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Calcium-Induced Fusion of Didodecylphosphate Vesicles: The Lamellar to Hexagonal II (H_{II}) Phase Transition

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Summary. Electron microscopic techniques have been employed to investigate the ability of didodecylphosphate vesicles (diameter approx. 900 Å) to fuse in the presence of Ca^{2+}. As revealed by negative staining, Ca^{2+} induces extensive fusion and large vesicles with diameters up to 7000 Å are formed. In a process *secondary* to fusion, the fused vesicles display a tendency to flatten and are subsequently transformed into extended tubular structures. Freeze-fracture electron microscopy, in conjunction with ^{31}P NMR and selected area electron diffraction measurements indicate that the tubes are packed in a hexagonal (H_{II}) array and that the amphiphiles are converted from the lamellar to the hexagonal H_{II} phase.

The relationship between membrane fusion and the lamellar-to-hexagonal phase transition is discussed in terms of formation and abundance of transiently stable inverted micellar intermediates at contact regions between two interacting membranes. A model for the conversion of the (vesicular) lamellar into the (tubular) hexagonal H_{II} phase is presented, taking into account the molecular shape of the amphiphile. The relevance of using simple synthetic amphiphiles as models for phospholipid bilayers and complex biomembrane behavior is briefly discussed.

Key Words didodecylphosphate · calcium · membrane fusion · lamellar phase · hexagonal phase

Introduction

In biological membranes, phospholipids normally reside in a bilayer structure. Yet, in almost any biological membrane, lipids can be found which, in isolation, prefer the hexagonal II (H_{II}) phase. In this conformation, the lipids are organized in hexagonally arranged cylinders in which the polar head groups of the lipid molecules surround a narrow aqueous channel [3, 4]. In particular, unsaturated species of phosphatidylethanolamine display this tendency. Furthermore, in the presence of divalent cations, several acidic phospholipids may also undergo a lamellar-to-hexagonal phase transition. Since lipid vesicles, containing these lipids, may also display membrane fusion at these conditions, a direct correlation between lipid polymorphism and fusion has been proposed [29], in spite of the fact that vesicles consisting of lipids that do not exhibit this polymorphic behavior also fuse [6, 32].

Freeze-fracture electron microscopy has revealed the presence of "lipidic particles" (inverted lipid micelles) on the surface of fused vesicles. These structures are thought to act as intermediates between lamellar and hexagonal phases and to represent the structural entity mediating fusion [29]. However, both kinetic [9, 33] and quick-freezing experiments [1] indicate that the appearance of these lipidic particles must be slow on the time scale of fusion and that these particles actually emerge after substantial fusion has occurred [1]. It would appear, therefore, that lipidic particles are thermodynamically stable structures, representing an equilibrium rather than a dynamic intermediate state. Nevertheless, it seems obvious that in order to render membranes susceptible to fusion a transient nonbilayer structure should be formed.

In recent work, we have shown [21, 22] that vesicles composed of simple synthetic amphiphiles mimic in many respects the properties of vesicles prepared from phospholipids. For example, vesicles formed from didodecyldimethylammonium bromide fuse upon addition of divalent anions, such as that derived from dipicolinic acid. Modulation of the bilayer/water interface triggers fusion, and such changes can be conveniently studied in these systems as, in contrast to phospholipid systems, the threshold anion concentrations for aggregation and fusion *per se* differ markedly. Furthermore, the molecular structure of synthetic amphiphiles can be readily modified, which provides an opportunity to study systematically the relationship between structure and function in processes such as membrane fusion.

Herein we report a study of the Ca^{2+}-induced fusion of vesicles consisting of didodecylphosphate (DDP), employing electron microscopic techniques.

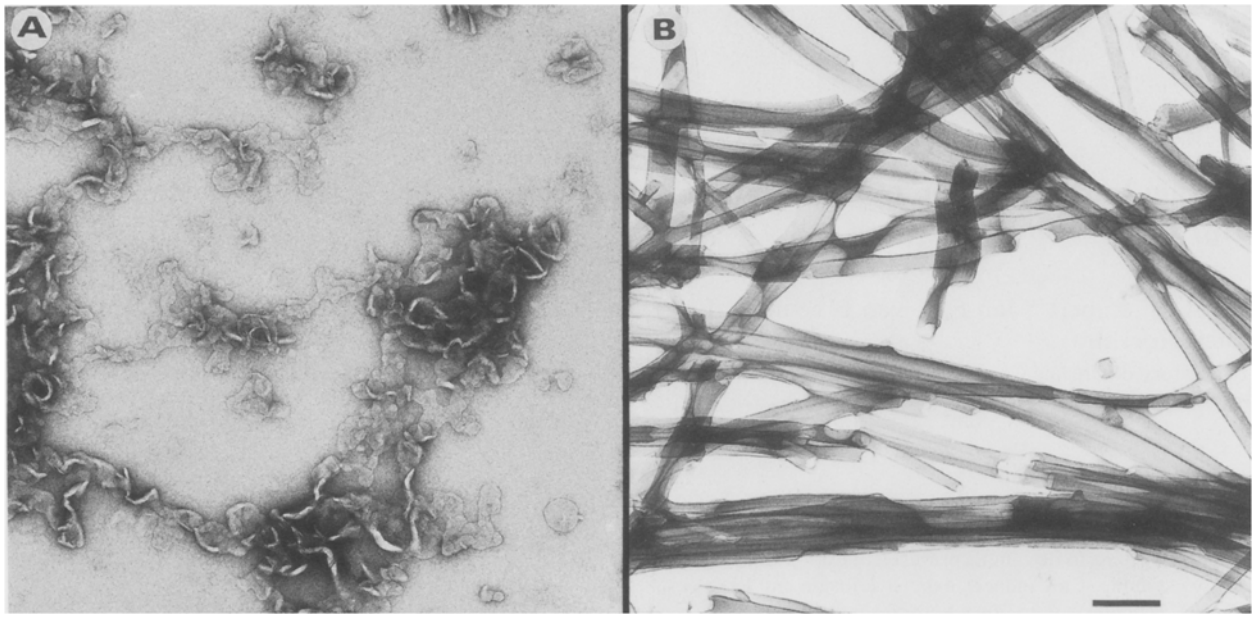

Fig. 1. Electron micrographs of DDP vesicles, before and after an incubation with Ca^{2+}. *A* shows the control vesicles, while *B* was obtained after incubating the vesicles (1.2 mM) with Ca^{2+} (3.2 mM) for 1 hr. Negative staining was carried out as described in Materials and Methods. The bar indicates 0.35 μm

It is shown that fusion of DDP vesicles results in the formation of larger vesicles, which in a process *secondary* to fusion rearrange into large tubular structures. These tubes appear to consist of a distinct number of amphiphilic cylinders in a hexagonal H_{II} array, as evidenced by ^{31}P NMR and electron diffraction measurements. A model is presented to account for these observations.

Materials and Methods

Didodecylphosphate (DDP)[1] was obtained from Alpha Chemicals (Danvers, MA) and was used as such. Vesicles were prepared by ethanol injection [13]. To this end, a stock solution of DDP was made by dissolving 10 mg of the amphiphile in 100 μl of ethanol, containing 0.3 M NaOH. With a prewarmed Hamilton syringe, 50 μl of the stock solution was injected into 5 mM HEPES/5 mM sodium acetate, pH 7.4 at 55°C. The final volume was 2 ml. Fusion was induced by adding the desired amount of $CaCl_2$ from a 0.1 M stock solution to the vesicle suspension at 40°C. Note that at pH 7.4 the gel/liquid crystalline phase transition temperature of DDP is 28°C (L.A.M. Rupert et al., *unpublished results*).

For the ^{31}P NMR measurements a lamellar dispersion of DDP was prepared by vortexing a film of DDP in 5 mM HEPES/5 mM sodium-acetate buffer (pH 7.4 and 20% (vol/vol) D_2O) above the phase transition temperature. The film was obtained by dissolving 90 mg of DDP in chloroform and then evaporating the solvent under a stream of nitrogen. The film was vacuum-dried overnight. A phase transition from the lamellar to the hexagonal H_{II} phase was induced by adding an equimolar amount of $CaCl_2$, relative to DDP, to the lamellar DDP dispersion.

For negative staining with 1% (wt/vol) uranyl acetate, the two-step droplet procedure was followed, using carbon-coated Formvar grids, pretreated by glow discharge in air, as supporting matrix. The samples were examined with a Philips EM 300 electron microscope, operating at 80 kV. For freeze-fracture experiments the vesicles were deposited on phosphor bronze specimen carriers, prepared by the method of Mueller and Pscheid [18] and immediately quenched in a slush of solid and liquid nitrogen. The samples were fractured at −160°C in a freeze-fracture device as described elsewhere [31]. The replicas were cleaned in a solution of sodium hypochlorite (6% wt/vol) and examined in a JEM 1200 EX electron microscope, operating at 80 kV.

The selected area electron diffraction patterns were obtained with a Philips EM 400 electron microscope operating at 80 kV. To obtain a well-defined diffraction pattern, the ED measurements were performed at a low electron dose. The camera length (608 mm) was calibrated with crystals of catalase.

^{1}H-decoupled ^{31}P NMR measurements were performed using a 10-mm tube at 81 MHz on a Nicolet NT200 instrument, equipped with a temperature controller and a deuterium lock. Chemical shifts (ppm) were determined relative to the external reference of hexachlorocyclotriphosphazene in $CDCl_3$ (+19.9 ppm downfield from 85% H_3PO_4). Accumulated free induction decays were obtained with a pulse time of 19 μsec, which corresponds to a pulse angle of circa 90°. Interpulse times of 2.3 and 21.5 sec were used to accumulate 10,000 and 5,000 transients for the lamellar and the hexagonal H_{II} dispersion, respectively.

[1] *Abbreviations:* DDP—didodecylphosphate; EDTA—ethylenediaminetetraacetic acid (sodium salt); ED—electron diffraction; PA—phosphatidic acid; PS—phosphatidylserine; NMR—nuclear magnetic resonance.

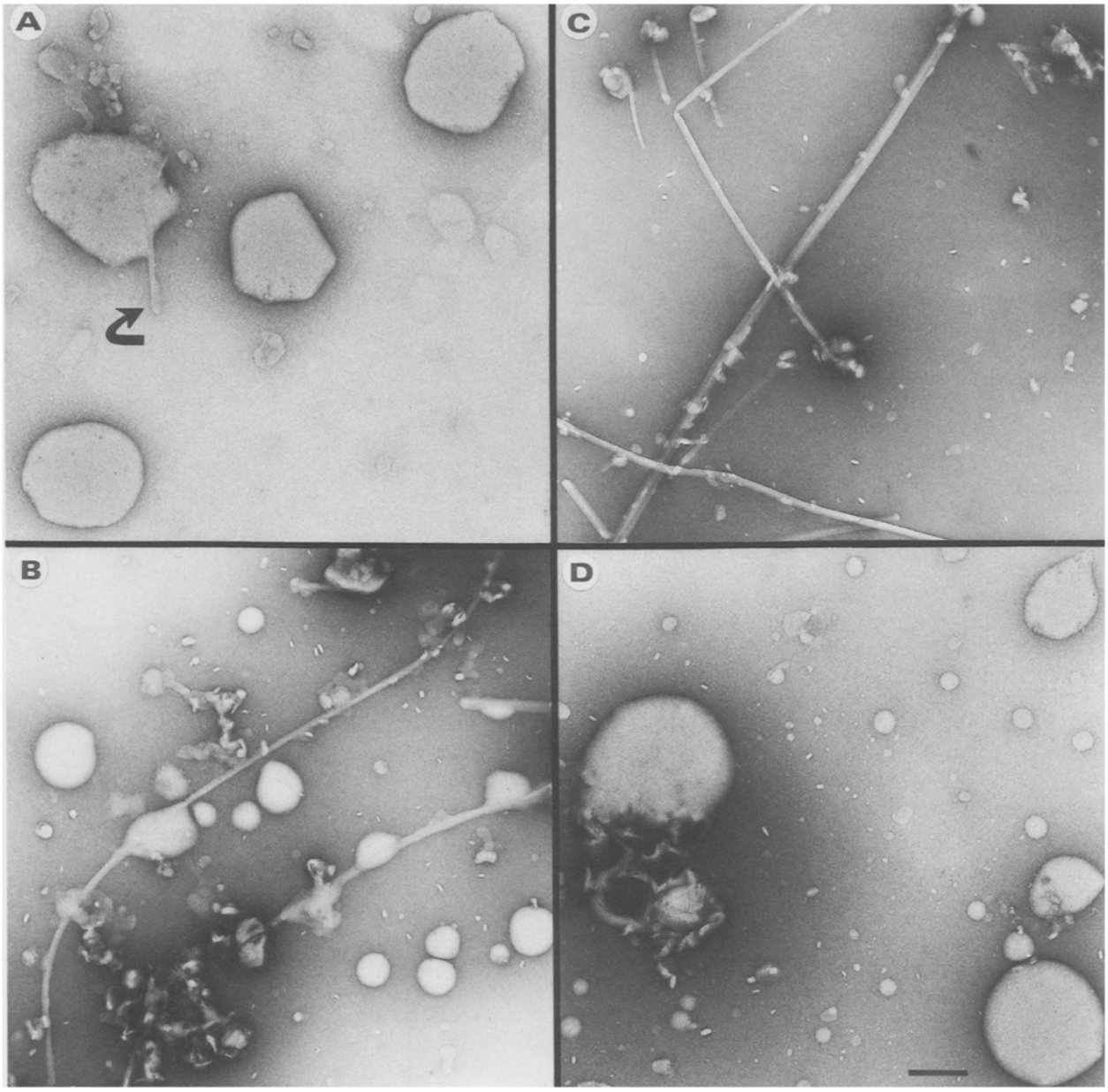

Fig. 2. Ca^{2+}-induced alterations of the DDP vesicle structure as a function of time. DDP vesicles (1.15 mM) were incubated with Ca^{2+} for various time intervals. After 1 (*A*), 3 (*B*) and 5 min (*C*) samples were taken, negatively stained, and examined in the electron microscope. The arrow in *A* denotes the appearance of a tubular structure. Micrograph *D* was obtained for vesicles incubated with Ca^{2+} for 5 min (*cf. C*), followed by addition of a twofold excess of EDTA. To allow for an accurate monitoring of the transition process, a relatively low Ca^{2+} concentration (0.5 mM) was used in this experiment. The bar is 0.35 μm

Results

When an ethanolic solution of sodium didodecylphosphate (DDP) is injected into an aqueous medium, vesicles are formed with an average diameter of ca. 900 Å (Fig. 1*A*). Their appearance as aggregates is artificially induced by the cationic stain uranyl acetate (*cf.* ref. 15), since only randomly dispersed vesicles were seen in nonstained preparations and in samples prepared by freeze fracture (*not shown*). Preliminary experiments indicated that addition of Ca^{2+} leads to fusion of the vesicles, as revealed by an assay based on resonance energy transfer [21, 22, 27]. However, when left for 1 hr in

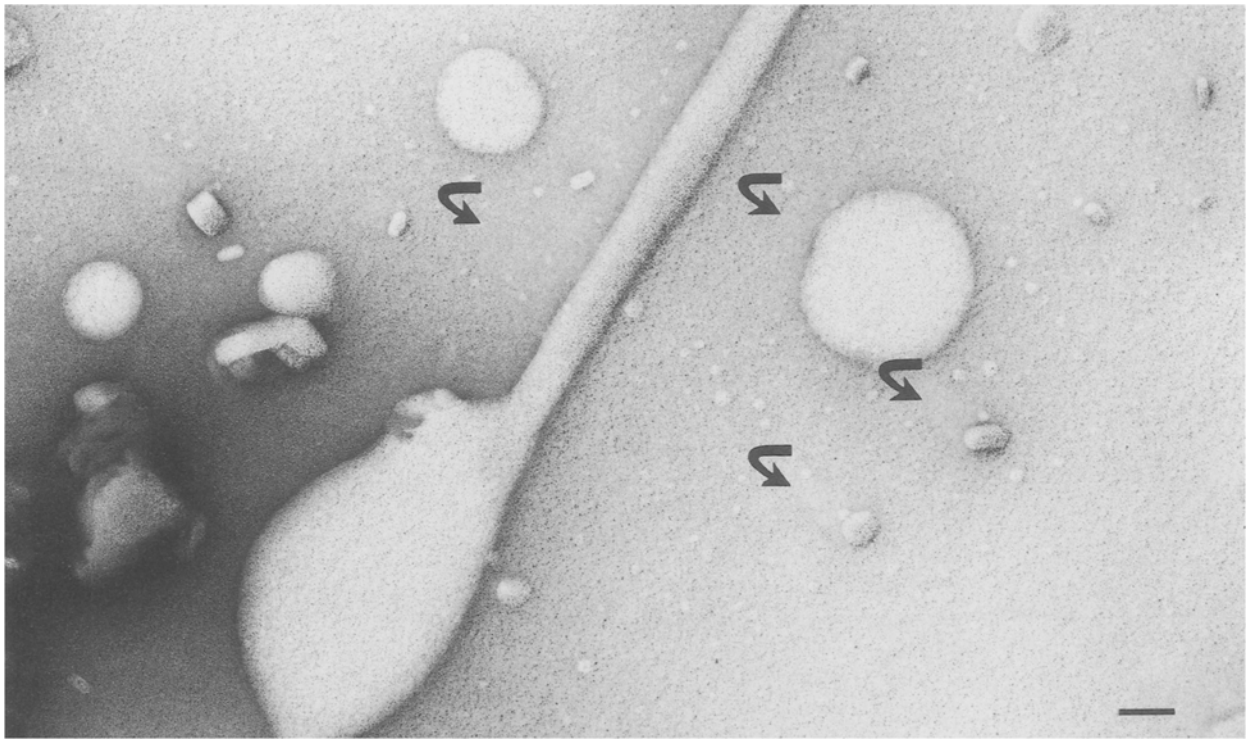

Fig. 3. Micrograph representing a sample which has been shadowed with Pt after negative staining. The arrows indicate the length of the shadow. The sample was taken 3 min after incubation with Ca^{2+} as in Fig. 2*B*. The bar represents 0.10 μm

the presence of Ca^{2+}, no large vesicular structures were observed but, instead, the vesicles had been transformed into a network of tubes (Fig. 1*B*).

To investigate more closely the time course of this transformation negatively stained samples were prepared 1, 3 and 5 min after addition of Ca^{2+}. As shown in Fig. 2*A*, when the vesicles were incubated with Ca^{2+} for 1 min, large fused vesicles were apparent with diameters up to approx. 7000 Å. Nonfused vesicles were also present. Interestingly, some of the fused vesicles in the preparation displayed a tendency to produce tubes (arrow, Fig. 2*A*), suggesting that tube formation is related to fusion. This concept is further supported by the images that were obtained after incubation of the vesicles with Ca^{2+} for 3 min (Figs. 2*B* and 3). Many of the large vesicles, originating from Ca^{2+}-induced fusion, were now engaged in the process of transformation into tubular structures which were evidently associated with vesicular remnants. By shadowing negatively stained preparations with platinum (12–15° angle) at this stage, it was inferred from the ultimate edges of the shadow (arrows) that the fused vesicles displayed a flattened (Fig. 3) rather than a spherical appearance, particularly when compared to their nonfused counterparts. After another 2 min, essentially all fused vesicles had dissolved into tubular structures (Fig. 2*C*). The only vesicle structures still present were apparently those that had never been involved in fusion, as their size is the same as that of the original vesicle preparation. Addition of EDTA to a preparation as that shown in Fig. 2*C* induced the reappearance of the large fused vesicles (Fig. 2*D*), providing additional support for the suggestion that the tubes originated from the fused vesicles. Moreover, when the chelator was added 1 min after the addition of Ca^{2+}, the transformation of fused vesicles into tubes was completely inhibited.

Figure 4 shows freeze-fracture images of the tubular structures, obtained after incubating DDP vesicles with Ca^{2+} for 3 min. On the surface of the tubes a substructure can clearly be distinguished, which in appearance closely resembles the structures identified in Ca^{2+}-treated systems of cardiolipin and phosphatidic acid as hexagonal phases [2, 5, 29].

To establish more firmly the hexagonal H_{II} character of the tubular structure, ^{31}P NMR measurements were performed. The vesicular suspensions, used in these experiments, consisted of multilamellar vesicles with a diameter of ca. 5000 Å

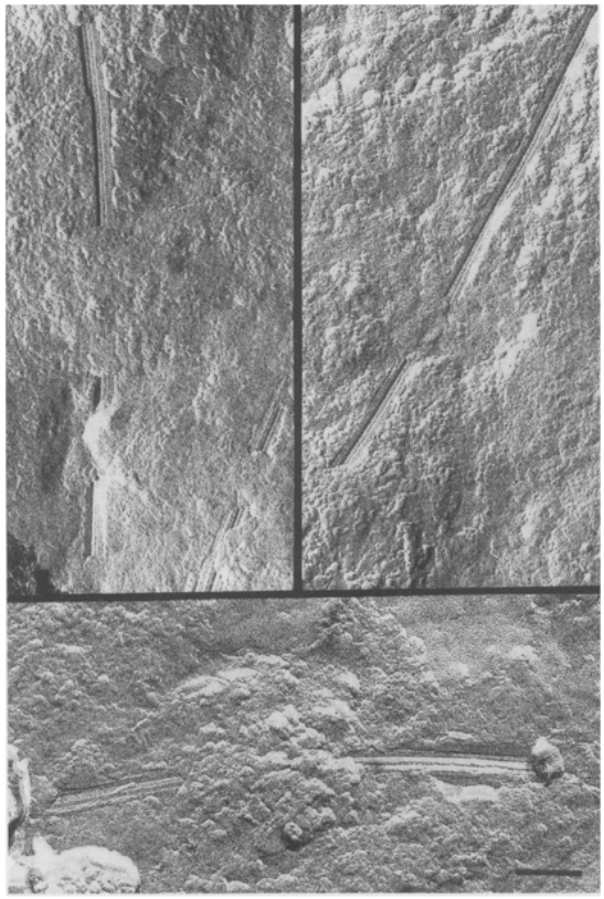

Fig. 4. Montage of freeze-fracture electron micrographs of DDP/Ca^{2+} tubes. DDP and Ca^{2+} concentrations were as described in the legend to Fig. 2. The incubation time was 3 min. The bar indicates 0.10 μm

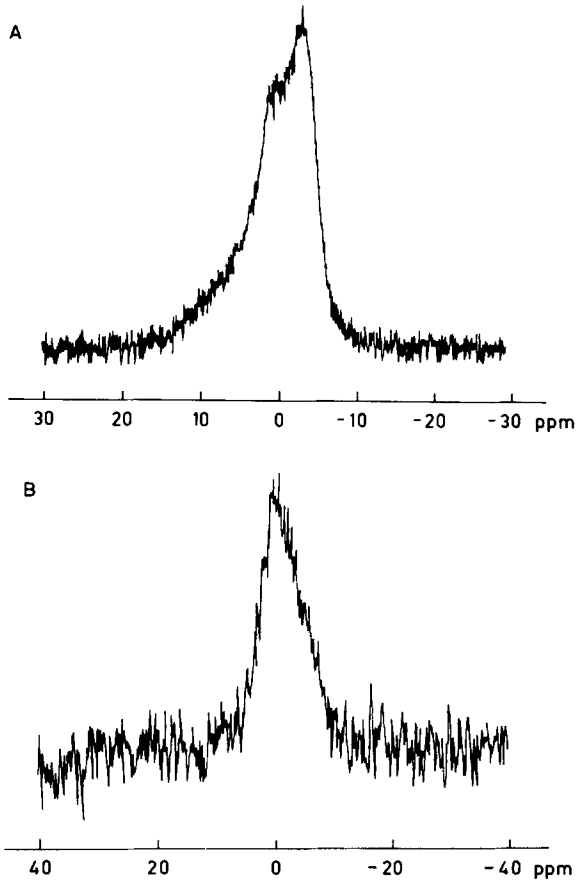

Fig. 5. ^{31}P NMR spectra recorded before (*A*) and after (*B*) the addition of Ca^{2+} (0.15 M) to DDP vesicles (0.15 M), incubated at 40°C and pH 7.4

as determined by negative staining electron microscopy (*not shown*). In the absence of Ca^{2+}, the spectrum (Fig. 5*A*) reveals the characteristic asymmetric lineshape of a lamellar phase with a shoulder at low field [3, 4, 8, 23]. Addition of an equimolar amount of Ca^{2+}, relative to DDP, causes the formation of tubes, identical in appearance to those obtained from the 900 Å vesicles upon addition of Ca^{2+} (*cf.* Fig. 1*B*). The ^{31}P NMR spectrum of the Ca^{2+}-treated suspension confirmed the hexagonal H_{II} character of the tubular structures. The asymmetric signal shows a shoulder on the high field side of the spectrum (Fig. 5*B*) [3, 4, 8, 23]. The small effective chemical shift anisotropy of the bilayer spectrum ($\Delta\sigma_{eff}$ is circa 16 ppm) is a result of the conformation of the phosphate head group and the motion of the DDP molecules in the bilayer (L.A.M. Rupert et al., *unpublished*). The effective chemical shift anisotropy for the hexagonal H_{II} arrangement (circa −8 ppm) is half of $\Delta\sigma_{eff}$ as would be expected [23, 26]. This also indicates that the DDP molecules are able to rotate rapidly around the aqueous channel on the NMR time scale, suggesting a substantial fluidity of the tubular structures. This is further supported by the fact that, despite a long interpulse time, no solid state spectrum was observed. It is interesting to note in this respect that such a long interpulse time did reveal a solid state spectrum for the system PS/Ca^{2+} [10]. The theory predicts that $\sigma_\perp^H = (\sigma_\perp^L + \sigma_\parallel^L)/2$ where $\sigma_\perp$ and $\sigma_\parallel$ are the chemical shifts with the magnetic field perpendicular (high intensity edge) and parallel to the directory axis (low intensity edge of the spectrum), respectively [26]. The subscripts *H* and *L* denote the hexagonal (*H*) and lamellar (*L*) phase. Experimentally, a value of 0.6 ppm, rather than the expected 5 ppm, was determined for $\sigma_\perp^H$. This shift in $\sigma_\perp^H$ is in accordance with a Ca^{2+}-induced upfield shift, conceivably related to a partial dehydration of the headgroups (L.A.M. Rupert et al., *submitted*).

To further investigate the structural details of

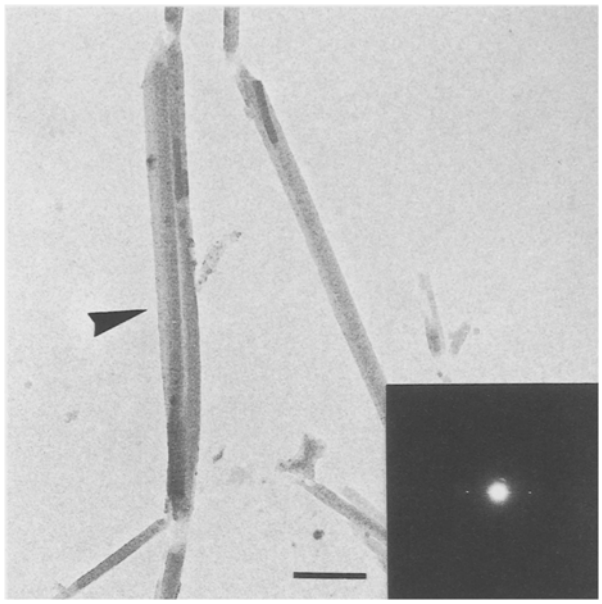

Fig. 6. Selected area electron diffraction pattern (inert) obtained from a region (arrow head) of the tubular structure. DDP and Ca^{2+} concentrations were as described in the legend to Fig. 2. The bar indicates 0.47 μm

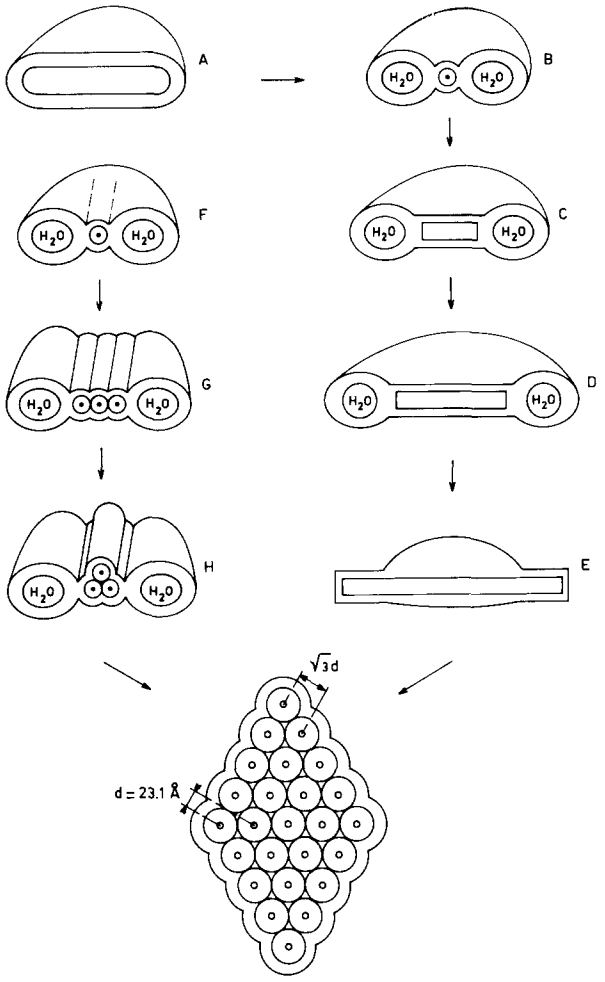

Fig. 7. Hypothetical mechanism of the lamellar to hexagonal H_{II} transition. In the presence of Ca^{2+}, DDP vesicles fuse to larger, flattened vesicles (*A*) which further collapse as Ca^{2+} presumably enters the vesicles (*B*). Interbilayer contacts are formed, allowing the formation of inverted micellar intermediates (IMI's) which subsequently merge into extended hexagonal cylinders (*C* and *D*, side view; *E*, top view; *F* to *H*, front view). Within minutes (*cf.* Fig. 2) the vesicles are thus transformed into an array of cylinders, surrounded by a monolayer of the amphiphile

the tubes, electron diffraction (ED) measurements were carried out on carefully defined, but randomly selected, areas on the tubes (Fig. 6). The correlation between the diffraction pattern and the spatial orientation of the tube is shown in the insert of Fig. 6. The reflection spots on the equator represent a periodicity of 23.1 Å. This repeat distance corresponds with the center-to-center distance of the two-dimensional projection of two hexagonally arranged cylinders (*cf.* Fig. 7, bottom). Since the length of a DDP molecule, in its *extended* form, is 19 Å [13], it follows that the radius of the channel in the hexagonal cylinders amounts to minimally 4 Å. Finally, it is relevant to note that when performing the ED experiments at room temperature, repeat distances other than 23.1 Å vanished before they could be recorded. Most likely, this should be attributed to electron radiation damage.

The Table summarizes a series of measurements of the tube diameters, that were most frequently seen in the samples. When taking into account that the boundary of the tubes is surrounded by a monolayer of the amphiphile with its hydrocarbon chains facing the interior of the tube, it can be readily deduced that the overall diameters correlate fairly well with being multiplications of 46 Å, i.e., the diameter of the cylinder. We conclude, therefore, that the tubes apparently consist of varying numbers of hexagonal (II) phase cylinders, ranging from 7–17 cylinders on the small axis of the tube.

Discussion

In this report we have shown that vesicles formed from didodecylphosphate fuse in the presence of Ca^{2+}. Large fused vesicles are formed which, however, display a transient stability as they subsequently transform into tubular structures. These tubes are composed of cylinders, varying between 7 to 17 cylinders on the small axis of the tube. The evidence, as obtained by ^{31}P NMR and electron dif-

fraction measurements, strongly supports the view that within the cylinders DDP has adopted the hexagonal H_{II} phase. Thus, the repeat distance of 23.1 Å, perpendicular to the long axis of the tube corresponds with the radius of the hexagonal cylinder, formed in the DDP/Ca^{2+} system. Moreover, by employing a novel approach based on cryo electron microscopy, we have been able to confirm and extend the information obtained from the electron diffraction pattern, thereby providing additional evidence for a hexagonal packing of the tubes (L.A.M. Rupert et al., *unpublished observation*). This conclusion is further supported by the images obtained with freeze-fracture electron microscopy (Fig. 4). Substructures on the surface of the tubes closely resemble the structures previously seen for ionotropically-induced lamellar-to-hexagonal H_{II} transitions in phospholipid systems [2, 5, 20, 29]. The fact that the different tube diameters are all multiplications of 23.1 Å (Table) strengthens the argument that the lamellar to hexagonal (H_{II}) phase transition in the tubular structures is essentially complete. Finally, it is emphasized that the selected area electron diffraction procedure offers notable advantages in the analysis of polymorphic behavior of membrane systems over the more classical approach of X-ray diffraction in conjunction with freeze fracture [11]. As demonstrated in Fig. 6, a direct correlation between the structure, as observed with transmission electron microscopy, and the corresponding diffraction pattern is obtained.

The cylinders of which the tubes consist presumably possess a structure in which the amphiphiles are arranged so that the hydrocarbon chains extend radially from the long axis of the cylinders. The polar head groups are facing inward, surrounding a channel in the center of each cylinder with a diameter of ca. 8 Å (Fig. 6). A similar diameter is obtained from samples prepared by cryo electron microscopy (L.A.M. Rupert et al., *unpublished observation*).

The diameter of the DDP cylinder correlates fairly well with diameters reported for the H_{II} cylinders in phospholipid systems [16, 20, 28, 30]. On the other hand, the diameter of the channel appears fairly small in comparison with that found for the cardiolipin/Ca^{2+} system, i.e., 8 Å *vs.* 15 Å, respectively [20]. Rather, there seems to be a close correspondence with the channel diameter of the Cd^{2+}/diethylphosphate complex, which is formed when this low molecular analogue of DDP is incubated with Cd^{2+}[17]. The equally sized Cd^{2+} and Ca^{2+} ions display equivalent fusion activities and have similar complex formations in phosphatidic acid/divalent-ion model systems [16]. Evidently the results on the interaction of Ca^{2+} with DDP vesicles, which we consider as a model for lipid bilayers, support the notion put forward by Miner et al. [17] that the coordination geometries found in simple phosphate complexes (i.e., diethylphosphate/Cd^{2+}) may extend even to complex lipid systems.

Table. Size distribution of hexagonal tubes

Tube diameter (Å)	N_H[a]
350 ± 19	6.8
405 ± 19	8.0
540 ± 19	10.9
630 ± 19	12.9
675 ± 19	13.8
825 ± 19	17.1

[a] N_H represents the number of hexagonal cylinders on the short axis of the tube. This number was calculated, using the formula: N_H = tube diameter −2× (outer monolayer thickness)/46 Å, in which the thickness of the outer monolayer is 19 Å, while 46 Å corresponds to the diameter of the clyinder.

The present observations differ from those observed for the phosphatidylserine/Ca^{2+} system where Ca^{2+}-phosphate interactions lead to the formation of cochleate cylinders which originate from spirally folded lipid bilayers [10, 19]. Importantly, in this system the bilayer structure is maintained, whereas for the DDP vesicles, the interaction of Ca^{2+} with the phosphate head group eventually (*see below*) leads to a bilayer/hexagonal phase transition. In this regard, it is pertinent that phosphatidic acid, which more closely resembles DDP, is also converted into a hexagonal phase upon addition of divalent cations [16], although a time-dependent transition to tubular structures as found for DDP vesicles (Fig. 2) has not been reported (*see below*). In previous work, we have shown that vesicles composed of didodecyldimethylammonium bromide (DDAB), which differs from DDP only in the structure and charge of the head group, fuse in the presence of divalent anions [21, 22]. However, in this case large stable vesicles are obtained and tube formation nor lamellar to hexagonal phase transitions are observed. The reason for these differences in behavior is clearly associated with the substantial difference in the head group structure. Of particular importance is the fact that the head group of DDAB is much bulkier than that of DDP. As outlined by Cullis and De Kruijff [3] and Israelachvili [12], the preference of certain lipids to adopt a particular phase can be understood in terms of the molecular shape of the lipid, as defined by the shape factor S (= volume of hydrocarbon chains/head group area × hydrocarbon chain length). This concept implies that molecules with an overall cylindrical shape ($0.5 < S < 1.0$) prefer a lamellar organization, whereas

cone-shaped molecules ($S > 1$, the exact value depending on pH or Ca^{2+}) prefer inverted phases such as the H_{II} phase. In terms of this concept, it seems reasonable to expect a bilayer preference of DDAB ($S = 0.95$) in the fused vesicles. By contrast, given (i) the small phosphate head group of DDP, (ii) a shape factor of 1.5 and (iii) its close resemblance with the phospholipid PA, it is not surprising that Ca^{2+} triggers hexagonal phases in this system. The divalent cation will dehydrate the head group upon binding (*see above*) while concomitantly interheadgroup electrostatic repulsions will diminish, both processes decreasing the effective cross-sectional area of the head group. Thus, electrostatic interheadgroup repulsions and hydration no longer stabilize the lamellar structure. Apparently, under such conditions the DDP/Ca^{2+} system prefers the H_{II} arrangement.

Interestingly, the observations reported here strongly suggest that membrane fusion represents an essential intermediate event in the lamellar-to-hexagonal phase transition in this system. This may be inferred from the images shown in Fig. 2, which indicate that on the time scale of fusion the appearance of the hexagonal phase is relatively delayed. The results therefore support the view that this morphological change takes place secondary to fusion and that the fused vesicles as such function as intermediate structures in the transition process. Furthermore, it is also clear that the hexagonal cylinders do not act as fusion intermediates. This is consistent with recent work of Ellens et al. [7], showing that a thermotropically-induced transition from the lamellar-to-hexagonal phase does not promote membrane fusion.

Thus far, the mechanism of hexagonal phase formation has remained largely an enigma. It has been proposed that "lipidic particles" might serve as intermediates [29]. However, their thermodynamic stability does not particularly favor such a suggestion. Furthermore, Borovjagin et al. [2] suggested that formation of cylindrical tubes of bilayers, so-called "complex hexagonal phases," is involved in the lamellar to hexagonal H_{II} phase transition. This view has also been criticized for thermodynamical inconsistencies [25]. It appears, however, that our results are best explained by a model recently proposed by Siegel [24, 25]. The nature and kinetics of lamellar to hexagonal transitions require interbilayer contacts [24, 25] and the subsequent formation of structures like inverted micellar intermediates, so-called IMI's. As outlined by Siegel, IMI's may transform into fusogenic structures, called interlamellar attachments, provided that the IMI's remain isolated and that their number in the contact area is low. If not, IMI's can assemble and subsequently collapse, which results in H_{II} phase transformation. It has been predicted [7, 24, 25] that in contact areas formed between two liposomes which have diameters of ca. 0.2–0.3 μm, i.e., twice to thrice the size of DDP vesicles, there may be as few as *one* IMI. This would explain why DDP vesicles initially can fuse to larger structures. Apparently, after 1–3 min (Fig. 2) a membrane surface area has been formed, large enough to permit the formation of a sufficient number of IMI's (upon close approach with an apposed membrane), required to allow the H_{II} phase to be formed. Evidently, these IMI's are formed upon collapse of the vesicles (Fig. 3), forcing out the internal contents, presumably as a result of the entry of Ca^{2+} into the vesicles (L.A.M. Rupert et al., *unpublished observations*), similarly as described for PS vesicles [19]. As the collapse proceeds, the area of closely apposed membranes increases and the hexagonal cylinders can subsequently grow, as depicted in Fig. 7. The length of the tubes containing the cylinders eventually extends beyond the dimension of the fused vesicle *per se* (Figs. 3 and 7*E*). This implies that insertion of newly formed IMI's takes place into pre-existing cylinders. At present, the mechanism by which such an insertion might be accomplished remains obscure.

Finally, in the present and previous work [21, 22] we have shown that there exists a striking similarity between vesicles prepared from simple synthetic amphiphiles and phospholipid vesicles with respect to their fusogenic and polymorphic behavior. Since the molecular structure of the synthetic amphiphiles is readily amenable to chemical modification, they may be of great advantage in attempts to improve the insight into the fundamental molecular mechanisms underlying bilayer to nonbilayer transitions as they occur in phospholipid systems.

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