Bilayer Morphologies of Self-Assembled Crew-Cut Aggregates of Amphiphilic PS-*b*-PEO Diblock Copolymers in Solution

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Received September 25, 1997; Revised Manuscript Received March 30, 1998

ABSTRACT: A wide range of bilayer aggregates, among them tubules, vesicles, large compound vesicles (LCVs), and lamellae, were prepared from various polystyrene-*b*-poly(ethylene oxide) (PS-*b*-PEO) diblock copolymers, and studied by transmission electron microscopy (TEM). The preparation method involved copolymer dissolution in DMF at room temperature, followed by the addition of water. In addition, it was found that aggregates of various morphologies can be prepared from an identical block copolymer by changing the solvent from DMF to a water−DMF mixture, by the addition of electrolytes, or by the use of subambient temperatures. All of these methods can be used to facilitate the formation of specific bilayer aggregates. When the preparation method involved copolymer dissolution in water−DMF mixtures, it was found that the morphologies of aggregates under certain conditions also depended on the annealing time. For example, the ratio of tubules to vesicles is related to the annealing time; only tubules appear at long annealing times, while vesicles and tubules coexist at short annealing times. Possible mechanisms for the formation of the bilayers are discussed. Tubules with oscillatory perturbations in their diameters are seen; these might be intermediates in the vesicle to tubule transition. Lamellae are observed frequently in the present system. In addition, bent lamellae have been observed for the first time; they may be intermediates in the lamella to vesicle transition, as suggested by some theories dealing with small molecule amphiphiles. The present system is believed to be the first to yield stable block copolymer amphiphile tubules, as well more complex tubular or vesicular aggregates, such as "plumber's nightmare", starfishlike vesicles, budding vesicles (i.e. a chain of vesicles), and vesicles consisting of one or more small internalized vesicles within a larger one. Some of the morphologies are biomimetic.

1. Introduction

Self-assembled aggregates of diblock copolymers in selective solvents, which thermodynamically favor one of the blocks, usually have core−corona structures. If the corona-forming block (soluble block) is much longer than the core-forming (or insoluble) block, the aggregates are spherical and are called "star" micelles or aggregates.[1] Such structures have been explored for several decades. If the corona-forming block is much shorter than the core-forming block, the aggregates are called "crew-cut".[2]

In recent years, much attention has been focused in this laboratory on the self-assembly of block copolymer amphiphiles with highly asymmetric structures, and crew-cut aggregates of various morphologies have been observed directly by transmission electron microscopy (TEM).[3,4] The first observation dealt with aggregates made of polystyrene-*b*-poly(acrylic acid) (PS-*b*-PAA) diblocks. More recently, we described in preliminary reports the preparation and observation of crew-cut aggregates of various morphologies from polystyrene-*b*-poly(ethylene oxide) (PS-*b*-PEO) diblock copolymers.[4] The morphologies include normal spheres, rods, lamellae, vesicles, tubules, large compound vesicles (LCVs), large compound micelles (LCMs), and large compound rod micelles (LCRMs). The method of preparation usually involves dissolution of the copolymer in a solvent good for both PS and PEO blocks, such as *N*,*N*-dimethylformamide (DMF), and the subsequent addition of a precipitant, in our case water, to induce aggregation of the long PS blocks.

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While multiple morphologies have also been observed in other block copolymers,[5] no tubular aggregates were seen in systems other than PS-*b*-PEO,[4] with the exception of the one very recent report of their observation in PS-*b*-PAA.[5d] Although aqueous solutions of PS-*b*-PEO star micelles have been studied extensively by a number of techniques in other laboratories,[6] no study other than the present work has focused on crew-cut PS-*b*-PEO aggregates. In addition, no detailed study on the self-assembled bilayer aggregates of block copolymers in solution has been reported.

In contrast, the self-assembly of small molecule amphiphiles in solution into bilayer aggregates has been a very active field of research. A range of bilayer aggregates of small molecule amphiphiles, such as flat sheets (lamellae), closed spherical aggregates (vesicles or liposomes), and tubules, has been explored extensively.[7−9] When the closed spherical bilayer aggregates are formed from phospholipids or synthetic surfactants, they are usually called liposomes or vesicles, respectively.[8] The liposomes appear to be closely related to biological membranes, which are examples of bilayer structures found in nature. To successfully model and utilize the self-assembled bilayer structures for potential applications, such as drug delivery vehicles,[7−10] it is necessary to understand the relationship between the original molecular structures and the resulting bilayer structures. This forms the basis of intense research in self-assembled bilayer aggregates. For some applications, the stability and mechanical rigidity of the small molecule systems would need to be enhanced.[7−10] If the tubules or vesicles were to be constructed directly from polymers, these shortcomings would be partially over-

S0024-9297(97)01419-8 CCC: $15.00
Published on Web 05/12/1998

come, and the systems might have broader applications because of their increased internal stability.

In this paper, we present a detailed study of the preparation of PS-*b*-PEO bilayer aggregates, including tubular structures, vesicles, large compound vesicles (LCVs), and lamellae, observed directly by TEM. A range of methods of preparation is described, including the use of DMF and water−DMF mixtures as solvents, the addition of electrolytes and variation of the aggregation temperature. The observation of tubular aggregates with a "plumber's nightmare" morphology, oscillatory perturbations in the diameter of tubules, and vesicles with various shapes, such as starfishlike vesicles, chains of vesicles, and small internalized vesicles within bigger ones (i.e. "pregnant" vesicles), as well as bent lamellae is presented. Evidence is given for a number of mechanisms for the formation of the bilayers, including the possibility that vesicles might be precursors of tubules and that lamellae might be precursors of large vesicles, as has been suggested for a small molecule system.

2. Experimental Section

2.1. Synthesis and Characterization of Block Copolymers. The polystyrene-*b*-poly(ethylene oxide) diblock copolymers were prepared by sequential anionic polymerization, generally following published procedures.[11] The synthesis was carried out under an ultrapure nitrogen atmosphere, and the solvent was freshly distilled tetrahydrofuran (THF). Cumylpotassium was used as the initiator, this was prepared from methyl cumyl ether and sodium−potassium alloy in THF, by a method described elsewhere.[12] The methyl cumyl ether, in turn, was prepared by the reaction of α-methylstyrene (Aldrich) with methanol in the presence of $HClO_4$, according to the procedure of Ziegler et al.[13]

The PS-*b*-PEO diblock copolymers were synthesized by first polymerizing the styrene monomer, followed by addition of ethylene oxide monomer. Before the addition of the EO monomer, an aliquot of the reaction medium was withdrawn for characterization of the PS block. The remainder of the reaction medium was then divided into several fractions, placed in flamed flasks using flamed stainless steel capillaries, and kept at −78 °C. A different quantity of purified EO was then added to each PS fraction at the same temperature. Each reaction flask was then allowed to warm gradually to ca. 40 °C, and the polymerization was allowed to proceed for ca. 24 h. This anionic polymerization method allows us to synthesize block copolymers which have the same PS block length but different PEO block lengths.

Size exclusion chromatography (SEC) was used to characterize the PS block length and polydispersity (M_w/M_n). The SEC measurements were performed at room temperature on a Varian 5010 liquid chromatography apparatus equipped with a refractive index detector. THF was used as the eluent at a flow rate of 1 mL/min. The columns were progel-TSK G4000 HXL and G2000 HXL from Supelco Inc. The system was calibrated with polystyrene standards. The concentration of injected polymer solution was about 2 mg/mL. The molecular weight and the polydispersity index were calculated by a Varian DS-604 computer with SEC application software.

The crude copolymers may contain some homopoly(ethylene oxide) and homopolystyrene. The elimination of the homopoly(ethylene oxide) from the copolymer samples was achieved by Soxhlet extraction with methanol for 4−6 days. The resulting products were then dried under vacuum overnight.

The composition of the purified copolymers was determined by nuclear magnetic resonance (^{1}H NMR). The ^{1}H NMR experiments were performed on a Varian XL-500 MHz NMR spectrometer with tetramethylsilane (TMS) as the internal standard and chloroform-*d* ($CDCl_3$) as the solvent. The PEO block lengths of the purified diblock copolymers were calculated from the ratio of the intensities of the OCH_2CH_2 signal (at $\delta = 3.6$) and of the aromatic signal (at $\delta = 6.4-7.2$). The copolymers used in this work are designated, for example, as PS(240)-*b*-PEO(15), showing that this particular copolymer contains 240 styrene repeat units and 15 ethylene oxide repeat units. The polydispersity of the PS block is 1.07.

2.2. Preparation of Micellar Solutions. Two methods were employed to prepare block copolymer stock solutions for experiments performed at room temperature without additives. In the first method, PS-*b*-PEO diblock copolymers were dissolved in pure *N*,*N*-dimethylformamide (DMF) by stirring for more than 4 h to give different copolymer concentrations (0.2−3.0 wt %). In the second method, the diblocks were dissolved in water−DMF mixtures of varying water contents (4.0−6.5 wt %) by stirring for a few days to give different copolymer concentrations. The stock solutions prepared by both methods were divided into several portions for further experiments.

For the additive effect experiments, aqueous solutions of different electrolytes (KF, KI, NaCl, etc.) were added to the fractions of the stock solution prepared by method one. The resulting solutions were stirred for about 4 h. Subsequently, deionized water was added dropwise to the solutions. For the temperature effect experiments, the copolymer solutions (prepared by method one) and deionized water containers were placed in a controlled-temperature bath for ca. 30 min. The solutions were stirred. Deionized water was added to the copolymer DMF solution at the preset temperature. If temperature is not mentioned, the micellar solutions were prepared and the addition of water was performed at room temperature also.

Generally, the addition of deionized water proceeded dropwise with stirring, with one drop added every 10−15 s. Each drop of added water was equal to ca. 0.25−0.5 wt % of the solution. When the water content reached ca. 25%, the solutions were placed in dialysis tubes (Spectra/Por; molecular weight (M_w) cutoff 50 000) and dialyzed against distilled water for about 4 days to remove the DMF. Distilled water was changed twice a day.

2.3. Sample Preparation for Transmission Electron Microscopy (TEM). After dialysis, the aqueous solutions were further diluted by a factor of 10−20, depending on the copolymer concentration in the stock solution. A drop of the diluted solution (ca. 0.05−0.5 mg/mL) was placed onto a copper EM grid. Before sample deposition, the copper EM grids were precoated with a thin film of Formvar (polyvinyl formaldehyde plastic, J. B. EM Services Inc.) by placing the grids between a microscope cover glass and the Formvar, and were coated with a thin film of carbon. After the drop had been in contact with the grid for a few minutes, excess solution on the sample grid was blotted away using a strip of filter paper. The sample grid was then dried in air (at room temperature and atmospheric pressure) for a few hours, and was subsequently shadowed with a palladium/platinum alloy at an angle of ca. 30°. The morphologies of aggregates were observed on a Phillips EM410 transmission electron microscope which was operated at an acceleration voltage of 80 kV.

3. Results and Discussion

The results and discussion section is presented in six parts. An explanation of the present method of preparation of stable colloid solutions is discussed in the first part. The second part consists of a description of the relationship between the preparation procedures and the morphologies of the resulting bilayer aggregates, i.e., tubules, vesicles, LCVs, and lamellae. The characteristics of each of the bilayer aggregates are also described, and representative figures of the morphologies are given. All the structures described in this study can be grouped within four categories, namely, tubules, vesicles, LCVs, and lamellae. Possible mechanisms for the formation of bilayer morphologies are presented in part three. Vesicle shapes, shape transformations, and

a comparison with small molecule amphiphile aggregates are presented in part four. Finally biomimetic aspects of the bilayer aggregates and potential applications are discussed briefly in the last two parts.

It is known that the morphology of crew cut aggregates in solution is a function of a number of factors, including the absolute lengths of both the hydrophobic and hydrophilic blocks, the ratio of the number of repeat units of the two blocks, the copolymer concentration in the stock solution, the temperature, the nature of the common solvent, the homo-PS content (if any), the process of the addition of water, and the presence of additives such as electrolytes.[3−5,14] All of these factors and the interplay between them influence the observed morphologies. We focus in this study on the effect of copolymer concentration in the stock solution, the water content in the water−DMF mixture, and the effects of added electrolytes and temperature variation insofar as they affect the bilayer morphologies. The PS-*b*-PEO block copolymers used in this study have the same PS block length but different PEO block lengths; thus the effect of changes in the hydrophilic PEO block lengths is also investigated.

3.1. Explanation of the Method of Preparation of Crew-Cut Aggregates. Spherical star micelles consisting of a relatively small core and relatively long corona are frequently prepared by direct dissolution of the AB-type block copolymer in a solvent which is good for the longer block. The core is thus composed of the shorter (and less soluble) block, and is usually swollen by the solvent. In contrast, the amphiphilic diblock copolymers used in the present study have short hydrophilic EO blocks, the weight fraction of which is less than 15%, but relatively long hydrophobic PS blocks. Therefore, it is impossible to prepare aggregates in aqueous solution by dissolving the PS-*b*-PEO samples in water directly. To make stable aqueous solutions, the PS-*b*-PEO copolymers are dissolved first in pure DMF or in a water−DMF mixture (with a water content less than 6.5%). DMF is a good solvent for both blocks, while water is a good solvent only for the PEO block but a precipitant for the PS block. After the dissolution of the block copolymer, deionized water is added.

During water addition to the copolymer DMF solutions, the quality of the solvent for the PS block decreases; when the water content reaches 3−6%, the appearance of turbidity in the solutions indicates the aggregation of the PS blocks. The water content at the onset of turbidity depends on the composition of the block copolymer and the copolymer concentration in DMF. The longer the hydrophilic EO block or the lower the copolymer concentration in DMF, the more water is needed to cause turbidity. These aspects were discussed in some detail in a previous publication describing the formation of crew-cut aggregates from PS-*b*-PAA in DMF.[15] To obtain aqueous solutions of crew-cut aggregates, the solutions containing ca. 25% water are then dialyzed against water to remove the DMF.

In the initial stages of self-assembly of the block copolymers, the relatively low water content in the system may allow the exchange of the copolymer chains between unimers and aggregates to proceed at a significant rate. Thus the aggregation would be controlled by thermodynamics. At relatively high water contents, the rate of single chain exchange becomes low due to the decreased solubility of the single chains.[15] Furthermore, during the addition of water, the DMF concentration in the PS cores decreases to the point that the aggregates become kinetically frozen. The decrease of the critical micellization concentration (CMC) with increasing water content has been demonstrated for PS-*b*-PAA copolymers in DMF.[16] The same trends are expected to hold in the present system. Thus, before dialysis, the morphologies of aggregates are already frozen.

3.2. Relationship between the Morphologies of the Bilayer Aggregates and the Preparation Procedures. 3.2.1. Copolymer Dissolution in Pure DMF. 3.2.1.1. Tubules. Figure 1A shows an example of a tubule obtained from a 1.5 wt % PS(240)-*b*-PEO(15) solution. The tubular nature is evident from the higher transmission in central part of the aggregate compared to its periphery, indicating that the structure is hollow, coupled with a measurement of the height from shadowing, showing that its width equals its height, which means that the aggregate is cylindrical. The tubules have a wall thickness of ca. 35 nm, and an outside diameter of ca. 150 nm; however, the lengths are quite polydisperse, varying from tens of nanometers to hundreds of micrometers. The tubules are closed cylinders, sometimes with vesicles attached at the end. Using this method of preparation (copolymer dissolution in pure DMF at room temperature), tubules can only be made from this PS(240)-*b*-PEO(15) sample, with the copolymer concentration at 1.5 or 2.0 wt %. No tubular morphologies are seen from the other two samples.

Branched tubules are also observed frequently (see Figure 1, parts B and C). Sometimes, holes are seen in the tubules, either in the linear part or at the branches (see Figure 1C). An interconnected tubular structure analogous to a "plumber's nightmare" is shown in Figure 1D. In this structure, the interconnected tubules form a single structure, with two continuous "inside" and "outside" surfaces separated by the styrene component, which forms the continuous wall. This morphology resembles a sponge with holes and channels through which water can move.[17]

3.2.1.2. Vesicles. Vesicles made from a 2.0 wt % PS(240)-*b*-PEO(15) solution are shown in Figure 2A. The vesicles are usually large (∼500 nm in diameter) and quite polydisperse in size, but the wall thickness is quite uniform (ca. 20−25 nm). The vesicular nature is evidenced from a higher transmission in the center of the aggregates compared with their periphery, which means that they are hollow, together with the measurement of the length of the shadowed region, which gives the height of the aggregates and which shows that they are spherical. (Although they are shadowed, the shadowed region cannot be seen here because the exposure time for printing this picture was such as to optimize the appearance of aggregates and not that of shadowed regions. The exposure time for printing the picture is too short for the shadowed region to appear. The same situation was encountered in some of the other pictures.)

Vesicles can be observed from this sample (PS(240)-*b*-PEO(15)) when its concentration is 1.5 or 2.0 wt % in DMF, and they coexist with the tubules. Vesicles are encountered occasionally (as a minority morphology) from a 1.5 wt % PS(240)-*b*-PEO(45) solution, some of which have protruding rods. No vesicles have been observed from this sample at other concentrations, and no vesicles whatsoever are observed from the PS(240)-*b*-PEO(80) sample.

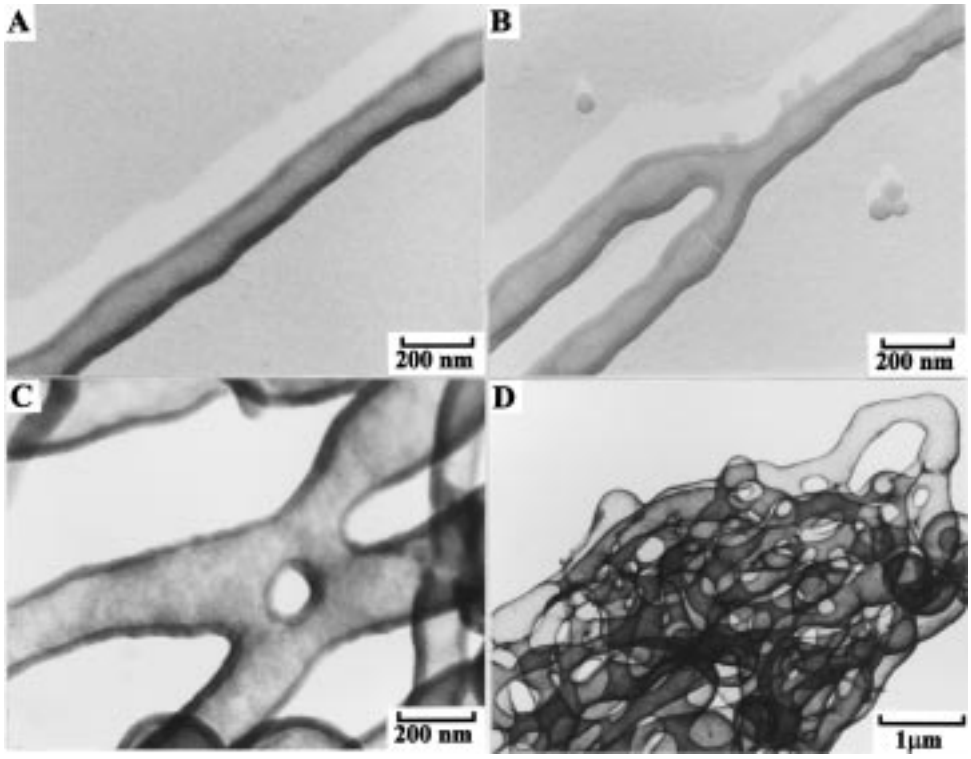

Figure 1. Tubular aggregates made from PS(240)-*b*-PEO(15) in DMF: (A) a linear tubule; (B) a branched tubule; (C) tubules with a hole at a branch; (E) a "plumber's nightmare".

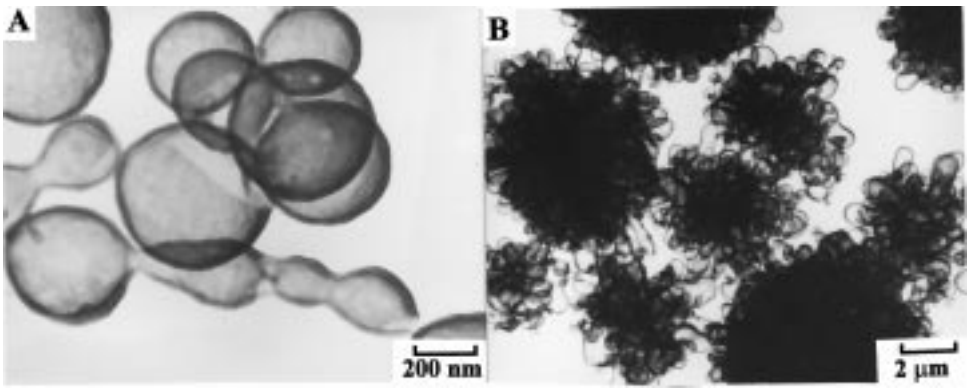

Figure 2. Vesicles (A) and large compound vesicles (B) made from PS(240)-*b*-PEO(15) in DMF.

3.2.1.3. LCVs. Figure 2B shows examples of the LCV aggregates obtained from a very dilute 0.5 wt % PS(240)-*b*-PEO(15) solution in DMF. The LCVs are generally near-spherical assembles of many vesicles of polydisperse sizes. The LCVs show some similarity to aggregated soap bubbles. The wall thickness is ca. 20−25 nm. Usually the LCVs settle in aqueous solutions, because of their large size, but they can be resuspended by gentle shaking. Thus the LCVs in powder form can be isolated by filtration and are easily stored, transported, and redissolved.

LCVs can be made from this sample (PS(240)-*b*-PEO(15)) in DMF when its concentration is between 0.2 and 1.0 wt %. At 0.2 and 0.5 wt %, LCVs are the dominant morphology, while at 1 wt %, mixtures of various aggregates are obtained. The formation of LCVs at such low copolymer concentration is noteworthy. No LCVs have been observed from the other two samples when this preparation method is employed.

3.2.1.4. Lamellae. Figure 3 shows a micrograph of lamellar aggregates made from a 1.5 wt % PS(240)-*b*-PEO(45) solution in DMF. The lamellar nature of the structure is evidenced by the uniformity of the light intensity over the central part of the aggregate, coupled with a measurement of the length of the shadowed region. The observed lamellae usually have protruding rods attached to them, and the thickness of the lamellae is ca. 40 nm. This morphology does not represent an equilibrium, but probably involves a trapped intermediate structure. When the PS(240)-*b*-PEO(45) copolymer concentration is appreciably higher or lower than 1.5 wt %, rodlike or pincushion-like aggregates are observed, respectively.[4,18]

Using the present method of preparation, lamellar aggregates were observed only with the PS(240)-*b*-PEO(45) sample, but they are observed frequently. It is possible that the PS(240)-*b*-PEO(45) sample is close to the composition at which the transition from rods to bilayer aggregates occurs under this method of preparation,[4] such that the observed lamellae are intermediate structures. This is supported by the fact that, for the PS(240)-*b*-PEO(80) sample, the predominant morphology of the aggregates is rodlike, and no bilayer aggregates are observed, while for the PS(240)-*b*-PEO(15)

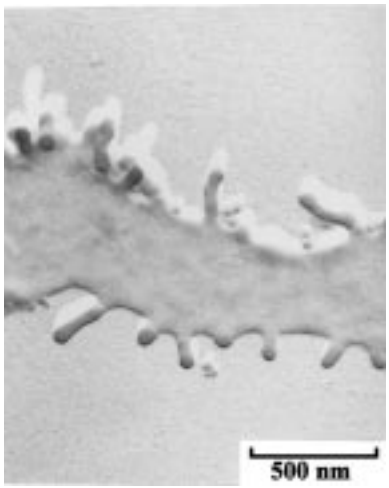

Figure 3. A lamella with protruding rods made from PS(240)-*b*-PEO(45) in DMF.

sample, bilayer morphologies (tubules and vesicles) are found.

3.2.1.5. Summary. The above information, together with the results of our preliminary reports,[3,4] shows that changing the block copolymer composition changes the morphologies of self-assembled aggregates. For a constant PS block length, a decrease in the PEO block length can change the morphology of the aggregates from spheres to rods, to bilayers.[4] In general, tubules, vesicles, and LCVs are prepared from PS(240)-*b*-PEO(15), and lamellae are prepared from PS(240)-*b*-PEO(45). Thus the copolymer composition plays a major role in determining the morphologies of the bilayer aggregates; however, the copolymer concentration is also relevant.

3.2.2. Copolymer Dissolution in Water−DMF Mixtures. In contrast to the preparation method involving copolymer dissolution in pure DMF, copolymer dissolution in water−DMF mixtures allows one to prepare aggregates of bilayer morphologies over a much wider range of block copolymer compositions. All of the bilayer morphologies, i.e., tubules, vesicles, LCVs, and lamellae, can be made from every one of the copolymer samples in certain water or polymer concentration regions. For example, LCVs are the dominant morphology when the aggregates are prepared from a 1.5 wt % PS(240)-*b*-PEO(45) solution in a 4.0 wt % water−DMF mixture. In general, the higher the water content in the mixture, the lower the copolymer concentration needed to prepare the bilayers. The results show that the copolymer concentration in the stock solution plays an important role in determining the morphology, along with the original water content in the water−DMF mixture and the copolymer composition. The results also show that changes in the composition of the water−DMF mixtures can induce morphological changes in aggregates of an identical block copolymer at one copolymer concentration, which proves that the nature of the solvent is an important parameter in determining the morphologies of self-assembled aggregates, as was also demonstrated experimentally in another system.[19]

Copolymer dissolution in water−DMF mixtures is a particularly good method for the preparation of tubules. The optimum conditions for tubule preparation are 1.5−2.0 wt % PS(240)-*b*-PEO(15) in a 4.5 wt % water−DMF mixture and 1.5 wt % PS(240)-*b*-PEO(15) in a 4.0 wt % water−DMF mixture. It is found that the annealing

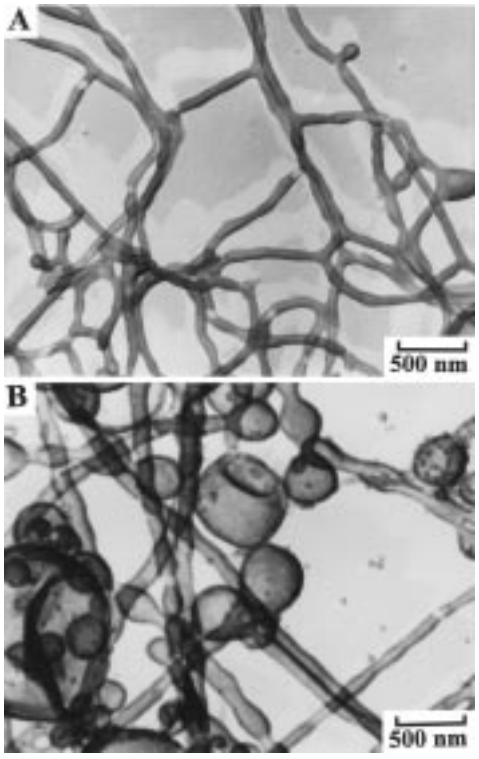

Figure 4. Tubules (A) and a mixture of tubules and vesicles (B) made from PS(240)-*b*-PEO(15) in a water−DMF mixture with annealing times of 10 and 3 weeks, respectively.

time (i.e. the length of time between copolymer dissolution in the mixture and the subsequent water addition) is important for the production of tubules as the dominant morphology. For example, when the annealing time was ca. 10 weeks, a 2.0 wt % PS(240)-*b*-PEO(15) solution in a 4.5 wt % water−DMF mixture gave tubules (as shown in Figure 4A) as the dominant morphology. However, when the annealing time was only three weeks, a mixture of vesicles and tubules (as shown in Figure 4B) was obtained. The longer the annealing time, the larger the proportion of tubules, which suggests that vesicles may be precursors of tubules. It should be noted that tubules with connected vesicles are sometimes observed.

The fact that the annealing time affects the morphologies was also seen in aggregates prepared from a 1.5 wt % PS(240)-*b*-PEO(45) solution in a 5.5 wt % water−DMF mixture.[18] Pincushions as the dominant morphology were seen with shorter annealing times, while mixtures of various morphologies, including lamellae and vesicles, were seen with longer annealing times.[18] In general, it is found that longer annealing times facilitate the formation of bilayer morphologies.

3.2.3. Addition of Electrolytes to Copolymer Solutions in DMF. As mentioned above, morphologies of aggregates made from a dilute copolymer solution in pure DMF are controlled by the copolymer composition. In a recent report,[14] we also showed that the addition of various electrolytes at very low concentrations (ca. 10^{-4}−10^{-3} M) can induce morphological changes in aggregates of an identical block copolymer and that the morphogenic effect of added ions is parallel to that of changing the length of corona chains. It is significant that morphological changes take place at such low added salt concentrations. A detailed study of the addition of electrolytes for PS-*b*-PAA system is described elsewhere.[20] It is shown that with increasing salt contents,

the morphologies of an identical PS-*b*-PAA block copolymer can change from small spheres to rods, to vesicles, and to LCVs.

The present section of the paper on the morphogenic effect of added inorganic ions to PS-*b*-PEO copolymer solutions is focused on finding the most effective salt which induces morphological changes. The inorganic salts used include KF, KI, NaCl, and LiCl, with *R* representing the molar ratio of the additives to EO repeat units in the block. The experiments are performed with *R* values between 0.005 and 0.1. Results are presented in order of increasing PEO contents in the samples.

Without additives, a 1.5 wt % PS(240)-*b*-PEO(15) solution in DMF gives vesicles and tubules as the dominant morphologies. When KF ($R = 0.05$) is added, LCVs are formed in the majority, while LiCl even at $R = 0.10$ has no significant effect on the morphologies.

A 1.5 wt % PS(240)-*b*-PEO(45) solution in DMF gives lamellar and rodlike aggregates without additives. When KF or KI ($R = 0.005-0.10$) is added, LCVs are in the majority. The degree of aggregation (i.e. the diameter of LCVs) produced by KF is higher than that by KI. When LiCl or NaCl is added, mixtures of various morphologies are observed, including LCVs.

A 0.5 or 1.0 wt % PS(240)-*b*-PEO(80) solution in DMF gives rodlike aggregates. When KF ($R = 0.10$) is added, vesicles and lamellae with protruding rods are observed, while KI ($R = 0.10$) has no obvious effect in the morphologies.

The above results indicate that KF is effective in inducing the formation of bilayer aggregates, such as LCVs. It is worth noting that morphogenic effects of added electrolytes have also been found in small molecule amphiphile systems; this is believed to be due to a decrease in the electrostatic repulsion among headgroups by the added ions.[21] However, in these systems, the concentration of added monovalent salt is often ca. 10^{-1} to 10 mol/L. The tendency of surfactant vesicles induced to aggregate upon the addition of ions was also described.[21]

The effects of neutral salts on the conformation of macromolecules have been studied.[22] If the over-all dimensions of polymer chains in solution are reduced by the addition of neutral salts, the effect is called "salting out". This effect is general. A study on the effect of inorganic electrolytes on the dimensions of homo-PEO chains in aqueous solutions has been performed.[23] Usually the concentrations of added electrolytes are ca. 10^{-1}mol/L. Some of the results suggest that due to an increase in the hydrophobic interaction of ethylene segments upon salt addition, the intramolecular association of PEO chains increases, in turn causing the contraction of polymer coils. The relative effectiveness of some ions on salting out was found to be $F^- > Cl^- > Br^- > I^-$ and K^+, $Na^+ > Li^+$. From this list, it is clear that KF would be the most effective in inducing the contraction of PEO coils among all of the electrolytes used in the present study.

Although it has been known that the addition of electrolytes decreases macromolecular dimensions in solution, the morphogenic effects of the addition of electrolytes on aggregates of block copolymers in solution represents a new finding.[14,20] The concentrations of added electrolytes which can induce morphological changes are much lower (ca. $10^{-4}-10^{-3}$ mol/L) than those used in previous studies of salting out effects in homopolymers.[22,23] It has previously been shown that water is preferentially distributed around the hydrophilic PAA blocks in copolymer DMF solutions.[20] The same trend is expected to hold in the present system. Thus, the local water concentration around the PEO block might be higher than that in the bulk solution, such that the PEO block in the present study might behave in a manner similar to that in water. In addition, the local electrolyte concentration around the PEO block may also be higher than that in the overall solution. Thus, the dimensions of the PEO chains may be reduced by the added electrolytes at a much lower electrolyte concentration than that in aqueous solution of the homopolymer. For certain individual electrolytes, the PEO dimensions may also be affected to some degree by specific ion−polymer interactions.

The contraction of the hydrophilic PEO coils leads to a decrease in the steric repulsion between the hydrophilic chains. It has been mentioned before that a decrease in the repulsion among the hydrophilic chains (i.e., effectively a shortening of the PEO blocks) can induce morphological changes, e.g., from spheres to rods or from rods to bilayer aggregates.[3,4] Therefore, one of the reasons why the addition of electrolytes induces the formation of bilayer aggregates is believed to be the decrease in the effective dimensions of the PEO chains. Other factors may also contribute, such as a possible change in the surface tension between the PS core and solvent.

3.2.4. Aggregation of Copolymer in DMF at Subambient Temperature. The effect of temperature was also explored briefly by adding water to DMF solutions (1.5 wt %) of all three copolymer samples at a subambient temperature. At 0.0 °C, LCVs were found to be the dominant morphology in all cases. The easy preparation of LCVs as a dominant morphology at 0.0 °C is noteworthy. At temperatures between room temperature and 0.0 °C, mixtures of various morphologies were observed. It should be recalled that, at room temperature, the dominant morphologies of the three samples, i.e., PS(240)-*b*-PEO(15), PS(240)-*b*-PEO(45), and PS(240)-*b*-PEO(80), are bilayers (tubules and vesicles), rods and lamellae, and rods, respectively. Thus, it appears that the decrease in temperature favors the formation of the bilayer aggregates. The coil dimensions of both the PS and PEO segments are affected by temperature; thus, it is conceivable that the ratio of the coil dimensions of PS to PEO segments increases as temperature decreases.

3.3. Possible Mechanisms for the Formation of Bilayer Morphologies. 3.3.1. Tubules. Since the preparation of tubules from block copolymer is new, there are no theoretical treatments which are directly relevant to this present work. However, theories dealing with the formation of tubules from small molecule amphiphiles do exist.[24,25] Because there is some similarity between the two types of amphiphiles, the existing theories may help us understand the present system. Thus, it is useful to review very briefly the formation mechanisms of small molecule amphiphile tubules.

Tubules were first observed from a chiral diacetylenic phospholipid in 1984.[9] Since then, they have been observed in a number of chiral organic small molecule systems, and extensive studies have been performed on such phospholipids.[9] Generally, two methods are used to prepare tubules: a precipitation method and a thermal method.[9] In the precipitation method, tubules

are formed by precipitation of the lipid alcohol solution by the addition of water at temperatures below the phase transition temperature of the lipid. In the thermal method, a solution of the lipid in water or in an alcohol−water mixture is first heated to above its hydrocarbon chain melting temperature, T_m, where liposomes are formed. Tubules, which might contain trapped liposomes, are formed when the solution is cooled below T_m. This method was studied in some detail.[9] When water is the solvent, the formation of tubules, which is a result of a quantitative conversion of the liposomes, is dependent on the size of the vesicle precursors. Large vesicles can convert to tubules when the temperature is lowered, while small vesicles, such as those formed by sonication, cannot. If the solvent is an ethanol−water mixture, the water content in the mixture and the lipid concentration strongly affect tubule formation.

Systematic studies show that molecular chirality is a required element for lipid tubule formation; the chirality of the tubules from *R* and *S* enantiomers of a chiral diacetylenic phospholipid is different.[9] Although the molecular packing is chiral in the lipid tubules, it is not in the spherical vesicles. While chirality is an important factor in lipid tubule formation, it is not an absolute requirement for the formation of nonlipid tubules, because tubules were also prepared from a polymerizable ammonium surfactant with no chiral center.[26] Clearly, chirality is not a requirement for the formation of block copolymer tubules, as can be seen from the present work.

The molecular factors involved in the process of self-assembly are complex, and more than one mechanism may be involved in the formation of tubules. The relevant theories reflect this complexity. Most theories invoke the presence of a chiral center;[24] there is only one theory which does not,[25] even though it was developed in connection with the tubule formation of a chiral diacetylenic phospholipid. In this theory, a general phase diagram incorporating various morphologies, including tubules, is proposed. It predicts that if the membrane of a spherical vesicle experiences a freezing transition, tubules might be formed from the vesicle by the development of in-plane orientational order, when the flexibility of the membrane is low enough. Besides, a transition from spheres to cylinders could occur as the flexibility of the membrane decreases upon cooling.

This approach may be valid for the present system, because the flexibility of the copolymer membranes decreases during the addition of water. As mentioned above, during the addition of water, the DMF comes out of the PS cores progressively so that the PS may even become glassy, i.e., the glass transition temperature, T_g, of the PS may be raised above room temperature. This procedure is therefore similar to the preparation of small molecule amphiphile tubules by the thermal method, with the T_g of the PS solution playing the role of the T_m of the hydrocarbon chains. Furthermore, the vesicles of the present system are also usually large. Thus, the possibility exists that the formation of block copolymer tubules might be a result of conversion from vesicles. In addition, in some of the present micrographs, it is seen that the wall thickness of the tubules is thinner than that of the vesicles, which means the degree of stretching of the PS chains in the tubules is lower than that in the vesicles. A discussion of the importance of a decrease in the degree of stretching the PS chains during morphological transition can be found elsewhere.[3,4] Thus, if tubules are produced from vesicles, one of the contributions to driving force might be a decrease in the degree of stretching of the PS chains.

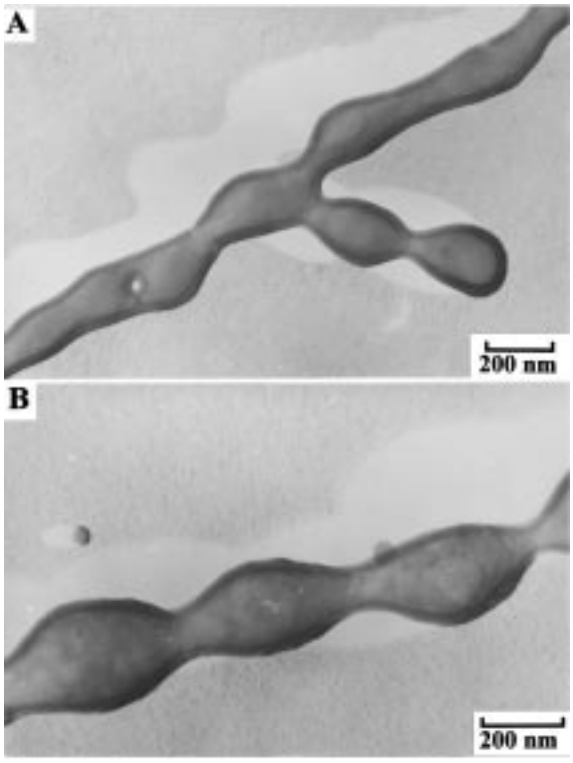

Figure 5. Possible intermediates of the vesicle to tubule transition: (A) a tubule with attached vesicles; (B) a tubule with an oscillatory perturbation in the diameter.

There is experimental evidence that vesicles are precursors of tubules, namely that the population ratio of tubules to vesicles is related to the annealing time when the tubule preparation involves copolymer dissolution in water−DMF mixtures. As was mentioned before, vesicles and tubules are seen to coexist with short annealing time, while only tubules appear with long annealing time. Besides, tubules with attached vesicles, which might be intermediates of the vesicle to tubule transition, are often seen. One example is shown in Figure 5A. Although block copolymer amphiphile tubules are usually straight, quite often an oscillatory perturbation state appears in the diameter of some parts of some tubules, as shown in Figure 5B. The wall thickness (δ) of membranes remains constant. The oscillatory perturbation in the diameter of tubules could be an intermediate state of the vesicle to tubule transition.

It is necessary to mention that although tubules made from small molecule amphiphiles are also straight cylinders,[9] similar oscillatory perturbation states (i.e. the peristaltic state or pearling state) in tubules are observed only when some external perturbations are performed on tubules, such as the use of laser tweezers[27] or anchoring hydrophilic polymers onto a lipid membrane by hydrophobic side groups.[28] The appearance of the oscillatory perturbation in block copolymer tubules without the imposition of external perturbations is therefore of note.

3.3.2. Vesicles. Bent lamellae, as shown in Figure 6, are often observed. The fact that a lamella is bent

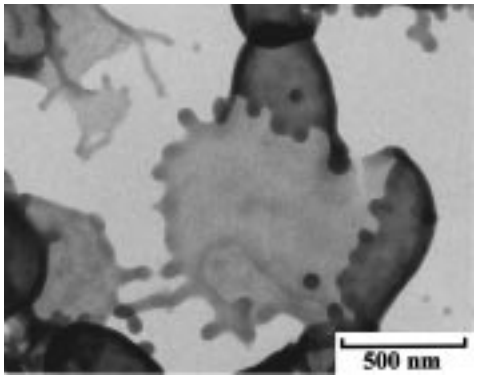

Figure 6. Bent lamellae, which might be the most direct evidence of the mechanism of the formation of large vesicles.

can be seen from the shape of the shadowed regions. The extent of bending is different from one lamella to another, and some bent lamellae have a nearly hemispherical appearance. A number of experiments involving the preparation of bilayer aggregates by copolymer dissolution in water−DMF mixtures have shown that the number of the bent lamellae depends on the storage time of the copolymer in the water−DMF mixture prior to the addition of water;[18] rods and pincushions are seen with short annealing times. The longer the copolymer is stored in the mixture, the more bent lamellae are observed, the greater the extent of bending, and the greater the concentration of closed vesicles. Therefore, the bent lamellae seem to be precursors of large vesicles.

Vesicle formation from lamellae is a mechanism which has been previously proposed for small molecule amphiphiles.[29] In such systems, vesicles can be made by several different procedures, but some authors suggest that large vesicles form from disks (lamellae) independent of the preparation procedure.[29−31] The formation of large vesicles from disk aggregates has been observed experimentally by microscopy.[30] In addition, estimations of vesicle sizes and size distributions based on this model are consistent with the experimental results.[31] However, no bent lamellae have ever been observed in small molecule surfactant systems.

The proposed mechanism suggests that if a bilayer is in the form of a large disk, the disk can bend due to thermal fluctuations, and a vesicle may form.[29] The reverse process is also possible. Whether a flat disk bends to produce a closed sphere (i.e., a vesicle), or a defect on a vesicle grows to produce a flat disk (i.e., a lamella) depends on the edge energy of the disk and the energy of bending. It has been shown that defects do form and disappear every now and then due to thermal fluctuation during self-assembly of surfactants.[29]

In the present system, holes on the bilayer membranes are seen occasionally, directly by TEM. In addition, the present microscopy results support this model, especially in view of the presence of intermediates of the lamella to vesicle transition. Bent lamellae are the first direct examples of this process observed by TEM.

3.3.3. LCVs. LCVs can be formed spontaneously in the present system under various conditions. The formation of LCVs may be due to different mechanisms: either from one lamella (i.e. by bending in several places) or by aggregation of individual vesicles and a subsequent fusion process, which might be under kinetic control[20] or a combination of both. Figure 6 shows some evidence for both mechanisms. When the individual vesicles aggregate, the repulsion between bilayers has to be minimal. It should be recalled that in the PS-*b*-PAA system, the repulsion between the PAA chains is large, and LCVs can only prepared from a copolymer solution in DMF when some electrolyte is added. The purpose of the addition of electrolytes is to reduce the repulsion among the PAA chains to allow the bilayers to approach each other. However in the present system, LCVs can be formed from a copolymer solution in DMF without the addition of electrolytes, because only weak steric repulsion is operative between PEO chains.

3.4. Vesicle Shapes and Shape Transformations. In addition to a normal spherical topology, PS-*b*-PEO diblock copolymer vesicles exhibit a variety of shapes under the present preparation conditions. Aggregates in which several vesicles are linearly connected, such as dimeric or linear trimeric vesicular aggregates, are often observed, as shown in Figure 7A. The aggregates of linearly connected vesicles of the present system might be examples of budding or vesicular transitions, which are used to describe the multistep process in which vesicles produce distinct daughter vesicles in small molecule amphiphile systems.[32]

Aggregates of nonlinearly connected vesicles are often encountered. For example, a starfishlike vesicular aggregate with three arms is shown in Figure 7B. A vesicle with smaller ones inside, as judged from the length of its shadow, is shown in Figure 7C. These

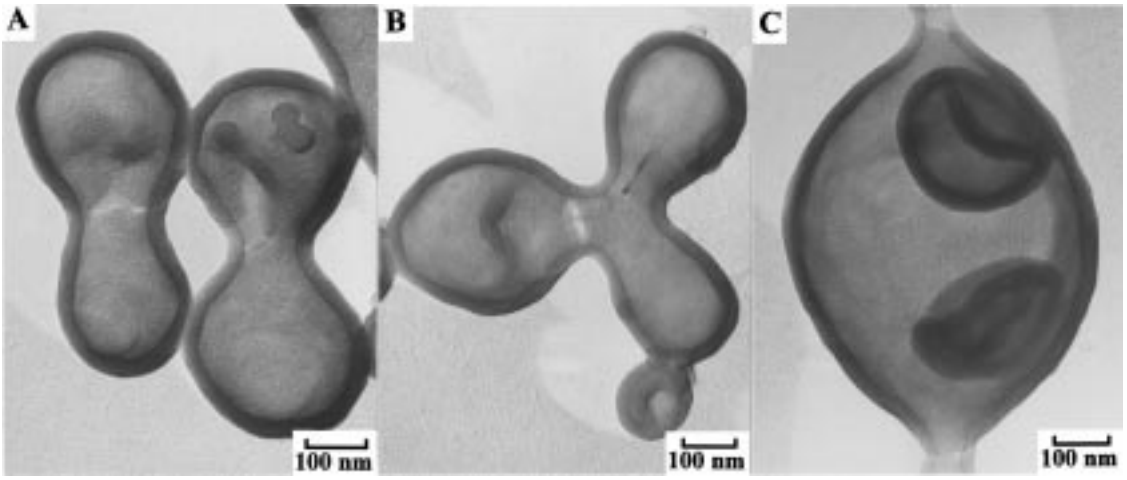

Figure 7. Various vesicle shapes: (A) budding transition vesicles; (B) a starfishlike vesicle; (C) a "pregnant" vesicle.

vesicles might be examples of inside budding or entrapment. Vesicles with one hole have also been observed.

Starfishlike vesicles and inside or outside budding transitions are also observed in small molecule systems but only as a result of changing external conditions, such as temperature, osmotic pressure, pH, partial polymerization of a lipid vesicle by UV irradiation, etc.[33−35] In contrast to small molecule vesicles, the formation of the unusual shapes in the block copolymer vesicles described here does not require external perturbations. Although the detailed mechanism of the formation is not known at this stage, it may be related to the lower mobility of the high molecular weight system. Compared with small molecule amphiphiles, it is easier to trap intermediates of morphological transitions in the block copolymer amphiphiles.

3.5. Biomimetic Aspects. Many of the bilayer aggregates observed here are biomimetic. For example, tubules resemble biological structures such as microfilaments and microtubules of the cytoskeleton existing in eukaryotic cells; these structures are responsible for many cell movements and cell shapes.[37,38] Structures similar to the "plumber's nightmare" are often seen around the area in the membrane where a cell is splitting or fusing with another cell and are involved in regulating the activity and stability of cell membranes. Some of the block copolymer vesicles, e.g., outside or inside budding vesicles, are reminiscent of the process of exocytosis or endocytosis, respectively. The formation of LCVs involves membrane adhesion and fusion, which are essential in some cellular processes, such as material transport. A study of block copolymer bilayer aggregates, which allows the determination of the factors which regulate the self-assembly and morphogenesis, may help our understanding of self-organization in some biological systems.

3.6. Potential Applications. In view of the increased thermal stability of block copolymer aggregates relative to those of small molecule systems, the former might have some advantages in a range of existing applications, as well as a broader range of potential applications where their increased stability is utilized. A number of applications have been suggested for small molecule tubules and vesicles, most of which may also be applicable to block copolymer bilayer aggregates. For example,[9] tubules can be coated with metals or alloys and then be cast into a composite. Such a composite could have unique electric and magnetic properties which would make it useful in various technological applications, such as high-dielectric materials, magnetic materials, and microcathodes. In addition, tubules, as well as vesicles and LCVs, can also serve as controlled-release systems for engineering and biomedical applications. A study of these structures could help us develop advanced materials such as artificial tissues and soft biomaterials.[8d]

4. Conclusion

Bilayer aggregates, i.e., tubules, vesicles, LCVs, and lamellae, are made from PS-*b*-PEO block copolymers in solution and are directly observed by transmission electron microscopy. The aggregates produced by the addition of water to copolymer solutions in pure DMF at room temperature are mainly controlled by the copolymer composition. For example, tubules, vesicles and LCVs can be prepared by this method mostly from sample PS(240)-*b*-PEO(15). To prepare bilayers from the other two samples, i.e., PS(240)-*b*-PEO(45) and PS(240)-*b*-PEO(80), different preparation methods are applied, namely, the use of a water−DMF mixture instead of pure DMF, the addition of electrolytes, and the use of subambient aggregation temperatures. It is found that by using these alternate preparation methods, aggregates of various morphologies can be prepared from one block copolymer sample. By copolymer dissolution in a water−DMF mixture, all four types of bilayer aggregates can be prepared from each of the samples used in the study. Specifically for the preparation of tubules, it is seen that vesicles and tubules coexist if the annealing time is short, while only tubules appear upon long annealing time. The addition of electrolytes, such as KF, and the use subambient aggregation temperatures (0 °C), are useful for the preparation of LCVs. The use of subambient temperatures in the preparation of bilayer aggregates of block copolymers is new. All these preparation methods provide us with additional ways to "engineer" the bilayer aggregates.

The preparation of block copolymer tubules, "plumber's nightmare," inside or outside budding vesicles, and starfishlike vesicles is also novel. The bilayer aggregates are stable and are easy to observe. Due to the relatively low mobility of polymers, intermediates of self-assembly are easily trapped. Some of the trapped intermediates, such as the bent lamellae, may provide us with a deeper insight into the nature of the self-assembly. The bent lamellae are the most direct evidence yet obtained relating to the mechanisms of the formation of large vesicles, i.e., lamellae as precursors of large vesicles. Tubules with an oscillatory perturbation state in their diameters might be one of the intermediates of the vesicle to tubule transition. No chiral center is needed for the formation of tubules from these block copolymers.

Bilayer aggregates of block copolymer amphiphiles may have many potential applications, including drug delivery vehicles. A detailed study of morphogenesis, especially the biomimetic structures, may help our understanding of corresponding biological systems.

Acknowledgment. We thank The Natural Sciences and Engineering Research Council of Canada (NSERC) for financial support of this research. We are also indebted to L. Zhang, G. Yu, and J. Cox for useful discussions.

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MA971419L