UV-Vis, Fluorescence, and EPR Studies of Porphyrins in Bilayers of Dioctadecyldimethylammonium Surfactants

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Received: February 8, 1994*

The incorporation characteristics of three simple tetraarylporphyrins into bilayers of dioctadecyldimethylammonium surfactants was studied by UV-vis, fluorescence, and EPR spectroscopy. The porphyrins used are tetraphenylporphyrin (TPP), tetrakis(4-(hexadecyloxy)phenyl)porphyrin (THPP), and tris(4-(hexadecyloxy)phenyl)(4-methylpyridinium)porphyrin tosylate (TrHPyP). At low porphyrin to surfactant molar ratios (<5 \times 10⁻⁴) the porphyrins show a strong fluorescence. Increasing this ratio to 5 \times 10⁻³ causes a change in the UV-vis spectra and a decrease of the fluorescence intensity. Time-resolved fluorescence measurements indicated that the latter is due to the formation of non-fluorescent porphyrin aggregates. The spectral characteristics of the porphyrin aggregates are discussed in terms of the formation of different types of aggregates. The location of the porphyrins within the bilayers was investigated by fluorescence quenching experiments using iodide, 9(10)-bromooctadecanoic acid, and 16-bromohexadecanoic acid. These experiments suggest that THPP is located in the middle of the bilayer and TrHPyP near the aqueous interface. For the parent compound TPP no well-defined position was found. EPR spectroscopy on cast films of dioctadecyldimethylammonium surfactants with the copper derivatives of the porphyrins incorporated (porphyrin-to-surfactant ratio = 5×10^{-3}) revealed a clear anisotropic distribution of the latter molecules in the cast bilayers. The angles between the porphyrin normals and the bilayer normal were determined by comparison of experiment with simulated spectra. In the case of THPP and TrHPyP these angles agree very well with an arrangement in which the long porphyrin axis lies parallel to the bilayer surface. The arrangement of TPP aggregates could not be established.

Introduction

The biomimetic chemistry of porphyrins is inspired by the central role of this molecule in important biological processes such as light energy conversion, oxygen transport, and catalysis. In these processes the porphyrin molecule has a variety of functions: it is involved in the process of charge separation, reversible binding of dioxygen, reductive activation of dioxygen, and electron transport. The diverse chemical and photophysical properties of porphyrins are partly the result of variations in the nature of the central metal ion. They are also due to the fact that these molecules are often located in very different environments. In the past a number of biomimetic systems have been described in which the properties of the porphyrin molecule are controlled by modification of this molecule and by changing the surrounding reaction medium.^{1,2}

Our interest is to study the biomimetic properties of synthetic porphyrins incorporated into micelles and lipid bilayers. It has been shown that incorporation of porphyrins in micelles dramatically alters the rate of metalation of these molecules.³ Porphyrins anchored to lipid bilayers have successfully been applied for reversible binding of dioxygen in aqueous solutions.⁴ The feature of supramolecular organization has been used to mimic the action of the cytochrome P_{450} enzyme system.⁵ In another biomimetic study a membrane-bound porphyrin has been used to achieve regioselective epoxidations.⁶ The electron carrier properties of porphyrins incorporated into synthetic membranes have been studied by several groups.⁷

It is clear that for a proper evaluation of the results of these studies knowledge of the location and orientation of the porphyrins in the lipid bilayer is essential. Furthermore, simple porphyrins are known to aggregate at higher concentrations which alters their catalytic and photophysical properties. In most studies CHART 1



assumptions on the aggregation and location of porphyrins in lipid membranes have been made based on a limited number of UV-vis, fluorescence,⁸⁻¹¹ or EPR^{6,15} measurements. More information is available about the orientation of porphyrins or heme proteins in bilayers. Various techniques like polarized UVvis, fluorescence,¹² and EPR spectroscopy^{6,15} in combination with computer simulations¹³⁻¹⁵ have been used. Although in these studies many properties of the porphyrin molecules in lipid bilayers are discussed, they do not give a complete description in terms of location, orientation, and aggregation of these molecules in the bilayers.

In this paper the incorporation characteristics of three tetraarylporphyrins in bilayers formed from the synthetic surfactant dioctadecyldimethylammonium chloride (DODAC) are reported. These porphyrins include 5,10,15,20-tetraphenylporphyrin (TPP), 5,10,15,20-tetrakis(4-(hexadecyloxy)phenyl)porphyrin (THPP), and 5,10,15-tris(4-(hexadecyloxy)phenyl)-20-(4-(*N*-methylpyridinium))porphyrin tosylate (TrHPyP, see Chart 1). Two of these porphyrins, THPP and TrHPyP, have hydrophobic substituents on the aryl groups, the latter has also a hydrophilic substituent. A combination of the above mentioned techniques is used to study the spatial distribution of these two porphyrins in the bilayers. The results are compared with those obtained for the parent compound tetraphenylporphyrin.

[•] Abstract published in Advance ACS Abstracts, April 15, 1994.



Figure 1. Incorporation efficiency ($R_{inc} = [\text{porphyrin incorporated}]/[DODAC]$) of TPP (\blacktriangle), THPP (\blacklozenge), and TrHPyP (\blacksquare) in DODAC vesicles. Incorporation was achieved by the sonication method. The dotted line is the theoretical curve for complete incorporation of the porphyrin initially present in the mixed film ($R_{add} = [\text{porphyrin added}]/[DODAC]$); [DODAC] = 5 mM, T = 50 °C.

Results

Incorporation. Upon dispersion in water DODAC forms welldefined bilayers with a gel to liquid-crystalline phase transition occurring at 37 °C.16 Since the porphyrins studied are insoluble in water, addition of these molecules to vesicular dispersions of DODAC did not result in incorporation into the bilayers, as was checked by centrifugation and gel permeation chromatography (GPC). Successful incorporation could however be achieved by first preparing a mixed film of the porphyrins and DODAC followed by dispersion of this film in water by sonication. Another procedure involved the injection of an ethanol/THF solution of the porphyrin and DODAC into water.^{17,18} Both methods resulted in the formation of opalescent solutions. In GPC experiments, DODAC vesicles with CuTPP incorporated have the same elution volume as vesicles without CuTPP incorporated. The waterinsoluble CuTPP did not elute at all, as was checked separately. The other porphyrin-DODAC dispersions showed similar elution profiles. In ultrafiltration experiments, the porphyrin-containing vesicle preparations were quantitatively retarded by membranes with cutoff values up to 100 000 MW. This indicates that incorporation of the porphyrins in the DODAC bilayers does not result in the formation of small micellar aggregates. Electron micrographs of the various preparations showed that closed spherical vesicles were present.

We observed differences in incorporation efficiency between the sonication and injection method. Figure 1 shows, for the sonication method, the amount of porphyrin that is incorporated after centrifugation of the samples, when the porphyrin to DODAC molar ratio (R) is increased. In the case of TPP only a small fraction of the porphyrin was solubilized. The maximum quantity of this molecule that could be incorporated amounted to R = 0.0004. Prolonged incubation of the samples did not increase the incorporation efficiency. THPP showed a similar behavior, but the maximum quantity incorporated into the bilayers was higher (R = 0.0008). TrHPyP could be incorporated up to R = 0.0036. These differences can be explained from the fact that THPP and TrHPyP contain long aliphatic substituents which increase their solubilities in the DODAC film. The presence of an additional positively charged substituent in TrHPyP makes this porphyrin almost completely soluble in the film.

With the injection method, no solubility limit was found over the concentration range studied, and all three porphyrins were quantitatively incorporated up to R = 0.005 (data not shown). The fact that the incorporation efficiency does not depend on the initial porphyrin to DODAC ratio makes the injection method more useful than the sonication method. The small fraction of



Figure 2. Absorption spectra of THPP in DODAC vesicles at R = 0.0005(A) and R = 0.005 (B); [porphyrin] = 1.8×10^{-6} M; T = 20 °C.

 TABLE 1: Spectroscopic Data of Porphyrins Incorporated in DODAC Bilayers

| porphyrin | R | abs | absorption spectrum ² λ_{max}/nm | | | | | | $\frac{fluorescence}{spectrum^b} \frac{\lambda_{max}/nm}{\lambda_{max}}$ | |
|-----------|--------|-----|---|-----|-----|-----|-----|-----|--|--|
| TPP | 0.0005 | 418 | | 514 | 546 | 591 | 648 | 652 | 717 | |
| | 0.005 | 420 | | 516 | 546 | 590 | 648 | 652 | 717 | |
| THPP | 0.0005 | 421 | | 516 | 552 | 592 | 650 | 653 | 716 | |
| | 0.005 | 402 | 436 | 517 | 554 | 592 | 650 | 653 | 716 | |
| TrHPyP | 0.0005 | 426 | | 520 | 556 | 594 | 652 | 660 | 720 | |
| • | 0.005 | 424 | | 520 | 557 | 595 | 652 | 660 | 720 | |

^a [porphyrin] = 10⁻⁶ M; T = 20 °C. ^b Excitation is at wavelengths corresponding to the B-band maxima of the UV-vis absorption spectra at R = 0.0005. [porphyrin] = 10⁻⁶ M; T = 50 °C.

solvent (1-2 vol %) that remained in the samples prepared by the injection method did not alter the results discussed in the following sections, as was checked separately by comparison with extensively dialyzed vesicle preparations.

Aggregation. In Figure 2 the visible absorption spectra of THPP incorporated into bilayers of DODAC vesicles at two porphyrin to lipid ratios are shown. At a low ratio $(R \le 5 \times 10^{-4})$ the spectrum has the same maxima as the spectrum of a homogeneous solution of the porphyrin in dichloromethane. Increasing the THPP concentration in the bilayer caused the absorption spectrum in the B-band region to change, whereas the Q-band region remained undisturbed (see also Table 1). Most notable is the splitting of the band at 421 nm into two new bands: one of lower intensity at 402 nm and one of higher intensity at 436 nm. For TPP and TrHPyP, the B-band maximum shifted to higher and lower wavelength, respectively, when R was increased. The Q-bands remained the same at higher R values (Table 1). Most likely, the observed changes are due to the formation of popphyrin dimers or higher aggregates. In the aggregates, a strong exciton coupling will exist between two neighboring porphyrins.^{19,20} The wavelength shifts due to this coupling are proportional to the oscillator strength of the respective transitions. Since the oscillator strengths of the B-bands are an order of magnitude greater than those of the Q-bands, this may explain why only the B-bands are disturbed. An alternative explanation, viz., that the porphyrins bind to regions of different polarity in the bilayer, cannot be excluded, although such a binding would have been expected to cause a shift of the absorption maxima of the Q-bands as well.^{21,22}

The changes in the absorption spectra were accompanied by a strong decrease of the fluorescence intensities (Figure 3), whereas no shifts of the emission wavelengths were observed (Table 1). This behavior also points to aggregation of the porphyrins in the bilayers when their concentrations are increased.^{9,19} Disposition of the porphyrins in regions of different polarity is unlikely, because



Figure 3. Self-quenching of the fluorescence of TPP (\diamond), THPP (\Box), and TrHPyP (Δ) in DODAC vesicles; I_0 is the fluorescence intensity at $R = 10^{-4}$; [porphyrin] = 10^{-6} M; T = 50 °C.

 TABLE 2:
 Fluorescence Lifetimes of Porphyrins in DODAC

 Bilayers
 Image: Comparison of Compar

| porphyrin | | lifetime ^a /ns | | |
|-----------|----------------------|---------------------------|-----------------|--|
| | benzene ^b | $R = 0.0005^{c}$ | $R = 0.005^{c}$ | |
| TPP | 12.0 | 11.2 | 11. | |
| THPP | 11.0 | 10.3 (0.87) | 11.0 (0.20) | |
| | | 1.6 (0.13) | 2.6 (0.80) | |
| TrHPyP | 8.4 | 8.6 | 8.5 | |

^a The number in parentheses is the preexponential factor, describing the relative contribution of the particular fluorescence process to the total fluorescence. ^b [porphyrin] = 5×10^{-7} M; T = 20 °C. ^c [porphyrin] = 10^{-6} M; T = 50 °C.

in that case the decrease of the fluorescence intensity should have been accompanied by a shift of the emission wavelength.

Time-resolved fluorescence measurements at low porphyrin concentration ($R = 5 \times 10^{-4}$) showed a monoexponential decay of the fluorescence for TPP and TrHPyP. The observed fluorescence lifetimes were similar to those of homogeneous solutions of the porphyrins in benzene (Table 2). At higher concentrations ($R = 5 \times 10^{-3}$), the fluorescence intensity decreased, but the decay was still monoexponential with the same lifetime. This indicates that the observed fluorescence originates from porphyrin molecules located in similar environments, most likely as monomers when the concentration is low. An increase in concentration leads to the formation of nonfluorescent species, probably aggregates of porphyrins.

A different behavior was observed for THPP. Both at low (R= 5 × 10⁻⁴) and high concentration ($R = 5 \times 10^{-3}$) of the porphyrin in the bilayer the fluorescence intensity decayed biexponentially with fluorescence lifetimes of approximately 11 and 2 ns, respectively (Table 2). The relative contributions of the two processes, however, changed. At low concentration the longer lifetime process dominated whereas at high concentration the shorter lifetime process was dominant. The observed lifetime of 11 ns is the same as the fluorescence lifetime of monomeric porphyrin dissolved in benzene. Furthermore, the excitation spectrum of THPP at high concentration is similar to the absorption spectrum of aggregated THPP. We may therefore conclude that the longer lived component originates from monomeric THPP and the shorter lived component from aggregates of this porphyrin. The fact that the behavior of THPP differs from that of TPP and TrHPyP can be explained by the formation of different types of aggregates for the three porphyrins. This issue will be discussed below. It should be noted that our fluorescence measurements were carried out above the phase transition temperature of the bilayers. Below this temperature the decays became multiexponential. This indicates that below the phase transition the three porphyrins are located in more heterogeneous environments.

The curve for TrHPyP in Figure 3 can well be described by a model that involves self-quenching within a complex of only dimers.²³ The association constant (K_a) for dimer formation was calculated to be 4.7×10^4 M⁻¹. In this calculation an overall (aqueous) concentration of the porphyrin was used. If this K_a value is corrected for the much higher local concentration of the porphyrin in the bilayer,²⁴ an association constant of approximately 254 M⁻¹ is obtained. For the quenching curves of TPP and THPP, the deviation from this dimer model was, especially at higher Rvalues, considerable. This suggests that higher aggregates are involved. The aggregation numbers for TPP and THPP were determined by a procedure described by Schick et al.²⁵ It involves the use of mixtures of zinc and copper porphyrins. In aggregates with one or more copper porphyrins the fluorescence of the zinc porphyrin is effectively quenched via intersystem crossing to a trip doublet state. Therefore, any observed fluorescence intensity originates from aggregates which contain only zinc porphyrins. Assuming a statistical distribution of zinc and copper porphyrins in the aggregates, the relative fluorescence intensity (I/I_0) is given by eq 1, in which I_0 is the fluorescence intensity of aggregates

$$I/I_0 = (n_{\rm Zn})^N \tag{1}$$

...

of pure zinc porphyrin, n_{Zn} is the mole fraction of zinc porphyrin, and N is the average aggregation number of the porphyrins. The experiments were carried out at a high porphyrin concentration $(R = 5 \times 10^{-3})$, thus almost all porphyrin molecules are aggregated. Plots of $\log(I/I_0)$ versus the logarithm of n_{Zn} were linear for both TPP and THPP. The aggregation numbers are given by the slopes of the plots and were found to be 3.5 ± 0.4 and 4.2 ± 0.3 for TPP and THPP, respectively. Wavelength shifts in aggregates of zinc or copper porphyrin showed similar trends as observed for the free-base porphyrins, indicating that aggregation is independent of the central metal ion.

Location. Quenching of the fluorescence of monomeric porphyrins in DODAC vesicles was studied with various hydrophilic and hydrophobic quenchers. This provided information on the location of the fluorophores within the bilayer.²⁶ As quenchers we used iodide ions and two brominated fatty acids, viz., 9(10)-bromooctadecanoic acid27 and 16-bromohexadecanoic acid. For comparison, we also performed experiments with hexadecanoic acid, which does not bear any quenching moiety. The iodide ion is strongly absorbed to the surface of the bilayer, due to strong electrostatic interaction with the quaternary ammonium head groups. This ion is therefore believed to quench only porphyrin molecules located near the aqueous interface. The fatty acids carry a bromine substituent either in the middle or at the end of the alkyl chain. Alignment of these alkyl chains with the amphiphile alkyl chains in the bilayer will result in positioning of the bromine substituents either halfway the aqueous interface and the center of the bilayer or near the center of the bilayer.²⁸ The quenching experiments were performed above the phase-transition temperature of the bilayers. Under this condition there is a fast lateral diffusion of the quenchers and the lipid molecules in the bilayer. Therefore, quenching of the fluorescence will be controlled by the relative position of the fluorophore and the quencher along the bilayer normal. Linear Stern-Volmer plots were obtained up to a quencher concentration of 0.2 mM (10% of the DODAC concentration). Above this concentration, precipitation of the surfactant and the porphyrin was observed in the case of the iodide. When the concentration of the brominated fatty acids was increased above 0.2 mM, the turbidity of the solutions decreased, most likely as the result of micellization of the bilayers.

The results of the quenching experiments are presented in Table 3. As can be seen the fluorescence of TrHPyP is strongly quenched by I^- , whereas the fluorescence of THPP is hardly affected by this ion. In contrast, the fluorescence of TrHPyP is hardly quenched by the brominated fatty acids. THPP on the other

TABLE 3: Uncorrected Stern-Volmer Quenching Constants (K_{SV}) for Various Porphyrins and Quencher Combinations inDODAC Bilayers^a

| | $K_{\rm SV}/{\rm M}^{-1}$ | | | | | |
|-----------|---------------------------|--------------------|---------------------|------------------|--|--|
| porphyrin | NaI | C9-Br ^b | C16-Br ^c | C16 ^d | | |
| TPP | 430 ± 67 | 156 ± 39 | 388 ± 35 | 64 ± 7 | | |
| THPP | 117 ± 25 | 78 ± 37 | 492 ± 144 | 130 ± 16 | | |
| TrHPyP | 1170 ± 62 | 60 ± 17 | 156 ± 3 | 92 ± 28 | | |

^a The K_{SV} quenching constants are not corrected for partitioning between the aqueous phase and the vesicles. The excitation and emission wavelengths are the B-band maxima and the emission wavelengths, respectively, given in Table 1 at R = 0.0005; [porphyrin] = 10⁻⁶ M; R= 0.0005; T = 50 °C. ^b 9(10)-Bromooctanoic acid. ^c 16-Bromohexadecanoic acid. ^d Hexadecanoic acid.



Figure 4. X-ray diffraction pattern of cast films of DODAM on a Mylar substrate (A) and a schematic representation of the packing of the amphiphilic molecules in the bilayers (B).

hand is effectively quenched by 16-bromohexadecanoic acid. Most likely, TrHPyP is located near the surface of the bilayer and THPP near the center of the bilayer. The less efficient quenching of TPP by iodide suggests that this porphyrin is situated in the hydrophobic part of the bilayer. Its position, however, is not well defined, as the two brominated fatty acids quench the fluorescence equally well.

Orientation. The orientation of the porphyrins in the bilayers was investigated by means of EPR spectroscopy on the copper containing derivatives incorporated in aligned bilayers of N,Ndioctadecyl-N,N-dimethylammonium methacrylate (DODAM). Aligned bilayers are easily obtained when a vesicular solution of the surfactant is left to dry out on a flat substrate such as glass or a synthetic film, e.g., Mylar.29 These so-called "cast bilayers" are more or less regular stacked with their planes parallel to the substrate surface. We were unable to obtain well-oriented structures from DODAC. This is probably due to poor binding of the chloride counterion to the positively charged bilayer surface of this amphiphile. As a result there will be strong electrostatic repulsion between the bilayers during the drying process which prevents regular stacking. When we used DODAM well-defined cast bilayers were obtained. Wide-angle X-ray diffraction showed the presence of a lamellar structure with a periodicity of 60 Å (Figure 4). The maximum molecular length of nonhydrated DODAM as estimated from CPK space-filling models amounts to approximately 27 Å. This value is in good agreement with the experimentally found periodicity of 60 Å for our bilayers. When the assumption is made that the hydration laver between two bilayers of DODAM does not exceed 6-8 Å, the tilt angle of the lipid molecules with the bilayer normal is smaller than 10°. Similar results have been reported for cast films prepared from the acrylate analogue of DODAM.³⁰ Scanning electron micrographs of our cast DODAM bilayers revealed that regularly stacked sheets were present with an orientation parallel to the surface (Figure 5). The thickness of the sheets is much larger than the thickness of a single bilayer, which means that each sheet consists of several bilayers.

The square-planar porphyrin ligand system around the copper-(II) ion gives rise to a strong anisotropy of both the **g** tensor and the copper hyperfine splitting tensor (A^{Cu}) . Incorporation





Figure 5. Scanning electron micrograph of a fractured cast film of DODAM (2000× magnification).



Figure 6. Coordinate systems for porphyrins and bilayers.

of the porphyrins in oriented bilayers and subsequent measurement of EPR spectra at various angles between these bilayers and the magnetic field will provide information on the orientation of the porphyrins.^{15,31} To describe this orientation, the coordinate system $(xyz)_p$ for the porphyrin molecule is defined as in Figure 6. In an independent coordinate system $(xyz)_b$ the bilayer normal is defined as the z axis and the plane of the bilayer as the xy plane (Figure 6). It is reasonable to suppose that there is no anisotropy of the porphyrins in the xy plane of the bilayers. Furthermore, we assume that the porphyrin molecules have a gaussian distribution σ around the angle θ between the bilayer normal (z_b) and the porphyrin normal (z_p) . The orientation of the porphyrins can now effectively be represented by $\theta \pm \sigma$.

The anisotropy of the EPR signal of a copper(II) porphyrin can be described in terms of g_x , g_y , g_z and A_x , A_y , A_z . EPR measurements on single crystals of CuTPP have revealed that the coordinate systems for the **g** and **A** tensors coincide with the coordinate system for the porphyrin molecule.³² Because of the 4-fold rotation axis in the porphyrin ligand the **g** and **A** tensors for the x and y direction are degenerate. They are denoted g_{\perp} and A_{\perp} , respectively. The g_z and A_z tensors are designated as g_{\parallel} and A_{\parallel} . Due to the coupling of the unpaired electron with the Cu nucleus (I = 3/2), g_{\parallel} is split into four lines with A_{\parallel} approximately 200 G. Since A_{\perp} (typically 20-30 G) is generally not resolved, g_{\perp} remains visible as a single line.

In the first series of experiments EPR spectra were recorded of the pure copper(II) porphyrins in a frozen CHCl₃ glass. These powder spectra of CuTPP and CuTHPP did not reveal any nitrogen hyperfine splitting, whereas in the powder spectrum of CuTrHPyP even the copper splitting was unresolved. However, upon magnetic dilution of the solutions with the corresponding zinc porphyrins or free-base porphyrins (ratio of copper porphyrin to zinc or free-base porphyrin = 0.1), both the copper and nitrogen hyperfine splittings became resolved. The loss of the nitrogen hyperfine splitting in the nondiluted samples is due to line broadening because of dipolar relaxation.^{33,34} This phenomenon can occur even at Cu-Cu distances up to 20 Å. This condition is met in very concentrated solutions or when aggregation of porphyrin molecules takes place. Although the g and A parameters can be measured directly from the experimental spectra, more accurate values are obtained when the spectra are simulated. Satisfactory simulations were obtained for the powder spectra of the three porphyrins. The optimal parameters are tabulated in Table 4. These parameters were used for the simulations of the spectra of the porphyrins in the oriented bilayers. Experimental spectra in which no nitrogen hyperfine splitting was observed, were simulated either with nitrogen hyperfine interaction and linewidth parameters in the order of 60 G or without nitrogen hyperfine interaction and larger linewidth parameters (up to 120G). Both approximations gave very similar results when the same values of the g and A^{Cu} tensors were used. The latter approximation however is less time consuming. For the simulation of the spectra of the porphyrins in the oriented bilayers the parameters θ and σ as well as the angle α between $z_{\rm b}$ and the magnetic field vector **B** must be known. This angle α follows from the experiment and can be adjusted by rotation of the cast bilayers between the magnet poles (see Experimental Section).

The EPR spectra of CuTPP in DODAM (R = 0.005) at different angles between the bilayer normal and the magnetic field showed a strong effect of the orientation (Figure 7). When the bilayer normal was placed parallel to the magnetic field (α = 0), a spectrum was obtained consisting of four broad lines with $g_{obs} = 2.20$ and a splitting of about 200 G (Figure 7A). These numbers correspond well with the g_{\parallel} and A_{\parallel} values measured for CuTPP in a CHCl₃ glass (Table 4). When the orientation of the bilayer normal with respect to the magnetic field was changed to $\alpha = 90^{\circ}$, a spectrum resulted consisting of one broad line with a g_{obs} similar to g_{\perp} for CuTPP in CHCl₃ (Figure 7B). These observations are consistent with an orientation of the porphyrin z_p axis approximately parallel with the bilayer normal.

Simulations of the experimental CuTPP spectra were carried out for a range of values of θ and σ at $\alpha = 60^{\circ}$ and $\alpha = 90^{\circ}$. Satisfying results were obtained for $\theta \pm \sigma = 30 \pm 30^{\circ}$ (Figure 7). Smaller angles of θ resulted in a significant discrepancy between the coupling pattern in the simulated spectra and the experimental spectra for $\alpha = 0^{\circ}$, whereas for larger values of θ the single line observed at $\alpha = 90^{\circ}$ became split. The shape of simulated spectra was found to depend strongly on the value of σ . The large value of σ has several origins. First an error is introduced by stacking the cast films on the quartz rod (see Experimental Section) and setting the orientation of the quartz rod in the spectrometer. The sum of these two factors is estimated not to exceed 5°. Irregular stacking of the bilayer sheets in the cast film may also introduce an error. Electron microscopy has revealed that this error does not exceed 5°. For CuTPP the main source, therefore, will be a less-defined orientation of the porphyrin molecules relative to the bilayer normal.

Incorporation of CuTHPP in the cast bilayers of DODAM at R = 0.005 also led to a dependency of the EPR spectra on α (Figure 8). At $\alpha = 90^{\circ}$ a spectrum was observed that resembled



Figure 7. EPR spectra of CuTPP in cast films of DODAM (R = 0.005) with the magnetic field parallel to the bilayer normal ($\alpha = 0^{\circ}, -, A$) and perpendicular to the bilayer normal ($\alpha = 90^{\circ}, -, B$) at T = 6 K. The simulated spectra are calculated with $\theta \pm \sigma = 30 \pm 30^{\circ}$ and $\alpha = 0^{\circ}$ (---, A) and $\alpha = 90^{\circ}$ (---, B). The values for $g_{\parallel}, g_{\perp}, A_{\parallel}^{Cu}$, and A_{\perp}^{Cu} are taken from Table 4, $W_{\parallel} = 120$ G, and $W_{\perp} = 140$ G. Spectrometer conditions: modulation amplitude 4.014 G, modulation frequency 100 kHz, microwave power 1 mW, microwave frequency 9.303 GHz, receiver gain 2 × 10⁴.

TABLE 4: EPR Data of Copper(II) Porphyrins^a

| porphyrin | 8⊥ | 81 | A_{\perp}^{Cu}/G | A_{\parallel}^{Cu}/G | $A_{\perp}^{\rm N}/{\rm G}$ | $A_{\parallel}^{\rm N}/{\rm G}$ |
|-----------------------|-------|-------|--------------------|------------------------|-----------------------------|---------------------------------|
| CuTPP | 2.063 | 2.197 | 20 | 195 | | |
| CuTHPP | 2.055 | 2.188 | 20 | 203 | | |
| CuTrHPyP ^b | 2.049 | 2.188 | 24 | 202 | 17 | 14.5 |

^a EPR spectra were recorded using 5 mM solutions of the porphyrins in a chloroform glass at 6 K. The g and A tensors were obtained by simulations of the experimental spectra. ^b CuTrHPyP was diluted with TrHPyP: [CuTrHPyP] = 5 mM; [TrHPyP] = 45 mM.

the powder spectrum of CuTHPP (Figure 8B). This points to an orientation of the porphyrin z_p axis roughly perpendicular to the bilayer normal. However, a precisely perpendicular orientation should result at $\alpha = 0^{\circ}$ in a spectrum consisting of a single line with a g_{obs} similar to g_{\perp} measured for CuTHPP in a CHCl₃ glass. We observed at this angle a spectrum consisting of four broad incompletely resolved lines (Figure 8A). The Cu hyperfine splitting was much smaller than in the powder spectra of CuTHPP. This observation indicates that the orientation of the porphyrin deviates from $\theta = 90^{\circ}$.

Simulation of the experimental spectra at $\alpha = 0^{\circ}$ and 90° confirmed this conclusion. Satisfactory results were obtained for $\theta \pm \sigma = 63 \pm 12^{\circ}$ (Figure 8). For smaller angles of θ the four lines in the simulated spectra at $\alpha = 0^{\circ}$ became unresolved and the spectrum at $\alpha = 90^{\circ}$ became too broad. Larger values of θ gave the opposite effect. An uncertainty in σ of 10° is likely on the basis of consideration of experimental conditions and the quality of the cast film. We may therefore conclude that the CuTHPP molecules have a well-defined orientation in the cast films.

In the spectra of both CuTPP and CuTHPP the hyperfine splitting due to coupling of the copper nucleus with the nitrogen ligands is not visible. The molar ratio of the porphyrins in the bilayers amounted to R = 0.005. As discussed above, at this concentration the porphyrin molecules are present mainly in the form of aggregates, which explains this result. The occurrence of aggregates was confirmed by optical absorption spectroscopy on the DODAM cast films. Although the strong background



Figure 8. EPR spectra of CuTHPP in cast films of DODAM (R = 0.005) with the magnetic field parallel to the bilayer normal ($\alpha = 0^{\circ}$, -, A) and perpendicular to the bilayer normal ($\alpha = 90^{\circ}$, -, B) at T = 6 K. The simulated spectra are calculated with $\theta \pm \sigma = 63 \pm 12^{\circ}$ and $\alpha = 0^{\circ}$ (--, A) and $\alpha = 90^{\circ}$ (--, B). The values for $g_{\parallel}, g_{\perp}, A_{\parallel}^{Cu}$, and A_{\perp}^{Cu} are taken from Table 4, $W_{\parallel} = 70$ G, and $W_{\perp} = 60$ G. Spectrometer conditions: modulation amplitude 4.014 G, modulation frequency 100 kHz, microwave frequency 9.330 GHz, receiver gain 2×10^4 .

scattering prohibited a detailed interpretation of the absorption spectra, the cast films with CuTHPP indeed showed a similar splitting of the porphyrin B-band as vesicular solutions of DODAM containing CuTHPP.

The effect of aggregation on the EPR spectra was particularly evident in the case of CuTrHPyP. EPR spectra of this porphyrin in DODAM cast films (R = 0.005) did not show any dependence on the angle α . This does not necessarily mean that the porphyrins are not oriented in the cast films, or that there is no anisotropy in the g and A tensors. Our interpretation is that such effects are not observed due to line broadening because of dipolar relaxation, as this phenomenon was also noted in the EPR spectra of the pure compound in the CHCl₃ glass. Upon magnetically diluting the porphyrin with TrHPyP the anisotropy of both the g and A tensors became visible. EPR spectra of cast films of DODAM containing CuTrHPyP/TrHPyP in a 1:4 molar ratio (total porphryin-to-lipid ratio R = 0.005) did reveal a clear effect of α (Figure 9). The spectra at $\alpha = 0^{\circ}$ and $\alpha = 90^{\circ}$ suggest that the CuTrHPyP molecules in the DODAM cast films have an orientation comparable to that of CuTHPP molecules. The spectrum at $\alpha = 0^{\circ}$, however, shows a less well resolved splitting pattern than the spectrum of the latter porphyrin. This indicates that θ is larger for CuTrHPyP than for CuTHPP.

Simulations of the experimental CuTrHPyP spectra at $\alpha = 0^{\circ}$ and 90° gave the best results for $\theta \pm \sigma = 73 \pm 12^{\circ}$ (Figure 9). The line shape of the simulated spectra differed considerably from the shape of the experimental spectra. The positions of the lines, however, agreed very well. Other values for θ and σ gave significant deviations of the positions of the lines in the simulated spectra, whereas the resemblance of the line shapes did not improve. It must be noted that the experimental spectra contain contributions from both TrHPyP-CuTrHPyP mixed dimers and CuTrHPyP dimers. If we assume that TrHPyP and CuTrHPyP are statistically distributed in the dimers and that the intensity of the EPR signal of one molecule of CuTrHPyP is the same in both species, the contribution of CuTrHPyP to CuTrHPyP molar ratio is 4:1. As mentioned above, the EPR spectrum of pure



Figure 9. EPR spectra of CuTrHPyP diluted with TrHPyP (molar ratio 1/4) in cast films of DODAM (total porphyrin to lipid ratio: R = 0.005) with the magnetic field parallel to the bilayer normal ($\alpha = 0^{\circ}, -$), A) and perpendicular to the bilayer normal ($\alpha = 90^{\circ}, -$), B) at T = 6 K. The simulated spectra are calculated with $\theta \pm \sigma = 73 \pm 12^{\circ}$ and $\alpha = 0^{\circ}$ (--, A) and $\alpha = 90^{\circ}$ (--, B). Values for $g||, g_{\perp}, A_{\parallel}^{Cu}, A_{\perp}^{Cu}, A_{\parallel}^{N}$, and A_{\perp}^{N} are taken from Table 4, $W_{\parallel} = 15.5$ G, and $W_{\perp} = 35.6$ G. Spectrometer conditions: modulation amplitude 4.014 G, modulation frequency 100 kHz, microwave power 1 mW, microwave frequency 9.203 GHz, receiver gain 2 × 10^4.

CuTrHPyP dimers consists of one broad, nearly isotropic line. The occurrence of CuTrHPyP dimers in the magnetically diluted sample might therefore account for the deviation in the line shape between the experimental and simulated spectra.

Ishikawa and Kunitake¹⁵ have shown that in the case of anionic porphyrins dispersed in bilayers of cationic amphiphiles, it is possible to observe the EPR spectrum of a monomeric copper porphyrin, with resolved nitrogen hyperfine splitting. In the present work, the fluorescence experiments (see above) show that at very low porphyrin-to-amphiphile ratios (R = 0.0005) the porphyrins are present mainly as monomers. however, attempts to obtain EPR spectra of monomeric copper porphyrins in DODAM cast film at these R values were unsuccessful. The spectra show a clear effect of the orientation but were similar in appearance to those at R = 0.005, i.e., they did not show any nitrogen hyperfine splitting. Apparently, the porphyrins in cast films are still present mainly as aggregated species. We explain the apparent discrepancy between EPR and fluorescence results by the difference in experimental conditions which may have influenced the aggregation of the porphyrins. The drying of the DODAM cast films for EPR took place at room temperature, wehre the bilayers are in the liquid-crystalline state, whereas the fluorescence spectroscopic measurements were carried out above the phase transition temperature of the DODAC bilayers. At lower porphyrin content, R = 0.00025, the signal-to-noise ratio of the EPR spectra became too low to observe any effect of the orientation or nitrogen hyperfine splitting effect.

Discussion

Our UV-vis and fluorescence studies show that at low concentrations the porphyrins in the bilayer are present mainly as monomeric species. The location of these species depends on the nature of the substituents on the porphyrin. The presence of four long lipophilic substituents as in THPP leads to a disposition of the porphyrin near the center of the bilayer, whereas one hydrophilic substituent as in TrHPyP is already sufficient to force Bilayers of Dioctadecyldimethylammonium Surfactants



Figure 10. Arrangement of porphyrins in aggregates (A); r_{cc} is the vector between the molecular centers of the porphyrins and φ_x , φ_y , and φ_z are the angles between r_{cc} and the porphyrin x_p , y_p , and z_p axes, respectively. Two types of aggregates are shown, viewed along z_p : a face-to-face type of aggregate, in which the displacements along the x_p and y_p axis are equal (B) and an edge-to-edge type of aggregate, in which the displacement along the x_p axis is larger than along the y_p axis.

this molecule to take a position near the interface. Without substituents the porphyrin molecule is relatively free to move. Its position along the bilayer normal is less well defined.

Increasing the concentration of the porphyrins in the bilayers leads to the formation of aggregates. As expected, the aggregation number of the charged porphyrin TrHPyP is smaller than that of the uncharged ones (2 versus ~4 at R = 0.005). This ability of porphyrins to form aggregates is often overlooked in studies that deal with porphyrins in bilayer systems. Only the use of sterically hindered porphyrins or porphyrins that have strong electrostatic interactions with the lipid molecules seems to prevent formation of aggregates up to high concentrations of the porphyrins in the bilayer.^{4,15}

In almost all porphyrin aggregates of which the structure is known, both in solution and in the crystalline state, the porphyrin xy planes are stacked in a cofacial arrangement with a interplanar distance ranging from 3.5 to 4 Å (Figure 10).^{35,36} The porphyrin rings are not rotated around any of the three axes, but there is a considerable displacement along the x and y direction. If we do not consider solvent effects or crystal packing forces, the formation of aggregates of porphyrins seems to be dominated by van der Waals interactions between the porphyrin rings. Electrostatic interactions are of minor importance in this respect.³⁷ These electrostatic interactions, however, do control the geometry of the aggregates. Information on this geometry comes from changes in the absorption and fluorescence spectra of the porphyrins. As was already mentioned in the Results section, these changes are the result of exciton coupling. Several approaches have been advanced to describe this phenomenon.^{19,20} Kasha's theory gives a good qualitative explanation. This theory predicts that interactions between localized transition dipole moments can cause a splitting of the absorption bands. For a dimer the resulting transition energy (E_{dimer}) is related to the transition energy of the monomer $(E_{monomer})$ by eq 2, in which D

$$E_{\text{dimer}} = E_{\text{monomer}} + D \pm \epsilon \tag{2}$$

$$\epsilon = (2q^2/r_{\rm cc}^{3})(1 - 3\cos^2\varphi) \tag{3}$$

is a dispersion energy term and ϵ is the exciton splitting energy. In case of cofacially arranged chromophores ϵ is related to the transition dipole moment (q) and the geometry of the aggregate, as given by eq 3.

In eq 3, r_{cc} is the center-to-center distance of the two chromophores in the aggregate and φ is the angle between the center-to-center vector and the transition dipole moment (Figure 10). Since one of the two resulting transitions is symmetry forbidden, exciton splitting usually results in only one absorption band. In porphyrins, however, the excited state is 2-fold degenerated, with one transition dipole moment aligned along the x axis and the other along the y axis. By use of eqs 2 and 3, changes in the absorbance spectra are easily interpred in terms of displacements of the porphyrin molecules along the x and y axes (see Figure 10).

 TABLE 5: Parameters (degrees) Describing the Structure of the Porphyrin Aggregates and the Orientation in DODAM Cast Films

| porphyrin | $\varphi_{\mathbf{x}}$ | φ_y | φ_z^c | θ | σ | ωa |
|-------------------|------------------------|-------------|---------------|----|----|----|
| TPPa | 56 | 56 | 52.4 | 30 | 30 | 0 |
| TrHPyP | 54.7 | 54.7 | 54.7 | 73 | 12 | 69 |
| THPP ^b | 49 | 61 | 55 | 63 | 12 | 56 |

^a These parameters have been taken from the crystal structure of TPP.³⁹ ^b These parameters have been taken from the structure of TOPP aggregates.²⁵ $c \varphi_z$ is related to φ_x and φ_y by the following equation: $\cos^2 \varphi_z = \sin^2 \varphi_x + \sin^2 \varphi_y - 1$.

Aggregation of THPP causes a splitting of the B-band into one band of lower intensity at 402 nm and one band of higher intensity at 436 nm. This splitting can be explained by an edge-to-edge orientation of the molecules (Figure 10). In this orientation there is a larger displacement along the x axis and a smaller displacement along the y axis. The red-shifted component in the visible spectrum originates from an interaction of the transition dipoles aligned along the x axis with φ_x being smaller than 54.7°. The blueshifted component originates from interactions of the transition dipoles aligned along the y axis, with φ_y larger than 54.7°. Similar changes in the absorption spectra have been reported for thin films of THPP³⁸ and for monolayers of 5,10,15,20-tetrakis(4-(octyloxy)phenyl)porphyrin (TOPP).²⁵ Apparently, the matrix has little effect on the spectroscopic properties of the porphyrin molecules in the aggregates. The structure of aggregates of the porphyrins in monolayers of TOPP is similar to that in Figure 10C. For the porphyrin molecules in these aggregates φ_x and φ_y amounted to approximately 49° and 61°, respectively.

In the case of TPP and TrHPyP the shift of the absorption maximum of the B-band is very small, and no splitting is observed. We may therefore conclude that the displacement of the porphyrin molecules in the aggregates must be nearly the same in the x and y directions. Because of the small shift φ_x and φ_y must be close to 54.7°. If it is assumed that the distance between porphyrin planes is 3.5–4 Å, this corresponds to a displacement along the x and y axis of 2.5–3 Å. In crystalline TPP the porphyrin molecules are displaced along the x and y axis by 3.3 Å. The center to center distance in the crystals is 5.9 Å; hence φ_x and φ_y amount to 56°.³⁹

We have already noted that the geometry of the aggregates is controlled mainly by electrostatic interactions between the porphyrin molecules. It is therefore reasonable to assume that the arrangement of the porphyrins in our aggregates will be similar to those reported in the literature. We will use these arrangements from the literature in the following discussion. The angles φ describing the structures of the aggregates are summarized in Table 5. For the aggregate structure of THPP and TPP we have adopted the values of φ that have been reported in the literature for monolayers of TOPP²⁵ and for crystals of TPP.³⁹ For TrHPyP no data are available. The angles φ_x , φ_y , and φ_z are therefore set to 54.7° (see above).

Recently, Ishikawa and Kunitake have reported on the orientation of porphyrins with hydrophobic and negatively charged substitunts in cast bilayers formed by positively charged amphiphiles.¹⁵ They showed that a porphyrin with four symmetrically distributed negatively charged groups is oriented with the porphyrin plane parallel to the surface of the bilayers. This orientation is most likely the result of ion pairing of the substituents with the amphiphile headgroups. On the contrary, a nonsymmetrically substituted porphyrin, bearing two negatively charged substituents on one side of the central porphyrin nucleus and two hydrophobic substituents on the opposite side, was oriented more or less perpendicular to the bilayer surface. The precise orientation of this porphyrin plane matched the tilt angle of the lipid chains. Our EPR measurements on the orientation of the porphyrins in DODAM cast films are more difficult to interpret than Ishikawa and Kunitake's measurements as our porphyrins



Figure 11. Coordinate system for aggregates of porphyrins (A) and the possible orientations of the aggregates with respect to the bilayer normal: z_a parallel to z_b (B), z_a at an intermediate angle to z_b (C) and z_a perpendicular to z_b (D).

are present as aggregates. The measured angles between the porphyrin normals and the bilayer normal should therefore be discussed in terms of the orientation of the aggregated species in the bilayer and not of the monomeric species as in the case of Ishikawa and Kunitake. To do so a coordinate system $(xyz)_a$ for the porphyrin aggregate is defined as indicated in Figure 11A. In this coordinate system the z_a axis coincides with the centerto-center vector r_{cc} between two porphyrin molecules in the aggregate (see also Figure 10). The porphyrin z_p axis is situated in the $(xz)_a$ plane. It is most convenient now to describe the orientation of the aggregated species in the DODAM bilayers by the angle between z_a and the bilayer normal z_b . We can distinguish three possible orientations: z_a is oriented parallel to z_b (Figure 11B), z_a is oriented perpendicular to z_b (Figure 11D), or z_a has an intermediate orientation (Figure 11C). A proper orientation of porphyrin molecules in the cast films requires that the aggregates are aligned along the surfactant molecules. We found that the tilt angle of the surfactant molecules in the cast bilayers is smaller than 10°. Alignment of the aggregates along the surfactant molecules therefore implies that z_a should be oriented either parallel to z_b or oriented perpendicular to z_b .

In the case of a parallel arrangement of z_a and z_b the measured angle θ should be equal to φ_z . Although the structures of the aggregates of the three porphyrins may differ, φ_z will remain more or less constant with a value of approximately 54° (Table 5). The measured values of θ (Table 5) differ significantly from this value for TrHPyP and TPP. A parallel orientation therefore is unlikely. Moreover, such an orientation would place only one of the positively charged porphyrins in the aggregates of CuTrHPyP near the surface of the bilayer.

In the case of THPP the difference between θ and φ_z is only 9°. This could point to an alignment of the porphyrin aggregates along the surfactant molecules which have a tilt angle of similar value (X-ray results, vide supra). In such an arrangement the aggregate z_a axis is no longer parallel to z_b but has an angle toward z_b equal to the tilt angle of the surfactant molecules (Figure 11C). In this case rotation of the aggregates around z_a does change the angle between z_p and z_b . One an easily see that the maximum and minimum angles between z_p and z_b are equal to the surfactant the tilt angle, respectively. The averaged angle between z_p and z_b , however, is still equal to φ_z . When rotation of the aggregate around its axis is not hindered an angle of 9° between z_a and z_b should have resulted in an overall

orientation of THPP toward z_b of 55 ± 9°. This still does not agree with the experimental value of $\theta \pm \sigma = 63 \pm 12^\circ$.

The alternative is a perpendicular orientation of z_a with respect to z_b . When x_a is chosen to be parallel with z_b , the measured angle θ is the complement of φ_z in the $x_a z_a$ plane: $(90^\circ - \varphi_z)$. Comparison of θ with this complement shows that they are different. Rotation of the aggregates around z_b is possible, but this does not change the orientation of z_p toward z_b . Rotation around z_a , however, does change the orientation of z_p toward z_b (Figure 11D). The relation between θ , φ_z , and the angle of rotation (ω_a) around z_a is given by eq 4.

$$\sin^2\theta = \cos^2\varphi_z + \sin^2\varphi_z \sin^2\omega_a \tag{4}$$

Rotation of the aggregate around z_a may result in preferred values for ω_a as the aggregates have no cylindrical symmetry. Certain discrete ω_a values will result in a more favorable disposition of the porphyrin molecules in the bilayer than other ω_a values. This will especially be the case for CuTrHPyP. The charged substituents in the aggregates of this porphyrin will preferentially be located near the aqueous interface. Calculation of ω_a for a perpendicular orientation of z_a and z_b using the values of θ and φ_z in Table 5 reveal that this angle of rottion must have values of 56° and 69° for CuTHPP and CuTrHPyP, respectively. The σ values found for CuTHPP and CuTrHPyP imply that rotation around z_a is strongly hindered, since variations in ω_a would have resulted in a higher value of σ . Taken together we may conclude that an orientation of the aggregates as depicted in Figure 11D nicely accounts for the experimental results for CuTHPP and CuTrHPvP.

For TPP the situation is less clear. As we already discussed above, a small tilt angle of the surfactant molecules of approximately 10° accompanied by a similar tilt angle between the aggregate axis and the bilayer normal causes a maximum change in the angle between z_p and z_b (equal to φ_z for a parallel arrangement of z_a and z_b) of 10°. This does not account for the observed value of $\theta \pm \sigma = 30 \pm 30^{\circ}$. A perpendicular arrangement of z_a and z_b also does not give a satisfying explanation for the experimentally found orientation between z_p and z_b . Evaluation of equation 5 gives for $\varphi_z = 54.7^{\circ}$ a minimum value of $\theta = 35.3^{\circ}$ if $\omega_{0} = 0^{\circ}$, whereas the experimentally determined value of $\theta \pm$ σ amounts to 30 ± 30°. The large value of σ could have explained the difference but as we have already mentioned only a minor part (approximately 10°) can be ascribed to the experimental error in the determination of the orientation. The major part of σ does represent a real variation of the angle between z_p and z_b . This is most likely due to an unhindered rotation of the aggregate around its axis. In the case of a perpendicular arrangement of z_a and z_b , rotation around z_a would have caused θ to range from $(90^{\circ} - \varphi_z)$, which is entirely different from the observed value of θ and σ . We therefore have to conclude that the observed orientation of CuTPP in cast films of DODAM cannot be adequately explained by any of our models.

In the case of CuTHPP and CuTrHPyP rotation around the aggregate z_a axis of 56° and 69°, respectively, makes the porphyrin x axis become approximately parallel to the $z_a z_b$ plane. A consequence of this is that two adjacent phenyl substituents of CuTHPP will be located in one of the monolayer halves and the two opposite phenyl substituents in the other monolayer half. This allows the porphyrin alkyl chains to be aligned with the lipid alkyl chains (Figure 12A). In the case of CuTrHPyP two (hexadecyloxy)phenyl chains will point to the hydrophobic part of the bilayer, whereas the positively charged substituent together with one (hexadecyloxy)phenyl will point toward the aqueous interface. The latter chain is most likely bent backward into the bilayer (Figure 12B). For TPP, as mentioned, the arrangement is unknown.



Figure 12. Arrangement of aggregates of THPP (left) and of TrHPyP (right) in a bilayer of DODAM.

Experimental Section

General Methods. ¹H NMR spectra were recorded on a Bruker WH 90 instrument (90 MHz). Chemical shifts are given in ppm downfield from tetramthylsilane. Abbreviations used are s = singlet, d = doublet, t = triplet, m = multiplet, and b = broad. Transmission and scanning electron microscopy were carried out with Philips EM 201 and JEOL 100 CX II instruments, respectively. A Branson 2200 sonication bath was used for the preparation of the vesicles by the sonication method. EPR spectra were recorded on a Bruker ESP 300 spectrometer, equipped with a Oxford flow cryostat and a goniometer. Infrared and UV-vis spectra were recorded on Perkin-Elmer 298 and Perkin-Elmer Lambda 5 spectrophotometers, respectively. Steady-state fluorescence measurements were carried out on a Perkin-Elmer MPF 4 fluorospectrometer. Time-resolved fluorescence measurements were performed in the group of Prof. J. Verhoeven (Laboratory of Organic Chemistry, University of Amsterdam, The Netherlands). The equipment used for these measurements is described elsewhere.40 The cuvettes for UV-vis and fluorescence measurements were thermostatted with an accuracy of 0.1 °C.

Materials. Technical grade solvents were used, unless otherwise indicated. DMF and THF were distilled before use. Column chromatography and TLC were performed on silica (Merk, silica gel 60H and precoated F-254 plates, respectively). For the preparation of the vesicles Uvasol grade solvents (Merck) and deionized and triple distilled water were used. 5,10,15,20-Tetraphenylporphyrin, copper(II) 5,10,15,20-tetraphenylporphyrin, and zinc(II) 5,10,15,20-tetraphenylporphyrin were prepared according to literature procedures.^{41,42} 4-(Hexadecyloxy)benzaldehyde and 16-bromohexadecanoic acid were prepared as described in the literature.^{43,44} Hydrobromination of 9-octadecenoic acid gives a reaction product which consisted of 9-bromooctadecanoic acid and 10-bromooctadecanoic acid.⁴⁵ This mixture (abbreviated as 9(10)-octadecanoic acid) was used in the fluorescence quenching experiments.

N,N-Dioctadecyl-*N,N*-dimethylammonium Chloride (DODAC). *N,N*-Dioctadecyl-*N,N*-dimethylammonium bromide (DODAB, purchased from the Eastman Kodak) was converted into the chloride form by ion-exchange chromatography using a 30 × 30 cm column packed with Amberlite IRA 410 resin (chloride form, eluent ethanol). The product was recrystallized three times from ethyl acetate: white crystals; TLC (silica gel, eluent 10 vol % of methanol in CHCl₃) $R_f = 0.1$, single spot (a mixture of DODAB and DODAC showed two spots). Anal. Calcd for C₃₈H₈₀NCl·H₂O: C 75.50%, H 13.67%, N, 2.32%, Found: C, 75.71%, H 13.61%, N 2.42%.

N,N-Dioctadecyl-N,N-dimethylammonium Methacrylate (DODAM). Freshly distilled methacrylic acid (20 mL) was dissolved in 100 mL of water and neutralized with a 2.5 M aqueous sodium hydroxide solution. This solution was passed over a $3 \times$ 30 cm column loaded with Amberlite IRA 410 anion exchange resin (chloride form). Following this, the column was eluted with 200 mL of water and with 200 mL of ethanol. DODAB (10 g, 0.016 mol) was dissolved in 50 mL of warm ethanol and passed over this column. After evaporation of the solvent the product was recrystallized from ethylacetate: white powder; yield 9.07 g (90%); TLC (silica gel, eluent 10 vol % of methanol in CHCl₃) $R_{\rm f} = 0.05$, single spot; mp 59–61 °C; Anal. Calcd for C₄₂H₈₅NO₂·2.5H₂O: C, 73.51%, H 13.95%, N 2.04%. Found: C 73.83%, H 13.76%, N 2.41%.

21,23-Dihydro-5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin. Freshly distilled pyrrole (12.5 g, 0.186 mol) was slowly added to 22.7 g (0.186 mol) of 4-hydroxybenzaldehyde in 425 mL of refluxing propionic acid.⁴¹ Refluxing was continued for 45 min. After standing overnight at room temperature the propionic acid was removed under vacuum. The porphyrin was isolated from the resulting black tar by flash chromatography (silica, eluent 10 vol % of ethanol in CHCl₃): purple powder; yield 2.9 g (9.2%); ¹H NMR (acetone- d_6) δ 9.0 (s, 8H, β -pyrrole), 8.1 (d, 8H, 2.6phenyl), 7.3 (d, 8H, 3.5-phenyl), -2.5 (br, 2H, NH); UV-vis (CH₂Cl₂) λ /nm 400, 514, 550, 592, 649; IR (neat) ν /cm⁻¹ 3400 (OH), 1610 (C=C), 1510 (C=C), 1170 (C–O), 800 (C–H).

21,23-Dihydro-5,10,15,20-tetrakis(4-(hexadecyloxy)phenyl)porphyrin (THPP). 21,23-Dihydro-5,10,15,20-tetrakis(4-hydroxyphenyl)porphyrin (200 mg, 0.3 mmol) and 200 mg of powdered sodium hydroxide were dissolved in 10 mL of DMF by stirring under a nitrogen atmosphere. Hexadecyl bromide (1.1 g, 3.6 mmol) dissolved in 20 mL of toluene was added, and stirring was continued at 70 °C for 4 h. After cooling to room temperature, the reaction mixture was poured into 100 mL of water from which the product was extracted with $CHCl_3$ (3 × 50 mL). The organic layers were combined, washed three times with 50 mL of water, dried (anhydrous sodium sulfate), and concentrated under vacuum. The crude product was purified by flash chromatography (silica, eluent 50 vol % of hexane in CHCl₃), followed by recrystallization from 1,4-dioxane: purple powder; yield 410 mg (88%); ¹H NMR (CDCl₃) δ 8.85 (s, 8H, β -pyrrole), 7.95 (d, 8H, 2,6-phenyl), 7.15 (d, 8H, 3,5-phenyl), 4.15 (t, 8H, OCH₂), 1.1-1.2 (b, 112H, CH₂), 0.9 (t, 12H, CH₃), -2.7 (b, 2H, NH); UVvis (CH₂Cl₂) λ /nm, (log(ϵ /M⁻¹ cm⁻¹)) 416 (5.03), 518 (4.23), $555 (4.09), 593 (3.68), 650 (3.91); IR (neat) \nu/cm^{-1} 1610 (C=C),$ 1510 (C=C), 1176 (C-O), 800 (C-H). Anal. Calcd for C108H158N4O4•C4H8O: C 80.82%, H 10.05%, N 3.37. Found: C 80.01%, H 10.18%, N 3.40%.

Zinc 5,10,15,20-Tetrakis(4-(hexadecyloxy)phenyl)porphyrin (ZnTHPP). 21,23-Dihydro-5,10,15,20-tetrakis(4-(hexadecyloxy)phenyl)porphyrin (100 mg, 0.063 mmol) was dissolved in a mixture of 10 mL of DMF and 15 mL of toluene and heated to reflux. Zinc acetate dihydrate (400 mg) was added to the boiling solution, and the course of the reaction was followed by UV-vis. Refluxing was continued until the absorption bands of the free-base porphyrin had vanished. After this cooled to room temperature, 25 mL of toluene and 50 mL of water were added. The organic layer, containing the zinc porphyrin, was washed three times with 50 mL of water and three times with 50 mL of 5 wt % aqueous sodium carbonate solution. The organic layer was dried (sodium sulfate) and concentrated in vacuum. The crude product was purified by flash chromatography (silica, eluent 50 vol % of hexane in CHCl₃): purple powder; yield 102 mg (98%); UV-vis (CH₂Cl₂) λ /nm, (log(ϵ /M⁻¹ cm⁻¹)) 423 (5.50), 545 (4.10), 592 (3.80); IR (neat) v/cm⁻¹ 1606 (C=C), 1510 (C=C), 1176 (C-O), 806 (C-H).

Copper 5,10,15,20-Tetrakis(4-(hexadecyloxy)phenyl)porphyrin (**CuTHPP).** This porphyrin was synthesized starting from THPP (100 mg) as described for ZnTHPP: purple powder; yield 98 mg (94%); UV-vis (CH₂Cl₂) λ /nm, (log(ϵ /M⁻¹ cm⁻¹)) 419 (6.30), 541 (5.10), 580 (3.30); IR (neat) ν /cm⁻¹ 1607 (C=C), 1510 (C=C), 1176 (C-O), 804 (C-H). Anal. Calcd for C₁₀₈H₁₅₆N₄O₄Cu: C 79.19%, H 9.60%, N 3.42%. Found: C 78.35%, H 9.52%, N 3.45%.

21,23-Dihydro-5,10,15-tris(4-(hexadecyloxy)phenyl)-20-(4pyridyl)porphyrin. Freshly distilled pyrrole (4 mL, 57 mmol) was slowly added to a solution of 4-(hexadecyloxy)benzaldehyde (5.1 g, 14 mmol) and 4-pyridinecarbaldehyde (4.5 mL, 42 mmol) in 240 mL of refluxing propionic acid.⁴⁶ Refluxing was continued for 2.5 h. A mixture of porphyrins was isolated by flash chromatography (alumina, eluent CHCl₃). Four different fractions were obtained from which one was identified as 21,23-dihydro-5,10,15-tris(4-(hexadecyloxy)phenyl)-20-(4-pyridyl)porphyrin: purple powder; yield 0.42 g (7%); ¹H NMR (CDCl₃) δ 8.8 (s, 8H, β -pyrrole), 8.7 (d, 2H, pyridyl), 7.9 (m, 8H, 2,6-phenyl and pyridyl), 7.1 (d, 6H, 3,5-phenyl), 4.05 (t, 6H, OCH₂), 1.1–2.1 (br, 84H, CH₂), 0.9 (t, 9H, CH₃); UV-vis (CH₂-Cl₂) λ /nm 421, 519, 554, 592, 649.

21,23-Dihydro-5,10,15-tris(4-(hexadecyloxy)phenyl)-20-[4-(1methylpyridinium)]porphyrin Tosylate (TrHPyP). 21,23-Dihydro-5,10,15-tris(4-(hexadecyloxy)phenyl)-20-(4-pyridyl)porphyrin (100 mg), 0.1 mL of methyltosylate, and 0.1 mL of 2,6dimethylpyridine were dissolved in a mixture of 6 mL of toluene and 3 mL of acetonitrile and stirred at 80 °C under an atmosphere of nitrogen for 16 h. After cooling to room temperature, the reaction mixture was poured over a glass filter filled with silica. After washing with 50 mL of CH₂Cl₂ the pure product was isolated by elution with 10 vol % of methanol in CHCl₃: purple power; yield 70 mg (60%); ¹H NMR (CDCl₃) δ 9.5 (br, 2H, pyridyl), 8.8 (s, 8H, β -pyrrole), 8.6 (br, 2H, pyridyl), 8.1 (d, 2H, tosylate), 7.9 (d, 6H, 2,6-phenyl), 7.3 (d, 2H, tosylate), 7.1 (d, 6H, 3,5phenyl), 5.0 (s, 3H, pyridyl-NCH₃), 4.2 (t, 6H, OCH₂), 2.2 (s, 3H, tosylate-CH₃), 1.1-2.1 (br, 84H, CH₂), 0.9 (t, 9H, CH₃); UV-vis $(CH_2Cl_2) \lambda/nm$, $(\log(\epsilon/M^{-1} cm^{-1})) 424 (5.15), 571 (4.01)$. 658 (3.79); IR (neat) v/cm⁻¹ 3318 (NH), 3095 (CH-Py), 3049 (CH-Py), 1607 (C=C), 1510 (C=C), 1244 (SO), 1175 (CO).

Copper 5,10,15-Tris(4-(hexadecyloxy)phenyl)-20-(4-pyridyl)porphyrin. This compound was synthesized starting from 21,-23-dihydro-5,10,15-tris(4-(hexadecyloxy)phenyl)-20-(4-pyridyl)porphyrin (46 mg), as described for ZnTHPP. The purple product gave a single spot on TLC: yield 48 mg (99%); UV-vis (CH₂Cl₂) λ /nm) 424, 546, 584.

Copper 5,10,15-Tris(4-(Hexadecyloxy)phenyl)-20-[4-(1-methylpyridinium)]porphyrin Tosylate (CuTrHPyP). This compound was synthesized starting from copper 5,10,15-tris(4-(hexadecyloxy)phenyl)-20-(4-pyridyl)porphyrin (48 mg), as described for TrHPyP. The product gave a single spot on TLC: yield 31 mg (60%); UV-vis (CH₂Cl₂) λ /nm 419, 546, 594.

Preparation of Vesicles. In a test tube the desired amount of a stock solution of the porphyrin in CH_2Cl_2 (1-5 mM) was added to a solution of 14.6 mg (25 mmol) of DODAC in 0.5 mL of CH_2Cl_2 . The solvent was evaporated under a stream of nitrogen to leave a homogeneous mixed film of the porphyrin and the surfactant. This sample was dried for 2 h in a desiccator at 0.2 mmHg. For samples prepared by the sonication method 5 mL of water (preheated at 50 °C) was added to the mixed film while vortexing. The resulting dispersion was placed in a bath-type sonicator and sonicated for 2 h at 50 °C. For samples prepared by the ethanol injection method the mixed film was solubilized in 100 μ L of ethanol/THF (1/2 v/v). Part of this solution (50 μ L) was injected into 2.5 mL of water (preheated at 50 °C) while vortexing. Samples prepared by the two methods were left to equilibrate at 50 °C for 16 h and were finally filtered over 0.2- μ m Millipore filters.

Measurements of the Incorporation Efficiency. Aqueous dispersions of 5 mM of DODAC and 2.5-25 mM of the porphyrin were prepared as described above. Aliquots (1 mL) of these dispersions were centrifugated for 30 min at 10000g. The amount of porphyrin present in the dispersion before centrifugation was determined by diluting 0.1 mL of the dispersion with 0.9 mL of THF and measuring the absorbance at the B-band maximum ($A_{dispersion}$). The amount of porphyrin solubilized by DODAC was measured similarly as $A_{supernatant}$ of the supernatant obtained after centrifugation of the dispersions. The fraction of porphyrin incorporated was calculated from $A_{supernatant}/A_{dispersion}$.

Fluorescence Measurements. Fluorescence measurements were carried out in duplicate and at least for two different vesicle preparations. Before measuring the samples where purged with argon for 20 min. Quenching of the fluorescence was studied by titrating 1 mL of a vesicle solution ($R = 5 \times 10^{-4}$, [porphyrin] = 10⁻⁶ M) with 5 μ L aliquots of a 5 mM stock solution of the quencher (aqueous solutions of sodium iodide or (brominated) fatty acids dissolved in ethanol). Ethanol had no effect on the fluorescence intensity, nor had sodium chloride, as was checked separately. The fluorescence intensity was recorded 3 min after each addition of the quencher during 1 min. The measured fluorescence intensities were corrected for volume increments.

Preparation of Cast Bilayers for the EPR Measurements. Vesicle dispersions of DODAM (5 mM) containing porphyrin were prepared by the ethanol injection method. Aliquots of these dispersions (2 mL) were left to dry on a Mylar film in an desiccator over sodium hydroxide. The resulting brittle films were carefully cut into 3×15 mm strips. In general 15-25 strips were stacked on a quartz rod and fixed with Sellotape. This rod was subsequently placed in the EPR spectrometer. The angle α could be set by the goniometer.

Electron Microscopy. Samples for transmission microscopy were prepared by the negative staining method. Carbon coted grids were made hydrophilic by exposure to an argon plasma for 2 min. A droplet of a 2.5 mM DODAC suspension was placed on the grid for 1 min after which it was drained with filter paper. Following this a droplet of a 1 wt % aqueous uranyl acetate solution was placed on the grid and also removed after 1 min.

Samples for scanning electron microscopy were prepared by fixing a piece of the cast film between two microscope slides and fracturing the film in liquid nitrogen at the edges of the slides. The sample was immediately covered with a moderate gold layer by ion sputtering.

Simulations of the EPR Spectra. For the simulations a modified version of a program written by Dr. W. R. Hagen was used. The analytical expressions used in this program are derived from perturbation theory up to second order for the central ion and first order for the ligand nuclear spins. All hyperfine tensors were assumed to be collinear with the g tensor. Quadrupole interactions were ignored and natural abundance distribution of the copper isotopes was assumed. The line shape was assumed to be Gaussian and via asymmetry parameters related to the metal nuclear spin quantum number.⁴⁷ For the simulation of the EPR spectra with an anisotropic distribution of the porphyrin in the magnetic field we used the expressions for the direction cosines from ref 15. The definitions of the coordinate system for the magnetic field, the cast film, and the porphyrin molecule used in this paper are consistent with those defined by Ishikawa and Kunitake.15

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