Directed Multi-Heme Self-Assembly and Electron Transfer in a Model Membrane

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Vectorial processes in biological systems such as the energy transduction strategies of respiration and photosynthesis occur in intricately constructed membrane assemblies.[1,2] We have sought to explore the deployment of artificial hemes in phospholipid vesicles to extend the limits of membrane-directed self-assembly[3] and to probe the nature of electron transfer within these assemblies.

Described here are strategies for the construction of model membrane systems. A synthetic membrane-spanning bis-heme assembly is shown to recruit cytochrome *c* to the outside surface of phospholipid vesicles. The structure of the construct has been probed by observing electron transfer between cytochrome *c* and redox centers embedded within a phospholipid bilayer at approximately known distances. The observed electron-transfer rate at a known driving force is suggestive that distances and structural information may be deduced from the surprisingly simple behavior of these complicated assemblies.

The steroidal Mn(III) porphyrin Mn^{III}ChP (**1**) has been shown by us to bind to phospholipid bilayers.[3] Spectroscopic probes and catalytic selectivities have placed the metalloporphyrin ring parallel to the aqueous–lipid interface and at the middle of the hydrophobic region of the membrane bilayer. A series of amphiphilic Zn porphyrins, ZnTCImP, **2**, have been prepared[4] and shown to bind to **1** with the imidazole tail to form the binary constructs **3**. Typically, small unilamellar vesicles (dipalmitoylphosphatidylcholine (DPPC) and dimyristoylphosphatidylcholine (DMPC), 80/20) containing **1** were prepared in the manner we have previously described.[3] Subsequent infusion of 1 equiv of **2** into these preparations afforded **3**. Size exclusion chromatography of the mixtures on Sepharose 4B showed that both components, **1** and **2**, eluted with the lipid fraction. Visible difference spectroscopy and a 36% decrease in the fluorescence intensity of **2** confirmed that a Mn(III)–imidazole complex had been formed.[5] By contrast, an amphiphilic Zn porphyrin lacking the imidazole ring (ZnTCAmP) showed only a 4% fluorescence quenching under otherwise identical conditions (Figure 1A).[5]

Vesicular suspensions of **3** were shown to recruit cytochrome *c* to the membrane surface. The interaction is due to specific ionic interactions between the carboxyphenyl anions of **2** and the positively charged lysine patch which is known to surround the heme edge of this peripheral membrane protein.[6] Fluorescence quenching data for **3** and visible difference spectroscopy[4] (Figure 1B) have confirmed that the stoichiometric binding property was

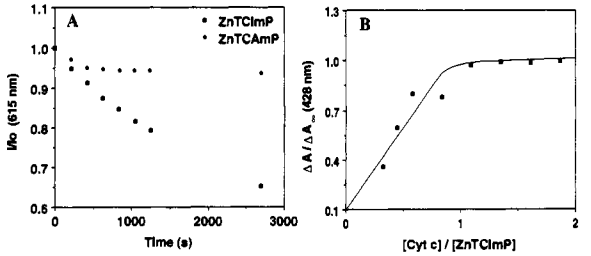
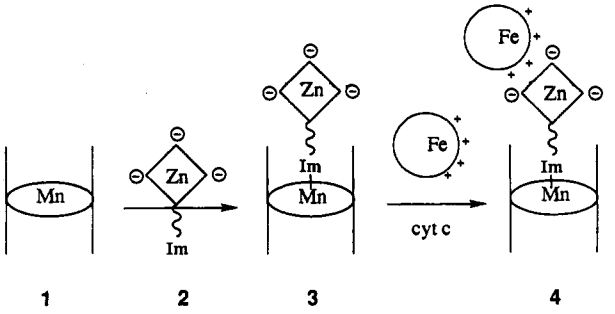


Figure 1. (A) Comparison of the time course of fluorescence intensity upon addition of ZnTCImP and ZnTCAmP to vesicles containing **1** (1 μM, pH 8, 25 °C). (B) UV difference (428 nm) titration of **2a** with cytochrome *c* in 5 mM phosphate, pH 8, 25 °C, indicating strong binding ($K_a \sim 3 \times 10^6$ M^{-1} to 3×10^7 M^{-1}).[6b]

retained for the three porphyrins **2a–c** while bound to **1**. Moreover, this binding was not observed at high ionic strength or with a *cationic N*-methylpyridinium porphyrin,[4] thus confirming the lysine–carboxylate nature of the association to form the ternary complex **4**.



Having established the formation of the termolecular complex **4**, we have examined electron-transfer reactions from Mn(II) in the membrane-spanning porphyrin **1** to ferricytochrome *c* tethered via **2** to the membrane periphery[7] by monitoring visible spectral changes for both hemes. The data showed that the electron transfer from Mn(II) to ferricytochrome *c* was cleanly first order over several half-lives (Figure 1, inset). The first-order rate constants obtained in this way for the three tethered porphyrins, **2a**, **2b**, and **2c**, were 2.88×10^{-3}, 2.60×10^{-3} and 1.82×10^{-3} s^{-1}, respectively. These results indicate that *the rate of electron transfer remained invariant despite the large changes in the tether length*. The well-behaved nature of this process is of interest since it represents a long-range electron transfer through a phospholipid medium to a well-studied electron acceptor.

We have compared the rates of electron transfer observed here to the set of rates in the literature[8] involving cytochrome *c and* having a similar driving force (~0.35 eV)[9] and reorganization energy.[6b] The plot of these data is nearly linear with distance,[10] thus inviting their extrapolation as an angstrom ruler. That extrapolation for the rate constants for **4** yields a predicted distance of ~22 Å between the Mn and Fe centers (Figure 2). This

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(1) Voet, D. B.; Voet, J. G. *Biochemistry*; Wiley: New York, 1990; pp 528–557.

(2) (a) Chance, B., DeVault, D. C., Frauenfelder, H., Marcus, R. A., Schrieffer, J. R., Sutin, N., Eds. *Tunneling in Biological Systems*; Academic: New York, 1979. (b) McLendon, G. *Acc. Chem. Res.* **1988**, *21*, 160. (c) Gust, D.; Moore, T. A. *Science* **1989**, *244*, 35.

(3) Groves, J. T.; Neumann, R. *J. Am. Chem. Soc.* **1987**, *109*, 5045. (b) Groves, J. T.; Neumann, R. *J. Am. Chem. Soc.* **1989**, *111*, 2900.

(4) (a) Ungashe, S. Ph.D. Thesis, Princeton University, 1991. (b) New compounds were characterized by ^1H and ^{13}C NMR, mass spectrometry, and UV spectra where appropriate.

(5) Axial ligation of nitrogenous bases to Mn porphyrins is well documented. For a review, see: Boucher, L. J. *Coord. Chem. Rev.* **1972**, *7*, 289.

(6) Small anionic molecules, including tetracarboxyphenylporphyrin and uroporphyrin, have been shown to form robust complexes with cytochrome *c* in homogeneous solution. (a) Clarck-Ferris, K.; Fisher, J. J. *J. Am. Chem. Soc.* **1985**, *107*, 5007. (b) Zhou, J. S.; Granada, E. S. V.; Leontis, N. B.; Rodgers, M. A. J. *J. Am. Chem. Soc.* **1990**, *112*, 5074.

(7) (a) The electron-transfer reactions were carried out at micromolar concentrations in phosphate buffer (5 mM, pH 8). Mn(II) was obtained by photoreduction using a xenon arc lamp and monitored by its appearance at 444 nm in the visible spectrum. (b) Hendrickson, D. N.; Kinnaird, M. G.; Suslick, K. S. *J. Am. Chem. Soc.* **1987**, *109*, 1243. The presence of **1** facilitated the reduction of **2**.

(8) (a) Kostić, N. M. Diprotein Complexes and Their Electron Transfer Reactions. In *Metal Ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker, Inc.: New York, 1991; Vol. 27. (b) English, A. M.; Cheung, E. *Inorg. Chem.* **1988**, *27*, 1078. (c) Elias, H.; Chou, M. H.; Winkler, J. R. *J. Am. Chem. Soc.* **1988**, *110*, 429. (d) McLendon, G.; Miller, J. R. *J. Am. Chem. Soc.* **1985**, *107*, 7811. (e) Meade, T.; Gray, H. B.; Winkler, J. R. *J. Am. Chem. Soc.* **1989**, *111*, 4353. (f) Konklin, K. T.; McLendon, G. *J. Am. Chem. Soc.* **1988**, *110*, 3345.

(9) The driving force for the Mn(II)ChPCl–ferricyt *c* electron transfer has been calculated to be 0.34 eV. See: Creager, S. E.; Murray, R. W. *Inorg. Chem.* **1987**, *26*, 2612.

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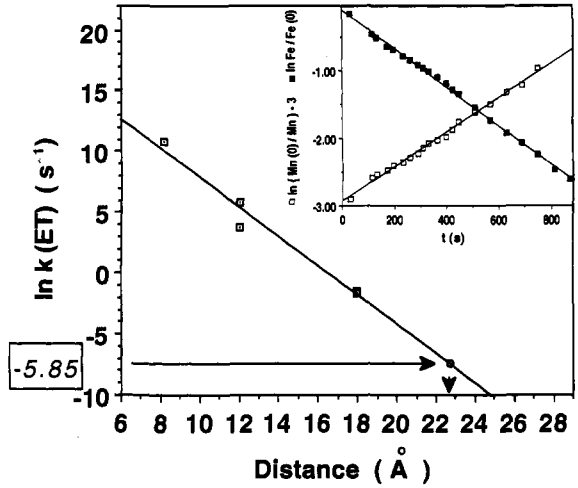

Figure 2. The rate versus edge-to-edge distance relationship for cyt *c* related systems having a driving force of ~0.35 eV and λ ~ 1 eV. The data points are taken, respectively, from refs 8d, 8c, 8e, 8b, and 8f. The inset shows a first-order plot of the appearance of *ferrocytochrome c* (550 nm) and disappearance of Mn(II)-**1** (444 nm): for **2a** (cyt *c*/**3** = 1:1.5), $k = 2.88 \times 10^{-3}$ s^{-1} ($R^2 = 0.997$); for **2b**, $k = 2.60 \times 10^{-3}$ s^{-1}; for **2c**, $k = 1.82 \times 10^{-3}$ s^{-1}. Controls with added superoxide dismutase present showed little effect on the rate.

distance suggests that cytochrome *c* in these constructs resides at the surface of the membrane, separated from the manganese porphyrin by the thickness of one leaflet of the phospholipid bilayer (~20 Å)[11] as depicted in Figure 3.[12] An extended geometry would have an unreasonably long separation between the Fe and Mn centers (ca. 36 Å) for the observed rate. Further, it can be deduced that the tether, per se, cannot be the main electron-transfer pathway[13] since one would expect a rate increase greater than 100-fold for each four carbons deleted from the chain.[14] Accordingly, a medium-mediated process is preferred for this long-range, thermal electron transfer within the construct **4**. An electron transfer from Mn(II) to Fe(III) through the steroidal superstructure and the intervening phospholipid at such a low driving force is expected to be near the limit of a weakly coupled process with many available pathways.[15]

We have sought to define a model membrane system which affords routes to large, ordered ensembles through designed intermolecular associations. The phospholipid membrane has served as a scaffolding for the erection of the ternary complex **4** in which cytochrome *c* has been induced to perform its peripheral membrane redox function with a synthetic membrane component.[16] The strategy of recruiting the protein to the outside surface of preformed vesicles has produced an ordered, vectorial array in which electrons flow only in one direction. Certainly, this

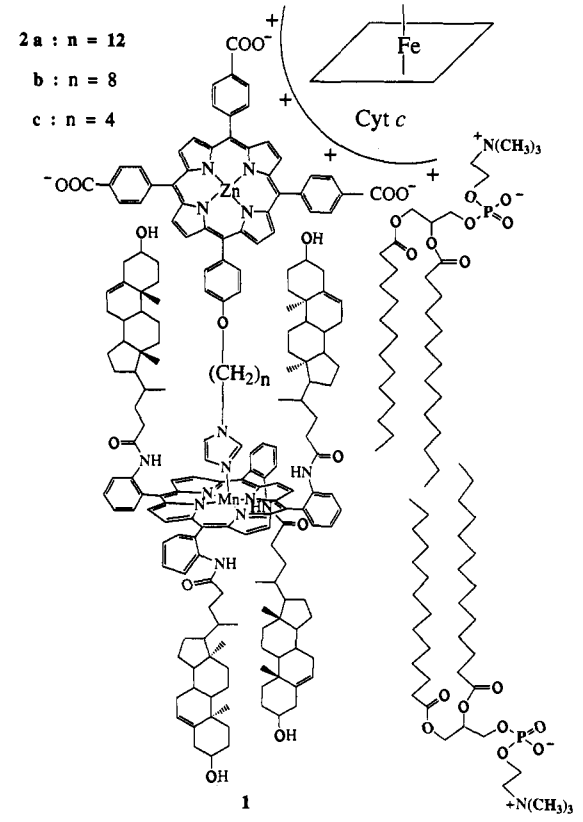

Figure 3. Structural representation of the electron-transfer assembly **4**, showing the membrane-spanning manganese porphyrin **1**, the amphiphilic zinc porphyrin **2**, and the solvent-exposed heme edge of cytochrome *c* in the vesicle bilayer. The orientation of the heme of cytochrome *c* is shown to lie parallel to the surface of the membrane bilayer.[12a,b] The orientation of the trianionic porphyrin moiety of the amphiphilic spacer is more flexible and is probably determined by its electrostatic association with the surface lysines and the membrane insertion of the hydrophobic tail.[12c] The heme plane of the cytochrome *c* is shown to be perpendicular to the porphyrin plane of **2** with a center to center distance of 12 Å.[12d,6b,4a]

assembly is conformationally exploratory. However, the membrane and the rigid steroidal appendages of **1** serve to restrict the range of possible distances between the termini of the construct **4**. Thus, while lengthening of the connecting tether may increase the flexibility of the ensemble, the closest approach of the redox partners and the nature of the intervening medium are not changed. These considerations predict that a shorter lipid and a commensurately redesigned steroidal porphyrin will increase the electron-transfer rate in a predictable manner. Thus, the design and comparison of similar systems could provide a flexible experimental approach for the dissection of integral membrane protein systems and for the understanding of biological electron transfer.

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Supplementary Material Available: Experimental details on the preparation of vesicular assemblies, the synthesis of the membrane-binding porphyrins, and the spectroscopic characterization of these compounds (5 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) (a) Dutton has shown that ln k_{ETmax} varies linearly with distance, where k_{ETmax} is the maximal electron-transfer rate. See: Moser, C. C.; Keske, J. M.; Warcke, K.; Farid, R. S.; Dutton, P. L. *Nature* **1992**, *355*, 796. (b) Wasielewski has produced a similar plot for synthetic porphyrin/quinone systems, using nonmaximal rates. See: Wasielewski, M. R. *Photoinduced Electron Transfer, Part A, Conceptual Basis*; Fox M. A., Chanon, M., Eds.; Elsevier: New York, 1988.

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(12) Studies with cytochrome *c* have shown that the heme plane lies approximately parallel to a membrane surface. (a) Pachence, J. M.; Amador, S.; Maniara, G.; Vanderkooi, J.; Dutton, P. L.; Blasie, J. K. *Biophys. J.* **1990**, *58* (2), 379. (b) Erecinska, M.; Blasie, J. K.; Wilson, D. F. *FEBS Lett.* **1977**, *76*, 235. (c) Ishikawa, Y.; Kunitake, T. *J. Am. Chem. Soc.* **1991**, *113*, 621. (d) Pelletier, H.; J. Kraut, J. *Science* **1992**, *258*, 1748.

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(14) The addition of four carbon atoms to the hydrophobic tail of **2** would lead to an increase in the path length by 4.59 Å. Expressing the rate of electron transfer as $k_{ET} \alpha \exp(-\beta r)$ and using a β value of 1.2 Å^{-1}, one can estimate the resulting change in the rate to be ~250 for every four atoms added or deleted from the tether.

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(16) For another example of protein–metalloporphyrin intercommunication, see: Groves, J. T.; Ungashe, S. B. *J. Am. Chem. Soc.* **1990**, *112*, 7796.