(Received June 1, 1994)

Redox Reaction across the Membrane Incorporating Porphyrin-Linked Polymer Beads

Satoko Okubayashi, Hirokazu Akahori, Jin Matsumoto, and Teruo Hori

Department of Applied Chemistry & Biotechnology, Faculty of Engineering, Fukui University,
3-9-1 Bunkyo, Fukui, 910 Japan

Abstract: Functional membranes, in which manganese *meso*-tetra (*o*-aminophenyl) porphyrin-spacer-linked polymer beads were incorporated, were prepared. The electron transfer function of the membranes was investigated by driving the conjugated redox reactions across the membranes. Consequently, it was shown that manganese porphyrin derivatives fixed to the side chains of polymer in form of beads acted as good electron carrier, while iron porphyrin, hemin, fixed to them did not. The kinetic investigation of electron transfer supported that the hydrophilic polymer matrix and rigid side chains (spacers) were of large advantage to electron transfer function of metalloporphyrin.

1. Introduction

Electron transfer reactions associated with biological membranes have been investigated to clarify the vital functions [1, 2] and to apply the energetical advantage to other fields by constructing artificial electron transfer systems [3-5]. In this regard, there are some reports on transmembrane redox reaction systems in which two conjugated redox reactions can occur across the polymer membranes or lipid bilayers mimicing biomembrane. In those cases, some synthetic redox species such as viologen [6], ferrocene [7], lipoamide [8], quinone [9] derivatives or natural products such as cytochromes and chlorophylls [1, 10, 11] have been introduced in the membrane as so-called electron carrier.

Recently, new attempts have been done by Nakamura and his coworkers [12, 13] to construct conjugated transmembrane oxidoreductase reaction system by incorporating intercellular component extracted from wool [14], a kind of natural electron carrier, and/or coenzyme NAD(P)⁺/NAD(P)H in the hydrogel membranes and adding two oxidoreductase enzymes in both reaction bathes divided by the membrane, separately.

On the other hand, we have presented a new method for preparing the functional membranes by incorporating the functional polymer beads in the other inactive polymer membrane by casting method [15, 16]. For prepar-

ing an electron transfer membrane, chloromethylated polystyrene (CMPS) beads for peptide solid phase synthesis were employed; they were first grafted by tetraethylene glycol (TEG) and appropriate structures of oligopeptides were introduced at the end groups of the grafted TEG side chains. The membranes were prepared by dispersing the TEG-peptide-introduced polymer beads in tetrahydrofuran solution of poly(vinylchloride) (PVC) and casting the dispersion on a glass plate. After the heat treatment the membranes were doped aqueous copper sulfate solution to make Cu²⁺ complexes with the oligopeptide chains introduced in the beads. It was shown that the membranes possessed an extremely high electron transfer function.

However, there are few reports concerning the electron transfer membrane correlating metalloporphyrin, in spite of its efficient electron transfer property.

In this paper, we apply metalloporphyrins as a electron carrier, which are well known as active center of cytochromes and chlorophylls, and are also important pigments as a photosensitizer catalysing photolysis of water from the viewpoints of investigating an artificial photosynthesis. An attempt has been done to construct a novel model system for the electron transfer membrane, which may be used, in future, for artificial photosynthesis system and so on. For this purpose the above described membrane preparation technique was employed;

polymer beads having spacer-linked porphyrin structure were incorporated in inactive polymer membranes. The effect of the sorts of polymer beads and the structure of spacer on the electron transfer rate were investigated.

2. Experimental

2.1 Materials

Chloromethylated polystyrene (Bio-RadTMS-X1, capacity 4.15 meq/g, CMPS) beads for peptide synthesis were purchased from Bio-Rad, and cellulose beads were supplied from Rengo Co. Ltd. respectively. The average diameter of the beads were ca.80 and 130 μ m, respectively.

Hemin was obtained from Sigma and used without further purification. The other commercially available reagents were used without further purification.

2.2 Preparation of Functional Beads

Meso-tetra-(nitrophenyl)porphyrin (abbreviated as H_2TNO_2PP) was synthesized by refluxing *o*-nitro-benzaldehyde and pyrrole in glacial acetic acid [17], and *meso*-tetra(*o*-aminophenyl) porphyrin (H_2TamPP) was prepared by reduction of H_2TNO_2PP with tin chloride in concentrated hydrochloride.

As supporting polymer beads, hydrophobic CMPS and hydrophilic cellulose beads were employed.

As spacer between polymer backbone and functional group, porphyrin two different types of dialcohol, tetraethylene glycol (TEG) and decamethylene glycol (DMG) were chosen; the former is amphiphilic and flexible, while the latter is hydrophobic and rigid.

The graft of TEG/DMG on CMPS via an ether linkage was catalysed by sodium hydride. After then glycine (Gly) and following succinic acid anhydrous (Suc) were reacted at the terminal -OH groups of CMPS-TEG (DMG) beads [16] to obtain the CMPS-TEG (DMG)-Gly-Suc beads (see Scheme 1).

Cellulose (Cell) beads introduced by TEG or DMG were prepared as follows; the beads (1.0 g) and Suc (3.1 g, 31 mmol) were added in 50 mL of distilled water and pH value of the mixture was then adjusted to 9-10 with sodium hydroxide. After stirring for 4 h at room temperature, the beads were filtrated through a sintered glass frit and filtrate cake was washed with distilled water, ethanol and acetone sufficiently, and dried *in vacuo* (Cell-Suc). Cell-Suc beads (1.0 g) were added in the mixture of 4-dimethylaminopyridine (DMAP; 0.75 g, 6.1 mmol) and

Scheme 1 Preparation pathways of beads-linked metalloporphyrin derivatives in the case of CMPS beads(a), and Cellulose beads(b).

N, N'-dicyclohexylcarbodiimide (DCC; 5.1 g, 25 mmol) in 20 mL of dimethyl sulfoxide (DMSO). The solution was stirred for 1 h at room temperature and then TEG (2.4 g, 12 mmol) or DMG (2.1 g, 12 mmol) was dissolved in the solution. The reaction mixture was shaken slowly for 7 days at room temperature, avoiding the break of beads, filtrated and washed with DMSO. Cell-Suc-TEG(DMG) beads were dried *in vacuo*. Carboxylation of the TEG- or DMG- introduced beads was also reacted with Suc by the same manner described above.

H_2TamPP was covalently bound to the resulted end carboxyl spacer groups via an amide linkage using DCC. For CMPS beads, the mixture of CMPS-TEG(DMG)-Gly-Suc beads (1.0 g), DCC (0.17 g, 0.8 mmol) and DMAP (0.074 g, 0.4 mmol) in 50 mL of dichloroethane was stirred for 2 h and H_2TamPP (0.71 g, 0.4 mmol) was added in the mixture. After reaction for 15 h, the mixture was filtrated and beads were washed with enough amounts of dichloroethane and dried to get CMPS-TEG(DMG)-Gly-Suc- H_2TamPP beads.

For Cell beads, H_2TamPP was fixed on the end groups of side chains of Cell-Suc-TEG(DMG)-Suc beads by similar method in the case of CMPS beads; DMSO was used as reaction solvent in stead of dichloroethane.

Manganese complexes of porphyrin derivatives were obtained by refluxing the mixture of manganese (II) 2, 4-pentanedionate and H_2TamPP -fixed polymer beads in pyridine/dichloromethane/chloroform [18]. The beads prepared by this method were abbreviated as CMPS-TEG(DMG)-MnTamPP, and Cell-TEG(DMG)-MnTamPP

beads.

Hemin was also fixed in each polymer beads in the similar process as described above and the details of the preparation procedure were omitted.

Introduction of DMG and TEG into polymer beads was confirmed by infrared spectroscopy. The concentration of porphyrin introduced onto polymer beads was estimated spectrophotometrically from that of released porphyrin by hydrolysis with 2 N of NaOH/DMF (1:1 v/v) at 60 °C for 3 h.

2.3 Membrane Preparation

Poly(vinylchloride) (PVC, Wako Pure Chemical Ind. Ltd., degree of polymerization:1,100) and plasticizer phthalic acid di(2-ethylhexyl) ester (DEPA) were dissolved in tetrahydrofuran (THF) in 2.0 and 0.4 wt%, respectively, and in this solution 1.0 wt % of the functional polymer beads were dispersed with vigorous stirring. It was then cast on a glass plate set on a horizontal plate in a incubator. The membrane was obtained by evaporation of solvent THF *in vacuo* at 30 °C for 3 h.

The each membrane was then interposed between two glass plates and treated in boiling water for 30 min to move the beads in the center of membrane so that the functional beads pass through the membrane from one surface to the other surface [15].

2.4 Electron Transfer Assay

The membranes prepared were used for the following measurements of the transmembrane redox reactions.

The membrane was set in a two-compartments diffusion cell with 0.785 cm² effective permeation area (Fig. 1). In a compartment I a solution of 200 mM Na₂S₂O₄ (100 mL) in imidazole/HCl buffer [18, 19] was placed and in the other compartment II, a solution of 1 mM K₃[Fe(CN)₆] (100 mL) in the same buffer solution was placed. Both solutions were agitated by introducing nitrogen gas. The reactions were driven at 10, 20, 30, and 40 °C. The rate of electron transport from S₂O₄²⁻ to Fe(CN)₆³⁻ was monitored by measuring the absorbance of the K₃[Fe(CN)₆] at 420 nm ($\lambda_{\max}$ of K₃[Fe(CN)₆]). It is well known that such rate of electron transfer obeys the first order reaction [8]; when the reactions at both membrane surfaces follows the pseudo-first order reaction, the whole reaction can also follow apparently the first order reaction. The apparent first order rate constants, k_{obs} (in min⁻¹) were estimated from the slope of the intermediate linear part of the variation of the absorption

through

$$-\ln(A_t/A_0) = k_{\text{obs}} \cdot t \quad (1)$$

where A_0 and A_t represent initial absorbance and that at time t at 420 nm, respectively [19]. The leakage of S₂O₄²⁻ and/or Fe(CN)₆³⁻ through membrane was checked for every system at the lowest 10 °C and the highest temperature 40 °C.

3. Results and Discussion

The UV/vis spectra of synthesized H₂TamPP were agreed with those by literature [17].

Fig. 2 shows IR charts of CMPS beads, its TEG- and DMG-derivatives(a), and Cell beads, its Suc- and Suc-TEG-derivatives(b). The peak at 1270 cm⁻¹ representing chloromethyl group becomes small and the peak at 1100 cm⁻¹ indicating ether linkage becomes large after TEG-grafting reaction, from which it is clear that TEG was introduced in CMPS beads. The reaction yield was estimated gravimetrically and the results were summarized in Table 1. In the case of cellulose beads, no apparent difference was found in IR charts between native cellulose and Suc- and Suc-TEG-introduced cellulose beads because native cellulose also has much ester, ether and carboxyl groups essentially.

Fig. 3 a)-d) show the time courses of the reduction of Fe(CN)₆³⁻ by S₂O₄²⁻ across the functional beads incorpo-

Fig. 1 Apparatus for driving of conjugated redox reaction across membrane. I and II phases contain 200 mM of Na₂S₂O₄ and 1 mM of K₃[Fe(CN)₆] in imidazole/HCl buffer (100 mM, pH 7.0), respectively.

(a)

(b)

Fig. 2 IR charts of grafted polymer beads. (a) CMPS derivatives; -CMPS, CMPS-TEG and CMPS-DMG, (b) Cellulose derivatives; Cell, Cell-Suc, and Cell-Suc-TEG.

rated PVC membrane (given with open marks). The fact that the slow reduction occurred also in the cases of blank membranes (given with painted marks) indicates that $\text{S}_2\text{O}_4^{2-}$ and/or $\text{Fe}(\text{CN})_6^{3-}$ can diffuse (leak) to the opposite compartment. This phenomenon is more remarkable in Cell beads system rather than in CMPS beads system. It is attributed to the fact that Cell beads is more hydrophilic and a little porous.

In any cases, reduction rate of $\text{Fe}(\text{CN})_6^{3-}$ across the functional beads at higher temperatures is much larger

Table 1 Reaction Yields of TEG, DMG and MnTamPP into Polymer Beads

Beads	Yield (mol % against monomer unit)	
	Spacer	MnTamPP
CMPS-DMG-MnTamPP	14.0	3.22
CMPS-TEG-MnTamPP	17.2	4.05
Cell-DMG-MnTamPP	-	4.11
Cell-TEG-MnTamPP	-	4.14

Fig. 3 Reduction of $\text{Fe}(\text{CN})_6^{3-}$ with $\text{S}_2\text{O}_4^{2-}$ across the prepared membrane: $-\ln(A_t/A_0)$ was plotted as a function of the reaction time for CMPS-DMG-MnTamPP(a), CMPS-TEG-MnTamPP(b), Cell-DMG-MnTamPP(c), Cell-TEG-MnTamPP(d) system, respectively. Temperatures of the reaction were 10(▽), 20(□), 30(△), and 40 °C (○). The blank reactions across the membrane without MnTamPP were checked 10(▼) and 40 °C (●).

than across the blank membranes which suggests that electron transfer is catalysed by metalloporphyrin. The electron transfer in this system can be considered to occur in a manner illustrated in Fig. 4; at the surface of the membrane in the compartment I $\text{S}_2\text{O}_4^{2-}$ ions reduce Mn^{2+} -complex of porphyrin to the Mn^+ -complex, which reduces then the adjacent porphyrin to reduced form. This process is repeated in the membrane and the reduced porphyrin on the opposite membrane surface reduces the $\text{Fe}(\text{CN})_6^{3-}$ in the compartment II. In such man-

ner the electrons are transported through the membrane.

On the other hand, using the membrane incorporating hemin-fixed polymer beads, reactions did not occur in every case. The difference in MnTamPP and hemin seems to be the experimental fact by Saveant and his coworkers [20] that porphyrin derivatives linked through ether bond cannot act as electron carrier while those linked through amide bond can. But, the reason for the difference is now not evident.

The yield of DMG or TEG grafting on polymer beads and that of porphyrin introduced on side chains were given in Table 1. From the table, it is clear that the amounts of the introduced MnTamPP were not in large quantities in every case. On the basis of these results, the distances between two adjacent porphyrins in a polymer chain can be calculated, assuming that the porphyrins were introduced uniformly in the polymer beads and the results were illustrated schematically in Fig. 5. In this system an electron could be transferred over the dis-

Fig. 4 Mechanism of conjugated redox reaction across the membrane for the system in $Na_2S_2O_4$ /functional membrane/ $K_3[Fe(CN)_6]$.

tance of ca. 80-100 Å. However, the electron transfer from one porphyrin on a polymer chain to that on the adjacent polymer chain is also possible, whose distance is perhaps closer than the distance 80-100 Å calculated above. Taking into consideration that in ferricytichromes a distance requiring for electron transfer from porphyrinateiron to nearby that is considered to be several dicades Angstrom [21], these distances are a little larger

Fig. 5 Schematic diagram of the distances between fixed adjacent porphyrins in a polymer chain. The distance was estimated from the data of the monomer ratio of copolymerization of styrene and chloromethylated styrene (2 : 1), yield of DMG or TEG grafting and fixation yield of metalloporphyrin shown in Table 1.

Table 2 The Kinetic and Thermodynamic Data for Transmembrane Redox Reactions

	$k_{obs}(\text{min}^{-1}) \times 10^4$				E_a (kJ/mol)	A (min^{-1})
	10°C	20°C	30°C	40°C		
CMPS-DMG-MnTamPP	-	9.75	10.1	34.0	47.1	2.00×10^5
CMPS-TEG-MnTamPP	-	1.95	15.5	23.4	95.5	2.58×10^{13}
Cell-DMG-MnTamPP	24.5	22.6	28.1	52.9	18.2	4.71
Cell-TEG-MnTamPP	2.10	6.73	-	9.57	34.4	612

k_{obs}' represents the difference in rate constant between k_{obs} and k_{obsb} obtained for functional membrane and for blank membrane. E_a and A are activation energy and frequency factor for electron transfer, respectively.

Fig. 6 Arrhenius plots of k_{obs}' for CMPS-DMG-MnTamPP (● in (a)), CMPS-TEG-MnTamPP (○ in (a)), Cell-DMG-MnTamPP (● in (b)), and Cell-TEG-MnTamPP (○ in (b)).

for efficient electron transfer. This may be one of the reasons of relatively slow electron transfer of these systems.

The values of k_{obs}' , obtained as the difference in observed rate constant between electron transfer measurements k_{obs} and blank measurements k_{obsb} , are summarized in Table 2. These values are a little smaller than that for viologen-fixed membrane systems reported by Nambu [6] et al. and similar to that for the porphyrin-contained membrane systems proposed by Dewa et al. [19].

Activation energies and frequency factors for electron transfer in the whole systems were calculated from Arrhenius plots of k_{obs}' and summarized also in Table 2. Relatively large scatter of Arrhenius plots is considered to be due to the fact that the transmembrane reactions were not complete first order reaction, and as results the k_{obs} obtained from the appropriate linear parts in the reaction curves (Fig. 6) contains some errors. It seems apparent that, activation energies in the DMG-introduced systems, are considerably smaller, compared with that in the TEG-introduced systems. Considering the flexibility of DMG and TEG spacer it is difficult to explain the difference. From the results, it can be said that the flexible motions are not required for electron transfer, especially for the hopping mechanism of electron [22].

It is also obvious that the activation energy for Cell beads systems is smaller than that for CMPS beads systems. This can be explained by the fact that the protons are also transferred accompanying with electrons to keep electron neutrality. For the proton transfer the rough and swollen structure of cellulose is of greater advantage

than the tight structure of CMPS.

The electron transfer of metalloporphyrin obeys hopping mechanism [22], thus, the rate of electron transfer across the beads-TEG-MnTamPP/PVC membrane which has large activation energy is small while that across the beads-DMG-MnTamPP/PVC membrane which has small activation energy is large independent on kinds of polymer beads. Moreover, it is also thought that the reason why in cellulose beads series E_a is small and k_{obs}' is large in comparison with that in CMPS beads series, is that a transport of proton through the membrane, which is rate-determining step of electron transfer system, is fast due to hydrophilic property of cellulose.

4. Conclusion

Although metalloporphyrin derivatives are well known as a good natural electron carrier, there were little investigations for constructing electron transfer membranes using these compounds. In this paper an attempt has been done to prepare efficient electron transfer membranes using metalloporphyrin derivatives by the functional polymer beads incorporating method, in the view points if the polymer matrix and spacer for porphyrin fixation influence on the electron transfer efficiency. The electron transfer property of the membranes prepared was estimated by driving the conjugated transmembrane redox reaction.

As results, in the cases of MnTamPP the relatively fast reactions were observed, while in the case of hemin no reactions occurred. This difference was considered to be attributed to the sorts of chemical linkage between spacers and porphyrin derivatives as pointed out by Saveant and his coworkers. The efficient electron transfer for MnTamPP systems could be explained by the reasonable order of redox species from electron donor to electron acceptor throughout the porphyrin derivatives bound in polymer beads in the membrane. Large advantage to electron transfer was found for hydrophilic cellulose beads and rigid DMG spacer. An artificial model system for biomembrane using metalloporphyrin derivatives was obtained.

This research was presented in part at the 13th Meeting of Textile Union Japan (Osaka, November, 1993) and at the Annual Meeting of the Society of Fiber Science and Technology Japan (Tokyo, July 1994).

References

1. D. G. Nocera, J. R. Winkler, K. M. Yocom, E. Bordignon, and H. B. Gray, *J. Am. Chem. Soc.*, **106**, 5145 (1984).
 2. T. Asakura, H. R. Drott, and T. Yonetani, *J. Biol. Chem.*, **244**, 6626 (1969).
 3. T. Kawai, K. Tanimura, and T. Sakata, *Chem. Lett.* 137 (1979).
 4. K. Takuma, M. Kajiwara, and T. Matsuo, *Chem. Lett.*, 1199 (1977).
 5. I. Okura, S. Kusunoki, and S. Aono, *Inorg. Chem.*, **22**, 3828 (1983).
 6. Y. Nambu, T. Endo, and K. Tashiro, *J. Polym. Sci., Polym. Lett., Ed.*, **23**, 409 (1985).
 7. T. Shinbo, K. Kurihara, Y. Kobatake, and N. Kamo, *Nature*, **270**, 277 (1977).
 8. Y. Nambu, T. Endo, and M. Okawara, *J. Polym. Sci., Polym. Chem., Ed.*, **23**, 223 (1985).
 9. T. Arai, S. Nishibori, T. Kato, and K. Takemoto, *Proc. Membrane Symp., No. 4, Kyoto*, p113 (1992).
 10. S. G. Boxer and K. A. Wright, *J. Am. Chem. Soc.*, **101**, 6791 (1979).
 11. Y. Toyoshima, M. Morino, H. Motoki, and M. Sugi-gara, *Nature*, **265**, 187 (1977).
 12. Y. Nakamura, N. Kamon, and T. Hori, *Bull. Chem. Soc. Jpn.*, **62**, 551 (1989).
 13. Y. Nakamura, A. Tanaka, S. Okubayashi, and T. Hori, *Bull. Chem. Soc. Jpn.*, submitted.
 14. Y. Nakamura, K. Hirota, T. Hori, and H. Hojo, *Proc. 8th Intern. Wool Text. Res. Conf., Chrystchurch*, Vol. **1**, 296 (1990).
 15. T. Hori, H. Hori, H. Tsujimura, and Y. Nakamura, *Proc. 29th Symp. Polym. Water*, p3 (1991).
 16. T. Hori, H. Naruse, H. Kitajima, S. Okubayashi, and Y. Nakamura, *in preparation*, T. Hori, H. Kitajima, and Y. Nakamura, *Proc. Membrane Symp. No.4, Kyoto*, p121 (1992).
 17. J. P. Collman, R. R. Gagne, C. A. Reed, T. R. Halbert, G. Lang, and W. Robinson, *J. Am. Chem. Soc.*, **97**, 1427 (1975).
 18. M. Nango, T. Dannhauser, D. Huang, K. Spears, L. Morrison, and P. A. Loach, *Macromol.*, **17**, 1898 (1984).
 19. T. Dewa, M. Satoh, and J. Komiyama, *Macromol. Chem. Phys.*, **195**, 1031 (1994).
 20. E. Anxolabehere, D. Lexa, and J. -M. Saveant, *J. Phys. Chem.*, **96**, 1266 (1992).
 21. For example, L. Stryer "Biochemistry", W. H. Freeman & Co., New York, p.408 (1988).
 22. T. J. Dannhauser, M. Nango, N. Oku, K. Anzai, and P. A. Loach, *J. Am. Chem. Soc.*, **108**, 5865 (1986).
-