 DOI: 10.1002/masy.200950717

pH- and Ionic-Strength-Induced Structural Changes in Poly(acrylic acid)-Lipid-Based Self-Assembled Materials

Anthony Crisci,[1,4] *Daniel N. T. Hay,*[1,3] *Sönke Seifert,*[2] *Millicent A. Firestone**[1]

Summary: The effect of a polyanion introduced as a lipid conjugate (poly(acrylic acid)-dimyristoyl-*sn*-glycero-3-phosphoethanolamine, PAA-DMPE) on the structure of a self-assembled, biomembrane mimetic has been evaluated using synchrotron small-angle X-ray scattering (SAXS). At high grafting density (8–11 mol.%), the PAA chains were found to produce significant changes in structure in response to changes in pH and electrolyte composition. At low pH and in the absence of salt (NaCl), the neutral PAA chains adopt a coil conformational state that leads to the formation of a swollen lamellar structure. Upon the addition of salt at low to intermediate pH values, two lamellar phases, a collapsed and an expanded structure, coexist. Finally, when the polymer is fully ionized (at high pH), the extended conformation of the polymer generates a cubic phase. The results of this study contribute to an understanding of how polyelectrolytes may ultimately be harnessed for the preparation of self-assembling materials responsive to external stimuli.

Keywords: poly(acrylic acid)-lipid conjugate; polyelectrolytes; self-assembly; small-angle X-ray scattering (SAXS)

Introduction

Polyelectrolytes, which possess ionizable functional groups on their backbone or side chains, are abundant in nature (e.g., DNA), often serving essential biological functions.[1] In addition, polyelectrolytes are well-established in many areas of practical application, including drug delivery, consumer products and industrial processing.[1] The macromolecular conformation of a polyelectrolyte can be altered by changes in pH, ionic strength or temperature, thus providing the basis for the development of stimulus-responsive materials. The control of the architecture and/or physical properties of colloidal materials (e.g., microemulsions, liposomes) by the addition of polyelectrolytes has been demonstrated as a means to prepare smart or stimuli-responsive materials.[2–7] Due to the complexity of interactions, however, considerable work is required to fully harness polyelectrolytes for predictable structural or functional regulation of materials. Commonly, the polyion is introduced to the colloid as a homopolymer.[2,5,6] An alterative approach is to incorporate a hydrophobe with the polyion, thereby promoting insertion and anchoring of the polymer to the colloid.[3,4,8]

In this report, we describe the synthesis of a weak polyacid-lipid conjugate, poly(acrylic acid) (PAA) - 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE), and determine how it incorporates into a lipid mesophase and influences its self-assembled structure. Prior work in our laboratory has demonstrated the potential

[1] Materials Science Division, Argonne National Laboratory, Argonne, IL 60439, USA
Fax: 001630-252-9151; E-mail: firestone@anl.gov
[2] X-ray Sciences Division, Argonne National Laboratory, Argonne, IL 60439, USA
[3] Current address: Nalco Chemical Co., Naperville, IL, USA
[4] Current address: Department of Chemistry, University of California, Santa Barbara, CA, USA

of polymer-lipid-based self-assembled materials as stimuli-responsive nanostructures and as model biological systems.[9–12] Our studies of these materials, which comprise ternary mixtures of a saturated phospholipid, a co-surfactant, and a polymer (commonly, a nonionic polymer such as poly(ethylene glycol), PEG, or poly(*N*-isopropylacrylamide), PNIPAM) dispersed in water, have shown that the polymer is the key component for tuning the physical and structural properties of the material.[9–13] Herein we evaluate how replacement of the nonionic polymer with a polyelectrolyte alters the structure of these materials. This work is important not only for the development of new classes of pH-responsive, self-assembled nanostructured materials but also (on a more fundamental level) in advancing our understanding of how macromolecules associate with lipid bilayers such as biological membranes. Such understanding may ultimately lead to their use as therapeutic agents for the treatment of a variety of diseases and injuries.

Materials and Methods

Materials

Carboxy-terminated poly(*tert*-butyl acrylate), PtBuACOOH, ($M_n = 4200$, $M_n/M_w = 1.25$, functionality = 0.95, Polymer Source Inc., Dorval, Quebec) was dried at 25 °C under vacuum overnight prior to use. N-hydroxysuccinimide, NHS (97%), 1,3-dicyclohexylcarbodiimide, DCC (99%), triethylamine, Et_3N (99.5%), methanol, MeOH (anhydrous), toluene (99.5%), activated alumina, Al_2O_3 (neutral, Brockmann I, *ca.* 150 mesh), and trifluoroacetic acid, TFA (≥99.0%), were used as received from Sigma-Aldrich (Milwaukee, WI). Diethyl ether, Et_2O (anhydrous) was used as received from Fisher Scientific (Pittsburgh, PA). Chloroform, $CHCl_3$ (99.8%, Aldrich) was freshly distilled from CaH_2 under argon atmosphere prior to use. DMPE was purchased from Avanti Polar Lipids (Alabaster, AL) and used as received. Synthetic procedures were carried out using standard Schlenk techniques.

Synthesis and Purification of PtBuACOOH-DMPE Conjugate (1)

PtBuACOOH (669 mg, 0.159 mmol) and NHS (24.3 mg, 0.211 mmol) were combined in a 100-mL two-neck round-bottom flask equipped with a magnetic stir-bar and a gas inlet valve. The flask was purged with argon and $CHCl_3$ was added, via cannula, dissolving the solids. The solution was cooled in an ice bath and DCC (42.9 mg, 0.208 mmol), dissolved in $CHCl_3$, was added via cannula. The reaction was kept on ice for an additional two hours, then warmed to room temperature and stirred for four days. The resulting clear colorless solution was taken to dryness under vacuum. The dried solid was treated with *ca.* 15 mL of toluene, forming a cloudy white mixture which was transferred to a glass centrifuge tube. The solid was separated by centrifugation; the supernatant was decanted and filtered through a 0.45 μm filter. The resulting clear/colorless toluene solution was added to a two-neck 100-mL round-bottom flask equipped with a magnetic stir bar and a gas inlet valve. Toluene was removed under vacuum. DMPE (102 mg, 0.160 mmol) was added to the reaction flask which was then purged with argon. Chloroform was added, via cannula, forming a cloudy white mixture. To the stirring reaction mixture Et_3N (*ca.* 45 μL, 32.7 mg, 0.323 mmol) was added via syringe and the reaction was heated at 35 °C for 11 h. The resulting clear solution was taken to dryness under vacuum, affording an off-white solid. The resulting dried product was dissolved in 1:1 (v/v) toluene/$CHCl_3$ and purified via Al_2O_3 column chromatography. The column was eluted with 25 mL (*ca.* 1 column volume) of 1:1 (v/v) toluene/$CHCl_3$, followed by 25 mL of $CHCl_3$, 25 mL of 1:1 (v/v) $CHCl_3$/MeOH, and finally with 25 mL of MeOH. Throughout the run, 2–3 mL fractions were collected and visualized via TLC (Al_2O_3, ethyl acetate/CH_2Cl_2). Collected fractions containing a single component (by TLC) were combined and the solvent was removed

under a stream of argon, then under vacuum via freeze-drying. The conjugate (269 mg, 34% yield based on PtBuA-COOH) was isolated from the 1:1 (v/v) $CHCl_3$/MeOH fractions as a white solid.

Synthesis and Purification of PAA-DMPE Conjugate (2)

In an argon-purged glove bag, 1 (269 mg, 0.0547 mmol, *ca.* 1.8 mmol *t*-Bu, based on 32 monomer units) was treated with ca. 1 mL of TFA (*ca.* 13 mmol). The solid dissolved over several minutes to form a clear, off-white solution. The solution was allowed to stand for 30–45 min with occasional manual agitation. Diethyl ether was then added to the solution, resulting in the formation of a white precipitate. The mixture was vortex-mixed, then centrifuged to separate the solid. The supernatant was decanted and the solid re-suspended in fresh Et_2O. The solid was isolated by centrifugation/decantation and residual bulk Et_2O was removed under vacuum. The resulting solid was dissolved in *ca.* 2 mL of methanol, forming a clear colorless solution. This solution was taken to dryness under a stream of argon, then under vacuum via freeze-drying yielding a white solid (173 mg, 64.4% recovery, compared with 64.7% recovery of PAA from P*t*BuA-COOH under similar conditions). ^{31}P NMR: δ 1.47 ppm; ^{1}H NMR δ 0.90 ppm (*t*, CH_3 lipid), 1.29 ppm (s, CH_2 lipid), 2.31 ppm (C-H polymer protons). ATR FT-IR: 1550 cm^{-1} (amide II, C-N-H stretch-bend mode), 1050 cm^{-1} (P-O-C or P-O stretch of the phosphate group).[14]

Complex Fluid Preparation

The quaternary compositions consisted of 0.674 ± 0.084 weight fraction of water (Φ_w), $\Phi_s = 0.0181 \pm 0.0022$ co-surfactant (LDAO), $\Phi_L = 0.213 \pm 0.080$ lipid (DMPC) and $\Phi_C = 0.0949 \pm 0.0091$ PAA_{32}-DMPE conjugate, yielding a polymer-lipid conjugate concentration of between 8–11 mol.%. Hydration of the solid components was accomplished by repeated cycles of heating (45–60 °C), vortex-mixing and cooling on an ice bath until thorough mixing was achieved.

Physical Methods

Proton-decoupled ^{31}P NMR spectra were obtained using a Varian Unity INOVA spectrometer on a 300 MHz magnet equipped with a NALORAC broad band Z-spec probe. All spectra were referenced to 85% H_3PO_4 (aq). ^{1}H NMR spectra were obtained using a Bruker Avance DMX spectrometer on a 500-MHz magnet equipped with a 10-mm BBO probe employing 5-mm sample tubes. FT-IR spectra were obtained using a Bruker Vertex 70 spectrometer run in ATR mode with a Pike ATR accessory. The spectral resolution was set at 4 cm-1 and averaged over 32 scans.

Small-angle X-ray scattering (SAXS) measurements were made using the instrument at undulator beamline 12ID-C of the Advanced Photon Source at Argonne National Laboratory. The sample-to-detector distance was such as to provide a detection range for momentum transfer of $0.025 < q < 0.6$ $Å^{-1}$. The scattering vector, q, was calibrated using a silver behenate standard at $q = 1.076$ $Å^{-1}$. The 2-D scattering images were first corrected for spatial distortion and detector sensitivity, then radially averaged to produce plots of scattered intensity, $I(q)$, versus scattering vector, q. The value of q is proportional to the inverse of the length scale, $Å^{-1}$. Samples were sealed in 1.5 mm quartz capillaries. Temperature control of samples was achieved using a custom-built Peltier cooler.

Results and Discussion

In this work, we first synthesized an anionic polymer-lipid conjugate of poly(acrylic acid) (PAA) and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) and then examined its insertion into lipid bilayers. Specifically, the conjugate 2 was prepared using the multi-step procedure outlined in Figure 1.[8] The terminally-grafted polymer-lipid conjugate was prepared using a carboxy-terminated poly(*tert*-butyl acrylate). The *tert*-butyl groups serve

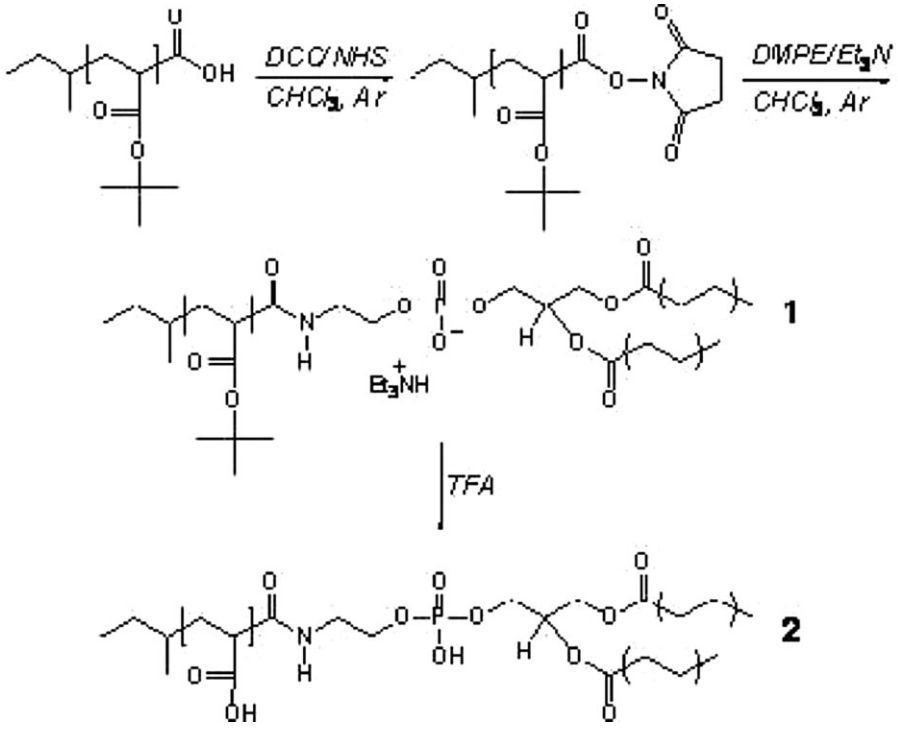

Figure 1.
Synthetic scheme outlining the procedure used to prepare the PAA-DMPE conjugate used in this study.

to protect the backbone of the polymer and left exposed a single attachment site for amide bond coupling to the lipid. A similar procedure has been previously demonstrated to provide a facile means of preparing a PNIPAM-DMPE conjugate.[11] First, dicyclohexylcarbodiimide, DCC, was used to prepare the active ester of the terminal carboxyl group of poly(*tert*-butyl acrylate) using *N*-hydroxysuccinimide, NHS. The isolated and purified NHS-ester of poly(*tert*-butyl acrylate) was then conjugated to the primary amine of the phospholipid, DMPE, via amide bond formation. The resulting conjugate was purified using column chromatography on alumina. Once the poly(*tert*-butyl acrylate)-DMPE conjugate 1 was prepared, the *tert*-butyl protecting groups were easily removed to yield a poly(acrylic acid)-DMPE conjugate 2. Cleavage of the *tert*-butyl groups using trifluoroacetic acid (TFA) afforded the PAA-DMPE conjugate. The conjugate was recovered by precipitation from diethyl ether.

PAA-lipid Conjugate Mesophase Structure

Polyelectrolyte-lipid conjugate-based complex fluids were prepared as mixtures of poly(acrylic acid) - 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (PAA_{32}-DMPE), a saturated phospholipid (dimyristoylphosphatidycholine, DMPC), and a zwitterionic co-surfactant (*N,N*-dimethyldodecylamine *N*-oxide) in water. The compositions are similar to those previously described but employed a variety of nonionic polymers (PEGylated lipids or PEO-based block copolymers) that have been shown to self-assemble into temperature-responsive nanostructures.[9,10] In these compositions, the ionization state of the polyelectrolyte was controlled by using a series of aqueous buffers of varying pH and NaCl concentrations. All of the quaternary mixtures prepared in this work

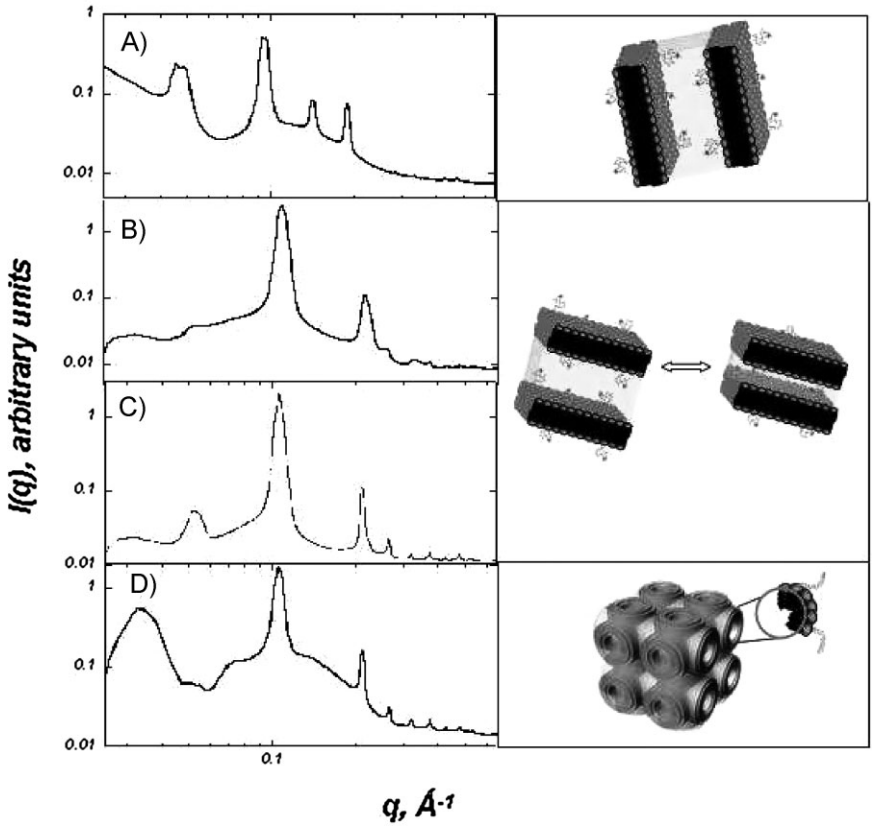

Figure 2.
Synchrotron small-angle X-ray scattering profiles collected at 25 °C for quaternary mixtures consisting of (A) a composition prepared with a strong acid, 0.1 M HCl, 0.758 water weight fraction, Φ_w; 0.133 DMPC weight fraction, Φ_L; 0.0888 PAA-DMPE conjugate weight fraction, Φ_p; and 0.0204 LDAO surfactant weight fraction, Φ_s. Schematic illustration of PAA-lipid brush induced formation of a swollen lamellar structure (B) a composition prepared with a weak acid, citrate 100 mM NaCl pH 3, 0.649 water weight fraction, Φ_w; 0.242 DMPC weight fraction, Φ_L; 0.0915 PAA-DMPE conjugate weight fraction, Φ_p; and 0.0175 LDAO surfactant weight fraction, Φ_s. Schematic illustration of two types of lamellar structures that coexistence under these experimental conditions. PAA-lipid brush induced formation of a swollen lamellar structure (C) a composition prepared at neutral pH, 20 mM Hepes, 100 mM NaCl pH 6.8, 0.649 water weight fraction, Φ_w; 0.238 DMPC weight fraction, Φ_L; 0.0952 PAA-DMPE conjugate weight fraction, Φ_p; and 0.0175 LDAO surfactant weight fraction, Φ_s. (D) a sample prepared employing a weak base, 10 mM sodium carbonate, 100 mM NaCl pH 9.8, 0.641 water weight fraction, Φ_w; 0.238 DMPC weight fraction, Φ_L; 0.104 PAA-DMPE conjugate weight fraction, Φ_p; and 0.0173 LDAO surfactant weight fraction, Φ_s. Schematic illustration of PAA-lipid uncoiled induced formation of a 3-D cubic structure.

were found to be milky white physical gels at room temperature and low-viscosity liquids when cooled on ice. Heating of the mixtures to ca. 50 °C yielded only a slight improvement in sample uniformity and optical transparency. The effect of pH and salt concentration on the self-assembled structures of the PAA-lipid-based mixtures (at 26 °C) was determined using small-angle X-ray scattering (SAXS), and representative scattering patterns are shown in Figure 2. The SAXS pattern collected on a composition prepared with a strong acid (0.1 M HCl) shows eight diffraction peaks positioned at $q = 0.0457$, 0.0953, 0.143, 0.191, 0.285, 0.333, 0.431, 0.447 Å^{-1} (Figure 2A). The diffraction peaks occur at integral order spacing with

respect to the first order peak ($q = 0.0457$ Å^{-1}) and are thus assigned to a multi-lamellar structure with a *d*-spacing of 137 Å. The repeat distance, *d*, indicates that the structure features a large interstitial water layer separating the lamellae. The formation of a ''swollen'' or expanded lamellar structure, such as the one observed here, is believed to arise from the covalently-grafted acrylic acid chains adopting a coiled conformation. Prior work on similar compositions employing a variety of nonionic polymers has shown that both the polymer grafting density and conformational state are important factors regulating the self-assembled mesophase architectures.[15] Moreover, studies employing PEGylated lipid conjugates have shown that when the PEG chains are in a brush state (i.e., polymer at high grafting densities), they produce sufficient steric repulsion between opposing bilayers to induce an increased water layer thickness, and an expansion of the lamellar structure.[15–17] These findings are consistent with prior work examining the conformational state of PAA in solutions at low pH (i.e., below the pK_a) and no salt, which has shown that when the polymer is neutral (fully protonated), the polymer chains adopt a coiled conformation.[18] In addition, it is further noted that the protonated PAA chain may be hydrogen bonding to the phosphodiester group of the lipids, leading to strong interaction/association with the membrane.[2,7] Thus, a grafted neutral polymer in a coiled conformational state with enhanced interactions with the lipid headgroups represent conditions in which PAA behaves similarly to grafted nonionic polymers, producing sufficient steric repulsion between lamellae to yield a swollen lamellar structure.

Compositions prepared under acidic conditions but with intermediate salt concentrations (20 mM sodium citrate; 100 mM NaCl; pH 3.8 buffer) exhibit a SAXS pattern (at 26 °C) with diffraction peaks positioned at $q = 0.0522, 0.108, 0.217, 0.263, 0.326, 0.373$ Å^{-1} (Figure 2B). The increased intensity of the diffraction peaks located at $q = 0.108$ and 0.217 Å^{-1} relative to the other reflections suggests the possible existence of two phases under these experimental conditions. The majority phase is a lamellar structure featuring a significantly reduced *d*-spacing of 58 Å, a distance comparable to that of aqueous dispersion of pure lipid in water. In addition, a minority component with the (001), (002), (004) and (005) reflections positioned at $q = 0.522$, 0.108, 0.217, 0.263 Å^{-1} is observed and indicates the coexistence of an expanded lamellar structure with a larger *d*-spacing of 120 Å. The coexistence of two (a collapsed and swollen) lamellar phases has been noted previously in other self-assembled mixed ionic and zwitterionic amphiphilic systems.[19,20] Furthermore, the work of Macdonald and coworkers has shown that polyelectrolytes can induce domain formation (i.e., regions enriched with a particular component) in lipid bilayer membranes.[21] Thus, it is possible that under these conditions, the swollen lamellar component (with the 120 Å repeat distance) contains the PAA. Here, however, the grafted polymer must be more tightly coiled and associated with the lipid headgroup region than under conditions of strong acid and no salt (Figure 2A). Prior studies have found that grafted PAA chains in the pH 3.5–5.0 region with 0.1 M electrolyte have an average degree of dissociation, α, on the order of 0.1–0.3, a regime where electrostatic screening is strong.[22] Under these conditions, the grafted PAA is said to be in the ''salted brush'' state, generating a decrease in polymer thickness.[22] This decrease would reduce the steric repulsion between opposing lamellae, thereby decreasing the water layer dimension and, accordingly, the interlamellar repeat distance in the swollen lamellar phase.

Compositions prepared with the aqueous component at or near neutral pH and intermediate ionic strength (20 mM HEPES; 100 mM NaCl; pH 6.8) form a self-assembled structure that generates a SAXS pattern exhibiting nine diffraction peaks at $q = 0.0527$, 0.107, 0.212, 0.267, 0.323, 0.375, 0.430, 0.484, 0.530 Å^{-1}

(Figure 2C). Such a pattern is consistent with formation of a well-ordered multilamellar structure with a *d*-spacing of 119 Å. The absence of the third-order reflection (003), which should be located at $q = 0.158$ Å^{-1}, is noted. The increased intensity of the diffraction peaks located at $q = 0.107$, 0.212 and 0.322 Å^{-1} relative to the other reflections is similarly noted, and signals the coexistence of a second collapsed lamellar structure with a *d*-spacing of 59 Å. The balance between the two phases was found to be temperature-dependent, with increasing temperature (40 °C) yielding a SAXS pattern showing three diffraction peaks and thus, a single lamellar phase with a repeat distance of 54 Å (Figure 3A). An increase in the diffuse scattering is also noted in the low *q*-region at 0.032 Å^{-1} as the temperature is increased. The observed change in the structure is believed to be due to the known subtle expansion of the compact coil conformation of the partially ionized PAA with increasing temperature.[23] While these changes are not significant in bulk solution, as noted

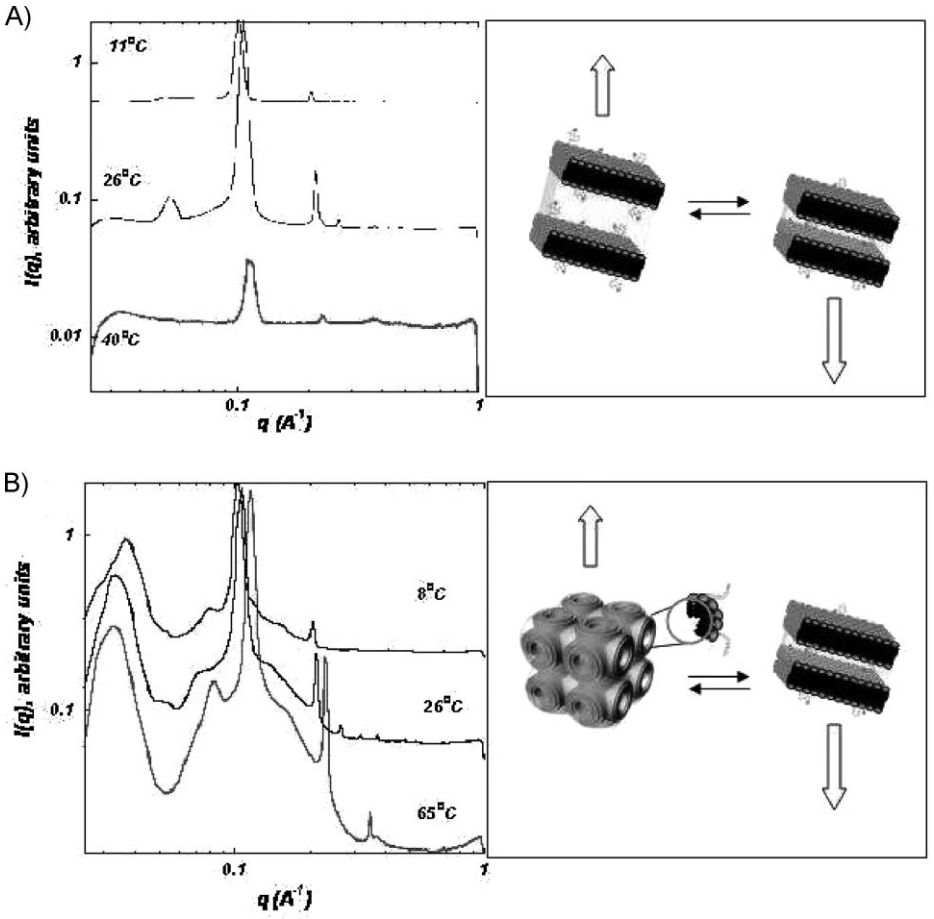

Figure 3.
Temperature-dependent SAXS patterns collected on a composition prepared at neutral pH (exact composition provided in Figure 2C caption). Schematic illustration showing how temperature changes the ratio of the mixed phase components, swollen and collapsed multilamellar structures; (B) at high pH (exact composition provided in Figure 2D caption). Schematic illustration showing how temperature changes the ratio of 3D cubic and collapsed lamellar structures.

earlier,[9] upon grafting and confinement, the temperature-induced conformational changes in the polymer produce substantial changes in the self-assembled structure and organization of the lipid bilayer.

Finally, at the highest pH examined, pH = 9.87 (100 mM sodium carbonate), the diffraction pattern differs from those observed at the other pH values (Figure 2D). First, in the low-q region, a large, broad peak at $q = 0.0324$ Å^{-1} is observed. In the mid-q region, two large diffraction peaks are observed at 0.106 and 0.211 Å^{-1}, clearly suggesting the presence of a collapsed lamellar structure with a d-spacing of 59 Å. In addition to these, lower-intensity peaks are observed within this region at $q = 0.0511$, 0.0552, 0.0723, 0.139 Å^{-1}. These Bragg peaks, along with the low-q peak (at 0.0324 Å^{-1}), can be indexed to the $\sqrt{2}$, $\sqrt{5}$, $\sqrt{6}$, $\sqrt{10}$ reflections of a 3-D cubic lattice. More specifically, these reflections suggest the formation of a cubic structure composed of discrete micelles organized on a primitive lattice (Figure 2D). The lattice spacing, a, determined from the slope of a plot q vs. $\sqrt{h^2 + k^2 + l^2}$, employing the observed Miller plane indices (110), (210), (211) and (310), is 413 Å. The co-existence of a lamellar phase with a swollen primitive cubic phase (a = 311 Å) has been observed previously in monoacylglyceride – water binary mixtures.[24] Lastly, in the high-q region, the peaks at $q = 0.322$ and 0.425 Å^{-1} can be assigned to the third- and fourth-order reflections of the collapsed lamellar phase. The co-existence and/or transformation of the self-assembled mesophase from a cubic to a lamellar structure is further established by examining how temperature changes alter the observed SAXS pattern (Figure 3B). As the temperature is reduced to 8 °C, the reflections assignable to the cubic phase become more apparent. Conversely, as the temperature is increased, the lamellar structure dominates. Temperature-induced changes in the structure again arise from modulation of the PAA conformational state.[25] More specifically, under alkaline and moderate ionic strength (~0.1 M) conditions, the charge density is close to that of a PAA chain in a dilute bulk solution, which adopts an extended conformation. Such an uncoiled polymer conformation may drive the formation of a cubic structure. Upon decreasing the temperature, the PAA chains would be expected to uncoil even further, leading to stabilization of the cubic component. The conversion to an ordered micelle structure serves to reduce the steric pressure. A similar effect has been observed to occur in the temperature-induced conformational extension of nonionic polymers such as PEG and PEO-based copolymers.[9]

Conclusion

A polyacrylic acid – lipid conjugate has been synthesized and its influence on the structure of lipid bilayers has been evaluated over a range of pH values and salt concentrations. At low pH and in the absence of salt, the protonated polymer adopts a coil (brush) conformation that promotes the formation of a swollen multilamellar structure. As the pH is raised, the polymer thickness is determined by a complicated balance between electrostatic interactions of the charged polymer (which induces stretching) and the conformational state of the chain, which opposes stretching.[22] At low to neutral pH values in the presence of moderate salt (0.1 M NaCl) concentrations, the coexistence of the two lamellar phases is observed. One is a collapsed lamellar structure with a repeat distance characteristic of a lipid bilayer without polymer. The other is a swollen lamellar structure that most likely contains the polymer. When the polymer is fully protonated (under alkaline conditions), the coexistence of both a lamellar and cubic structure is observed. Collectively, these results reveal the sensitivity of polyacrylic acid to its environment and how changes in the state of the PAA induces significant changes in the lipid matrix in which it is embedded. Ultimately, these studies may aid in understanding the biochemical

implications of the formation of polyelectrolyte-induced domains, membrane regions enriched in a particular component which are believed to be important for the regulation of protein activity and cell signaling.[21] Although these initial experiments demonstrate the feasibility of constructing a pH-responsive, self-assembled soft nanostructure, considerable additional work will be required to develop monophasic materials with suitable optical and mechanical characteristics.

Acknowledgements: This work was supported by the Office of Basic Energy Sciences, Division of Materials Sciences, United States Department of Energy under Contract No. DE-AC02-06CH11357 to the UChicago, LLC.

[1] Z. Adamczyk, A. Bratek, B. Jachimska, T. Jasinski, P. Warszynski, *J. Phys. Chem. B* **2006**, *110*, 22426–22435.
[2] K. Seki, D. A. Tirrel, *Macromolecules* **1984**, *17*, 1692–1698.
[3] H. Kitano, Y. Akatsuka, N. Ise, *Macromolecules* **1991**, *24*, 42–46.
[4] H. Kitano, Y. Maeda, S. Takeuchi, K. Ieda, Y. Aizu, *Langmuir* **1994**, *10*, 403–406.
[5] M. Fujiwara, R. H. Grubbs, J. D. Baldeschwieier, *J. Coll. Interface Sci.* **1997**, *185*, 210–216.
[6] E. Buhler, J. Appell, G. Porte, *J. Phys. Chem. B* **2006**, *110*, 6415–6422.
[7] S.-M. Lee, H. Chen, C. M. Dettmer, T. V. O'Halloran, S. B. T. Nguyen, *J. Am. Chem. Soc.* **2007**, *129*, 15096–15097.
[8] D. N. T. Hay, P. G. Rickert, M. A. Firestone, *Polym. Prepr. (American Chemical Society, Division of Polymer Chemistry)* **2005**, *46*, 816–817.
[9] M. A. Firestone, P. Thiyagarajan, D. M. Tiede, *Langmuir* **1998**, *14*, 4688–4698.
[10] M. A. Firestone, A. C. Wolf, S. Seifert, *Biomacromolecules* **2003**, *4*, 1539–1549.
[11] D. N. T. Hay, P. G. Rickert, S. Seifert, M. A. Firestone, *J. Am. Chem. Soc.* **2004**, *126*, 2290–2291.
[12] M. A. Firestone, S. Seifert, *Biomacromolecules* **2005**, *6*, 2678–2687.
[13] P. D. Laible, R. F. Kelley, M. R. Wasielewski, M. A. Firestone, *J. Phys. Chem. B* **2005**, *109*, 23679–23686.
[14] N. B. Colthup, L. H. Daly, S. E. Wiberley, *Introduction to Infrared and Raman Spectroscopy*, 3 ed., Academic Press, Inc., New York **1990**.
[15] B. Lee, M. A. Firestone, *Biomacromolecules* **2008**, *9*, 1541–1550.
[16] K. Hristova, D. Needham, *Macromolecules* **1995**, *28*, 991–1002.
[17] T. Masui, M. Imai, K. Nakaya, T. Taniguchi, *J. Chem. Phys.* **2006**, *124*, 74904–74912.
[18] D. F. Anghel, J. L. Toca-Herrera, F. M. Winnik, W. Rettig, R. von Klitzing, *Langmuir* **2002**, *18*, 5600–5606.
[19] G. Montalvo, A. Khan, *Langmuir* **2002**, *18*, 8330–8339.
[20] J. Gustafsson, G. Oradd, M. Almgren, *Langmuir* **1997**, *13*, 6956–6963.
[21] P. Mitrakos, P. M. Macdonald, *Biomacromolecules* **2000**, *1*, 365–376.
[22] E. P. K. Currie, A. B. Sieval, G. J. Fleer, M. A. Cohen Stuart, *Langmuir* **2000**, *16*, 8324–8333.
[23] M. Sakurai, T. Imai, F. Yamashita, K. Nakamura, T. Komatsu, T. Nakagawa, *Polym. J.* **1993**, *25*, 1247–1255.
[24] C. E. Conn, O. Ces, X. Mulet, S. Finet, R. Winter, J. M. Seddon, R. H. Templer, *Phys. Rev. Lett.* **2006**, *96*, 108102-1-4.
[25] Y. Zhao, J. Kang, T. W. Tan, *Polymer* **2006**, *47*, 7702–7710.

