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SPECTROSCOPIC STUDIES ON THE SYNTHETIC MAGNESIUM PORPHYRINS IN THE VESICLES AS TRANS-MEMBRANE ELECTRON TRANSPORT SENSITIZERS

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Synthetic magnesium porphyrins, Mg-P^{3+} ** and Mg-P *** were solubilized into DPPC **** vesicles and the trans-membrane electron transport from EDTA·2Na to MV^{2+} was studied in the concentration range where the porphyrins did not aggregate. Mg-P^{3+} was found to photo-catalyze the reaction about 30 times as efficiently as Mg-P.

In the studies of some functions of chlorophylls which are unstable toward oxygen and light, various types of amphiphilic metal complexes[1,2] solubilized into bilayer lipid membranes, micelles, and vesicles have been reported.[3,4] Chlorophyll molecules are considered to be solubilized into the thylakoid membrane in vivo by inserting their phytyl groups as anchors into the hydrophobic region of the membrane. The mechanism of the solubilization of synthetic amphiphilic complexes into artificial membranes may be analogous. In the present study, we have compared the efficiencies of the trans-membrane electron transport from EDTA·2Na to MV^{2+} photocatalyzed by Mg-P^{3+} and Mg-P and found that Mg-P^{3+} was much more efficient than Mg-P as a photosensitizer (vide infra). The difference may be due either to the deactivation of the excited porphyrin by aggregation or to the different orientation of the porphyrin rings in the vesicle membrane. In order to preclude the former possibility, we have determined first the concentration range spectroscopically[5] where Mg-P^{3+}, Mg-P, and MgTPyP ***** exist stably as monomers in the vesicles and conducted the electron-transport experiments in that concentration range.

The organic solvents and other reagents were spectrosol or special grade (Wako or Dojindo). Methylviologen (MV^{2+}) from Nakarai and DPPC from Sigma were purchased and used without further purification. The ion-exchanged distilled water was used and Tris-Cl buffer (pH 7.50) was prepared.[6] MgTPyP was synthesized from its free base from Strem and purified by column chromatography.[7] Stock solutions of Mg-P^{3+} (CH_3OH), Mg-P ($CHCl_3$), MgTPyP (CH_3OH - $CHCl_3$, 1 : 1), and DPPC (C_2H_5OH) were prepared. In order to analyze the concentration dependence of the absorption spectra, the experiments

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** 5,10,15-tris(1-methylpyridinium-4-yl)-20-[4-(octadecyloxy)phenyl]porphinatomagnesium trichloride

*** 5,10,15-tris(4-pyridyl)-20-[4-(octadecyloxy)phenyl]porphinatomagnesium

**** Dipalmitoyl L-α-phosphatidylcholine

***** 5,10,15,20-tetrakis(4-pyridyl)porphinatomagnesium

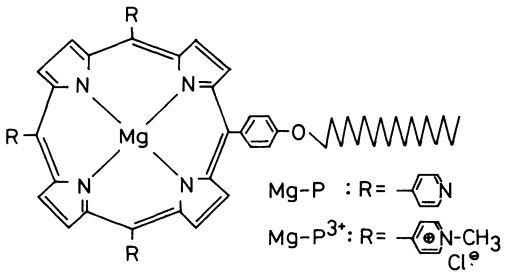

Fig. 1 Synthetic magnesium porphyrins

were conducted as follows. Aliquots of the porphyrin and DPPC stock solutions were mixed in a glass tube and dried *in vacuo* for 2 h. Tris-Cl (4 cm^3) was added to this sample and the sonication was operated for 5 - 10 min at 60 °C (Ohtake 5200 , 100 W). The molar ratio [f = porphyrin/DPPC] was changed by altering the amount of the porphyrin, and the final concentration of DPPC was *ca.* 3.34×10^{-4} M in all samples. Absorption spectra were measured at 25 °C using a Hitachi 340 spectrometer with a 1 cm (or 1 mm) path-length quartz cell. The background due to the vesicles was obtained from the spectra of the dispersion without porphyrin and this value was subtracted from the observed absorbances. The procedures of the study of the electron transport reactions were similar to those for the zinc porphyrin complex.[6] EDTA 2Na (0.02 M) was entrapped in the vesicles and MV^{2+} (2.0×10^{-3} M) was dissolved in the outer aqueous phase of the vesicles. The sample dispersion was bubbled with argon for 15 min in the dark and illuminated with a 750 W tungsten lamp through an IRA-25S filter.

Concentration Dependence of the Absorption Spectra[5,8]

The spectral changes in the f-value of 1/300 through 1/10 are shown in Figs. 2, 3, and 4. The shape of the absorption spectra of the $Mg\text{-}P^{3+}$ vesicles did not change greatly, but the half-height width of the Soret band at 442 nm increased from 34 nm to 38 nm and the peak shifted to 438 nm in the f-value over 1/20. In the Mg-P vesicles, the half-height width of the Soret band at 426 nm gradually changed from 13 nm (f = 1/300) to 34 nm (f = 1/14). A shoulder (*ca.* 444 nm) also appeared in the Soret band concomitant with increasing of the amount of porphyrin. But in the vesicles containing MgTPyP which has no long alkyl chain in the porphyrin ring, the marked spectral change was observed. Over the f-value of *ca.* 1/50, the Soret band splitted into two components (426 and 462 nm).[9] These changes are considered to be due to the different orientations of the porphyrin ring in the vesicle. The absorbances at the Soret band were plotted against the f-value and the range of f where the spectral data obey the Lambert-Beer's law was studied (Fig. 5). This law is obeyed almost completely in

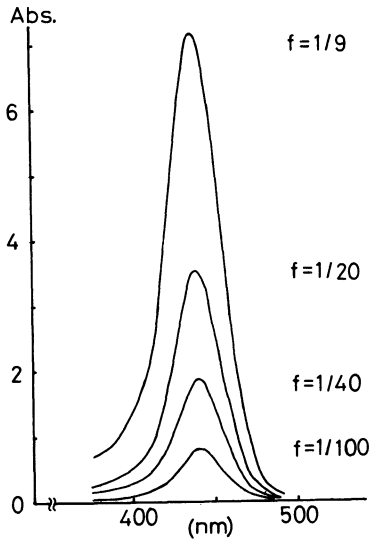

Fig. 2 $Mg\text{-}P^{3+}$ vesicles

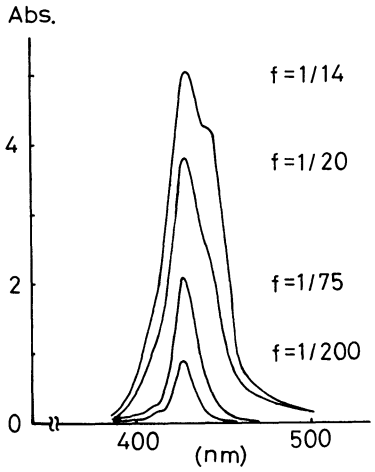

Fig. 3 Mg-P vesicles

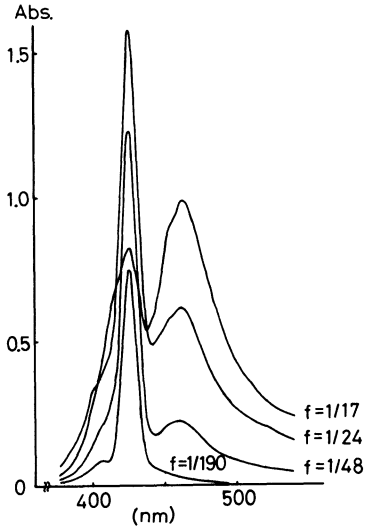

Fig. 4 MgTPyP vesicles

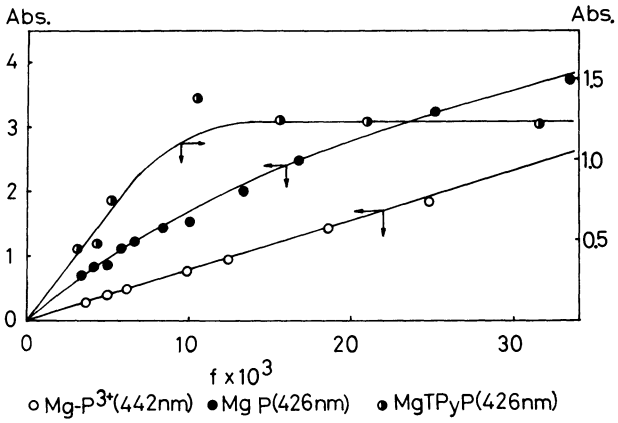

Fig. 5 Plots of abs. vs. f

the $Mg\text{-}P^{3+}$ vesicles, but only partially in other two cases. In the MgTPyP vesicles, this law holds in the low range of f but the absorbance becomes constant over the f-value of ca. 1/70. The molar extinction coefficients at the Soret band in the vesicles (ε) are calculated to be 2.30×10^5 $M^{-1}cm^{-1}$ ($f < 1/50$, correlation coefficient is 0.999) for the $Mg\text{-}P^{3+}$ vesicles, 4.63×10^5 $M^{-1}cm^{-1}$ ($f < 1/100$, 0.991) for the Mg-P vesicles, and 3.94×10^5 $M^{-1}cm^{-1}$ ($f < 1/100$, 0.992) for the MgTPyP vesicles. In order to study these phenomena in detail, A/f (where A is the absorbance of the porphyrin at the Soret band) are plotted against f (Fig. 6). If the porphyrin molecules dissolve homogeneously in the lipid phase of the vesicles, A/f should be parallel to the f-axis. This was observed in the $Mg\text{-}P^{3+}$ vesicles ($f <$ ca. 1/40) and the MgTPyP vesicles ($f <$ ca. 1/160). To investigate whether the decrease of A/f is due to the exclusion of the excess porphyrin molecules into the aqueous phase, the dispersions showing the spectral changes were gel-filtered with Sephadex G-100. This experiment showed that the amount of the porphyrin in the vesicles was almost the same before and after the gel-filtration for the Mg-P and MgTPyP vesicles but that the amount decreased in the $Mg\text{-}P^{3+}$ vesicles.

$Mg\text{-}P^{3+}$ vesicles Because of the high solubility of $Mg\text{-}P^{3+}$ in water,[10] excess $Mg\text{-}P^{3+}$ molecules are considered to be transferred into the aqueous phase over the f-value of ca. 1/40. As ε in the vesicles is similar to that in dimethyl sulfoxide (DMSO) (2.29×10^5 $M^{-1}cm^{-1}$), the porphyrin ring may exist in the neighborhood of the Stern phase. The solubility limit of $Mg\text{-}P^{3+}$ is calculated to be f = 1/24 from the extraporation of two straight lines ($f > 1/20$ and $f < 1/50$).[5]

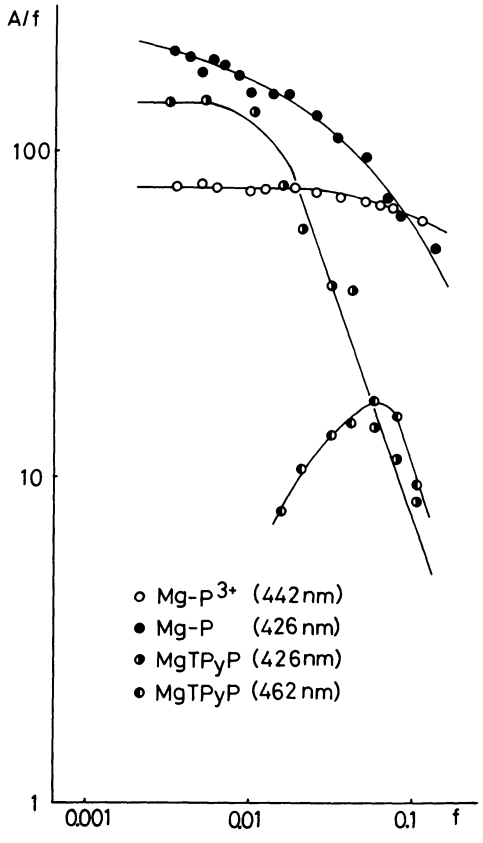

Fig. 6 Plots of A/f vs. f

MgTPyP vesicles The A/f at 426 nm is proportional to the reciprocal of f over the f-value of ca. 1/60 and A/f at 462 nm takes the maximum at the f-value of ca. 1/17. These results indicate that MgTPyP molecules are solubilized and aggregate in the lipid phase up to the f-value of ca. 1/17, but that over this value excess MgTPyP molecules precipitate within the MgTPyP domain on the vesicles.[5]

Mg-P vesicles The porphyrin ring is assumed to exist in the neighborhood of the membrane surface by the aid of the long alkyl chain, because the spectra of the porphyrin in the vesicle resemble that in DMSO (5.04×10^5 $M^{-1}cm^{-1}$). The appearance of a shoulder in the Soret band and the reduction of ε when the f-value increased may be accounted for by the interaction between the porphyrin rings.[9] The smaller gradient of the curve of A/f vs. f as compared to that of the MgTPyP may be due to the long alkyl chain which makes the porphyrin more soluble into the lipid phase. In the non-polar solvents such as benzene, the Soret band of Mg-P splitted (a peak appeared at 444 nm) in the concentration range where the band of MgTPyP did not change. When trace amount of pyridine (v/v 0.13%) was added to the benzene solution of Mg-P,[11] the absorption spectrum changed into the one similar to the

spectrum of DMSO solution showing isosbestic points. This result suggests that the long alkyl chain causes the porphyrin ring to aggregate. But in the case of Mg-P^{3+}, the intermolecular coulombic repulsion of the pyridinium groups may hinder the aggregation of the porphyrins.

Trans-membrane Electron Transport

The difference of the catalytic abilities of Mg-P^{3+} and Mg-P in the trans-membrane electron transport was studied from the standpoint of the difference of the polarity in the porphyrin ring. After the illumination for 30 min, the concentrations of the MV^{+} were analyzed quantitatively by measuring the absorbance at 393 nm [12] to be (4.6 ± 0.28) x 10^{-5} M for the Mg-P^{3+} vesicles and (0.16 ± 0.06) x 10^{-5} M for the Mg-P vesicles (f = ca. 1/120; both porphyrins do not aggregate in this concentration (vide supra)). In the Mg-P vesicles, a new peak at 410 nm appeared by illumination. In the Mg-P^{3+} vesicles, a new peak at 416 nm was observed by illumination in the absence of EDTA·2Na. These new peaks are assigned to the cation radicals of the porphyrins by referring to the spectra of zinc tetraphenylporphyrin and its cation radical.[13] Therefore the reactions are interpreted in terms of the concerted two step mechanism proposed by Matsuo.[2] The concentration depencence of the absorption spectra indicates that the porphyrin molecules exist as monomers in the reactions, and the catalytic difference may not be due to the difference of aggregation of the porphyrins. Previously we suggested that the orientation of the porphyrin ring in the membrane affected the electron transport reaction greatly in the asymmetrical vesicles containing the zinc porphyrins.[14] Similar arguments may be applied for the present experiments, namely, Mg-P^{3+} moleculaes exist in the more polar region of the membrane than that where Mg-P molecules exist because of the hydrophilicity of the porphyrin ring due to three pyridinium groups and this facilitates Mg-P^{3+} to transport electrons from the electron donor to the acceptor.

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