Interaction between Poly(acrylic acid) and Phospholipid Vesicles: Effect of pH, Concentration, and Molecular Weight

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Interactions between phospholipid vesicles and poly(acrylic acid) (PAA) are of fundamental importance for pH-sensitive delivery systems in pharmaceutical applications. In this study, we investigated the behavior of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) at varied concentrations of PAA with low and high molecular weights. Differential scanning calorimetry and fluorescence microscopy experiments suggested that hydrogen bonding between the carboxyl group of PAA and the phosphodiester group of the lipid is the main driving force for association under acidic conditions. Complexation between DPPC and PAA depends on the PAA molecular weight and concentration. At high enough concentrations of the large molecular weight PAA, the polymer can completely disrupt the bilayers of DPPC. For the small molecular weight PAA, in contrast, the complexation leads to vesicle aggregation without destroying the bilayers. The difference in complex structure was detected by SEM and a UV−visible spectrophotometer. At alkaline pH, complexation did not occur because of strong dissociation of PAA.

1. Introduction

Phospholipid vesicles have attracted much attention as drug carriers in the biomembrane research due to their structural similarity to cell membranes. Apart from drug delivery systems, they also show a potential use in biochemical reaction control, imaging, sensing, and medical diagnostics.[1−4] Despite their diverse applications, a major drawback of vesicles is the limited physical, chemical, and biological stability. These limitations cause premature drug leakage before reaching target sites, induced aggregation of vesicles to form larger particles, and acidic hydrolysis of phospholipid molecules.[5−10] As known, a very small change of pH in the human body system can affect the cell environment and function. From a biomedical point of view, this poses an intractable problem for the drug carrier system since the pH in the human body can vary in a wide range, for example, 5.5 (saliva), >4.4 (esophagus), 7.4 (blood), 2−4 (stomach), 8 (duodenum), 4 (small intestine), and 5.5−6.0 (urine).[11] In addition, the more acidic microenvironment of tumor cells than that of normal cells usually will destroy most of the phospholipid carriers before any significant reaction takes place.[12] Apparently, how carriers respond to pH is therefore critically important for drug delivery and release.

Poly(acrylic acid) (PAA) is a weak polyelectrolyte in water solution at neutral or acidic pH. Owing to its pH modulating ability, PAA has been widely exploited in the fabrication of layered films, ultrafine fibers, and nanotubes as well as in the coating of nanoparticle suspensions. It was reported that PAA could either stabilize or flocculate the particles by changing the pH.[13−17] Moreover, interpolymer complexation of PAA with many other polymers has been well-documented in the literature.[18−26] The formed complexes are relatively stable and can lead to novel materials with unique properties that are potentially useful for drug delivery.

For phospholipid vesicles, PAA and its derivatives at low concentrations have been found to complex with the bilayer upon acidification, thereby modifying the membrane properties.[27−29] This associative behavior has recently led to attractive applications particularly in the dermatological field.[30−34] Prior studies focused largely on hydrophobically modified PAA, poly(2-ethylacrylic acid) (PEAA), because it can bind and solubilize phospholipid bilayers to form polymer−phospholipid mixed micelles and facilitate release of the entrapped contents in a pH-dependent manner.[35−39] Hydrogen bonding between the polymer's unionized carboxyl groups and the lipid's phosphodiester groups is thought to be the main driving force for the association and complexation.[40]

PAA and poly(methylacrylic acid) (PMAA), in contrast, are much less explored, probably because of their inability to solubilize phospholipid membranes at any pH in the polymer concentration ranges that have been investigated so far. Under an acidic condition (pH < 5), these polymers interacting with bilayers primarily via hydrogen bonding can increase the main phase transition temperature of the bilayers and trigger vesicle coagulation to form large polymer−lipids complexes at low polymer concentrations.[41,42] Despite these reported findings, a thorough investigation is still lacking regarding the structural and physical properties of the complexes formed, the molecular weight, and concentration dependences. It is thus interesting and important to understand how PAA interacts with lipid membranes over a wide range of concentrations, in particular, for high concentrations, and whether the polymer molecular weight influences the complexation.

In this study, we aim to investigate and elucidate the interaction mechanisms for lipid membranes with both low and high molecular weight of PAA covering a wide range of concentrations primarily using calorimetry and fluorescent microscopy. The physical properties and structural changes of PAA−lipids complexes are studied by varying the pH. We also explore the possibility of solubilization of PAA−lipids complexes at low pH by manipulating the polymer concentration in an attempt to widen their potential applications. The obtained results herein might shed light on how to design a PAA−lipid system with desired stability and pH sensitivity.

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2. Materials and Methods

Materials. Poly(acrylic acid) from Sigma Aldrich with average molecular weight $M_w = 2000$ and 450 000 g/mol were used as received. From the manufacturer's data and our measured intrinsic viscosity, we find $M_w/M_n = 1.22-1.57$. 1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) with ≥99% purity from Sigma Aldrich (Singapore) was used to prepare phospholipid vesicles. High-purity sodium chloride (BDH Anala), sodium hydroxide pellets (NaOH), chloroform (analytical reagent grade), and methanol (ACS grade) were purchased from Merck. Tetrahydrofuran (HPLC 99.8% purity) was obtained from Tedia, U.S.A. 1,6-Diphenyl-1,3,5-hexatriene (DPH) for fluorescence and UV−vis spectrophotometry with ≥97.5% purity was from Fluka. Deionized water with a resistivity of 18.2 MΩ·cm was obtained from a PureLab Maxima water purification system (ELGA).

Preparation of Poly(acrylic acid) Solution. Poly(acrylic acid) solutions ranging from 0.005 to 15 wt % were prepared at room temperature. Deionized water or 20 mM NaCl buffer solution was slowly added to a weighed amount of PAA, and the sample was then gently stirred for 12 h. The dissolved PAA solutions were then allowed to hydrate and be dispersed overnight at 4 °C in a refrigerator. All samples were brought to room temperature before use. For PAA solutions with varying pH values, an appropriate amount of NaOH was slowly added to the solution, stirred, and measured using a pH meter (Lab 960, Schott Instruments). This procedure was repeated until the desired pH value was obtained.

Preparation of Phospholipid Vesicles−PAA Solution. Phospholipid vesicles were prepared based on the conventional thin-film hydration method. An appropriate amount of DPPC was dissolved in chloroform/methanol with a volume ratio of 2:1. The solvent was thoroughly evaporated to dryness in a water bath under a continuous flow of nitrogen. The dry lipid films that formed in the flask bottom were then resuspended in an aqueous buffer solution (20 mM NaCl) with or without PAA. Samples were vortexed several times until all of the lipid molecules were well dispersed at a temperature above the main phase transition temperature of the lipids. The final concentration of lipids prepared was fixed at 0.5 mg/mL, unless otherwise stated.

Fluorescence Microscopy. Hydrophobic dye, DPH (λ_{ex} = 350 nm/λ_{em} = 428 nm) was used as the fluorescent probe. Fluorescent micrographs were obtained using a Leica fluorescence microscope (DMLM microscope, Germany) equipped with a mercury lamp (ebq 100 isolated). Filter cube type A suitable for DPH was used for excitation between 340 and 380 nm and emission above 400 nm. The molar ratio of lipid to DPH was kept constant at 100:1 for all preparations. To incorporate the DPH into the lipid bilayer, an appropriate amount of DPH was dissolved in tetrahydrofuran and dried together with phospholipids (chloroform/methanol = 2:1) under a continuous flow of nitrogen. To test if PAA has any effect on DPH fluorescence, predetermined amounts of DPH in tetrahydrofuran were injected into pure PAA solutions at different concentrations and vigorously stirred for 1 h at room temperature. The experiment was performed in the dark at all times. Each sample solution was pipetted onto a microscope slide, which was then covered with a glass coverslip (22 mm × 22 mm, thickness 0.13−0.17 mm) before analysis on the microscope using the reflected mode of observation. All experiments were conducted at least twice for reproducibility at 25 °C.

Differential Scanning Calorimetry (DSC). DSC experiments were performed using a Mettler Toledo DSC822 differential scanning calorimeter. A 40 μL aluminum sealed pan was filled with approximately 10 mg of sample solution, with an empty pan as the reference. The data were collected upon heating, cooling, and heating thermal cycles from −10 to 60 °C at a scan rate of 2.5 °C/min. For analysis and comparison purposes, DSC curves at the second heating run were used in order to remove the influence of thermal history.

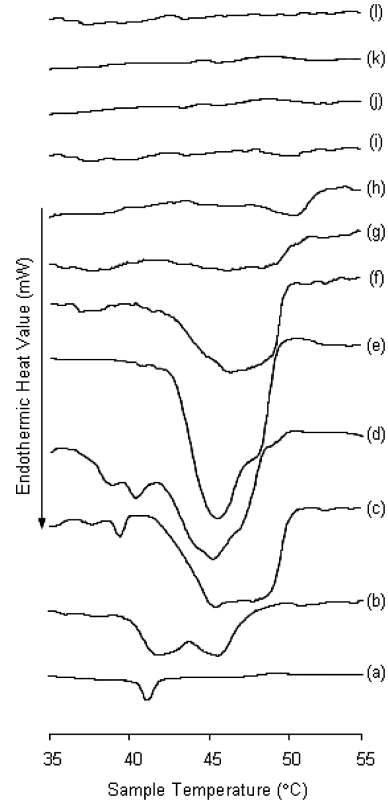

Figure 1. DSC heating curves of DPPC with different PAA (450 000 g/mol) concentrations, (a) 0.0, (b) 0.005, (c) 0.02, (d) 0.04, (e) 0.1, (f) 0.3, (g) 0.5, (h) 0.7, (i) 1.0, (j) 5.0, (k) 10.0, and (l) 15.0 wt %.

Rheometry. The rheological properties of samples at 25 °C were measured using a Haake RheoStress 600 rheometer with a DC50 water circulating bath temperature controller and a cone−plate sensor system (C60/4, diameter 60 mm and angle 4°).

UV−Visible Spectrophotometer. UV−vis absorbance spectra were measured using a Shimadzu UV-1601 spectrophotometer in the range of 200−600 nm at 25 °C. The hydrophobic dyes DPH will show high absorbance at 343, 358, and 378 nm when dyes are partitioned into a hydrophobic environment, such as the hydrophobic core of a micelle, the hydrophobic interior of a lipid bilayer, and so forth.[43−45] All of the samples were prepared via a dye solubilization method similar to that for the fluorescence microscopy characterization. All of the samples were equilibrated in the dark for 12 h before measurement.

3. Results and Discussion

High Molecular Weight PAA (450 000 g/mol). The effect of PAA concentration on the phase behavior of hydrated DPPC bilayers was investigated using DSC, with the results shown in Figure 1 and Table 1. It should be noted that varying the PAA concentration changes the solution pH. As seen in Table 1, the measured pH decreases from 4.2 to 1.7 when the PAA concentration increases from 0.005 to 15 wt % in the prepared samples. In Figure 1, the signals observed in the thermogram

TABLE 1: Thermal Data and pH for DPPC and PAA (450 000 g/mol)

PAA (wt%)	pH	T_m (°C)	HHW (°C)	ΔH (J/g)
0.000	6.3	41.2	0.75	26.1×10^{-3}
0.005	4.2	41.8; 45.9	1.92; 1.67	64.5×10^{-3}; 49.7×10^{-3}
0.020	3.6	39.5; 45.7	0.89; 5.60	15.1×10^{-3}; 0.39
0.040	3.2	40.5; 45.6	1.03; 3.98	18.7×10^{-3}; 0.46
0.100	2.9	45.9	4.52	0.88
0.300	2.5	46.8	5.42	0.52
0.500	2.3	48.8	4.94	0.16
0.700	2.2	48.4	2.30	64.1×10^{-3}
1.000	2.1	46.3		6.50×10^{-3}
5.000	2.0			
10.00	1.9			
15.00	1.7			

are associated with DPPC because there is no peak for pure PAA solutions in the investigated temperature range from our testing.

As can be seen from the figure, the addition of a small amount of PAA (at 0.005 wt %) caused the appearance of two broad peaks in the thermogram, as opposed to a sharp single peak (T_m = 41.2 °C) for DPPC vesicles in the absence of PAA. A similar bipeak behavior was reported by Fujiwara et al. for 1.04 mg/mL DPPC and 0.1 wt % PAA (M_w = 250,000 g/mol) with the pH maintained at 4.6.[41] When the pH was decreased to 3.8, they observed only one peak. The peak at the lower temperature is thought to represent the main phase transition of lipid bilayers, while the other peak suggests the existence of lipid−PAA complexes. It can be seen from Figure 1 that the peak at the lower temperature slightly shifts to the left with increasing PAA concentration in the range of 0.005−0.04 wt %. It reflects a disturbed arrangement of lipid molecules in the bilayers caused by PAA chains for a small number of lipid vesicles. The broadening of the main transition peak, as evidenced by the increased half-height width (HHW), indicates lower cooperativity between the acyl chains of the lipid molecules when undergoing the transition. When the PAA concentration is progressively increased from 0.02 to 0.1 wt %, the original transition peak weakens substantially and vanishes eventually, whereas the other peak grows in both magnitude and breadth and becomes dominant, indicating the prevalence of the PAA−lipid complexes. We speculate that the structural heterogeneity and compactness of PAA−lipid complexes is very strong, resulting in the large, broad transition peak and the higher main phase transition temperature. Since PAA is a weak polyelectrolyte, most of the carboxyl groups remain protonated at acidic pH. These carboxyl groups behave as the proton donor to interact with lipid's phosphodiester groups as the acceptor. The resulting hydrogen bonding allows a long PAA chain to associate with more than one phospholipid vesicle and hence induce vesicular aggregation for the formation of PAA−lipid complexes. Apart from hydrogen bonding, hydrophobic interaction between PAA and the lipid's hydrocarbon acyl chains may also contribute to the complexation because PAA becomes more hydrophobic at low pH. The growing width and the large transition enthalpy (ΔH) for the peak associated with the PAA−lipid complexes (see Table 1) indicate a great microstructural difference from lipid bilayers. We try to make comparison with the result obtained by Fujiwara et al. for samples having similar compositions.[41] They reported HHW = 1.58 °C for 1.04 mg/mL DPPC and 0.1 wt % PAA (M_w = 250 000 g/mol) at pH 3.8, while we obtained HHW = 5.6 and 3.98 °C for 0.5 mg/mL DPPC with PAA (M_w = 450 000 g/mol) at 0.02 (pH 3.6) and 0.04 wt % (pH 3.2), respectively. Our larger HHW implies that the complexation behavior is dependent on the PAA molecular weight and the concentrations of both species, not just their mass ratio.

Interestingly, by further increasing the PAA concentration from 0.3 to 1.0 wt %, the broad peak for the PAA−lipid complexes gradually weakened and completely disappeared at 5−15 wt %. As mentioned earlier, a high PAA concentration leads to a lower pH. The degree of dissociation can be roughly estimated to decline from 0.08 (0.3 wt %) to 0.01 (15 wt %). This reduction appears to affect the association, leading to a certain change in the complexes. What can be clearly inferred from our DSC results is disruption of lipid bilayers at sufficiently high PAA concentrations, evidenced by the disappearance of the signature peak for the bilayer main transition. Our finding reveals for the first time that PAA can solubilize vesicles under appropriate conditions, in contrast to the claimed inability in the literature where the tested PAA concentrations were not high enough.[27,28,40]

Fujiwara et al. reported that complexation of PAA and vesicles becomes weaker at higher polymer concentrations due to the competition of PAA for binding to the vesicle surface and the steric repulsion of extending PAA chains.[41] In their experimental study, the fluorescence anisotropy of acenaphthylene-modified PAA (M_w = 43 000 g/mol) in the dispersion of preformed egg PC vesicles was determined for very dilute solutions at pH 3.8 (about 48 ppm lipid, and the mass ratio of PAA to lipid ranging from 0.1 to 1.5). Since the anisotropy was observed to decrease with increasing polymer concentration, they made the aforementioned claim for the concentration dependence. This trend, nevertheless, is limited to very low PAA and lipid concentrations with pH control. Increasing the PAA concentration with the pH maintained constant essentially does not decrease the degree of dissociation. Although the electrostatic repulsion between each pair of PAA does not change much because of the unchanged charge density, the molecular encounter can become frequent at the high enough PAA concentrations. For our presented cases, the degree of dissociation decreases with increasing PAA concentration because pH is not controlled, and also, the concentrations of the lipid and polymer are much higher. Therefore, the interaction and complexation between PAA and the lipid becomes stronger, as experimentally reflected by the increase of HHW and the transition enthalpy of the main phase transition peak at intermediate PAA concentrations, as shown in Table 1.

In order to seek more evidence for the aforementioned interaction mechanisms, we employed fluorescence microscopy because it is a simple and convenient approach allowing direct visualization. The hydrophobic dye DPH can only bind to and be located between acyl chains of the lipid molecules since our testing has found no fluorescence for pure PAA solutions with concentrations spanning from 0.005 to 15 wt %. The micrograph of multilamellar DPPC vesicles in the absence of PAA is presented in Figure 2a. One can clearly see from Figure 2b that addition of a very small amount of PAA (0.005 wt %) induces significant vesicular aggregation, leading to the formation of large PAA−lipid complexes coexisting with a small number of lipid vesicles. The complex looks like a loose cluster of aggregates. When the PAA concentration is increased to 0.1 wt %, as demonstrated in Figure 2c, only aggregates are present in the solution. By further increasing the PAA concentration to 0.5 and 1.0 wt %, we observed complexes decreasing progressively in size, as shown in Figure 2d and 2e. This behavior persists until the PAA concentration reaches 10 wt % or higher,

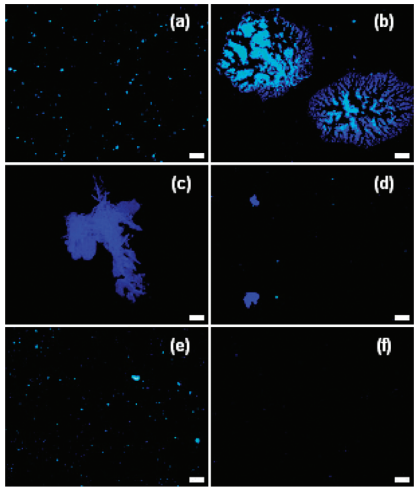

Figure 2. Fluorescence image of DPPC with PAA (450,000 g/mol) at (a) 0.0, (b) 0.005, (c) 0.1, (d) 0.5, (e) 1.0, and (f) 10 wt %. Bar = 200 μm. Magnification = 50.

where no fluorescence was observed (see Figure 2f). This finding suggests that with increasing polymer concentration, the microstructure of the complexes changes gradually, and they ultimately become too small to be seen under the fluorescent microscope.

In this study, dynamic light scattering is not suitable to examine the size change of complexes with varying PAA concentration because of (1) precipitation of the formed large complexes at low to intermediate PAA concentrations and (2) significant interference in light scattering from the polymer at high PAA concentrations.

To further support the proposed interaction mechanisms, the absorbance of hydrophobic dye DPH was measured using a UV−vis spectrophotometer, with results shown in Figure 3. Note that for samples with precipitates formed, their supernatants were measured. As mentioned in section 2, DPH will only show three sharp peaks at wavelengths 343, 358, and 378 nm when partitioned into a hydrophobic domain. To find out if PAA affects the absorbance, we test pure PAA solutions with the DPH concentration kept constant. It can be seen from Figure 3a that no peak was observed when the PAA concentration ranged from 0.1 to 1 wt %. However, when the PAA concentration was further increased, the absorbance at 343, 358, and 378 nm could be detected and increased with PAA concentration, as illustrated by the case of 10 wt % in the figure. It indicates the formation of hydrophobic domains at high PAA concentrations of PAA due to the increased hydrophobicity under acidic condition. As for the DPPC solution without addition of PAA, DPH was partitioned into the hydrophobic interiors of bilayers, leading to the absorbance as shown by curve v in Figure 3b. In the presence of PAA at low to intermediate concentrations, no peak was detected because large complexes with PAA and vesicles for these samples formed and precipitated, and they hardly existed in the supernatants. Interesting results were obtained when the PAA concentration was raised to 1 and 10 wt %. The former contained a small amount of precipitates, while no precipitation was observed for the latter. As seen from Figure 3b, the three detectable peaks with very weak intensity at 1 wt % (cf. curves iii and v) signify the onset of formation of dispersible complexes containing hydrophobic microdomains to accommodate DPH. In contrast, no peak was detected for pure 1 wt % PAA, as mentioned earlier. The absorbance was substantially increased at 10 wt % PAA, implying that more DPH was partitioned into the hydrophobic microenvironment. The absorbance intensity can be estimated by the difference between that at 378 and 400 nm ($A_{378} - A_{400}$) to eliminate the turbidity and scattering effect of PAA.[43,44] It is found that the absorbance intensity for DPPC with 10 wt % PAA (0.3491) is higher than that for the pure DPPC solution (0.2229) and 10 wt % PAA (0.0992) as well as their sum. Since the DSC characterization has suggested no bilayers for these samples, we speculate that the vesicle bilayers are completely disrupted and the solubilized lipid molecules complex with PAA in a form of mixed micelles to accommodate DPH, as illustrated in Figure 4b.

To further test the behavior for DPPC at high PAA concentrations, we measured the solution viscosity and moduli at 25 °C. All samples tested showed a typical shear thinning behavior, with the viscous modulus greater than the elastic modulus in the investigated frequency range. The presence of DPPC increased the zero shear viscosity considerably to 1.68 Pa s for 10 wt % and 23.8 Pa s for 15 wt % as compared to 1.07 Pa s and 17.4 Pa s for the corresponding pure PAA solutions. The moduli were also enhanced in a similar way. However, at lower PAA concentration (5 wt %), the viscosity increase was only 2.3% (from 42.6 to 43.6 mPa s). These results do support the interpolymer bridging facilitated by the phospholipid.

The aforementioned solubilization and destruction of vesicles at high enough PAA concentrations can happen when one does not deliberately adjust pH. This behavior is very different from that of Pluronic F-127, for which the solubilized vesicles form bilayer patches in the mixed micelles at high polymer concentration.[46] Since pH is an important parameter affecting the mixture behavior, we chose the case with 10 wt % PAA and varied the sample pH by adding NaOH. PAA is a weak polyelectrolyte, and its secondary structure is sensitive to pH change. PAA molecules become more extended upon neutralization[40] due to the increased intramolecular electrostatic repulsion between the ionized carboxyl groups. Interesting results were obtained from the DSC characterization when varying the pH for the samples with 10 wt % PAA, as shown in Figure 5.

PAA−lipid complexes prevail at pHs from 1.9 up to 5.7 because of the absence of the main transition peak. At higher pH (6.9 and 8.3), however, a peak emerges, which is located nearly at the main transition temperature of pure DPPC vesicles, despite a larger breadth. This is ascribed to sufficient dissociation of the carboxyl groups, thereby decreasing the PAA association with lipid via hydrogen bonding. Moreover, the phosphodiester groups of the lipid have been reported to show a tendency of ionizing to become negatively charged at high pH.[47] As such, the PAA−lipid complexation should be weakened substantially, so the typical bilayer behavior resumes and dominates. The broader peaks at pH 6.9 and 8.3 as compared to those of pure DPPC vesicles suggest decreased transition cooperativity of the lipid molecules in the bilayers. The change in transition enthalpy is +2.7% at pH 6.9 and −10.7% at pH 8.3. According to the literature,[48] PAA becomes almost completely dissociated and highly charged at pH 6.5 or above. Therefore, its association with the lipid via hydrogen bonding is very unlikely. We speculate that it may have to do with the weak electrostatic interaction between the PAA charge and dipole moment of the lipid head group.

Low Molecular Weight PAA (2000 g/mol). To investigate the effect of PAA molecular weight on the interaction and complexation with DPPC, low molecular weight PAA (M_w = 2000 g/mol) is also used in this study. DSC results for PAA concentrations from 0.005 to 15 wt % are shown in Figure 6. In this concentration range, the sample pH changes from 4.0

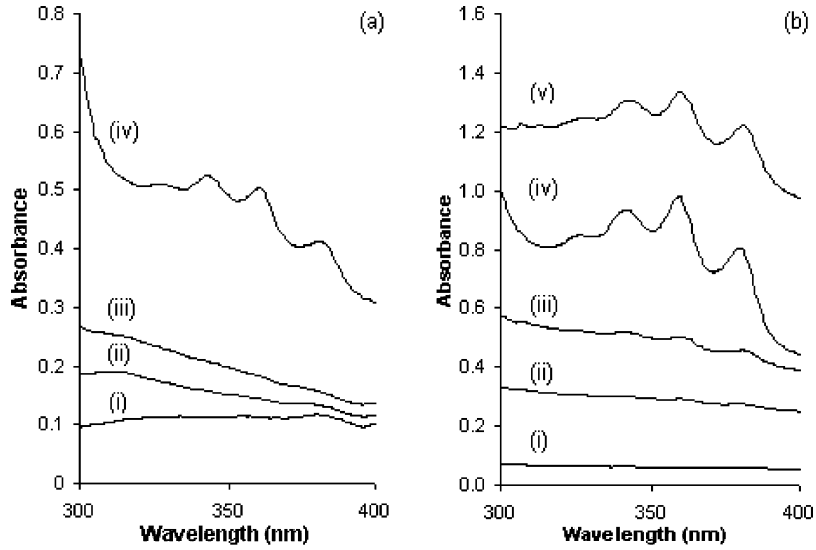

Figure 3. (a) UV−vis spectra of DPH for PAA (450k Da) at (i) 0.1, (ii) 0.5, (iii) 1.0, and (iv) 10 wt %. (b) UV−vis spectra of DPH for 0.5 mg/mL DPPC with PAA at (i) 0.1, (ii) 0.5, (iii) 1.0, (iv) 10, and (v) 0 wt %.

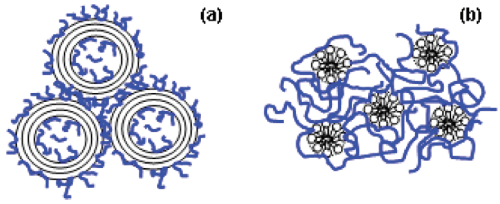

Figure 4. Complexes for DPPC and 10 wt % PAA with molecular weights of (a) 2000 and (b) 450 000 g/mol.

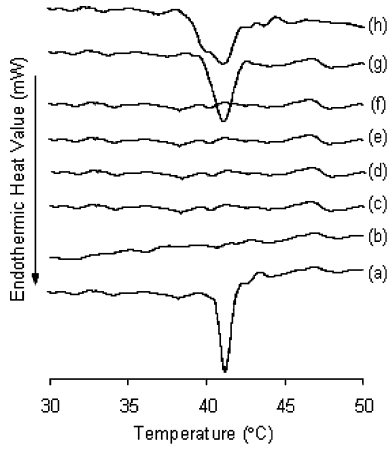

Figure 5. DSC thermogram of (a) DPPC vesicles (pH 6.3) and 10 wt % PAA/DPPC with different pHs of (b) 1.9, (c) 2.6, (d) 3.5, (e) 4.4, (f) 5.7, (g) 6.9, and (h) 8.3.

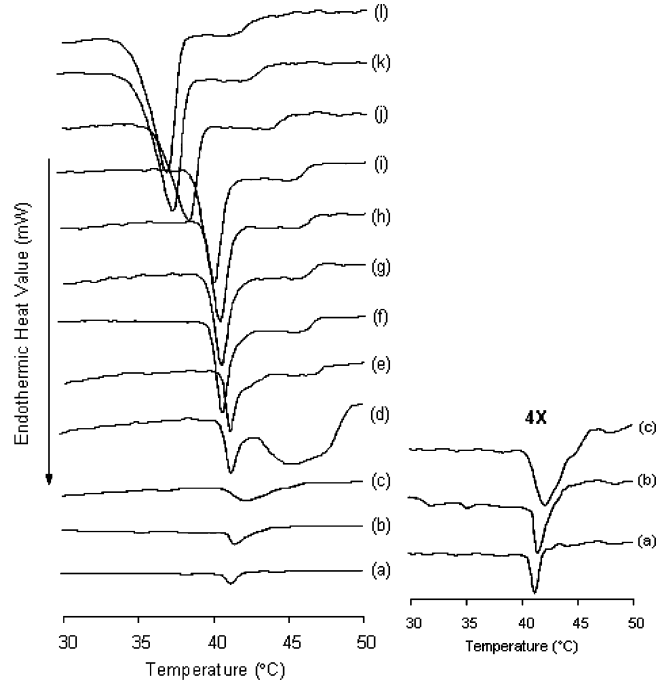

Figure 6. DSC heating curves of DPPC with different PAA (2000 g/mol) concentrations of (a) 0.0, (b) 0.005, (c) 0.02, (d) 0.04, (e) 0.1, (f) 0.3, (g) 0.5, (h) 0.7, (i) 1.0, (j) 5.0, (k) 10.0, and (l) 15.0 wt %.

down to 1.9, as seen in Table 2, which is only slightly different from that for M_w = 450 000 g/mol. At a very low PAA concentration (0.005 wt %), in contrast to the high molecular weight counterpart, the polymer hardly disturbs the DPPC vesicles since there is nearly no difference in the main phase transition peak, as shown by curve b. The PAA effect becomes noticeable when the concentration is increased to 0.02 wt % (see curve c) because the peak broadens considerably at this concentration. It is attributable to the same mechanism, adsorption of PAA on the bilayer surfaces via hydrogen bonding, leading to lower cooperativity. The very slight increase in the transition temperature could be due to the enhanced attraction between some lipid molecules via hydrogen bonding with short PAA. The appearance of two peaks starts from 0.04 wt %, which is much higher than the onset concentration of 0.005 wt % for the high molecular weight PAA. This comparison suggests that the PAA−lipid interaction is stronger for longer polymer chains. As pointed out earlier, the new peak (at the higher temperature) is associated with the formation of PAA−lipids complexes. At 0.04 wt % PAA, vesicles coexist with a small number of lipid−PAA complexes. However, further increasing the PAA concentration gives rise to a behavior very different from that for M_w = 450 000 g/mol. The new peak subsides to become a shoulder to the other peak, which, in contrast, grows and shifts to lower temperatures. As can be seen from Table 2, both HHW and ΔH of this major peak increase with PAA concentration and appear to level off to 1.83 °C and about 0.75 J/g. The breadth of this peak, however, is smaller than that for the high molecular weight PAA at intermediate concentrations. The presence of the strong peak at high PAA concentration could

TABLE 2: Thermal Data and pH for DPPC and PAA (2000 g/mol)

PAA (wt%)	pH	T_m (°C)	HHW (°C)	ΔH (J/g)
0.000	6.3	41.2	0.75	26.1×10^{-3}
0.005	4.0	41.5	0.79	26.7×10^{-3}
0.020	3.6	42.1	2.33	0.10
0.040	3.5	41.3; 46.3	1.07; 4.65	90.2×10^{-3}; 0.14
0.100	3.2	41.05	0.72	0.26
0.300	2.9	40.7	0.96	0.40
0.500	2.8	40.5	1.13	0.40
0.700	2.7	40.4	1.17	0.41
1.000	2.6	40.2	1.33	0.55
5.000	2.2	38.3	1.83	0.53
10.00	2.1	37.5	1.83	0.78
15.00	1.9	36.8	1.83	0.73

imply the possible existence of modified bilayers or a bilayer-like structure in the PAA−lipid complexes (see Figure 4a), although the peak differs considerably from that for pure DPPC vesicles. This is because pure PAA solutions, regardless of the PAA molecular weight, cannot exhibit any peak in the DSC thermogram for our investigated temperature range. Moreover, no DPH absorbance peak was obtained in UV−vis spectrometry for the supernatant of DPPC with 10 wt % PAA as most of the lipid/PAA complexes were large and precipitated in the solution (data not shown). Therefore, it seems appropriate to assert that the low molecular weight PAA can partially solubilize vesicles instead of completely destroying bilayers.

As mentioned earlier, without adjusting the acidity, the solution pH at a given PAA mass concentration is almost the same, irrespective of the PAA molecular weight. It means that the degree of dissociation of PAA is nearly independent of its molecular weight in the investigated concentration range, and thereby, the number of interaction sites on each polymer molecule available for hydrogen bonding increases with the PAA chain length.[48−50] Accordingly, longer PAA molecules can render a stronger correlation for lipid molecules either in the same bilayer or in different bilayers. The former can lead to changes in the lipid arrangement in a bilayer and thus affect the cooperativity, whereas the latter can give rise to bridging between vesicles and the consequential aggregation and complex formation. At the lowest PAA concentration (0.005 wt %) in this study, the formation of large complexes observed for M_w = 450 000 g/mol, but not for M_w = 2000 g/mol, could be explained by the bridging effect, which is much stronger for the former. For short PAA, a higher concentration is required for sufficient polymer adsorption on the surface of each vesicle to form a thin layer. Since PAA is rather hydrophobic at acidic pH, the thin layers can make vesicles less stable for aggregation. This behavior is observed for PAA concentrations from 0.04 to 15 wt %. The fluorescent images shown in Figure 7 can complement the DSC results to support this argument. For high molecular weight PAA, in contrast, the vesicular aggregation and complexation appears very strong via both bridging and hydrophobic interactions, leading to complete disruption of the bilayer structure at high enough PAA concentrations, as evidenced by the disappearance of the main transition peak in the DSC thermogram.

As seen from Table 2 and Figure 6, the peak shifts to lower temperatures and becomes about 4 °C lower for 15 wt % PAA than that for pure DPPC. According to Garidel et al.,[51] the main transition temperature for pure DPPC vesicles is nearly identical at pH 2 and 7. Therefore, a change in pH alone cannot explain the observed peak shift upon increasing the PAA concentration. We speculate that when vesicles aggregate, some adsorbed PAA

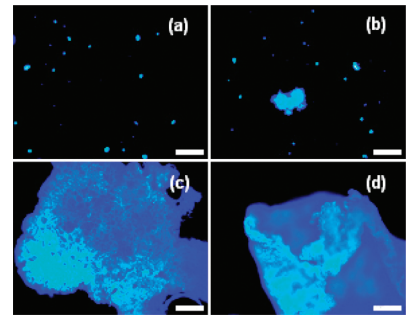

Figure 7. Fluorescence image of DPPC with PAA (2,000 g/mol) at (a) 0.005, (b) 0.04, (c) 0.1, and (d) 15 wt %. Bar = 100 μm. Magnification = 200.

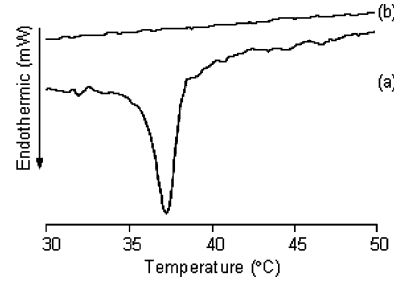

Figure 8. DSC thermogram of 10 wt % PAA/DPPC prepared by the addition method with PAA molecular weights of (a) 2000 and (b) 450 000 g/mol.

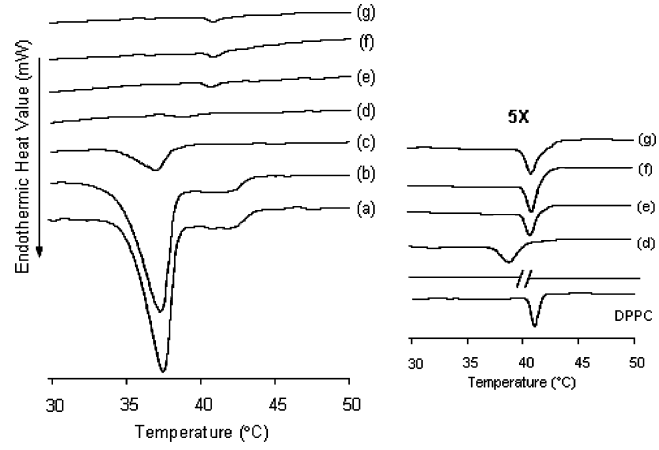

Figure 9. DSC thermogram of 10 wt % PAA/DPPC with different pHs of (a) 2.1, (b) 3.2, (c) 4.1, (d) 5.0, (e) 6.2, (f) 7.1, and (g) 8.0.

molecules can be incorporated into the hydrophobic interior of the bilayer, disturbing the arrangement of the acyl chains of the lipid molecules. This insertion can abate the van der Waal attraction for the acyl chains, thereby causing the decrease in the main transition temperature.

It has been known that complexation between the polymer and vesicles may be affected by how the polymer is introduced during the sample preparation. For Pluronic, insertion of the hydrophobic block into the bilayer requires using the polymer solution for film hydration to synthesize the vesicles directly rather than adding the polymer to the preformed vesicle dispersion.[52−54] One may thus wonder whether this preparation issue affects the PAA−DPPC systems studied here because PAA becomes more hydrophobic under an acidic condition. To investigate this matter, we added PAA into a preformed DPPC vesicle solution to carry out the DSC characterization. The results for 10 wt % PAA of two different molecular weights are shown in Figure 8, where we cannot find an observable

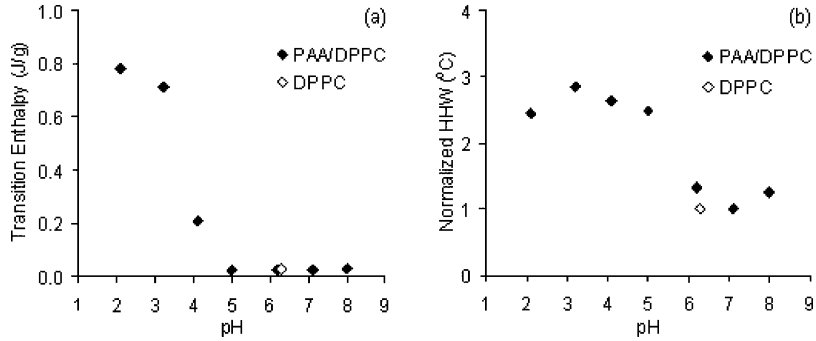

Figure 10. Transition enthalpy (a) and HHW (b) for the main phase transition of DPPC with 10 wt % PAA (2000 g/mol) as functions of pH. The results of the pure DPPC solution are also shown for comparison.

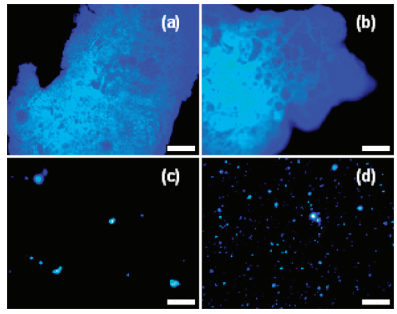

Figure 11. Fluorescence image of 10 wt % PAA/DPPC (2000 g/mol) with pHs of (a) 2.1, (b) 3.2, (c) 4.1, and (d) 8.0. Bar = 100 μm. Magnification = 200.

difference in the thermogram when compared to Figure 1 and 6. For PAA (M_w = 2000 g/mol), the peak is located at 37.3 °C, with ΔH and HHW values very similar to those by the incorporation method, while there is no peak for M_w = 450 000 g/mol. It can therefore be concluded that the behavior of PAA−DPPC solutions is independent of the preparation methods.

For comparison purpose, the pH effect is also investigated for 10 wt % PAA (M_w = 2000 g/mol) using DSC, with the results shown in Figure 9. One can see that with the increasing pH, the peak progressively shrinks and shifts right toward 41 °C. Figure 10 exhibits the variations of ΔH and HHW with pH. We find that at pH 5 and above, the transition enthalpy is very close to the value for pure DPPC vesicles. The peak breadth also decreases to a value similar to that for pure DPPC vesicles when the pH becomes 7.1 and shows a weak variation with pHs ranging from 6 to 8. The fluorescent images for varying pHs are presented in Figure 11. Large PAA−lipids complexes at low pH can be observed. The amount and size of the complexes are drastically reduced when the pH is increased to 4.1 (Figure 11c). For pHs higher than 6.0 (see Figure 11d), we observed objects that were visually indistinguishable in size from those in the absence of PAA. These results indicate that the PAA effect becomes very weak at pH 6−8, similar to that for its high molecular weight counterpart. At large pH, highly negatively charged PAA becomes quite extended and can thicken the dispersion if its molecular weight is large. The enhanced viscosity along with steric hindrance to vesicle diffusion can expectedly reduce the probability of vesicles approaching one another for aggregation.

One may wonder whether the PAA chain end structure affects the interaction and complexation, in particular, for short PAA. According to the literature,[55−58] the end group could be phenyl, thiol, azide, or amine, depending on the polymerization method. These end groups are relatively stable and not expected to lead to other significant interactions and effects, as implied by the fact that the main transition temperature hardly changes at a high enough pH, where carboxylic groups all dissociate and can no longer be H donors (see Figures 5 and 9).

4. Conclusions

The interaction and complexation between PAA and phospholipid vesicles depends on the medium pH, polymer concentration, and molecular weight. At acidic pH, hydrogen bonding is the major associative interaction between the two species. At low enough concentrations, adsorption of PAA molecules on the lipid bilayer surfaces via hydrogen bonding can increase the cooperativity of the acyl chains of the lipid molecules, thereby leading to a higher transition temperature for the complexes. At high PAA concentrations, the polymer can interconnect different vesicles to induce aggregation through bridging for high molecular weight or hydrophobic attraction for low molecular weight. The different mechanisms bring about different microstructures of the formed complexes. High molecular weight PAA can completely disrupt the bilayer structure, evidenced by the absence of a phase transition peak. This interesting vesicle solubilization by PAA at high molecular weight and concentration is discovered for the first time from the present study. In contrast, low molecular weight PAA induces vesicle aggregation to form PAA−lipid complexes without destroying the basic nature of bilayer. The transition temperature is lowered, probably because of PAA insertion into the bilayer, changing the arrangement of lipid molecules and their acyl chains' interaction. At neutral or alkaline pH, PAA is highly charged and can hardly associate with phospholipid vesicles. Our finding can potentially be instrumental for applications in pH-sensitive carriers and their content release.

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