http://dx.doi.org/10.5012/bkcs.2012.33.7.2187

A Facile Synthesis of Discoidal Lipid Bilayer Nanostructure by Association of a Cationic Amphiphilic Polyelectrolyte

Eun Chul Cho

Department of Chemical Engineering, Division of Chemical and Bioengineering, Hanyang University, Seoul 133-791, Korea
E-mail: enjoe@hanyang.ac.kr
Received March 5, 2012, Accepted March 28, 2012

This study presents a facile way synthesizing disc-like lipid bilyaer nanostructures with a cationic amphiphilic polyelectrolyte. The cationic amphiphilic polyelectrolyte was in a form of partially quarternized copolymer and was synthesized with 2-(dimethylamino)ethyl methacrylate and stearyl methacrylate. At some concentration ranges of the polymer, the addition of the polymer to lipid components during the preparation of bilayer nanostructures resulted in discs with a fairly high yield (~99%). The mechanism for the formation of the nanostructures was discussed based on the physical properties of these nanostructures and by comparing the nanostructures obtained with an anionic amphiphilic polyelectrolyte.

Key Words : Lipid bilayer nanostructures, Cationic amphiphilic polymer, Hollow hemisphere, Disc

Introduction

Liposomes, vesicular forms of lipid bilayer, have been used as carriers to efficiently deliver therapeutic agents into cells and skins.[1-6] The agents are mostly water-soluble, and water-insoluble agents can be also delivered from their association with lipid bilayer in the liposomes.[7,8] To extend their applications, liposomes modify their surfaces with genes[9,10] or polymer electrolytes through various processes such as a multilayer assembly.[11,12] However, for some applications, the shape of the lipid bilayers should not be necessarily spherical or vesicular: the morphology and surface chemistry of the lipid bilayers sometimes need to be tailored for their proper uses.

It has been known that disc-like nanostructures can be synthesized by mixing lipid components with some additives.[13] The additives include bile salts,[14] amphiphilic lipids having different charges in the headgroups,[15] proteins,[16,17] poly(2-ethyl acrylate) at some pHs,[18,19] and poly(ethylene glycol)-modified lipids,[20,21] and amphiphilic block copolymers.[22,23] Although this form is an intermediate produced during the transformation of micelle to vesicles,[24] the disc-like nanostructures could be stabilized by the additives and thus exist in a long period of time. The yields and the surface chemistries of the discs rely on the types of additives. However, it is still necessary to develop the disc-like nanostructures having various ligands on the surfaces for their various applications to biomedicine.

This study presents a facile method synthesizing disc-like lipid bilayer nanostructures. As depicted in Figure 1, the disc-like lipid bilayer nanostructures are obtainable by introducing a cationic amphiphilic polyelectrolyte during the preparation of lipid bilayers. Amphiphilic polymer electrolytes have been mostly introduced with lipid components not only to increase the stability of liposomes, but also to give the liposomes responsiveness to external stimuli such as temperature and pH.[25-27] From this study, it has been newly found that the vesicular shape of lipid bilayers can be successfully transformed into the disc-like nanostructures by introducing the cationic amphiphilic polyelectrolyte provid-

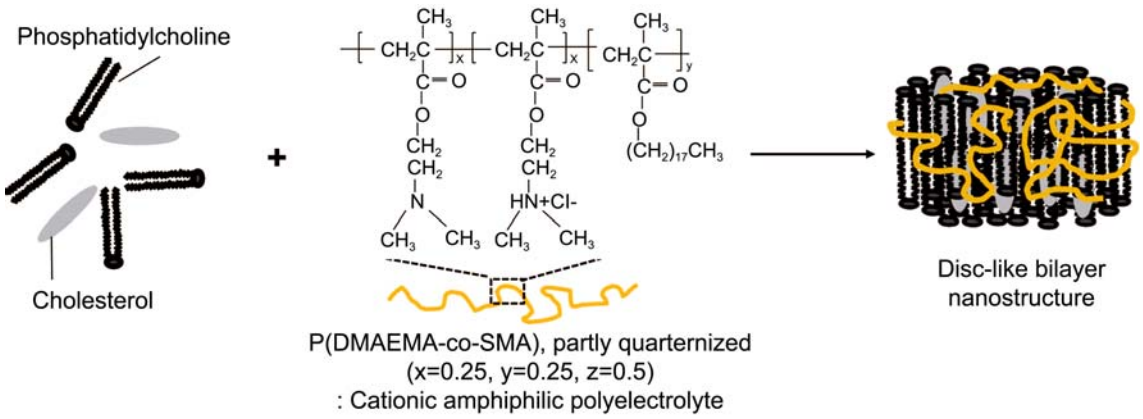


Figure 1. A schematic showing the synthesis of disc-like lipid bilayer nanostructures with a cationic amphiphilic polyeletrolyte, a partially quarternized poly(DMAEMA-co-SA).

ed that its charge densities and hydrophilic/hydrophobic balances were properly regulated. The mechanisms for the formation of the disc-like nanostructures are discussed on the basis of their physical properties and a comparative study with the lipid bilayer nanostructures with an anionic amphiphilic polyelectrolyte.

Experimental

Materials. 2-(Dimethylamino)ethyl methacrylate (DMAEMA), stearyl methacrylate (SA), methacrylic acid, hydrochloric acid (37%) aqueous solution, and Cholesterol (99%) were obtained from Sigma-Aldrich. Azobisisobutyronitrile (AIBN) was obtained from Wako Chemicals. Absolute ethanol, methanol, tetrahydrofuran (THF), and acetonitrile were obtained from Fisher Scientific. Phosphatidylcholine (PC, > 96% pure) was obtained from Lipoid GMBH, Germany.

Synthesis of a Cationic Amphiphilic Polyelectrolyte. All the chemical compounds were used as received. 20% (w/w in ethanol) of monomers comprising DMAEMA and SA, with a molar ratio of 1:1, and AIBN (1/100 mole relative to total mole of monomers) were solubilized in 200 mL of ethanol. Then the mixture was heated up to 75 °C to copolymerize the two monomers for 18 hrs under the flow of N_2 gas. The resulting polymer solution was transferred very slowly to the excess amount of methanol-deionized water (2/8 by v/v) mixture for harvesting. During the transfer, the co-solvent should be kept below 10 °C with excessive stirring. The polymer was re-solubilized in ethanol, and this purification process was conducted two more times. After purification, the polymer was dried in a vacuum oven at 60 °C. For the quartenization, 5 wt % of this tacky polymer was solubilized in THF, and HCl aqueous solution was dropped into the solution. The concentration of HCl was calculated in a way that the 50 mol % of the DMAEMA in the polymer should be quaternized. During the quarternization, the solution was kept stirring. After 2 h, the polymer was transferred to excess amount of acetonitrile for the precipitation of polymer. Then, the polymer was re-solubilized in ethanol and the solution was transferred again to excess amount of acetonitrile. The purification process was conducted one more time. The collected polymer was vacuum dried at 60 °C. The resulting polymer became glassy, different from the polymer before the quarternization.

Preparation of the Lipid Bilayer Nanostructures with the Cationic Amphiphilic Polyelectrolyte. The lipid bilayer nanostructures were next prepared by using the partially quaternized poly(DMAEMA-co-SA). PC and cholesterol were used as lipid components. The molar ratio of the PC and cholesterol was kept constant (4:1) for all the preparations to exclude the possibilities that a morphological change of bilayer was caused by the compositions of lipids. Table 1 shows the compositions for the lipid bilayer nanostructures in the present studies. Typically, the lipid components and the cationic amphiphilic polyelelctrolyte were co-solubilized in ethanol at 50 °C, and the solution was transferred to deionized water under homonization (with T.K. Homomixer Mark II, Takushu Kika Kogyo Ltd., Japan) at 7000 rpm. After 5 min, the mixture was going through a microfluidizer (Microfluidics Corp., USA), and the ethanol was finally eliminated by using a rotary evaporator. The concentration of the cationic amphiphilic polyelectrolyte was changed to investigate the concentration effects of the polymers on the shape of the lipid bilayer nanostructures. As a control experiment, a lipid bilayer was synthesized with only lipid components (without polymer). The preparation procedure was the same as above-described protocol.

Synthesis of an Anionic Amphiphilic Polyelectrolyte and the Lipid Bilayer Nanostructure Made with the Polymer and Lipid Components. For a comparative study, an anionic amphiphilic polyelectrolyte was also synthesized *via* a free radical polymerization of methacrylic acid and SA

Table 1. Compositions for the preparation of lipid bilayer nanostructures with the cationic amphiphilic polyelectrolyte synthesized in the present work

Polymer wt %	Composition (g)			Ethanol (mL)	DI water (mL)	Final concentration* (mg/mL)
	PC	Cholesterol	Polymer			
0	2.66	0.34	0	40	300	33
2.0	3.47	0.43	0.08	40	300	50
3.8	4.27	0.54	0.19	40	300	50
8.5	4.87	0.62	0.51	40	300	50

*Final concentration of lipid bilayer nanostructures after evaporation of ethanol.

Table 2. Compositions for the preparation of lipid bilayer nanostructures with poly(methacrylic acid-co-SA)

Polymer wt %	Composition (g)			Ethanol (ml)	DI water (mL)	Final concentration* (mg/mL)
	PC	Cholesterol	Polymer			
10	3.20	0.40	0.40	80	600	50
20	2.80	0.35	0.80	80	600	50
30	1.86	0.24	0.90	80	600	50

*Final concentration of lipid bilayer nanostructures after evaporation of ethanol.

in ethanol.[28] The molar ratio of methacrylic acid and SA was 7:3. For the synthesis of the lipid bilayer nanostructures, the molar ratio of PC:cholesterol is 4:1, identical to that for the nanostructures using the cationic lipid bilayer nanostructures. The method for the synthesis of the lipid bilayer nanostructure was the same as the method for the nanostructures with a cationic amphiphilic polyelectrolyte. Table 2 shows the compositions for the preparation of lipid bilayer nanostructures with the anionic amphiphilic polyelectrolyte.

Cryo-Transmission Electron Microscopy (TEM) Studies for the Morphologies of the Lipid Bilayer Nanostructures. The shape of the lipid bilayer nanostructures were observed from a cryo-TEM (Phillips TECNAI12). For this experiment, a drop of lipid nanostructure aqueous dispersion (about 7 μL) was placed on a grid with holes. The grid was then immediately plunged into liquid ethane. The frozen grids were stored in liquid nitrogen and transferred in a cryo-transfer (Gatan model 630, Gatan, Inc., Warrendale, PA) under liquid nitrogen at approximately −185 °C. Samples were observed at approximately −170 °C and the acceleration voltage of 120 kV, and the images were acquired with a Multiscan 600W CCD camera (Gatan, Inc., Warrendale, PA).

Microcalorimetry Studies on the Lipid Bilayer Nanostructures. The structures of the lipid bilayer nanostructures were investigated by using a microcalorimetry (VP-DSC, MicroCal, Northampton, MA, USA). 0.6 mL of a lipid bilayer nanostructure aqueous dispersion was filled into a sample cell, and an equal volume of deionized water was injected into a reference cell. The cells were closed tightly (25-30 psi) so that water could not evaporate during the experiment. After thermal equilibrium to 5 °C, the aqueous dispersions were scanned from 5 to 80 °C at a scanning rate of 1 °C/min. During the scan, the heat capacity difference between the sample cell and the reference cell was plotted as a function of temperature.

Anisotropy Ratios of the Lipid Bilayer Nanostructures. The fluidity of lipid bilayer nanostructures was estimated from steady state fluorescence anisotropy ratios according to previously described principles.[29] The lipid bilayer nanostructures were diluted in deionized water for the concentration of nanostructures being equal to 1 mg/mL. Next, 15 μL of 2 mM diphenyl hexatriene (Aldrich) in THF was added to the aqueous dispersion of diluted nanostructures, the mixture was sonicated for 30 min, and then the mixture was incubated overnight at 60 °C. Fluorescence emission spectra were measured at room temperature using a model F-4500 fluorescence spectrophotometer (Hitachi), with excitation of membrane-doped diphenyl hexatriene at 360 nm and emission at 430 nm. The slit widths of both the excitation and emission windows were 5 nm. The fluorescence intensity of the emitted light polarized parallel (I_{II}) and perpendicular ($I_{\perp}$) to the excited light was recorded, and the fluorescence anisotropy ratio (r) was calculated using Perrin's equation:

$$r = \frac{I_{II} - GI_{\perp}}{I_{II} + 2GI_{\perp}}$$

, where G is the instrumental correction factor.

Surface Charges of the Lipid Bilayer Nanostructures. The surface charges of lipid bilayer nanostructures were determined using photocorrelation spectroscopy (PCS; Malvern Instruments 3000HS) equipped with a zetapotential analyzer.

Results and Discussion

The present study first synthesized a new cationic amphiphilic polymer, suitable for producing the disc-like lipid bilayer nanostructures, *via* a free radical polymerization. Typically, two monomers, 2-(dimethylamino)ethyl methacrylate (DMAEMA) and stearyl methacrylate (SA) with a molar ratio of 1:1, and AIBN (initiator) were polymerized in ethanol. DMAEMA was selected as a hydrophilic part of polymer, and it was necessary to further quaternize a part of DMAEMA to effectively regulate the hydrophilicity of the poly(DMAEMA-co-SA). For this reason, after some purification procedures, approximately a half of the DMAEMA in the polymer was quarternized by the addition of HCl.

Putting this polymer together with lipid components, the optimal condition for the synthesis of the disc-like lipid bilayer nanostructures established (see the experimental section and Table 1). It was found that the feed molar ratio of DMAEMA to SA and the degree of quarternization played a critical role in the formation of disc-like bilayer nanostructures. When the feed molar ratio of DMAEMA and SA was 1:1, and all the DMAEMA were quartenized, the polymer was very soluble in water (the aqueous solution looked transparent). Increasing the concentration of DMAEMA also made the polymer very soluble in water even if a small part of DMAEMA was quarternized. When these polymers were introduced, even with a small amount, the lipids-polymer mixtures in water looked clear, indicating that the lipids were solubilized or in the form of tiny micelles (it was too small to measure the hydrodynamic sizes using a photocorrelation spectroscopy). From a trial and error, the optimal molar DMAEMA to SA ratio was found to be 1:1 (feed mole basis), and a half of the DMAEMA should be quarternized for the production of the disc-like lipid bilayer nanostructures other than vesicular forms. In addition, the concentration range of polymer was determined for the synthesis of disc-like bilayer nanostructures. After optimization, the aqueous mixture of lipid and polymer mostly looked bluish or light milky (depending on the concentration of the polymer).

Figure 2 shows cryo-transmission electron microscopy (cryo-TEM) images of the lipid bilayer nanostructures for different concentrations of the cationic amphiphilic polyelectrolyte. Without the polymer, the lipid bilayers had uni- or multi-vesicular nanostructures (Figure 2(a)), as found in most literatures. At 2.0 wt % of the polymer (Figure 2(b)), some vesicles began to deform, and the other vesicle had a line in the center. At 3.8 wt % (Figure 2(c)), interestingly, all of the vesicular bilayers were transformed into ellipsoids or rods. Similar image was obtained at 8.5 wt % of the polymer

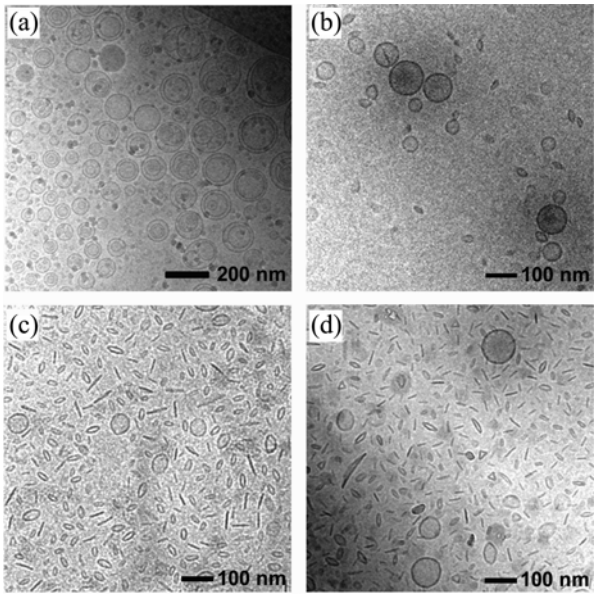
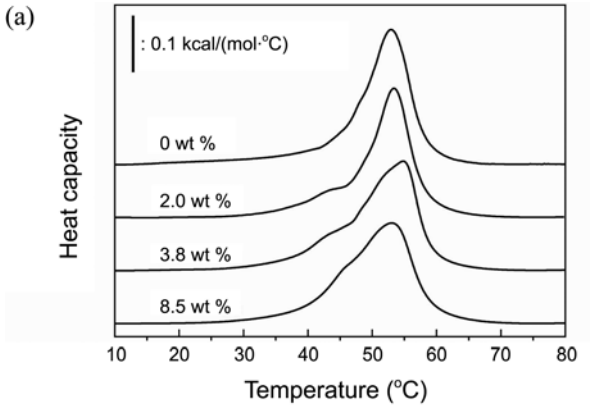


Figure 2. Cryo-TEM images of (a) PC-cholesterol bilayer nanostructures, and lipid nanostructures made with (b) 2.0 wt %, (c) 3.8 wt %, and (d) 8.5 wt % of the cationic amphiphilic polyelectrolyte.



(b)

Concentration of polymer in the nanostructures (wt %)	Anisotropy ratios	Zetapotentials (mV)
0	0.375	-0.9
2.0	0.371	+9.6
3.8	0.373	+20.8
8.5	0.375	+34.7

Figure 3. (a) Microcalorimetry thermograms and (b) aniotropic ratios and zetapotentials of the lipid bilayer nanostructures made with the cationic amphiphilic polyelectrolyte for their different concentrations.

(Figure 2(d)). From the image analysis (based on the numbers), 99% of the lipid bilayer nanostructures were rods or ellipsoids and only 1% of the bilayers are the vesicular shape for the polymer concentrations of 3.8 and 8.5 wt %. There was no clear dependence of the concentration on the percentage of ellipsoids and rods at the latter two concentrations. The diameters or lengths (for ellipsoids, length of long axis) of the nanostructures were changed with the polymer concentration. Without the polymer, the diameter of the vesicles were 105 ± 33 nm. For 3.8 wt %, the sizes of ellipsoids and rods were 46 ± 11 and 45 ± 11 nm, respectively. Meanwhile, for 8.5 wt%, the sizes of ellipsoids and rods were decreased to 37 ± 16 and 36 ± 5 nm, respectively. Within the same polymer concentration, there was essentially no difference in size between the rods and ellipsoids. When the polymer concentration was further increased (*e.g.*, > 10 wt %), the aqueous mixture of polymer and lipid became quite transparent from the bluish or milky appearance at low concentrations, indicating the nanostructures would be in the form of micelles, as Johnsson and Edwards reported.[20]

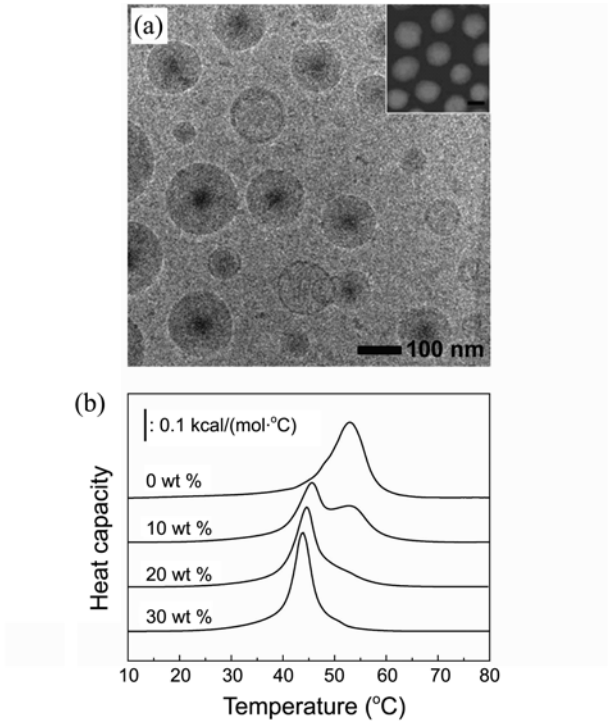
Johnsson and Edwards reported lipid bilayer discs by mixing poly(ethylene glycol)-grafted lipid with phosphatidylcholine.[20] At some concentrations, the vesicular form of the lipid bilayers was transformed into the disc-like bilayers. In their cryo-TEM studies, they commented that the disc-like bilayer nanostructures were shown as rods or ellipsoidal shapes. The different morphologies were attributable to the configurations of the discs on a cryo-TEM sample grid: for the edge-on viewpoint, the discs looked rods whereas they looked ellipsoids for the face-on viewpoint. From their interpretation, it was suggested that the rods and ellipsoidal shapes of the lipid bilayer nanostructures from the present study came from discs. As such, with 3.8 and 8.5 wt % of the cationic amphiphilic polyelectrolyte, it could be said that the vesicular forms of the lipid bilayers were successfully transformed into the disc-like bilayer nanostructures.

To understand the formation and structure of the disc-like lipid bilayer nanostructures, the physical properties of the lipid bilayers nanostructures were further investigated. Figure 3(a) shows microcalorimetry thermograms of the bilayer nanostructures constructed with the cationic amphiphilic polyelectrolyte for its various concentrations. Without the polymer, the gel to fluid transition temperature of vesicles was displayed around 53 °C. At 2.0 wt %, a shoulder appeared in the thermogram around 43-45 °C, and this shoulder became prominent with the concentration of the polymer. However, the two peaks were not clearly resolved: instead, there showed the broadening of the peak. The broadening of the peak might indicate that there were various lipid bilayers differently associated with the cationic amphiphilic polyelectrolyte. In the meantime, the peak position at 53-54 °C, typical of pure lipid bilayers, was more or less the same regardless of concentrations. The results suggested the discs had a pure lipid bilayer domain together with other lipid bilayer domains mixed with the cationic amphiphilic polyelectrolyte. In addition, it can be also said that the domain of lipid bilayers without the polymer was still dominant even after the complete conversion of vesicles into the discs. Meanwhile, the membraine fluidities of the bilayer nanostructures, expressed as anisotropy ratios of the bilayers (Figure 3(b)),[29] were essentially the same regardless of the cationic amphiphilic polyelectrolyte. This indicates that the addition of the cationic amphiphilic polyelectrolyte did not significantly affect the mobilities of the bilayer

nanostructures. As for the surface charges, the bilayer nanostructures were changing their surface charges very sharply with increasing the concentration of the polymer, indicating that the cationic amphiphilic polyelectrolyte preferentially covers the surface of the bilayer nanostructures even at low concentration.

It was suggested that discs of bilayers can be produced as intermediates during the preparation of vesicles in various conditions.[24] That is to say, these forms are quite unstable so that they are eventually transformed rapidly into the vesicular forms. On the other hand, Zemb *et al.* reported that the disc-like bilayers could be stably constructed with increasing the concentration of a positively charged lipid.[15] In their studies, the positively charged lipid was more concentrated in the outer parts of the disc, thereby stabilizing the highly curved edges of their lipid bilayers. Similar nanostructures were recently found by the proper combination of scaffold proteins and lipid components by stabilizing the lipid bilayers with the proteins in the outside.[16,17] The stabilization of the bilayer discs can be also achieved by covering the outer surface of the lipid bilayers with poly(ethylene glycol)-grafted lipids[20,21] or amphiphilic block copolymers.[22,23] Similarly, the current study demonstrated that the addition of the novel cationic amphiphilic polyelectrolyte also offered the lipid components a chance to stay in the form of disc by mostly covering the outside of the lipid bilayers.

One intriguing question in the present study is how the current cationic amphiphilic polyelectrolyte can induce and stabilize disc-like forms of lipid bilayers. To explore this problem, the morphologies and physical properties obtained in this work were compared with those obtained from the lipid bilayer nanostructures with poly(methacrylic acid-co-SA), an anionic amphiphilic polyelectrolyte (Figure 4). For this case, all the lipid bilayers were in the form of unilamellar vesicles (Figure 4(a)). In addition, within the polymers we tested, there was no indication of transformation of vesicles into discs, as in the case of the lipid bilayers with the cationic amphiphilic polyelectrolyte, even we increased the hydrophilicity (*e.g.*, by increasing the concentration of methacrylic acid) of the anionic amphiphilic polyelectrolyte. Moreover, the microcalorimetry thermograms and anisotropy ratios for the lipid bilayer nanostrcutres were quite different from those obtained with the cationic amphiphilic polyelectrolyte (Figure 4(b) and (c); also see the discussion in the supplementary material). A new peak at 45 °C was clearly developed and it was separated from the peak of pure lipid bilayer at low concentrations (10 wt %) of the anionic amphiphilic polyelectrolyte. In addition, the intensity of the new peak became increased with the polymer concentration while the pure lipid bilayer peak disappeared. Membrane fluidities were much increased at 10 wt % (anisotropy ratio was decreased; the decrease in the anisotropy ratio was also observed even at 5 wt % of the polymer). Putting together, it can be said that the way of association of the anionic amphiphilic polyelectrolyte with lipid bilayers seemed different from the way with the present cationic amphiphilic polyelectrolyte. When a stearyl group in a polymer is well-



(c)

Polymer used	Concentration of polymer (wt %)	Anisotropy ratios	Zetapotentials (mV)
None		0.375	-0.9
Poly(methacrylic acid-co-SA)	10	0.357	-13.4
	20	0.344	-18
	30	0.343	-27

Figure 4. (a) A cryo-TEM image of the lipid bilayer nanostructures made with the lipid components and poly(methacrylic acid-co-SA), an anionic amphiphilic polyelectrolyte. The concentration of the polymer was 30 wt % in the nanostructures. Inset shows the conventional TEM image of this nanostructure by using a conventional TEM (H-7600, Hitachi) after staining the sample with 1% phosphotungstic acid aqueous solution. The scale bar was 100 nm. (b) Microcalorimetry thermograms of the lipid bilayer nansotructures made with lipid components and poly(methacrylic acid-co-SA) for the different concentrations of the polymer. (c) The anisotropy ratios and surface charges of the lipid bilayer nanostructures made with lipid components and the anionic amphiphilic polyelectrolytes. For all the samples, the molar ratio of PC:cholesterol of the lipid bilayer nanostructures were 4:1.

associated with the bilayer components, as in the case of poly(methacrylic acid-co-SA), more stable vesicles were formed with a sharp and new gel to fluid transition temperature and with different membrane fluidities. In contrast, in the present study, it is thought that there was a poor association between the stearyl group in the cationic amphiphilic polyelectrolyte and the lipid bilayer, resulting in the broad peak in the thermograms and no change in the fluidity. From the cryo-TEM images, it might be that such a poor association of the polymer broke the vesicles into the discs, and these bilayer nanostructures could be further stabilized by the cationic amphiphilic polyelectrolyte. In fact, the conclusion seemed similar to the work by Tirrell and co-workers who made the discoidal lipid nanostructures in poly(2-ethyl

acrylic acid) aqueous solution at low pHs.[14,15] In their studies, the disc type of bilayers were obtained by first binding the alkyl groups at low pHs, followed by the buckling or rupture of lipid layers. Since the polymer has very short alkyl groups, the association of the alkyl group with the lipid components would be poor, thereby triggering the transformation of the vesicles. For the same reason, it is possible to successfully synthesize the shape of the disc-like bilayer nanostructures with the present cationic amphiphilic polyelectrolyte.

Conclusions

In conclusion, the present study suggests a novel and facile way for synthesizing disc-like lipid bilayer nanostructures by introducing the positively charged amphiphilic polyelectrolyte. The nanostructures could be synthesized in fairly high yields (~99%) by properly adding the polymer presented in this work to the lipid components during the preparation of lipid structures. While most discoidal bilayer nanostructures with polymers had neutral or negative charges,[18-23] the present disk-like nanostructures have the positive charge in their surfaces. Therefore, it may be suggested that the current discoidal lipid bilayer nanostructures be more useful in gene delivery system than the other type of discoidal bilayer nanostructures. Especially, disk-like gene carriers would be expected to deliver differently from the spherical gene carriers because nanostructures having different shapes with their surface ligands being regulated showed different delivery mechanism to cell membranes,[30-32] In this sense, this research give an important insight to those who wish to design a lipid nanostructure for their numerous applications such as to biomedicines and template-based multilayer assemblies.

Acknowledgments. This work is supported by the startup grant from Hanyang University (201100000000226).

Supplementary Material. Supplementary Table and discussion associated with this article can be found in the supplementary material.

References

1. Allen, T. M.; Cullis, P. R. *Science* **2004**, *303*, 1818.
2. Lian, T.; Ho, R. J. Y. *J. Pharm. Sci.* **2001**, *90*, 667.
3. Gregoriadis, G. *Trends in Biotechnol.* **1995**, *13*, 527.
4. Schreier, H.; Bouwstra, J. *J. Control. Rel.* **1994**, *30*, 1.
5. Marin, A.; Sun, H.; Husseini, G. A.; Pitt, W. G.; Christensen, D. A.; Rapoport, N. Y. *J. Control. Rel.* **2002**, *84*, 39.
6. Mei, Z.; Chen, H.; Weng, T.; Yang, Y.; Yang, X. *Eur. J. Pharm. Biopharm.* **2003**, *56*, 189.
7. Mason, R. P.; Rhodes, D. G.; Herbette, L. G. *J. Med. Chem.* **1991**, *34*, 869.
8. Gaede, H. C.; Gawrisch, K. *Biophy. J.* **2003**, *85*, 1734.
9. El-Aneed, A. *J. Control. Rel.* **2004**, *94*, 1.
10. Mahato, R. I.; Tomlinson, E. *Pharm. Res.* **1997**, *14*, 853.
11. Haidar, Z. S.; Hamdy, R. C.; Tabrizian, M. *Biomaterials* **2008**, *29*, 1207.
12. Katagiri, K.; Hamasaki, R.; Ariga, K.; Kikuchi, J.-I. *Langmuir* **2002**, *18*, 6709.
13. Carmona-Ribeiro, A. M. *Current Med. Chem.* **2006**, *13*, 1359.
14. Mazer, N. A.; Benedek, G. B.; Carey, M. C. *Biochemistry* **1980**, *19*, 601.
15. Zemb, Th.; Dubois, M.; Deme, B.; Gulik-Krzywicki, Th. *Science* **1999**, *283*, 816.
16. Skar-Gislinge, N.; Simonsen, J. B.; Mortensen, K.; Feidenhans'l, R.; Sligar, S. G.; Møller, B. L.; Bjørnholm, T.; Arleth, L. *J. Am. Chem. Soc.* **2010**, *132*, 13713.
17. Shih, A. Y.; Freddolino, P. L.; Sligar, S. G.; Schulten, K. *Nano Lett.* **2007**, *7*, 1692.
18. Borden, K. A.; Eum, K. M.; Langley, K. H.; Tan, J. S.; Tirrell, D. A.; Voycheck, C. L. *Macromolecules* **1988**, *21*, 2649.
19. Thomas, J. L.; Tirrell, D. A. *Acc. Chem. Res.* **1992**, *25*, 336.
20. Johnsson, M.; Edwards, K. *Biophy. J.* **2003**, *85*, 3839.
21. Sandstrom, M. C.; Johansson, E.; Edwards, K. *Langmuir* **2007**, *23*, 4192.
22. Johnsson, M.; Silvander, M.; Karlsson, G.; Edwards, K. *Langmuir* **1999**, *15*, 6314.
23. Rangelov, S.; Edwards, K.; Almgren, M.; Karlsson, G. *Langmuir* **2003**, *19*, 172.
24. Lasic, D. D. *Biochem. J.* **1988**, *256*, 1.
25. Torchilin, V. P.; Trubetskoy, V. S. *Adv. Drug Deliv. Rev.* **1995**, *16*, 141.
26. Zignani, M.; Drummond, D. C.; Meyer, O.; Hong, K.; Leroux, J.-C. *Biochim. Biophys. Acta* **2000**, *1463*, 383.
27. Polozova, A.; Winnik, F. M. *Langmuir* **1999**, *15*, 4222.
28. Lim, H. J.; Cho, E. C.; Kim, J.; Chang, I.-S. *Colloids Surf. A* **2007**, *294*, 71.
29. Bernsdorff, C.; Winter, R. *J. Phys. Chem. B* **2003**, *107*, 10658.
30. Chithrani, B. D.; Ghazani A. A.; Chan, W. C. W. *Nano Lett.* **2006**, *6*, 662.
31. Leroueil, P. R.; Hong, S.; Mecke, A.; Baker, J. R., Jr.; Orr, B. G.; Holl, M. M. B. *Acc. Chem. Res.* **2007**, *40*, 335.
32. Verma, A.; Uzun, O.; Hu, Y.; Hu, Y.; Han, H.-S.; Watson, N.; Chen, S.; Irvine, D. J.; Stellacci, F. *Nature Mater.* **2008**, *7*, 588.