CHEMISTRY LETTERS, pp. 1157-1160, 1994. © 1994 The Chemical Society of Japan

Transmembrane Electron Transfer across a Keratin Membrane and Lipid Bilayers Catalyzed by Manganese Porphyrin Dimers

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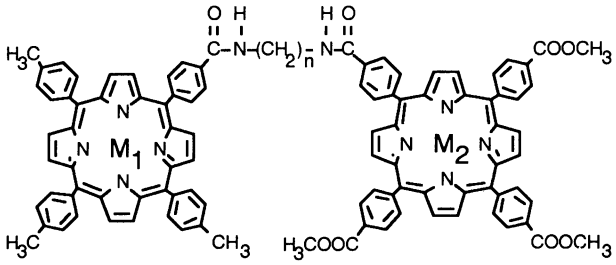
Transmembrane electron transfer across S-cyanoethylated keratin (SCEK) and liposomal membranes catalyzed by (**1**), MnTTP-$(CH_2)_n$-MnP(COOMe)$_3$, n=2,3,12 showed that an enhanced electron transfer was observed especially when n=2 or 3 on (**1**) in these membranes and imidazole was present.

Synthetic porphyrin models can be very helpful in studying the effect of distance and orientation in electron transfer reactions of photosynthesis and other biological processes.[1-7] Porphyrin pigments play a key role in these electron transfers.[8-11] However, there has been little study of ground state electron transfer to provide an insight into the effect of porphyrin structure in the electron transfer.[1,2,5] We now present transmembrane electron transfer across S-cyanoethylated keratin (SCEK) and liposomal membranes catalyzed by manganese complexes of covalently-linked porphyrin dimer with spacer methylene groups (**1**). We reasoned that the covalently-linked two porphyrin centers should allow more possibilities to the path of electron transfer in these membranes than monomer porphyrins.[4] Furthermore, the keratin membrane is not only chemically stable to continuously examine the electron transfer in comparison to that in the lipid bilayers but also is useful to examine the effect of the porphyrin structure on the electron transfer in the lipid bilayers, in which the porphyrin dimers are fixed in the keratin membrane while the porphyrins are mobile in the lipid bilayers.

The porphyrin, (**1**), (**2**), (**3**) and Manganese 5, 10, 15, 20-tetra-p-tolylporphyrin (MnTTP) were prepared as described in our previous papers.[1,4-7,12] ^{1}H NMR and mass spectra of the porphyrins support unambiguously the assigned structure.[12] SCEK was prepared from Merino 64' wool according to the procedure of the literature.[6-13] The SCEK membrane was prepared by casting 5 cm^3 of 3 wt% SCEK / formic acid solution containing manganese porphyrins on cylindrical cavity with 5 cm diameter in teflon block at room temperature for 3 days. SCEK membranes were cross-linked by immersing the cast membranes in 33% formaldehyde solution at 30 °C for 3 h. The thickness is 20-30 μm, measured by Elecont micrometer (Mitsutoyo Co.). Liposomal membranes were prepared by sonication of egg york phosphatidylcholine (PC) as described previously.[2]



M_1TTP-Cn-M_2P(COOMe)$_3$
(**1**) M_1, M_2 =Mn, n=2, 3, 12
(**2**) M_1=Mn, M_2 =H_2, n=2, 12
(**3**) M_1, M_2 =H_2, n=2, 3, 12

The absorption spectra of (**1**) and MnTTP in CH_2Cl_2-10% ethanol, PC vesicle and a SCEK membrane were identical at Soret band; λmax/nm, 468 (CH_2Cl_2-10% ethanol), 465 (PC vesicle) and 471 (SCEK) for (**1**)(n=2), 470(CH_2Cl_2-10% ethanol), 467 (PC vesicle) and 472 (SCEK) for (**1**)(n=3), 471(CH_2Cl_2-10% ethanol), 468 (PC vesicle) and 472 (SCEK) for (**1**)(n=12), and 471(CH_2Cl_2-10% ethanol), 471 (PC vesicle) and 471 (SCEK) for MnTTP and all showed the presence of a normal porphyrin chromophore from the absorbance. The data imply that the porphyrin dimers can be embedded into these membranes. The fluorescence spectra of (**2**), (**3**) and TTP were also recorded in CH_2Cl_2 -10% ethanol, PC vesicle and poly-γ-methyl-L-glutamate (PMLG) membrane,[14] in which the PMLG membrane was prepared by casting the dichloroethane solution as described in the preparation of SCEK membrane. The excitation of Soret band of (**2**) or (**3**) shows a normal porphyrin fluorescence in these membranes as well as in CH_2Cl_2 -10% ethanol; λmax/nm 655, 716 (CH_2Cl_2 -10% ethanol), 651, 716 (PC vesicle), 652, 715 (PMLG) for (**2**)(n=2), 654, 718 (CH_2Cl_2 -10% ethanol), 656, 716 (PC vesicle), 651, 715 (PMLG) for (**2**)(n=12), 655, 717 (CH_2Cl_2 -10% ethanol), 655, 717 (PC vesicle), 653, 715 (PMLG) for (**3**)(n=2), 657, 717 (CH_2Cl_2 -10% ethanol), 657, 717 (PC vesicle), 655, 717 (PMLG) for (**3**)(n=3), 656, 715 (CH_2Cl_2 -10% ethanol), 657, 718 (PC vesicle), 654, 715 (PMLG) for (**3**)(n=12). A higher quenching occurred in these membranes when the length of linker (n) on the porphyrin dimers is 2 for (**2**), where relative fluorescence yields of (**2**) are 9.11 % for n=2 and 25 % for n=12, respectively. Electron transport from a reductant, reduced indigotetrasulfonic acid, $ITSAH_2$, $1x10^{-5}$ mol dm^{-3} to potassium ferricyanide (0.1 mol dm^{-3}) was measured anaerobically at pH 7.0, 0.4 mol dm^{-3} imidazole buffer as mediated by catalyzed of (**1**) (n=2,3,12) and (**3**) (n=2) incorporated in liposomal membrane as described previously.[2] The oxidized form of $ITSAH_2$ has an intense absorbance band at λ=600 nm. The rate (V_0) was determined from the initial slope for the change of absorbance band. Figure 1 illustrates the plots of the rate vs. the concentration of manganese complex incorporated in the liposomal membrane. An enhanced rate was observed especially when n=2 or 3 on (**1**) in comparison to n=12 on (**1**) and (**2**)(n=2), indicating that the length of linkage on the compound and manganese dimer have an important role on the electron transfer. Furthermore, electron transfer catalyzed by the porphyrin dimers in SCEK membrane was measured to gain more information on the effect of the porphyrin structure in the electron transfer in which the porphyrin dimers are fixed in the keratin membrane. Electron transport from an reductant, sodium hydrosulfite 0.05 mol dm^{-3} to potassium ferricyanide ($7 x10^{-4}$ mol dm^{-3}) was measured anaerobically at 35 °C, either imidazole or phosphate buffer as mediated by catalysts of (**1**) incorporated in a SCEK membrane. The membrane was set in a two compartment cell under an atmosphere of nitrogen gas as described previously.[7] Figure 2 shows an example of the reduction of $[Fe(CN)_6]^{3-}$ by SCEK membrane containing manganese porphyrin dimer (**1**) (n=2 and n=3). The potassium ferricyanide has an intense absorbance

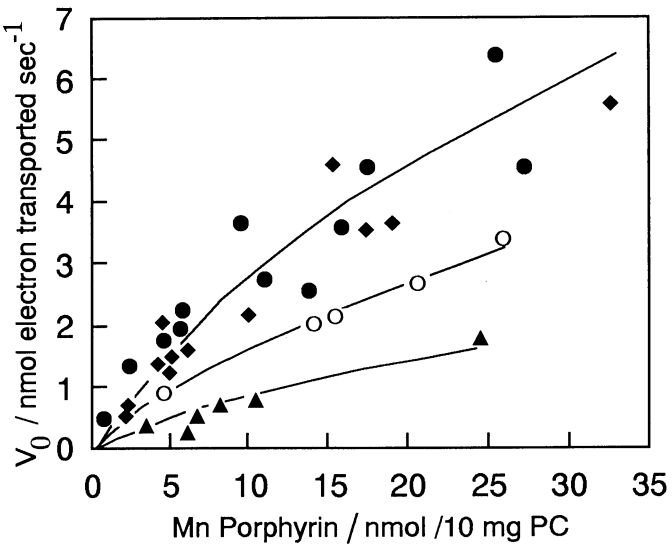


Fig. 1. The rate (V_0) of transmembrane electron transfer catalyzed by manganese porphyrin dimers as a function of porphyrin concentration in PC vesicle at 25 °C.
(◆) : (**1**) (n=2), (●) : (**1**) (n=3), (▲) : (**1**) (n=12), (○) : (**2**) (n=2).

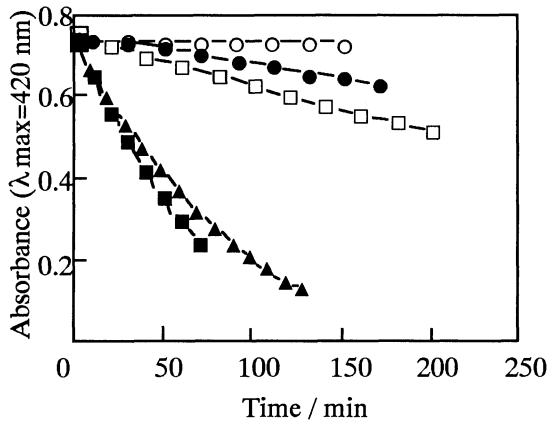


Fig. 2. Reduction of $[Fe(CN)_6]^{3-}$ by SCEK membrane containing Manganese porphyrin dimer at pH 7.0. (○): control membrane - 0.1 mol dm^{-3} Phosphate buffer, (●): control membrane - 0.1 mol dm^{-3} Imidazole buffer, (□): (**1**) (n=2) , 216 μmol/g SCEK membrane - 0.1 mol dm^{-3} Phosphate buffer, (■) (**1**) (n=2), 216 μmol / g SCEK membrane-0.1 mol dm^{-3} Imidazole buffer. (▲) (**1**) (n=3), 259 μmol/g SCEK membrane-0.1 mol dm^{-3} Imidazole buffer.

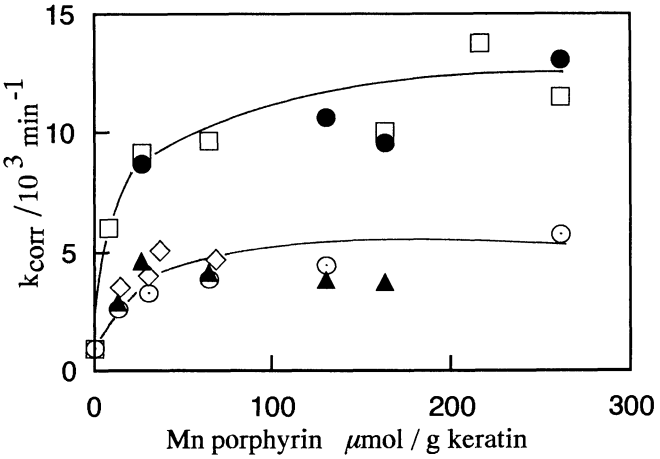


Fig.3. The rate (k_{corr}) of transmembrane electron transfer catalyzed by manganese porphyrin dimers as a function of porphyrin concentration at pH 7.0, 0.1 mol dm^{-3} imidazole buffer, 35 °C. (□):(**1**)(n=2), (●):(**1**)(n=3), (▲): (**1**)(n=12), (◇):(**2**)(n=2), (⊙) : MnTTP .

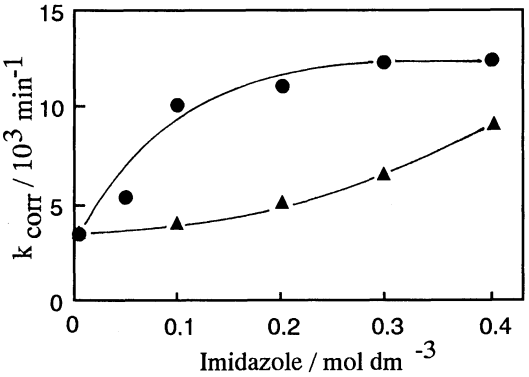


Fig.4. The rate (k_{corr}) of transmembrane electron transfer catalyzed by manganese porphyrin dimers as functions of the concentration of imidazole 35 °C, pH 7.0, 0.1 mol dm^{-3} phosphate [(●) : (**1**) (n=3), (▲) : (**1**)(n=12)].

band at λ=420 nm. The rate was determined from the change of absorbance band and pseudo-first order rate constant (k_{obs}) was calculated from the variation of $\log(A_\infty - A_t)$ vs. time (t) by use of the least-squares methods, in which the rate (k_{corr}) is corrected for uncatalyzed transmembrane redox, $k_{corr} = k_{obs}$ (SCEK membrane containing (**1**)) - k_{obs} (SCEK control membrane). The leakage of $S_2O_4^{2-}$ or $[Fe(CN)_6]^{3-}$ for each membrane system was not detected even after 5 h, in which the leakage was monitored spectroscopically by the change of Fe(II)/(III) for $[Fe(CN)_6]^{3-}$. As is apparent from Fig. 2, the rate of the reduction of $[Fe(CN)_6]^{3-}$ in (**1**)(n=2)/SCEK membrane-phosphate buffer was very slow in comparison to that in (**1**)(n=2)/SCEK-imidazole buffer, implying that the leakage of $S_2O_4^{2-}$ or $[Fe(CN)_6]^{3-}$ through the SCEK membrane was negligible and thus electron transfer across the SCEK membrane containing (**1**) (n=2) was observed especially when imidazole is present. Figure 3 illustrates the plots of the rate (k_{corr}) vs. concentration of manganese complex incorporation in a SCEK membrane at pH 7.0, 0.1 mol dm^{-3} imidazole buffer. As is apparent from Fig. 3, an enhanced rate was observed especially when n=2 or 3 on (**1**), in comparison to that when n=12 on (**1**), n=2 on (**2**) and MnTTP. The data imply that the length of linkage between the porphyrin dimers as well as the manganese dimer have a crucial effect on the electron transfer, in which the porphyrin dimers are fixed in the keratin membrane. Thus, these results indicate that a number of

spacer methylene groups between porphyrins as well as manganese dimer play an important role on these electron transfers which may involve inter- or intramolecular electron transfer between porphyrins. The mechanism of electron transfer across a SCEK membrane and lipid bilayers catalyzed by manganese porphyrins is likely to that electron transfer from one manganese (II) porphyrin to another manganese (III) porphyrin can occur only when they approach each other. Quantitative comparison and further interpretation must wait more detail direct studies. Figure 4 illustrates the plots of k_{corr} vs. concentration of imidazole at pH 7.0, 0.1 mol dm^{-3} phosphate buffer. The rate increases with increasing concentration of imidazole, indicating that imidazole plays a crucial role on the electron transfer. Similar results on the imidazole effect were observed in the transmembrane electron transfer across lipid bilayers catalyzed by manganese porphyrin dimers and in the electron transfer on electrode modified with keratin membrane.[2,3,5,6] In conclusion, these electron transfers across a SCEK membrane and lipid bilayers are the first reported system in which manganese porphyrin dimers enhanced catalytic electron transfer in these membranes especially when n=2 or 3 on (**1**) and imidazole is present.

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12) More detailed synthetic and analytical data will be reported elsewhere. Mass data, 1804 (M) for (**1**)(n=12), 1611 (M) for (**2**)(n=2), 1750 (M) for (**2**)(n=12), 1558 (M+1) for (**3**)(n=2), 1572 (M+1) for (**3**)(n=3), and 1698 (M+1) for (**3**)(n=12). ^{1}H NMR data, δ -2.80 (4H, d, pyrrole-NH), 2.63 (6H, s, tolyl), 2.70 (3H, s, tolyl), 4.05 (4H, bs, methylene), 4.08 (6H, s, carbomethoxy), 4.11 (3H, s, carbomethoxy) 7.45 - 8.12 [12H, m, aromatic(tolyl)], 8.25 - 8.45 (20H, m, aromatic), 7.62 (1H, t, amide), 7.78 (1H, t, amide), 8.76 - 8.90 (16H, m, β-pyrrole) for (**3**)(n=2), -2.80 (4H, d, pyrrole-NH), 2.10 (6H, bs, methylene), 2.63 (6H, s, tolyl), 2.70 (3H, s, tolyl), 3.97 (4H, bs, methylene),4.08 (6H, s, carbomethoxy), 4.11 (3H, s, carbomethoxy), 7.45 - 8.12 [12H, m, aromatic(tolyl)], 8.21 - 8.43 (20H, m, aromatic), 7.62 (1H, t, amide), 7.74 (1H, t, amide), 8.76 - 8.90 (16H, m, β-pyrrole) for (**3**)(n=3), -2.80 (4H, d, pyrrole-NH), 2.50-3.20 [33H, m, methylene and methyl (tolyl)], 4.08 - 4.10 (9H, s, carbomethoxy), 7.45 - 8.45 (32H, m, aromatic), 7.62-7.74 (2H, t, amide), 8.76 - 8.90 (16H, m, β-pyrrole) for (**3**)(n=12).
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14) The reason why we used the PMLG membrane is that ZnTTP and TTP could be inserted into the PMLG membrane but not inserted into the SCEK membrane.

(Received March 17, 1994)