

Langmuir

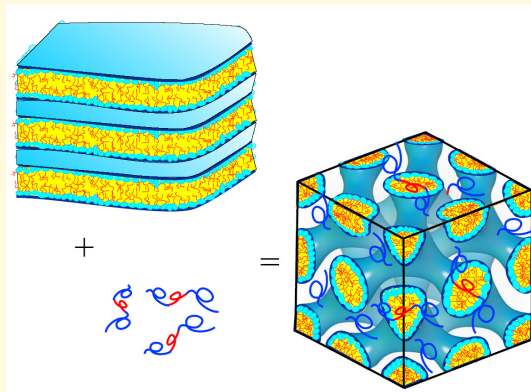


Langmuir 2005, 21, 1766-1775

**Lamellar-to-Cubic Phase Change in
Phospholipid Bilayer Systems
Incorporated with Block Copolymers:
DMPC and PEO-PPO-PEO (P85)**

Torben Ishøy and Kell Mortensen

(Research Article) DOI: [10.1021/la048206r](https://doi.org/10.1021/la048206r)



Lamellar-to-Cubic Phase Change in Phospholipid Bilayer Systems Incorporated with Block Copolymers: DMPC and PEO–PPO–PEO (P85)

Torben Ishøy and Kell Mortensen*

Danish Polymer Center, Risø National Laboratory, DK-4000 Roskilde, Denmark

Received July 16, 2004. In Final Form: December 2, 2004

We have used small-angle X-ray scattering and calorimetric methods to investigate the temperature-dependent phase behavior of ternary systems of phospholipid (DMPC), amphiphilic PEO–PPO–PEO block copolymer (Pluronic P85), and water. It is shown that a relatively small amount of block copolymers (~3 wt %) results in a lamellar-to-cubic phase transition. Still, both the bilayer-characteristic main transition, associated with chain melting, and the pretransition, associated with in-plane modulations, are preserved for copolymer concentrations up to 50–70 wt %, indicating the preservation of a bilayer type of lipid organization also within the cubic phase. The main transition splits up into two transitions upon the addition of copolymers, one resembling the high cooperativity of the main transition and one broad transition which may reflect complex formation with the copolymers. Parallel studies incorporating poly(ethylene glycol) into the DMPC multilamellar vesicles do not give analogous structural changes. It is concluded that the major effect on the molecular scale of adding PEO–PPO–PEO block copolymers is not only due to the hydration of the membrane but also due to the incorporation of the PPO block into the bilayer structure.

1. Introduction

There has within the past decades been significant research in materials for controlled drug delivery systems and drug carriers. These studies include soluble polymers, lipoproteins, microparticles made of insoluble or biodegradable natural and synthetic polymers, microcapsules, micelles, and liposomes. Each of these carrier types offers its own advantages but has also serious shortcomings. Phospholipid liposomes are promising drug delivery systems, due to their biocompatibility and ability to entrap and transport hydrophilic species. Pure liposomes, on the other hand, show already after few times of consumption a highly reduced lifetime in the body. A route to control and increase this liposome lifetime in vivo may be to control the steric stability by incorporating surface active polymers into the system. Poly(ethylene oxide) (abbreviated as PEO) and polymeric surfactants are among the promising candidates.

In an attempt to be able to design a specific drug delivery or transport system, it is essential to develop a better basic understanding of the physical–chemical effect that the surface active polymers might have on the thermodynamic properties of the lipid bilayer systems. There have already been some reports concerning liposomes incorporated with the poly(ethylene oxide) type of copolymers, but with somewhat diverging conclusions. There is therefore a need for further systematic studies of such systems.

A number of studies have concerned aqueous mixtures of lipids and the amphiphilic “Pluronic” type of triblock copolymers,¹ PEO–PPO–PEO, which is also the type of polymer we have investigated. PEO and PPO are the abbreviations for poly(ethylene oxide) and poly(propylene oxide), respectively. Some papers have addressed the question of the miscibility between PEO copolymers and

lipids: Bryskhe et al. recently conducted a systematic study on aqueous mixtures of soybean phosphatidylcholine and EO₅PO₆EO₅ copolymers (abbreviated as Pluronic L121), focusing on the ambient-temperature phase behavior.² The PEO–PPO–PEO block copolymer used is itself bilayer-forming at ambient temperature. Bryskhe et al. found that L121 block copolymers and lecithin are immiscible under ambient conditions. Chandaroy et al.,³ Johnsson et al.,^{4,5} and Oradd et al.⁶ have reported a corresponding immiscibility but on the mesoscopic length scale in terms of micelle formation when mixing lipids and poly(ethylene oxide) copolymers. Chandaroy et al. studied the effect of adhesion between cells and liposomes incorporated with EO₉₉PO₆₇EO₉₉ copolymers (abbreviated as Pluronic F127).³ Chandaroy et al. found a major temperature dependency, which was attributed to the block copolymer micelle formation. Landh found by mixing the same F127 triblock copolymer with lipids that the micellar L₂ phase is restricted to copolymer concentrations beyond 20 wt %. Johnsson et al. found in mixtures of PEG–lipids and respectively distearoyl phosphatidylcholine and dipalmitoyl phosphatidylcholine similar characteristics, namely, a transition from a dispersed lamellar liposome phase to a micellar phase.^{4,5} Johnsson et al. used cryogenic transmission electron microscopy and dynamic light scattering to characterize the aggregate structure and phase behavior. The applied PEG–lipids contained PEG with a molecular weight of either 2000 or 5000. The onset of micelle formation was found to take place at PEG–lipid concentrations of <5 mol %.

Oradd et al. argued that the micelles arrange themselves into close packed ordered structures.⁶ Sadaghiani et al. found, using NMR, that solutions of poly(ethylene oxide)–

* To whom correspondence should be addressed. E-mail: kell.mortensen@risoe.dk.

(1) Pluronic is a BASF trade name for PEO–PPO–PEO triblock copolymers; see *Pluronic Surfactants Technical Brochure*; BASF-Corporation: Parsippany, NJ, 1989.

(2) Bryskhe, K.; Schillen, K.; Lofroth, J.-E.; Olsson, U. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1303.

(3) Chandaroy, P.; Sen, A.; Alexandridis, P.; Hui, S. W. *Biochim. Biophys. Acta* **2002**, *1559*, 32.

(4) Johnsson, M.; Silvaner, M.; Karlsson, G.; Edwards, K. *Langmuir* **1999**, *15*, 6314.

(5) Johnsson, M.; Edwards, K. *Biophys. J.* **2003**, *85*, 3839.

(6) Oradd, G.; Lindblom, G.; Fontell, K.; Ljusberg-Wahren, H. *Biophys. J.* **1995**, *68*, 1856.

octylphenyl ether (abbreviated as Triton X-100) can solubilize lecithin up to 10 wt %.^{7,8} Sadaghiani et al. found micelles or lamellar phases, depending on composition. Khahn et al. studied the solubilization of poly(ethylene oxide) homopolymer into the lamellar phase of soybean lecithin, using NMR and polarizing microscopy methods.⁹ The solubilization of polymers is significant in the lamellar phase only with a water content of <10%. Between 20 and 33 wt % water, the liquid crystalline lamellar phase is unable to solubilize any appreciable amounts of polymers. Upon the addition of ~0.5% polymers, the lamellar mesophase is destabilized. Khahn et al. concluded that the binding of water to the interface and the molecular organization of the choline headgroup are little affected by the presence of polymer molecules in the mesophase.

The investigations concluding the miscibility of lipids and PEO copolymers are rather divergent and include both the remaining lamellar phases and copolymer driven formation of novel cubic and hexagonal phases. Baekmark et al. studied unilamellar vesicles of dipalmitoyl phosphatidylcholine (DPPC) mixed with different poly(ethylene oxide)-based block copolymers: DSPE-EO₄₅, DSPE-EO₁₁₀, and EO₄₂S₄₂EO₄₂, where DSPE is the abbreviation for distearoyl phosphatidylethanolamine and S₄₂ is polystyrene.¹⁰ Baekmark et al. showed that the main phase transition temperature changes only by a few degrees upon the addition of polymers, but an additional enthalpic peak was observed at temperatures slightly above the main transition. Kostarelos et al.¹¹ found, based on dynamic light scattering and electrophoresis, results indicating that the Pluronic PEO-PPO-PEO block copolymers are physically adsorbed to the vesicle surface. This conclusion was partly based on a marked increase in vesicle size upon the addition of copolymers.

Landh,¹² Funari et al.,¹³ and Oradd et al.⁶ found a wide range of miscibility between lipids and related copolymers. Landh¹² used the same F127 triblock copolymer as Chandaroy and found in aqueous mixtures of single-chained lipid-monoacylglyceride four different bilayer-based cubic bicontinuous phases: the *Ia3d* gyroid phase (G), the *Pn3m* double diamond phase (D), the Schwarz primitive structure (P), and the Neovius periodic minimal surface structure (C).¹² At copolymer concentrations higher than 20 wt %, the cubic P phase is transformed to a reversed micellar (L₂) phase, as mentioned above. The phase diagram possesses in addition two lamellar (L_α) phases, of which Landh suggested one to originate from the monoacylglyceride/water system. Funari et al. found in another system, POPE-C₁₂EO₂, where POPE is the abbreviation for phosphatidylethanolamine, similar structural characteristics.¹³ Using X-ray scattering, Funari et al. found the lamellar L_α phase at 20 °C, the cubic *Ia3d* gyroid phase at intermediate temperatures, and the cubic *Pn3m* double diamond phase at 39 °C. Oradd et al. established, using X-ray and NMR, that soybean phosphatidylcholine together with diacylglycerol and water form either lamellar, reversed hexagonal, or cubic phases depending on the concentration and temperature.⁶ In opposition to the bilayer model of Landh, Oradd et al.

argued that the cubic phase is built up of close packed reversed micelles.

The present paper reports experimental calorimetric and small-angle X-ray structural studies of aqueous systems of dimyristoyl phosphatidylcholine (DMPC) lipids incorporated with Pluronic P85 triblock copolymers, EO₂₇-PO₃₉-EO₂₇. The studies include variations in the polymer-to-lipid ratio, the content of water, and the temperature. The studies conclude that there is a wide range of macroscopic miscibility between the copolymers and the lipids. At temperatures above the bilayer-associated *main transition* temperature, the mixtures remain in lamellar organization as pure DMPC in water. Below *T_m*, on the contrary, the incorporation of small amounts of block copolymers results in a transition to a cubic phase, but with a preserved bilayer type of lipid organization. It is shown that the major effect of adding PEO-PPO-PEO block copolymers involves incorporation of the PPO block into the bilayer structure.

2. Materials and Methods

The experimental studies concern aqueous mixtures of dimyristoyl phosphatidylcholine (DMPC) lipids incorporated with Pluronic P85 triblock copolymers, EO₂₇-PO₃₉-EO₂₇. The characteristics of both DMPC lipids and P85 copolymers are in aqueous suspension by themselves rather rich in phase behavior. A brief summary may be relevant for the comparison to the mixed systems.

The phase behavior of a pure aqueous suspension of DMPC phospholipid is characterized by various lamellar phases:^{14,15} Two low-temperature solid (gel) phases with conformationally ordered acyl chains, denoted respectively by L_{β'} and P_{β'}, and at high temperatures the liquid crystalline smectic phase, L_α, with conformationally disordered acyl chains. The P_{β'} phase is characterized by in-plane modulations and is accordingly often named the ripple phase. The transition at *T_m* = 24 °C between the P_{β'} gel phase and the L_α liquid phase is the so-called main phase transition. At lower temperatures, *T_p* ~ 10 °C, the bilayers undergo a solid-solid pretransition between the low-temperature, planar, lamellar L_{β'} phase and the intermediate-temperature P_{β'} ripple phase. A fourth 3d crystalline L_c phase is stabilized after a long storage time below *T_c* = 16 °C.¹⁵

Aqueous suspensions of P85 triblock copolymers, EO₂₇PO₃₉EO₂₇, are dominated by micelle formation and micellar ordering.¹⁶ At low temperatures and low concentrations, the block copolymers are dissolved as unimers. Upon either raising the temperature or increasing the block copolymer concentrations, the PPO blocks tend to associate, resulting in the formation of spherical micelles. The volume fraction of these micelles depends on both temperature and copolymer concentration. At a sufficiently high polymer concentration (above roughly 20%), the hard-sphere interaction of the micelles causes a body centered cubic micellar structure. Close to 50% copolymer concentrations, the micelles transform into cylinderlike structures, resulting in a hexagonally ordered phase. At even higher concentrations, the micelles form platelike objects, forming a lamellar phase. In pure P85 block copolymers, the PEO crystallizes in thin lamellae, also resulting in an overall block copolymer lamellar phase. Pluronic-type micelles are used for drug delivery also in the absence of phospholipids; see, for example, the review by Torchilin.¹⁷

2.1. Materials and Sample Preparations. Dimyristoyl phosphatidylcholine (abbreviated as DMPC) with a purity higher than 99% was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). The poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) triblock copolymer (Pluronic P85) with a mean composition of EO₂₇-PO₃₉-EO₂₇ was a gift from the BASF Corporation. The poly(ethylene glycol) homopolymers PEG1000

(7) Sadaghiani, A. S.; Khan, A.; Lindman, B. *J. Colloid Interface Sci.* **1989**, *132*, 352.

(8) Sadaghiani, A. S.; Khan, A. *Langmuir* **1991**, *7*, 898.

(9) Khan, A.; Zhang, K. W.; Mendonca, C. *J. Colloid Interface Sci.* **1994**, *165*, 253.

(10) Baekmark, T. R.; Pedersen, S.; Jørgensen, K.; Mouritsen, O. G. *Biophys. J.* **1997**, *73*, 1479.

(11) Kostarelos, M.; Luckham, P. F.; Tadros, T. F. *J. Chem. Soc., Faraday Trans.* **1998**, *94*, 2159.

(12) Landh, T. *J. Phys. Chem.* **1994**, *98*, 8453.

(13) Funari, S. S.; di Vitta, C.; Rapp, G. *Acta Phys. Pol., A* **1997**, *91*, 953.

(14) Tardieu, A.; Luzatti, V.; Reman, F. C. *J. Mol. Biol.* **1973**, *75*, 711.

(15) Mortensen, K.; Pfeiffer, W.; Sackmann, E.; Knoll, W. *Biochim. Biophys. Acta* **1988**, *945*, 221.

(16) Mortensen, K. *J. Phys.: Condens. Matter* **1996**, *8*, A103.

(17) Torchilin, V. P. *J. Controlled Release* **2001**, *73*, 137.

($\sim\text{EO}_{23}$) and PEG4000 ($\sim\text{EO}_{91}$) are gifts from Hoechst AG. Other chemicals were purchased from Sigma Chemical Co and used without further purification.

The lipid mixtures were made by mixing DMPC with P85 or PEG in a solvent consisting of an equal amount of chloroform and methanol. After being mixed, the solvent was evaporated under a clean dry nitrogen stream and subsequently dried under vacuum for at least 12 h, to remove traces of solvent and water. The lipid/polymer mixtures were successively dissolved in a desired amount of aqueous salt buffer (50 mM KCl and 1 mM NaN_3) and sealed. This buffer is in the following for simplicity referred to as solvent, water, or buffer.

The samples were hydrated for a minimum of 2 h at $T \approx 35$ °C, which is ~ 10 °C above the main melting transition, $T_m \approx 24$ °C, of the lipids. The samples were shaken vigorously with a vortex mixer and cycled through T_m every 15 min.

The samples are in the following described in terms of water content and relative polymer concentration, with the latter identity defined as $w_{\text{P85}} = M_{\text{P85}}/(M_{\text{P85}} + M_{\text{DMPC}})$, where M_{P85} and M_{DMPC} are the total masses of P85 block copolymers and DMPC lipids, respectively.

To ensure that the samples with high DMPC/P85 concentrations had reached equilibrium, they were stored at room temperature for at least 2 weeks.

2.2. Differential Scanning Calorimetry. The phase transitions of the ternary DMPC/P85/water systems were determined using differential scanning calorimetry (DSC). Measurements on samples of low DMPC/P85 concentration were carried out on a Microcal MC-2 liquid-DSC instrument. The samples with high DMPC/P85 content are too viscous to be transferred into that sample well. The measurements of the high-concentration samples were therefore carried out using a Perkin-Elmer Pyris 1 differential scanning calorimeter, to which ~ 20 mg of the samples was transferred onto small aluminum wells and sealed. After being equilibrated for 12 h at 5 °C, each sample was scanned successively two times, with scan rates of 1 and 2 °C/min, respectively. Before the second scan, the samples were equilibrated for 50 min at 5 °C.

2.3. Small-Angle X-ray Scattering. The samples used in the small-angle X-ray scattering (SAXS) experiments were transferred to 2 mm diameter borosilicate capillary tubes (W. Müller, Germany) sealed with wax.

The SAXS measurements were performed on the Risø-SAXS camera, which is equipped with a two-dimensional gas-filled detector (Gabriel EMBL, France). The beam is the Cu $K\alpha$ radiation generated by a rotating anode X-ray generator (Rigaku, Japan) and monochromized using a pyrolytic graphite crystal, giving the wavelength $\lambda = 1.54$ Å. The collimation of the beam is determined by three sets of slits: one just after the source and two close to the sample. The slits give a square shaped beam with a width of 1 mm at the sample position and 2 mm at the detector position. With a sample-to-detector distance of 1 m, the range of momentum transfer ranges from $q = 0.02$ Å $^{-1}$ to $q = 0.5$ Å $^{-1}$. The X-ray acquisition time was typically 1 h. The sample temperature was regulated by a homemade computer controlled oven with a precision better than 0.5 °C.

3. Results and Discussion

3.1. Differential Scanning Calorimetry (DSC). The thermodynamic characteristics of the ternary DMPC/P85/water system were studied using differential scanning calorimetry (DSC). The thermodynamic data are extracted by fitting Gaussian functions to the thermograms. Within experimental resolution, the spectra are well described by the Gaussian functions

$$C_p(T) = \frac{\Delta H}{\Delta T \sqrt{\pi/2}} e^{-2(T-T_{\text{trans}})^2/\Delta T^2} + C_{p,0} \quad (1)$$

where ΔH is the transition enthalpy, T_{trans} is the transition temperature, ΔT is the full temperature width at half-maximum, and $C_{p,0}$ is an offset parameter.

From Figure 1, it is seen that the specific heat capacity, C_p , shows several characteristics. The most dominant

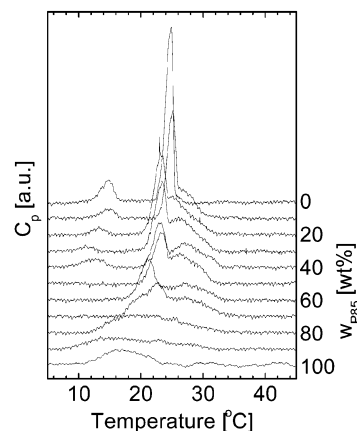


Figure 1. Specific heat capacity (arbitrary units) of the DMPC/P85/water system. The solvent content is kept at 70 wt % in all samples. The individual curves have been shifted proportional to P85 concentration for clarification.

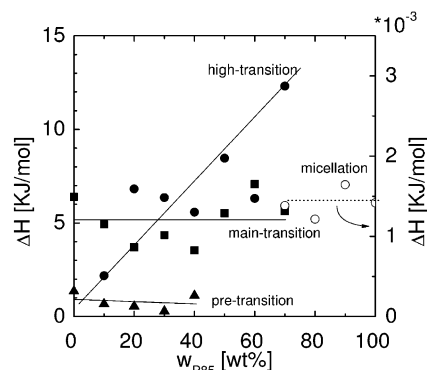


Figure 2. Transition enthalpy, ΔH (kilojoules per mole), for the different transitions of the DMPC/P85/water system. The solvent content is kept at 70 wt % in all samples, while the relative copolymer concentration is varied: (■) the main transition; (▲) the pretransition; (●) the high-temperature transition. The enthalpies are normalized to the concentration of DMPC. The micelle formation transition is, in contrast to the other transitions, linked to the right axis and is shown with the symbol ○. The micelle formation transition is normalized to the concentration of P85.

feature is the change in the enthalpy peak near $T_m \sim 24$ °C. In the pristine aqueous DMPC system, $T_m(w_{\text{P85}} = 0) = T_m^0$, this is the main phase transition due to combined chain melting and the order-to-disorder arrangement of the lipid molecules, separating the low-temperature $P_{\beta'}$ gel phase from the high-temperature L_{α} fluid phase. In the DMPC/P85 mixed samples, two peaks are observed in the same temperature regime, revealing two distinct transitions. One relatively sharp transition resembles the characteristics of the lipid main transition being rather sharp and continuously changing from the value of pure DMPC. The other transition is significantly broader and distinctly different from the DMPC main transition characteristics.

It seems that the two transitions both evolve from the pristine DMPC main transition, indicating that both transitions reflect some lipid chain melting characteristics. The temperature of the sharp transition decreases slightly with P85 content, while the broader transition increases with P85 concentration. The broad transition is, though, experimentally observed only for the P85 content $w_{\text{P85}} \sim 10$ wt %.

Figure 2 shows the integrated enthalpy of the observed transitions (Figure 1). It appears from Figures 1 and 2 that while the temperature $T_m(w_{\text{P85}})$ of the sharp transition

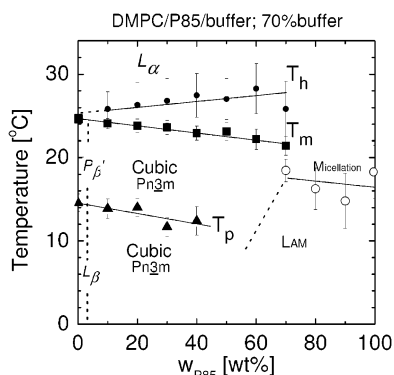


Figure 3. Transition temperatures, T , for the (■) main transition, (▲) the pretransition, (●) the high-temperature transition, and (○) the micelle formation transition of the DMPC/P85/water system. The error bars indicate the width of $\Delta T/2$.

near T_m° decreases with an increasing amount of P85, the integrated enthalpy remains basically unaffected up to 70 wt % P85 (corresponding to $\sim 11\%$ mol fraction). For $w_{P85} > 70$ wt %, the peak cannot be resolved. It is plausible to associate this transition to chain melting in analogy with pristine DMPC, thus indicating that the local lipid structure is preserved even for copolymer concentrations as high as $w_{P85} = 70$ wt %. The observed transition temperatures are summarized in the T - w_{P85} phase diagram shown in Figure 3.

Baekmark et al. found corresponding DSC results in DPPC vesicles incorporated with PEO related polymer surfactants.¹⁰ With the width of the sharp peak relatively constant, they concluded that the DSC peak corresponds to the main phase transition. This is in accordance with the high mutual order between the acyl chains, resulting in high cooperativity in the acyl-chain melting behavior.

The preserved lipid bilayer structure is further supported by the enthalpy peak that is observed near $T_p \sim 14$ °C, where the pretransition, T_p° , of the pure DMPC system occurs, separating the lamellar L_{β} gel phase from the lamellar P_{β} ripple phase. The pretransition enthalpy peak is visible up to $w_{P85} = 40$ wt %. From the spectra, it can furthermore be seen that the pretransition peak shows the same development as the main transition peak, both regarding temperature and integrated enthalpy. The enthalpy involved in the pretransition (Figure 2) is relatively low (~ 1 kJ/mol) and within statistics independent of P85 content, like that of the main transition.

The broad heat capacity peak occurring at temperatures slightly above the main transition of pristine DMPC has a width that increases linearly with increasing P85 weight fraction (Figures 1 and 2). The transition temperature corresponding to this peak increases as the P85 weight fraction increases, as shown in Figure 3. For $w_{P85} > 70$, the high-temperature enthalpic peak cannot be resolved in our experiments. Baekmark et al. found in DPPC vesicles incorporated with other PEO polymers very similar behavior, observing an enthalpic peak at temperatures slightly above the main transition.¹⁰ The position of this peak increases as well with increasing polymer concentration, and the temperature difference between the main and high-temperature peaks increases with polymer concentration in perfect agreement with our observations.

Baekmark et al. argued that the broad peak on the high-temperature side of the main phase transition might not come from a lateral separation of lipids and polymers into two phases within the bilayer. To describe their system, Baekmark et al. therefore suggested a simple two-phase

model as originally proposed by Hristova and Needham.^{18,19} At high lipid concentrations, it was argued that the free energy of the bilayer system should dominate. As the polymer concentration increases, Hristova and Needham argued that the bilayers destabilize, whereby micellar aggregates consisting of both copolymers and lipids start to form, but while coexisting with the bilayers. Both the sharp and broad peaks might then reflect lipid ordering, the sharp enthalpic peak originating from lipids in the lamellar phase and the broad peak from lipids incorporated in the polymeric micelles. The broad high-temperature enthalpic peak was accordingly used as an argument for micelle formation including lipids with small cooperativity. Low cooperativity between acyl chains is not, however, restricted to micellar phases.

The formation of complexes between P85 and lipid molecules will most probably cause reduced cooperativity between the acyl chains also when present in bilayer systems. The presence of lipids with low cooperativity between acyl chains does accordingly not prove the existence of micelles but is only evidence of some degree of DMPC/P85 complex formations, for example, as micelles or in nanoscopic domains within a thermodynamic single phase. Moreover, if the enthalpic peaks are only related to the lipid organization, one should expect that the integrated enthalpy observed as the sum of the two reflections would be constant. In the DMPC/P85/water system, this sum clearly increases with the amount of P85, indicating tight lipid/polymer complex formation.

We have in our system no independent indications of macroscopic phase separation into a system of dominating pure lipid/water and a system of dominating P85/water (or lipid/P85/water), and there is no structural indication of polymer micelles for P85 concentrations up to $w_{P85} \approx 50$ wt %. The X-ray data presented here, on the contrary, strongly indicate mixing of polymers and lipids within one structural ordering but possibly with some microphase separation as seen in other lipid systems.¹⁵ We therefore find it more likely that the broad peak reflects melting and disordering of lipids in the near vicinity of P85 polymers, or perhaps even complexed with those, thus giving rise to significantly reduced mutual cooperativity between these lipid molecules.

Only for P85 concentrations of $w_{P85} \geq 70$ wt %, the DSC data are dominated by a very broad peak that reasonably can be assigned to micelle formation. The enthalpy peak spans the temperature range between 10 and 15 °C (Figure 3). This peak is assigned to dehydration of the PPO blocks and the consequent formation of micelles. Similar properties are well established from pure aqueous P85 systems.²⁰

3.2. Temperature-Dependent SAXS Pattern. A detailed study on the temperature-dependent structure was made on the DMPC/P85/water system using small-angle X-ray scattering. The sample used is a DMPC/P85/water mixture with $w_{P85} = 10$ wt %, corresponding to 1.6 mol % copolymers. The water content was 70 wt %, as in the calorimetric studies described above. The temperature scan was made by increasing the temperature from 10 to 35 °C, in steps of 1°. After equilibration for 5 min, the SAXS pattern was acquired for 1 h. The resulting SAXS spectra are shown in Figure 4.

The peak positions of the SAXS spectra have been determined by fitting a Lorentzian line shape to the experimental scattering functions corrected for powder

(18) Hristova, K.; Needham, D. *J. Colloid Interface Sci.* **1994**, *168*, 302.

(19) Hristova, K.; Needham, D. *Macromolecules* **1995**, *28*, 991.

(20) Brown, W.; Schillen, K.; Almgren, M.; Hvidt, S.; Bahadur, P. *J. Phys. Chem.* **1991**, *95*, 1850.

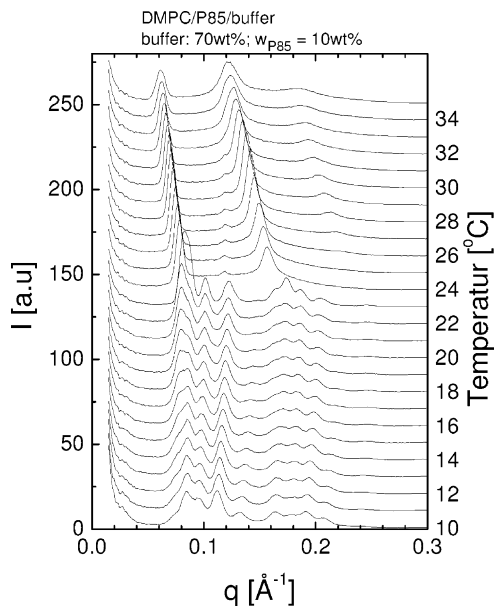


Figure 4. Scattering function, $I(q)$, versus temperature for DMPC mixed with $w_{P85} = 10$ wt % P85 copolymers. The water content is 70 wt %. Each SAXS spectrum is obtained by exposing the sample for 1 h. A phase transition is clearly seen to occur around 23 °C. The curves have been shifted proportional to temperature for clarification.

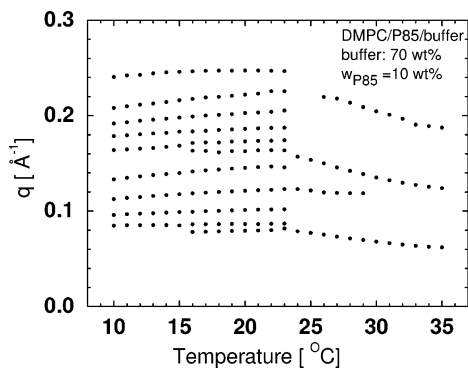


Figure 5. Observed Bragg reflections, $q(hkl)$, of the DMPC/P85/water system. The q -values are determined by fitting Lorentzian peaks to the experimental scattered intensities, corrected for the powder averaging by multiplying with q^2 .

averaging by multiplying with q^2 . The resulting peak positions are plotted in Figure 5.

The temperature-dependent scattering patterns have distinct differences relative to those of pristine DMPC vesicles but also similarities. In analogy with the calorimetric DSC results presented above, a dominating feature of the temperature-dependent scattering patterns is the changes observed near the main transition, $T_m^\circ = 24$ °C, of pristine DMPC vesicles. The transition is associated with chain melting. Above this main transition temperature, the SAXS data reveal structural characteristics that are in agreement with the lamellar L_α phase in analogy with pristine DMPC.

3.2.1. High-Temperature Lamellar Phase. The high-temperature L_α phase is dominated by equally spaced Bragg reflections, as required for the lamellae structure. Three orders of reflections are seen: (0,0,1), (0,0,2), and (0,0,3).

The periodicity of the aqueous DMPC/P85 high-temperature phase, as obtained from the position of the Bragg peaks (Figure 5), is shown versus temperature as closed symbols in Figure 6. Both the absolute value of the periodicity and the large temperature dependence are

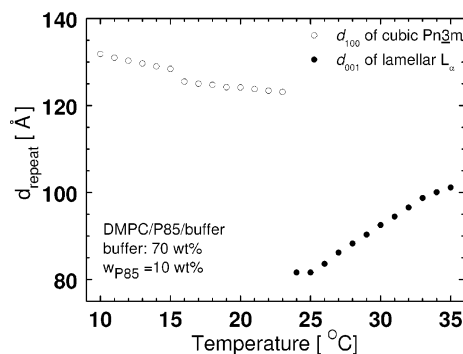


Figure 6. Resulting unit cell parameter versus temperature, as determined from SAXS spectra of the DMPC/P85/water system. Open circles represent the unit cell parameter, d_{100} , of the $Pn3m$ phase up to 23 °C, while closed circles represent the repeat distance, d_{001} , for the L_α lamellar phase above 23 °C.

significantly different from those of the pure DMPC lamellar L_α phase. There is no signature of the anomalous swelling observed in pure DMPC multilamellar vesicles.²¹

Above the main phase transition by 2°, where the anomalous swelling of pure DMPC can be neglected,²¹ pure DMPC vesicles have a periodicity equal to $d = 67$ Å, while the DPMC/P85 ($w_{P85} = 10$ wt %) sample has $d = 84$ Å. At 35 °C, the pure DMPC lamellar periodicity has decreased to $d = 65$ Å, while that of the DPMC/P85 sample has increased to $d = 101$ Å. The difference approaches a value that is close to 2 times the gyration radius of P85 unimers, $R_g = 17$ Å,²² which could suggest that the copolymers in the fluid DMPC phase are expelled from the inner part of the lamellae and make up polymer layers on both sides of the lipid bilayer membrane. It is also possible, though, that the polymers have a large softening effect on the bilayer bending modulus, resulting in an enhanced Helfrich repulsion and consequently a larger equilibrium interlamellar distance.

There are no major changes in the observed lamellar scattering pattern near 26 °C, where a broad enthalpic peak was observed in the DSC. The third-order reflection does seem to vanish exactly at this temperature, but our temperature resolution is too coarse to verify such a relationship between structure and measured enthalpy.

Even though the high-temperature phase seems quite equivalent to the L_α phase of pure DMPC multilamellar vesicles, we do observe an additional but relatively weak peak beyond the lamellae reflections, namely, at $q = 0.12$ Å⁻¹. The origin of this reflection is still not resolved. The peak is clearly visible up to ~30 °C, which is the temperature where the transition enthalpy of the high-temperature (26 °C) transition vanishes, as mentioned above. One may therefore speculate that this structural property is related to the P85-induced high-temperature transition. Beyond 30 °C, however, the Bragg reflection merges with that of the third-order lamellar peak, and it is no longer possible to resolve the peak with the experimental setup used.

In contrast to the three strong lamellar reflections, the q -value of the extra peak is rather independent of the temperature. These distinct different temperature dependencies clearly show that the $q = 0.12$ Å⁻¹ peak is not directly related to the lamellar thickness and periodicity. One may speculate in some in-plane order within the lamellae, perhaps correlation between the copolymer chains. Alternatively, the system may not, at least at high

(21) Hønger, T.; Mortensen, K.; Ipsen, J. H.; Lemmich, J.; Bauer, R.; Mouritsen, O. G. *Phys. Rev. Lett.* **1994**, *72*, 3911.

(22) Mortensen, K.; Pedersen, J. S. *Macromolecules* **1993**, *26*, 805.

Table 1. Observed Position of Bragg Peaks Associated with $Pn\bar{3}m$ Cubic Symmetry of the DMPC/P85/Water System at 16 °C^a

$Pn\bar{3}m$	110	111	200	211	220	221	310	311
observed peak no.	1	2	3	4	5			6
q -value (\AA^{-1})	0.0813	0.0896	0.103 18	0.123 41	0.147 08			0.174 75
$Pn\bar{3}m$	222	321	400	322	330	331	420	422
					411			
observed peak no.		7	8			9		10
q -value (\AA^{-1})		0.188 75	0.206 17			0.2298		0.2561

^a The water content is 70%, and the relative copolymer concentration is $w_{P85} = 10$ wt %.

temperatures, be a true single-phase system with excess water but may have a minority component different from the DMPC/P85-dominating lamellae.

We conclude that the high-temperature structural properties are dominated by a lamellar phase which resembles the fluid lamellar L_α phase of pure DMPC multilamellar vesicles, but with a significant renormalized periodicity and perhaps some weak in-plane correlations.

3.2.2. Low-Temperature Cubic Phase. From Figures 4 and 5, it is seen that the scattering pattern in the 10–23 °C low-temperature range is dominated by a large number of closely spaced Bragg reflections. The reflection resolved at the lowest q -value, $q \approx 0.08 \text{\AA}^{-1}$, and the reflection near 0.16\AA^{-1} vanish both below $T \approx 15 \text{ °C}$, which is where the pretransition peak in DSC is observed.

The intensities of the peaks observed between $q = 0.15$ and 0.23\AA^{-1} vary significantly in the temperature regime between 10 and 23 °C, which principally may be caused by changes in both the form factor and the structure factor. Four peaks can be resolved within the $q = 0.15$ – 0.23\AA^{-1} range in the whole 10–23 °C low-temperature regime. The peak positions move slightly toward higher q -values with increasing temperature. Only the peak positioned around 0.25 – 0.26\AA^{-1} exhibits a complex evolution in the position.

The low-temperature phase of the DMPC/P85/water system cannot be described as a simple coexistence of lamellar ordered DMPC and cubic ordered P85 micelles in terms of the published results of DMPC vesicles²³ and aqueous P85 systems, respectively.¹⁶ This proves that the low-temperature phase is a new phase where both lipids and copolymers are components.

The large number of reflections observed in the low-temperature scattering pattern is in agreement with a one-phase system of cubic space group $Pn\bar{3}m$, the double diamond structure. All observed reflections are reasonably well associated with this phase, but some reflections allowed in the $Pn\bar{3}m$ structure factor are not observed. This absence of reflections, on the other hand, is likely caused by a form factor minimum near 0.16\AA^{-1} from the lipid bilayer basic structure. Table 1 shows the measured q -values at 16 °C and the associated index according to the cubic symmetry. It should be noticed that between 15 and 16 °C the ninth peak changes index from (331) to the two Bragg peaks (411) and (330) with the same square sum.

As discussed above, the two reflections positioned at $q \approx 0.08 \text{\AA}^{-1}$ and $q \approx 0.16 \text{\AA}^{-1}$, respectively, vanish below $T \approx 15 \text{ °C}$, exactly where the enthalpic pretransition peak is observed. One may thus speculate that these reflections reflect correlations within the planes of the bilayers in analogy with the ripples of the P_β phase of DMPC vesicles. In analogy with the significant difference between the lamellar periodicity of pure DMPC vesicles and DMPC

incorporated with P85, the first-order peak at $q \approx 0.08 \text{\AA}^{-1}$ is significant different from the first-order ripple reflection of pure DMPC vesicles, $q = 0.045 \text{\AA}^{-1}$.¹⁵

It is not clear, yet, whether these in-plane correlations are intrinsic features of the cubic phase or should be viewed as modulations on top of the $Pn\bar{3}m$ structure. The remaining basic cubic structure observed also below $T \approx 15 \text{ °C}$ argues that the two peaks reflect some higher-order modulations. The absence of the two reflections at low temperature may of course just be a matter of vanishing intensity in the temperature-dependent product of form and structure factor. In any case, the DSC measurements show evidence of the pretransition, and thereby of in-plane correlations at intermediate temperatures.

The temperature-dependent cubic unit cell parameter, $d_{100}(Pn\bar{3}m)$, is shown in Figure 6. The basic lattice parameter decreases by $\sim 10 \text{\AA}$ in the temperature range 10–23 °C, going from 132 to 123 Å, while the $d_{110}(Pn\bar{3}m)$ parameter is close to that of the lamellar periodicity. A small but distinct jump in the lattice parameter is seen at the pretransition temperature, revealing structural correspondence to the enthalpic data.

3.2.3. Cubic-to-Lamellar Epitaxy. The scattering pattern observed at 23 °C, which is at the boundary between the lamellar and cubic phases, shows characteristics of both phases, even though the cubic $Pn\bar{3}m$ spectrum dominates with the large number of reflections (Figure 4). A characteristic feature of the 23 °C pattern is a drastic increase in the intensity of the 0.085\AA^{-1} peak, which in the cubic phase is the (111) Bragg reflection. The intensity approaches that of the (100) lamellar Bragg peak, thus suggesting a simple epitaxy relationship between the corresponding two crystal planes.

3.3. Ternary DMPC/P85/Water System. Representative scattering data obtained on ternary DMPC/P85/water systems are shown in Figures 7–9. The figures show the scattering patterns as obtained at two temperatures, $T = 20 \text{ °C}$ and $T = 35 \text{ °C}$, measured on samples with respectively varying water content measured with a fixed P85 concentration of $w_{P85} = 10$ wt % (Figure 7), on samples with varying relative block copolymer concentrations obtained with a fixed 70 wt % water concentration (Figure 8), and on samples with varying relative block copolymer concentrations obtained with a fixed 30 wt % water concentration (Figure 9). Even relatively small concentrations of P85 cause significant changes in the SAXS spectra. The low-temperature ($T = 20 \text{ °C}$) scattering patterns given in Figure 7 show major changes between the 30 and 53 wt % water concentrations. Corresponding changes are seen in the spectra of the 70% water samples (Figure 8) for block copolymer concentrations between 0 and 10 wt %, while the samples with 30% water show no corresponding changes. The peak positions of these scattering data obtained for water contents above roughly 50 wt % and copolymer contents above 3 wt % are in agreement with the cubic $Pn\bar{3}m$ phase discussed above. Further changes in scattering pattern are seen between the 70

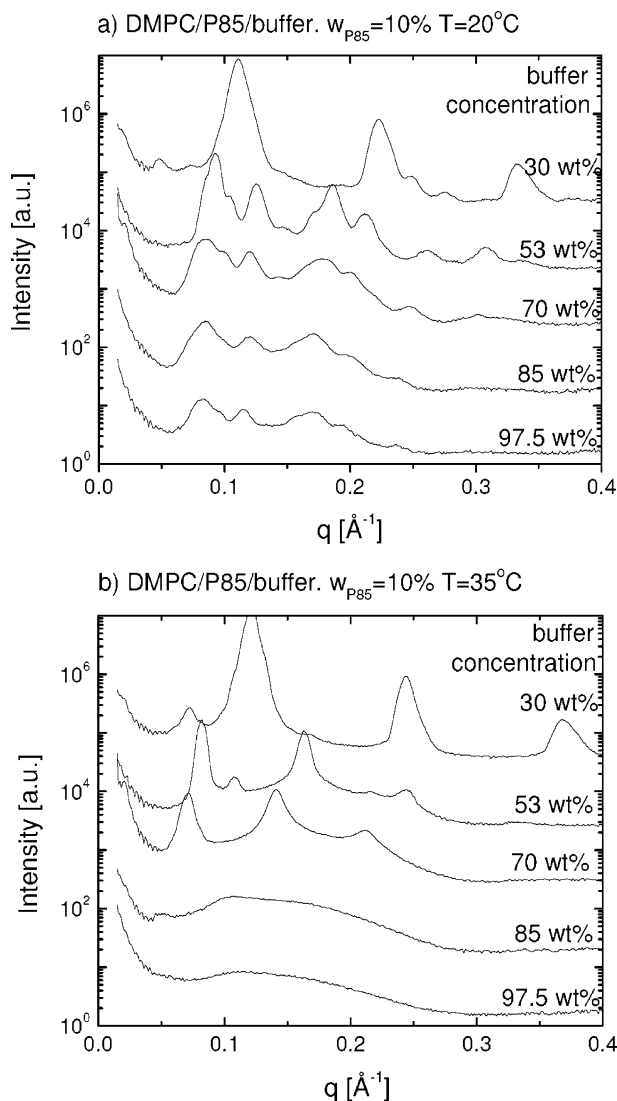


Figure 7. $I(q)$ SAXS spectra versus water content on samples with a fixed copolymer concentration of $w_{\text{P85}} = 10$ wt %, as obtained at (a) 20°C and (b) 35°C , respectively. The curves have been shifted proportional to water concentration for clarification.

and 80 wt % P85 spectra of the sample with 70 wt % water, pointing to an upper P85 limit of the cubic phase. The variation in spectra of the samples with 30 wt % water, shown in Figure 9, is less pronounced.

The high-temperature spectra ($T = 35^\circ\text{C}$) seem to be dominated by the lamellar phase, as discussed above in relation to the $w_{\text{P85}} = 10$ wt % sample. Also here, however, we see peaks beyond those of the simple planar lamellar phase.

The lattice spacing obtained from the SAXS spectra decreases generally at the main phase transition, when going from the low-temperature gel phase to the high-temperature fluid phase. This is the case for both pure DMPC multilamellar vesicles and for samples including the block copolymers.

It should be further noticed that variation in the content of solvent in the samples incorporated with copolymers causes marked changes in the lattice parameter. At 20°C , for example, a change in the solvent content from 53 to 97.5 wt % with the P85 concentration fixed at $w_{\text{P85}} = 10$ wt % causes an increase in the lattice parameter of ~ 14 \AA , going from 119.1 to 132.8 \AA (Table 2). This is in clear contrast to the observations for the pure DMPC

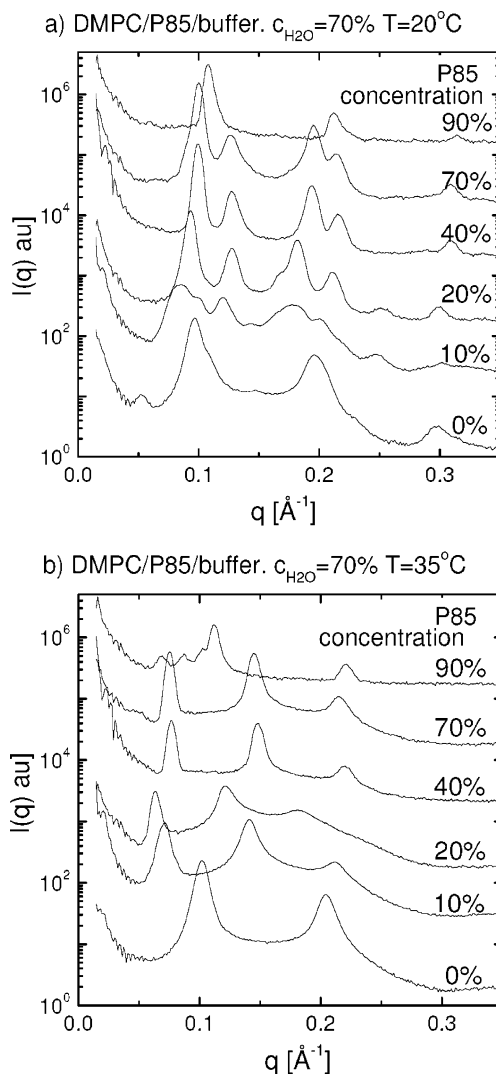


Figure 8. $I(q)$ SAXS spectra versus copolymer concentration on samples with a fixed water content equal to 70 wt %, as obtained at (a) 20°C and (b) 35°C , respectively. The curves have been shifted proportional to copolymer concentration for clarification.

vesicles, where the repeat distance shows only small variations with water content in both the P_β and L_α phases. The lattice parameter changes from respectively 64.5 to 66.7 \AA at 20°C (P_β) in the regime of 53–98% water and from 61.6 to 64.5 \AA at 35°C (L_α) in the same water regime.

3.3.1. Low-Temperature Cubic Gel Phase. The $\text{Pn}\bar{3}m$ cubic phase is observed at low temperature in the regime of copolymer concentrations between $w_{\text{P85}} \sim 3$ wt % and $w_{\text{P85}} \sim 90$ wt % and water concentrations larger than 30–50 wt % (see Figure 10).

For water concentrations below 30 wt %, the scattering patterns are in agreement with the P_β ripple phase, which is well established in the pure DMPC multilamellar systems. The presence of copolymers apparently stabilizes the ripple phase, as seen in Figure 9, showing SAXS spectra obtained on samples with 30 wt % water at 20°C . This may well be analogous to other additives, like cholesterol, which has also been shown to stabilize a modulated type of structure.¹⁵ In ref 15, it was suggested that cholesterol forms complexes at boundaries between local solid and liquid characteristics of the lipid chains, that is, at the top of the ripple structure. The scattering patterns of the DMPC/P85/water systems are in agreement with an analogous modulated structure.

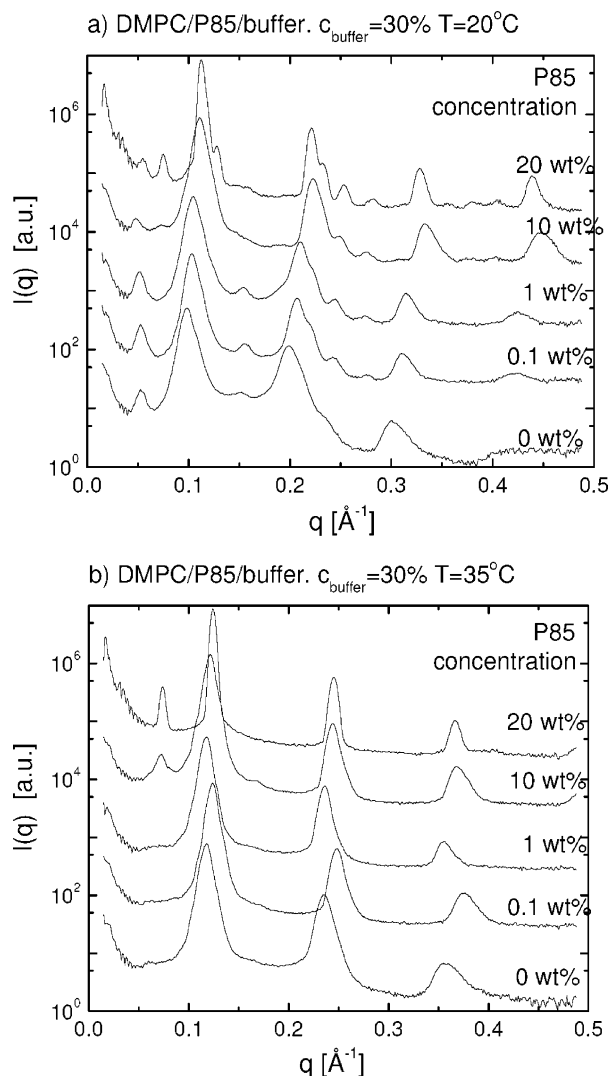


Figure 9. $I(q)$ SAXS spectra obtained on samples with 30 wt % water, at (a) 20 °C and (b) 35 °C, respectively. The curves have been shifted proportional to copolymer concentration for clarification.

Table 2. Reciprocal Unit Cell Parameter, a , and the Corresponding Unit Cell Length, $d_{100}(Pn\bar{3}m)$, for Samples with $w_{P85} = 10$ wt % and Solvent Contents of 53, 70, 85, and 97.5 wt %^a

w_{P85} temp phase	10 wt % 20 °C $Pn\bar{3}m$		0 wt % 20 °C $P_{\beta'}$		0 wt % 35 °C L_{α}	
	a (Å ⁻¹)	d_{100} (Å)	a (Å ⁻¹)	d_{001} (Å)	a (Å ⁻¹)	d_{001} (Å)
53 wt %	0.052 74	119.13	0.095 66	65.69	0.1003	62.67
70 wt %	0.050 05	125.54	0.097 45	64.47	0.1020	61.60
85 wt %	0.048 48	129.60	0.097 38	64.52	0.1005	62.51
97.5 wt %	0.047 30	132.84	0.094 17	66.72	0.09749	64.45

^a The values are listed together with the corresponding values for the $P_{\beta'}$ and L_{α} Phases of the Pure DMPC MLV system.

For P85 copolymer concentrations lower than 3–10 wt %, the scattering patterns are dominating lamellar. For P85 concentrations beyond roughly 80 wt %, the patterns reflect highly swollen and disordered lamellae. The observed phases are summarized in the diagram shown in Figure 10a.

3.3.2. High-Temperature Phase. The cubic $Pn\bar{3}m$ phase cannot be observed in any of the spectra obtained at 35 °C. Instead, a dominating lamellar structure is observed.

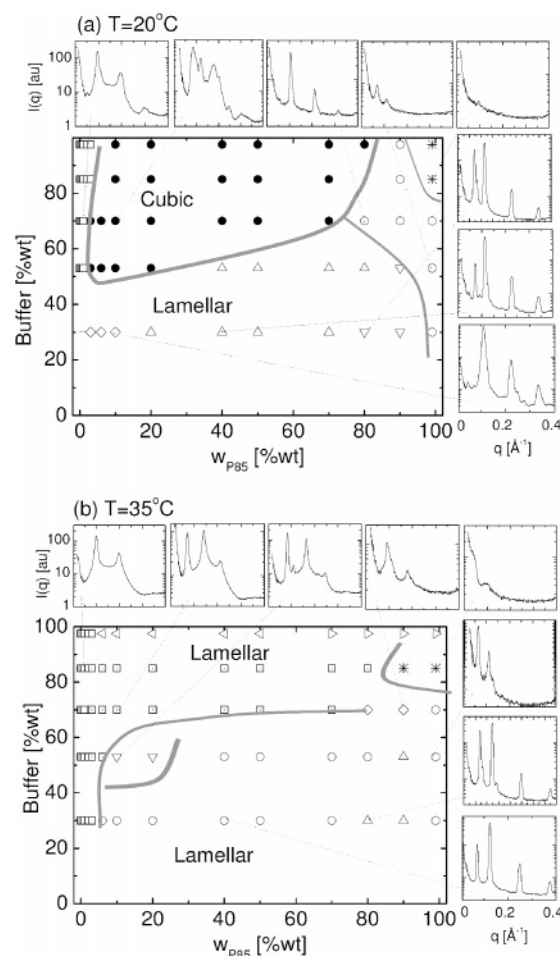


Figure 10. Phase diagrams of the DMPC/P85/water ternary system, with water content shown versus w_{P85} , as obtained at (a) $T = 20$ °C and (b) $T = 35$ °C, respectively. The various symbols represent scattering patterns with similar characteristics, as given by the examples in the insets. The gray lines represent borderlines between characteristic scattering patterns and are in some cases direct phase boundaries.

The lamellar repeat distance, d_{repeat} , varies significantly with water content. For the $w_{P85} = 10$ wt % samples, d_{repeat} changes from 88.5 to 76.8 Å as the water content changes from 53 and 70 wt %. The variation of the repeat distances may be due to dehydration of the lamellar phase, as caused by the PEO segments of the P85 copolymers.

The apparent high-temperature lamellar phase includes additional Bragg peaks that are not intrinsic to the lamellar order. In the $w_{P85} = 10$ wt % system with a water content of 53 wt %, for example, two additional peaks are observed at 0.11 and 0.22 Å⁻¹, respectively. This supports the suggestion made above, that in-plane modulation is stabilized by the presence of P85 copolymers.

3.3.3. Phase Diagram. Parts a and b of Figure 10 summarize in diagrams the regimes where similar structural characteristics are observed at 20 and 35 °C, respectively.

At $T = 20$ °C, the ternary DMPC/P85/water system forms a bilayer ripple phase for low P85 copolymer concentrations and all water contents studied. As the copolymer concentration is increased beyond $w_{P85} \sim 3$ wt %, the cubic $Pn\bar{3}m$ phase is stabilized for water contents above 30–50 wt %. A further increase in the copolymer concentration brings the ternary system into a regime of coexisting phases. Only at very high copolymer concentrations are phases resembling that of the pure P85 aqueous system formed.

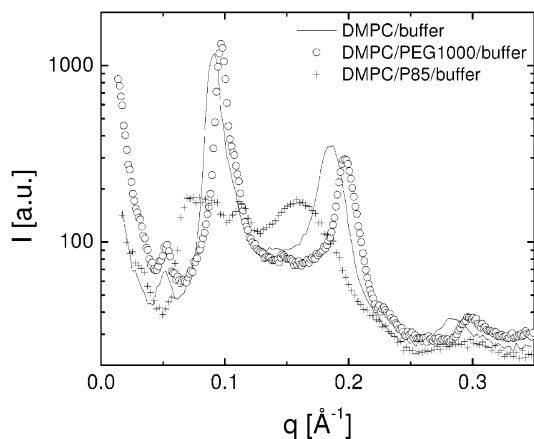


Figure 11. SAXS spectra obtained at 20 °C for aqueous systems of (—) pure DMPC multilamellar vesicles, (+) DMPC mixed with P85 block copolymers, and (○) DMPC mixed with PEG1000 homopolymers. The DMPC concentration is for the pure system 300 mM and for the DMPC/P85 and DMPC/PEG systems 297 mM. The molar concentration of P85 and PEG1000 with respect to lipids are respectively 1.00 and 1.98 mol %.

At $T = 35$ °C, the lamellar phase dominates, although with some variation in the characteristics of the scattering patterns. The characteristics of the L_{α} phase of pure DMPC/water are recognized at low copolymer concentrations. As the block copolymer concentration, w_{P85} , is increased, keeping the water content below 70 wt %, the ternary system undergoes a transition to a lamellar phase with distinctly different characteristics. Only for very high copolymer concentrations the ternary system begins to form phases dominated by the pure P85 copolymers.

3.4. Comparison with PEO Homopolymers. In the attempt to judge whether the P85 hydrophobic parts of the P85 block copolymers are truly incorporated into the lipid bilayer system, we made a comparative study including systems of DMPC/water mixed with poly(ethylene oxide) homopolymers (abbreviated PEG for poly(ethylene glycol)). For optimal comparison, we have studied the incorporation of two PEG homopolymers, PEG1000 and PEG4000, which have a degree of polymerization similar to each of the two PEO blocks, respectively, in P85 and the full P85 copolymer. The direct comparison between P85 and PEG1000 should be where the molar concentration of PEG1000 is 2 times that of P85, that is, same molar concentrations of PEO chains.

Figure 11 shows that the presence of 1 mol % P85 (corresponding to 6.4 wt %) results in a dramatic change in the scattered intensity of the DMPC system, whereas 1.98 mol % PEG1000 only slightly alters the scattering function relative to that of the pure DMPC multilamellar vesicle system. A small increase in the position of the Bragg peak upon the addition of PEG may be explained by dehydration of the bilayer caused by the presence of poly(ethylene glycol).²⁴ The addition of PEG4000 homopolymers to the DMPC systems (not shown) has a qualitatively exact similar effect on the lamellar system as PEG1000.

The positions of the observed Bragg peaks for the ternary DMPC/P85/water and the two DMPC/PEG/water systems are plotted in Figure 12. It is seen that while P85 block copolymers cause dramatic changes in both the positions and number of observed peaks at both 20 and 35 °C, as discussed in detail above, the incorporation of PEG homopolymers has only minor effects. The main effect of P85 is observed when increasing w_{P85} from 0.5 to 1.0 mol

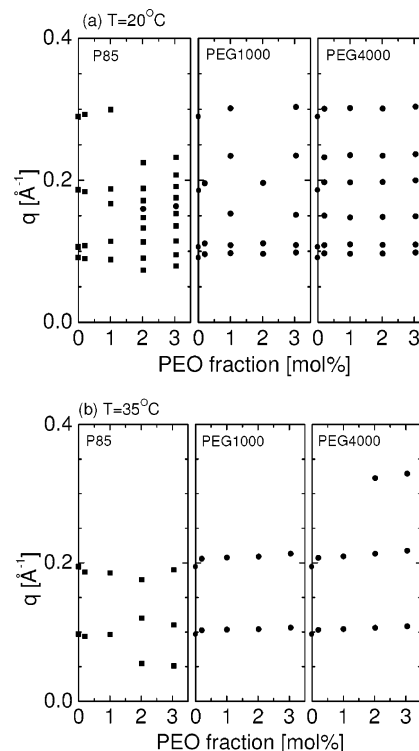


Figure 12. Peak positions for spectra obtained on DMPC samples mixed with either P85 or PEG1000, as obtained at (a) $T = 20$ °C and (b) $T = 35$ °C. The concentration of PEG1000 is the double of P85 but equals the concentration of PEO chains in the P85 samples.

Table 3. Mean Values of the Lattice Parameters for Different DMPC Mixtures at 20 and 35 °C^a

concn (mol %)	P85		PEG1000		PEG4000			
	20 °C	35 °C	20 °C	35 °C	20 °C	35 °C		
0.0	67.10	64.94	0.0	67.10	64.94	0.0	67.10	64.94
0.1	67.45	67.19	0.2	64.82	61.12	0.2	64.21	60.77
0.5	66.79	66.45	1.0	64.06	60.59	1.0	64.46	60.13
1.0	118.83	108.97	2.0	64.61	60.18	2.0	64.27	58.89
1.5	113.62	111.15	3.0	63.49	59.00	3.0	63.39	57.64

^a For DMPC mixed with P85, the parameters are the repeat distance, d_{001} , for P85 concentrations ≤ 0.5 mol %, and the unit cell axis, d_{100} , for P85 concentrations ≥ 1 mol %. For both PEG types, the lattice parameter is d_{001} .

% P85. At 20 °C, this reflects the phase transition from the lamellar to cubic $Pn\bar{3}m$ phase, but also at 35 °C, we observe a significant change, even though the system seems to remain lamellar. The scattering patterns of the DMPC/PEG/water systems, on the contrary, show that the presence of PEG homopolymers only slightly modifies the lamellar morphology. Except for a small jump in the position of the lamellar Bragg peaks for the lowest PEG content, the lattice parameter seems to vary only slightly and gradually with polymer concentration.

The lattice parameter of the DMPC/PEG/water systems decreases with increasing concentration of PEG, which is attributed to dehydration of the bilayer²⁴ (Table 3). The data reveal that the presence of PEG causes a relatively large number of higher-order Bragg peaks related to the ripple structure of the DMPC/PEG/water systems in the P_{β} phase. The comparative studies of the effect of P85 block copolymers and of PEG homopolymers on DMPC bilayer systems strongly indicate that not only is the effect of P85 block copolymers to modify the degree of hydration,

(24) Lehtonen, J. Y. A.; Kinnunen, P. K. J. *Biophys. J.* **1995**, *68*, 525.

but also the hydrophobic PPO blocks of P85 copolymers are incorporated into the interior of the lipid bilayer structure.

Summary and Conclusions

In summary, it has been shown that incorporating P85 triblock copolymers into DMPC/water vesicular systems causes marked structural changes already with relatively low copolymer concentrations. The changes can not only be addressed to the dehydration of the system, since parallel studies incorporating PEO homopolymers have only relatively minor effects on the basic lamellar structure. We therefore conclude that P85 copolymers are incorporated into the bilayer.

Both the pretransition and the main phase transition are preserved in samples with PEO-PPO-PEO block copolymers. The phase transition around 24 °C splits up into two, one with high cooperativity defined here as the main transition and a second rather broad transition of low cooperativity at slightly higher temperatures. The dominant high-cooperative transition remains visible in the system until at least $w_{\text{P85}} = 70$ wt % block copolymers. It shows a minor but systematic decrease in temperature upon increasing P85 content. The remaining presence of this sharp enthalpic peak enables us to conclude that there is in the presence of block copolymers remains a dominating high cooperativity between the acyl chains. The pretransition peak remains visible until at least $w_{\text{P85}} = 40$ wt %, further indicating that the bilayer structure somehow is kept present in the system.

These observations lead to the conclusion that the lipid molecules to a large extent arrange themselves with a high degree of mutual order also in the low-temperature nonlamellar phase (cubic $Pn\bar{3}m$); that is, the basic bilayer structure is preserved. The microscopic structure might be related to that proposed by Funari et al., namely, a $Pn\bar{3}m$ phase where the lipids are arranged in rods with a radius equal to half of the bilayer thickness.¹³

The additional broad enthalpic peak emerging at temperatures slightly above T_m is designated to the chain melting of lipid molecules with low cooperativity between the acyl chains and may reflect the melting of lipid molecules that form direct complexes with the P85 copolymers, or at least have the copolymers in the direct neighborhood, thus indicating a form of phase separation on the molecular scale. Such "microscale" phase separations have previously been proposed in DMPC bilayer systems incorporated with cholesterol.¹⁵ The observed transition temperatures are summarized in the T - w_{P85} phase diagram shown in Figure 3.

The X-ray scattering studies show that the aqueous DMPC/P85 system above the main phase transition temperature is in a lamellar phase. The repeat distance, d_{001} , is significantly larger than that of the corresponding lamellar L_α phase for the pure aqueous DMPC MLV system. The lamellar phase of the DMPC/P85/water system shows further a marked increase in d_{001} with increasing temperature, going from 78 Å at 24 °C to 97 Å at 35 °C. The pure DMPC/water system has a repeat distance of $d_{001} \approx 65$ Å.

Within the high-temperature phase, however, an additional small Bragg reflection was systematically ob-

served in all samples investigated. The origin of this peak is still unclear to us, but one may speculate in some in-plane order within the lamellae, perhaps correlation between the copolymer chains.

At low temperatures, $T \leq 23$ °C, the X-ray scattering patterns are in agreement with $Pn\bar{3}m$ cubic symmetry. This is the case for copolymer concentrations up to at least $w_{\text{P85}} = 40$ wt % relative to the amount of lipid and water contents up to 70 wt %.

On the basis of the results from the different experimental observations, it is possible to draw phase diagrams of the ternary system at 20 and 35 °C, respectively. At $T = 20$ °C, the ternary system is in a bilayer ripple phase for low copolymer concentrations and all water contents studied. As the copolymer concentration increases beyond $w_{\text{P85}} \sim 3$ wt %, the cubic $Pn\bar{3}m$ phase is stabilized for water contents above 30–50 wt %. A further increase in the copolymer concentration brings the ternary system into a regime of coexisting phases, while it only at very high copolymer concentrations forms phases resembling that of the pure P85 aqueous system.

At $T = 35$ °C, the lamellar phase dominates. The characteristics of the L_α phase of the pure DMPC/water system are recognized at low copolymer concentrations. As w_{P85} increases for water contents below 70 wt %, the ternary system undergoes a transition to a lamellar phase with somewhat different characteristics. Only for very high copolymer concentrations, the ternary system begins to form phases dominated by the pristine P85 copolymers.

The possible structure of incorporated PPO polymer chains within the bilayer systems might be considered. The gyration radius is for polymeric systems given by $R_g = (Nbl/6)^{1/2}$, where N is the number of monomers, l is the length of the monomers, and b is the Kuhn length. Assuming l is 2 Å and b is 10 Å for PPO,¹⁶ R_g is determined to be 12 Å. This gives a diameter of 24 Å, which is significantly less than the hydrophobic thickness of the bilayer, thus indicating that either the PPO chain is stretched, having the two PEO blocks on each side of the bilayer, or the PPO chain is more relaxed, having the two PEO blocks entering at the same side of the DMPC lamellae. The latter structure could explain the marked curved bilayer structures leading to the bicontinuous cubic structure.

The cubic phase induced by small amounts of block copolymers in the lipid lamellar state may have attractive applications, not only in terms of drug delivery systems but also, for example, as gels used for dressing components in the treatment of burns. Pure Pluronics systems have previously been proposed for such use.²⁵ Such gels can control the water loss and the heat loss, and additives such as antibiotics can be applied via the gel.

Acknowledgment. The studies have been supported by the Danish Natural Research Council (SNF) via the Biophysics Research School and the Danish Technical Research Council (STVF) via the Danish Polymer Centre.

LA048206R