Partial Stacking of a Water-Soluble Porphyrin in Complex Monolayers with Insoluble Lipid

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Partial Stacking of a Water-Soluble Porphyrin in Complex Monolayers with Insoluble Lipid

M. T. Martín,*,†,‡ I. Prieto,‡ L. Camacho,‡ and D. Möbius†

Max-Planck-Institut für biophysikalische Chemie, Am Fassberg 11, D-37077 Göttingen, Germany, and Departamento de Química Física y Termodinámica Aplicada, Facultad de Ciencias, Universidad de Córdoba, Avda. S. Alberto Magno s/n, 14004 Córdoba, Spain

Mixed monolayers of a water-soluble tetracationic porphyrin (TMPyP) and an insoluble lipid matrix with anionic head groups (DMPA) were formed by the cospreading method. A ratio TMPyP:DMPA = 1:4 was selected by investigation of π−area isotherms and stability of the cospread monolayer. Reflection spectroscopy has been used to infer the molecular organization of the porphyrin molecules in the complex monolayer at the air−water interface. A blue shift in the Soret band was observed with increasing surface pressure. The mixed monolayer of TMPyP:DMPA = 1:4 was transferred onto a glass substrate, and the molecular organization of the porphyrin was studied by transmission spectroscopy with plane polarized light (s and p) under various angles of incidence. The analysis of the reflection spectra at the air−water interface and absorption spectrum of the mixed monolayer on glass leads to a model of partial stacking of the porphyrin molecules in the monolayer at the air−water interface when the surface pressure is increased. On the glass substrate, the TMPyP molecules attached to DMPA are monomeric. Further, indirect evidence for the partial stacking model in the case of the mixture TMPyP:DMPA = 1:4 was obtained from the investigation of the mixed monolayer TMPyP:DMPA:PME = 1:4:16 (PME, methyl palmitate) at the air−water interface and transferred on glass. The presence of the neutral lipid molecules facilitates dense packing of the insoluble monolayer matrix and the matching of surface charge densities of the matrix and the porphyrin moiety. In this way, the intermolecular interactions between porphyrin molecules at the air−water interface under high surface pressure are reduced and the stacking is avoided.

Introduction

Porphyrins and their derivatives, due to the close structural similarity between porphyrins and chlorins, have been extensively studied as models of biological systems since chlorins participate in numerous and important biological functions like energy transfer systems, as prosthetic groups on various proteins such as hemoglobin or the cytochromes, or in the photosynthetic process where the chlorophylls are primary energy receptors as well as involved in the primary charge separation processes. Spectroscopic techniques have provided very detailed information concerning porphyrin chemical reactivity and structure.[1,2] Much attention has been devoted to water-soluble porphyrins since they may be regarded as models of native porphyrin derivatives in the physiological state. The structural simplicity, in comparison to their native analogs, facilitates the understanding of structure−biological activity relations.

In recent years, the architecture and molecular engineering of artifical supermolecular assemblies at surfaces or interfaces, in particular of complex monolayers, with desired structure and physical properties have attracted great interest. These complex monolayers consist of various components, and their structure and the properties of particular components have to be known before the monolayers can be used as parts of even more complex monolayer organizates. On this basis, a large variety of systems have been organized to investigate intermolecular interactions such as molecular association as well as energy and electron transfer processes. New techniques of monolayer organization and manipulation have also

been developed and reviewed.[3−5] The association of functional molecules like porphyrins in solution have been investigated;[6,7] however, the environment and orientation of such molecules may be controlled by incorporating them in molecular assemblies. The Langmuir−Blodgett (LB) method has been used as one of the most versatile techniques for fabricating organic thin films with well-controlled composition, structure, and thickness. Due to these features, the LB technique is considered particularly suited to handle and assemble different molecules with various functions in a desired manner[8] with the aim of, e.g., fabricating molecular devices or investigating physicochemical processes occurring at the monolayer/subphase interface as models for biological systems.[9,10] Typical LB films consist of amphiphilic molecules with long alkyl chains. Well-defined organized monolayers have also been prepared using lightly-substituted molecules or molecules without long alkyl chains. Such non-amphiphile molecules have been incorporated in monolayers of amphiphilic molecules like fatty acids by adsorption from the aqueous subphase[10−12] or by the cospreading technique.[13−16] The structure and organization of such mixed monolayers as well as the photophysical

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properties of the non-amphiphile functional molecules in ordered molecular assemblies have to be determined.

This paper presents the results of an investigation of the interactions and distribution of a water-soluble cationic porphyrin (TMPyP), in a complex monolayer with a lipid having an anionic head group (DMPA). The mixed monolayer TMPyP/DMPA was formed by the cospreading method,[17] and the expected optimal ratio of porphyrin: matrix was determined by investigation of surface pressure−area isotherms and stability of the cospread monolayer. Reflection spectroscopy was used to infer the molecular organization of the porphyrin in the complex monolayer at the air−water interface. Subsequently, the mixed monolayer of TMPyP/DMPA was transferred onto a glass substrate, and the molecular organization of the porphyrin was obtained by transmission spectroscopy with plane polarized light (s and p) under various angles of incidence. The results are interpreted by a model of partial stacking of the porphyrin molecules at the air−water interface in the case of compressed monolayers TMPyP: DMPA = 1:4.

Three-component mixed monolayers including methyl palmitate (PME) as an additional lipid with an uncharged head group, TMPyP/DMPA/PME, were studied as a reference system on water and on glass substrates to provide more support for the proposed model of the cospread TMPyP/DMPA monolayer.

Experimental Section

Materials. L-α-Dimyristoylphosphatidic acid, DMPA, was purchased from Sigma Chemical Co. and used as received. Methyl palmitate, PME (E. Merck), was used without purification. 5,10,15,20-Tetrakis(1-methyl-4-pyridyl)-21H,23H-porphine, TMPyP, was purchased from Aldrich Chemical Co. and used as received. A mixture of trichloromethane and methanol, ratio 3:1 (v/v), was used as spreading solvent. The pure solvents were obtained without further purification from Panreac (Spain) for the measurement of the isotherms of TMPyP/DMPA at different molar ratios, and from Baker Chemicals (Germany) for the other experiments. The water for the subphase was prepared with a Milli-Q filtration unit of Millipore Corp.
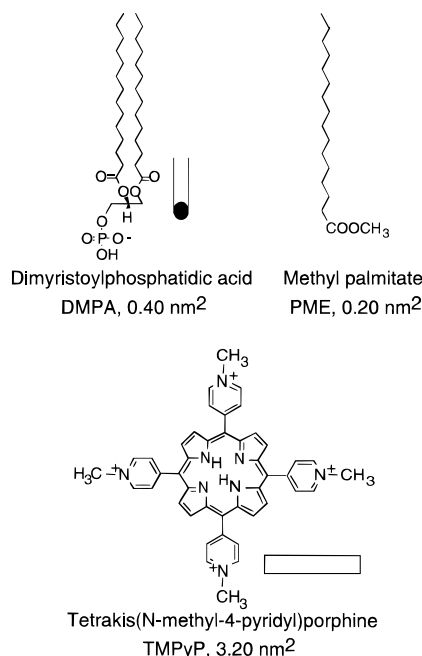
The structures and area per molecule of DMPA, TMPyP, and PME are shown in Chart 1.

Monolayers at Air−Water Interface. Monolayers of TMPyP/DMPA and TMPyP/DMPA/PME were prepared by cospreading on a Lauda Filmwaage FW2 and a circular trough provided with a filter paper Wilhelmy plate,[18] respectively, for measurement of surface pressure (π)−area (A) isotherms, for stability of monolayers, and for reflection spectroscopy at normal incidence.[11,19] The difference in reflectivity, ΔR, of the monolayer-covered water surface and the bare water surface is determined. Details of the reflection spectrometer have been described elsewhere.[11]

The subphase was Milli-Q water at pH 5.6, and the temperature was kept constant at room temperature, 21 °C. The monolayers were compressed by a movable Teflon barrier with a compression velocity of 0.05 nm² min⁻¹ molecule⁻¹.

Monolayers on Solid Substrates. The transfer of monolayers onto transparent hydrophilic substrates was done by vertical dipping at a surface pressure of 35 mN m⁻¹ with the lifting speed of 5 mm min⁻¹. To infer the molecular orientation of the porphyrin in the system, absorption spectra were measured with plane-polarized light (s polarization, electric vector oscillating perpendicular to the plane of incidence, and p polarization in the plane of incidence) under angles of incidence of 0°, 30°,

Chart 1

Dimyristoylphosphatidic acid
DMPA, 0.40 nm²

Methyl palmitate
PME, 0.20 nm²

Tetrakis(N-methyl-4-pyridyl)porphine
TMPyP, 3.20 nm²

and 45°. Details of the spectrometer have been described elsewhere.[20,21]

Results and Discussion

Surface Pressure−Area Isotherms. Mixed monolayers of TMPyP/DMPA were prepared at the air−water interface by the cospreading method which presents considerable advantages[13,14] as compared to adsorption techniques,[22] for the organization of the non-amphiphilic water-soluble component, TMPyP, and an amphiphilic anchor, DMPA, into well-defined monolayers at the air−water interface. In order to characterize the interactions of the tetracationic porphyrin TMPyP with DMPA, surface pressure−area (π−A) isotherms of monolayers of different composition were measured. The influence of the binding of TMPyP to DMPA, at various ratios of the two components on π−A isotherms, is clearly seen in Figure 1.

The π−A isotherms of cospread monolayers of TMPyP and DMPA at different molar ratios of TMPyP:DMPA (1: 1, curve 2; 1:4, curve 3; 1:6, curve 4; 1:8 curve 5) are markedly different from the reference DMPA isotherm (curve 1). This is clear evidence for the presence of the porphyrin at the interface. An expansion at low π and relative convergence at high π are observed for all mixtures. The observed expansion at low π depends on the molar ratio of TMPyP/DMPA and increases with the fraction of TMPyP in the mixture. However, when the fraction of the porphyrin is larger than 0.2, no further expansion is seen. This behavior is in agreement with proposed models for an anionic water-soluble porphyrin[23] and a cyclic bypiridinium tetracation,[24] respectively, attached electrostatically to lipid monolayers. Consequently we conclude that the TMPyP molecules are retained in the head group region of the DMPA monolayer predominantly by electrostatic interactions. Optimal

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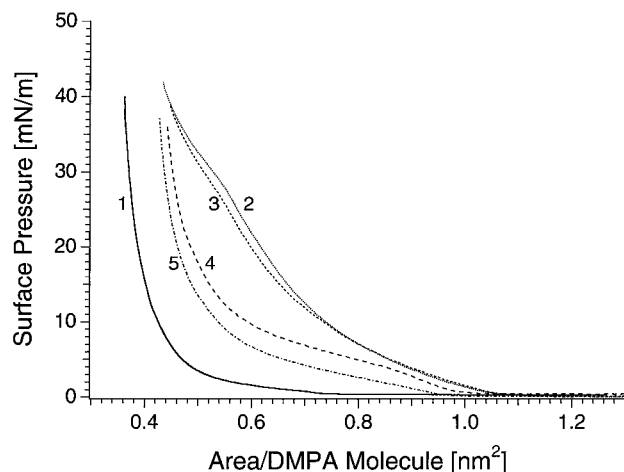(19) Möbius, D.; Orrit, M.; Grüniger, H.; Meyer, H. *Thin Solid Films* **1985**, *132*, 41.

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Figure 1. Surface pressure−area (π−A) a monolayer of the DMPA (curve 1) and the cospread TMPyP/DMPA monolayers, molar ratios 1:1 (curve 2), 1:4 (curve 3), 1:6 (curve 4), and 1:8 (curve 5), all curves are plotted vs area per DMPA molecule; subphase water, 21 °C.

Chart 2
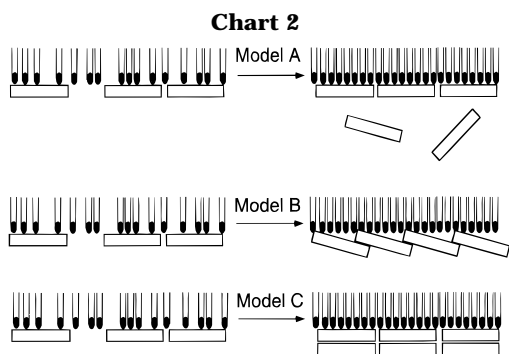
binding requires four negative charges, i.e., four molecules of DMPA, to interact with the four positive charges of one molecule of TMPyP. A larger fraction of TMPyP in the mixture with DMPA may cause a loss of the surplus from the mixed monolayer to the aqueous subphase. This means that the optimal ratio for a mixed TMPyP:DMPA monolayer should be 1:4.

Three possible models (Chart 2) for the organization of the mixed TMPyP/DMPA monolayer in a molar ratio of 1:4 may be considered. In model A (Chart 2), a fraction of the porphyrin molecules located underneath the polar lipid head groups leaves the mixed monolayer with increasing surface pressure since the area per four molecules of DMPA, i.e., 1.60 nm², is much smaller than the area of one flat lying molecule of porphyrin (approximately of 3.20 nm²,[2,17,22]). Models B and C (Chart 2) show two possibilities to keep all molecules of TMPyP bound to the lipid matrix. In both cases, the area per molecule of TMPyP is reduced with respect to 3.20 nm². In model B, the molecules of TMPyP are tilted with respect to the surface with increasing surface pressure. Consequently, in this case the average area per TMPyP molecule is smaller than that expected for a single flat-lying molecule.

In the case of the model C two layers of porphyrin are present under the lipid matrix. The first layer of porphyrin is bound by electrostatic interactions between four molecules of DMPA (=four negative charges) and one molecule of porphyrin with four positive charges. In the second layer of porphyrin, the TMPyP molecules have still access to DMPA head groups and simultaneously interact with the porphyrin molecules of the first layer thus forming particular dimers. In this case, a flat orientation of the
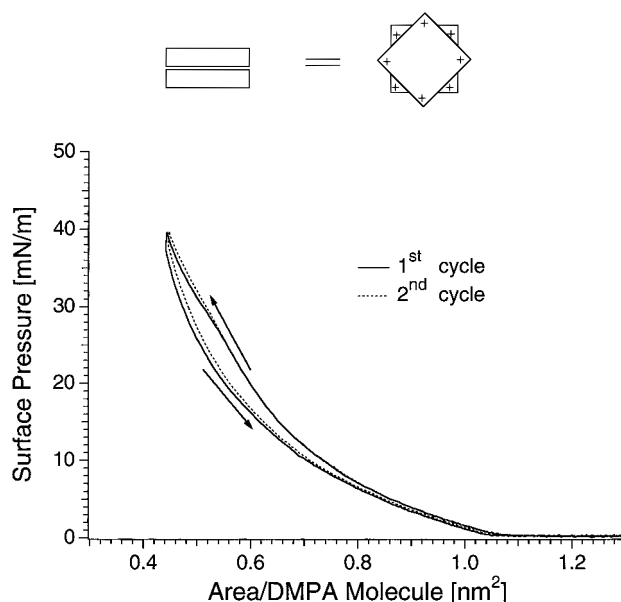
Chart 3

Figure 2. Hysteresis of a cospread monolayer of TMPyP:DMPA = 1:4: first compression−decompression (solid line); second cycle (dashed line); T = 21 °C.

porphyrin molecules with respect to the surface is assumed with a particular stacking as shown in Chart 3 (right).

In order to detect a possible loss of TMPyP molecules to the aqueous subphase, the monolayer stability at the air−water interface was tested (Figure 2). Figure 2 shows the π−A isotherms obtained for a monolayer of TMPyP/DMPA, molar ratio 1:4, during two successive cycles of compression and expansion of the monolayer by the movable barrier. A slight hysteresis between the compression and expansion isotherms is seen in both cycles. However, the hysteresis disappears at low pressure.

In addition, the monolayer of TMPyP:DMPA = 1:4 was compressed to a surface pressure of π = 35 mN/m, and the relaxation was followed with π kept constant by a feedback system during 4 h. The area per molecule of DMPA after 4 h was 0.39 nm², which is a small reduction of area compared to 0.41 nm² at time zero due to relaxation.

The result shown in Figure 2 indicates that the loss of TMPyP molecules from the mixed monolayer at the air−water interface to the aqueous subphase is negligible. The monolayer at the air−water interface is also very stable at high surface pressure corresponding to the solid condensed phase of the lipid monolayer.

The molecular organization of the porphyrin in the complex monolayer with DMPA requires a more detailed characterization. The structure of this mixed monolayer is governed by surface density, orientation of porphyrin relative to the monolayer plane, and porphyrin aggregation.

In order to discriminate between these structural models, additional information is required regarding density, stability, orientation, and nature of the interaction of TMPyP with DMPA molecules.

Reflection Spectroscopy at the Air−Water Interface. Direct evidence for the presence of TMPyP in the mixed monolayer with DMPA is obtained by measuring the reflection spectrum of the monolayer. This method[11] detects only those molecules which are at the interface and contribute to enhanced reflection from the air−water interface. Thus, to infer the molecular organization of the porphyrin in the cospread TMPyP:DMPA = 1:4 monolayer with increasing surface pressure, the reflection spectra were measured. The results are shown in Figure
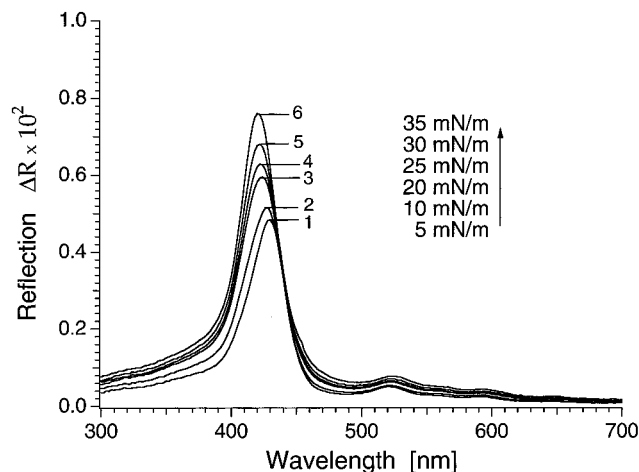
Figure 3. Reflection spectra of a monolayer of TMPyP:DMPA = 1:4 formed by cospreading at the air−water interface at different surface pressures: 5 mN/m (curve 1), 10 mN/m (curve 2), 20 mN/m (curve 3), 25 mN/m (curve 4), 30 mN/m (curve 5), and 35 mN/m (curve 6); $T = 21$ °C.

3. The reflection spectra of TMPyP are typical of a porphyrin; i.e., they consist of an intense Soret band and four weak Q bands. A blue shift of the Soret band with the increasing surface pressure is observed in the series of spectra. However, the shapes and positions of the Q bands are not changed. The maximum of the Soret band for the porphyrin in a TMPyP:DMPA = 1:4 monolayer at 35 mN m^{-1} at the air−water interface is at 420 nm, which is a shift of 10 nm to shorter waves with respect to the maximun wavelength in organic solution (430 nm) as well as in the monolayer at low surface pressure. This fact may be due to interactions between porphyrin molecules, which increase with increasing surface density when the surface pressure is enhanced.

The reflection ΔR is related to the absorption A by $\Delta R = R_i^{1/2}A$, according to a simplified model, where R_i is the reflectivity of the interface at normal incidence. ΔR has been shown to be proportional to the surface density at low values of ΔR[11]. The reflection ΔR at the maximum of the Soret band may be calculated from the surface density σ of the porphyrin in the mixed monolayer using the extinction coefficient at the wavelength of the maximum, λ_{max}, in solution provided that the shape of the absorption spectrum in solution is very similar to that of the reflection spectrum.

$$\Delta R = 2.303 \, f_{orient}\left[\frac{\epsilon}{\text{L mol}^{-1}\text{ cm}^{-1}}\right]1.66 \times 10^{-7} \times$$

$$\left[\frac{\sigma}{\text{nm}^{-2}}\right]R_i^{1/2} = 1.605 \times 10^{-2}\left[\frac{\sigma}{\text{nm}^{-2}}\right] \quad (1)$$

In eq 1 $f_{orient} = 1.5$ is a numerical factor that takes into account the different orientation of TMPyP in solution as compared to the monolayer at the air−water interface, $\epsilon = 2 \times 10^5$ L mol^{-1} cm^{-1} is the extinction coefficient of TMPyP in solution, and the value of $R_i^{1/2}$ is ≈0.14. The factor $f_{orient} = 1.5$ refers to a horizontal orientation of the porphyrin ring. The surface density of the porphyrin is taken from the π/A isotherm (Figure 2) assuming that no loss into the bulk occurs. We are using the decompression isotherm since the reflection spectra are measured for relaxed monolayers. At $\pi = 5$ mN/m, $\sigma = 0.29$ nm^{-2}, and from eq 1 the reflectivity $\Delta R = 0.477 \times 10^{-2}$ is calculated in excellent agreement with the experimental value 0.476×10^{-2}. The experimental results for different surface pressures are collected in Table 1.

Table 1. Values of ΔR at λ_{max} and Surface Densities of TMPyP in the TMPyP:DMPA = 1:4 Monolayer, at Various Surface Pressures

π (mN/m)	λ_{max} (nm)	ΔR (%)	A/TMPyP[a] (nm^2)	σ^a (nm^{-2})
5	430	0.4760	3.37	0.297
10	428	0.5082	2.82	0.355
20	424	0.5835	2.20	0.454
25	422	0.6162	2.02	0.495
30	422	0.6667	1.89	0.529
35	420	0.7416	1.79	0.558

[a] Area per molecule and σ, respectively, obtained from the curve upon decompression, first cycle, Figure 2.

The agreement of experimental and calculated reflectivity clearly shows that all molecules of porphyrin stay at the interface after spreading ($\pi = 0$ mN m^{-1}) in a flat orientation through compression to the surface pressure of 5 mN m^{-1}.

Since both, the hysteresis in Figure 2 and the agreement between experimental reflectivity ΔR and the value of ΔR calculated from eq 1 for a flat orientation of the porphyrin ring, indicate that all of porphyrin molecules remain at the interface, model A (Chart 2) for the structure of the mixed monolayer can be excluded.

Thus, an association of the porphyrin molecules in the head groups region of the complex monolayers at the air−water interface has to be considered that gives rise to the blue shift observed in Figure 3.

Analysis of Reflection Spectra during Compression. We assume an equilibrium between porphyrin monomers and particular dimers with a Soret band shifted to blue with respect to the monomers. According to the extended dipole model,[25] a blue shift of ≈9 nm is obtained for a porphyrin dimer with a twist of the parallel lying rings of 45° as shown in Chart 3, right (using eqs 3 and 8 of ref 25 with a dipole length of $l = 3.8$ Å, a charge of $\epsilon = 0.22e$, a dielectric constant of $D = 2.5$, and a thickness of the porphyrin of 3.5 Å). The fraction of monomers depends on the surface density of the porphyrin. Up to $\sigma_{TMPyP} = 0.31$ nm^{-2}, corresponding to an area per porphyrin of $A_{TMPyP} = 3.2$ nm^2, this fraction is $f_m = 1$ and the spectrum is that of monomeric porphyrin. We select the spectrum taken at 5 mN/m as that of the monomers. When the surface density σ of the porphyrin is larger than A_{TMPyP}^{-1}, a new phase II with porphyrin dimers is formed in equilibrium with the phase I of porphyrin monomers. Thus, the surface density of the porphyrin is

$$\sigma_{II} = 2(\sigma - \sigma_{TMPyP}) \quad (2)$$

and

$$\sigma_I = 2\sigma_{TMPyP} - \sigma \quad (3)$$

where σ is the average surface density of TMPyP as calculated from the area per porphyrin molecule (transformation of $\pi-A$ isotherm Figure 2).

The spectrum at 5 mN/m represents the monomer spectrum for a surface density $\sigma_1 = 0.29$ nm^{-2}. We determine the contributions of phases I and II to the measured spectra by first calculating the monomer spectrum for the values of σ_1 at each surface pressure as obtained from eq 3 and subtracting that contribution from the measured spectra; as an example σ_1 (20 mN/m) = $[0.62 - 1/(4 \times 0.56)]$ nm^{-2}, and therefore, the monomer contribution to the spectrum is ΔR (5 mN/m) $\times \sigma_1$ (20 mN/m)/σ_1 (5 mN/m). In this way we obtain the contribution of phase II (dimers). The result of this evaluation is

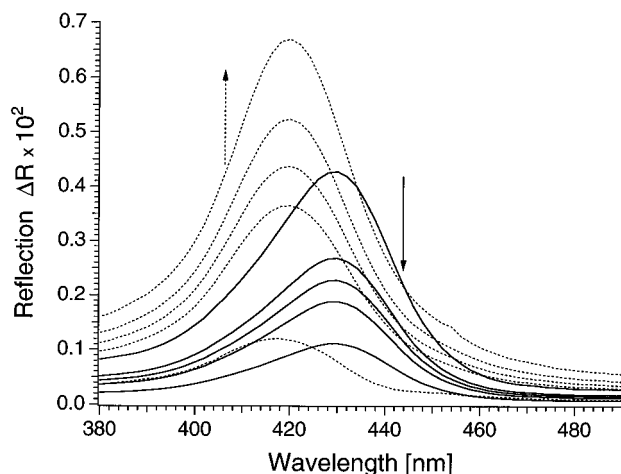(25) Czikkely, V.; Försterling, H. D.; Kuhn, H. *Chem. Phys. Lett.* **1970**, *6*, 207.

Figure 4. Contributions of phases I, porphyrin monomers (solid lines), and II, porphyrin dimers (dashed lines), at different surface pressures to the measured reflection spectra of a mixed monolayer TMPyP:DMPA = 1:4 shown in Figure 3. With increasing surface pressure, the monomer contribution diminishes and the dimer contribution increases.
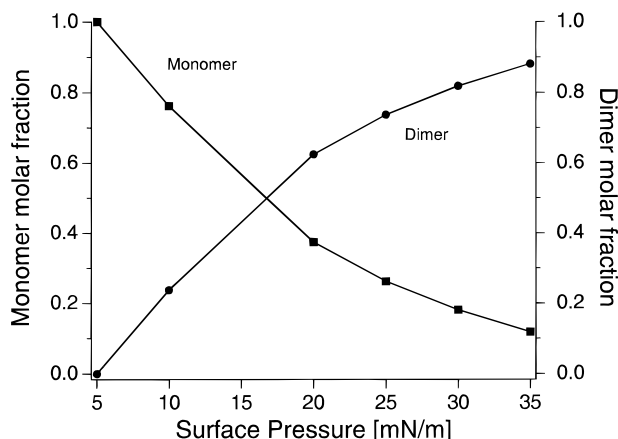

Figure 5. Molar fractions of monomers (■) and dimers (●) in the mixed monolayer TMPyP:DMPA = 1:4 plotted versus surface pressure.

shown in Figure 4 where the solid lines represent the monomer contribution and the dotted lines the dimer contribution. It is amazing that the shape of the evaluated contribution of the dimers is independent of the surface pressure.

Then, the fractions of porphyrin in the phases I (monomers) and II (dimers), respectively, are

$$ f_m = \frac{\sigma_I}{\sigma} = 2\,\frac{A_\pi}{A_{TMPyP}} - 1 \tag{4} $$

$$ f_d = \frac{\sigma_{II}}{\sigma} = 2\left(1 - \frac{A_\pi}{A_{TMPyP}}\right) \tag{5} $$

The molar fractions of the two species, monomers and dimers, are plotted in Figure 5 versus the surface pressure. Figure 5 shows the transformation of phase I to phase II with increasing surface pressure in the monolayer TMPyP:DMPA = 1:4 at the air−water interface. One interesting technique to investigate different phases at the air−water interface is the Brewster angle microscopy (BAM). Preliminary experiments have shown the coexistence of two phases at a surface pressure of 12 mN m⁻¹. Details about them will be published separately.

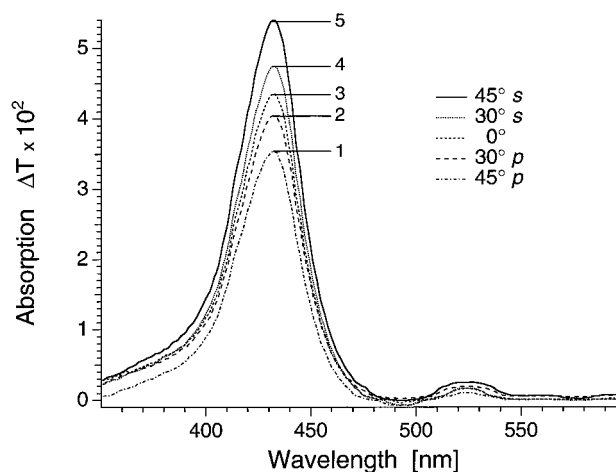We have assumed a horizontal orientation of the TMPyP molecules in the monolayer at the air−water interface in

Figure 6. Absorption spectra of one monolayer of TMPyP/DMPA (1:4) transferred on a glass plate at 35 mN m⁻¹ with plane polarized light under various angles of incidence with respect to the surface normal: 45° incidence, p polarization (electric vector the plane of incidence) (curve 1); 30° incidence, p polarization (curve 2); 0° incidence unpolarized (curve 3); 30° incidence, s polarization (electric vector perpendicular to the plane of incidence) (curve 4); 45° incidence, s polarization (curve 5).

this successful analysis in agreement with model C (Chart 2 and Chart 3, right). The interactions of the porphyrin rings in a tilted arrangement (model B) should have a shift of the Soret band to longer waves depending on the slip angle.[25]

Transferred Monolayer on Glass Substrate. An important aspect of the Langmuir−Blodgett technique is the preparation of a complex monolayer at the air−water interface and its subsequent transfer to a solid substrate. Thus, a single mixed monolayer of TMPyP/DMPA, molar ratio 1:4, was transferred to a clean hydrophilic glass plate by dipping the slide into the water, spreading and compressing the monolayer to π = 35 mN/m, and lifting the slide at a speed of 5 mm/min. In this way, the hydrophilic groups of porphyrin in the mixed monolayer stick to the hydrophilic glass surface. The transfer ratio was close to 1.
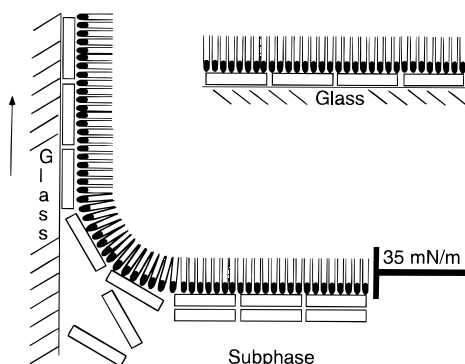
Absorption spectroscopy of transferred monolayers under oblique incidence is a useful tool to infer the average organization of the porphyrin in the mixed monolayers. The absorption spectra of transferred single monolayers on transparent support were measured as difference ΔT in transmission between a reference section and the sample section of the slide[20] with plane polarized light (s and p) under 0°, 30°, and 45° incidence (with respect to the surface normal). From such measurements the average orientation of the transition moments can be determined.[21] The results are shown in Figure 6.

For all spectra the maximum of the Soret band is at 432 ± 2 nm, very close to the value for TMPyP in CHCl₃/CH₃OH solution (430 nm). However, the maximum is shifted by ≈12 nm with respect to the wavelength for the monolayer TMPyP/DMPA, molar ratio 1:4, at 35 mN m⁻¹ at the air−water interface obtained by reflection measurement (see curve 6 in Figure 3). It is remarkable that the porphyrin in the transferred monolayer is predominantly in the monomer form.

According to Orrit et al.,[21] the ratios of the measured transmission difference ΔT_i (with i = s for s-polarized, i = p for p-polarized, and i = n for unpolarized light at normal incidence), $\Delta T_s/\Delta T_p$ and $\Delta T_s/\Delta T_n$ for 30° and 45° incidence, were calculated and the average orientation of the porphyrin in the transferred monolayer was determined. The results are shown in Table 2. The calculated

Table 2. Comparison of Measured and Calculated (for an Orientation of the Porphyrin Ring Parallel to the Monolayer Plane, Parentheses) Values of ΔT Ratios

	α	
	30°	45°
$\Delta T_s/\Delta T_p$	1.20 ± 0.10 (1.19)	1.51 ± 0.10 (1.50)
$\Delta T_s/\Delta Tn$	1.07 ± 0.05 (1.1)	1.19 ± 0.05 (1.24)

Chart 4

Figure 7. Reflection spectra of a mixed monolayer of TMPyP: DMPA:PME = 1:4:16 formed by cospreading at the air−water interface at different surface pressures: 5 mN/m (curve 1), 10 mN/m (curve 2), 30 mN/m (curve 3), and 35 mN/m (curve 4); subphase water, $T = 21$ °C.

Figure 8. Reflection and absorption spectra of a mixed monolayer of TMPyP:DMPA:PME = 1:4:16. ΔR of monolayer formed by cospreading at the air−water interface, curve a (solid line), and ΔT of a monolayer on glass transferred at π = 35 mN m^{-1}, curve b (dashed line); $T = 21$ °C.
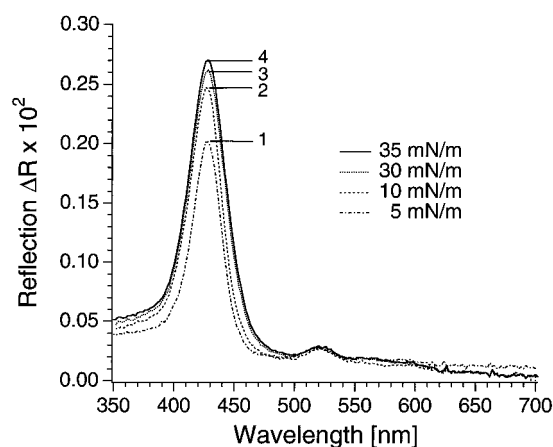
values for the ΔT ratios (in parentheses) are those expected for a flat orientation[21] of the porphyrin in the monolayer.

The transferred monolayer on glass shows the spectrum of the porphyrin monomers only. Consequently, when the mixed monolayer is transferred on glass, only those molecules of TMPyP that are directly underneath the head groups of DMPA are transferred. This means that approximately 50% (see model C in Chart 2) of the porphyrin is lost to the subphase during the transfer process (Chart 4), and we expect that the density of the porphyrin in the mixed monolayer at the air−water interface is approximately twice the density of the transferred monolayer on glass.

The relation between reflection and absorption is obtained by applying the model of Grüniger et al.[11] according to which $\Delta R = A R_i^{1/2}$, where R_i is the reflectivity of the clean interface; i.e. $R_i^{1/2}$ for air/water in the visible range. Approximating A with ΔT, we expect the ratio $\Delta T/\Delta R \approx 8.5$. The value of the ratio $\Delta T/\Delta R$ of the reflection and absorption at the maximum of the Soret band (see Figure 3, 35 mN/m, and Figure 6, 0°) for TMPyP:DMPA = 1:4 monolayer at 35 mN m^{-1}, is $\Delta T/\Delta R = 5.73$. This value clearly deviates from the value 8.5 expected for complete porphyrin transfer.

Organization of TMPyP in TMPyP/DMPA/PME, Ratio 1:4:16. In order to avoid the formation of monolayer phase with porphyrin dimers, the charge density of the dense-packed lipid part should be equal to the charge density of dense-packed porphyrin molecules.[23] By incorporation of the uncharged lipid methyl palmitate (PME) in the DMPA matrix, the charge density can be easily adjusted, and the mixed monolayer TMPyP:DMPA:PME = 1:4:16 was investigated, thereby matching the surface charge density of lipid part and porphyrin part of the mixed monolayer.[23] A value of $\Delta T/\Delta R$ close to 8.5 in this case would indicate complete transfer of the porphyrin, indirectly supporting the model C (Chart 2 and 3) for the mixed monolayer TMPyP/DMPA, molar ratio 1:4.

The reflection spectra of the mixed monolayer TMPyP/DMPA/PME, molar ratios 1:4:16, at the air−water interface at different surface pressures are shown in Figure 7. The position and shape of the Soret band remains
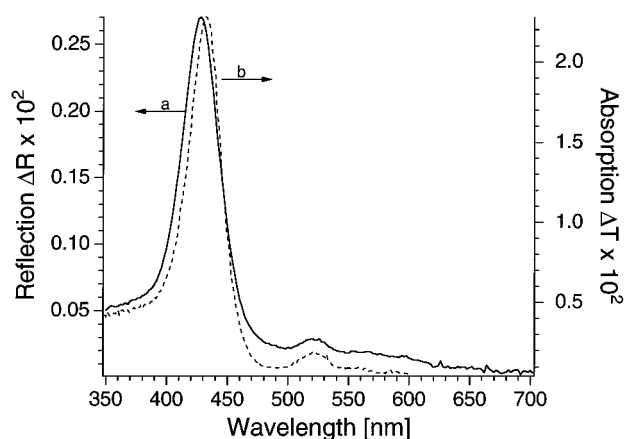
practically constant with maximum at 428 nm when increasing the surface pressure. No evidence for formation of porphyrin associates is observed in this mixed monolayer.

The mixed monolayer TMPyP:DMPA:PME = 1:4:16 was transferred on a glass plate under the same conditions as those used for TMPyP:DMPA = 1:4 ($\pi_{transfer}$ = 35 mN/m, speed$_{transfer}$ = 5 mm/min, T = 21 °C). The absorption spectrum is shown in Figure 8, along with the reflection spectrum taken at 35 mN/m from Figure 7. The ratio $\Delta T_{max}/\Delta R_{max} \approx 9$ is obtained in good agreement with the theoretically expected value of 8.5. The slight blue shift of the reflection spectrum (curve a in Figure 8) with respect to the absorption spectrum (curve b in Figure 8) may be related to the different refractive index of glass as compared to water.

Conclusions

The organization of the water-soluble tetracationic porphyrin, TMPyP, in complex monolayers with the anionic phospholipid, DMPA, formed by the cospreading method depends on the molar ratio of TMPyP/DMPA and the surface pressure. Different to a system of a tetracationic bis(bipyridinium) salt and DMPA in cospread monolayers,[24] the tetracationic porphyrin studied here forms a phase with porphyrin dimers. We attribute this

to a strong tendency of porphyrins to associate and to the accessibility of the lipid head groups to the second porphyrin layer by a particular stacking of the porphyrin rings. During the transfer of such complex monolayers, this second layer is released to the aqueous bulk phase. The molecular organization of the porphyrin may be controlled by addition of the third component with neutral head group. In a cospread mixed monolayer of neutral (PME) and anionic (DMPA) lipids and the cationic porphyrin (TMPyP) the charge densities of the lipid part and the porphyrin moiety can be matched, and dense-packed porphyrin monomers are then observed.

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