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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(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,

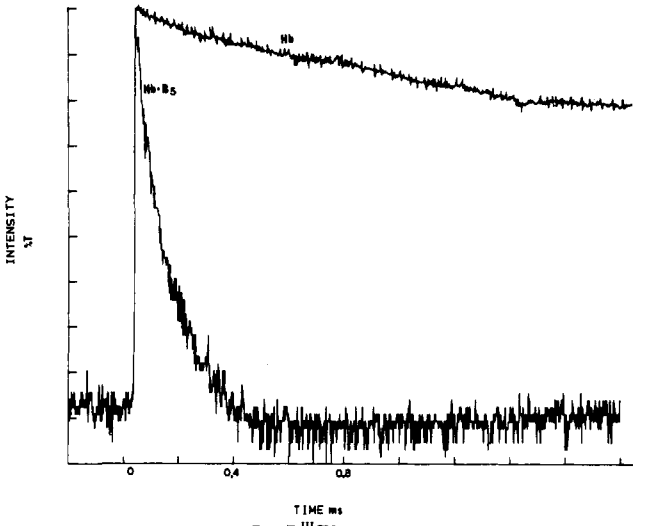

Figure 1. Triplet decay of $\alpha_2^{Zn}\beta_2^{Fe^{III}CN}$ hemoglobin monitored at 475 nm ([Zn] = 2×10^{-5} M) in the presence and absence of ferricytochrome b_5 (4.5×10^{-5} M: 0.001 M phosphate (μ = 0.0012 M), pH 6.2, T = 299 K, nitrogen atmosphere. Under these conditions, >81% of the Zn-containing 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.

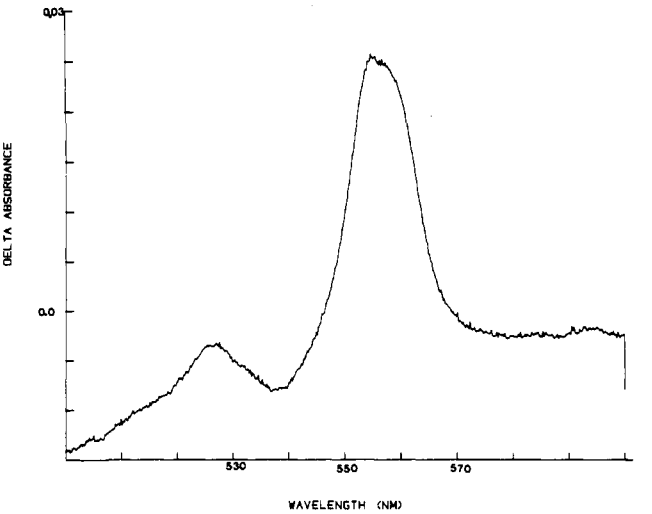

Figure 2. The difference spectrum (irradiated Hb·b_5 complex − unirradiated complex) obtained on irradiating the sample described in the legend of Figure 1 for 10 min with the filtered 436 line of a 200-W Hg lamp. This difference spectrum precisely corresponds to that expected for reduction of Fe^{III}cyt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

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).

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$$^3(ZnHb)^*\cdot Fe^{III}(cyt\ b_5) \rightarrow (ZnHb^+)\cdot Fe^{II}(cyt\ b_5) \qquad \Delta E \equiv 1.0\ V^{13} \quad (1)$$

Results of laser flash photolysis experiments are shown in Figure 1.[14] The Hb derivative $\alpha_2^{Zn}\beta_2^{Fe^{III}CN\ 5,12}(Zn_2Hb)$ has a long-lived triplet excited state, with $^3k = 60\ s^{-1}$. However, when this derivative of Hb is bound to ferricytochrome b_5, the Zn lifetime is dramatically reduced: $^3k = 8 \times 10^3\ s^{-1}$ (Figure 1). *No* significant decrease in zinc porphyrin triplet lifetime is observed when Hb is bound to ferrocytochrome b_5 or under conditions of pH and ionic strength that are incompatible with formation of a complex between the two proteins. As shown elsewhere,[6] this decrease cannot be explained in terms of simple (dipolar) energy transfer. Moreover, on irradiation, a persistent increase in [Fe^{II}cyt b_5] is observed[16] (Figure 2), and the rate of formation of ferrocytochrome b_5 equals the rate of zinc(II) porphyrin triplet decay. These observations demonstrate that the observed enhancement of triplet decay rate in the Hb·cyt b_5 complex occurs by a direct electron transfer deactivation of the porphyrin triplet. The formation of a stable and detectable level of ferrocytochrome b_5 is in accord with observation by McGourty[5] of analogous redox chemistry on irradiating the $\alpha_2^{Fe(III)}\beta_2^{Zn(II)}$ Hb hybrid. This directly demonstrable reduction implies[5] that the zinc(II) porphyrin cation radical that is formed after electron transfer decays (by a yet uncharacterized pathway) in competition with the recombination reaction:

$$(HbZn^{II}\text{–porphyrin}^{+}\cdot) + Fe^{II}cyt\ b_5 \rightarrow (HbZn^{II}\text{–porphyrin})\cdot Fe^{III}cyt\ b_5 \quad (2)$$

Two interesting rate comparisons can be made with this result. First, the rate of intramolecular electron transfer within the complex of $a_2^{Zn}\beta_2^{Fe^{III}CN}$ and ferricytochrome b_5 ($k \sim 8 \times 10^3\ s^{-1}$; heme edge-to-heme edge distance ~7 Å[11]) far exceeds that reported[5] for intrasubunit electron transfer in Hb ($k \sim 60\ s^{-1}$; heme edge-to-heme edge distance ~ 20 Å[5]). This difference is not unexpected given the difference in separation between the donor and acceptor sites. If anything, the dependence of rate on distance is weaker than might have been expected from studies of nonadiabatic electron-transfer reactions in glasses.[18]

Second, although the physiological complexes of Hb·cyt b_5 and cyt c·cyt b_5 are thought to be structurally similar,[11,17] large differences are observed between the electron-transfer rates of $\alpha_2^{Zn}\beta_2^{Fe^{III}CN}$/cyt b_5 ($k \sim 8 \times 10^3\ s^{-1}$) and Zn(cyt c)/cyt b_5 ($k \sim 4 \times 10^5\ s^{-1}$).[6] Clearly, (subtle) structural differences between these complexes are sufficient to cause large rate differences. These differences may reflect direct differences in the "conductivity" of Hb vs. cyt c or may reflect a difference in protein flexibility, which allows stronger coupling between the cyt c/cyt b_5 hemes. These alternatives can be tested, and such tests are in progress.

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(13) $^3E_{00}$ = 1.8 V for zinc(II) protoporphyrin, $E^\circ(ZnP^+/ZnP)$ = 0.85 V, E°(cyt b_5) = 0.05 V. Therefore, ΔE_{rxn}^2 = 1.8 - 0.85 + 0.05 = 1.0 V.

(14) The laser flash system is similar to that described elsewhere.[7] It includes a Quanta Ray DCR-2 Nd YAG laser operated at 532 nm (160 mJ/pulse), with standard 6 stage P.M.T. (1P28) detection and input to a biomation 6500 transient recorder for signal averaging. The entire system is controlled by a PDP 11/23 processor.

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(16) The quantum yield for formation of ferrocytochrome b_5 is low ($\Phi \sim 0.02$) compared with that for Zn/Fe^{III}Hb ($\Phi \sim 0.15$) and is consistent with a more rapid back recombination in the $\alpha_2^{Zn}\beta_2^{Fe^{III}CN}$·cyt b_5 complex.

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Facile Deuterium Exchange of Alkyl and Methine Protons in Octaalkylporphyrins

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Selectively deuterated porphyrins and metalloporphyrins are of considerable utility for physicochemical studies of this important class of compounds. The deuterium-substituted species provide a direct means for resolving or identifying vibrational bands. In addition to clarifying peak assignments in NMR spectroscopy, deuterated metalloporphyrins permit in situ deuterium NMR spectroscopy of highly reactive electrochemically oxidized and reduced metalloporphyrin species.[1-3]

Deuterium incorporation through total synthesis is possible, but new procedures for deuterium exchange on preformed porphyrins would clearly be desirable. Such procedures have been developed for methine deuteration of porphyrins and metalloporphyrins. The simplest of these techniques involves refluxing the porphyrin or metalloporphyrin in deuteroacetic acid.[4] However, the assumption of methine specificity is called into question by the results presented herein (vide infra). The remainder of these techniques are limited to vinyl-substituted porphyrins,[5] metalloporphyrins which tend to demetalate in a side reaction,[6,7] those requiring sealed-tube reactions,[8] and base-catalyzed exchange on porphyrins with strong electron-withdrawing substituents.[9]

Among the techniques for methine deuteration is the report that naturally occurring porphyrins may be deuterated at the methine position by deuterio-*p*-toluenesulfonic acid in an *o*-dichlorobenzene reflux in 2-4 days.[10] While employing octaethylporphyrin deuterated by this procedure (in the presence of sodium chloride) we fortuitously discovered that a small fraction of the methylene protons had been exchanged. By increasing the concentration of the acid and the time of reflux, significant and useful levels of deuteration of the ring-adjacent protons can be achieved, as well as nearly quantitative methine deuteration. Furthermore, we have found that a melt of deuterio-*p*-toluenesulfonic acid serves to deuterate octaalkylporphyrins exclusively at the methine position in hours rather than days.

For simultaneous ring alkyl and methine hydrogen exchange typically 1.5 g of *p*-toluenesulfonic acid sodium salt was dissolved in 15 mL of deuterium oxide and 1.5 mL of 6 M DCl and rotoevaporated to dryness. To the flask were added 80 mL of dry *o*-dichlorobenzene and 75 mg of etioporphyrin (80/1 mol ratio of acid/porphyrin). The mixture was allowed to reflux under nitrogen for 4-8 days. The solution was filtered and shaken twice with 75 mL of water to extract the sodium chloride and acid. After freezing at -10 °C, most of the water was removed by decanting, and the solution was rotoevaporated to dryness. The porphyrin was redissolved in chloroform, the solution was filtered on a medium-porosity glass frit, and the porphyrin was precipitated by addition of heptane. Both deuterium and proton NMR spectra were recorded. The extent of deuteration was determined by integration of proton NMR peaks using the methyl peak of the ethyl group as a reference. Four days of reflux resulted in 92% methine deuteration and 45% deuteration of the ring-adjacent

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