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X-ray Diffraction Study of the Effect of the Detergent Octyl Glucoside on the Structure of Lamellar and Nonlamellar Lipid/Water Phases of Use for Membrane Protein Reconstitution

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The phase behavior and structural parameters of dioleoylphosphatidylethanolamine (DOPE), monoolein (MO), and dipalmitoylphosphatidylcholine (DPPC) hydrated in excess aqueous phase containing micellar octyl glucoside (OG) were investigated by means of synchrotron X-ray diffraction in a wide range of temperatures. The lipid-to-detergent molar ratios were selected to be equal to those in a recent membrane-protein-reconstitution study. It was established that lipids displaying either nonlamellar (DOPE and MO) or lamellar propensities (DPPC) form mixed supramolecular structures with the detergent OG. The phase states and structures of these mixed assemblies are essentially different from those of the pure lipid components. At full hydration and at the investigated lipid-to-detergent molar ratios, OG destabilizes the inverted-hexagonal (H_{II}) phase organization of DOPE and the inverted cubic (Q^{224}) structure of MO, transforming them into a lamellar liquid crystalline (L_α) phase. The fluid DOPE/OG and MO/OG bilayers totally accommodate the micellar OG from the aqueous phase, as evidenced by the absence of diffuse scattering of OG or lipid/OG micelles. In the temperature interval from 0 to 52 °C, the investigated DOPE/OG/water and MO/OG/water systems are in the lamellar L_α state and do not undergo structural phase transitions. The DPPC/OG/water system undergoes a phase transition from a gel to a liquid-crystalline phase state. Due to the particular molar amount of OG in this system, a coexistence of mixed DPPC/OG bilayers and DPPC/OG micelles was found. The structural results suggest that the DOPE/OG and MO/OG systems, which are of lamellar rather than of nonlamellar organizations, are suitable for reconstitution of membrane proteins, as was established for DPPC/OG membranes.

Introduction

In the development of techniques for membrane-protein reconstitution involving the steps of membrane solubilization and protein delipidation and purification followed by subsequent incorporation into lipid membranes of defined composition and properties, the role of detergents such as octyl glucoside, Triton X-100, sodium cholate, CHAPS, and so forth is of crucial importance.[1−4] The detergent should be properly selected as to provide solubilization of the native biological membrane without denaturation of the intrinsic protein structure. In addition, it should be able to be reversibly removed from the lipid/protein system without distorting its physical and functional properties. To enhance the degree of membrane solubilization and the effectiveness of the processes of protein delipidation and reconstitution, a detergent is progressively added to a lipid/protein mixture to concentrations exceeding its critical micelle concentration (cmc). Under such conditions, detergents could cause considerable structural changes in the organization of the host lipid membranes.[5−7]

Because of its nonionic character and high cmc (∼25 mM),[8] octyl glucoside (OG) appears to be an effective surfactant in membrane-protein reconstitution.[1−3a] For bilayer membranes, the reversibility of the lamellae-to-micelle transition and the success of protein reconstitution when using of OG as a membrane-solubilizing detergent has been demonstrated.[8−10]

The fact that lipids hydrated in water may form not only bilayer membranes but also nonlamellar lipid phases,[11−13] which could affect the functioning of embedded

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Figure 1. CPK molecular models of *n*-octyl β-D-glucopyranoside (OG), monoolein (MO), dioleoylphosphatidylethanolamine (DOPE), and dipalmitoylphosphatidylcholine (DPPC). The bar corresponds to 1 nm.

or adsorbed proteins, has attracted significant research interest.[14−16] More often, techniques for the encapsulation of water-soluble (rather than of membrane-soluble) proteins into lyotropic phases formed by nonlamellar lipids have been reported. Thus, a variety of purified proteins, such as cytochrome c,[11,17] lysozyme,[18,19] casein,[20] α-chymotrypsin,[21] hemoglobin,[22] lipase from *Humicola lanuginosa*,[23] and so forth have been directly encapsulated into lipid/water phases of cubic symmetries without using detergents.

Recently, reconstitution of the membrane protein bacteriorhodopsin (BR) in nonlamellar lipid/water phases has been investigated with the aim of achieving protein crystallization[24] or of modulating the spectroscopic properties of the protein in organized lipid media.[25,26] In these BR reconstitution studies, OG has been employed as the most appropriate[9] detergent. To our knowledge, the influence of OG on the stability of nonlamellar lipid/water phases has not been investigated by structural methods so far. It is important to find out whether this detergent alters the nonlamellar structures characteristic of the host lipids or if it influences their stability at the concentrations employed for the membrane solubilization and protein reconstitution.

In the present study, the influence of OG on the structural organizations of three types of lipid/water phases of nonlamellar (cubic and inverted hexagonal) or lamellar bilayer periodicities is examined. The investigated lipids, monoolein (MO), dioleoylphosphatidylethanolamine (DOPE), and dipalmitoylphosphatidylcholine (DPPC), were typical representatives, in fully hydrated states and at a wide range of temperatures, of lipid species forming bicontinuous cubic Q^{224} ($Pn3m$), inverted hexagonal (H_{II}), and lamellar bilayer phases, respectively. (The supramolecular organizations of the pure lipids dispersed in water are presented in the left portion of Figure 6.) These lipids were hydrated in an excess of OG aqueous solution at lipid and detergent concentrations appropriate for membrane−protein reconstitution. The lipid-to-detergent molar ratios have been previously optimized[26] based on the reconstitution of the membrane protein BR.

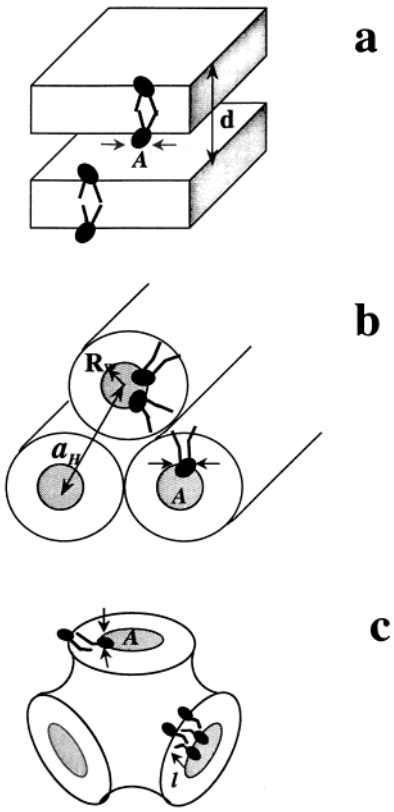

Figure 2. Schematic drawings of the structural elements of lamellar (a), inverted hexagonal (H_{II}) (b), and bicontinuous cubic Q^{224} (c) lyotropic phases. Notations: A, area per amphiphilic molecule at the headgroup/water interface of an amphiphilic assembly; d, repeat spacing of a lamellar phase; a_H, lattice parameter of an H_{II} phase; R_W, radius of the water tubes in an H_{II} phase; and l, thickness of a lipid monolayer. The shaded areas in schemes (b) and (c) represent cross-sections of aqueous channels. The molecular orientation at the headgroup/water interfaces is schematically indicated.

Here, synchrotron X-ray diffraction was applied for structure determination of the investigated lipid/detergent/water mixtures. X-ray diffraction data were collected during thermal scanning of the samples within a wide temperature range in order to study the eventual structural phase transitions of the hydrated MO/OG and DOPE/OG systems. It was established that OG essentially destabilizes the nonlamellar lyotropic phases of MO and DOPE, causing their transformation into lamellar ones. The critical molar fraction of OG in the lipid/OG mixtures, above which the detergent is expected to modify the

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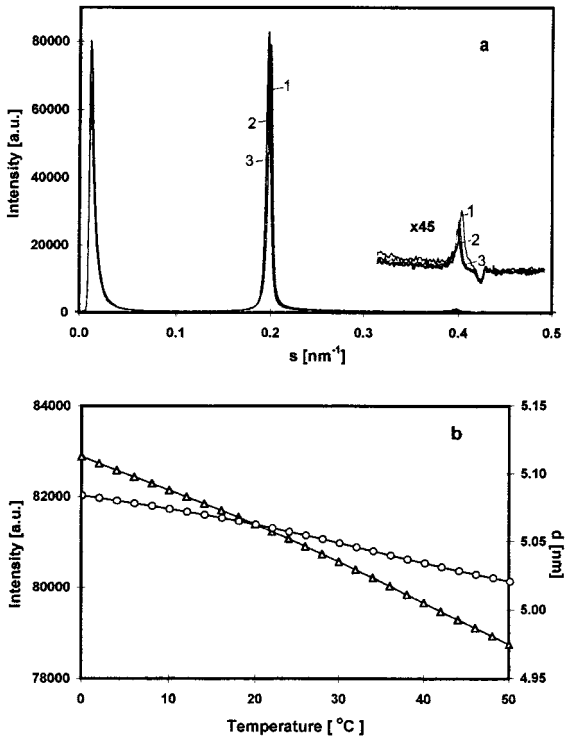

Figure 3. (a) X-ray diffraction patterns of DOPE hydrated in an excess of OG phosphate-buffer solution. The presented patterns were recorded at temperatures of 0 °C (1), 20 °C (2), and 50 °C (3). The lipid-to-detergent molar ratio is 1:1.55. (b) Temperature dependences of the intensity (triangles) of the first-order lamellar diffraction peak and of the *d* spacing (circles) of the lamellar lattice of the L_α-phase DOPE/OG/water system. The lamellar spacing, *d*, was determined as $d = 1/s$. The values plotted were obtained as a mean of the results for the first- and second-order Bragg peaks.

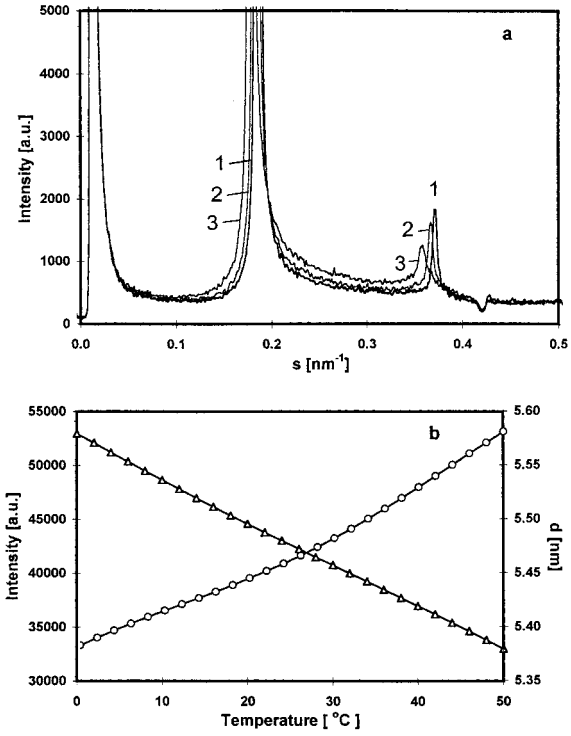

Figure 4. (a) X-ray diffraction patterns of MO fully hydrated in OG phosphate-buffer solution. The presented patterns were recorded at temperatures of 0 °C (1), 20 °C (2), and 50 °C (3). The lipid-to-detergent molar ratio is 1.36:1. (b) Temperature dependences of the intensity (triangles) of the first-order lamellar diffraction peak and of the *d* spacing (circles) of the lamellar lattice of the L_α-phase MO/OG/water system. The lamellar spacing, *d*, was determined as $d = 1/s$.

nonlamellar organizations of MO and DOPE, was estimated on the basis of the "molecular shape" concept.[27,28]

Methods and Materials

Chemicals and Sample Preparation. Lipid/detergent/water mixtures were prepared using chemicals from the following sources. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) (MW 734.05) was supplied from Avanti Polar Lipids Co. (Alabama). l-α-Dioleoylphosphatidylethanolamine [C18:1, *cis*-9] (DOPE) (MW 744.0), 1-monooleoyl-*rac*-glycerol [C18:1, *cis*-9] (MO) (MW 356.5), and *n*-octyl β-D-glucopyranoside (OG) (MW 292.4) were from Sigma Co. (France). Phosphate buffer with a concentration of 10^{-2} M was prepared by dissolving inorganic salts (NaH_2PO_4/Na_2HPO_4 (1:1 mol/mol) p.a. grade (Merck)) in doubly distilled pure water.

The molar ratios between the lipid and the detergent components in the investigated samples were chosen to be equal to the optimized values employed in the membrane-protein reconstitution study,[26] namely, DOPE/OG 1:1.55 (mol/mol) (system 1), MO/OG 1.36:1 (mol/mol) (system 2), and DPPC/OG 1:1.97 (mol/mol) (system 3). The corresponding detergent-to-lipid molar ratios, $R_{OG/Lipid}$, are given in Table 1. Samples were prepared by hydration of dry lipid powders (DOPE, MO, and DPPC) in excess aqueous phase containing OG phosphate-buffer solutions. The concentration of the solid lipid in the aqueous dispersions was 20 wt % (i.e., in the range from 0.3 to 0.7 M for the different lipid types). The concentration of OG in the aqueous phase was determined by the desired lipid-to-OG molar ratios and fell in the range from 13.1% to 16.6 wt % (i.e., about 0.5−0.7 M). Hydration of the lipid powders, to yield dispersions of lipids in the OG solution, was performed at temperatures of 10 °C (system 1), 24 °C (system 2), and 50 °C (system 3). For every system, eight cycles of vortexing (for 1 min) and incubation (for 5 min) at these temperatures were applied. After the homogenization, the hydrated samples were equilibrated for 24 h at 4 °C and sealed in glass X-ray capillaries with a diameter of 1.4 ± 0.1 mm. They were stored in a refrigerator at 4 °C before measurements were made.

X-ray Diffraction Measurements and Data Processing. The structure of the lipid/detergent/water mixtures was investigated by means of X-ray diffraction. Synchrotron X-ray diffraction measurements were performed at the D24 beam line at LURE in Orsay, France. The principle of the experimental setup was analogous to that described in ref 29. Two detectors covering the small-angle (SAX) and the wide-angle (WAX) diffraction regions were used. Data were collected using the OTOKO software.[30]

The structural measurements were performed as follows. The samples were positioned in a X-ray setup holder,[31] the temperature of which was continuously controlled by means of a Peltier element during thermal scanning from 0 °C to 52 °C. The capillaries were continuously heated from 0 to 52 °C at a heating scan rate of 1 Kelvin/min. The acquisition of the X-ray diffraction data was performed at definite time intervals, which corresponded to a temperature difference of 2 K between subsequent measurements. It was suggested that the employed heating rate be sufficiently slow to allow equilibration of the samples at every measurement. The exposure time was 60 s. For the DOPE/OG/water and the MO/OG/water mixtures, 26 diffraction patterns

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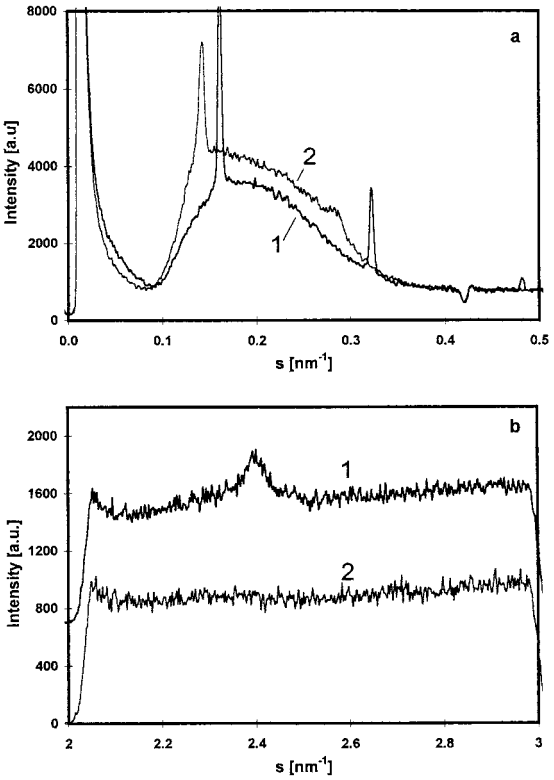

Figure 5. (a) X-ray diffraction patterns of DPPC hydrated in an excess of OG phosphate-buffer solution. The patterns were recorded at temperatures of 20 °C (1) and 30 °C (2). The lipid-to-detergent molar ratio is 1:1.97. The corresponding *d* spacing values are given in Table 1. (b) WAX patterns of the DPPC/OG/water system ($R = 1.97$) at temperatures of 20 °C (1) and 30 °C (2).

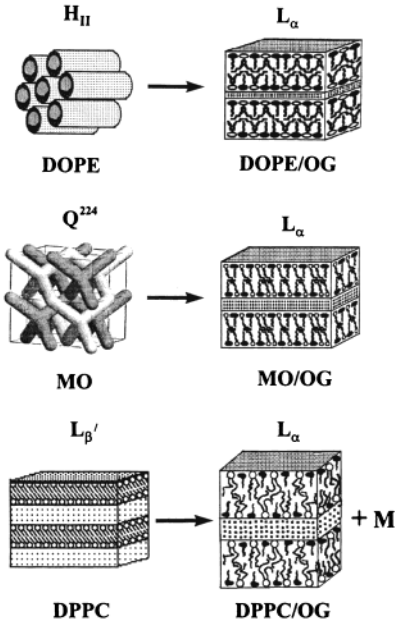

Figure 6. Scheme of the structural phase changes of lyotropic phases of DOPE, MO, and DPPC upon mixing of these lipids with the detergent OG at a temperature of 30 °C. The polar groups of OG are drawn in black. Notations: Q^{224}, inverted bicontinuous cubic phase (space group $Pn3m$) of MO hydrated in excess water; H_{II}, inverted hexagonal phase of DOPE hydrated in excess water; $L_{\beta'}$, gel phase of fully hydrated DPPC with ordered, tilted hydrocarbon chains; L_α, lamellar liquid-crystalline phase of fluidlike hydrocarbon chains.

were recorded on thermal scanning from 0 to 52 °C. The diffraction patterns of the DPPC/OG/water mixture were recorded at temperatures of 20 and 30 °C, which were respectively below and above the main phase transition temperature of the system, determined in the study of Dahim.[5]

Using OTOKO software, the recorded one-dimensional X-ray diffraction data were presented as intensities versus reciprocal spacings (*s*). The latter were defined as $s = (2/\lambda) \sin \theta = 1/d$, where 2θ is the scattering angle, λ is the X-ray wavelength, and *d* is the repeat spacing. For determination of the *s* values, from which the structural parameters of the hydrated lipid/detergent mixtures could be estimated, the stable β form of tristearin (obtained from solvent recrystallization) was employed as a calibration sample. Its characteristic lamellar spacing, $d_{001} = 4.497$ nm, was used for the calibration in the SAX region. In the region covered by the WAX detector, the tristearin calibration sample exhibited three strong peaks of *d* spacings equal to 0.459, 0.385, and 0.370 nm. The negative peaks emerging in the recorded X-ray diffraction patterns at $s \sim 0.42$ nm^{-1} (see Figures 3a, 4a, and 5a) were due to a detector artifact.

Structural Elements of Nonlamellar and Lamellar Lyotropic Phases and Structural Formalism for the Determination of Molecular Parameters from X-ray Diffraction Results. To define the lattice and molecular parameters determined from the X-ray diffraction data, the structural elements of lamellar, inverted hexagonal, and bicontinuous cubic phases are schematically presented in Figure 2. The dimensions of the structural elements of the lipid/water phases, from which the values of the molecular areas at the lipid headgroup/water interfaces were deduced, were estimated according to the structural formalism developed previously[32−35] and the X-ray diffraction data for DPPC, DOPE, and MO hydrated in water.

The repeat unit of a lamellar lattice contains an amphiphilic bilayer and a thin aqueous layer, that is, the periodicity of the lattice is one-dimensional (Figure 2a). In X-ray diffraction patterns, lamellar lattices display Bragg X-ray peaks with *s* values spaced in the ratio 1:2:3:4: .... The repeat *d* spacing of a lamellar structure could be determined from X-ray diffraction plots as a mean value of the spacings $d_i = i/s_{00i}$, where *i* denotes the Bragg peak's order number.

The area per lipid molecule, *A*, in a lamellar phase structure (in our case DPPC) is given by

$$A = 2M/(df_L N_A v_L) \quad (1)$$

where *M* is the molecular weight of the lipid, *d* the repeat spacing of the lamellar lattice, N_A Avogadro's number, v_L the partial specific volume of lipid, and f_L the lipid volume fraction defined as

$$f_L = \left[1 + \frac{(1 - c_L)v_W}{c_L v_L}\right]^{-1} \quad (2)$$

In eq 2, c_L is the lipid weight fraction and v_W the partial specific volume of water (= 1.0 cm^3/g). For DPPC, v_L was taken as 0.99 cm^3/g at 20 °C. In estimating the dimensions of the structural elements of the lipid phases, the values of f_L were taken at the limiting hydration[32] of the lipids.

In an inverted hexagonal (H_{II}) phase, the amphiphilic molecules are organized as monolayers around nearly cylindrical aqueous tubes. The cross section of the tubes is given in Figure 2b. The H_{II} phase is of a two-dimensional periodicity, and its lattice parameter, a_H, defines the nearest-neighbor distances of the water-core centers of the cylindrical lipid aggregates, which are periodically arranged in a hexagonal packing. The *s* values of the Bragg X-ray peaks displayed by an H_{II} phase structure are spaced in the ratio $1:\sqrt{3}:\sqrt{4}:\sqrt{7}$: ... The lattice parameter could be estimated from the first-order Bragg X-ray peak using the relationship $a_H = d\ (2/\sqrt{3})$, where $d = 1/s_{10}$.

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Table 1. Structural Parameters of the Investigated Lipid/Detergent/Water Mixtures at Selected Temperatures[a]

lipid/OG ratio (mol/mol)		$R_{OG/Lipid}$	d [nm]	temperature [°C]			
				0	20	30	50
DOPE/OG	1:1.55	1.55	d	5.083	5.062	5.048	5.020
MO/OG	1.36:1	0.73	d	5.382	5.445	5.482	5.581
DPPC/OG	1:1.97	1.97	d	n.m.	6.206	7.008	n.m.
			d_{wax}	n.m.	0.417 0.403[w]	none	n.m.
			d_M	n.m.	5.464	5.814	n.m.

[a] Samples were prepared by hydrating DOPE, MO, or DPPC powders in excess aqueous phase (phosphate-buffer solution) containing micellar OG. d, d_{wax}, and d_M denote, respectively, the long spacing of the lipid lamellae (SAX region), the short spacing of the lateral lipid packing (WAX region), and the spacing corresponding to the peak maximum of the micellar phase in Figure 5a. The values of d were determined as $d = 1/s$. Notations: $R_{OG/Lipid}$, detergent-to-lipid molar ratio; n.m., not measured; w, weak reflection.

The area per molecule, A, at the lipid headgroup/water interface of an H_{II} phase (here DOPE) is determined by the equation

$$A = \frac{4}{\sqrt{3}}\pi R_W M/(a_H^2 f_L N_A v_L) \quad (3)$$

where a_H is the lattice parameter of the inverted hexagonal structure, R_W is the radius of the water cores of the curved lipid monolayers that are assumed to form cylindrical tubes in an H_{II} phase, and M, f_L, N_A, and v_L are as defined above. Using a known value of a_H for the DOPE dispersion at a constant temperature (e.g., 20 °C), the radius of the aqueous tubes arranged in a hexagonal packing could be estimated from the equation

$$R_W = a_H\sqrt{\sqrt{3}(1 - f_L)}/\sqrt{2\pi} \quad (4)$$

and employed in the determination of the surface area per molecule in an H_{II} phase (eq 3).

In a bicontinuous cubic Q^{224} phase, two independent networks[11a] of aqueous tubes are separated by a continuous bilayer of lipid molecules. A schematic cross-sectional view of the rodlike structural elements, connected at tetrahedral angles in the water-channel junctions of the structure, is shown in Figure 2c. The three-dimensional arrangement of the aqueous channels is of a double-diamond type. The periodicity of the cubic Q^{224} lattice is defined by the cubic lattice parameter, a_Q, which is determined using characteristic X-ray diffraction peaks with reciprocal spacings spaced in the ratio $\sqrt{2}$:$\sqrt{3}$:$\sqrt{4}$:$\sqrt{6}$:$\sqrt{8}$:$\sqrt{9}$:$\sqrt{10}$:$\sqrt{12}$:$\sqrt{14}$....

The area per lipid headgroup, A, at the lipid/water interface in a bicontinuous Q^{224} cubic phase is given by eq 5.[35] For calculation of A (e.g., in the MO cubic structure), the cubic lattice parameter, a_Q, of the fully hydrated lipid should be known. In the equation

$$A = 2M(\sigma a_Q^2 + 2\pi\chi l^2)/(a_Q^3 f_L N_A v_L) \quad (5)$$

χ and σ are constants ($\chi = -2$ and $\sigma = 1.919$ for the Q^{224} cubic phase), and l is the thickness of the lipid monolayers. The lipid length, l, was calculated from the equation[35]

$$f_L = 2\sigma(l/a_Q) + \frac{4}{3}\pi\chi(l/a_Q)^3 \quad (6)$$

The partial specific volume of MO, v_L, was taken as 1.06 cm^3/g at 20 °C, and that of water, v_W, was 1.0 cm^3/g.

Micellar phases are known to display broad diffuse bands in their X-ray patterns.[36] The inverted spacing ($s = 1/d_M$), corresponding to the maximum of the observed X-ray diffraction peak, could be used for determination of the mean intermicellar distance between micelles (center-to-center spacing). The detection limit of micellar phase organizations in synchrotron X-ray diffraction experiments is very low and depends on the concentration of the detergent (and/or lipid) and the exposure time.[31b]

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Results

Structural Organizations of the Pure Lipids DOPE, MO, and DPPC Fully Hydrated in Water. Our X-ray diffraction experiments on the organization of the pure lipids hydrated in water yielded structural results which are in a good agreement with those reported by other authors for hydrated phases of DOPE,[37] MO[11a,38,39] and DPPC.[3b,40,41] The recorded X-ray diffraction patterns of the pure lipids in excess water and their temperature dependences are not shown here. When fully hydrated in the aqueous phase, pure DOPE undergoes a lamellar-to-hexagonal (L_α-H_{II}) phase transition within the temperature range from about 5 to 9 °C. At nonzero temperatures, L_α and H_{II} phases coexist, and the lamellar L_α phase is destabilized above 5 °C. The inverted hexagonal H_{II} phase is characteristic of the lipid dispersion at temperatures higher than ∼ 9 °C and is stable upon heating to very high temperatures (at least 90 °C). At 20 °C, the lattice parameter (a_H) of the inverted hexagonal phase of DOPE in water was determined to be 7.6 nm.

Upon hydration in the excess water phase, pure MO spontaneously self-assembles into a bicontinuous cubic phase Q^{224} structure (of the space group $Pn3m$). The cubic phase is stable over a wide temperature interval from 0 to about 94 °C. Above this temperature, a phase transition from the Q^{224} cubic phase to an inverted hexagonal (H_{II}) phase occurs. MO does not form lamellar bilayer phases in its fully hydrated state. The cubic-cell-lattice parameter (a_Q) of the Q^{224} cubic structure of MO in excess water is 10.8 nm at a temperature of 20 °C.

At full hydration, DPPC undergoes temperature-induced phase transitions with the following sequence of lamellar phases in pure water: crystalline sub-gel (L_c) ↔ gel ($L_{\beta'}$) ↔ ripple gel ($P_{\beta'}$) ↔ liquid crystalline (L_α) phase. The phase transition temperatures in the above sequence are 17 °C (subtransition), 35 °C (pretransition), and 41.5 °C (main transition). The bilayer repeat spacings, d, of the lamellar phases of DPPC at selected temperatures are: 5.97 nm (L_c phase at 15 °C), 6.4 nm ($L_{\beta'}$ phase at 20 °C), 7.2 nm ($P_{\beta'}$ phase at 40 °C), and 6.65 nm (L_α phase at 43 °C). The short, in-plane spacings of the $L_{\beta'}$ gel-phase DPPC measured at 20 °C in the WAX region are $d_1 = 0.42$ nm and $d_2 = 0.41$ nm.

In the following sections, X-ray diffraction results are presented on the structural organization of the investi-

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gated three-component lipid/detergent/water systems at chosen lipid-to-detergent molar ratios.

DOPE/Octyl Glucoside/Water System. X-ray diffraction patterns of DOPE fully hydrated in the OG phosphate-buffer solution were recorded in the interval from 0 to 52 °C with a step of 2 °C. They showed monotonic thermal behavior with an absence of phase transitions in the investigated temperature range. The patterns corresponding to 0, 20, and 50 °C are presented in Figure 3a. They demonstrate that, at the investigated DOPE/OG molar ratio of 1:1.55, the detergent totally alters the phase behavior of pure DOPE. The first two orders of diffraction peaks observed in the SAX region determine a lamellar structure for the hydrated DOPE/OG mixture. The DOPE/OG bilayers are in the fluid, liquid crystalline (L_α) state, as indicated by the absence of WAX reflections typical for gel and crystalline lipid phases (patterns in the WAX region in the interval 0−52 °C are not shown here).

The *d*-spacing variation of the lamellar lattice of the DOPE/OG dispersion is plotted versus temperature in Figure 3b (circles). The values of *d* were determined as $d = 1/s$ and were averaged for the first- and second-order Bragg peaks. Upon rising the temperature from 0 to 50 °C, the *d* spacing of the bilayer structures decreases from 5.08 to 5.02 nm (Table 1). This is accompanied by a decrease of the intensity of the diffraction peaks. Figure 3b (triangles) shows that the total reduction of the first-order-peak intensity with temperature is about 5% within the investigated temperature range. The decrease, Δd, of the *d* spacing on heating is also slight: $\Delta d = 0.06$ nm over a temperature interval of 50 °C.

At the same temperature (e.g., 0 °C), the *d* spacing of the liquid-crystalline L_α-phase DOPE/OG bilayers (Table 1) is about 0.2 nm smaller compared to the *d* spacing of pure DOPE L_α-phase bilayers formed in excess water below 5 °C ($d = 5.3$ nm). Obviously, incorporation of the short-chain surfactant OG into the DOPE supramolecular organization leads to thinning of the lipid lamella.

The X-ray diffraction patterns of the DOPE/OG mixture showed structural features distinct from those of the individual, lipid, and detergent components. Peaks characteristic of an inverted hexagonal (H_{II}) phase of pure DOPE, as well as diffuse scattering typical for the micellar phase of OG, were missing in the recorded patterns in the entire interval from 0 to 52 °C. This indicates that the DOPE and OG components of a molar ratio 1:1.55 form mixed bilayer structures rather than coexisting as phase-separated domains in the investigated aqueous dispersion.

Monoolein/Octyl Glucoside/Water System. Figure 4a shows X-ray diffraction patterns of a MO/OG/water system recorded at temperatures of 0, 20, and 50 °C and a lipid-to-detergent molar ratio of 1.36:1. With this three-component system, phase transitions were not established upon thermal scanning of the samples within the interval from 0 to 52 °C (with a temperature step of 2 °C). At every temperature studied, the structural organization of the MO/OG system hydrated in excess aqueous phase was lamellar. The MO/OG bilayers were of a fluid nature in the entire range from 0 to 52 °C. No gel-phase reflections were recorded in the WAX region covered by the detector (WAX patterns in the range from 0 to 52 °C are not shown here).

The change of the *d* spacing of the L_α-phase MO/OG bilayers with temperature is presented in Figure 4b (circles). The lamellar spacing increases from 5.38 to 5.58 nm as the temperature is raised from 0 to 50 °C (Table 1). This implies swelling of the MO/OG bilayer structure upon heating. The established thermal expansion behavior shows an opposite tendency compared to that of the DOPE/OG system (Figure 3b, circles), which displayed a decrease of lamellar spacing with a rise in temperature. The intensity of the diffraction peaks of the lamellar L_α-phase MO/OG structures decreased with temperature, as shown in Figure 4b (triangles). Within experimental scattering, the ratio of the intensities of the second-order to first-order Bragg peaks was almost constant, $I_{002}/I_{001} = 0.037 \pm 0.001$, and was invariant of temperature. The total decrease of intensity was about 37% in the interval from 0 to 50 °C. This percent was larger compared to the case of the DOPE/OG system (Figure 1b, triangles), confirming the different thermal behavior of the two kinds of lipid/detergent bilayers. The differences in the thermal expansion behavior might result from different hydration of the headgroup regions of the MO/OG and DOPE/OG bilayers. Owing to a strong hydrogen bonding affinity, the phosphatidylethanolamine headgroups of DOPE interact weakly with water, and the bilayer period is slightly temperature dependent. At 20 °C, the *d*-spacing of the MO/OG bilayers is about 0.4 nm larger compared to that of the DOPE/OG lamella (Table 1).

The structural results in Figure 4 indicate that MO forms mixed structures with OG upon its hydration in the micellar solution of the detergent under excess water conditions. Although in the investigated MO/OG system, the detergent was present in a lower molar amount compared to the lipid ($R_{OG/Lipid} = 0.73$), structural features characteristic of the excess component, MO, were not observed. In the recorded X-ray patterns, reflections of a cubic-phase structure, characteristic of pure MO in excess water, were absent within the entire temperature range studied, from 0 to 52 °C. The established bilayer supramolecular periodicity of the MO/OG mixtures presents structural evidence that MO does not separate in domains of a cubic-phase organization and that OG is not present as distinct micelles in the dispersion system. This implies that the nonlamellar lipid MO and the detergent OG are homogeneously mixed in the L_α phase bilayers formed and are in equilibrium with monomeric OG from the aqueous phase (the MO solubility in water is very low and could be considered as negligible).

DPPC/Octyl Glucoside/Water System. The lipid-to-detergent molar ratio in the investigated system was 1:1.97. The phase diagram and thermal behavior of DPPC/OG/water mixtures have been studied in detail by Dahim.[5] Because the composition of the aqueous buffer and the *R* value in the membrane-protein-reconstitution system[26] were not exactly the same as those in the experiments of Dahim,[5] we have independently measured the precise structural parameters of the DPPC/OG/water system at $R_{OG/Lipid} = 1.97$ and at particular temperatures. It was taken into account that the gel-to-fluid phase transition of the DPPC/OG/water mixture occurs in a narrow temperature range[5] around 25 °C (at $R = 2.17$) to 27 °C (at $R = 1.35$). Thus, the structural results presented here refer to two temperatures, which are below (20 °C) or above (30 °C), the main phase transition temperature of the lipid/detergent mixture in excess water.

Figure 5 shows X-ray diffraction patterns of the DPPC/OG/water system ($R = 1.97$) at temperatures of 20 and 30 °C. The X-ray patterns recorded in the SAX region (Figure 5a) reveal structural features suggesting a coexistence of lamellar and micellar phases. At both temperatures, the lamellar peaks are superimposed on a diffuse micellar band in the patterns.

At 20 °C, three Bragg peaks of a lamellar bilayer phase were recorded (Figure 5a, curve 1). The *d* spacing determined for the DPPC/OG bilayers at 20 °C was 6.206 nm (Table 1). Figure 5b (curve 1) shows the diffraction

pattern recorded in the WAX region. A well-defined WAX reflection with an s value of 2.396 nm^{-1} and a weaker one with $s = 2.484$ nm^{-1} were observed. They indicate that the DPPC/OG bilayer is in a gel-phase state at 20 °C. The short spacings, d_{wax}, of the gel-phase lamellar structure are 0.417 and 0.403 nm (Table 1). The peaks obtained may arise from a two-dimensional distorted hexagonal[3b] lattice of the lipid hydrocarbon chains.

The intensity of the lamellar peaks decreased upon increasing the temperature, which resulted in the observation of a strong first-order Bragg peak and a weak second-order peak at 30 °C (Figure 5a, curve 2). The d spacing of the mixed DPPC/OG bilayers at this temperature was 7.008 nm. The absence of WAX peaks at 30 °C (Figure 5b, curve 2) is a result of a phase transition to a fluid-phase-state bilayer structure of disordered hydrocarbon chains. The lattice parameter, d, of the fluid L_α-phase DPPC/OG bilayer is larger in comparison to that of the gel-phase DPPC/OG bilayer (Table 1).

At both investigated temperatures, 20 and 30 °C, diffuse scattering typical for the presence of micellar aggregates in the DPPC/OG dispersion system was established in the recorded X-ray diffraction patterns (Figure 5a). The micellar peaks were broad and asymmetrical in shape, as were those for pure OG.[5] The s values of the peak maxima were determined by numerical fitting of the experimental curves. The corresponding d spacings, d_M, of the micellar-phase DPPC/OG aggregates were 5.464 nm at 20 °C and 5.814 nm at 30 °C (Table 1).

The diffraction patterns in Figure 5 indicate that DPPC and OG form mixed DPPC/OG bilayers at $R = 1.97$. The excess amount of OG, which could not be accommodated in the lamellar amphiphilic matrix, is separated in the form of micellar aggregates ("M" phase) containing equilibrium amounts of lipid. The coexisting mixed DPPC/OG bilayers and DPPC/OG micelles are in equilibrium with OG monomers in the aqueous phase and result in a three-phase system.

Discussion

Questions of major importance for the feasibility and effectiveness of detergent-mediated membrane-protein reconstitution into lyotropic phases formed by nonlamellar lipids are: (i) whether the detergent (OG) is capable of mixing with the nonlamellar-phase forming lipids (DOPE and MO) upon their hydration in OG micellar solutions or whether OG remains in the form of phase-separated micelles in the aqueous dispersion; and (ii) what kind of structural organization adopts the mixed lipid/OG assemblies, into which the reconstitution of membrane protein occurs: nonlamellar, lamellar, or micellar.

Depending on their concentrations in lipid/detergent/water systems, detergents could considerably affect the phase states and structural parameters of lipid membranes and amphiphilic lipid assemblies; for example, for DPPC/OG[5] and egg PC/OG[6,7] systems, phase transitions between lamellar, micellar, and mixed phases with coexisting lamellar and micellar domains have been reported with variation of the OG concentration. With another nonionic surfactant, diethyleneoxidemonododecyl ether[42a] ($C_{12}EO_2$), induction of two types of cubic phases (Q^{230} and Q^{224}), an inverted hexagonal (H_{II}) phase as well as lamellar bilayer phases (L_α and $L_{\beta'}$), has been established[42b] upon mixing with palmitoyloleoylphosphatidylcholine (POPC) at diverse molar ratios. The above examples demonstrate the complex influence of surfactants on the phase behavior of host lipid systems and the necessity of precise structural characterization of mixed lipid/surfactant dispersions of particular compositions. Taking this into account, the reconstitution of membrane proteins into nonlamellar lipid/water phases cannot be presumed a priori in the cases where detergents are used as solubilizing agents. Structural measurements should establish whether the membrane protein reconstitution indeed occurs into nonlamellar lipid phases or into lyotropic systems of other supramolecular organizations.

The present X-ray diffraction study revealed that the addition of the detergent OG to lipid systems of nonlamellar structures could essentially modify their organizations and structural parameters. The structural changes induced by OG in lipid/detergent aqueous dispersions at constant temperature are schematically shown in Figure 6. The structural dimensions of the investigated lipid/OG mixtures at selected temperatures are summarized in Table 1. Transformations from inverted hexagonal (H_{II}) and inverted cubic (Q^{224}) phases into a lamellar bilayer L_α phase were established for DOPE and MO, respectively, in the presence of OG. The structural results obtained (Figures 3 and 4) demonstrate that DOPE and MO, hydrated in excess aqueous phase containing OG micelles, form mixed lipid/detergent structures of a lamellar supramolecular periodicity and scattering of a coexisting micellar phase of OG is lacking in the recorded patterns. This means that the detergent is accommodated in the mixed lipid/OG lamellae, which are in equilibrium with OG monomers dissolved in the aqueous phase. As a result, the hydrated DOPE/OG and MO/OG systems display a single lyotropic (L_α) phase at the investigated lipid-to-detergent molar ratios and temperature ranges.

The X-ray diffraction results in Figure 5 indicate that the bilayer-forming lipid DPPC assembles with the detergent OG into mixed bilayer structures, which coexist with mixed DPPC/OG micelles and with dissolved OG monomers. The structural dimensions of the mixed DPPC/OG bilayers were compared to those of a pure DPPC dispersion. The d spacing of gel-phase DPPC/OG bilayers at 20 °C (6.206 nm) is smaller compared to that of DPPC in the $L_{\beta'}$-gel phase state (6.4 nm). Due to the incorporation of the short (C_8) chain surfactant OG, the mixed DPPC/OG lamellae are thinner, which might be associated with a more tilted organization of the lipid acyl chains. The presence of OG also affects the lateral packing and order of the lipid tails. The diffraction peaks in the WAX region of the mixed DPPC/OG bilayer (Figure 5b, curve 1) suggest the formation of a 2D distorted hexagonal lattice of different in-plane parameters compared to those of the single component DPPC in the $L_{\beta'}$-gel-phase state. The gel phase formed by the mixed DPPC/OG bilayers has been denoted[5] as an L_S phase. Obviously, the structural organization of the L_S phase is different from that of the $L_{\beta'}$-gel phase of pure DPPC.

Whereas the DPPC/OG bilayers are in a gel phase at 20 °C, they are in a fluid (L_α) phase at 30 °C. The structural studies showed that, at a given temperature and at $R = 1.97$, the DPPC/OG/water system displays a coexistence of two lyotropic phases (a lamellar phase and an "M" phase) (Figure 6).

Application of the "Molecular Shape" Concept to Suggest the Supramolecular Organization of Mixed Lipid/Detergent Assemblies. The established structural transformations (Figure 6) could be understood analytically in terms of geometrical packing factors, considering the molecular shapes and dimensions of the investigated lipid and detergent molecules (Figure 1).

(42) (a) Funari, S.; Mädler, B.; Rapp, G. *Eur. Biophys. J.* **1996**, *24*, 293−299. (b) Funari, S.; Rapp, G. *J. Phys. Chem.* **1997**, *101*, 732−739.

According to the self-assembly theory of lipid aggregates,[27] the type of supramolecular organization, which an amphiphile will adopt in water could be suggested on the basis of its molecular shape through a geometrical "packing" parameter defined as

$$\eta_i = V_{hc}/l_{hc}A \quad (7)$$

where V_{hc} is the volume of the hydrocarbon chains of the molecule, l_{hc} is the maximum, extended-state hydrocarbon chain length, and A is the optimal cross-sectional area per molecule at the polar headgroup/water interface of the lipid assembly. Thus, on the basis of the ratio of the lipid acyl-chain and headgroup areas, lamellar bilayer organization should be typical for amphiphiles of a "cylindrical" molecular shape,[27] characterized by an η_i value less than unity ($0.5 < \eta_i < 1$). Inverted nonlamellar phases of negative mean curvatures would be feasible for "wedge-shaped" lipid molecules with a larger packing parameter: $\eta_i > 1$ for an inverted-cone shape. Micellar phases are anticipated to form upon hydration of amphiphiles characterized by $\eta_i < 0.5$.

The supramolecular structure, which may form upon hydration of a *mixture* of amphiphiles in water, could be qualitatively predicted by defining a "mean" packing parameter[27,28] for the mixed amphiphilic system, η_m:

$$\eta_m = \sum_i x_i \eta_i \quad (8)$$

where x_i denotes the molar fraction of the i^{th} component in the mixture and η_i its individual "packing" parameter (eq 7).

The above approach for evaluation of the mean packing parameter of a mixture of amphiphiles is applicable to systems in which the components assemble into a single ("mixed") lyotropic phase, that is they do not coexist as separated phases in the aqueous dispersion. In our case, the mixed DOPE/OG (1:1.55 mol/mol) and MO/OG (1.36:1 mol/mol) systems fulfill this condition.

However, it should be taken into account that the definition of η_m (eq 8) is based on "ideal" mixing of the components and does not account for specific intermolecular interactions between them. Quite often, mutual influence of the components in binary or multicomponent amphiphilic systems leads to nonideality of interfacial mixing.[43,44] As a result of modified hydration, electrostatic attraction or repulsion, steric confinement, or hydrogen-bonding interactions, the mean area per molecule in a mixed amphiphilic assembly could essentially deviate in magnitude from a weighted-averaged value and could not be predicted simply by the above approach. Another approximation in the definitions of η_i and η_m (eqs 7 and 8) is that the molecular shapes are assumed to be intrinsic to the lipid species. In fact, the hydrophobic volumes of the lipid tails, as well as the optimal headgroup areas at the polar/apolar interfaces of the supramolecular lipid assemblies, depend on the experimental conditions (such as temperature, degree of hydration, lipid composition of the mixtures) and could not be known unless prior measurements are done. Thus, lipid species may homogeneously mix at certain molar ratios, whereas phase separation between them could occur at other molar fractions. Depending on the presence of charges, hydrogen bonds, or particular substituent groups in the mixed systems, the same lipid molecule could be characterized by considerably different surface molecular areas. The phase state of the lipid supramolecular assembly is another factor influencing the optimal area exposed by an amphiphile at the headgroup/water interface. Thus, for geometrical and steric reasons, the area for the same lipid molecule at the lipid/water interface may be essentially different in lamellar and nonlamellar phases (see eqs (1), (3), and (5) for the determination of the mean molecular areas in diverse types of lyotropic phases).

Table 2. Molecular Parameters of the Investigated Lipids and Detergent and "Packing" Parameters, η_i, of the Amphiphiles Estimated According to Eq 7[a]

lipid	DOPE	MO	DPPC	OG
l_{hc} [nm]	1.86	1.86	1.76	0.93
l [nm]	2.61	2.71	2.80	1.50
M [g/mol]	744.04	356.5	734.05	292.4
v_L [cm^3/g]	1.00	1.06	0.99	0.84
V_{hc} [nm^3]	0.953	0.476	0.871	0.245
A [nm^2]	0.46 ± 0.03	0.24 ± 0.02	0.60 ± 0.05	0.37−0.55
η_i	1.11 ± 0.07	1.07 ± 0.09	0.83 ± 0.07	0.48−0.71

[a] Notations: l, total length in the extended-state molecular conformation, determined from molecular models, l_{hc}, extended-state hydrocarbon chain length; M, molecular weight, v_L, partial specific volume of an amphiphile, V_{hc}, hydrophobic volume of the hydrocarbon tails; A, area per amphiphilic molecule at the headgroup/water interface. All parameters are given at 20 °C. Data for the specific volumes v_L were collected from refs 34, 37, and 46.

Taking into account the approximations involved in eqs 7 and 8, more recent theoretical approaches have suggested[45] that the supramolecular organization that a given amphiphilic system adopts is such that its geometry minimizes the sum of the monolayer curvature, hydrocarbon chain packing, and electrostatic and surface hydration free energies. The "shape" concept,[27] which is based on geometrical parameters, could not fully account for specific forces in the polar headgroup region as well as for the competition of forces in the polar and apolar portions of the lipid assemblies. According to Gruner,[45] it is the balance between curvature and packing energies (which are different in magnitudes for lamellar and nonlamellar phases) that determine the equilibrium organization and phase behavior of hydrated amphiphilic systems.

Because the precise theoretical estimation of the complex energetic balance, which determines the lipid supramolecular organization, is still under development for lyotropic phases of diverse lattice dimensionalities (1D, 2D, 3D) and crystallographic space group symmetries, in this study, calculations were performed, on the basis of structural formalism[32−35] and the "molecular shape" concept,[27] for evaluation of the types of supramolecular structure, which could be predicted for mixed lipid/detergent/water systems of the investigated amphiphiles. An idealized situation was assumed, in which eq 8 was applied for computation of the average parameter η_m of the mixed systems in a geometrical manner, without considering "excess" intermolecular interactions of the components, that may cause nonideality of mixing.[43,44]

Table 2 presents the values of molecular and structural parameters used in the estimation of the "packing" factor,

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(45) Gruner, S. M. *J. Phys. Chem.* **1989**, *93*, 7562−7570.

η_i, for every amphiphilic type. The extended-state chain lengths were determined from the stereochemical molecular models in Figure 1. The hydrophobic volumes occupied by the hydrocarbon chains of the lipids, V_{hc}, were calculated by summing the volumes of the constituent CH_3, CH_2, and CH groups. According to ref 34, $V_{CH_3} = 0.0535$ nm^3, $V_{CH_2} = 0.0273$ nm^3, and $V_{CH} = 0.0203$ nm^3 at 20 °C. The areas per lipid molecule at the headgroup/water interfaces of the amphiphilic lipid assemblies were determined on the basis of eqs 1, 3 and 5 for lamellar-phase DPPC, H_{II}-phase DOPE, and cubic Q^{224}-phase MO, respectively. The estimations of η_i in Table 2 refer to a temperature of 20 °C (at which the membrane-protein reconstitution was performed[26]).

As expected, DOPE is characterized by $\eta_i > 1$, according to Table 2. The molecule is considered to be of an inverted-cone shape, resulting from its small headgroup size. This lipid has been known to display a high propensity to form nonlamellar lyotropic phases.[37] The molecular area of MO obtained from eqs 5 and 6 is in good agreement with results from studies on monoglyceride monolayers at the air/water interface.[47] The presence of only a single, cis-unsaturated chain in its structure results in a smaller value of V_{hc} with $\eta_{MO} < \eta_{DOPE}$ (Table 2). The estimated η_{MO} (= 1.07 at 20 °C) suggests that, as a wedge-shape molecule, MO is capable of assembling in inverted nonlamellar structures ($\eta_i > 1$). For DPPC, the calculated η_i (< 1) confirms that this is a typical lamellar-forming lipid.

Table 2 demonstrates that the extended-chain length of the OG molecule is considerably smaller compared to those of the lipids studied. Another stereochemical peculiarity of OG is that its polar headgroup occupies a larger area with respect to the projection of its short (C_8) hydrophobic tail. The area per headgroup, A, of the OG molecule was taken from ref 7 ($A = 0.37$ nm^2/molecule) and also as an average value of the areas estimated for hydrated amphiphiles with β-D-glucopyranosyl-*rac*-glycerol headgroups ($A = 0.55$ nm^2/molecule).[34,48] With this possible variation of the OG molecular area (which depends on the hydration and phase state of the amphiphile), the estimated packing parameter, η_i, for OG falls in the range from 0.48 (at $A = 0.55$ nm^2/molecule) to 0.71 (at $A = 0.37$ nm^2/molecule).

The "mean" packing parameters of the mixed lipid/detergent systems, η_m, were estimated on the basis of eq 8, taking into account the molar compositions of the mixtures and the η_i values for the individual lipid and detergent components (Table 2). The parameters η_m are presented in Table 3 as values that range between two limits, corresponding to the minimum (0.48) and the maximum (0.71) value of the OG packing parameter, η_{OG}, which determines the widest feasible range of η_m for the mixed systems. Table 3 shows that the η_m values calculated at the investigated lipid-to-detergent molar ratios are smaller than unity, revealing that the "shape" concept predicts lamellar supramolecular organizations for all of the three mixed amphiphilic systems. The theoretical prediction is in agreement with the experimental results for the DOPE/OG and MO/OG systems. It appears that, for the DPPC/OG mixture, the "shape" concept is not applicable because of the coexistence of lamellar and micellar phases in that system. The employed model is also not applicable to systems with strong specific intermolecular interactions or organizations, such as the interdigitated phase observed[5] with a DPPC/OG mixture at $R = 0.44$.

Table 3. Mean "Packing" Parameter, η_m eq 8, of the Investigated Lipid/Detergent Mixtures Containing OG Molar Fraction x_{OG} and the Estimated Critical Molar Fraction of OG for Induction of the Nonlamellar-To-Lamellar Phase Transition, x^{crit}_{OG}, Based on the "Molecular Shape" Concept[a]

system	DOPE/OG	MO/OG	DPPC/OG
lipid/OG molar ratio	1:1.55	1:0.73	1:1.97
x_{OG}	0.61	0.42	0.66
η_m	0.73−0.87	0.82−0.92	0.60−0.75
x^{crit}_{OG} (at $\eta_m = 1$)	0.18−0.28	0.11−0.19	−
lipid/OG molar ratio at $\eta_m = 1$	1:0.22−1:0.39	1:0.13−1:0.23	−

[a] The "packing" factors, η_i, of the individual lipid components are 1.11 (DOPE), 1.07 (MO), and 0.83 (DPPC). For OG, two limiting values, $\eta_{OG} = 0.48$ and $\eta_{OG} = 0.71$, were considered (Table 2), which yielded the presented range of values for η_m and x^{crit}_{OG}.

Conclusion

When hydrated in excess aqueous phase containing the detergent OG, the nonlamellar lipids DOPE and MO form lamellar liquid-crystalline (L_α) phases at optimized lipid-to-detergent molar ratios that are suitable for membrane-protein reconstitution. The results obtained suggest that the structural effects caused by the detergent OG on the supramolecular organizations of the lipids DOPE, MO, and DPPC are predominantly due to its steric and geometrical molecular peculiarities (short hydrophobic length and large headgroup size), rather than to dipolar effects. Octyl glucoside is a nonionic molecule, known as a mild surfactant. The established absence of micelles in the DOPE/OG and MO/OG dispersions implies that the OG micelles are capable of forming mixed lipid/OG membranes and that the detergent is accommodated in the L_α-phase lipid/OG bilayers. After mixing, the detergent OG does not remain as a separate micellar phase from the lipid assembly. When mixed with the lipids DOPE and MO at the employed lipid-to-detergent molar ratios, OG totally modifies their nonlamellar organizations. The resulting structures are of a different phase state compared to that of the pure lipids, as evidenced by the X-ray diffraction patterns obtained of lamellar (L_α) fluid lipid/OG phases. It was found that the nonlamellar lipids DOPE and MO are capable of accommodating OG molecules in their structures to a greater extent than the bilayer-forming lipid DPPC. The DPPC bilayers are composed of relaxed, zero-curvature monolayers. Upon addition of OG, these bilayers could accommodate only equilibrium amounts of OG molecules. Hence, in the planar DPPC bilayers, OG does not play the role of a stabilizer of a lamellar structural organization, as it does in the case of the two other lipid structures.

In applying the "molecular shape" concept to predict the average packing parameters η_m of a mixed amphiphilic system, it cannot be known a priori whether the components of the system will mix homogeneously or will phase-separate. Experimental data are necessary to determine the mixing behavior of the considered components. In addition, evaluation of the optimal molecular areas at the polar/apolar interface of the amphiphilic assemblies requires the structural parameters of the supramolecular organizations of the pure components to be known from experiment in order for eqs 1, 3, and 5 to be applied.

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Appendix

Estimation of the "Critical" Molar Fraction of OG in Lipid/OG Mixtures for Induction of Nonlamellar-to-Lamellar Structural Transformations in DOPE/OG/Water and MO/OG/Water Systems. The transition of the mixed lipid/OG systems from nonlamellar to lamellar structures or to coexisting nonlamellar and lamellar or lamellar and micellar phases is expected to depend on the lipid-to-detergent molar ratio in the systems under consideration. Above a critical aqueous concentration, the detergent OG will begin to penetrate into the nonlamellar lipid structures and destabilize them by changing the lipid monolayer curvature. The hydrophilic OG headgroups should be accommodated at the lipid headgroup/water interface of the mixed lipid/detergent assemblies (Figures 2 and 6). Thus, upon increasing the amount of the accommodated OG molecules, the nonlamellar structures will progressively transform into less-curved structures.[45] To be stable, the bilayer structures formed should accommodate a sufficient amount of spacer OG molecules. The appearance of lipid/OG micelles in the dispersion system could be expected at detergent concentrations, ensuring an excess amount of OG in the mixed bilayers.

The "critical" molar fraction, x^{crit}_{OG}, at which OG added to the nonlamellar phases of DOPE and MO will induce structural transformation of the mixed systems from nonlamellar ($\eta_m > 1$) to lamellar ($\eta_m < 1$) organization was estimated from eq 8, assuming that the nonlamellar-to-lamellar transition would occur at $\eta_m = 1$. The range of x^{crit}_{OG} presented in Table 3 was obtained at the following η_i values of the individual components: $\eta_{DOPE} = 1.11$, $\eta_{MO} = 1.07$, and $\eta_{OG} = 0.48$ (or 0.71). On the basis of the "shape" concept, molar fractions of OG in the MO/OG mixtures $x_{OG} > 0.11-0.19$ are anticipated to cause transformation of the nonlamellar MO into lamellar MO/OG structures. At x_{OG} in the range 0−0.11(or 0.19), either a cubic or a coexistence of cubic and lamellar phases may be supposed. For the DOPE/OG system, the critical OG molar fraction, x^{crit}_{OG}, causing a nonlamellar-to-lamellar phase transition, is around 0.18−0.28. At x_{OG} in the range 0−0.18(or 0.28), one may suggest a complex phase behavior of the DOPE/OG mixture, in which a pure H_{II} phase or a coexistence of H_{II} and cubic phases or of H_{II} and/or cubic and lamellar phases could be present for $1 < \eta_m < 1.11$.

It appeared from the performed calculations that the concentration of OG that is sufficient to induce transformation of nonlamellar DOPE and MO phases into lamellar ones is rather low. This fact requires that the nonlamellar organizations of amphiphilic systems employed for membrane-protein reconstitution be proven by direct structural methods.

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