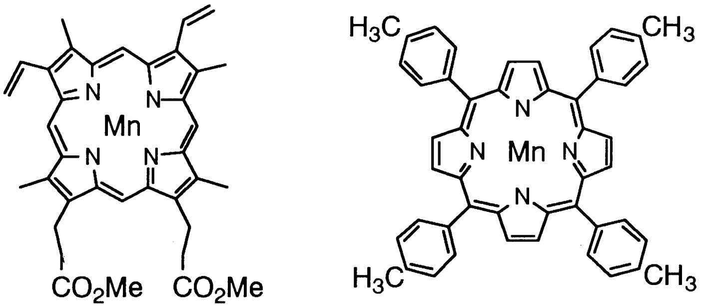
CHEMISTRY LETTERS, pp. 753-756, 1994.

Electron Transfer on Electrode Modified with Keratin Membranes Containing Manganese Porphyrins

Kouji IIDA, Mamoru NANGO,* Masaya HIKITA, Ayako HATTORI, Keiji YAMASHITA,
Kiyoshi YAMAUCHI,† and Kazuichi TSUDA
Department of Applied Chemistry, Nagoya Institute of Technology, Gokiso-cho, Showa-ku, Nagoya 466
†Department of Applied Biochemistry, Osaka City University, Sugimoto-cho, Sumiyoshi-ku, Osaka 558

Enhanced electron transfer between porphyrins in membranes cast on a glassy carbon electrode was observed especially in hydrophilic keratin membranes containing manganese protoporphyrin dimethyl esters (MnPPDME). An imidazole derivative played an important role on the electron transfer in hydrophobic S-cyanoethylated keratin membranes containing MnPPDME.

Synthetic porphyrin models can be very helpful in studying the effects of porphyrin structure and an axial ligand on electron transfer reactions of photosynthesis and other biological processes.[1-5] Porphyrin pigments play a key role in these electron transfers. We now report the study of electron transfer on electrodes modified with a cross-linked keratin (S-keratin) , S-cyanoethylated keratin (SCEK), poly(γ-methyl-L-glutamate) (PMLG) and dipalmitoylphosphatidylcholine (DPPC) membranes containing a manganese porphyrin complex of protoporphyrin dimethyl esters (PPDME) or tetratolylporphyrin (TTP) (Scheme 1) to provide an insight into the structural effects of porphyrins and membranes on the electron transfer. A large number of chemists have identified that electroactive species such as porphyrins, flavins, viologens, ferrocenes, and quinones can be immobilized on electrode surfaces.[6,7] However, there has been little study of electron transfer on electrodes modified with proteins and lipid bilayers containing porphyrins. The keratin membranes are not only chemically stable to continuously examine the electron transfer but also the constituting amino acid residues of the keratin is easily modified. MnPPDME and MnTTP were prepared as described in our previous papers.[1)] S-Keratin was prepared from wool by the originally developed method[8)] and SCEK was prepared from Merino 64'wool according to the procedure of the literature.[9)] Proteins or DPPC membranes were prepared by casting 0.05 ml of 1 wt% keratin / formic acid, PMLG/$CHCl_3$ or DPPC/$CHCl_3$ solution containing manganese porphyrins on a glassy carbon (GC) (45-60 x 10^{-4} W/cm , Tokai Carbon Co.) or transparent SnO_2 electrode (9.9 W/cm, gifted from Nippon Itagalasu Co.) (surface area 2 cm^2) at room temperature for 1 h. Then, the S-keratin membrane was cross-linked by heating for 30 min. at 80 °C. SCEK and PMLG membranes were used without further



MnPPDME **MnTTP**

Scheme 1.

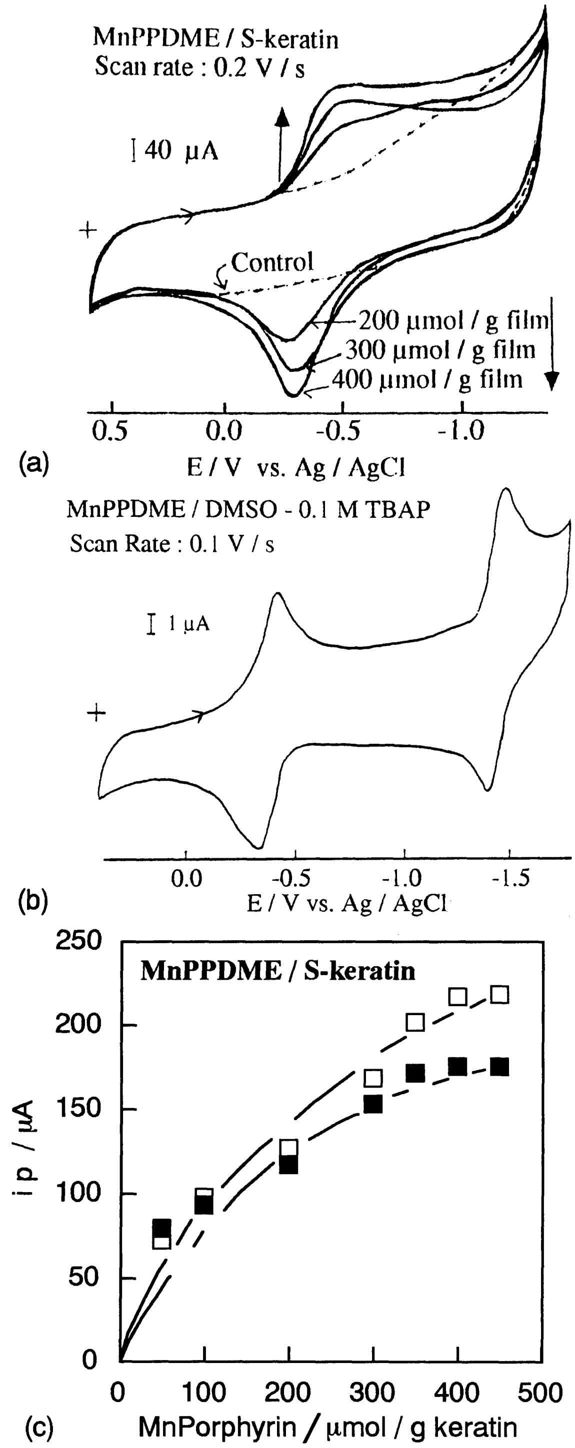


Fig. 1. The cyclic voltammetric response of a GC electrode covered with 200-400 μmol MnPPDME/ g S-keratin membrane (a), that in DMSO containing MnPPDME (6.88×10^{-4} M) and 0.1 M TBAP (b), and the anodic (□) and cathodic (■) peak currents of MnPPDME / S-keratine membrane as a function of the concentration (c) at 25 °C.

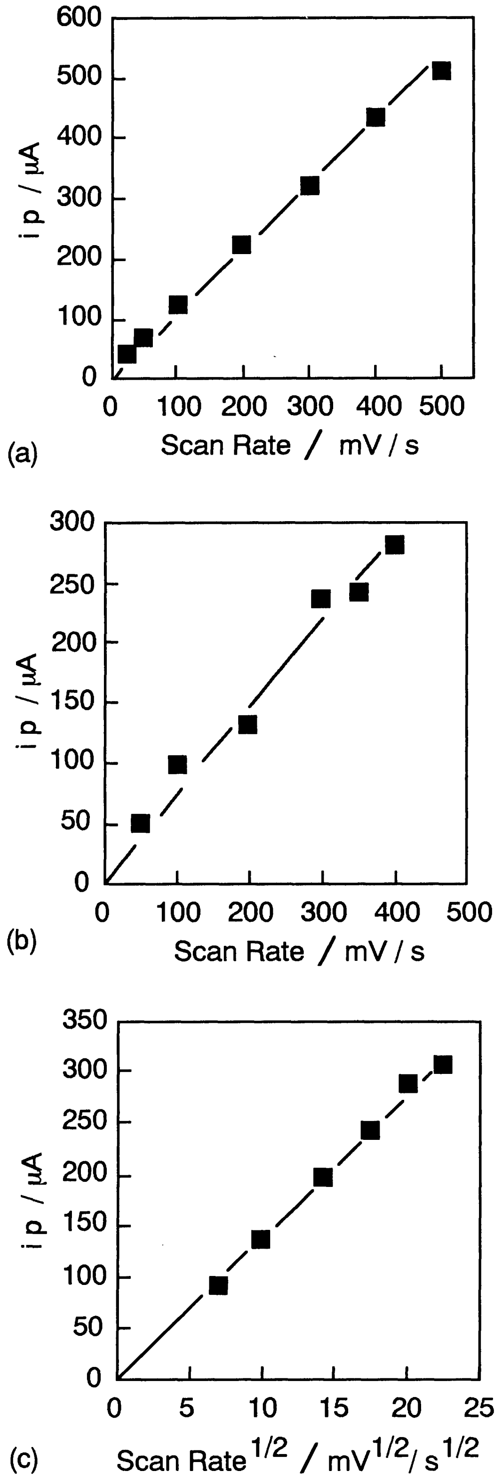


Fig. 2. The cathodic peak currents for the reduction of Mn(III)PPDME (400 μmol/g S-keratin, 400 μmol/g SCEK and 200 μmol/g DPPC) as a function of the scan rate. (a) SCEK (b) S-keratin (c) DPPC.

treatment. This level of coverage results in the proteins or DPPC membrane having a thickness of approximately 8-10 μm, measured by Elecont micrometer (Mitsutoyo Co.). The degree of swelling of the SCEK membrane for pH 5.5, 7.0 and 8.5 was 24, 35 and 45%, respectively,[10] while that of the S-keratin membrane is 60% at pH 7.0, implying that the S-keratin membrane is more hydrophilic than the SCEK one. The UV/Vis. absorption spectra of MnPPDME and MnTTP in CH_2Cl_2-ethanol, S-keratin and DPPC membranes cast on a SnO_2 electrode were identical at Soret band, 463 nm, 466 nm, 461 nm for MnPPDME and 478 nm, 473 nm, 476 nm for MnTTP, respectively, and all showed the presence of a normal Mn(III) porphyrin absorbance. Fig. 1 shows the cyclic voltammetric responses of a GC electrode covered with S-keratin membrane containing MnPPDME and immersed in an aqueous phosphate buffer solution and that in DMSO containing MnPPDME at 25 °C. As is apparent from Fig. 1a), one set of waves is visible corresponding to the consecutive monoelectronic reductions of the manganese porphyrin unit [Mn(III)/Mn(II)] that is similar to those in Fig. 1b). Thus, the redox process of MnPPDME in S-keratin membrane exhibits a characteristic behavior of a reversible redox couple, and the anodic and cathodic peaks currents increase with increasing the concentration of MnPPDME as shown in Fig. 1c). Similar result was observed for the cyclic voltammetric response of MnPPDME in SCEK membrane cast on the GC electrode(the data not shown). The currents in S-keratin membranes were 1.5 times larger than those in SCEK membrane which has more hydrophobic property than the S-keratin membrane. However, the voltammetric behaviors of MnPPDME in PMLG membrane and of MnTTP in keratin membranes showed no characteristic reversible redox couple. Furthermore, MnPPDME yields reversible voltammetric waves in a nonaqueous environment, like in DPPC membrane cast on the electrode that is similar to those in Fig. 1. The electron transfer on the electrode modified with DPPC membrane containing MnPPDME was observed only above the phase transition temperature (>42 °C). The redox potentials for MnPPDME vs. Ag/AgCl increase in the order of SCEK(-0.22 V) ≥ DPPC (-0.24 V) > DMSO (-0.39 V) ≥ S-keratin (-0.40 V). The cathodic peak currents for MnPPDME in the keratin membranes increase linearly with the scan rate rather than the square root of the scan rate, while those for the reduction of Mn(III) of MnPPDME in DPPC lipid bilayer at 42 °C increase linearly with the square root of the scan rate as shown in Fig. 2. These facts indicate that the

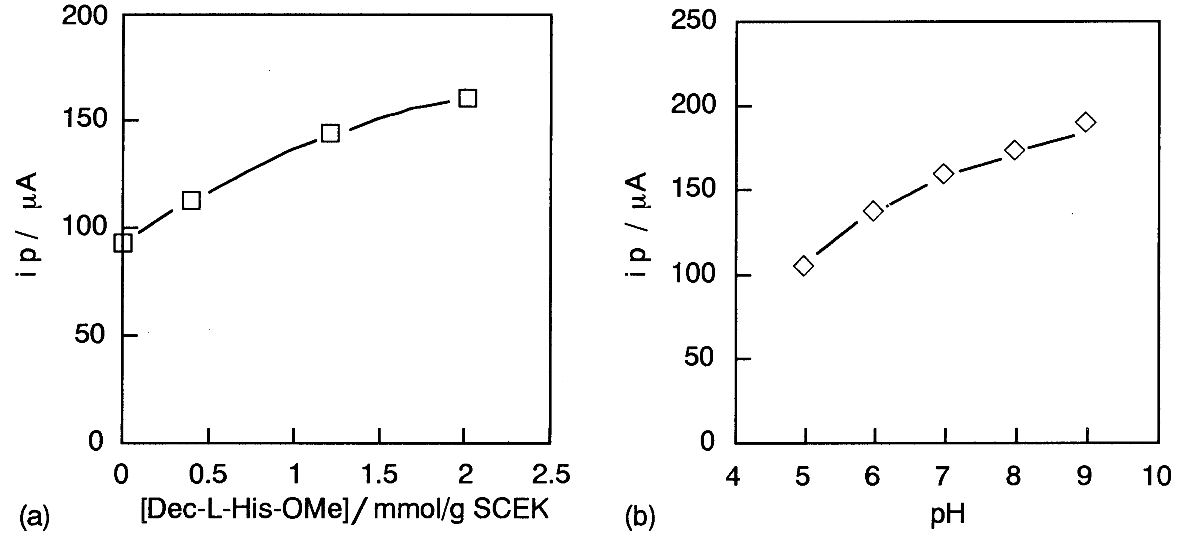


Fig. 3. The anodic peak currents of MnPPDME as a function of the concentration of N-decanoyl-L-histidine methyl ester (Dec-L-His-OMe) (a) and of pH change (b) in the case of a GC electrode covered with 400 μmol MnPPDME/g SCEK in the presence of Dec-L-His-OMe (2.0 mmol/g SCEK) at 25 °C.

electrochemistry of MnPPDME inside the keratin membranes is not diffusion controlled while that of MnPPDME in the DPPC membrane is diffusion controlled,[11)] implying that electron transfer between porphyrins on the electrode modified with protein membrane occurred. Fig. 3 illustrates anodic peak currents of MnPPDME as a function of the concentration of N-decanoyl-L-histidine methyl ester (Dec-L-His-OMe) a) and of pH change b) in the case of a GC electrode covered with 400 mmol MnPPDME/g SCEK membrane and immersed in an aqueous phosphate buffer solution at 25 °C. The peak current increases with increasing the concentration of Dec-L-His-OMe and with increasing pH in the presence of Dec-L-His-OMe, indicating that the imidazole moiety on the Dec-L-His-OMe has an enhanced effect on the passage of ionic currents driven by electrochemical conversion in the membrane. However, the enhanced effect of Dec-L-His-OMe on the peak currents was not observed in the case of the S-keratin membrane which is more hydrophilic than the SCEK one. The SCEK membrane was impermeable to ferricyanide ions with increasing pH, at least in the absence of ionophores, carriers, or channel-forming species in comparison to S-keratin membrane.[11)] It is likely that the structural change of the SCEK membrane in the range of pHs 5-9 is not large because the SCEK has an isoelectric point at pH 4 and contains about 10% of basic amino residues such as lysine (pKa = 10.5) and arginine (pKa = 12.5).[12)] The observation of sizable faradaic currents in the voltammetric experiments with GC/SCEK electrodes containing the imidazole derivative indicates that the imidazole is likely to provide substantial proton currents passed through the SCEK membrane in order to maintain the electroneutrality condition, in response to the electrochemical conversions especially at high pH. In conclusion, electron transfer between porphyrins on the electrode modified with protein membranes was reported and an enhanced electron transfer was observed especially in hydrophilic S-keratin membranes containing MnPPDME. The imidazole derivative enhanced the electron transfer in the hydrophobic SCEK membrane. This procedure is of interest to provide insight into the structural effects of porphyrins and proteins on electron transfer in biological membranes.

References

1) M. Nango, M. Higuchi, H. Gondo, and M. Hara, *J. Chem. Soc., Chem. Commun.*, **1989**, 1550 ; M. Nango, A. Mizusawa, T. Miyake, and J. Yoshinaga, *J. Am. Chem. Soc.*, **112**, 1640 (1990) ; M. Nango, K. Iida, T. Kawakita, M. Matsuura, Y. Harada, K. Yamashita, K. Tsuda, and Y. Kimura, *J. Chem. Soc., Chem. Commun.*, **1992**, 545.
2) J. Barber, "Topics in Photosynthesis," Elsevier/North-Holland Biochemical Press , New York (1978), Vol.3.
3) J. Anton, P. Loach, and Govinjee, *Photochem. Photobiol.*, **28**, 235 (1978).
4) J. Cowan, J. Sanders, G. Beddard, and R. Harrison, *J. Chem. Soc., Chem. Commun.*, **1987**, 55.
5) J. Rodriguez, C. Kirmaier, M. Johnson, R.Friesner, D. Holten, and J. Sessler, *J. Am. Chem. Soc.*, **113**, 1652 (1991).
6) R. W. Murray, "Molecular Design of Electrode Surfaces," Techniques of Chemistry Series, Wiley-Interscience: New York (1992), Vol.22.
7) T. Osa and M. Fujihira, *Membrane*, **6**, 321 (1981) ; N. Nakashima, *ibid.*, **13**, 335 (1988).
8) K. Yamauchi, Abstract from National Meeting of Chemical Society of Japan, Spring, 1992, p.1177. More detailed synthetic and analytical data will be reported elsewhere.
9) I. O'Donnell and P. Thompson, *Aus. J. Biol. Sci.*, **17**, 973 (1964).
10) T. Dewa, M. Satoh, J. Komiyama, M. Nango, M. Hikita, and K. Tsuda, in preparation.
11) M. T. Rojas, M. Han, and A. E. Kaifer, *Langmuir*, **8**, 1627 (1992).
12) E. G. Blendid and M. Fueghelman, "Encyclopedia of Polymer Science,"Wiley, New York (1968), Vol. 8.

(Received December 24, 1993)