Stages of the Bilayer-Micelle Transition in the System Phosphatidylcholine-$C_{12}E_8$ as studied by Deuterium- and Phosphorous-NMR, Light Scattering, and Calorimetry

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ABSTRACT The perturbation of phospholipid bilayer membranes by a nonionic detergent, octaethyleneglycol mono-*n*-dodecylether ($C_{12}E_8$), was investigated by ^2H- and ^{31}P-NMR, static and dynamic light scattering, and differential scanning calorimetry. Preequilibrated mixtures of the saturated phospholipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphorylcholine (DMPC), and 1,2-dilauroyl-*sn*-glycero-3-phosphorylcholine (DLPC) with the detergent were studied over a broad temperature range including the temperature of the main thermotropic phase transition of the pure phospholipids. Above this temperature, at a phospholipid/detergent molar ratio 2:1, the membranes were oriented in the magnetic field. Cooling of the mixtures below the thermotropic phase transition temperatures of the pure phospholipids led to micelle formation. In mixtures of DPPC and DMPC with $C_{12}E_8$, a narrow calorimetric signal at the onset temperature of the solubilization suggested that micelle formation was related to the disorder-order transition in the phospholipid acyl chains. The particle size changed from 150 nm to ~7 nm over the temperature range of the bilayer-micelle transition. The spontaneous orientation of the membranes at high temperatures enabled the direct determination of segmental order parameters from the deuterium spectra. The order parameter profiles of the phospholipid acyl chains could be attributed to slow fluctuations of the whole membrane and to detergent-induced local perturbations of the bilayer order. The packing constraints in the mixed bilayers that eventually lead to bilayer solubilization were reflected by the order parameters of the interfacial phospholipid acyl chain segments and of the phospholipid headgroup. These results are interpreted in terms of the changing average shape of the component molecules. Considering the decreasing cross sectional areas in the acyl chain region and the increasing hydration of the detergent headgroups, the bilayer-micelle transition is the result of an imbalance in the chain and headgroup repulsion. A neutral or pivotal plane can be defined on the basis of the temperature dependence of the interfacial quadrupolar splittings.

INTRODUCTION

Solubilization is a prerequisite for an investigation of the components of biological membranes at the molecular level. The transformation of membranes into mixed phospholipid/detergent micelles involves a number of intermediary states that have been studied by techniques such as static and dynamic light scattering (Goni et al., 1986; da Graca et al., 1989; Ollivon et al., 1988; Levy et al., 1990; Edwards and Almgren, 1991), electron microscopy (Edwards and Almgren, 1991; Vinson et al., 1989), differential scanning calorimetry (Schullery et al., 1981; Lewis et al., 1987; Goni et al., 1986; Alonso and Goni, 1983), resonance energy transfer (Goni et al., 1986; da Graca et al., 1989; Ollivon et al., 1988; Levy et al., 1990; Edwards and Almgren, 1991), and nuclear magnetic resonance (van Echteld et al., 1981; Jackson et al., 1982; Ulmius et al., 1982; Nilsson et al., 1987). These transition states are of particular interest for the choice of proper conditions both for solubilization and reconstitution of membrane proteins.

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Emphasis has been placed in earlier work on the structural, thermodynamic, and kinetic aspects of the micellization process. It is customary to use unilamellar egg lecithin vesicles in model studies as a target for detergent binding and solubilization. Nonionic detergents such as octylglycoside (Stubbs and Litman, 1978; Jackson et al., 1982; Vinson et al., 1989; da Graca et al., 1989; Eidelman et al., 1988; Ollivon et al., 1988) and the oxyethylene-type detergents (Goni et al., 1986; Edwards et al., 1989; Nilsson et al., 1987; Edwards and Almgren, 1991; Levy et al., 1990) have been studied in most detail, because these amphiphiles are frequently being used for biomembrane work because of their inoffensiveness to integral membrane proteins (le Maire et al., 1983; Møller et al., 1986; Helenius et al., 1979). With increasing detergent/lipid ratio, the detergent/vesicle interaction usually involves uptake of the detergent into the vesicle bilayer membrane, opening of the vesicles and, eventually, the formation of spheroidal micelles. Intermediary structures such as open bilayers and cylindrical micelles appear after vesicle opening, and large aggregates may form before complete micellization of the phospholipid/detergent mixture (Vinson et al., 1989; Edwards et al., 1989; Edwards and Almgren, 1991).

The thermal stability of detergent saturated phospholipid bilayers has not received much attention so far, although this aspect may be of interest both in a practical sense, i.e., for membrane protein reconstitution, and from a theoretical point of view. In the present study, membrane solubilization

was observed with decreasing temperature in aqueous phospholipid/detergent mixtures with a twofold molar excess of the phospholipid. We have focused on the intermediary states of the membrane-micelle transition in the system DMPC/$C_{12}E_8$. For an understanding of the chain and headgroup packing that leads to the bilayer-micelle transition, it was also necessary to include phospholipids with longer or shorter acyl chains. Saturated phospholipids were studied exclusively to avoid complications due to the variable degree of unsaturation and chain lengths of natural phospholipids such as egg lecithin. Although construction of a phase diagram was not intended here, our results can be helpful for an understanding of the complicated phase relationships in related systems (Mädler et al., 1994).

Bilayer breakdown and micelle formation as observed by ^{2}H- and ^{31}P-NMR, light scattering, and calorimetry can be qualitatively understood in terms of the molecular shape concept that considers average molecular properties such as chain length, chain volume, and interfacial area (Israelachvili et al., 1980). We have demonstrated the usefulness of this concept in a recent study on the lamellar-hexagonal (H_I) phase transition in the liquid crystalline DMPC/$C_{12}E_8$ system (Thurmond et al., 1994). The motional restrictions in the liquid crystalline phospholipid/detergent mixture (at low water content) and in dilute aqueous solution are reflected by the ^{2}H quadrupolar splittings of the selectively deuterated phospholipids and, equivalently, by average acyl chain lengths and cross sectional areas that can be derived from the quadrupolar splittings (Schindler and Seelig, 1975; Salmon et al., 1987; Jansson et al., 1992; Thurmond et al., 1994). For an evaluation of the packing constraints within a mixed phospholipid/detergent structure, it is necessary to consider repulsive interactions in the headgroup domain *and* among the hydrophobic chains. Thus, the balance of forces between the hydrophobic and hydrophilic domains determines the phase structure that the system will assume.

Enhanced bilayer elasticity (Helfrich, 1973) in the lamellar lipid/detergent mixture and the anisotropy of the alkyl chain magnetic susceptibility lead to almost perfect orientation of the aggregates in the magnetic field with the director axis perpendicular to the field. This phenomenon is useful for a direct evaluation of the composite ^{2}H-NMR spectra of chain perdeuterated phospholipids. Close to gel-to-liquid crystalline phase transition temperature of the pure phospholipid components, the field orientation is partially lost. Acyl chain crystallization in C-14 and C-16 phosphatidylcholines probably contributes to the sudden rearrangement into the micellar state.

MATERIALS AND METHODS

Materials

The non-ionic detergent $C_{12}E_8$ was obtained from the Kouyoh Trading Co. (Tokyo, Japan) or from Fluka (Neu-Ulm, Germany). Fully saturated phospholipids (acyl chain lengths from C-8 to C-18) were from Sigma Chemical Co. (Deisenhofen, Germany). The deuterated phospholipids were purchased from Avanti Polar Lipids (Alabaster, AL). The percentage of deuteration was checked by high resolution proton NMR. Deuterium-depleted water was from Sigma. The required amounts of phospholipid and detergent were dissolved in chloroform and dried under a stream of nitrogen. Drying was completed on a vacuum line overnight. The mixtures were suspended in doubly distilled water or in deuterium-depleted water by vortex mixing at temperatures above the main phase transition of the phospholipid. Before the measurements, the samples were equilibrated for several days. In the ^{2}H- and ^{31}P-NMR experiments, the total amount of phospholipid was typically 5 mg in 90 μl of water, i.e., the water content of the NMR samples was about 90% of the total sample weight.

Experimental methods

Measurements were performed with a Varian VXR 400*S* spectrometer operating at a magnetic field strength of 9.4 T. ^{2}H-NMR spectra (^{2}H frequency of 61.4 MHz) were collected using a horizontal solenoid coil of 5 mm diameter. A phase-cycled quadrupolar echo sequence was applied (Davis et al., 1976) with a 90° pulse of 2.9 μs duration, 20 μs pulse separation, a recycle time of 0.5 s, and a digitization dwell time of 2 μs. The time domain signals were Fourier-transformed using both quadrature channels, taking care to initiate the transform at the top of the echo. ^{31}P-NMR spectra were obtained at 161.9 MHz without proton decoupling using a 2.1 μs, 90° pulse and a recycle time of 3 s. All phosphorus spectra were referenced to external H_3PO_4. Spectra were taken in order of increasing temperature, allowing the samples to equilibrate at the given temperature for at least 30 min before data acquisition.

Optical density was measured at λ_{Hg} = 578 nm with an Eppendorf 1101 M photometer (Eppendorf, Germany). Dynamic light scattering was performed at at an angle of 90° with a ALV5000 instrument (ALV, Langen, Germany). Differential Scanning Calorimetry (DSC) was performed with a MC-2 from MicroCal (Northampton, MA) at a scan rate of 15°C/h. The data in Fig. 4 show the second upwards scan out of five measurements with increasing and decreasing temperature. With respect to the NMR samples, the mixtures were diluted by a factor of 1:5 (optical density), 1:50 (dynamic light scattering), and 1:14 (DSC).

Analysis of ^{2}H-NMR spectral results

For lipids in the liquid crystalline phase, the bilayer normal is an axis of motional averaging, and the shape of the ^{2}H-NMR spectra corresponds to axially symmetric motion. In the case of a planar bilayer, the quadrupolar splitting, $\Delta\nu_Q$, between the two spectral transitions is related to the C—^{2}H bond segmental order parameter, by (Seelig, 1977)

$$\Delta\nu_Q = 3/2\chi\, P_2(\cos\theta)\, |S_{CD}^{(i)}|. \tag{1}$$

The static quadrupolar coupling constant χ has a value of 170 kHz for a C—^{2}H bond (Seelig, 1977), P_2 is the second Legendre polynomial, and θ is the angle between the bilayer normal (director axis) and the static magnetic field. The order parameter of the carbon-deuterium bond, S_{CD}, is defined by

$$S_{CD}^{(i)} \equiv \langle P_2(\cos\beta_i)\rangle = 1/2\langle 3\cos^2\beta_i - 1\rangle, \tag{2}$$

where β_i is the time-dependent angle between the direction of the ith C—^{2}H bond and the bilayer normal and the brackets indicate a time or ensemble average.

A further reduction of the quadrupolar splittings will be obtained, if the local director axis fluctuates rather than being fixed in space. Thus, the order parameter obtained from the observed quadrupolar splitting can be decomposed by

$$|S_{CD}^{(i)}|_{obs} = |S_{CD}^{(i)}|\, S_{dir}, \tag{3a}$$

with

$$S_{dir} = \langle P_2(\cos\zeta)\rangle = 1/2\langle 3\cos^2\zeta - 1\rangle, \tag{3b}$$

where ζ describes the angular excursion of the director axis from its

mean direction. The order parameter specifies rapid symmetric motions about the local director axis or, equivalently, about the instantaneous bilayer normal (see Eq. 2), and S_{dir} accounts for the motion of the local director axis due to fluctuation of the aggregate surface and for whole-body motion of the aggregates. This factorization is only justified for motions on strictly different time scales, i.e., if S_{CD} and S_{dir} are statistically independent (Jansson et al., 1992).

The experimental ^{2}H-NMR spectra for the pure lipid phase were numerically deconvoluted using the de-Pakeing algorithm (Bloom et al., 1981). For the pure lipid phase, the C—^{2}H bond segmental order parameters were evaluated from the de-Paked subspectra. For the mixed phases, which were magnetically oriented, line positions and signal areas were obtained by fitting Lorentzian lines. The chain segment assignments were made by integrating the intensities of the spectral lines and by comparison with previous results for DPPC with specifically deuterated and perdeuterated acyl chains (Seelig and Seelig, 1975). Because of the different orientations with respect to the bilayer surface, the two acyl chains are inequivalent, leading to separate profiles for the *sn*-1 and *sn*-2 chains (Engel and Cowburn, 1981; Salmon et al., 1987).

The segmental order parameters of phospholipids in a bilayer membrane can be used to obtain an estimate of the average projection of the acyl chain length $\langle L_{chain}\rangle$, along the bilayer director (Schindler and Seelig, 1975; Salmon et al., 1987):

$$\langle L_{chain}\rangle = l_0\left[\frac{n-m+1}{2} + \sum_{i=m}^{n-1} |S_{CD}^{(i)}| + 3|S_{CD}^{(n)}|\right], \quad (4)$$

where the index i refers to the numbering of the acyl chains beginning with the ester carbon ($i = 1$) and ending with the methyl carbon ($i = n$), e.g., $n = 14$ for DMPC. The effective acyl chain length is taken as extending from the C_1 carbon to the methyl carbon in the case of the *sn*-1 chain, i.e., $m = 2$. In the case of the *sn*-2 chain, the contribution from the C_2 carbon is neglected so that $m = 3$. The length of one carbon bond projected onto the long axis of the all-*trans* reference state is $l_0 = 1.25$ Å (Salmon et al., 1987). The half-thickness $\langle t\rangle$ in a bilayer of N chains with an average area $\langle A\rangle$ and a volume V per chain is by definition $N\langle A\rangle = NV/\langle t\rangle$. The average end-to-end distance $\langle L_{chain}\rangle$ as measured by ^{2}H-NMR is expected to be smaller than the average bilayer half-thickness $\langle t\rangle$ obtained by diffraction techniques. This is due to end effects, which are also reflected by the "plateau" usually observed in the order parameter profile.

The calculation of an average interfacial area per chain from the ^{2}H order parameters hinges on the assumption that the experimentally accessible inverse of the average projected acyl chain length is equivalent to the average of the inverse of the chain length, i.e., $1/\langle L\rangle \approx \langle 1/L\rangle$ (Jansson et al., 1992; Thurmond et al. 1994). Further, consideration of the whole length of the chains,

$$\langle A_{chain}\rangle = V_{chain}/\langle L_{chain}\rangle, \quad (5)$$

can be assumed to yield an upper limit value, whereas including only the plateau values of the order parameter profile into the area calculation

$$\langle A\rangle' = V_{plat}/\langle L_{plat}\rangle \quad (6)$$

gives a lower limit, i.e., $V_{plat}/\langle L_{plat}\rangle \le \langle A\rangle \le V_{chain}/\langle L_{chain}\rangle$ (Jansson et al., 1992; Thurmond et al., 1993, 1994). V_{chain} is given by $n_{CH2}V_{CH2} + V_{CH3}$, with $V_{CH2} = 28.0$ Å^3 and $V_{CH3} = 2V_{CH2}$ (Nagle and Wilkinson, 1978). Thus, the true average interfacial area that follows by definition from the bilayer thickness $\langle t\rangle$ as determined by x-ray or neutron scattering can be only approximated by the above deuterium order parameter evaluation (see Thurmond et al. (1994) for a detailed discussion).

Spectral moments can be calculated using half of the symmetric ^{2}H-NMR spectrum (Davis, 1983). For a quadrupolar powder pattern, which is typical for nonoriented phospholipid bilayers, the kth moment is given by

$$M_k = A_k\,(6\pi/4)^k\,\chi^k\langle|S_{CD}|^k\rangle, \quad (7)$$

where $A_1 = 2/(3\sqrt{3})$ and $A_2 = 1/5$ for the first and second moments, which are related to the mean order parameter and to the mean-squared order parameter, respectively (Davis, 1979; Davis, 1983). The relative mean-square deviation from the mean, Δ_2, is given by

$$\Delta_2 = \frac{\langle|S_{CD}|^2\rangle - \langle|S_{CD}|\rangle^2}{\langle|S_{CD}|\rangle^2} = \frac{M_2}{1.35M_1^2} - 1 \quad (8)$$

which characterizes the width of the distribution of order parameters. In a sample oriented with the membrane director perpendicular to the magnetic field ($\theta = 90°$) $A_k = 1/2^k$, $k = 1, 2, \cdots$, and $\Delta_2 = M_2/M_1^2 - 1$.

^{31}P-NMR

The lineshape of ^{31}P-NMR spectra at high magnetic field strengths is dominated by the chemical shielding tensor. Because of the axially symmetric motions of the phosphate group around the bilayer normal, the orientation dependence is again given by the second Legendre polynomial (see Eq. 1). For randomly distributed lipid bilayers, this results in a powder spectrum with a high frequency shoulder for $\theta = 0°$ and a low frequency peak for $\theta = 90°$ (Seelig, 1978). Lipids in micellar solutions and small bilayer vesicles experience fast isotropic averaging resulting in narrow symmetric spectra.

RESULTS

Stages of the isothermal bilayer-micelle transition as detected by ^{2}H- and ^{31}P-NMR in the DMPC/$C_{12}E_8$ system

The effect of increasing detergent concentration was studied by ^{2}H- and ^{31}P NMR spectroscopy in mixtures of DMPC-d_{54} and $C_{12}E_8$ as shown in Fig. 1. The mixtures were equilibrated in the NMR spectrometer at 40°C for at least 30 min before recording the spectra. No further changes of the ^{2}H- and ^{31}P-NMR lineshapes were observed after this incubation time (Fig. 1). The ^{2}H-NMR spectrum of pure fully hydrated DMPC-d_{54} (Fig. 1 *a*) displays the powder-type lineshape that is typical for multi-lamellar phospholipid dispersions in the liquid crystalline state (Seelig, 1977). The spectral intensity on the high frequency and low frequency sides of the main signal envelope corresponds to bilayer orientations close to $\theta = 0°$, where θ denotes the polar angle between the bilayer normal and the magnetic field. The unusually low intensity of these shoulders indicates that the liposomes are somewhat distorted in the strong magnetic field (9.4 T).

Several stages of the isothermal transformation of the DMPC bilayers into DMPC/$C_{12}E_8$-mixed micelles can be detected in the ^{2}H- and ^{31}P-NMR spectra of the mixtures as shown in Fig. 1. The overall breadth of the ^{2}H-NMR spectrum and the spectral intensity arising from the $\theta = 0°$ orientation decrease on going from the pure phospholipid dispersion to a phospholipid/detergent molar ratio of 2:1 (Fig. 1 *a–c*). In the 2:1 mixture, the shoulders are no longer visible and deuterium signals of individual DMPC-d_{54} acyl chain segments are particularly well resolved, indicating that at this composition the aggregates are almost perfectly oriented in the magnetic field (vide infra). The lineshape of the ^{2}H-NMR spectrum is less well defined in the range of phospholipid/detergent molar ratios from 2:1 to 1:1, where the overall width of the spectrum is further reduced. The largest quadrupolar splittings in the spectrum corresponding with carbon-deuterium bonds in the interfacial region decrease more rapidly than the smaller splittings originating from the

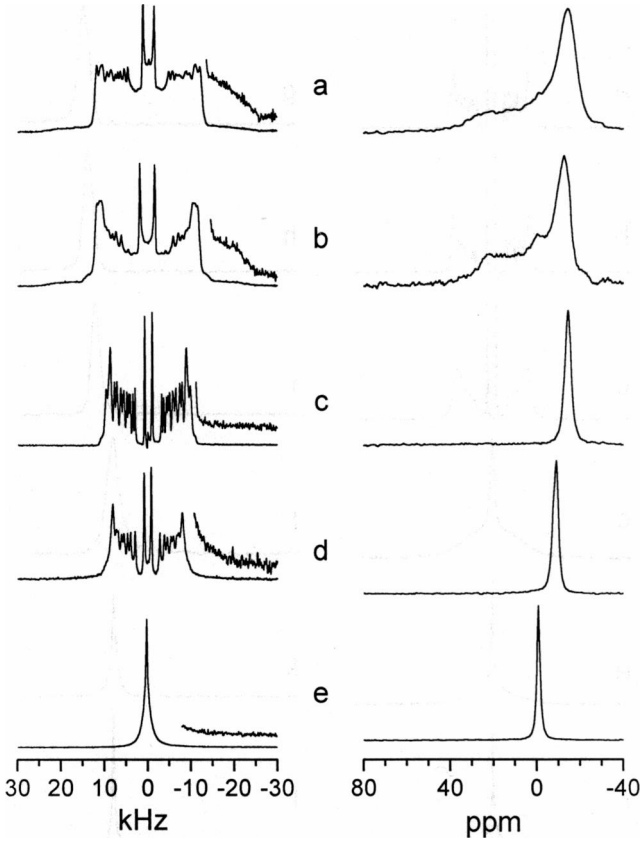

FIGURE 1 ^{2}H-NMR spectra (*left* column) and ^{31}P-NMR spectra (*right* column) of pure DMPC-d_{54} (*a*) and of DMPC-d_{54}/$C_{12}E_8$ mixtures at molar ratios 3:1 (*b*), 2:1 (*c*), 1.5:1 (*d*), and 1:1 (*e*). Temperature, 40°C. Water content, 95% w/w. In the ^{2}H-NMR spectra the eightfold vertical enhancement of the low frequency spectral region shows the almost perfect field orientation of the 2:1 mol/mol sample. The phosphorous spectra were referenced to the signal of an external sample of 85% phosphoric acid.

terminal segments, including the terminal methyl group. Eventually, close to an equimolar ratio, the spectrum merges into a single line (Fig. 1 *e*), which is probably due to rapid isotropic tumbling of the relatively small phosholipid-detergent aggregates.

The detergent-induced transitions in the binary phospholipid-detergent mixtures as seen by ^{2}H-NMR also appear in the ^{31}P-NMR spectra. The powder type ^{31}P spectra of pure DMPC (Fig. 1 *a*) and of a mixture of DMPC and $C_{12}E_8$ at a molar ratio of 3:1 (Fig. 1 *b*) consist of a high frequency shoulder and a maximum at low frequency. This lineshape signifies rapid reorientation of the phosholipid headgroup about the local director axis and random distribution of the directors with respect to the magnetic field (Seelig, 1978). Considerable line narrowing results upon addition of detergent. At a 2:1 molar ratio of DMPC and $C_{12}E_8$, the low field shoulder (corresponding to $\theta = 0°$) is completely absent and the symmetric phosphorus signal is centered at −13 ppm. This signal position indicates that the local director axis about which phospholipid diffusion occurs is perpendicular to the magnetic field. Further addition of $C_{12}E_8$ leads to a continuous shift of this signal from −13 ppm obtained at 2:1 molar ratio to −1 ppm in the equimolar mixture (Fig. 1, *d* and *e*).

The continuous shift of the ^{31}P-NMR signal may be due to progressive loss of aggregate orientation or to the decreasing average size of the aggregates. Moreover, the ill-defined lineshapes of the ^{2}H-NMR spectra at DMPC/$C_{12}E_8$ compositions other than 2:1 mol/mol (see Fig. 1) may be the result of some polydispersity of the aggregates. Notably, the broad wings in the ^{2}H-spectra obtained below the 2:1 ratio (Fig. 1 *e*) suggest that micelles of very different size coexist. Size reduction and polydispersity have indeed been observed during the titration of egg lecithin vesicles with $C_{12}E_8$ (Edwards and Almgren, 1991). Such interpretations from the NMR spectra alone are not unique, however, because they do not consider the possibility of conformational changes in the phospholipid headgroup and altered phospholipid acyl chain packing. These ambiguities prompted us to concentrate on the bilayer perturbation and bilayer-micelle phase transition at 2:1 phospholipid/detergent molar ratio. The resolution of individual resonances in the ^{2}H-NMR spectrum and the homogeneous upfield ^{31}P-NMR signal indicate that the 2:1 mol/mol mixture represents large, well oriented bilayers or bilayer fragments. The presence of bilayers rather than oriented tubes or cylinders is also borne out by the continuity of the ^{2}H-NMR spectra in Fig. 1 *a–c* (see Discussion). Small aggregates such as micelles or vesicles are definitely absent in this sample. Thus, chain packing and thermal stability were studied further in phosholipid-detergent mixtures at this particular molar ratio.

Thermal transitions in the DMPC/$C_{12}E_8$ system

The 2:1 mol/mol DMPC/$C_{12}E_8$ mixture undergoes a transition from the bilayer state into the mixed micellar state with decreasing temperature. This was directly observed by following the optical density at 578 nm over a broad temperature range (Fig. 2 *a*) including the temperature of the thermotropic phase transition of pure DMPC (23°C). During a slow cooling scan (0.05°C/min), a dilute sample shows a rather constant turbidity between 50 and 26°C. Below 26°C the turbidity increases by about 50% with a maximum at 25°C. Further cooling then results in a sudden decrease of the optical density, indicating that mixed micelles have formed (Fig. 2 *a*).

An estimate of the particle size was obtained by dynamic laser light scattering (Fig. 2 *b*). Mean values of the diffusion constant, D, were determined by the method of cumulants assuming a single-exponential time autocorrelation function. The mean hydrodynamic radii of the aggregates were calculated from the Stokes-Einstein relation: $R_h = kT/6\pi\eta D$. In agreement with the static light scattering experiment, there is a sudden change of R_h at 25°C. Below 20°C and above 30°C, R_h is rather invariable, indicating that the average hydrated particle radius is approximately 7 and 150 nm in the low and high temperature regions, respectively. It must be noted that the data around 25°C are less reliable because

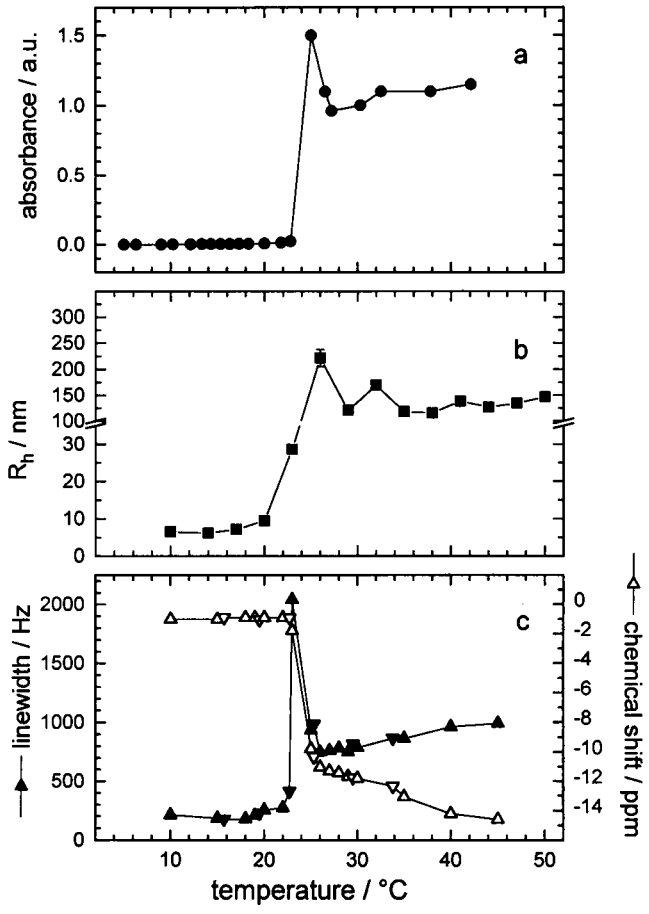

FIGURE 2 Temperature dependence of the aggregate size of a 2:1 mol/mol DMPC/$C_{12}E_8$ sample as detected by absorbance measurements, dynamic light scattering, and by phosphorus-NMR. (*a*) Absorbance measurement at 578 nm. (*b*) Average hydrodynamic radius obtained by dynamic light scattering at 632.8 nm. (*c*) ^{31}P-NMR line width (*filled* symbols) and chemical shift (*open* symbols). Panel *c* displays scans from low to high (*upward triangles*) and from high to low temperature (*downward triangles*).

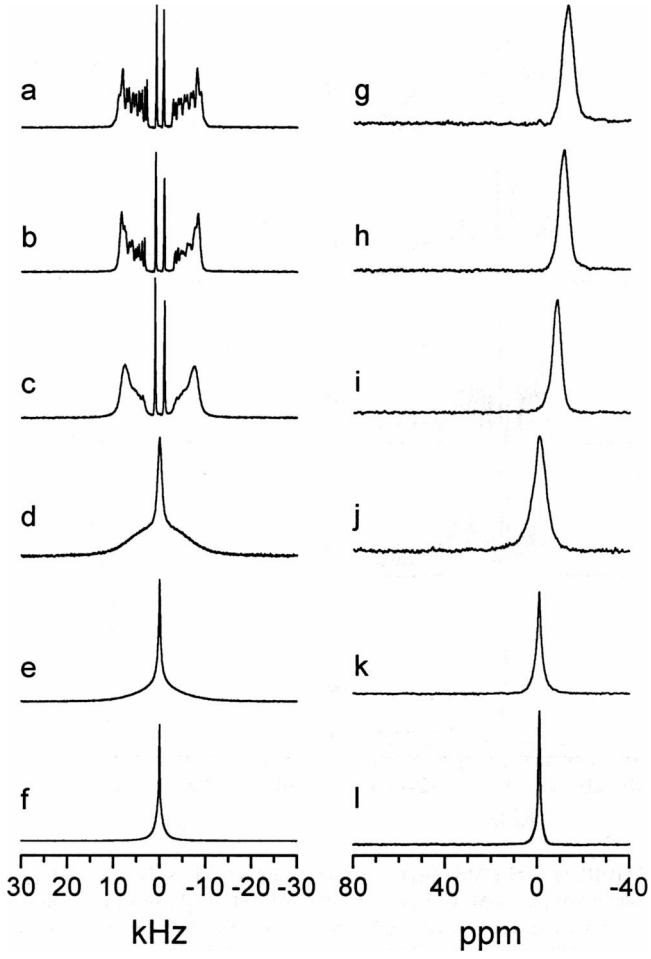

FIGURE 3 Temperature dependence of ^{2}H-NMR (*a–f*) and ^{31}P-NMR (*g–l*) spectra of a DMPC-d_{54}/$C_{12}E_8$ mixture at molar ratio 2:1. Temperatures: 45.7°C (*a, g*); 37°C (*b, h*); 28.4°C (*c, i*); 22°C (*d, j*); 19.7°C (*e, k*); 11.7°C (*f, l*).

multiple scattering may occur because of the enhanced sample turbidity as shown in the static scattering experiment.

Deuterium- and phosphorus-NMR spectra of a DMPC-d_{54}/$C_{12}E_8$ mixture at 2:1 molar ratio revealed changes of aggregate size in agreement with the light scattering results (Fig. 3). In the ^{2}H-NMR spectra above 35°C (Fig. 3 *a*), almost perfect field orientation can be deduced as outlined above (cf. Fig. 1). Below 35°C the individual ^{2}H-NMR signals undergo significant broadening that seems to be more effective for the spectral components exhibiting large quadrupolar splittings (Fig. 3, *b* and *c*). In the temperature range from 24 to 20°C, i.e., close to the $P_{\beta'}$-L_α phase transition temperature of pure DMPC-d_{54} (19.5°C), the ^{2}H-NMR quadrupolar powder pattern breaks down and individual quadrupolar splittings are completely blurred (Fig. 3 *d*). Below 20°C the total spectral intensity merges into a single residual resonance line (Fig. 3 *e*). The shape of this signal is uncertain considering its broad, slowly decaying wings. Incubation of the sample below 16°C eventually results in a narrow Lorentzian signal, which is indicative of isotropic phospholipid motion (Fig. 3 *f*).

The above sequence of transitions can also be observed in the ^{31}P-spectra of the same sample. The homogeneous phosphorus signal at −13 ppm (Fig. 3 *g*), resulting from almost perfect field orientation above 40°C, moves downfield with decreasing temperature toward −1 ppm, which is the chemical shift typical for isotropic diffusion of the phospholipid phosphodiester moiety. In the temperature range from 30 to 20°C, the ^{31}P-NMR signal significantly broadens (Fig. 3, *i* and *j*). At the same time, a low intensity shoulder appears at the lowfield side of the main signal, indicating that the field orientation is partially lost in this temperature region. Isotropic phospholipid tumbling occurs below 16°C according to the narrow Lorentzian ^{31}P-line (Fig. 3, *k* and *l*), in agreement with the corresponding ^{2}H-NMR spectra.

The changes in the phosphorus line width and resonance frequencies shown in Fig. 3 are summarized in Fig. 2 *c*. The signal continuously moves from −13 to −1 ppm on going from 50 to 20°C, whereas the line broadening shows a more complicated behavior in this temperature range. Between 40 and 30°C, the line width slightly decreases and increases again at 25°C. Between 25 and 18°C, i.e., in the temperature

range where isotropic tumbling sets in, the phosphorous signal considerably narrows approaching a value of 100 Hz at 16°C, in accordance with the breakdown of the ^2H quadrupolar splittings.

Changes in heat capacity over the temperature range of the transition as observed by NMR and light scattering measurements were determined by differential scanning calorimetry. Fig. 4 *b* shows a heating scan of a 2:1 mol/mol DMPC/$C_{12}E_8$ sample. Heating scans obtained in 1.8:1 and 2.25:1 mixtures are included in Fig. 4, *a* and *c* for comparison. The 2:1 sample displays a rather narrow transition (actually a poorly resolved double peak) centered at 23.5°C and a broad endotherm with a maximum at 20.5°C. The former calorimetric signal is considerably broadened at sample compositions slightly different from the 2:1 molar ratio as shown in Fig. 4, *a* and *c*, respectively. Integration and deconvolution of the calorimetric curve for the 2:1 mixture yields a total transition enthalpy of 10.5 kJ/mol (with respect to the phospholipid component), of which 31% is due to the narrow calorimeter signal.

The results summarized in Figs. 2–4 show that a bilayer-micelle transition in the 2:1 DMPC/$C_{12}E_8$ system occurs over a temperature range of approximately 11°C. It is important to note that the changes observed with light scattering, ^2H- and ^{31}P-NMR, and calorimetry are completely reversible. A temperature scan in the opposite direction, i.e., from low to high temperature, yielded the same NMR spectra, optical densities, hydrodynamic radii, and calorimeter signals.

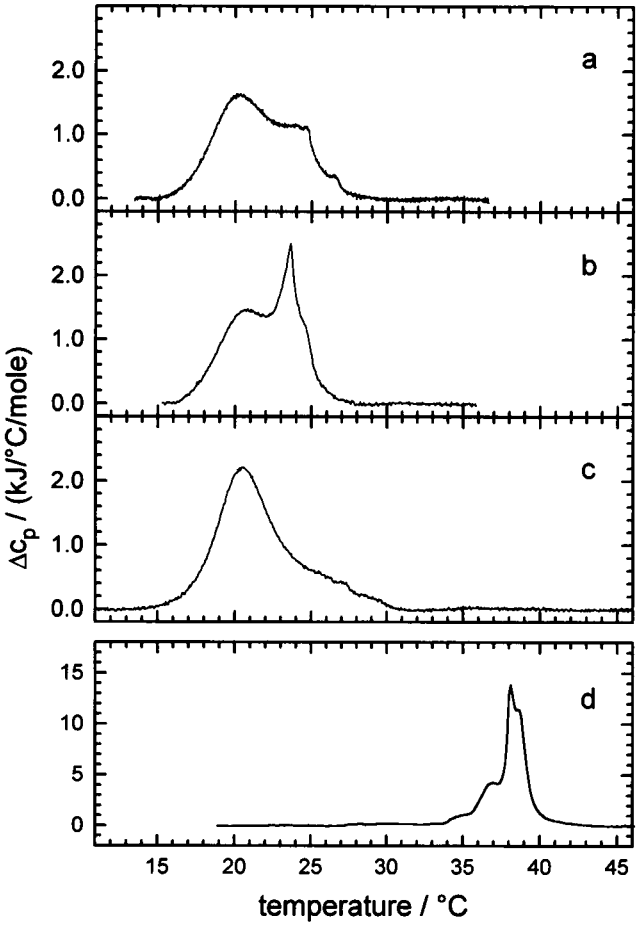

FIGURE 4 Differential scanning calorimetry in DMPC/$C_{12}E_8$ mixtures (*a–c*) and in the system DPPC/$C_{12}E_8$ (*d*). Molar ratios DMPC/$C_{12}E_8$, 1.8:1 (*a*), 2.0:1 (*b*), 2.2:1 (*c*). In *d* the molar ratio was 2:1. Heating scans, scan rate, 15°C/h.

Chain length dependence of phospholipid micellization

The temperature dependence of the aggregational state in the 2:1 mol/mol phospholipid/detergent system was also monitored by ^{31}P-NMR with variation of the phospholipid acyl chain lengths from C-8 to C-18. Homogeneous micellar solutions were obtained with 1,2-dioctanoyl-*sn*-phosphatidylcholine (C-8) and 1,2-Didecanoyl-*sn*-phosphatidylcholine (C-10) between 0 and 50°C as shown by the narrow ^{31}P-NMR signal close to 0 ppm, by the low sample viscosity, and by the absence of sample turbidity. A thermally induced bilayer-micelle transition was observed, however, with 1,2-dipalmitoyl-*sn*-phosphatidylcholine (DPPC; C-16) and 1,2-dilauroyl-*sn*-phosphatidylcholine (DLPC; C-12). Mixing of the homologous 1,2-distearoyl-*sn*-phosphatidylcholine (DSPC) with $C_{12}E_8$ was not successful, probably because of the high thermotropic phase transition temperature of this phospholipid (55°C), which is close to the lower consolute phase boundary of the detergent (~60°C; Mitchell et al., 1983).

The DPPC/$C_{12}E_8$ system behaves in a similar way as the DMPC/$C_{12}E_8$ system. A calorimetric transition occurs two degrees below the gel-to-liquid crystalline phase transition temperature of the pure phospholipid (Fig. 4 *d*). As in the DMPC/$C_{12}E_8$ mixture (cf. Fig. 4 *b*), there is a poorly resolved double peak on the high temperature side and a broad transition at the low temperature side of the calorimetric signal. The total enthalpy of the transition is about 3 times larger, however, and the width is considerably narrower by approximately 50%. Again, the aggregates were oriented in the magnetic field above 45°C as shown by ^{31}P-NMR. However, unlike the DMPC/$C_{12}E_8$ mixture, at 40°C there was an additional resonance at −1 ppm in the ^{31}P-NMR spectrum due to the presence of small nonoriented aggregates corresponding to approximately 5% of the oriented material (spectra not shown). Incubation below 38°C resulted in an isotropic signal due to the formation of micelles. It is important to note that in the case of DPPC, in contrast to the DMPC/$C_{12}E_8$ and DLPC/$C_{12}E_8$ mixtures, pure gel phase phospholipid slowly precipitates from the solution upon incubation of the sample for several hours below 40°C.

In the DLPC/$C_{12}E_8$ mixture, the chemical shift and lineshape of the ^{31}P-NMR signal was indicative of magnetic field orientation of the aggregates above 24°C (spectra not shown). Similar to the DPPC/$C_{12}E_8$ mixture, there was a minor "isotropic" ^{31}P signal in addition to the signal from oriented structures. The temperature dependence of the ^{31}P line broadening was different from the DMPC/$C_{12}E_8$ and DPPC/$C_{12}E_8$ systems. Between 24 and 12°C, the phosphorous signal

moved toward the isotropic position, whereas the line width of the major signal component continued to increase. A broad transition then occurred upon cooling from 12 to 0°C according to the drastic narrowing of the phosphorous signal. The optical density of the mixture had a maximum at 12°C and gradually decreased with further decreasing temperature, indicating that mixed micelles appeared in the mixture (not shown). In contrast to the DMPC and DPPC mixtures, the transition had a very low enthalpy (<0.3 kJ/mol), i.e., too small for an accurate determination.

Local order and segmental dynamics

The local disturbance of the phospholipid bilayer as a result of detergent incorporation was studied by ^{2}H-NMR (Fig. 5, *a* and *b*). In the field-oriented DMPC-d_{54}/$C_{12}E_8$ mixture, it is particularly easy to obtain a profile of segmental quadrupolar splittings directly from the spectra (cf. Fig. 3 *a*). A profile of effective segmental order parameters $|S_{CD}^{(i)}|_{obs}$ can be calculated from the splittings as defined in Eq. 3. In the pure DMPC sample (Fig. 5 *a*), which is randomly distributed, individual quadrupolar splittings and corresponding order parameters were determined by deconvolution of the ^{2}H-NMR spectra (Bloom et al., 1981). It is customary to assume that the quadrupolar splittings increase monotonically from the terminal methyl group toward the interfacial chain segments (Seelig, 1977). The only exception from this rule is the C_2-position in the *sn*-2 chain, which has reduced quadrupolar splittings (Seelig and Seelig, 1975; Engel and Cowburn, 1981) as a consequence of the inherent asymmetry of the phospholipid molecule (Olsson et al., 1990).

Comparing Figs. 5 *a* and 6 *b*, it can be seen that the order parameter profile in the oriented mixture has a similar shape as in the pure phospholipid sample, i.e., there is a plateau of roughly constant order parameter values up to C_7 followed by continuously decreasing values toward the chain termini. The magnitudes of the effective order parameters along the whole length of the fatty acyl chains, however, are significantly reduced in the phospholipid/detergent mixture. This may be ascribed (i) to the local perturbation introduced by the detergent into the DMPC acyl chains at the level of individual CD_2-segments, and (ii) to the whole body fluctuation of the oriented aggregate. Thus, the effective order parameter $|S_{CD}^{(i)}|_{obs}$ can be factorized according to Eqs. 3a

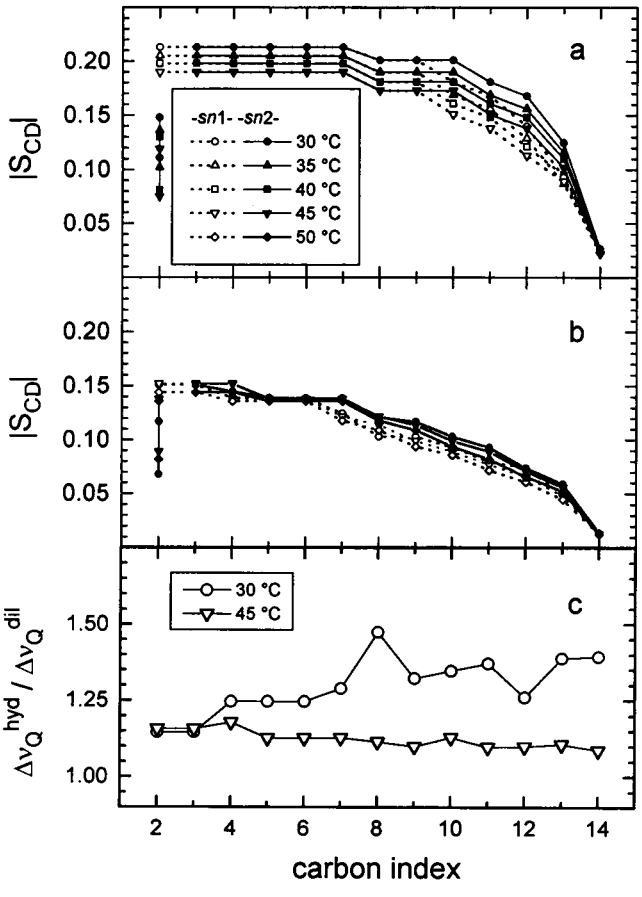

FIGURE 5 Order parameter profiles for the *sn*-1 chains (*open* symbols, - - - -) and of the *sn*-2 chains (*closed* symbols, ——) of pure DMPC-d_{54} (*a*) and of a DMPC-d_{54}/$C_{12}E_8$ 2:1 mol/mol mixture (*b*). Panel *c* shows the ratios of segmental quadrupolar splittings, $\Delta\nu_Q^{(i)}{}_{hyd}/\Delta\nu_Q^{(i)}{}_{dil}$ of 2:1 mol/mol mixtures containing 50 wt% (Thurmond et al., 1994) and >95 wt% water, respectively.

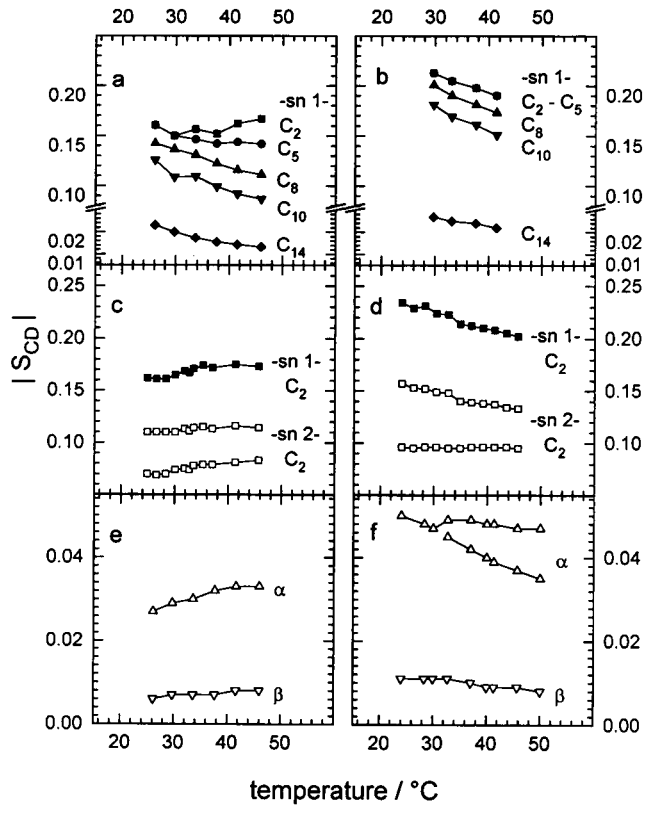

FIGURE 6 Temperature dependence of selected segmental acyl chain order parameters and headgroup quadrupolar splittings of the DMPC/$C_{12}E_8$ 2:1 mol/mol mixture (*a*, *c*, *e*) and of pure DMPC (*b*, *d*, *f*). (*a*, *b*) Order parameters of the *sn*-1 acyl chain. (*c*, *d*) Order parameters of selectively chain deuterated DMPC (α, α′-d_4-DMPC). (*e*,*f*) Quadrupolar splittings of DMPC selectively deuterated in the α and β methylene positions of the choline headgroup. Note that the numerical deconvolution in *f* for the nonoriented pure lipid sample was performed for $\theta = 90°$.

and 3b using the notation $|S_{CD}^{(i)}|$ for the rapid symmetric motion about the local director axes and S_{dir} for fluctuations of the local director axes and rotational diffusion of the whole aggregate.

Recently, order parameter profiles of DMPC/$C_{12}E_8$ mixtures at much higher concentration (50% w/w water content) have been obtained (Thurmond et al., 1994). It can be expected that whole-body motion of the aggregates is restricted at such low water content due to interaggregate interactions. The aggregate size may also increase as a result of the dense packing in these liquid crystalline mixtures. Thus, the ratios of quadrupolar splittings obtained at 50% w/w water content, $\Delta\nu_Q^{(i)}{}_{hyd}$, and in the dilute aqueous solution, $\Delta\nu_Q^{(i)}{}_{dil}$, provides an estimate of S_{dir}. As shown in Fig. 5 *c*, this ratio is almost invariable over the length of the fatty acyl chains of DMPC-d_{54} at 45°C (1.10–1.15), suggesting that $|S_{CD}^{(i)}|$ and $|S_{dir}|$ are indeed statistically independent as required for the factorization in Eq. 3a. Thus, $|S_{dir}| \approx 0.87$ can be obtained from the average decrease of the quadrupolar splittings in the dilute solution. At 30°C the ratio increases toward the chain ends from 1.15 to 1.40. According to the phosphorus chemical shift (Fig. 2 *c*) and to dynamic laser light scattering (Fig. 2 *b*), field orientation is less perfect and the particle size is less well defined at 30°C. Thus, a direct comparison of the order parameter profiles at 50% hydration and in excess water may be feasible only at temperatures >40°C.

It can be concluded from Fig. 5 that the segmental order parameters decrease more rapidly with temperature in the pure phospholipid than in the phospholipid/detergent mixture. This can be more directly demonstrated when segmental order parameters are plotted against temperature using a DMPC sample that is perdeuterated in the *sn*-1 chain only. The temperature dependence of $|S_{CD}^{(i)}|_{obs}$ for selected chain positions are compared in Fig. 6, *a* and *b*. The interfacial segments show a significantly different temperature dependence in the mixture than in the pure phospholipid. Most notably $|S_{CD}^{(i)}|_{obs}$ for $i = 2$ increases with increasing temperature in the mixed system. Even the order parameter for the $i = 5$ segment remains almost constant in the temperature range 26 to 48°C in the phospholipid/detergent mixture, in contrast to pure DMPC-d_{54}. In pure DMPC-d_{54} there is an order parameter plateau comprising C_2—C_7. This plateau value decreases monotonically with temperature (Fig. 6 *b*). The temperature dependence of the interfacial order parameters can be even better recognized when the α-carbons in the acyl chains are selectively deuterated (α,α'-d_4-DMPC; Fig. 6, *c* and *d*). In the mixture the corresponding order parameters of both the *sn*-1 and *sn*-2 chains increase with increasing temperature. Note that in the *sn*-2 position there are two quadrupolar splittings due to the inequivalence of the C_2-deuterons (Engel and Cowburn, 1981; Olsson et al, 1990). The temperature dependence of the order parameters of interfacial segments was also studied at the level of the phospholipid headgroup using DMPC, selectively deuterated in the α- and β-position of the choline moiety. Slightly increasing values were obtained for both positions with increasing temperature in the 2:1 mol/mol mixture, in contrast to the pure phospholipid samples.

The quadrupolar splittings have been further evaluated for an estimate of the average projected chain lengths (Eq. 4) and average areas of the hydrocarbon chains $\langle A\rangle$ or, alternatively, the interfacial area $\langle A\rangle'$, respectively, using Eqs. 5 and 6 (Table 1). The corresponding results for pure DMPC-d_{54} are included for comparison in Table 1. It may be noted that in pure DMPC both $\langle A\rangle$ and $\langle A\rangle'$ increase with increasing temperature, whereas in the mixture $\langle A\rangle$ increases whereas $\langle A\rangle'$ decreases slightly. Thus, the temperature dependence of the interfacial area must be considered separately at the level of different chain segments.

Order parameters are not available in the thermal transition region. Instead, the distribution of resonance frequencies can be characterized by moment analysis of the ^{2}H-NMR spectra (Fig. 7). In the lamellar state, the first and second moments, M_1 and M_2, give the mean quadrupolar splittings and the mean-square quadrupolar splittings, respectively. The parameter Δ_2 characterizes the mean-square deviation from the mean quadrupolar splitting, i.e., the width of the distribution of quadrupolar splittings (see Eqs. 7 and 8). Above the bilayer-micelle transition, the temperature dependence and the absolute values of M_1, M_2, and Δ_2 are rather similar to those obtained in the pure phospholipids (Davis, 1979; Barry et al., 1991). With decreasing temperature, M_1 and M_2 increase, whereas Δ_2 decreases slightly as a result of the increasing order in the membrane. In the transition region, M_1 decreases abruptly by 29% between 28 and 12°C for the DMPC-d_{54}/$C_{12}E_8$ mixture and by 70% between 36 and 30°C for the DPPC-d_{62}/$C_{12}E_8$ mixture. The transitions as detected by the deuterium spectral moments are in good agreement with the calorimetric scans shown in Fig. 4. The total width of the DMPC bilayer-micelle transition is approximately 11 and 10°C in the DSC scan and in the deuterium first moment, respectively. In the DPPC/$C_{12}E_8$ system, the transition extends over approximately 6°C as judged from the calorimetric scan and from M_1. In the latter system, the onset of the transition is approximately by 4°C lower in the first moment than in the DSC scan. This temperature shift is due to the deuteration of the phospholipid acyl chains. Deuteration results in a 4°C shift of the gel-to-liquid crystalline transition

TABLE 1 Average length ⟨L⟩, area ⟨A⟩, and interfacial area ⟨A⟩ of the sn-1 chain of pure DMPC-d_{54} and of a 2/1 mol/mol DMPC-d_{54}/$C_{12}E_8$ mixture

Sample	Temperature (°C)	⟨L⟩ (Å)	(corr)	⟨A⟩ (Å²)	(corr)	⟨A⟩′ (Å²)	(corr)
DMPC-d_{54}	30	11.1		35.5		30.6	
	35	10.9		35.9		31.0	
	40	10.8		36.3		31.5	
	45	10.7		36.7		31.6	
DMPC-d_{54}	30	9.86		39.8		33.9	
/C12E8,	45	9.84	(10.1)	39.8	(38.8)	33.3	(32.5)
2/1 mol/mol	50	9.78	(10.0)	40.1	(39.2)	33.3	(32.1)

⟨L⟩ was calculated from Eq. 4, and ⟨A⟩ and ⟨A⟩′ were calculated from Eqs. 5 and 6, respectively. The corrected values in brackets were calculated according to Eq. 3.

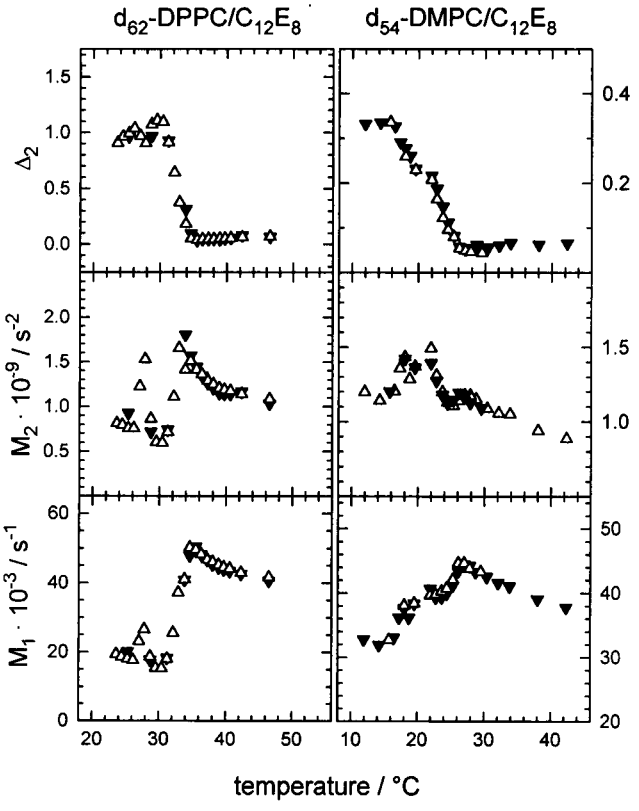

FIGURE 7 Temperature dependence of the first and second moments M_1 and M_2, respectively, and of the mean-square deviation from the mean of the quadrupolar splittings, Δ_2, of 2:1 mol/mol DPPC-d_{62}/$C_{12}E_8$ and DMPC-d_{54}/$C_{12}E_8$ mixtures. Water content 95 wt%. Open (closed) symbols indicate increasing (decreasing) temperature. Note that the total width of the transition is smaller in the DPPC mixture compared with the DMPC mixture (cf. Fig. 4).

temperature of pure DPPC (Davis, 1979). In the DMPC/$C_{12}E_8$ system, the broader transition probably prevents the detection of a shift in the transition temperature.

DISCUSSION

Structure of the magnetically oriented aggregates

The detergent-induced bilayer-micelle transition involves structural changes of considerable complexity. Solubilization of a phospholipid bilayer entails a number of stages that may be roughly divided into gross changes of bilayer size and molecular rearrangements resulting in bilayer breakdown and micellization. A succession of distinct morphological changes can be observed upon detergent titration of a bilayer membrane even in the most simple case of a two-component system consisting of a single phospholipid species and a detergent in an aqueous suspension. Smaller aggregates such as rod-like and spheroidal micelles may appear with increasing detergent concentration. Although such morphological transitions have been directly observed with different nonionic detergents by electron microscopy (Edwards et al., 1989; Edwards and Almgren, 1991; Vinson et al., 1989), the physical basis of these changes is far from understood.

In the present phospholipid/detergent systems, far above the thermotropic phase transition temperature of the pure phospholipid components, the acyl chains are oriented perpendicular to the field as shown by deuterium- and phosphorus-NMR. This observation alone does not allow an unambiguous determination of the aggregate shape, i.e., oblate or prolate ellipsoid. These principal aggregate shapes may originate from large unilamellar vesicles by distortion in the magnetic field or, alternatively, from extremely nonspherical micelles. Thus, flattened (oblate) or cylindrical (prolate) bilayer vesicles or micelles may account for the observed magnetic field orientation. A comparison with previous ^{2}H-NMR results strongly argues against rod-like micelles, however. At low water content (50% by weight) a 2:1 DMPC/$C_{12}E_8$ mixture forms a lamellar structure in the temperature range of interest (Beyer, 1983; Thurmond et al., 1994). The ^{2}H-NMR order parameter profile in Fig. 5 resembles the profile obtained in the latter sample. Specifically, the first segments of both chains have rather similar quadrupolar splittings, which results in the order parameter plateau typical for lamellar liquid crystalline phospholipids. In contrast, in a H_I-type hexagonal DMPC-d_{54}/$C_{12}E_8$ mixture, rapidly decreasing order parameters were found for the initial chain segments (Thurmond et al., 1994). Thus, the continuity with the structure at 50% water content and the chain packing as reflected by the order parameter profile are indicative of magnetically oriented *lamellar* structures.

The structural transitions occurring upon addition of $C_{12}E_8$ to small sonicated egg lecithin vesicles have been previously studied by Edwards and Almgren (1992). After addition of 35 mol% of $C_{12}E_8$, corresponding to a lecithin/detergent molar ratio of approximately 2:1, these authors observed a considerable enlargement of the vesicles. Between 30 and 40 mol% of $C_{12}E_8$, the vesicles remained essentially unilamellar and had a narrow size distribution in the range from 200 to 600 nm, depending on the sample composition. The same authors obtained large vesicles in equilibrium with open vesicles and bilayer fragments in a mixture of egg lecithin and Triton X-100 (Edwards et al., 1989). In these studies, cryo-transmission electron microscopy was performed after several days of equilibration. Before examination, the specimens were quenched from 25°C or above. It can be assumed that for the high temperature range our results are comparable with these earlier experiments. In the present system, equilibration was achieved by the sample preparation, which involves premixing of the components before hydration and long-term equilibration before measurement. Thus, large distorted vesicles may exist in the magnetically oriented DMPC/$C_{12}E_8$ mixture at 2:1 molar ratio as seen in the ^{2}H- and ^{31}P-NMR spectra, in agreement with the results obtained with egg lecithin (Edwards and Almgren, 1991).

Origin of the thermal bilayer-micelle transition

It is customary to explore the intermediary stages of the bilayer-micelle transition by controlled stepwise addition of detergent to phospholipid bilayer vesicles (da Graca et al.,

1989; Levy et al., 1990). In the present study, it is shown that in phospholipid/$C_{12}E_8$ mixtures the transition can also be induced by decreasing the temperature at *constant* phospholipid/detergent molar ratio. This behavior is somewhat surprising because one may naively assume that bilayer breakdown will be more likely at elevated rather than at low temperatures. Bilayer-micelle transformation with increasing temperature has indeed been observed in mixed chain phospholipids with one acyl chain being half the size or smaller than the other (Huang and Mason, 1986). In the system egg lecithin/octylglucoside micelle formation is also favored with increasing temperature. In the latter case, however, the micellization is due to the strong temperature dependence of the aqueous monomer concentration (cmc) of octylglucoside (da Graca et al., 1989). The monomer concentrations of octylglucoside at the breakpoint where bilayer vesicles transformed into cylindrical structures were 21.8 and 14.9 mM at 5 and at 35°C, respectively. The monomer concentration of $C_{12}E_8$ in the presence of egg lecithin vesicles is 0.07 mM at 25°C, which is close to the cmc value of the pure detergent (Edwards and Almgren, 1991; Meguro et al., 1987). In the present NMR experiments, the phospholipid concentration was typically 81 mM, i.e., the free monomer concentration of $C_{12}E_8$ was approximately 0.2% of the concentration of the membrane bound detergent. Even in the dilute samples used in dynamic light scattering experiments, the total detergent concentration was more than 10 times higher than the cmc value (Meguro et al., 1987). Thus, the bilayer-micelle transition in the present phospholipid/detergent system must be attributed to the monomer packing *within* the mixed aggregates rather than to the partition equilibrium of $C_{12}E_8$.

The average properties of the binary mixture must be considered for a qualitative understanding of the transition because the phospholipids and the detergent individually form bilayers or micelles in the temperature range under consideration. The packing constraints within a liquid crystalline mixture may be understood, at least qualitatively, using molecular shape arguments, a concept that has been introduced by Israelachvili et al. (1980). This idea emphasizes the importance of the average molecular shape for the stability of liquid crystalline phase structures such as spherical or nonspherical micelles and bilayers. According to these authors, the average molecular shape is determined by the volume V of the hydrophobic chains, the interfacial area A_o per molecule, and the critical length L_c of the chains. The interfacial area A_o is a measurable quantity that corresponds to the minimum in the interfacial free energy, whereas L_c is operationally defined as the maximum length the chains can assume. The critical length L_c is taken to be the same or somewhat less than the all-*trans* length of the hydrocarbon chains, and the chain volume is the sum of the segmental volumes assuming that the chains are incompressible. A critical packing parameter $p = V/A_oL_c$ specifies the stability range for spherical micelles ($p < \frac{1}{3}$), cylindrical structures with positive curvature, e.g., nonspherical micelles or hexagonal aggregates of type H_I ($\frac{1}{3} < p < \frac{1}{2}$), and bilayers ($\frac{1}{2} < p < 1$).

The average shape of the $C_{12}E_8$ molecule alone obviously satisfies the conditions for curved structures such as spherical or nonspherical micelles, i.e., it resembles an inverted truncated cone (Thurmond et al., 1994). An estimate of the total volumes of the hydrated detergent headgroup and of the detergent alkyl chain can be obtained allowing for the headgroup hydration of 15 water molecules per headgroup at 40°C as determined by neutron scattering (Zulauf et al., 1985). Taking account of the partial volumes of chain segments and water, a total volume of 959 $Å^3$ for the oxyethylene headgroup and 328 $Å^3$ for the detergent alkyl chain can be calculated (Zulauf et al., 1985). Thus, the headgroup/alkyl chain volume ratio of 3:1 is in agreement with the assumption of a truncated cone shape.

We have shown previously by deuterium NMR (Thurmond et al., 1994) that increasing $C_{12}E_8$ concentrations in lamellar liquid crystalline DMPC/$C_{12}E_8$ mixtures at 50 wt% hydration result in decreasing segmental order parameters in the phospholipid acyl chains. Eventually, at DMPC/$C_{12}E_8$ molar ratios <1:6, the bilayer undergoes a transition into a hexagonal (H_I) phase. It was also shown that at intermediate phospholipid/detergent ratios between 1:1 and 1:6 mol/mol, lamellar and hexagonal structures coexist and that the lamellar phase is favored at higher temperatures. Moreover, the order parameters of the interfacial segments (C_2 to C_4) were significantly higher in the hexagonal state than in the lamellar phase. These results are in line with the bilayer-micelle transformation of the DMPC/$C_{12}E_8$ system observed here at *constant* phospholipid/detergent molar ratio, assuming that the same principles govern the bilayer-hexagonal and the bilayer-micelle transitions. In the lamellar state in excess water, i.e., at temperatures above the micellization temperature, the phospholipid acyl chains are disordered, which is reflected by the deuterium order parameter profiles (Fig. 5). The detergent-induced chain perturbations translate into shorter average projected acyl chain lengths and larger average acyl chain areas (Table 1). From the quadrupolar splittings of the whole acyl chains, an average area can be calculated that represents an upper limit of the true interfacial area per chain (Eq. 5). Alternatively, the quadrupolar splittings of the plateau segments can be related to an average interfacial area $\langle A \rangle'$ (Eq. 6), yielding a lower limit value (Jansson et al., 1992; Thurmond et al., 1993, 1994). The existence of the lamellar state at higher temperatures can then be attributed to the compensating molecular shapes in the binary mixture. It can be assumed that the cone-like average shape of the phospholipid that is due to the experimentally observed acyl chain perturbation matches with the inverse truncated cone-shape of the detergent as derived from the above-mentioned neutron scattering data (see Fig. 8 in Thurmond et al., 1994).

With decreasing temperature, a more cylindrical shape of the hydrocarbon chains and, simultaneously, decreasing lateral pressure within the hydrophobic core of the structure ensues from the increasing chain ordering. At the same time, the hydration of the $C_{12}E_8$ oxyethylene moiety increases from 5 to 20 mol of water per detergent headgroup on going from

60 to 30°C, as shown by neutron scattering in a micellar detergent solution (Zulauf et al., 1985). More headgroup hydration leads to a larger effective headgroup volume and, correspondingly, to enhanced headgroup repulsion. Both factors, decreasing repulsion in the hydrocarbon layer and increasing repulsion among the detergent headgroups, are expected to force the membrane toward a transition into a structure with *positive* spontaneous curvature, which means convex with respect to the bulk water phase. Thus, the bilayer will eventually rupture as a consequence of the changing balance of forces in the headgroup and in the hydrocarbon regions. With increasing temperature, starting in the micellar state (below the micellization temperature), the same sequence of changes will be obtained in opposite order. Reversibility is borne out by the absence of hysteresis effects in the temperature scans performed with NMR spectroscopy, light scattering, and calorimetry.

The temperature dependence of the segmental acyl chain order parameters in Fig. 6 confirms the balance of repulsive forces in the lamellar DMPC-d_{54}/$C_{12}E_8$ mixture. Most notably, the mixture differs from pure DMPC-d_{54} in that the slope of the order parameter versus temperature curves change from positive to negative at the level of C_4 or C_5 (Fig. 6 *a*), suggesting that there is a neutral or pivotal plane where, by definition, the increasing chain repulsion (due to thermal isomerization of the chains) and the decreasing headgroup repulsion (due to thermal dehydration of the detergent headgroups) neutralize each other. As a consequence, the average area per molecule will change least in this plane during the transition (Gawrisch et al., 1992; Thurmond et al., 1993).

A relation among headgroup size, interfacial area, and bilayer thickness has been directly demonstrated by low angle x-ray scattering in a series of pure lamellar liquid crystalline oxyethylene detergents (Carvell et al., 1986). For $C_{12}E_3$, $C_{12}E_4$, and $C_{12}E_6$ at 25°C, the areas at the alkylpolyoxyethylene interface were 36.8, 42.6, and 51.6 Å^2, respectively. In this series, assuming constant partial specific volumes of the hydrocarbon and of the water regions, the corresponding thickness of the hydrocarbon layer was calculated to be 19.1, 16.5, and 13.6 Å. Thus, a cylindrical average molecular shape is maintained as required for the lamellar state by an increasing average area per molecule. An analogous behavior can be deduced from the interfacial ^{2}H order parameters in the lamellar phospholipid/detergent mixture. With decreasing temperature, when the *detergent* takes up more water, the increasing size of the hydrated oxyethylene headgroup creates an increasing average area at the level of the interfacial segments of the *phospholipid*, resulting in enhanced motional freedom. The temperature dependence of the corresponding segmental order parameters (Fig. 6) as well as the related average interfacial area per chain, $\langle A \rangle'$, reflect this gain in motional freedom in the water/hydrocarbon interface (Table 1). In the DMPC/$C_{12}E_8$ mixture $\langle A \rangle'$ slightly increases with decreasing temperature, in contrast to the pure phospholipid where both $\langle A \rangle'$ and $\langle A \rangle$ decrease. The order parameters of the phospholipid headgroup segments in the mixed system also point at the decreasing packing density in the phopholipid headgroup region (cf. Fig. 6, *e* and *f*).

Relation between bilayer micellization and thermotropic phase transition

A thermal transition between bilayer and micellar states has been observed here in mixtures of $C_{12}E_8$ with DPPC, DMPC, and DLPC. The temperature range, however, where the transition occurs is extremely different for phosphatidylcholines with different acyl chain lengths. It may be argued that in DPPC and DMPC the transition is related to the gel-to-liquid crystalline phase transition temperatures, T_c, of the pure phospholipids at 41 and 23°C, respectively. The bilayer-micelle or micelle-bilayer transformation (depending on the direction of the temperature scan) commences close to T_c for these lipids as detected by ^{2}H- and ^{31}P-NMR and by calorimetry. In DLPC, however, the bilayer-micelle transition was observed at 12°C, which is 13°C above the pure lipid phase transition (Cevc and Marsh, 1987). Phospholipids with still shorter saturated acyl chains, 1,2-didecanoyl-*sn*-phosphorylcholine and 1,2-dioctanoyl-*sn*-phosphorylcholine, were found in a micellar state over the whole temperature range from 0 to 50°C.

The simplifying shape model, therefore, may not be sufficient for a full comprehension of all details of the bilayer-micelle transition observed in the present system. The relatively narrow signal on the high temperature side of the calorimeter curve in the DPPC and DMPC mixtures can be attributed to a moderately cooperative phase transition of the phospholipid acyl chains. The appearance of this signal strictly depends on the composition of the phospholipid/$C_{12}E_8$ mixtures. At a phospholipid/detergent molar ratio 2:1, the width of the transition is <2°C. Even a small deviation from the 2:1 composition in either direction (cf. Fig. 4) leads to substantial broadening of the signal. Similarly, a well defined ^{2}H-NMR spectrum was only obtained at 2:1 molar ratio (Fig. 1), which can be attributed to microscopic homogeneity at this particular composition. These observations suggest that a 2:1 "complex" forms that probably involves hexagonal lateral order in the plane of the membrane with each detergent being surrounded by six phospholipids. Complex formation has been reported for mixtures of various fatty acids (Mabrey and Sturtevant, 1977; Schullery et al, 1981; Marsh and Seddon, 1982; Koynova et al, 1988; Cevc et al, 1988) and long chain alcohols (Pringle and Miller, 1979; Eliasz et al., 1976) with phospholipids. It is worth noting that in mixtures of palmitic acid and stearic acid with DPPC congruent melting occurs at a phospholipid/fatty acid molar ratio of 1:2, i.e., at the inverse ratio as observed in the present system. The congruent melting of phospholipid/fatty acid mixtures at this molar ratio was first attributed to a transition from a gel state into the L_α phase (Schullery et al., 1981). Later it was shown that the melting of the gel state complex involves a *direct* transition from the L_β to the H_{II} phase (Marsh and Seddon, 1982; Koynova et al., 1988) without an

intermediate lamellar liquid crystalline state. Thus, at the transition temperature, the interfacial curvature of the aggregate goes from zero to negative. This result is remarkable in the context of the present observations in the phospholipid/$C_{12}E_8$ system. Here the system responds to increasing temperature with a transition from a micellar state (which topologically corresponds with the H_I rather than with the H_{II} phase) into the lamellar state, i.e., the surface curvature changes from positive to zero.

In the DLPC sample, where chain crystallization is not involved, the transition proceeds less abruptly than in the DPPC and DMPC mixtures. The acyl chain volume of DLPC and the temperature-induced chain disordering suffice, however, to force the curved mixed micellar structure into the lamellar state above 18°C. In contrast, even at 50°C not enough lateral pressure is developed by the shorter chains of the C-8 to C-10 phospholipids to initiate the transition into the lamellar state. X-ray work will be necessary to substantiate this simple model through the determination of chain average interfacial areas in the transition region in the different phospholipid/detergent systems.

The origin of the broad calorimetric signals at 20.5 and 37°C in the DMPC and DPPC/$C_{12}E_8$ mixtures, respectively, is not clear. It may be assumed that hydration or dehydration of the oxyethylene chains that accompanies the structural change in either temperature direction contribute to the enthalpy of this transition. In the 1:2 phospholipid/fatty acid system, such a broad transition has not been observed. The total enthalpy of this signal corresponds to 3–3.5 kJ/mol (with respect to the detergent component). This may be compared with the micellar-lamellar transition enthalpy in pure $C_{12}E_4$ of 0.8 kJ/mol (Mädler et al., 1994). A direct comparison between pure detergent and the present phospholipid/detergent mixture may not be justified, however. Phase separation may occur in the mixture over the temperature range of the calorimetric signal (approximately 8 and 5°C in the presence of DMPC and DPPC, respectively). Polydispersity in the sample may also result in the ill-defined lineshape of the ^{2}H-NMR spectra (cf. Fig. 3 *d*) and in the ^{31}P-NMR line broadening (Fig. 2 *c*) in the transition region. Alternatively, assuming that the oriented aggregates are flattened vesicles with an approximate hydrodynamic radius of 150 nm, a loss of field orientation is expected to result in a ^{2}H-NMR lineshape similar to that in Fig. 3 *d* due to combined vesicle tumbling and lateral diffusion (Fenske, 1993). The magnetic field orientation will be lost if the bilayer bending rigidity increases with decreasing temperature (see below).

The first and second deuterium spectral moments obtained from a multiply deuterated phospholipid membrane are related to the average carbon-deuterium orientational order parameter and to the mean-squared order parameter, $\langle |S_{CD}| \rangle$ and $\langle |S_{CD}|^2 \rangle$, respectively. This holds true for nonoriented and oriented membranes (regardless of the slightly different numerical factors involved), provided that the phospholipid motion is axially symmetric in the membrane director system (cf. Eq. 7). In the oriented phospholipid/detergent mixtures, i.e., in the high temperature state of the system, the order of magnitude and the temperature dependence of the moments closely resemble those obtained in pure phospholipid multilamellar dispersions (Davis, 1979; Barry et al., 1991; Trouard, 1992). The M_1 and M_2 values show the increasing ordering of the acyl chains with decreasing temperature. At the same time, the slight decrease of Δ_2 most probably reflects the increasing statistical weight of the order parameter plateau values. A quantitative comparison with previous work may be difficult, however, because the moments critically depend on experimental parameters (Davis, 1983). After the onset of membrane solubilization, the spectral moments are no longer related to membrane order. Instead, they merely reflect the distribution of resonance frequencies. In pure lipid dispersions, there is a sudden increase of M_1 and M_2 at T_c and a continuing increase in the gel phase. In the present system, solubilization is indicated by the abruptly decreasing spectral moment values adjacent to T_c. The total width of the lamellar-micellar phase transition can be accurately monitored by the deuterium spectral moments. Moreover, the spectral moments reveal details in the frequency distribution such as the appearance of a very broad, unresolved spectral component in the DPPC mixture at 26.5°C and a sudden change in line broadening in the DMPC system at 17°C (cf. Fig. 7), which are not immediately obvious from the spectra. Although an interpretation is not possible on the basis of the NMR results alone, these features could be useful in combination with an electron microscopic study of the intermediary stages of the solubilization process.

A complete phase diagram was reported recently for the system DPPC/$C_{12}E_4$ (Mädler et al., 1994). This system shows homogeneous mixing in the L_α phase. In contrast to the phospholipid/$C_{12}E_8$ mixtures, however, the DPPC/$C_{12}E_4$ system does not transform into a mixed micellar state close to T_c of pure DPPC, except for very low DPPC concentrations (DPPC/$C_{12}E_4$ < 0.75 mol/mol). Rather, complicated phase equilibria, including peritectic and eutectic behavior, were found at low temperatures. This difference is not surprising because the shorter oxyethylene chain of $C_{12}E_4$ will exert less steric repulsion than the bulky hydrated headgroup of $C_{12}E_8$. Accordingly, $C_{12}E_4$ displays in its phase diagram a broad lamellar and a rather small micellar phase above and below 20°C, respectively (Mitchell et al., 1983).

Magnetic field orientation and bilayer elasticity

Magnetic field orientation of bilayers has been observed occasionally after the high field strength of superconducting magnets became available (Maret and Dransfeld, 1977; Seelig et al., 1985). Mixtures of different lipids were found to be particularly prone to orientation in the magnetic field (Forrest and Reeves, 1981; Speyer et al., 1987; Jansson et al., 1990). This phenomenon is often a nuisance in wide line NMR studies because incomplete orientation leads to lineshape distortions that render the deconvolution ("de-Pakeing") (Bloom et al., 1981) of deuterium-NMR spectra impossible. In the present case of a mixed phospholipid/detergent system, the orientation seems to be nearly perfect

so as to enable the determination of the quadrupolar splittings of individual chain segments directly.

The extent of magnetic field orientation in a phospholipid aggregate is proportional to $\exp(-N\Delta\chi H^2/kT)$, where H is the magnetic field strength, $\Delta\chi$ is the anisotropy of the diamagnetic susceptibility of the constituent phospholipids (assuming an axially symmetric susceptibility tensor), and N is the average number of the lipid molecules in the aggregate (Maret and Dransfeld, 1977; Speyer et al., 1987). Negative $\Delta\chi$ values have been determined for crystalline fatty acids and phosholipids, i.e., $-26 \cdot 10^{-6}$ and $-68 \cdot 10^{-6}$ EMU/mol in stearic acid and DPPC, respectively (Lonsdale, 1939; Sakurai et al., 1980). Therefore, it can be anticipated that phospholipid aggregates will orient with their long molecular axis perpendicular to the external field. Field orientation requires that the lipid molecules are parallel correlated in an essentially flat bilayer structure. The opposite membrane orientation with the bilayer normal parallel to the magnetic field has been achieved by addition of the polar aromatic compound α-naphthol. This can be ascribed to the approximately 10-fold larger diamagnetic susceptibility of the aromatic ring system as compared with the fatty acyl chains of the phospholipids (Sanders et al., 1993).

The free energy necessary to overcome the bending rigidity of curved structures must be compensated by the gain in free energy due to field alignment. It has been shown that even small additions of lysolecithin (Jansson et al., 1990) or Triton X-100 (Sanders et al., 1993) result in spontaneous orientation in the magnetic field. Thus, the presence of single-chain amphiphiles in the bilayer can be assumed to attenuate the bilayer rigidity. To our knowledge, no systematic study of the detergent effect on the bilayer elastic modulus has been presented thus far, however. Membrane disordering seems to be essential for the orientation effect because it was shown that membranes do not orient spontaneously in the magnetic field below the order-disorder transition temperature (Qiu et al., 1993; Speyer et al., 1987). Thus, it can be assumed that the segmental order parameter profile obtained by ^{2}H-NMR spectroscopy is in some way related to the the elastic modulus of the membrane.

The magnetic field orientation of liposomes, as expected, largely depends on the field strength *and* on the aggregate size. Nonvortexed multibilayer liposomes of 1–5 μm diameter are almost perfectly oriented at 11.7 T, whereas smaller vesicles or multibilayers prepared by appropriate procedures will only be partially oriented (Qiu et al., 1993). The particle size obtained in the DMPC/$C_{12}E_8$ mixture above the micellization temperature (Fig. 2) was approximately 0.2 μm, suggesting that the detergent-induced reduction of the bending rigidity governs the orientability rather than the aggregate size.

It is interesting to note that α-helices in peptides and proteins tend to orient with their long axis parallel to the magnetic field direction (Neugebauer et al., 1977). In most membranes, however, compensation of the opposing magnetic susceptibilities of lipids and proteins prevents the membranes from orienting in a magnetic field at all. Reconstitution into an appropriate lipidic environment including both phospholipids and detergents may be useful for the homogeneous orientation of certain membrane proteins in strong magnetic fields.

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