U.S.A* **1975**, *72*, 3382–3386.

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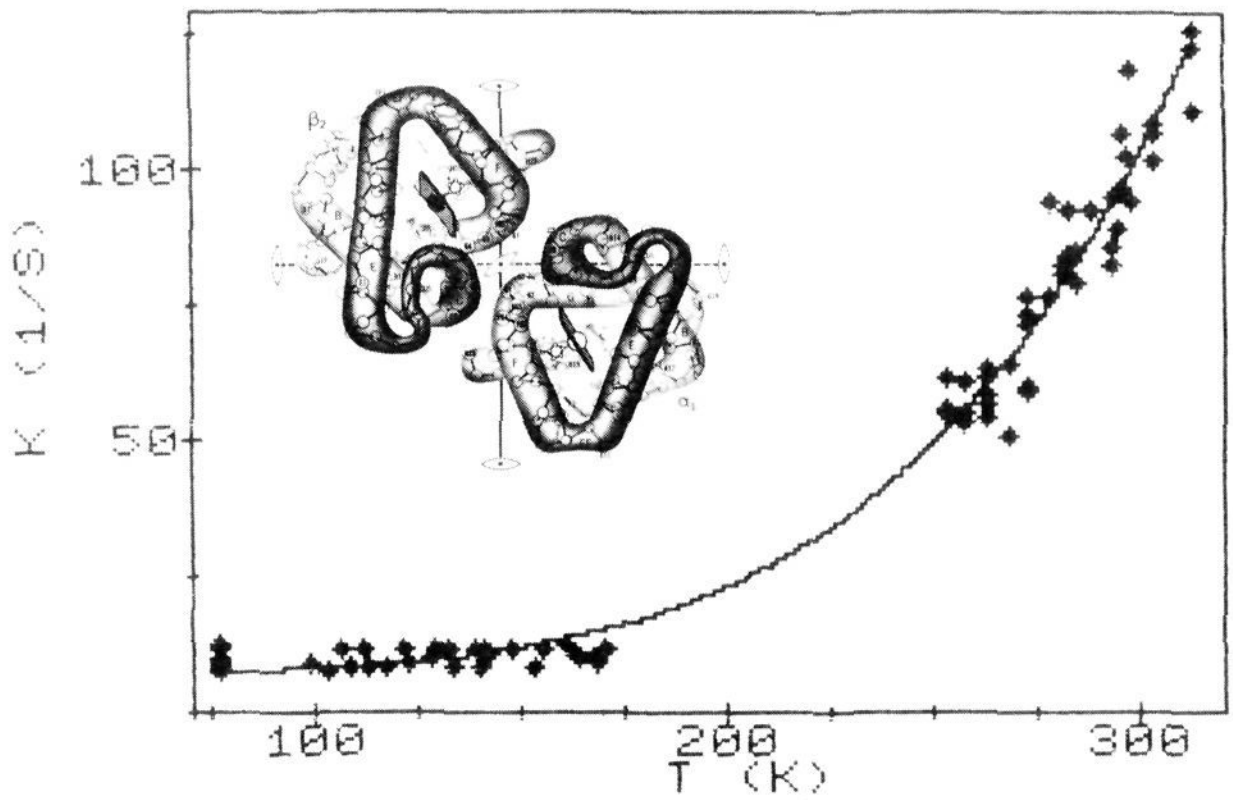

Figure 2. Temperature dependence of the electron-transfer rate, k_t, in the $[\alpha(Fe^{II}H_2O);\beta(Zn)]$ hybrid hemoglobin. The solid line is a plot of eq 1, employing the parameters given in the text. Conditions are given in ref 6. Inset: Representation of the (α_1,β_2) subunit pair, electron-transfer complex within the T state $[\alpha^{Fe},\beta^{Zn}]$. The distance between Fe (O) and Zn (●) atoms is 24.7 Å. Adapted from: Dickerson, R. E.; Geis, I. "The Structure and Action of Proteins"; Harper and Row; New York, 1969.

A ^{3}ZnP in an [Zn,Fe^{III}] hybrid hemoglobin decays with an observed rate constant, k_{obsd}, that is the sum of the intrinsic decay rate constant, k_D, and of the rate constant for electron transfer: $k_{obsd} = k_D + k_t$. The temperature dependence of k_t is determined experimentally as the difference between the observed ^{3}ZnP decay rates for the [Zn,Fe^{III}] and [Zn,Fe^{II}] hybrids. For the [Zn,Fe^{II}] hybrid, electron transfer from ^{3}ZnP is blocked and thus we assign $k_{obsd} = k_D$. For example,[6] at room temperature, k_{obsd} $[\alpha(Fe^{III}H_2O);\beta(Zn)] = 155 \pm 10\ s^{-1}$, k_{obsd} $[\alpha(Fe^{II});\beta(Zn)] = k_D = 55 \pm 3\ s^{-1}$, and thus $k_t = 100 \pm 10\ s^{-1}$. Figure 1 presents the temperature responses of the ^{3}ZnP decay rate constants, k_{obsd}, for the oxidized $[\alpha(Fe^{III}H_2O);\beta(Zn)]$ and the reduced $[\alpha(Fe^{II});\beta(Zn)]$ hemoglobin hybrids.[6] Figure 2 presents the resulting temperature variation of k_t for the $[\alpha(Fe^{III}H_2O);\beta(Zn)]$ hybrid.[7] For all temperatures, $k_h \gg k_t$, so k_h cannot be measured accurately.

The electron-transfer rate, k_t, falls smoothly from the room-temperature value to a nonzero value, $k_t = 9 \pm 4\ s^{-1}$, which is effectively invariant from 170 K down to 77 K. Data 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/mol. The low-temperature rate constant is comparable to that reported to occur over comparable distance in frozen glasses.[8] 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*.[9,3] Only in these two systems has the full temperature response, including observation of a low-temperature plateau,[10] been observed, and only with the [Zn,Fe] hybrid is the molecular architecture known (see Figure 2, inset).

The regime of temperature independence indicates that the transfer process involves nonadiabatic electron tunnelling in which the accompanying nuclear rearrangement proceeds by nuclear tunneling; the temperature dependence indicates coupling of the transfer process to thermal vibrations and/or fluctuations.[3,11] Further work on Hb hybrids is needed to clarify the nature of the nuclear motions involved in the transfer process, but for purposes of comparison to *C. vinosum*, the temperature dependence of k_t (Figure 2) can be described either by the semiclassical[11] or quantum mechanical[12,11b] treatments of thermally assisted, nonadiabatic electron transfer.[3] The simplest expression is that of the semiclassical model incorporating vibronic coupling to oscillators of a single frequency, ω; it may be written in terms of three parameters[3a]

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

Here, $T_c \equiv \hbar\omega/2k_B$, and the other parameters are interpreted as follows: $\beta \equiv E^\dagger/k_\beta T_c \equiv (\Delta E_0' - \lambda)^2/2k\omega\lambda$, where the activation energy in the high-temperature, classical limit[3a] is $E^\dagger = (\Delta E_0' - \lambda)^2/4\lambda$ and λ is the reorganization energy; $\alpha = (2\pi/\hbar)\,|H|^2/(2\pi\hbar\omega\lambda)^{1/2}$, where H is the electron tunnelling matrix element. The solid curve in Figure 2 represents the result of a nonlinear least-squares fit of the data to eq 1 and employs the parameters $T_c = 275$ K, $\alpha = 2.14 \times 10^5\ s^{-1}$, $\beta = 10.3$ K. Calculations with β and T_c give $E^\dagger = 0.244$ V, and employing $\Delta E_0' = 0.8$ V[13] one obtains $\lambda \approx 2.3$ (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.[3] Taking either value of λ, one obtains from α the tunnelling matrix element for the Hb hybrid, $|H| \approx 3\text{–}4 \times 10^{-6}$ eV, which is comparable to values expected on the basis of simple parametrization schemes.[3a]

The observation of tunnelling within the well-defined environment of the Hb hybrids opens the way to modulation of the energetic and vibronic aspects of the process through chemical variation of the macrocycles, metals, and ligands of donor and acceptor chromophores. As a most straightforward example,

(6) ^{3}ZnP decays were monitored at 415 nm, the (Fe^{III}P–Fe^{II}P) isosbestic. To obtain low-temperature data, samples were prepared in 50% (v/v) glycerol/0.01 M KP_i, of pH 6, at room temperature. The pH was chosen so that pH ≈7 at low temperatures (Douzou, P. "Cryobiochemistry: An Introduction"; Academic Press: New York, 1977). The solution also contained 10 μM inositol hexaphosphate and ~1.0 μM tetramer. The rates are independent of concentration (ref 1). Samples were also prepared in 0.01 M KP_i, pH 7 at room temperature, 10 μM inositol hexaphosphate, 1 μM tetramer. At temperatures above ~273 K, there was no variation in k_{obsd} as a function of pH or glycerol concentration. Unpredictable, violent shattering of the glycerol/H_2O glass (and the cuvette) occurring for temperatures between 170 and 250 K prevented measurements in this range. The use of alternate solvent systems will address this problem.

(7) Room-temperature results from α^{Zn} hybrids are similar to those for β^{Zn} reported here and in ref 1, except that the autoreduction noted in ref 1 does not occur for α^{Zn}.

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(10) Work is in progress to confirm this by measuring the electron-transfer rate at liquid-helium temperatures.

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(13) Use of this value (ref 5b) would be appropriate only if the immediate products of the reaction are in their ground state. The experimental situation is ambiguous, since the H_2O bound to Fe^{III}P is unbound in Fe^{II}P.

preliminary measurements indicate that ligation of the ferriheme by CN^- or F^- reduces $\Delta E_0'$ for reaction a and also reduces k_t.[14]

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Registry No. Zn, 7440-66-6.

(14) To be published. Note that the difference in behavior of the aquo- and fluoroferriheme derivatives, both of which are in the $S = {}^5/_2$ states, confirms that paramagnetic quenching of ^{3}ZnP by the ferriheme is not a major factor in these measurements.

Photoinduced Electron Transfer within a Protein-Protein Complex Formed between Physiological Redox Partners: Reduction of Ferricytochrome b_5 by the Hemoglobin Derivative $\alpha_2^{Zn}\beta_2^{Fe^{III}CN}$

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Despite considerable theoretical activity,[2] there is little systematic experimental data on rates of intramolecular electron transfer within proteins. Three separate approaches have been undertaken to provide such data. Gray,[3] Iseid,[4] and co-workers have used coordinative chemical modification to bind redox active $Ru^{III}(NH_3)_5$ moieties to specific histidyl imidazole groups of cytochrome c[3a,b,4] and other proteins.[3c] With such derivatives, photoinduced intramolecular electron transfer between the Ru label and the active site of the protein can be studied. In another approach, McGourty et al.[5] and McLendon et al.[6] have used hemeproteins reconstituted with photoactive heme groups to investigate long distance electron transfer in metal hybrid hemoglobins[5] or in stable complexes formed between interacting cytochromes.[6] A third line of investigation has involved analysis of intramolecular electron transfer between redox-active centers in proteins containing two such sites per protein molecule (e.g., cytochrome cd_1, from *P. aeruginosa*[7]). We now report a unique example of electron transfer within the protein complex formed between two physiological[8] redox partners: (Zn-substituted) hemoglobin (Hb) and cytochrome b_5 (cyt b_5).

This complex is of particular interest for several reasons. First, cyt b_5 and Hb form a stable and *specific* noncovalent complex ($K_A = 3.4 \times 10^5$ at $\mu = 2$ mM, pH 6.2, and 25 °C).[9] Second, the three-dimensional structures of both proteins are known at high resolution.[10] Finally, Poulos and Mauk[11] have recently proposed a detailed three-dimensional model for the complex formed between hemoglobin and cytochrome b_5, which predicts both the distance between (~7 Å edge-edge) and relative orientation of (~coplanar) the heme moieties of the two proteins within this complex.

When redox photoactive hemes (e.g., zinc(II) protoporphyrin IX) are substituted into hemoglobin,[5,12] it is possible to photoinititate electron transfer directly within this complex (eq 1).

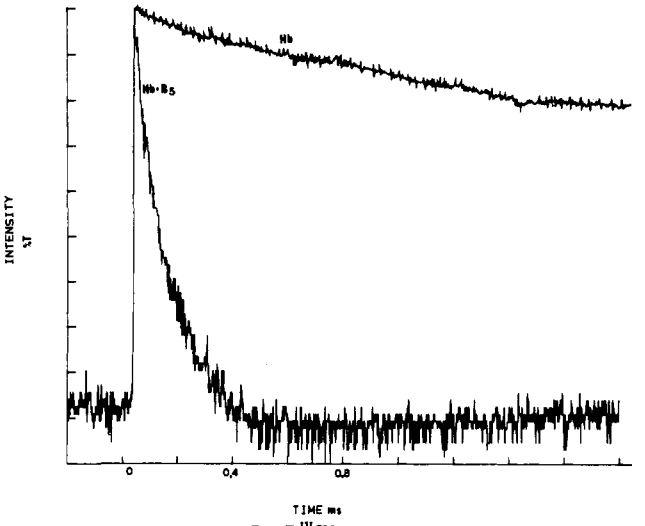

Figure 1. Triplet decay of $\alpha_2^{Zn}\beta_2^{Fe^{III}CN}$ hemoglobin monitored at 475 nm ([Zn] = 2×10^{-5} M) in the presence and absence of ferricytochrome b_5 (4.5×10^{-5} M: 0.001 M phosphate (μ = 0.0012 M), pH 6.2, T = 299 K, nitrogen atmosphere. Under these conditions, >81% of the Zn-containing subunits should have cyt b_5 bound. The tryptic fragment of bovine liver cytochrome b_5[14] and the mixed-metal hemoglobin hybrid[12] were prepared as described previously.

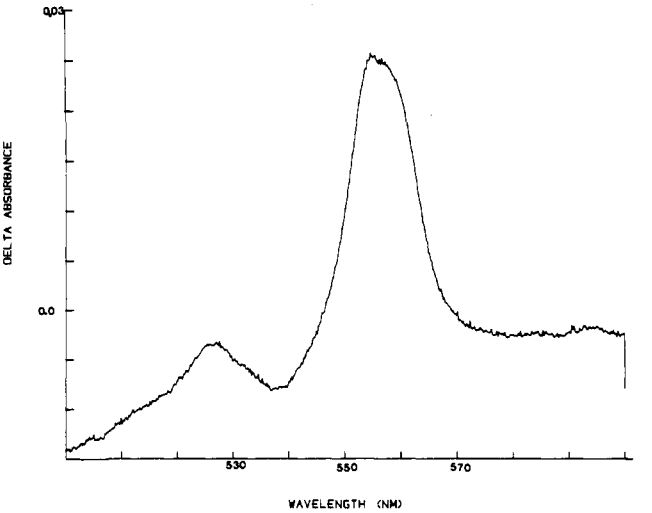

Figure 2. The difference spectrum (irradiated Hb·b_5 complex − unirradiated complex) obtained on irradiating the sample described in the legend of Figure 1 for 10 min with the filtered 436 line of a 200-W Hg lamp. This difference spectrum precisely corresponds to that expected for reduction of Fe^{III}cyt b_5 to Fe^{II}cyt b_5.

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