Synthesis and Characterization of Water-Soluble/Phospholipid Bilayer Active, Polymer-Linked Porphyrins†

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ABSTRACT: The synthesis of porphyrins covalently linked to polyethylenimine (PEI) was accomplished by reacting an activated 5-(4-carboxyphenyl)-10,15,20-tri-*p*-tolylporphyrin either directly with PEI or after first attaching an ω-amino acid of selected length. To introduce a linking chain between PEI and the porphyrin moiety, aminoaliphatic acid methyl esters were reacted with the acid chloride of the above porphyrin and the products were purified by column chromatography on silica gel followed by preparative thin-layer chromatography. Base-catalyzed hydrolysis of the methyl ester derivative gave the free acid. The carboxy functionality was then converted to the acid chloride by reaction with thionyl chloride followed by reaction with PEI in chloroform to give a nearly quantitative yield of the PEI-linked porphyrin. The polymer-linked porphyrins were purified by gel exclusion chromatography. Characterization and verification of structures were done by using NMR, UV/vis absorbance, and fluorescence emission spectroscopy. The resulting complexes are of considerable interest in model studies as the porphyrin portion readily inserts into lipid bilayer systems.

Introduction

Our laboratory has been involved in the synthesis of porphyrin complexes both as models for the primary photochemical events of photosynthesis[1-6] and also as models for secondary electron transport.[6,7] In pursuit of this goal we have synthesized covalently linked porphyrin dimers and trimers[1,2] as well as covalently linked porphyrin–quinone complexes.[3-5] One of the limitations of our previously synthesized model complexes, as well as those synthesized by others,[8-16] has been their limited solubility in water.

In this paper we report the preparation of water-soluble polyethylenimine (PEI)-linked porphyrin complexes and characterize their interaction with lipid bilayers. We reasoned that the highly charged PEI polymer (at pH 7.0) would confer water solubility upon the complex, while the hydrophobic porphyrin, covalently attached to the polymer via an ω-amino aliphatic acid, should readily insert into the lipid bilayer. Our ideas as to how they might then be utilized in our model studies are shown schematically in Figure 1. For the reaction center model, if polyethylenimine-anchored zinc porphyrins with variable-length linkages are prepared, the optimal distance for light-induced charge separation between these zinc porphyrins and surface-bound quinone derivatives on the opposite side of the bilayer may be evaluated. In the model system for electron transport, anchored polyethylenimine manganese porphyrins will be useful to evaluate the maximal distance an electron may travel between such centers within the bilayer.

Polyethylenimine can be prepared by polymerization of ethylenimine to give a highly branched rather than a linear macromolecule. Approximately 25% of nitrogens are primary amines, 50% secondary, and 25% tertiary. The molecular weight of polyethylenimine used in these studies was 1800 and thus the number of ethylenimine units in the product was about 43. The primary amine groups form a very suitable locus for the attachment of apolar groups to the polymer.[17] Thus, a wide range of local environments can be created on this water-soluble macromolecule. Furthermore, this polymer has good solubility properties in several solvents. This type of design flexibility is very useful to study model systems in vitro.[18]

Methods and Materials

All reactions and chromatographic separations were carried out in minimum room light. Benzene, chloroform, and pyridine were distilled and stored over molecular sieves. Other solvents used were spectral grade or better quality. Manganese(II) 2,4-pentanedionate and zinc acetate were obtained from Ventron Co. (Alfa Division) and Fisher Scientific Co., respectively. The silica gel used for dry column chromatography was Woelm Silica TSC, activity III/30 mm obtained from ICN Pharmaceuticals. Analtech silica gel GF Uniplates of 1000- and 250-μm thickness were used for preparative and analytical thin-layer chromatography, respectively. All analytical samples of porphyrin analogues synthesized were recrystallized and dried in vacuo at 70 °C for 24 h.

PEI-18, a polyethylenimine derivative with an average molecular weight of about 1800 was a gift from Dr. I. M. Klotz of the Department of Chemistry, Northwestern University (originally purchased from Dow Chemical Co.). This material was purified by ultrafiltration in an Amicon Diaflo ultrafiltration apparatus, using an Amicon UM-2 membrane. The primary amine content of PEI-18 was 25% by the TNBS (trinitrobenzene sulfonic acid) method.[21]

Nuclear Magnetic Resonance Spectra. NMR spectra were taken with a Hitachi Perkin-Elmer R20-B instrument, operating at 60 MHz with a probe temperature of 35 °C. Samples were prepared at a concentration of approximately 5% by weight in $CDCl_3$ (99.8% D, Aldrich Chemical Co.) or in D_2O (99.96% D, Aldrich Chemical Co.). Tetramethylsilane was used as internal standard.

UV and Visible Spectra. Absorption spectra were taken with either a Cary 14 or a Cary 219 recording spectrophotometer. Solutions were prepared in 0.01 M Bistris buffer solution (pH 7.0), CH_2Cl_2 containing 10% ethanol, and egg yolk PC vesicles in 0.01 M potassium phosphate dibasic buffer containing 0.1 M KCl (pH 7.0). All measurements were at room temperature.

Fluorescence Emission Spectra. Corrected fluorescence emission spectra were taken with an SLM 4800 spectrofluorometer. All the emision spectra were also corrected for the contribution of the polymer backbone measured as a control with PEI-18 in each solution used. Excitation was usually at the wavelength of the Soret band which was adjusted to an absorbance of 0.20 at the respective maxima. The absorption spectra were found to be unchanged after conducting the fluorescence measurements.

Measurement of Fluorescence Decay Kinetics. The fluorescence decays were measured with a laser-based, time-correlated photon counting technique. The method and sample handling were very similar to our previously published work on porphyrins.[5] With the tunable, 3-ps duration laser excitation source, accurate measurements of lifetimes as short as 50 ps were possible using computer deconvolution calculations. The calcu-

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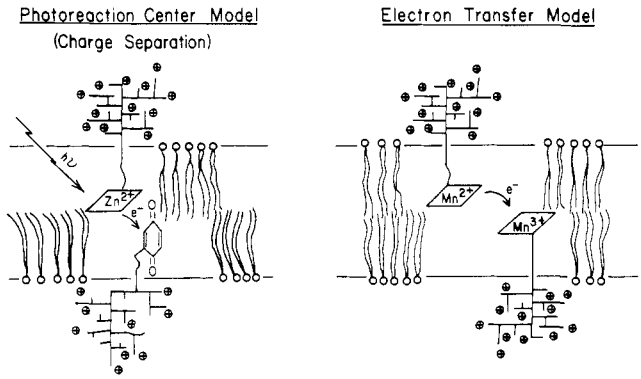

Figure 1. Schematic representation of proposed model for reaction centers of photosynthetic systems (left) or electron transfer (right). In the reaction center scheme a zinc porphyrin is shown anchored to one surface of a lipid bilayer by covalent attachment to the cationic polymer while a 1,4-benzoquinone is anchored to the other surface of the lipid bilayer, also by covalent attachment to a cationic polymer. In the electron-transfer scheme a Mn(II) porphyrin is anchored to one surface of the lipid bilayer by covalent attachment to a cationic porphyrin while a Mn(III) porphyrin is anchored to the other side. The length of the attachment arm can be varied in each case.

lations allowed us to easily analyze for nonexponential decay kinetics which might include components of very short duration. The accuracies of the experimental results are within 2–5%, depending on the quality of the exponential fit. It was observed that the PEI–porphyrin derivatives were much more readily dissolved in water when imidazole (0.01 M) or Bistris (0.01 M) were used as buffers in place of potassium phosphate (0.01 M, 0.1 M KCl). Therefore, these buffers were used for all the studies reported here.

Preparation of PC Vesicles Containing PEI-Linked Porphyrins. Liposomes containing PEI-linked porphyrin derivatives were prepared by previously reported methods for incorporating metalloporphyrins into egg yolk phosphatidylcholine vesicles.[7] For an example, egg yolk phosphatidylcholine (type III-E, Sigma Chemical Co.) (100 mg) in 1 mL of hexane and a polymer-linked porphyrin derivative (1 mg) in 5 mL of CH_2Cl_2–10% ethanol solution were mixed and evaporated onto the glass surface of a 100-mL round-bottom flask. The lipid containing the polymer was removed from the glass surface by suspending in 3 mL of aqueous buffer. The sample was then sonicated for 8 min (Branson Sonifier, standard tip, power = 3, 4 °C) to produce unilamellar liposomes and then centrifuged at 140000*g* for 30 min. When the sonication had been efficient, only a very small pellet formed (typically, less than 5% of the total material). The samples were generally composed of a phospholipid to polymer–porphyrin weight ratio of 200:1 or greater.

Synthetic Procedures

12-Aminododecanoic acid methyl ester and 6-aminocaproic acid methyl ester monohydrochloride were prepared by a literature method (mp 159–160 °C and mp 121–123 °C, respectively).[19,20] 5-(4-Carboxyphenyl)-10,15,20-tri-*p*-tolylporphyrin ((TTPP)-COOH, I) and the acid chloride II were prepared as described previously.[1,4,22,23]

5-[4-(((5-Carboxypentyl)amino)carbonyl)phenyl]-10,15,20-tri-*p*-tolylporphyrin, (TTPP)CONH$(CH_2)_5$COOH (III). (TTPP)COOH (I, 0.2 g) was dissolved in a mixture of benzene (100 mL) and thionyl chloride (12 mL). The solution was brought to reflux for 3 h and the solvent removed under reduced pressure. The acid chloride II was redissolved in benzene (30 mL) and once again taken to dryness under reduced pressure to remove traces of thionyl chloride. The acid chloride was redissolved in $CHCl_3$ (30 mL) and added dropwise to a solution of 6-aminocaproic acid methyl ester (52 mg) in $CHCl_3$ (20 mL) and pyridine (10 mL). The resulting solution was refluxed overnight and the reaction quenched by addition of water (60 mL). The chloroform layer was then separated and dried over magnesium sulfate. The chloroform was removed under reduced pressure and the sample purified by silica gel chromatography with 3–4% acetone in chloroform as eluent. The yield of the methyl ester of III, 5-[4-(((5-carbomethoxypentyl)amino)-carbonyl)phenyl]-10,15,20-tri-*p*-tolylporphyrin (IV), was 50% (117 mg).

^{1}H NMR (deuteriochloroform): δ 9.00–8.75 (m, 8 H, β-pyrrole), 8.2–7.95 (AB quartet, 4 H, carbomethoxyphenyl-2,3,5,6 and d, 6 H, tolyl-2,6), 7.6–7.4 (d, 6 H, tolyl-3,5), 3.6 (s, 3 H, OCH_3), 3.5–3.2 (m, 2 H, CCH_2CO), 2.6 (s, 9 H, CH_3), 2.5–2.1 (m, 2 H, CCH_2NH, 1.7–1.2 (m, 6 H, CCH_2C), –2.65 (s, 2 H, pyrrole NH).

The methyl ester derivative IV (107 mg) was dissolved in THF (20 mL) and 2 N KOH in H_2O (13 mL) added. The mixture was stirred at room temperature overnight in the dark. The cooled solution was then diluted with water (60 mL) and acidified with 2 N HCl (13 mL). A purple precipitate appeared and was extracted into chloroform (20 mL). The combined extracts were washed twice with water and dried over $MgSO_4$, and the solvent was removed under reduced pressure. The sample was purified by dry column silica gel chromatography with 10% pyridine–benzene as eluent. The yield of III was 81% (75 mg).

Anal. Calcd for $C_{54}H_{47}N_5O_3$: C, 79.68; H, 5.82; N, 8.60. Found: C, 79.03; H, 5.50; N, 8.21.

5-[4-(((11-Carboxyundecyl)amino)carbonyl)phenyl]-10,15,20-tri-*p*-tolylporphyrin, (TTPP)CONH$(CH_2)_{11}$COOH (V). This derivative was prepared in a similar way as that described for III; that is, the acid chloride II was reacted with 12-aminododecanoic acid methyl ester. The yield of the methyl ester of V, 5-[4-(((11-carbomethoxyundecyl)amino)carbonyl)-phenyl]-10,15,20-tri-*p*-tolylporphyrin (VI), was 33% (42 mg).

^{1}H NMR (deuteriochloroform): δ 9.05–8.80 (m, 8 H, β-pyrrole), 8.3–8.0 (AB quartet, 4 H, carbomethoxyphenyl-2,3,5,6 and d, 6 H, tolyl-2,6), 7.75–7.50 (d, 6 H, tolyl-3,5), 3.65 (s, 3 H, OCH_3), 3.6–3.4 (m, 2 H, CCH_2CO), 2.65 (s, 9 H, CH_3), 2.45–2.25 (m, 2 H, $NHCH_2C$), 1.8–1.2 (m, 18 H, CCH_2C), –2.8 (s, 2 H, pyrrole NH).

The methyl ester derivative VI (40 mg) was hydrolyzed with 2 H KOH (aqueous) in THF. The yield of V was 51% (20 mg).

Anal. Calcd for $C_{60}H_{59}N_5O_3$: C, 80.24; H, 6.62; N, 7.80. Found: C, 79.66; H, 6.42; N, 7.12.

PEI-TTPP (IX). The acid chlorides VII and VIII were prepared by dissolving III or V, respectively, in a mixture of chloroform and thionyl chloride in a similar way as that described for II.[1] The acid chloride was then used without further purification.

A solution of 0.2 g of (TTPP)COCl (II) in chloroform (15 mL) was added to PEI-18 (0.11 g) in chloroform (15 mL) at room temperature and stirred overnight. The modified polymer was isolated by passage of the reaction mixture through Sephadex LH-20, using absolute ethanol as eluent. The polymer-containing solution was dialyzed against 30% ethanol–water and then against water, using an Amicon Diaflo apparatus with a UM-2 membrane. The yield was generally over 90% on the basis of the weight of PEI-18 starting material. The aqueous solution was frozen and lyophilized to dryness. Integration of the proton magnetic resonance (^{1}H NMR) spectrum of the product in $CDCl_3$ indicated 1.11 mol of porphyrin per mol of polymer. Thus, the modified polymer may be represented by the stoichiometric formula; $(C_2H_4N)_m(TTPP)_{0.026m}$ where m = 42.86 or PEI(NHCO-(TTPP))$_{1.11}$.

PEI-C_5-TTPP (X) and PEI-C_{11}-TTPP (XI). These PEI derivatives were prepared in a similar way as that described for IX; that is, PEI-18 was reacted with the acid chloride VII or VIII in chloroform. Integration of the ^{1}H NMR spectrum of X or XI in $CDCl_3$ indicated the following stoichiometric formula: X, $(C_2H_4N)_m(TTPP)_{0.029}$ where m = 42.86 or PEI[NHCO-$(CH_2)_5$NHCO(TTPP)]$_{1.23}$; XI, $(C_2H_4N)_m(TTPP)_{0.030}$ where m = 42.86 or PEI[NHCO$(CH_2)_{11}$NHCO(TTPP)]$_{1.29}$.

Zinc Complex of PEI-Linked Porphyrins: PEI-Zn(TTPP) (XII), PEI-C_5-Zn(TTPP) (XIII), and PEI-C_{11}-Zn(TTPP) (XIV). For an example, the synthesis of XII will be described. Zinc acetate (45 mg) in methanol (4 mL) was added with stirring to a solution of IX (44 mg) in chloroform–dichloromethane (1:2.5) (35 mL). The solution was refluxed for 4 h. Progress of conversion to the zinc complex was followed by visible spectroscopy. The zinc complexes were purified by the method described for IX.

Manganese Complex of PEI-Linked Porphyrins: PEI-Mn(TTPP) (XV), PEI-C_5-Mn(TTPP) (XVI), and PEI-C_{11}-Mn(TTPP) (XVII). As an example, the synthesis of XV will be described. Manganese(II) 2,4-pentanedionate (35 mg) in pyridine (15 mL) was added with stirring to a solution of IX (45

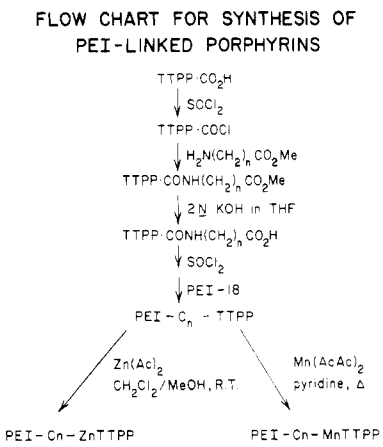

Figure 2. Outline of methods used for synthesis of PEI-linked porphyrin derivatives.

mg) in chloroform-methanol (1:1) (14 mL). The solution was refluxed overnight. A quantitative conversion of manganese complex was confirmed by visible spectroscopy. The manganese complexes were purified by the method described for IX.

Preparation of Uncharged PEI-TTPP (XVIII). PEI-TTPP (IX) (5 mg) was dissolved in a mixture of methanol (5 mL) and 10% NH_4OH (2 mL). The resulting solution was evaporated under reduced pressure to remove the methanol and then freeze-dried.

Results and Discussion

Polyethylenimine (PEI)-linked porphyrin and metalloporphyrin derivatives were prepared as outlined in Figure 2. The porphyrin moiety employed was 5-(4-carboxyphenyl)-10,15,20-tri-*p*-tolylporphyrin ((TTPP)COOH, I) and PEI of molecular weight 1800 (PEI-18) was covalently bound to the porphyrin monocarboxy functional group via an amide linkage to a primary amino group of the polymer. An intervening linking chain can be used to connect I with the PEI polymer. (TTPP)COOH (I) was synthesized by the porphyrin condensation reaction with *p*-tolualdehyde, *p*-carboxybenzaldehyde, and pyrrole according to a useful method reported previously by this laboratory.[4,22,23] Porphyrin I was esterified with diazomethane and purified as the methyl ester. The carboxyporphyrin was then obtained in good yield by base-catalyzed hydrolysis of the carbomethoxyporphyrin and subsequent acidification. To introduce the linking chain between PEI and the porphyrin moiety, aminoaliphatic acid methyl esters were reacted with the acid chloride of I and the products were purified by column chromatography on silica gel followed by preparative thin-layer chromatography. The resulting compounds, (TTPP)$CONH(CH_2)_nCOOMe$, were characterized by ^1H NMR. Base-catalyzed hydrolysis of (TTPP)$CONH(CH_2)_nCOOMe$ in an aqueous potassium hydroxide/tetrahydrofuran solvent system followed by acidification gave the free carboxyporphyrin (TTPP)-$CONH(CH_2)_nCOOH$. The porphyrin carboxy functionality was converted to the acid chloride by reaction with thionyl chloride followed by reaction with PEI in organic solvent. The linkage reactions between PEI and the acid chloride of the tetraarylporphyrins were almost quantitative at room temperature as indicated by thin-layer chromatography. The polymer-linked porphyrins were purified by gel exclusion chromatography on Sephadex LH-20 with ethanol as the solvent, followed by dialysis against 30% ethanol–water and then water. These complexes are very soluble in water at pH 7 since PEI-18 has an average of 10 cationic charges per molecule. Characterization and verification of structures were done by means of NMR, UV–visible absorbance, and fluorescence emission spectroscopy. The NMR spectrum of PEI-linked porphyrin X, as an example, is shown in Figure 3. The chemical shifts for all peaks were as expected and integration of NMR spectra indicated slightly more than one porphyrin group per polymer.

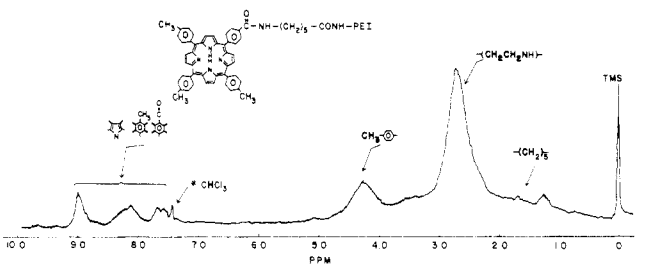

Figure 3. ^1H NMR spectrum for PEI-C_5-TTPP in deuteriochloroform.

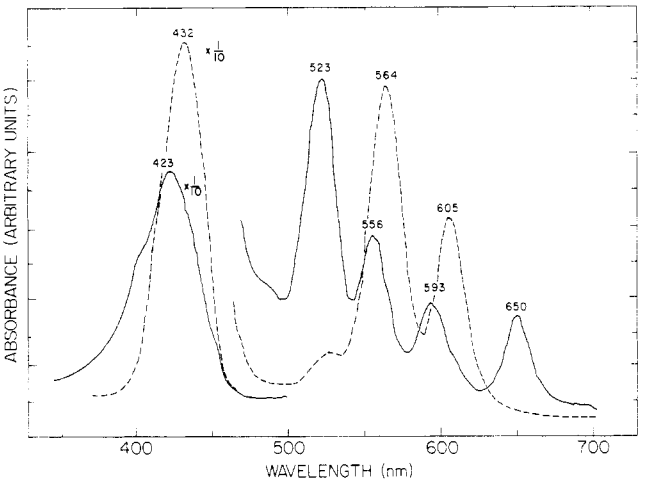

Figure 4. Absorbance spectra of PEI-TTPP (solid line) and PEI-Zn(TTPP) (dashed line) in 0.01 M Bistris buffer, pH 7.0, at room temperature. Extinction coefficients are given in Table I.

Zinc acetate and manganese pentanedionate were used to prepare the zinc- or manganese-substituted PEI-linked porphyrin derivatives. Proof of incorporation was obtained by visible spectroscopy. Typical examples of the visible spectra of the polymer-linked porphyrin or metalloporphyrin derivatives in water are shown in Figure 4 (complexes IX and XII). The UV–visible absorbance spectra in aqueous solution reveal characteristic porphyrin or metalloporphyrin absorbances. The data for the PEI-linked porphyrin or metalloporphyrin derivatives agree well with previous spectral data for tetraphenylporphyrin and zinc(II) tetraphenylphorphyrin.[1-3] One difference observed with the polymer derivatives is that the molar extinction coefficients may be slightly lower than those of the corresponding free porphyrins (see Table I), possibly due to the effect of the PEI backbone or to aggregation to form micelles.

The visible spectra of the polymer-linked porphyrin complex IX in water and in vesicles are also compared in Table I. We believe the slight red shift of the λ_{max} values in water in comparison to those in vesicles is real but close to experimental error. A large decrease in the molar absorptivity of the band near 420 nm (the Soret band) can be seen in water in comparison to those in vesicles. By contrast, the spectrum of IX in CH_2Cl_2–10% EtOH was found to be more nearly like that in the vesicles (see Table I). Thus, the absorbance spectra data indicate that when the PEI-linked porphyrin complexes are added to vesicle suspensions, the environment of the porphyrin is more like that of an apolar environment, consistent with the bilayer

Table I
Spectral Properties[a]

PEI–porphyrin, derivatives	H_2O (Bistris) pH 7.0					CH_2Cl_2–10% EtOH					PC vesicle (phosphate) pH 7.0				
PEI-TTPP (IX)	650	593	556	523	423	646	590	552	516	419	648	592	552	516	420
	(2.1)	(2.4)	(3.9)	(7.3)	(49)	(2.4)	(2.7)	(4.4)	(8.5)	(149)	(1.9)	(2.4)	(4.3)	(7.6)	(132)
PEI-C_5-TTPP (X)	648	592	556	520	420	647	592	553	517	420	648	593	551	516	421
	(2.5)	(2.7)	(4.6)	(8.4)	(99)	(3.1)	(3.5)	(5.8)	(11)	(185)	(2.0)	(2.4)	(4.7)	(9.0)	(180)
PEI-C_{11}-TTPP (XI)	650	594	555	520	422	647	592	552	517	418	649	593	551	516	421
	(1.9)	(2.0)	(3.4)	(6.6)	(70)	(2.4)	(2.7)	(4.2)	(8.9)	(183)	(1.9)	(2.4)	(4.3)	(7.8)	(149)
PEI-Zn-TTPP (XII)	605	564			432	604	563			430	603	561			429
	(5.6)	(9.2)			(95)	(6.3)	(10.0)			(202)	(4.4)	(8.7)			(172)
PEI-C_5-Zn(TTPP) (XIII)	605	564			430	605	564			430	602	561			429
	(2.7)	(4.4)			(49)	(5.3)	(8.3)			(179)	(4.0)	(7.9)			(156)
PEI-C_{11}-Zn(TTPP) (XIV)	604	564			432	604	563			429	602	562			429
	(2.0)	(3.6)			(37)	(2.0)	(3.4)			(87)	(1.9)	(3.4)			(85)
PEI-TTPP[b] (XV)						647	591	552	517	419					
						(2.3)	(2.6)	(4.2)	(8.3)	(156)					
(TTPP)COOH (XVI)						648	594	553	516	419					
						(3.5)	(4.5)	(8.4)	(10.9)	(275)					

[a] The wavelengths listed are in nm. The values in parentheses are extinction coefficients per TTPP (mM^{-1} cm^{-1}) based on the determination of the weight fraction of each preparation due to porphyrin as determined by NMR. [b] Uncharged PEI (this sample was from the preparation of the covalently linked derivative before exposing it to pH 7 in water).

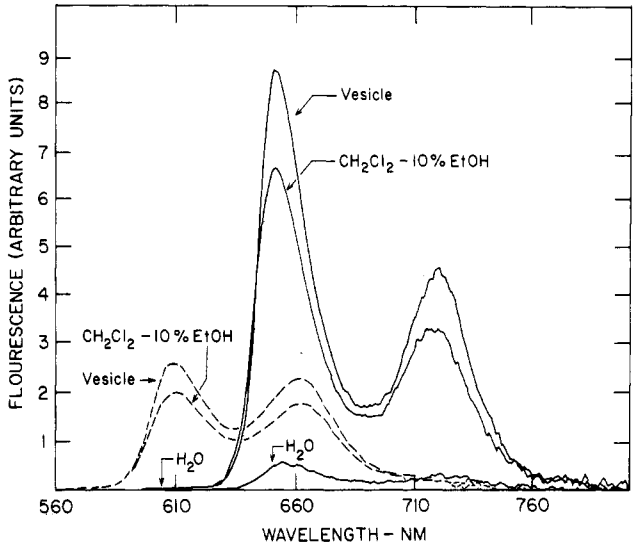

Figure 5. Corrected fluorescence emission spectra of PEI-C_5-TTPP (solid curves) and PEI-C_5-ZnTTPP (dashed curves) in water, CH_2Cl_2-10% ethanol and PC vesicles as indicated. Bistris buffer, pH 7.0, room temperature.

interior, than that of an aqueous environment. The spectroscopic behavior obtained for the rest of PEI-linked porphyrin and metalloporphyrin derivatives showed very similar trends to those just described.

Fluorescence emission spectra were measured for polymer-linked porphyrins and zinc porphyrin derivatives in water, CH_2Cl_2–10% EtOH and in vesicles. As an example, fluorescence emission spectra for X and XIII are shown in Figure 5. The solutions containing polymer-linked porphyrin derivatives were excited at wavelengths corresponding to the maxima of their respective Soret bands. As is apparent in Figure 5, the zinc porphyrin derivative (excitation 429 nm) gave an emission spectrum in vesicles with peaks at 610 and 670 nm, and the free base porphyrin derivative (excitation 420 nm) gave an emission spectrum in vesicles with peaks at 660 and 722 nm. The relative intensities of the two peaks in the emission spectra for the individual porphyrins were independent of the wavelength used for excitation. Table II summarizes relative fluorescence intensities for the emission bands for PEI-linked porphyrin and zinc porphyrin derivatives in water, CH_2Cl_2–10% EtOH, and vesicles. It can be seen that the highest fluorescence yields are observed in vesicles for both

Table II
Relative Fluorescence Yields[a]

PEI–porphyrin derivative	H_2O (Bistris) pH 7.0	CH_2Cl_2–10% EtOH	PC vesicles (phosphate) pH 7.0
PEI-TTPP	3.2	88	100
PEI-C_5-TTPP	7.3	85	100
PEI-C_{11}-TTPP	4.4	90	100
PEI-Zn(TTPP)	≤3	60	100
PEI-C_5-Zn(TTPP)	≤3	78	100
PEI-C_{11}-Zn(TTPP)	≤1	94	100

[a] Solutions of the porphyrin derivatives were adjusted to have equal absorbances of 0.20 at the Soret band λ_{max}. Values are normalized to the highest yields which were found for the vesicle samples and set at 100.

PEI-linked porphyrins and PEI-linked zinc porphyrins, again more nearly similar to the yield in CH_2Cl_2–10% EtOH. On the other hand, a very low fluorescence yield was observed in water. The data, therefore, imply that in the vesicle systems the porphyrin moiety is not at the surface in an aqueous environment but rather is immersed within the hydrophobic interior of membrane.

The kinetics of fluorescence decay can also be used to gain information on the environment of the porphyrin molecules. For example, while the fluorescence decay of PEI-C_{11}-TTPP in methylene chloride (containing 10% ethanol) appears to fit a simple exponential with a lifetime of 6.9 ns, that in water is shorter (2.4 ns), and the data often are not fit well with a single exponential. In comparison, PEI-C_{11}-TTPP added to liposomal solutions in water had a decay lifetime of 5.6 ns, and the data were well fit with a single exponential. Again, this strongly supports the conclusion that the porphyrin is immersed in the hydrocarbon region of the bilayer. It should be noted, however, that our measurements with shorter bridged complexes (PEI-TTPP and PEI-C_5-TTPP) when added to liposomes exhibited more complex decay behavior with lifetimes between those found in water and in methylene chloride.

Gel Filtration Experiments. To further examine the interaction of the PEI-linked porphyrin complexes with the lipid bilayer, we attempted to remove the polymer–porphyrin complexes from the external vesicle surface. The PEI–porphyrin complexes were first allowed to bind to the external surface of the bilayer of preformed vesicles. This was accomplished by simply mixing an aqueous solution of the polymer–porphyrin in 10 mM imidazole

buffer, pH 7.0 with a solution of vesicles. Proof of insertion into the bilayer was noted by the sharpening and increased intensity of the porphyrin Soret absorbance band, and an increase in fluorescence yield. A total of 2–3 mL of these externally loaded vesicles was then applied to a Sephadex G-50 column (45 cm × 1.0 cm i.d.), eluted with 10 mM imidazole buffer, pH 7.0, and the vesicle fraction was collected. The visible spectra of the vesicles were measured before and after gel filtration. In general, as would be expected, the PEI–porphyrin complexes in which the porphyrin was linked to the polymer with a long aliphatic linker were more tightly bound to the vesicles; only 10–20% PEI-C_{11}-TTPP was removed from the vesicle surface by gel filtration while PEI-TTPP was almost completely removed. Similar results were obtained with PEI-C_{11}-Mn(TTPP) and PEI-Mn(TTPP) with PEI-C_5-Mn(TTPP) giving an intermediate percent retention.

Conclusions

Our goals in synthesizing the PEI-linked porphyrin and metalloporphyrin complexes were to construct model systems which would have the following characteristics: (1) good solubility in water as well as in apolar solvents in monomeric form; (2) amphipathic properties such that the water soluble form would readily associate with the surface of lipid bilayers without diffusing across them; (3) unique physical properties such that characterization of behavior is facilitated. The complexes we have prepared meet these goals. Porphyrin complexes are particularly advantageous with regard to point 3 because of the variety of metals that may be complexed thus changing their photochemical, electrochemical, and spectroscopic properties without substantially changing their solubility properties (for example, their compatibility with lipid bilayers). Thus the fluorescence and triplet state properties of PEI-C_{11}-Zn(TTPP) (XIV) may be used to evaluate the likely location (that is, within the lipid bilayer) of the corresponding Mn derivative.

In addition, selection of structural properties so that the tolylmethyl hydrogen (porphyrin), ethylene hydrogen (PEI), and linker hydrogen (bridge) would have distinct locations when measured by ^{1}H NMR (Figure 3) allows this spectroscopic tool to be used both to verify the composition of the polymer–porphyrin and also to substantiate that the rings were not likely to be interacting (for example, as might be expected in a sandwich complex or intermolecular aggregation) in the vesicle systems used in this study.

Thus, appropriate analogues of these compounds are being utilized to systematically examine photochemical and electron transport activity in selected liposomal systems. Results of these studies will be reported elsewhere.

Registry No. I, 78265-42-6; II, 61449-64-7; III, 91879-40-2; IV, 91879-41-3; V, 91879-42-4; VI, 91879-43-5; VII, 91879-44-6; VIII, 91879-45-7; methyl 6-aminocaproate, 2780-89-4; methyl 12-aminodecanoate, 53005-24-6.

References and Notes

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