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ELECTRON TRANSPORT ACROSS LIPID MEMBRANES PHOTOSENSITIZED BY AN AMPHIPHILIC ZINC PORPHYRIN

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Electron transport reaction from EDTA·2Na to methylviologen (MV^{2+}) across the membrane of single-lamella vesicles of dipalmitoyl-L-α-phosphatidylcholine (DPPC) is photosensitized by an amphiphilic zinc porphyrinato complex, 5,10,15-tris-(1-methylpyridinium-4-yl)-20-(4-stearoxyphenyl)porphyrinatozinc(II) trichloride (Zn-P). This porphyrin complex can be incorporated into the vesicles symmetrically (on both sides of the membrane) or asymmetrically (only on the outer side). The symmetrical system facilitates the electron transport even in the absence of electron mediators.

Photoinduced electron transport across lipid membranes has been studied in order to understand photosynthesis and to construct its model systems.[1] As photosensitizers, an amphiphilic tris-(2,2'-bipyridine)ruthenium(II) derivative,[2] chlorophylls,[3] and other pigments[4] have been employed. Recently Matsuo and his coworkers proposed a concerted two-step activation of photoinduced electron transport across lipid membrane by using an amphiphilic zinc porphyrinato complex as a photosensitizer.[5] We have also developed the synthesis of a new type of surface-active porphyrins[6] to investigate the photochemical role of the sensitizers in photosynthesis and now report the photoinduced electron transport reaction across the vesicle membranes of DPPC.

One of the synthesized porphyrins, 5,10,15-tris(1-methylpyridinium-4-yl)-20-(4-stearoxyphenyl)-porphyrinatozinc(II) trichloride (Zn-P) (Fig. 1), has three hydrophilic pyridinium groups and one hydrophobic stearoxyphenyl group, is soluble both in aqueous and organic solvents and can be readily incorporated into lipid vesicles. We have prepared two types of DPPC vesicles, which entrap an aqueous solution of ethylenediamine-N,N,N',N'-tetraacetic acid disodium salt (EDTA·2Na) by the following procedures.

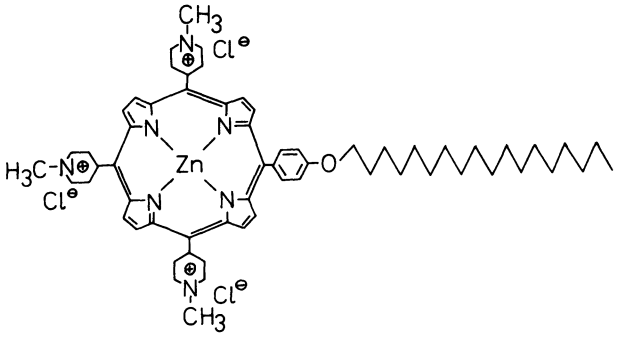

Fig. 1 5,10,15-tris(1-methylpyridinium-4-yl)-20-(4-stearoxyphenyl)porphyrinatozinc(II) trichloride (abbreviated as Zn-P)

The co-lyophilized mixture of DPPC and Zn-P was dispersed in 0.13 M EDTA·2Na buffer solution (0.2 M Tris-Cl and 0.155 M KCl, pH 7.50) by ultrasonication, and the dispersion was gel-filtered with Sephadex G-100 column equilibrated with Tris-Cl buffer (pH 7.50) to remove free DPPC and porphyrin. The intensity of an absorption band at 444 nm indicated that the molar ratio of Zn-P incorporated into the vesicles was ca. 1/100 of DPPC. The vesicle appears to have the porphyrin molecules incorporated on both sides of the vesicle membrane and so we call it symmetrical vesicle (Fig. 2).

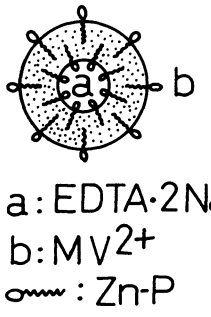

Fig. 2 Symmetrical vesicle

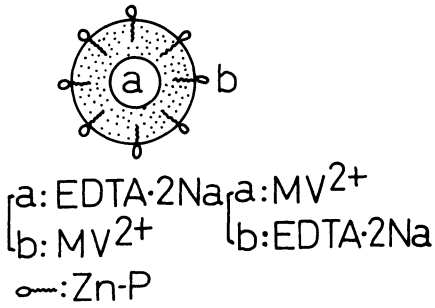

Fig. 3 Asymmetrical vesicle

We could also incorporate the porphyrin only on the outer side of the membrane (Fig. 3) by the following procedures. Co-lyophilized DPPC from ethanol solution under vacuum was dispersed with a 0.155 M KCl aqueous solution by ultrasonication. Into this dispersion, a 0.155 M KCl aqueous solution containing Zn-P (ca. 1/50 mole of DPPC) was added and gel-filtered with a Sephadex G-200 column to obtain a green fraction. The porphyrin incorporation was measured from the absorbance at 444 nm and the amount of DPPC in the dispersion was analyzed by the method of Barlett.[7] The temperature dependence of the incorporation is shown in Fig. 4. The molar ratio of the incorporated Zn-P to DPPC was about 1/200 after 2 h contact of the DPPC vesicles with the Zn-P solution at 60 °C. The amount of Zn-P in the vesicles at room temperature was analyzed after the released porphyrin into the aqueous phase was removed by gel-filtration (Fig. 5) to check the stability of the Zn-P vesicles. The porphyrin molecules were probably incorporated only on the outer side of the vesicle membrane, and so we call it asymmetrical vesicle (Fig. 3). To confirm this, we prepared symmetrical and asymmetrical vesicles containing the same amounts of Zn-P and compared the reactivities of the porphyrin in both types of the vesicles toward N-bromosuccinimide (NBS).[8] When the reaction of the porphyrin in the vesicles was monitored within a short time (ca. 15 min), the amount of Zn-P that reacted with

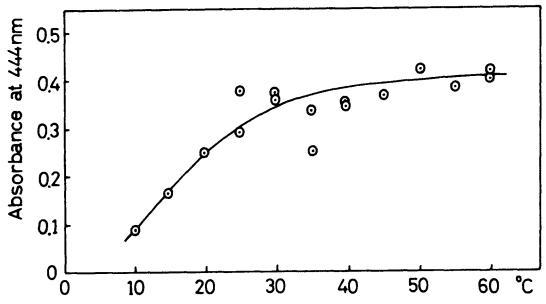

Fig. 4 The temperature dependence of the asymmetrical incorporation of Zn-P.

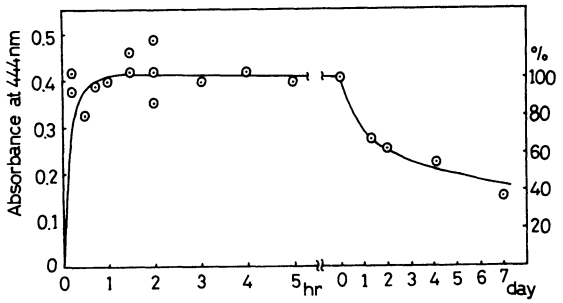

Fig. 5 The time dependence of the asymmetrical incorporation of Zn-P at 60 °C and the amount remaining in the vesicle at r.t.

NBS in the symmetrical system was about a half of that in the asymmetrical system. Namely, penetration of NBS into the vesicle membranes was slow enough for the outside porphyrin to react with NBS preferentially. The result supports the assumption that the asymmetrical system incorporates Zn-P only on the outer side of the membrane.

The photochemical behavior of these two kinds of vesicles was markedly different. After a 0.02 M solution of MV^{2+} was added to the dispersion of the symmetrical vesicles, it was transferred into a set of matched 1-cm path-length cuvettes and bubbled with argon for 30 min in the dark. One sample was illuminated with a 750 W tungsten lamp through a ND filter (IRA-25S). Difference spectra were recorded at each interval of illumination time, with the sample in the reference cell kept unilluminated (Fig. 6). The concentration of the methylviologen cation radical ($MV^{+\cdot}$) produced by illumination was estimated by the absorption at 390 nm (ε_{390} = 3.73 x 10^4 $M^{-1}cm^{-1}$),[9] and it was 1.10 x 10^{-4} M after 60 min illumination with the concentration of Zn-P (7.3 x 10^{-6} M). The result indicates that the porphyrin acted as a photocatalytic agent for the electron transport and was as effective as the zinc porphyrin reported by Matsuo.[5] The system without EDTA·2Na did not reduce MV^{2+}, so it is most likely that EDTA.2Na was the electron donor.[10] When 2-methyl-1,4-naphthoquinone (vitamin K_3)(VK_3, 8.0 x 10^{-5} M) or its derivative vitamin K_1 (VK_1, 1.0 x 10^{-4} M) was added as a constituent of the vesicles, VK_3 enhanced slightly (ca. 10%) the reduction of MV^{2+} but VK_1 retarded the production of $MV^{+\cdot}$. These facts imply that VK_3 played a role of an electron carrier but VK_1 did not under the present conditions.

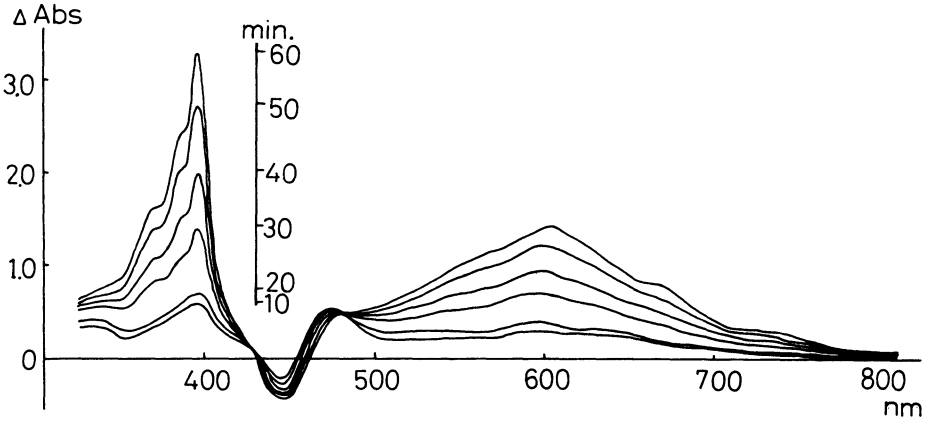

Fig. 6 The difference spectra of the illuminated and unilluminated vesicles of the symmetrical system shown in Fig. 2.

As described above, the trans-membrane electron transport takes place both in the presence and in the absence of VK_3. However, the asymmetrical system was inactive for the MV^{2+} reduction. When the photoreaction was carried out by use of the asymmetrical vesicles under similar conditions, the electron transport from EDTA·2Na entrapped in the vesicles to MV^{2+} in the outer solution did not occur. This was also the case when MV^{2+} was encapsulated in the vesicles and EDTA·2Na solution was placed in the outer continuous phase.

Since the electron transport in the symmetrical system may not be accounted for by tunnelling mechanism, and since the existence of Zn-P on both sides of the membrane seems to be necessary, it is likely that the porphyrin molecules act cooperatively for the electron transport from EDTA·2Na to MV^{2+} just like in the concerted two-step activation proposed by Matsuo.[5] The relationship between the electron transport and the symmetry of the membranes is intriguing in that it provides a clue to the mechanism of the electron transport reaction in photosynthesis.

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