Characterization of Amphipathic Fluorinated Porphyrin Derivatives

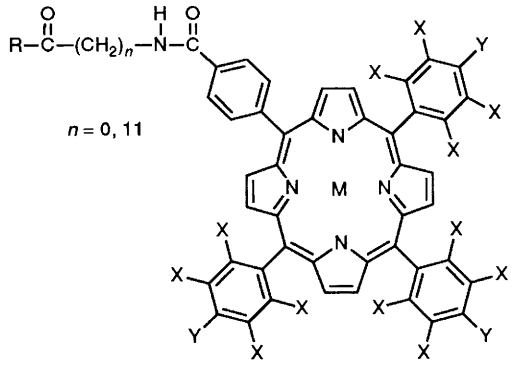
Mamoru Nango,*[a] Kouji Iida,[a] Tomoki Kawakita,[a] Mitsutaka Matsuura,[a] Yukiko Harada,[a] Keiji Yamashita,[a] Kazuichi Tsuda[a] and Yoshikazu Kimura[b]

[a] Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso, Showa-ku, Nagoya 466, Japan
[b] Ihara Chemical Co. Ltd., Shizuoka 421-33, Japan

An efficient energy transfer from zinc porphyrin **4** to the porphyrin portion of poly(ethylenimine) [PEI]-linked fluorinated porphyrin derivatives **1**, **2** anchored onto a lipid bilayer is observed; the manganese complexes of **1** show catalytic transmembrane electron transfer, depending upon the length of the methylene linkage.

Synthetic porphyrin model compounds might provide insight into the possible reactions involved in photosynthesis and mitochrondrial processes.[1–5] Porphyrin derivatives play a crucial role in such electron-transfer systems.[6] Here, we report the preparation of poly(ethylenimine) [PEI]-linked fluorinated porphyrins **1**, **2** (Scheme 1) and the characterization of their interactions with a lipid bilayer. We reasoned that not only is the fluorinated porphyrin easily inserted into the lipid bilayer because of its miscibility with the hydrophobic lipid but it is also chemically stable owing to the steric effect of the fluorines.[7] Furthermore, the PEI moiety on the compounds confers water solubility, while the porphyrin portion is hydrophobic. The porphyrin group could thus be anchored onto the lipid bilayer, providing the possibility of vectorial electron transfer between porphyrin centres in a biological membrane and thus modelling the natural processes.[1]

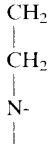
Compounds, **1**, **2** were prepared as follows. 5-(4-Methoxycarbonylphenyl)-10,15,20-tri-*p*-pentafluorophenylporphyrin, **PFPP**CO_2Me, was prepared by the condensation of pyrrole with a mixture of 4-methoxycarbonylbenzaldehyde and pentafluorobenzaldehyde in refluxing chloroform containing BF_3, followed by oxidation with *p*-chloranil (yield 29%). The porphyrin, **PFPP**CO_2Me, was converted into its acid form, **PFPP**CO_2H, by base-catalysed hydrolysis of **PFPP**CO_2Me and subsequent acidification. To introduce the linking chain between PEI and the porphyrin moiety, $NH_2(CH_2)_{11}CO_2H$, was treated with the acid chloride of **PFPP**CO_2H and the product, **PFPP**$CONH(CH_2)_{11}CO_2H$, was purified by column chromatography on silica gel. The ^{1}H NMR and MS spectra of **PFPP**$CONH(CH_2)_nCO_2H$ support unambiguously the assigned structure. The manganese complex of **PFPP**$CONH(CH_2)_nCO_2H$ (n = 0,11) was synthesized as described in an earlier paper.[1] 5-(4-[{(5-Carboxyalkyl)-amino}carbonyl]-phenyl)10,15,20-tri-*p*-pentafluorophenylporphyrin, **PFPP**$CONH(CH_2)_nCO_2H$, (n = 0,11) and their manganese complexes, Mn^{III}–**PFPP**$CONH(CH_2)_nCO_2H$, were treated with ethyl chloroformate in chloroform at low temperature and reacted with either PEI (M_w 1800) or cetylated PEI [cetylPEI] overnight to give polymer-linked porphyrins, PEI-C_n-**MPFPP** or cetylPEI-C_n-**MPFPP** (yields 60–70%), isolated by chromatographic separation (Sephadex LH-20 gel, EtOH). cetylPEI was prepared by reaction with cetylchloride and PEI as described in the previous paper.[8] The ^{1}H NMR spectra of PEI-C_n-H_2**PFPP** and cetylPEI-C_n-H_2**PFPP** support the assigned structure. The chemical shifts of NMR spectra for all peaks were as expected. The ^{1}H NMR spectra of PEI-C_n-H_2**PFPP** and cetylPEI-C_n-H_2**PFPP** indicated one porphyrin per PEI or cetylPEI. The absorption spectra of PEI-C_n-**MPFPP** and cetylPEI-C_n-**MPFPP** in CH_2Cl_2–10% EtOH and egg yolk phosphatidylcholine (egg PC, Nippon Fine Chemical Co.) were identical and all showed the presence of a normal porphyrin chromophore. Relative fluorescence intensities for PEI-C_n-H_2**PFPP** in several media at 25 °C showed that higher fluorescence yields were observed in egg PC vesicle and CH_2Cl_2 10% EtOH, 100 and 98%, respectively, and a lower fluorescence yield (55%) was observed in aqueous solution (values are normalized to yields for the sample in egg PC vesicle). Similar results were obtained for cetylPEI-C_n-H_2**PFPP**. The data, therefore, imply that in the vesicle systems the porphyrin moiety is immersed within the hydrophobic interior of the membrane. To further examine the interaction of the polymer-linked porphyrin complexes with the lipid bilayer, we attempted to remove the polymer-linked porphyrin complexes, PEI-C_0-H_2**PFPP** and PEI-linked tetratolylporphyrin [PEI-C_0-H_2**TTP**] **3** (Scheme 1) from the external vesicle surface by gel filtration.[1] Compound **3** was prepared as described in the previous paper.[1] The visible spectra of the vesicle were measured before and after gel (Sephadex G-150) filtration indicated that the fluorinated porphyrin portion of PEI-C_0-H_2**PFPP** was completely bound



PEI-C_n-**MPFPP**	R : PEI, X = F, Y = F, M = H_2, Mn	**1**
cetylPIE-C_n-**MPFPP**	R : cetylPIE, X = F, Y = F, M = H_2, Mn	**2**
PEI-C_0-**MTTP**	R : PEI, X = H, Y = Me, M = H_2, n = 0	**3**
PE-C_{11}-**MPFPP**	R : PE, X = F, Y = F, M = Zn, n = 11	**4**

PEI: H_2N-(CH_2CH_2NH)$_x$-(CH_2CH_2N)$_y$-(CH_2CH_2NH)$_z$–

x: 25% y: 25% z: 50%

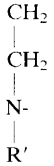


PE: -$NHCH_2CH_2O$–P(=O)(O$^-$)–$OCH_2CH(OCOR'')CH_2OCOR''$

R''=$(CH_2)_{14}$-Me

cetylPEI: H_2N-(CH_2CH_2NH)$_x$-(CH_2CH_2N)$_y$-(CH_2CH_2NH)$_z$–

x: 25% y: 25% z: 50%



R' = $(CH_2)_{15}$-Me

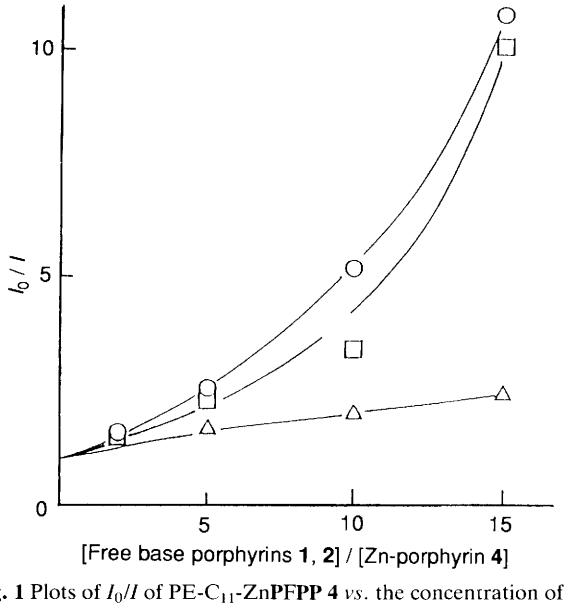


Fig. 1 Plots of I_0/I of PE-C_{11}-Zn**PFPP** **4** *vs.* the concentration of PEI- or cetylPEI-linked free base porphyrins **1**, **2** [free base porphyrins], in egg yolk phosphatidylcholine vesicle, 0.01 mol dm^{-3} Tris, pH 8.0 at 25 °C. The x-axis is represented by [free base porphyrins **1**, **2**]/[PE-C_{11}-Zn**PFPP** **4**]. (○): PEI-C_{11}-H_2**PFPP**, (□): cetylPEI-C_{11}-H_2**PFPP**, (△): PEI-C_0-H_2**PFPP**.

to egg PC vesicle while the porphyrin portion of PEI-C_0-H_2**PFPP** was almost removed. These data imply that in the vesicle systems the fluorinated porphyrin moiety is easily incorporated into the hydrophobic interior of the membrane.

Furthermore, energy transfer between porphyrins was measured to gain more information on the environment of the porphyrin molecules for **1**, **2** in the lipid bilayer. Energy transfer from a previously incorporated zinc porphyrin complex on dipalmitoylphosphatidylethanolamine-linked zinc porphyrin PE-C_{11}-Zn**PFPP** **4** (Scheme 1) in the vesicle to externally added free base porphyrin on the compounds **1**, **2**, in the same vesicle was measured. Compound **4** was prepared as described previously.[5] With addition of free base porphyrins **1**, **2**, the fluorescence emission of zinc porphyrin on **3** at 613 nm was decreased and in contrast, the emission of the free base porphyrins at 660 nm dramatically increased, indicating that the energy transfer from the zinc porphyrin to the free base porphyrins in compounds **1**, **2** had taken place. The efficiency of the energy transfer is expressed by the intensity change of the fluorescence emission of the zinc porphyrin at 613 nm, represented by I_0/I. Fig. 1 illustrates the change of (I_0/I) with addition of **1**, **2**. Both PEI-C_{11}-H_2**PFPP** and cetylPEI-C_{11}-H_2**PFPP** show a large increase in I_0/I, indicating a large energy transfer between **4** and these porphyrins. In contrast, PEI-C_0=H_2**PFPP** shows a lower energy transfer. These results of energy transfer support the conclusion that all the fluorinated porphyrin portions on **1**, **2** are immersed in the hydrocarbon region of the bilayer in the following order: PEI-C_{11}-H_2**PFPP** ≥ cetylPEI-C_{11}-H_2**PFPP** > PEI-C_0-H_2**PFPP**, indicating that the porphyrins linked to the polymer with a long aliphatic linker were well immersed in the bilayer but that the hydrophobic cetyl group on **2** has no significant effect on binding PEI-**PFPP** to the vesicle.

Electron transfer from an external reductant (reduced indigotetrasulfonic acid, $ITSAH_2$, 1 × 10^{-5} mol dm^{-3}) to potassium ferricyanide (0.1 mol dm^{-3}) trapped within a phospholipid liposome (egg PC) was measured anaerobically at 0.4 mol dm^{-3} imidazole buffer at pH = 7.0 as mediated by a

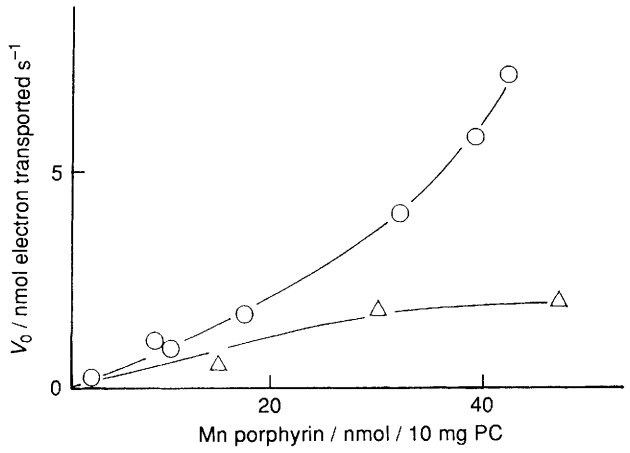


Fig. 2 The rate of transmembrane electron transfer catalysed by PEI-C_n-Mn**PFPP** (n = 0,11) as a function of porphyrin concentration in egg yolk PC vesicles at 25 °C. (○): PEI-C_{11}-Mn**PFPP**, (△): PEI-C_0-Mn**PFPP**.

catalyst of PEI-C_n-Mn**PFPP** incorporated into the vesicle bilayer.[2] Fig. 2 illustrates the rate of electron transport (V_0) across egg PC liposomes at 25 °C with increased complex concentration. As is apparent from Fig. 2, PEI-C_{11}-Mn**PFPP** catalysed transmembrane electron transport in egg PC vesicle. In contrast, PEI-C_0-Mn**PFPP** showed little or no catalytic activity in the vesicle system. The mechanism of electron transfer catalysed by PEI-C_{11}-Mn**PFPP** is assumed to be electron transfer from this manganous porphyrin to a manganic porphyrin tethered to a PEI molecule on the opposite side of the bilayer.[2]

Thus, appropriate analogues of **1** and **2** are being utilized to systematically examine the photochemical and electron transport activity between porphyrins in the lipid bilayer.

Received, 5th November 1991; Com. 1/05630A

References

1 M. Nango, T. Dannhauser, D. Huang, K. Spears, E. Morrison and P. A. Loach, *Macromolecules*, 1985, **17**, 1898.
2 T. Dannhauser, M. Nango, N. Oku, K. Anzai and P. A. Loach, *J. Am. Chem. Soc.*, 1986, **108**, 5865.
3 M. Nango, H. Kryu and P. A. Loach, *J. Chem. Soc., Chem. Commun.*, 1988, 697.
4 M. Nango, M. Higuchi, H. Gondo and M. Hara, *J. Chem. Soc., Chem. Commun.*, 1989, 1550.
5 M. Nango, A. Mizusawa, T. Miyake and J. Yoshinaga, *J. Am. Chem. Soc.*, 1990, **112**, 1640.
6 *Photosynthesis*, ed. Govindjee, Vol. 1, Academic Press, New York, 1982.
7 P. Pattioni, J. P. Renaud, J. F. Bartoli, M. Reina-Artiles, M. Fort and D. Mansuy, *J. Am. Chem. Soc.*, 1988, **110**, 8462.
8 N. Oku, S. Shibamoto, F. Ito, H. Gondo and M. Nango, *Biochemistry*, 1987, **26**, 8145. Integration of the ^{1}H NMR spectrum indicated the following stoichiometric composition: $(C_2H_4N)_m(C_{16}H_{33})_{0.05m}$, m = 43.