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PHOTOSENSITIZED ELECTRON TRANSPORT IN THE ASYMMETRICAL VESICLES INCORPORATING A SURFACE-ACTIVE ZINC PORPHYRIN AND AN ELECTRON MEDIATOR

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Photosensitized electron transport from ethylenediaminetetraacetic acid disodium salt (EDTA•2Na) or sodium ascorbate (Asc•Na) to methylviologen (MV^{++}) across vesicle membranes of dipalmitoyl-L-α-phosphatidylcholine (DPPC) which incorporate 5,10,15-tris(1-methylpyridinium-4-yl)-20-[4-(octadecyloxy)phenyl]porphinatozinc(II) trichloride (Zn-P) asymmetrically (only on the outer side of the membrane) is facilitated by an electron mediator such as ubiquinone Q_{10} (UQ_{10}) or tetraphenylporphine (TPP). Zn-P plays an important role in the redox reaction at the interface of the vesicles and the efficiency can be correlated with the redox potentials of the mediators.

The vectorial electron transport in the thylakoid membrane is effected by the orderly array of redox units.$^{1)}$ As the simple models of the thylakoid membrane, single-lamellar vesicles containing various kinds of pigments have often been employed, in which the pigments apparently exist on both sides of the membranes.$^{2-5)}$ In the previous letter,$^{6)}$ we reported that a synthetic surface-active porphyrin complex of zinc (Zn-P) could be incorporated either into both sides or only into outer side of the DPPC vesicle membranes and called them symmetrical or asymmetrical vesicles, respectively. Although photosensitized electron transport from EDTA•2Na to MV^{++} via the DPPC membranes occurred in the case of the symmetrical vesicles, the asymmetrical vesicles were inactive for the electron transport$^{6)}$ so long as we used no electron mediator.

The present communication will show that the addition of an appropriate electron mediator to the asymmetrical vesicle facilitates the photosensitized electron transport from a donor to an acceptor via DPPC membrane. p-Benzoquinone (BQ), vitamin K_1 (VK_1), vitamin K_3 (VK_3), ubiquinone Q_{10} (UQ_{10}), H_2TPP, ZnTPP, and octaethylporphinatozinc (ZnOEP) were tested as possible mediators. The quinones were of commercial grade and the porphyrins were prepared by the reported methods.$^{7)}$ After an Asc•Na or an EDTA•2Na solution was encapsulated inside the vesicles as an electron donor, a methylviologen solution was added to the aqueous dispersed solution of the vesicles as an electron acceptor. The dispersion of the asymmetrical vesicles (Fig. 1) buffered with 0.2 M Tris-Cl and 0.15 M KCl (pH 7.50) was prepared as reported previously$^{6)}$ and all operations were carried out under argon atmosphere. A mediator was introduced into the vesicles by co-lyophilizing the DPPC ethanol solution with the mediator. Lack of the leak of the electron donor encapsulated in the vesicles was checked by the control run using the vesicles without a mediator. After illuminating with a 750 W tungsten lamp through an ND filter (IRA-25S), the difference spectra of the illuminated and unilluminated samples were recorded. The electron transport was evaluated by the absorbance of methylviologen cation radical ($MV^{+\bullet}$) at 393 nm (ε 37300).

In the asymmetrical vesicles where the incorporated Zn-P is distributed only at the outer interface of the vesicles,6) its direct contact with the electron donor (inside of the vesicles) is unlikely and the electron transport through the lipid bilayer should depend on the reaction between the electron donor and the mediator. From thermodynamic point of view, the redox potentials of the donor and the mediator determine whether the electron transport to the mediator is possible or not. Also the diffusion of the mediator in the lipid bilayer is considered to be an important factor.

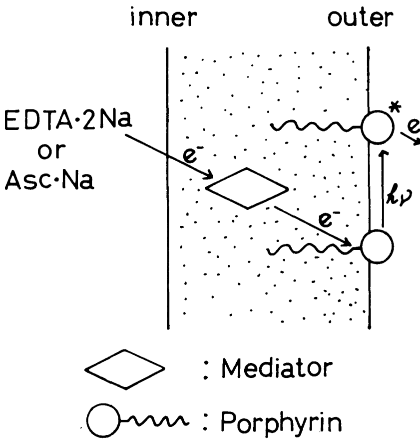

Fig. 1 Schematic illustration of the electron transport in the asymmetrical vesicle containing a mediator.

Quinones The properties of quinones as mediators have been investigated in the liquid membrane,8,9) monolayers,10) BLM,11) and vesicles.12) The reaction rate and activation energy of the electron transport reaction via quinones in the vesicles have been reported.12) The redox potentials of the quinones are as follows.13-15)

-0.106 (VK_1) $<$ -0.005 (VK_3) $<$ 0.06 (ascorbic acid) $<$ 0.098 (UQ_{10}) $<$ 0.26 (BQ) (volt vs. NHE, pH 7.50)

From the redox potentials, UQ_{10} and BQ should mediate electrons,16) but only UQ_{10} was effective in the present experiments. In the system entrapping Asc•Na (0.2 M) as an electron donor, $MV^{+\bullet}$ (1.30×10^{-5}M) was produced from MV^{++} (2×10^{-3} M) after 60 min illumination by means of the asymmetrical vesicles incorporating Zn-P (ca. 2 μM, Zn-P/DPPC = ca. 1/500) and UQ_{10} (6×10^{-6} M).

The difference spectra of the illuminated and unilluminated system of the vesicles containing only UQ_{10} and no Zn-P showed absorption decrease at 275 nm and increase at ca. 300 nm, which we interpreted as the indication of the reduction of UQ_{10}.17) Incorporation of Zn-P retarded the reduction of UQ_{10} and the electron transport is likely to have occurred in the manner illustrated in Fig. 1. In general, diffusion in the lipid bilayer as well as the matching of redox potentials seems to be important as rate-controlling factor for the simple quinones, but the mechanism will not be so simple for VK_1 and UQ_{10} which have long side chains.1,12) The fact that BQ is rather inactive may be accounted for by its reluctance to access to the hydrophilic region and the resultant slowness of its reaction with Asc•Na. However, UQ_{10} has a large structure which enables the transport of electrons by such a manner as the anchored flip-flop mechanism.1)

Porphyrins In the lipid bilayer, porphyrins can act both as a mediator and a photosensitizer.18) The present study shows that ZnTPP and H_2TPP actually play the both parts (Table 1). However, probably due to the hydrophobic nature of the porphyrins, the across-membrane electron transfer to MV^{++} are slow just as the quinone's case. Although the redox potentials of ZnTPP and H_2TPP are different with each other the productions of $MV^{+\bullet}$ are almost of the same low level. The effect of incorporation of Zn-P for the increase of electron transport was remarkable (note the ratio in Table 1). In order to check whether this effect results from the distribution of Zn-P in the hydro-

Table 1 $MV^{+\bullet}$ production in the asymmetrical vesicles incorporating a mediator with or without Zn-P (ca. 2 μM, Zn-P/DPPC = ca. 1/500).

Mediator(M)	$[MV^{+}]\times10^{5}M$ ‡		Ratio*
	(1)mediator	(2) mediator +Zn-P	
H_2TPP (4.0×10^{-6})	0.047	6.20	132
ZnTPP(4.4×10^{-6})	0.40	6.30	16
ZnOEP(4.4×10^{-6})	—	—	—

Electron donor: EDTA•2Na (0.13 M). Electron acceptor: MV^{++} (2×10^{-3} M). ‡After 60 min illumination. * (2)/(1)

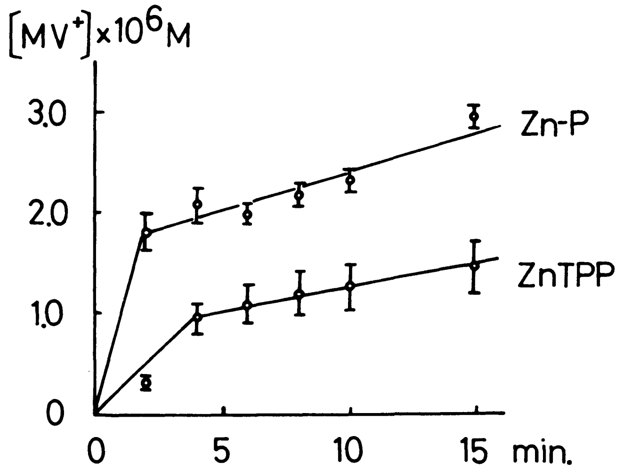

Fig. 2 $MV^{+\bullet}$ production in the homogeneous reaction in methanol dissolving ascorbic acid, MV^{++}, and ZnTPP or Zn-P.

philic region due to the N-methylpyridinium groups or from the intrinsic electrochemical properties of Zn-P, the homogeneous reaction among ascorbic acid (6.7×10^{-4} M), ZnTPP or Zn-P (4.0×10^{-6} M), and MV^{++} (7.0×10^{-4} M) was investigated in methanol solution under argon in a similar way to the case of the vesicle dispersion (Fig. 2). The Zn-P sytem reduced MV^{++} twice as much as the ZnTPP system did, but the ratio was much smaller than 16 in the Table. Thus the increase of the electron transport in the presence of Zn-P in the vesicle systems may be explained by taking the distribution of Zn-P in the hydrophilic region of the vesicle into account.

The production of $MV^{+\bullet}$ was not observed in the vesicles containing ZnOEP as the mediator. The result is in accord with the order of the half-wave potentials of the porphyrins ($E^{+/0}$) (volt vs. NHE).[14,19)]

$0.76\ (\mathrm{ZnOEP}) < \mathrm{ca.}\ 0.94\ (\mathrm{EDTA}) < 0.99\ (\mathrm{ZnTPP}) < 1.29\ (H_2TPP)$

Just as the quinones, the matching of the redox potentials is essential for the electron transport. Supporting evidence of the scheme (Fig. 1) was obtained from the difference spectra of the asymmetrical vesicles incorporating ZnTPP and no EDTA•2Na. The spectra indicated the increase of the absorbance at 410 nm which is assignable to the cation radical of ZnTPP[20)] but the presence of the electron donor retarded the change.

The above results suggest that the asymmetrical vesicles are convenient systems to survey potential electron mediators and to study their roles in photosynthesis.

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