*Experimental Section*

The alkylation in the presence of (*P*)-(+)-**2** with ≈0.13 % *ee* is representative (Table 1, entry 5). The *ee* value of **2** was determined by comparison of its specific rotation ($[\alpha]_D^{28} = +4.8^\circ$ ($c = 1.68$, $CHCl_3$)) with that of an enantiomerically pure sample (lit. [16] $[\alpha]_D^{20} = +3707^\circ$ ($c = 0.082$, $CHCl_3$)). A solution of $i Pr_2Zn$ in toluene (1M, 0.15 mL) was added dropwise over a period of 30 min at 0 °C to a solution of aldehyde **1** (9.4 mg, 0.05 mmol) and (*P*)-(+)-**2** (36.9 mg, 0.11 mmol) in toluene (2.5 mL). After the mixture was stirred for 16 h, toluene (0.8 mL), a solution of $i Pr_2Zn$ in toluene (1M, 0.48 mL), and aldehyde **1** (37.6 mg, 0.2 mmol) in toluene (1.5 mL) were added successively. After 7 h, toluene (4.7 mL), a solution of $i Pr_2Zn$ in toluene (1M, 1.92 mL), and aldehyde **1** (151 mg, 0.8 mmol) in toluene (2 mL) were added, and the mixture was stirred for an additional 16 h. The reaction was quenched by the addition of hydrochloric acid (1M, 5 mL), and made alkaline by the addition of saturated aqueous sodium bicarbonate (15 mL). The mixture was filtered through celite, and the separated aqueous layer was extracted with ethyl acetate. The combined organic layers were dried over sodium sulfate and concentrated. Purification of the residue by thin-layer chromatography on silica gel (developing solvent: hexane/ethyl acetate 2:1) gave pyrimidyl alkanol **4** (215 mg, 88 %). The *ee* value was determined to be 56 % by HPLC analysis using a chiral stationary phase (Daicel Chiralcel OD).

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[1] a) *Comprehensive Asymmetric Catalysis* (Eds.: E. N. Jacobsen, A. Pfaltz, H. Yamamoto), Springer, Berlin, **1999**; b) *Catalytic Asymmetric Synthesis* (Ed.: I. Ojima), 2nd ed., Wiley, New York, **2000**.

[2] a) R. H. Martin, *Angew. Chem.* **1974**, *86*, 727 – 738; *Angew. Chem. Int. Ed. Engl.* **1974**, *13*, 649 – 660; b) R. H. Janke, G. Haufe, E.-U. Würthwein, J. H. Borkent, *J. Am. Chem. Soc.* **1996**, *118*, 6031 – 6035; c) S. Grimme, S. D. Peyerimhoff, *Chem. Phys.* **1996**, *204*, 411 – 417; d) J. M. Schulman, R. L. Disch, *J. Phys. Chem. A* **1999**, *103*, 6669 – 6672.

[3] a) M. T. Reetz, E. W. Beuttenmüller, R. Goddard, *Tetrahedron Lett.* **1997**, *38*, 3211 – 3214; b) A. Terfort, H. Görls, H. Brunner, *Synthesis* **1997**, 79 – 86.

[4] S. D. Dreher, T. J. Katz, K. C. Lam, A. L. Rheingold, *J. Org. Chem.* **2000**, *65*, 815 – 822.

[5] T. Shibata, S. Yonekubo, K. Soai, *Angew. Chem.* **1999**, *111*, 749 – 751; *Angew. Chem. Int. Ed.* **1999**, *38*, 659 – 661.

[6] For a review, see: K. Soai, S. Niwa, *Chem. Rev.* **1992**, *92*, 833 – 856.

[7] Racemic [6]helicene (R. H. Martin, M.-J. Marchant, M. Baes, *Helv. Chim. Acta* **1971**, *54*, 358 – 360) was resolved by preparative HPLC using a chiral stationary phase (Chiralcel OD).

[8] Racemic [5]helicene (F. Dubois, M. Gingras, *Tetrahedron Lett.* **1998**, *39*, 5039 – 5040) was resolved by preparative HPLC using a chiral stationary phase (Chiralcel OD).

[9] a) K. Soai, T. Shibata, H. Morioka, K. Choji, *Nature* **1995**, *378*, 767 – 768; b) T. Shibata, H. Morioka, T. Hayase, K. Choji, K. Soai, *J. Am. Chem. Soc.* **1996**, *118*, 471 – 472; c) T. Shibata, J. Yamamoto, N. Matsumoto, S. Yonekubo, S. Osanai, K. Soai, *J. Am. Chem. Soc.* **1998**, *120*, 12157 – 12158; d) K. Soai, S. Osanai, K. Kadowaki, S. Yonekubo, T. Shibata, I. Sato, *J. Am. Chem. Soc.* **1999**, *121*, 11235 – 11236; e) I. Sato, K. Kadowaki, K. Soai, *Angew. Chem.* **2000**, *112*, 1570 – 1572; *Angew. Chem. Int. Ed.* **2000**, *39*, 1510 – 1512; f) K. Soai, T. Shibata, I. Sato, *Acc. Chem. Res.* **2000**, *33*, 382 – 390.

[10] For reviews on asymmetric autocatalysis with amplification of *ee*, see: a) K. Soai, *Enantiomer* **1999**, *4*, 591 – 598; b) K. Soai, T. Shibata, *Yuki Gosei Kagaku Kyokaishi* **1997**, *55*, 994 – 1005; c) H. Buschmann, R. Thede, D. Heller, *Angew. Chem.* **2000**, *112*, 4197 – 4199; *Angew. Chem. Int. Ed.* **2000**, *39*, 4033 – 4036; d) C. Bolm, F. Bienewald, A. Seger, *Angew. Chem.* **1996**, *108*, 1767 – 1769; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1657 – 1659; e) M. Avalos, R. Babiano, P. Cintas, J. L. Jiménez, J. C. Palacios, *Chem. Commun.* **2000**, 887 – 892; for reviews of non-autocatalytic and autocatalytic reactions with amplification of *ee*, see: f) C. Girard, H. B. Kagan, *Angew. Chem.* **1998**, *110*, 3088 – 3127; *Angew. Chem. Int. Ed.* **1998**, *37*, 2922 – 2959; g) K. Mikami, M. Terada, T. Korenaga, Y. Matsumoto, M. Ueki, R. Angelaud, *Angew. Chem.* **2000**, *112*, 3676 – 3701; *Angew. Chem. Int. Ed.* **2000**, *39*, 3532 – 3557; h) K. Soai, T. Shibata in *Comprehensive Asymmetric Catalysis* (Eds.: E. N. Jacobsen, A. Pfaltz, H. Yamamoto), Springer, Berlin, **1999**, pp. 699 – 725.

[11] For a review, see: B. L. Feringa, R. A. van Delden, *Angew. Chem.* **1999**, *111*, 3624 – 3645; *Angew. Chem. Int. Ed.* **1999**, *38*, 3418 – 3438.

[12] J. M. Brown, I. P. Field, P. J. Sidebottom, *Tetrahedron Lett.* **1981**, *22*, 4867 – 4870.

[13] H. Ihara, T. Sagawa, K. Nakashima, K. Mitsuishi, Y. Goto, J. Chowdhury, S. Sakaki, *Chem. Lett.* **2000**, 128 – 129.

[14] When benzene was added to a solution of **1** in $CDCl_3$ (0.031M), the $\Delta\delta$ values of the signals for the aldehyde proton and for the protons of the pyrimidine ring are −1.5 Hz and −0.9 Hz, respectively, per molar equivalent of benzene.

[15] a) A. Moradpour, J. F. Nicoud, G. Balavoine, H. B. Kagan, G. Tsoucaris, *J. Am. Chem. Soc.* **1971**, *93*, 2353 – 2354; b) W. J. Bernstein, M. Calvin, O. Buchardt, *J. Am. Chem. Soc.* **1972**, *94*, 494 – 497; for a review, see: Y. Inoue, *Chem. Rev.* **1992**, *92*, 741 – 770.

[16] *Dictionary of Organic Compounds* (Ed.: J. Buckingham), 5th ed., Chapman and Hall, New York, **1983**.


## Interprotein Electron Transfer Reaction Regulated by an Artificial Interface**

Yutaka Hitomi, Takashi Hayashi,* Kenji Wada, Tadashi Mizutani, Yoshio Hisaeda, and Hisanobu Ogoshi

Electron transfer (ET) reactions are fundamental to numerous important biological processes such as respiration, photosynthesis, and redox reactions of intermediary metabolism. In these systems, interprotein ET reactions are regulated by a specific interaction between two redox proteins. A unique charge distribution on the protein surface is responsible for this recognition. For example, cytochrome *c*, an electron carrier between complexes III and IV of the respiratory chain, has several highly conserved lysine residues surrounding the slightly exposed heme edge, while redox partners have a recognition domain consisting of anionic residues with the same topological symmetry as the lysine

[*] Prof. Dr. T. Hayashi, Prof. Dr. Y. Hisaeda
Department of Synthetic Chemistry and Biochemistry
Graduate School of Engineering, Kyushu University
Fukuoka 812-8581 (Japan)
Fax: (+81) 92-632-4718
E-mail: thayatcm@mbox.nc.kyushu-u.ac.jp
Dr. Y. Hitomi, K. Wada, Prof. Dr. T. Mizutani
Department of Synthetic Chemistry and Biological Chemistry
Graduate School of Engineering, Kyoto University
Kyoto 606-8501 (Japan)
Prof. Dr. H. Ogoshi
Fukui National College of Technology
Sabae, Fukui 916-8507 (Japan)

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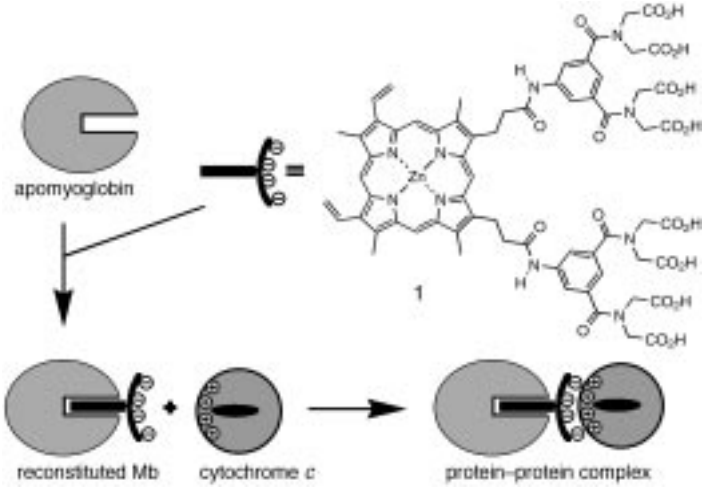
residues of cytochrome *c*. A series of studies utilizing mutagenesis, kinetics, X-ray crystal-structure analysis, and computational docking show that the electrostatic interaction between charged patches on each protein surface guides them to an optimal orientation of the reactant complex for facile ET.[1–4] Over the last decade, a large number of synthetic models have been developed to elucidate the factors that determine the biological ET reaction rate; however, ET model studies on the interfacial recognition of interprotein ET reactions are largely unknown.[5–7]

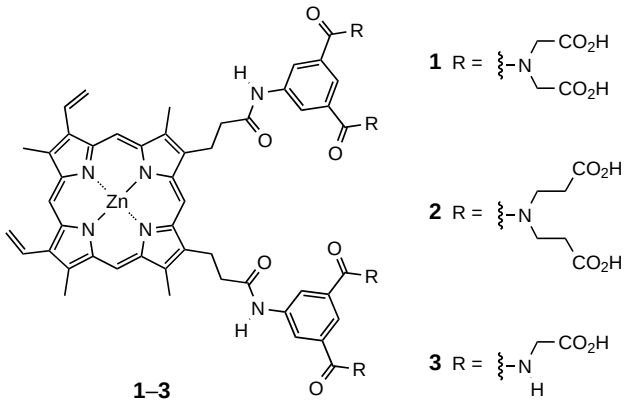
Herein we report on an interprotein ET reaction regulated by artificial recognition interfaces between cytochrome *c* and a series of reconstituted myoglobins (rMbs). Recently, we reported on rMb(**1**), having a functionalized zinc porphyrin **1** as a prosthetic group, which can form a protein–protein complex with cytochrome *c* through electrostatic interactions as shown in Scheme 1.[7] The cytochrome *c* recognition unit

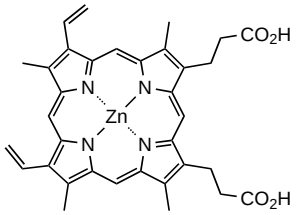



Scheme 1. Preparation of a reconstituted myoglobin bearing a cytochrome *c* receptor and the formation of a protein–protein complex with cytochrome *c*.

introduced on the myoglobin (Mb) surface has a total of eight carboxylates around two rigid and hydrophobic benzene rings at the terminal of the heme propionates. On this surface the negative charges should spread over approximately 15 Å$^2$ to match the situation in the lysine residues in the binding domain of cytochrome *c*. We found that rMb(**1**) works as an artificial ET protein, which efficiently photochemically reduces cytochrome *c* by forming the stable protein–protein complex. The facile construction of various recognition interfaces on the protein surface might provide a novel ET model which can be used to systematically understand the role of recognition interfaces in interprotein ET reaction.

Based on rMb(**1**), we designed the zinc myoglobins rMb(**2**) and rMb(**3**) which are a new type of cytochrome *c* receptor. Zinc porphyrins **1** and **2**, with the same number of carboxylates, differ in the flexibility of the attached receptor units. Zinc porphyrin **3** has four carboxylates, which provide a control for probing the charge number required for cytochrome *c* recognition. Zinc porphyrins **1**–**3** were synthesized according to the reported procedure, and then incorporated




into apoMb.[7] The resultant species rMb(**1**)–rMb(**3**) were purified on cation-exchange and gel columns. Similarly, a native zinc myoglobin, rMb(**4**) with the protoporphyrin zinc complex **4** was prepared as a reference protein. The UV/Vis spectra of rMb(**1**)–rMb(**3**) are virtually identical to that of rMb(**4**) (see Experimental Section).

Binding features and interprotein ET between cytochrome *c* and rMb(**1**)–rMb(**4**) were investigated using flash photolysis analysis. The excitation by laser of the porphyrin π system produces a triplet excited state of rMb(**1**)–rMb(**4**), $^3$rMb(**1**)–$^3$rMb(**4**), with intersystem crossing. The natural decay of $^3$rMb(**1**)–$^3$rMb(**4**), a return to the ground state, was studied by monitoring the absorbance at 460 nm which corresponds to the transient absorbance difference between $^3$rMb and rMb (Figure 1a). In the absence of cytochrome *c*, the decay profiles of $^3$rMb(**1**)–$^3$rMb(**3**), have single exponen-

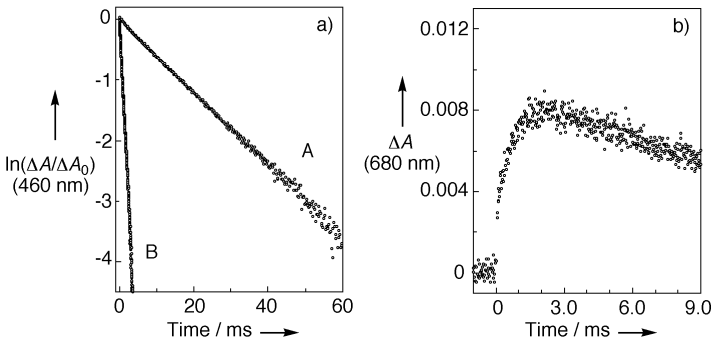



Figure 1. a) Triplet decay traces for rMb(**2**) in the absence (trace A) and presence (trace B) of cytochrome *c*. b) Transient absorption for the ET intermediate of rMb(**2**) under the same conditions as for trace B. Conditions: Phosphate buffer (μ = 10 mM, pH 7.0), 293 K; [rMb(**2**)] = $3.8 \times 10^{-6}$ M; [cytochrome *c*] = $1.0 \times 10^{-6}$ M.

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tial functions with $k_D = 59 \pm 1\ s^{-1}$, which is identical to the value observed for $^3$rMb(**4**). Adding cytochrome *c*(III) to solutions containing rMb resulted in a dramatic acceleration of the decay rate of $^3$rMb with a single exponential remained. In contrast to that, no acceleration was observed upon addition of cytochrome *c*(II), which indicates that the quenching is the result of an ET reaction from rMb to cytochrome *c*(III). Furthermore, the appearance and disappearance of ET intermediates as well as of the zinc porphyrin cation radical of rMb and cytochrome *c*(II), were observed at 680 nm (Figure 1b) and 550 nm, respectively. The observed decay rate constants ($k_{obs}$) of $^3$rMb(**1**) – $^3$rMb(**4**) are plotted for the concentration of cytochrome *c*(III) in Figure 2. The quenching

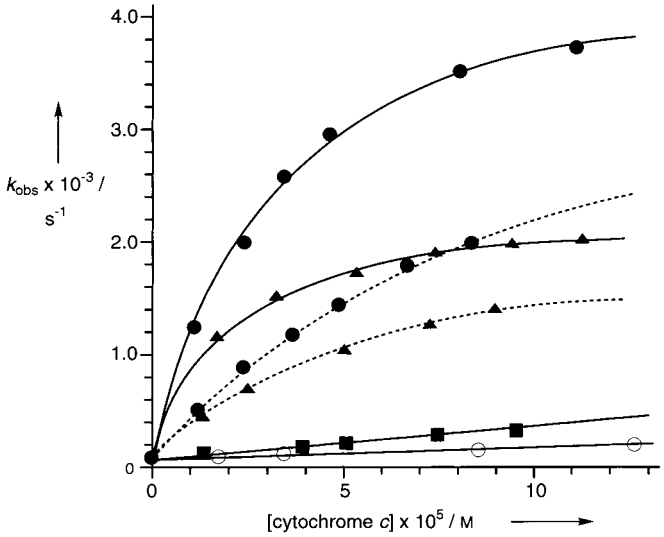


Figure 2. Decay rate of the triplet state of rMb(**1**) – rMb(**4**) as a function of cytochrome *c* concentration, in phosphate buffer at pH 7.0 and 293 K. ▲ = rMb(**1**); ● = rMb(**2**); ■ = rMb(**3**); ○ = rMb(**4**). The initial concentration of rMb is $4.3 \times 10^{-6}$ M, the lines are fitting curves and the ionic strengths are 10 mM (solid line) and 20 mM (dashed line).

of $^3$rMb(**3**) and $^3$rMb(**4**) is enhanced linearly with the concentration of cytochrome *c*(III). This linearity is consistent with the simple collisional quenching mechanism, which does not necessarily require the existence of a stable protein – protein complex. In this case, the triplet quenching rate ($k_q$) is given by [Eq (1)], where *C* represents the concentration

$$k_{obs} = k_D + k_q C \qquad (1)$$

of cytochrome *c*(III). From the fitting of the slope by [Eq (1)], the values of $k_q$ are determined to be $(2.2 \pm 0.2) \times 10^6\ M^{-1} s^{-1}$ and $(8.0 \pm 0.3) \times 10^5\ M^{-1} s^{-1}$ for rMb(**3**) and rMb(**4**), respectively.[8] The $k_q$ of rMb(**3**) is 2.8-fold faster than that observed for native rMb(**4**), which clearly indicates that the artificially introduced anionic recognition unit can serve as an attractive interaction site for cytochrome *c*. However, the charge introduced to rMb(**3**) is not enough to make a stable complex with cytochrome *c* through electrostatic interactions. On the other hand, $^3$rMb(**1**) and $^3$rMb(**2**) were very efficiently quenched by cytochrome *c*(III). The quenching rates increase nonlinearly with the concentration of cytochrome *c*(III) and level off at higher concentrations. This saturation can be accounted for by a quenching mechanism involving a fast equilibrium between the complex and its components. The electron transfer rate within the intracomplex ET ($k_{intra}$) and binding affinity ($K_a$) between cytochrome *c* and rMb are given by [Eq (2)], where *f* and *M* represent the fraction of

$$k_{obs} = k_D + k_{intra} f = k_D + k_{intra}[M + C + 1/K_a - \{(M + C + 1/K_a)^2 - 4MC\}^{1/2}]/2M \qquad (2)$$

complexed rMb and the total concentration of rMb, respectively. The experimental data at 10 and 20 mM ionic strength were fitted to [Eq (2)] by an iterative, nonlinear least-squares routine to yield a rate constant for intracomplex ET and binding affinities between cytochrome *c*(III) and rMb(**1**), and between cytochrome *c*(III) and rMb(**2**). The values of $K_a$ and $k_{intra}$ are summarized in Table 1. The binding affinities of

Table 1. The ET rate constants and binding constants between rMb and cytochrome *c*.[a]

| | Ionic strength | $k_{intra} \times 10^{-3}$ [$s^{-1}$] | $K_a \times 10^{-4}$ [$M^{-1}$] |
|---|---|---|---|
| rMb(**1**) | $\mu = 10$ mM | $2.2 \pm 0.1$ | $6.5 \pm 0.3$ |
| | $\mu = 20$ mM | $2.2 \pm 0.1$ | $1.5 \pm 0.6$ |
| rMb(**2**) | $\mu = 10$ mM | $4.8 \pm 0.2$ | $3.1 \pm 0.2$ |
| | $\mu = 20$ mM | $4.8 \pm 0.2$ | $0.83 \pm 0.1$ |

[a] All data were obtained at 293 K in phosphate buffer, pH 7.0.

rMb(**2**) are 2.1- and 1.8-fold smaller than those of rMb(**1**) at 10 mM and 20 mM ionic strength, respectively. This difference in the affinities for cytochrome *c* ($\Delta\Delta G = 1.8$ and 1.4 kcal mol$^{-1}$ at 10 and 20 mM ionic strength, respectively) arises from the difference in flexibility of recognition interfaces between rMb(**1**) and rMb(**2**). In contrast, $k_{intra}$ was greater for rMb(**2**) than for rMb(**1**).

Previous NMR spectroscopic studies revealed that the rMb recognizes the same region of cytochrome *c* that its physiological partners do.[7, 9] In the docking complex of the rMb and cytochrome *c*, therefore, the heme of cytochrome *c* is expected to be in the vicinity of the recognition units. This expectation is also supported by a small difference between the binding constants observed for rMb(**1**) and rMb(**2**). Accordingly the ET rates should not be greatly altered, even though the additional methylene groups of **2** could slightly decrease the electron coupling and the ET rate. However, $k_{intra}$ for rMb(**2**) is 2.2-fold larger than that observed for rMb(**1**). This result may be because the interface of the rMb(**2**) – cytochrome *c* complex is more flexible than that of rMb(**1**). This difference cannot be explained simply from the static structural viewpoint.

Recent discussion on ET reactions has pointed out that the observed ET rate is not a real ET rate constant when the redox reaction is controlled by a non-ET process.[10c, 11] If a protein – protein complex is undergoing interconversion between several conformational forms, then this process influences the observed ET rate constant. In fact, a protein – protein complex often adopts multiple conformational states. In some cases it has been reported that an interprotein ET reaction is

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regulated by configurational rearrangement in the complex.[10, 12, 13] These considerations lead us to one possible interpretation for the observed data: the rMb–cytochrome *c* complex exists in several conformational states. The most stable conformation, which contributes the most to the apparent binding constant, is not the most reactive conformation. The interfacial dynamics within the complex induce the most reactive conformation for ET, for example, with the heme of cytochrome *c* and the recognition units in contact. The more flexible recognition interface in the rMb(**2**)–cytochrome *c* complex facilitates this rearrangement. As a consequence, an apparent ET rate in the rMb(**2**)–cytochrome *c* system is faster than that of the rMb(**1**)–cytochrome *c* complex. At the moment, however, we do not exclude other possibilities. Further work will be done to reveal the detail mechanism of the ET reaction.

In summary, we have designed and constructed a novel *synthetic model* of interprotein ET to evaluate the importance of protein–protein recognition at a molecular level. Binding properties and overall ET rates observed for rMb(**1**) and rMb(**2**) clearly demonstrate the importance of the interfacial dynamic feature of the interprotein ET reaction. Although recently temperature and viscosity dependence of interprotein ET revealed that the ET reaction can be regulated by configurational changes,[12, 13] to our knowledge this is the first study that directly shows the control of the interprotein ET reaction by the flexibility of the recognition interface. Moreover, the data presented here show that a strict recognition does not necessarily result in efficient ET, although a proper recognition domain is required for an interprotein ET reaction.

## *Experimental Section*

Preparation of reconstituted myoglobins. ApoMb was prepared from horse heart metMb (Sigma) by Teale's 2-butanone method.[14] The solution of apoMb (0.4 mM) was mixed with **2** or **3** (1.0 equiv) and allowed to stand over 12 h at 4 °C. The mixture was passed through Sephadex G-25 and CM-cellulose columns; rMb(**1**): UV/Vis (phosphate buffer, pH 7.0) ($\lambda_{max}$ [nm] (relative intensity)): 428 nm (1.0), 555 (0.065), 595 (0.051); rMb(**2**): UV/Vis (phosphate buffer, pH 7.0) ($\lambda_{max}$ [nm] (relative intensity)): 428 (1.0), 555 (0.056), 595 (0.044); rMb(**3**): UV/Vis (phosphate buffer, pH 7.0) ($\lambda_{max}$ [nm] (relative intensity)): 428 (1.0), 555 (0.061), 595 (0.051); rMb(**4**): UV/Vis (phosphate buffer, pH 7.0) ($\lambda_{max}$ [nm] (relative intensity)): 428 (1.0), 555 (0.065), 595 (0.051). Further preparative and analytical data can be found in the Supporting Information.

Laser flash photolysis studies: the nanosecond laser photolysis studies were carried out with a Q-switched Nd:YAG laser, which delivered 6-ns pulses at 532 nm. The incident energy was about 10 mJ. The probe source was a continuous 150-W xenon arc lamp passed through a monochrometer. Signals were detected in transmission using a photo multiplier (Hamamatsu Photonics, R2949) and the transient signals were digitized using Tektronix TDS 320 oscilloscope. Signals were averaged 1000 to 5120 times. The data were transferred to a NEC PC9821Ae computer for further data analysis. No smoothing artifact affected the results of the data analysis. The sample solution containing Mb and cytochrome *c* (horse heart, Sigma) in a 10-mm quartz cell was prepared in a glove box. The rMb concentration was about $3.8-4.3\times10^{-6}$ M. The temperature was maintained at 293 K using a NESLAB circulation system.

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[1] *Cytochrome c* (Eds.: R. A. Scott, A. G. Mauk), University Science Books, Herndon, VA, **1996**.
[2] For recent reviews: a) G. McLendon, R. Hake, *Chem. Rev.* **1992**, *92*, 481–490; b) J. M. Nocek, J. S. Zhou, S. D. Forest, S. Priyadarsy, D. N. Beratan, J. N. Onuchic, B. M. Hoffman, *Chem. Rev.* **1996**, *96*, 2459–2489; c) B. Durham, F. Millett in *Comprehensive Supramolecular Chemistry*, *Vol. 5* (Ed.: K. S. Suslick), Pergamon, Oxford, **1996**, pp. 219–247.
[3] For example, see: a) T. L. Poulos, J. Kraut, *J. Biol. Chem.* **1980**, *255*, 10322–10330; b) H. Pelletier, J. Kraut, *Science* **1992**, *258*, 1748–1755; c) V. A. Roberts, M. E. Pique, *J. Biol. Chem.* **1999**, *274*, 38051–38060; d) H. Tian, R. Sadoski, L. Zhang, C. A. Yu, L. Yu, B. Durham, F. Millett, *J. Biol. Chem.* **2000**, *275*, 9587–9595.
[4] Hydrophobic interactions also make an important contribution to complex stability for interprotein ET. For example, see: J. K. Hurley, M. F. Fillat, C. Gómez-Moreno, G. Tollin, *J. Am. Chem. Soc.* **1996**, *118*, 5526–5531.
[5] a) J. L. Sessler, B. Wang, S. L. Springs, C. T. Brown in *Comprehensive Supramolecular Chemistry*, *Vol. 4* (Ed.: Y. Murakami), Pergamon, Oxford, **1996**, pp. 311–336; b) T. Hayashi, H. Ogoshi, *Chem. Soc. Rev.* **1997**, *26*, 355–364; and refs therein.
[6] Tsukahara and his co-workers reported the first artificial protein–protein recognition and photoinduced ET reaction between cytochrome *c* and a zinc myoglobin modified with diethylenetriaminepentaacetic acid: a) K. Tsukahara, C. Kimura, *J. Electroanal. Chem.* **1997**, *438*, 67–70; b) K. Tsukahara, K. Kiguchi, M. Matsui, N. Kubota, R. Arakawa, T. Sakurai, *J. Biol. Inorg. Chem.* **2000**, *5*, 765–773.
[7] a) T. Hayashi, Y. Hitomi, H. Ogoshi, *J. Am. Chem. Soc.* **1998**, *120*, 4910–4915; b) T. Hayashi, Y. Hitomi, T. Takimura, A. Tomokuni, T. Mizutani, Y. Hisaeda, H. Ogoshi, *Coord. Chem. Rev.* **1999**, *190–192*, 961–974; c) T. Hayashi, Y. Hitomi, T. Ando, T. Mizutani, Y. Hisaeda, S. Kitagawa, H. Ogoshi, *J. Am. Chem. Soc.* **1999**, *121*, 7747–7750.
[8] S. Aono et al. reported $k_q = 8.5\times10^5\,\text{M}^{-1}\text{s}^{-1}$ for rMb(**4**) in 10 mM phosphate buffer at pH 7.0: S. Aono, S. Nemoto, A. Ohtaka, I. Okura, *J. Mol. Catal. A* **1995**, *95*, 193–195.
[9] a) S. J. Moench, S. Chroni, B.-S. Lou, J. E. Erman, J. D. Satterlee, *Biochemistry* **1992**, *31*, 3661–3670; b) Q. Yi, J. E. Erman, J. D. Satterlee, *Biochemistry* **1993**, *32*, 10988–10994.
[10] a) B. M. Hoffman, M. A. Ratner, *J. Am. Chem. Soc.* **1987**, *109*, 6237–6243; b) B. S. Brunschwig, N. Sutin, *J. Am. Chem. Soc.* **1989**, *111*, 7454–7465; c) V. L. Davidson, *Acc. Chem. Res.* **2000**, *33*, 87–93.
[11] An ET step can be gated by, or coupled with, some non-ET process. When the equilibrium is faster than the subsequent ET step but unfavorable, the observed rate is influenced by the equilibrium constant (coupled ET). In contrast, when the preceding non-ET step is rate-limiting, the observed rate is that for a non-ET event (gated ET). See ref. [10c].
[12] a) M. M. Ivković-Jensen, N. M. Kostić, *Biochemistry* **1997**, *36*, 8135–8144; b) H. J. Lee, J. Basran, N. S. Scrutton, *Biochemistry* **1998**, *37*, 15513–15522; c) H. Mei, K. Wang, N. Peffer, G. Weatherly, D. S. Cohen, M. Miller, G. Durharn, F. Millett, *Biochemistry* **1999**, *38*, 6846–6854; d) E. V. Pletneva, D. B. Fulton, T. Kohzuma, N. M. Kostić, *J. Am. Chem. Soc.* **2000**, *122*, 1034–1046.
[13] After this work was completed an elegant study on electrostatic control of interprotein ET between Mb and cytochrome $b_5$ was reported by Hoffman and co-workers: Z.-X. Liang, J. M. Nocek, I. V. Kumikov, D. N. Beratan, B. M. Hoffman, *J. Am. Chem. Soc.* **2000**, *122*, 3552–3553.
[14] F. W. Teals, *Biochim. Biophys. Acta* **1959**, *35*, 543.

  1433-7851/01/4006-1101 $ 17.50+.50/0 