

Formation Kinetics of Polymer Vesicles from Spherical and Cylindrical Micelles Bearing the Polyelectrolyte Complex Core Studied by Time-Resolved USAXS and SAXS

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ABSTRACT: Polymer vesicles (polymersomes) have received considerable attention in the last several decades, but little has been known about the kinetics of the formation process, despite their importance both scientifically and in practical applications. Here, we reported the formation kinetics of polymer vesicles in aqueous NaCl solutions of anionic-neutral and cationic-neutral block copolymers when they transform from micelles with a polyelectrolyte complex core. Vesicle formation from spherical or cylindrical micelles was initiated by fast mixing with a stopped-flow device. We then employed time-resolved ultrasmall-angle X-ray scattering with millisecond resolution, which enabled monitoring the kinetic process of vesicle formation. An in-depth analysis of the scattering data elucidated the following processes: in the case that the initial state was spherical micelles, they grew with time to form short cylindrical micelles. These short cylindrical micelles then transformed into disklike micelles, which subsequently closed to form vesicles. On the other hand, when the initial state involved cylindrical micelles, the disklike micelles similarly assembled, which then transformed into vesicles. The bilayer membrane of vesicles used in this study was 4 times thicker than that of vesicles formed from conventional low-molecular-weight surfactants; hence, the bending rigidity of the bilayer was approximately 16 times higher. Due to this feature, the kinetic process was different from that of conventional low-molecular-weight surfactants, in particular, an increase in the size of the disklike micelles was not observed. Instead, the weight fraction of the disklike micelles gradually decreased, and concomitantly, the weight fraction of vesicles increased since the larger transient disklike micelles or bowl-like micelles were unstable. This behavior could be well reproduced by our proposed kinetic model. This is the first report on the self-assembly kinetics of polymer vesicles probed by time-resolved small-angle scattering.

INTRODUCTION

Polymer vesicles, sometimes referred to as polymersomes, have attracted great interest from diverse viewpoints, including applications as nanocarriers for more than three decades.^{1–11} Polymer vesicles are advantageous because the membrane is thick and robust and can be easily functionalized compared to liposomes. In contrast to such advantages, amphiphilic block copolymers do not usually form vesicles by simply dissolving in water, whereas mixing of oppositely charged low-molecular-weight surfactants is well known to lead to spontaneous vesicle formation.^{12–14} Thermal or mechanical treatments or the use of an organic solvent is necessary to make polymer vesicles in

an aqueous solution. This is one of the major bottlenecks in the applications of polymer vesicles. $^{\rm 2-7}$

Koide et al.¹⁵ reported that cationic—neutral and anionic neutral block copolymers spontaneously form vesicles bearing a polyelectrolyte complex core in aqueous solutions without any thermal or mechanical treatments. Since then, such kinds

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of vesicles have been extensively studied.¹⁶⁻²⁵ Recently, we²⁶⁻²⁸ investigated the morphologies of polyelectrolyte complex micelles formed from anionic–neutral and cationic–neutral double-hydrophilic block copolymers (AP and MP, respectively; Chart 1) at different added NaCl



^aBlock copolymer composed of poly[sodium 2-(acrylamido)-2methylpropanesulfonate] (PAMPS) and poly[2-(methacryloyloxy)ethyl phosphorylcholine] (PMPC). ^bBlock copolymer composed of poly{[3-(methacryloylamino)propyl]trimethylammonium chloride} (PMAPTAC) and PMPC.

concentrations (C_s) and mixing ratios of AP and MP (x_+) defined by

$$x_{+} \equiv \frac{C_{+}}{C_{+} + C_{-}} \tag{1}$$

where C_+ and C_- denote the molar concentrations of the cationic and anionic monomer units, respectively. We^{26–28} have found that various morphologies (spherical micelles, cylindrical micelles, and vesicles) were formed depending on $C_{\rm S}$ and x_+ , as summarized in Figure 1. The $C_{\rm S}$ -induced morphological transition is caused by a change in the packing parameter (balance between the core and corona volume)²⁹



Figure 1. Morphological diagram of the AP–MP mixture in aqueous NaCl solution (the ratio of the charged to neutral block chain lengths is ca. 10) in which the total polymer concentration is 0.005 g/cm^{3.26–28} The arrows represent the changes in C_S and x_+ to induce vesicle formation and then to monitor the transformation processes in the present study.

due to the reduction of the attractive interaction between the cationic and anionic block chains in the micelle core.²⁶ Meanwhile, the x_+ -induced morphological transition can be interpreted in terms of electrostatic instability (i.e., the vesicles can form only in cases that the polyelectrolyte complexes are almost neutralized).^{27,28}

The present study is concerned with the kinetics of vesicle formation, which is important from both scientific and application perspectives because it may shed light on making vesicles in a facile manner such as simple mixing. Furthermore, when drugs are loaded in vesicles, the kinetic pathway influences the loading efficiency of drugs. However, the kinetics of the formation of vesicles has rarely been investigated due to the difficulty in the preparation as mentioned above. Furthermore, the formation process is too fast to be observed by *ex situ* techniques (such as electron microscopy).

The kinetic pathways can be categorized into three different broad types predicted (Scheme 1): $^{6,7,30-32}$ (a) *via* semivesicle pathway: in this pathway, spherical micelles are initially formed and then flip-flops of the copolymer chains take place to make a hollow at the center of spherical micelles, followed by enlargment of the hollow (Scheme 1a). However, this pathway might be atypical; there are only a few experimental reports with relatively indirect evidence.33,34 The computational simulation reproducing this pathway was performed in weakly amphiphilic copolymers.³⁵ (b) By disk closure: here, the disklike micelles are first formed from spherical or cylindrical micelles and then the disklike micelles grow and close to form vesicles to reduce the line tension in the disk-edge portion (Scheme 1b). There have been many reports that agree with this pathway in low-molecular-weight surfactants using timeresolved small-angle X-ray scattering (SAXS) coupled with fast mixing by a stopped-flow device.^{12,13,36-41} However, concerning the kinetics of vesicle formation in block copolymers, little has been known compared to that in low-molecular-weight surfactants, as described above.7 Computational simulations can reproduce this pathway. 30,42,43 (c) Via phase separation:^{32,44} a block copolymer solution undergoes liquid–liquid phase separation. Within the polymer-rich droplet phase, the polymer chains gradually move to the interface of the polymerrich phase to form vesicles (Scheme 1c). Recently, Ianiro et al.³² found poly(ethylene oxide)-*b*-poly(caprolactone) forming vesicles upon phase separation. They stated that this pathway could be interpreted as the intermediate mechanism between the semivesicle and disk closure pathways. As far as the authors know, there has been only one report³² observing this pathway by time-resolved in situ monitoring technique, and computational simulations have not reproduced this pathway so far. After the formation of vesicles, they may grow to larger vesicles through the fusion of small vesicles or by Ostwald ripening (Scheme 1d).⁴³ It is noted that there is no thermodynamically stable size distribution for the disklike micelles and vesicles in contrast with that for the spherical and cylindrical micelles.⁴⁵ Thus, the vesicle may continuously grow unless there is a kinetic barrier.

It is noteworthy that Armes and co-workers studied the vesicle formation process during polymerization-induced self-assembly (PISA) of block copolymers by electron microscopy and time-resolved SAXS.^{46,47} The results seemingly agree with the disk closure pathway (Scheme 1b). However, the kinetics of structural development during PISA is governed by the kinetics of polymerization⁴⁸ because the time scale of the

Scheme 1. Schematic Illustrations of Possible Pathways of Vesicle Formation^{a 6,7,30-32}



^aIn the case of polyelectrolyte complex micelles or vesicles, the purple (core) region consists of polycation and polyanion block chains.

sample	$M_{1,n}^{a}$	$M_{1,w}/M_{1,n}^{b}$	$M_{1,w}^{c}$	$M_{1\pm,w}{}^d$	$M_{1-,w}^{e}$	$M_{1+,w}^{f}$	$N_{\pm 0}^{g}$	N_{-0}^{h}	N_{+0}^{i}
PMPC	6210	1.03	6400						
AP	48 500	1.07	51 900	6400	45 500		22	200	
MP	49 500	1.05	52 000	6400		45 600	22		210

^{*a*}Number-average molecular weight determined by ¹H nuclear magnetic resonance (NMR) spectroscopy. ^{*b*}Dispersity index determined by sizeexclusion chromatography (Figure S1). ^{*c*}Weight-average molecular weight calculated from $M_{1,n}$ and $M_{1,w}/M_{1,n}$. ^{*d*}Weight-average molecular weight of the neutral block (PMPC) determined by ¹H NMR. ^{*c*}Weight-average molecular weight of the anionic block (PAMPS) determined by ¹H NMR. ^{*f*}Weight-average molecular weight of the cationic block (PMAPTAC) determined by ¹H NMR. ^{*s*}Weight-average degree of polymerization of the PMPC block. ^{*h*}Weight-average degree of polymerization of the PAMPS block. ^{*i*}Weight-average degree of polymerization of the PAMPTAC block.

micelle formation/growth is much shorter than that of polymerization and the packing parameter²⁶ slowly changes during PISA.

In the present study, we have investigated the formation kinetics of the vesicles from the AP–MP mixture by timeresolved (ultra)small-angle X-ray scattering (USAXS and SAXS) with a stopped-flow device. AP and MP with similar block lengths as in previous studies^{26–28} were used in this work. In particular, we examined vesicle formation (1) from cylindrical micelles induced by NaCl addition and (2) from spherical micelles induced by changing x_+ . These changes are indicated as two arrows in the morphological diagram (Figure 1).

EXPERIMENTAL SECTION

Materials. Table 1 lists the molecular weight characteristics of AP and MP. The same AP and MP samples as in the previous investigations were used.²⁸ The detailed descriptions for the synthesis and characterizations are given elsewhere.⁴⁹ NaCl (>99.5%) was purchased from Wako Pure Chemical Industries, Osaka and used without any purification. Water was deionized by a Synergy system (Merck Millipore Corporation, Darmstadt).

Preparation of the Solution. AP and MP were separately dissolved in NaCl-free water or aqueous NaCl solution of $C_{\text{NaCl}} = 0.1$ M, wherein the polymer concentration (*c*) was 0.005 g/cm³. The AP solution was poured into the MP solution to obtain the AP–MP

mixture solution at $x_+ = 0.35$ and $C_{\text{NaCl}} = 0.1$ M or at $x_+ = 0.55$ and $C_{\text{NaCl}} = 0.1$ M followed by stirring with a vortex mixer. These solutions were used for the following stopped-flow experiments. The solutions used in this study were prepared and stored at room temperature and did not undergo any thermal or mechanical treatment.

Mixing by a Stopped-Flow Device. Test solutions for the timeresolved USAXS/SAXS experiments were prepared by mixing the following two pairs of solutions using an SFM-400 stopped-flow device (Bio-Logic Science Instruments, France).

- (1) A solution of the AP–MP mixture of $x_+ = 0.35$, $C_{\rm S} = 0.1$ M, and c = 0.005 g/cm³ and an aqueous NaCl solution of MP ($C_{\rm NaCl} = 0.1$ M and c = 0.005 g/cm³) were filled in the last two syringes of the stopped-flow device. The two solutions were mixed at a flow rate of ca. 7 mL/s to prepare a solution of $x_+ = 0.55$, $C_{\rm NaCl} = 0.1$ M, and c = 0.005 g/cm³. This mixing process is referred to as the "S \rightarrow V" process, as indicated in Figure 1.
- (2) An aqueous solution of AP-MP of $x_{+} = 0.55$, $C_{\text{NaCl}} = 0$, and $c = 0.01 \text{ g/cm}^3$ was mixed with an equivolume of an aqueous NaCl solution ($C_{\text{NaCl}} = 0.2$) by the stopped-flow device to prepare the solution with the same x_{+} , C_{NaCb} and c as in the case of (1). We refer to this procedure as the "C \rightarrow V" process (Figure 1).

The cumulative dead time of mixing and transfer time is calibrated to be <2.5 ms by mixing pure water and an aqueous solution of potassium bromide and monitoring the transmitted X-ray intensity.⁵⁰ With AP–MP solutions, a similar dead time is expected, as we did not detect any sample heterogeneities typical of incomplete mixing. We also checked that shear-induced assembly did not occur by the following control experiments. The two identical solutions of AP–MP were mixed by the stopped-flow device, followed by the time-resolved SAXS measurement; the obtained scattering profile remained unchanged with time.

Time-Resolved USAXS/SAXS. Time-resolved USAXS/SAXS measurements were performed at the ID02 beamline, ESRF, Grenoble, France. $^{\rm S1}$ The solutions were mixed rapidly using the stopped-flow apparatus and transferred to the measurement capillary kept a temperature of ca. 23 °C within 2.5 ms. In a given acquisition sequence, the X-ray exposure time was 5 ms and the separation between two frames was increased in a geometric progression. The first frame of the sequence was placed at the end of the mixing phase when the sample was in a continuous flow. Therefore, the corresponding kinetic time was 2.5 ms, the dead time of the stopped-flow device. We used an MX170 CCD detector (Rayonix, L.L.C., Evanston, IL) with 4×4 binning in this study. The excess scattering intensity was normalized to the differential scattering cross section per unit volume $[d\Sigma/d\Omega(q)]$ as a function of the magnitude of the scattering vector (q) by water calibration,⁵¹ where q is defined by $q \equiv (4\pi/\lambda_0)\sin(\theta/2)$ with the scattering angle θ and the wavelength of the incident X-ray $\lambda_0 = 0.0995$ nm. The data obtained at the two sample-to-detector distances (30.7 and 3.5 m) at each time point were merged. The normalized background of the stopped-flow cell filled with water or aqueous NaCl solution was subtracted from each sample scattering profile. The setup and procedure of data treatments were similar to those in a previous study.²⁸ This setup could access the q range of 1.5×10^{-3} –2.2 nm⁻¹. However, in some conditions, the scattering intensity was not high enough to accurately subtract the background scattering; thus, the q range with a subtraction problem was removed.

The weight-average molar mass (M_w) and the apparent z-average square radius of gyration $(\langle S^2 \rangle_z)$ for scattering particles in a sufficiently dilute solution were obtained by the Guinier plots with the following equation

$$\ln\left[\frac{1}{K_{\rm e}c}\frac{\mathrm{d}\Sigma}{\mathrm{d}\Omega}(q)\right] = \ln(M_{\rm w}) - \frac{1}{3}\langle S^2 \rangle_z q^2 + O(q^4) \tag{2}$$

where K_e is the optical constant (related to the contrast factor, calculated as described in the Supporting Information of ref 52). In eq 2, excess (free) MP chains in the solutions of $x_+ = 0.55$ were ignored because almost all AP and MP chains formed the polyelectrolyte complexes.²⁷

Transmission Electron Microscopy (TEM). Each AP–MP solution ($c = 0.005 \text{ g/cm}^3$) was dropped on a copper grid, and excess solution was blotted. An aqueous solution of sodium phosphotungstate (0.2 wt %) was similarly dropped and blotted. After the sample was dried in vacuo, TEM observations were performed using a JEM-3010 (JEOL Ltd., Tokyo) at an accelerating voltage of 100 kV.

RESULTS AND DISCUSSION

USAXS/SAXS Profiles in the Initial States and at t = 65 000 s. Before showing the kinetic data, the USAXS/SAXS profiles in the initial states and at t = 65 000 (a long time after the preparation) are shown in Figure 2a, along with the profiles for aqueous solutions of AP and MP alone. The blue and red circles indicate SAXS profiles for the solutions at $(x_+, C_{\text{NaCl}}) = (0.35, 0.1 \text{ M})$ and (0.55, 0 M), where the polyelectrolyte complex forms spherical micelles and cylindrical micelles, respectively. On the other hand, the green circles represent the profile for the solution at $(x_+, C_{\text{NaCl}}) = (0.35, 0.1 \text{ M})$ and 65 000 s after changing from $(x_+, C_{\text{NaCl}}) = (0.35, 0.1 \text{ M})$; the stopped-flow apparatus was not used to prepare this solution, but the solutions were mixed by pipetting and shaking. From the plateau values of profiles in the low-*q* region (cf. eq 2), the



Figure 2. (a) USAXS/SAXS profiles for the AP–MP mixtures in the initial state of the S \rightarrow V process ($C_{\text{NaCl}} = 0.1$ M and $x_+ = 0.35$; blue markers), in the initial state of the C \rightarrow V process ($C_{\text{NaCl}} = 0.01$ M and $x_+ = 0.55$; red markers) and at $t = 65\ 000\ \text{s}$ in the S \rightarrow V process ($C_{\text{NaCl}} = 0.1$ M and $x_+ = 0.55$; green markers). SAXS profiles for the individual AP ($C_{\text{NaCl}} = 0.1$ M and $x_+ = 0;^{26}$ orange markers) and MP ($C_{\text{NaCl}} = 0.1$ M and $x_+ = 1;^{26}$ purple markers). (b) USAXS/SAXS profiles at $t = 65\ 000\ \text{s}$ in the S \rightarrow V process (green markers) with the fitted curves by the vesicle alone model (broken red curve) and the vesicle + disk model (solid black curve). The previously obtained SAXS profile at (x_+ , $C_{\text{NaCl}} = (0.6, 0.1 \text{ M})$ (gray circles)²⁶ with the fitted curves by the vesicle alone model (broken red curve) is also shown.

aggregation numbers of AP and MP chains of the scattering particles in the solutions at $(x_+, C_{\text{NaCl}}) = (0.35, 0.1 \text{ M})$, (0.55, 0 M), and (0.55, 0.1 M) are ca. 100, 1000, and more than 10000, respectively. As already demonstrated, ^{26,27} the SAXS profiles at $(x_+, C_{\text{NaCl}}) = (0.35, 0.1 \text{ M})$ (blue circles) and (0.55, 0 M) (brown circles) can be fitted by the spherical and cylindrical micellar models, of which scattering functions are calculated by equations in the Supporting Information. The solid curves for the two profiles indicate the fit results, and the parameters obtained are listed in Table 2. See the Supporting Information for definitions of parameters.

In Figure 2b, the SAXS profile at $(x_+, C_{\text{NaCl}}) = (0.55, 0.1 \text{ M})$ and $t = 65\,000 \text{ s}$ (green circles) is compared with the previously obtained SAXS profile of the solution with $(x_+, C_{\text{NaCl}}) = (0.6, 0.1 \text{ M})$, which was prepared by directly mixing the AP and MP solutions with $C_{\text{NaCl}} = 0.1 \text{ M}$ (gray circles). Both profiles agree at an intermediate-*q* region but not in the low- and high-*q* regions. The previously obtained profile is fitted by the vesicle alone model (the solid black curve in the figure, calculated by the scattering function in the Supporting

Table 2. Parameters Obtained from the Fitting of the USAXS/SAXS Profiles in the Initial States and at $t = 6$)5 000 s	، ۲
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process	micelle type <i>i</i>	w_i^b	$M_{ m wi}/ m g\ mol^{-1c}$	size (nm)	${D_i}^d$	$\langle R_{\rm core} \rangle / {\rm nm}$	$\sigma_i/\langle R_{\rm core} \rangle^e$
$S \rightarrow V$ (initial)	sphere	1	$(7.5 \pm 0.3) \times 10^{6}$		1.2 ± 0.05	19 ⁱ	
$C \rightarrow V$ (initial)	cylinder	1	$(6.0 \pm 0.5) \times 10^7$	255 ^f	2^i	17 ± 0.2^{k}	0.15
$S \rightarrow V (65\ 000\ s)$	vesicle	0.4	$(1.6 \pm 0.2) \times 10^9$	253 ^g	1.5	12 ± 0.2^{l}	0.18
	disk	0.6	$(4.0 \pm 1) \times 10^{7}$	45 ^{<i>h</i>}	2	12 ± 0.2^{l}	0.18

^{*a*}The other parameters were fixed for all of the morphologies: $c_{core} = 0.38 \text{ g/cm}^3$, $\langle S^2 \rangle_{corona}^{1/2} = 1.5 \text{ nm}$, $f_{core} = 0.87$ (see the Supporting Information and ref 26). ^{*b*}Weight fraction of each micelle *i*. ^{*c*}Weight-average molar mass. ^{*d*}Dispersity index of molar mass. ^{*c*}Standard deviation σ_i with respect to R_{core} (see eq S17). ^{*f*}Average cylinder length calculated by eq S8 with $M = M_{w,C}$ (see the fourth column). ^{*g*}Average center-to-core radius of the vesicle calculated by eq S14 with $M = M_{w,V}$ (see the fourth column). ^{*b*}Average disk radius calculated by eq S11 with $M = M_{w,D}$ (see the fourth column). ^{*i*}Average radius of the spherical core. ^{*k*}Average cross-sectional radius of the cylindrical core. ^{*l*}Half of the thickness of the bilayer membrane core.

Information), as demonstrated in refs 26 and 27. However, it was impossible to fit the present profile obtained through the S \rightarrow V process by the vesicle alone model by adjusting values of the weight-average molar mass $M_{w,PCM}$ and the molar mass distribution \mathcal{D}_{PCM} (the broken red curve in Figure 2b), probably due to the different method of preparing the vesicles.

Weiss et al.³⁶ and Bressel et al.³⁸ reported that both disklike micelle and unilamellar vesicle coexist during the self-assembly process of the latter in low-molar mass surfactant solutions. Thus, we also assume that our system contains both disklike micelle and vesicle in the $S \rightarrow V$ process. As indicated by the solid black curve in Figure 2a, we succeed a satisfactory fit to the present profile in the $S \rightarrow V$ process ($t = 65\ 000\ s$) by the vesicle-disk mixture model, of which fitting parameters are listed in Table 2 (see the Supporting Information showing the calculation of the scattering function for this model). Interestingly, the late-stage morphology of the polyelectrolyte complex micelle depends on the mixing route of AP and MP solutions.

The vesicle formation was also confirmed by TEM observation; the obtained image is displayed in Figure 3.



Figure 3. Representative TEM images for the solutions at $C_{\text{NaCl}} = 0.1$ M and $x_+ = 0.55$, dried at $t \sim 65\,000$ s after preparation of the solution. The mean radius of 20 vesicles in TEM was 120 nm, and the standard deviation was 30 nm. In the right micrograph, a darker area is observed inside the vesicles, where the stain may be soaked in through the ripped area and trapped within the vesicles during the preparation of the TEM sample.

The solution for the TEM observations was similarly prepared to that for the SAXS measurements at $t \sim 65\,000$ s. The radius of vesicles in the TEM images is smaller but close to that in the result of USAXS/SAXS (R = 152 nm by eq 3 explained later; the *z*-averaged form factor obtained by USAXS/SAXS weighs the larger components).

Time Evolution of the USAXS/SAXS Profiles. To monitor the kinetic process of vesicle formation from the spherical and cylindrical micelles, the time-resolved USAXS/SAXS measurements were performed. Figure 4 shows time evolutions of the Guinier plots constructed by low angle data in the processes of S \rightarrow V and C \rightarrow V, obtained by USAXS. In



Figure 4. Guinier plots obtained by USAXS at the different time points (*t*) after changing the mixing ratio (x_+) from 0.35 to 0.55 at $C_{\text{NaCl}} = 0.1$ M (the S \rightarrow V process; panel a) and after changing NaCl concentration (C_{NaCl}) from 0 to 0.1 M at $x_+ = 0.55$ (the C \rightarrow V process; panel b). The total polymer mass concentration c = 0.005 g/ cm³ in both panels. The intercept and initial slope for each plot were estimated using the solid line indicated.

the S \rightarrow V process (panel a), data points at each *t* except at *t* = 65 000 s obey a straight line over almost all q^2 range shown, and from the intercept and slope of the line, we have estimated the weight-average molar mass M_w and the *z*-average radius of gyration $\langle S^2 \rangle_z^{1/2}$ of the polyelectrolyte complex micelles (cf. eq 2). Although the Guinier plots for the C \rightarrow V process in panel b exhibit nonlinearity in higher- q^2 region at shorter *t*, we have estimated M_w and $\langle S^2 \rangle_z^{1/2}$ from intercepts and initial slopes of the lines (within the range of $q \langle S^2 \rangle_z^{1/2} < 1$) indicated in panel b. (We have also checked the M_w and $\langle S^2 \rangle_z^{1/2}$ values by the Berry plot.)

The results of M_w and $\langle S^2 \rangle_z^{1/2}$ obtained are plotted against the elapsed time after mixing the two solutions in Figure 5. In the $S \rightarrow V$ process, both M_w and $\langle S^2 \rangle_z^{1/2}$ abruptly increased just after changing x_+ from 0.35 to 0.55, indicating that two or three spherical micelles merge to form a larger micelle. On the other hand, in the $C \rightarrow V$ process, both M_w and $\langle S^2 \rangle_z^{1/2}$ slightly decreased just after increasing the C_{NaCl} from 0 to 0.1 M. Thus, the cylindrical micelles in the solution may have split into small micelles just after changing the C_{NaCl} . After the initial abrupt changes, M_w and $\langle S^2 \rangle_z^{1/2}$ are almost constant in both $S \rightarrow V$ and $C \rightarrow V$ processes up to $t \sim 1$ s. At t > 1 s, M_w



Figure 5. Time evolutions of the weight-average molar mass M_w (a) and z-average radius of gyration $\langle S^2 \rangle_z^{1/2}$ (b) in the S \rightarrow V (blue markers) and C \rightarrow V (red markers) processes. The leftmost points represent the data in the initial states. The error bars are not shown in the cases that they are smaller than the size of symbols.

started increasing but $\langle S^2 \rangle_z^{1/2}$ remained constant or even slightly decreased, and at $t \gtrsim 50$ s, $\langle S^2 \rangle_z^{1/2}$ tended to increase slightly, especially in the C \rightarrow V process.

The double logarithmic plots of $\langle S^2 \rangle_z^{1/2}$ vs M_w in the S \rightarrow V and C \rightarrow V processes are shown in Figure 6. Although the M_w



Figure 6. Double logarithmic plots of $\langle S^2 \rangle_z^{1/2}$ vs M_w for the polyelectrolyte complex micelle in the S \rightarrow V and C \rightarrow V processes. The error bars are not shown in the cases that they are smaller than the size of symbols.

dependencies are not so strong, data points in the $C \rightarrow V$ process in the later stage (t > 50 s) obey the line with a slope of $M_w^{0.5}$, as indicated by the dotted line. This is the slope expected for disklike micelles and vesicles. Data points in the S \rightarrow V process in the same time range follow a much weaker M_w dependence $(M_w^{0.2})$ (see below).

Figure 7 displays the Kratky plots, $\langle S^2 \rangle_z q^2 P(q)$ vs $\langle S^2 \rangle_z^{1/2} q$, for the polyelectrolyte complex micelle in the S \rightarrow V and C \rightarrow V processes, constructed by SAXS data over all q range investigated (0.01 $\leq q/\text{nm}^{-1} \leq 1$), beyond the Guinier region. Here, P(q) is the particle scattering function calculated from $d\sum/d\Omega(q)$ divided by $d\sum/d\Omega(q)$ at q = 0 (determined from the intercept of the Guinier plot, as shown in Figure 4). We



Figure 7. Time-resolved USAXS data presented as the Kratky plots in the S \rightarrow V (a) and C \rightarrow V (b) processes. See also Figure S2, which shows theoretical curves with different morphologies in Kratky plots.

could qualitatively figure out the morphological transformations by the Kratky plots; as depicted in Figure S2, the main peak height of the Kratky plot can be used to judge the micellar morphology. The peak height of the plot increased in the *t* range from 0 to 0.0025 s and then decreased with *t* in the $S \rightarrow V$ process, whereas the maximum was at t = 0 s and decreased with *t* in the $C \rightarrow V$ process. Therefore, the Kratky plots in Figure 7a,b indicate that the spherical micelles merge to form a larger cylindrical micelle in the $S \rightarrow V$ process, and cylindrical micelles split to reduce their axial ratio (aspect ratio) in the $C \rightarrow V$ process, just after changing the solution condition to (x_{+} , C_{NaCl}) = (0.55, 0.1 M).

Figure 8 shows the time evolutions of the USAXS/SAXS profiles in the S \rightarrow V and C \rightarrow V processes over all q range investigated. The oscillation above $q \sim 0.2 \text{ nm}^{-1}$ characteristic of the profiles for spherical and cylindrical micelles at t = 0 disappeared in the early stage after stopped-flow mixing. This disappearance indicates that the micellar structure rapidly changed after mixing. On the other hand, profiles at t = 42.8 s and 269 s show a new inflection point at $q \sim 0.1 \text{ nm}^{-1}$ in the S \rightarrow V process and at $q \sim 0.05 \text{ nm}^{-1}$ in the C \rightarrow V process. Moreover, the new oscillation in the S \rightarrow V process shifted to $q \sim 0.02 \text{ nm}^{-1}$ at t = 65000 s and the oscillation at $q > 0.2 \text{ nm}^{-1}$ reappeared in the late-stage profile.

The slope in the middle-q range (~0.05 nm⁻¹) reflects the morphology. In the S \rightarrow V process, the slope was almost horizontal in the initial state and became q^{-1} at t = 0.0025 s, indicating the transformation from spherical to cylindrical micelles. With time, the slope reached q^{-2} , which signifies the formation of a thin layer (i.e., disklike micelle or vesicles). Meanwhile, in the C \rightarrow V process, the slope of q^{-1} gradually changed to q^{-2} ; thus, we can infer that the cylindrical micelles became disklike micelle or vesicles.



Figure 8. Time evolutions of USAXS/SAXS profiles in the S \rightarrow V (a) and C \rightarrow V (b) processes. Solid curves represent the fitting results (see below and the Supporting Information). The data points are shifted vertically by a factor A for clarity; the A values of 1, 10, 10², 10³, 10⁴, 10⁵, and 10⁶ are used for the data at t = 0, 0.0025, 1.326, 6.766, 42.80, 268.7, and 65 000 s, respectively.

The form factor of a vesicle (eq S12) shows an inflection point in the low-q region, which enables identifying the vesicle formation. Moreover, the position (q^*) of the inflection point is related to the average of the outer and inner radii of the bilayer for the vesicle (\overline{R}) by

$$\tan(q^*\bar{R}) = q^*\bar{R} \tag{3}$$

This equation is derived from the differential of the form factor of a vesicle (eq S12). Figure 9 shows the obtained \overline{R} values as a function of time. It is worth noting that the \overline{R} values changed



Figure 9. Growth of the vesicle in the S \rightarrow V and C \rightarrow V processes, where \overline{R} is the average of the outer and inner radii of the bilayer for the vesicle, calculated from q^* (the inflection point of the USAXS profile) by eq 3. The error bars are smaller than the size of symbols.

only slowly in both $S \rightarrow V$ and $C \rightarrow V$ processes. \overline{R} became much larger at $t = 65\,000$ s, indicating that the growth of the vesicle (see Scheme 1d) takes place at a very slow rate.

Fitting of the SAXS Profiles in the S \rightarrow V and C \rightarrow V Processes. From the results of Figures 2, 3, 6, and 7, we can say that the micellar morphology changed through steps of spherical micelles \rightarrow cylindrical micelles \rightarrow disklike micelles \rightarrow vesicles in the S \rightarrow V process and through steps of cylindrical micelles \rightarrow shorter cylindrical micelles \rightarrow disklike micelles \rightarrow vesicles in the C \rightarrow V processes. The theoretical particle scattering functions for spherical, cylindrical, and disklike micelles and vesicles^{53,54} are given by eqs S1–S17 in the Supporting Information, and we can compare the experimental SAXS profiles quantitatively with the theory to determine the model parameters.

We assume that the elementary reaction steps of spherical micelles \rightarrow cylindrical micelles, cylindrical micelles \rightarrow shorter cylindrical micelles, and cylindrical micelles \rightarrow disklike micelles occur in the all-or-none fashion and then the weight-average molar masses $M_{w,C}$ of the (shorter) cylindrical micelles and $M_{w,D}$ of the disklike micelles are constant in both S \rightarrow V and C \rightarrow V processes. As shown in Figure 5, the values of M_{w} obtained by the Guinier plots are almost constant in the early stage of both S \rightarrow V and C \rightarrow V processes, so that the spherical micelle \rightarrow cylindrical micelle step and the cylindrical micelle \rightarrow shorter cylindrical micelle step have finished during the mixing prior to the beginning of the USAXS measurements, and the constant $M_{\rm w}$ values can be identified with $M_{\rm w.C}$. The $M_{\rm w,D}$ value (=4.0 × 10⁷; see Table 2) has been already determined using the USAXS profile at $t = 65\ 000\ s$ of the S \rightarrow V process shown in Figure 2a. Furthermore, we can calculate the (weight-average) molar mass $M_{w,V}$ of the vesicle by eq S14 in the Supporting Information.

USAXS profiles at t > 30 s in both S \rightarrow V and C \rightarrow V processes can be fitted by the mixture of disklike micelles and vesicles, just like that at $t = 65\,000$ s in the S \rightarrow V process shown in Figure 2a, by adjusting the time-dependent weight fraction w_D of the disklike micelles (or $w_V = 1 - w_D$). On the other hand, USAXS profiles at t < 30 s in both S \rightarrow V and C \rightarrow V processes were fitted by adjusting two time-dependent weight fractions $w_{\rm C}$ of the cylindrical micelles and $w_{\rm D}$ ($w_{\rm V} = 1$ $-w_{\rm C} - w_{\rm D}$). The theoretical scattering functions for cylindrical and disklike micelle and vesicle (eqs S1-S14) weakly depend on the dispersity of the molar mass *D*, the mean value $2\langle R_{core} \rangle$ of the hydrophobic core thickness (Figure S6). The rootmean-square radius of gyration of the corona chain $\langle S^2 \rangle_{corona}$ (=1.5 nm), the mass concentration inside the hydrophobic core domain c_{core} (=0.38 g/cm³), and the contrast ratio f_{core} (=0.87) were fixed (see the Supporting Information and ref 26). The variances σ of the hydrophobic core thickness in eq S17 were also fixed to 0.23 for all of the morphologies, which is slightly larger than those listed in Table 2.

Solid curves in Figure 8 represent the fit curves, and Figure 10 shows the main parameters obtained by the analysis of the USAXS/SAXS profiles as a function of time. In both $S \rightarrow V$ and $C \rightarrow V$ processes, w_C gradually decreased while w_V increased. That is, in the $S \rightarrow V$ processes, the spherical micelles in the initial state evolved to cylindrical micelles. The process occurred within the dead time of the stopped-flow mixing (2.5 ms), as the initial growth by electrostatic attraction is known to be quite fast in polyelectrolyte complex micelles. ^{52,55,56} These cylindrical micelles then became disklike morphology. The disklike micelles finally closed to form



Figure 10. Time evolution of the parameters obtained by the fitting of the USAXS/SAXS profiles. Weight fraction w_i (a) and weight-average molar mass $M_{w,i}$ (b) of the cylindrical micelles (\bullet), disklike micelles (\bullet), and vesicles (\blacksquare). The leftmost points represent the data in the initial states. In the initial state for the S \rightarrow V process, the morphology is spherical micelles, although the marker of " \bullet " is used. The blue and red markers represent the data in the S \rightarrow V and C \rightarrow V processes, respectively. The solid black curves in panel (a) represent the fitted model curves (eq 19). In panel (b), the error bars are smaller than the size of symbols. See also Figure S6, where $2\langle R_{core} \rangle$ values are shown as a function of *t*.

vesicles. In the $C \rightarrow V$ process, the initial cylindrical micelles similarly become disklike micelles followed by the closure of the disklike micelles to form the vesicles. These results, therefore, indicate that both the $S \rightarrow V$ and $C \rightarrow V$ processes involved the disk closure pathway (Scheme 1b). More detailed kinetic analysis is shown later. It is noted that $M_{w,V}$ in the $C \rightarrow V$ process is considerably higher than that in the $S \rightarrow V$ process. Thus, the M_w dependence of $\langle S^2 \rangle_z^{1/2}$ in Figure 6 reflects the vesicles in the $C \rightarrow V$ proves but the mixture of the vesicles and disklike micelles with different growth rates in the $S \rightarrow V$ process.

Transformation Kinetics of Micellar Morphology. As shown in Figure 10a, AP–MP mixture solutions in both $S \rightarrow V$ and $C \rightarrow V$ processes involved only the cylindrical micelles in the early stage and then the disklike micelles and the vesicles grew in the solutions, but the vesicles grew later than the disklike micelles. The weight fraction $w_{\rm D}$ of the disklike micelles increased first and then decreased over $t \gtrsim 10$ s. Both disklike micelles and vesicles remained in the solutions in the late stage. These time evolutions of $w_{\rm C}$, $w_{\rm D}$, and $w_{\rm V}$ indicate that the cylindrical micelles transformed into disklike micelles and a part of the disklike micelles converted to vesicles. As shown in Figure 10b, $M_{w,D}$ gradually increased with t in both processes in the late stage, where $w_{\rm D}$ and $w_{\rm V}$ almost reached asymptotic values, indicating that several smaller vesicles assembled to form a larger vesicle. Because the disklike micelles remained in the late stage of both processes, we have to assume that there are two components of disklike micelles, which can and cannot be transformed into vesicles. In what follows, the two components of disklike micelles are denoted D^* and D^{∞} , respectively.

From the above consideration, we assume the following elementary reaction steps in the $S \rightarrow V$ process

$$\frac{m_{\rm C}}{m_{\rm S^{\circ}}} {\rm S}^{\circ}_{m_{\rm S^{\circ}}} \rightarrow {\rm C}_{m_{\rm C}}$$

 $(spherical micelles \rightarrow cylindrical micelles) \tag{4}$

$$\frac{m_{\rm D}}{m_{\rm C}} {\rm C}_{m_{\rm C}} \xrightarrow{k_{\rm D}^{\infty}} {\rm D}_{m_{\rm D}}^{\infty} \quad (\text{cylindrical micelles} \to {\rm D}^{\infty})$$
(5)

$$\frac{m_{\rm D}}{m_{\rm C}} {\rm C}_{m_{\rm C}} \xrightarrow{k_{\rm D}^*} {\rm D}_{m_{\rm D}}^* \quad (\text{cylindrical micelles} \to {\rm D}^*)$$
(6)

$$\frac{m_{\rm V}}{m_{\rm D}} {\rm D}_{m_{\rm D}}^* \xrightarrow{k_{\rm V}} {\rm V}_{m_{\rm V}} \quad ({\rm D}^* \to {\rm vesicles})$$
(7)

where S° represents a spherical micelle (before starting the S \rightarrow V process), C is a cylindrical micelle, D[∞] is a disklike micelle that does not transform to the vesicle, D* is a disklike micelle that can be transformed into the vesicle, and V is a vesicle. m_{S° , m_C , m_D , and m_V are the aggregation numbers of the micelles indicated by the subscripts ($m_D \equiv m_D^{\infty} = m_{D^*}$). The reaction rate constant at each step is represented by k_D^{∞} , k_D^* , or k_V . It is noted that $m_D/m_C = 1.2$ and $m_V/m_D = 2.3$ from $M_{w,C} = 3.3 \times 10^7$ g/mol, $M_{w,D} = 4.0 \times 10^7$ g/mol, and $M_{w,V} = 9.0 \times 10^7$ g/mol, as seen in Figure 10b.

The step of spherical micelles to cylindrical micelles in the early stage was finished in the mixing process before starting USAXS/SAXS measurements (at t < 0.0025 s) in the present study, and we cannot discuss these steps. It is worth noting that the spherical micelles \rightarrow cylindrical micelles step was very slow at $C_{\text{NaCl}} = 0.01$ M, as demonstrated in the previous study.²⁸ The screening effect on electrostatic repulsion among weakly charged spherical micelles at $C_{\text{NaCl}} = 0.1$ M may speed up the spherical micelles \rightarrow cylindrical micelles step.

The kinetic equations for the reaction steps given by eqs 5-7 can be written as

$$\frac{\mathrm{d}[C_{m_{\rm C}}]}{\mathrm{d}t} = -(k_{\rm D}^{\infty} + k_{\rm D}^{*})[C_{m_{\rm C}}]^{m_{\rm D}/m_{\rm C}}$$
(8)

$$\frac{\mathrm{d}[D_{m_{\mathrm{D}}}^{\omega}]}{\mathrm{d}t} = k_{\mathrm{D}}^{\infty} \frac{m_{\mathrm{C}}}{m_{\mathrm{D}}} [C_{m_{\mathrm{C}}}]^{m_{\mathrm{D}}/m_{\mathrm{C}}}$$

$$\tag{9}$$

$$\frac{d[D_{m_{\rm D}}^*]}{dt} = k_{\rm D}^* \frac{m_{\rm C}}{m_{\rm D}} [C_{m_{\rm C}}]^{m_{\rm D}/m_{\rm C}} - k_{\rm V} [D_{m_{\rm D}}^*]^{m_{\rm V}/m_{\rm D}}$$
(10)

$$\frac{d[V_{m_{\rm V}}]}{dt} = k_{\rm V} \frac{m_{\rm D}}{m_{\rm V}} [D_{m_{\rm D}}^*]^{m_{\rm V}/m_{\rm D}}$$
(11)

where [...] denotes the molar concentration of the component indicated in the parentheses, $m_D/m_C = 1.2$, and $m_V/m_D = 2.3$. Solving the differential eqs 8 and 9, we have

$$\begin{bmatrix} C_{m_{\rm C}} \end{bmatrix} = \left[(k_{\rm D}^{\infty} + k_{\rm D}^{*}) \frac{m_{\rm D} - m_{\rm C}}{m_{\rm C}} t + \left(\frac{M_{\rm w,C}}{c} \right)^{(m_{\rm D} - m_{\rm C})/m_{\rm C}} \right]^{-m_{\rm C}/(m_{\rm D} - m_{\rm C})}$$
(12)

$$[D_{m_{\rm D}}^{\infty}] = \frac{k_{\rm D}^{\infty}m_{\rm C}}{(k_{\rm D}^{\infty} + k_{\rm D}^{*})m_{\rm D}} \left(\frac{c}{M_{\rm w,C}} - [C_{m_{\rm C}}]\right)$$
(13)

(5')

where $M_{w,C}$ is the weight-average molar mass of the cylindrical micelles (=3.3 × 10⁷ g/mol). The differential equations (eqs 10 and 11) can be solved by numerical integration to obtain $[D_{m_0}^*]$ and $[V_{m_v}]$.

In the $C \rightarrow V$ process, the following reaction steps occur

$$\frac{m_{\rm C}}{m_{\rm C^{\circ}}} {\rm C}^{\circ}_{m_{\rm C^{\circ}}} \to {\rm C}_{m_{\rm C}}$$

(scission of cylindrical micelles in the early stage) (4')

$$C_{m_{C}} \xrightarrow{k_{D}^{\infty}} \frac{m_{C}}{m_{D}} D_{m_{D}}^{\infty}$$

(cylindrical micelles $\rightarrow D^{\infty}$ in the intermediate stage)

$$C_{m_{\rm C}} \xrightarrow{k_{\rm D}^*} \frac{m_{\rm C}}{m_{\rm D}} D_{m_{\rm D}}^*$$

(cylindrical micelles $\rightarrow D^*$ in the intermediate stage) (6')

where C° and $m_{C^{\circ}}$ are the cylindrical micelle (before starting the $C \rightarrow V$ process) and its aggregation number, respectively. The "D* \rightarrow vesicle" and "vesicle growth" steps are the same as the S \rightarrow V process. It is noted that m_C is larger than m_D in the C \rightarrow V process, opposite to the S \rightarrow V process, as seen in Figure 10. It is worth noting that the average molar mass of the cylindrical micelle in the equilibrium state is known to be proportional to the square root of the surfactant (copolymer) concentration. Thus, m_C must be smaller than $m_{C^{\circ}}$ because the original copolymer solution was diluted in the stopped-flow experiment (from c = 0.01 to 0.005 g/cm³).

The kinetic equations for the reaction steps given by eqs 5' and 6' are written as

$$\frac{d[C_{m_{\rm C}}]}{dt} = -(k_{\rm D}^{\infty} + k_{\rm D}^{*})[C_{m_{\rm C}}]$$
(14)

$$\frac{\mathrm{d}[\mathrm{D}_{m_{\mathrm{D}}}^{\infty}]}{\mathrm{d}t} = k_{\mathrm{D}}^{\infty} \frac{m_{\mathrm{C}}}{m_{\mathrm{D}}} [C_{m_{\mathrm{C}}}]$$
(15)

$$\frac{d[D_{m_{\rm D}}^*]}{dt} = k_{\rm D}^* \frac{m_{\rm C}}{m_{\rm D}} [C_{m_{\rm C}}] - k_{\rm V} [D_{m_{\rm D}}^*]^{m_{\rm V}/m_{\rm D}}$$
(16)

where $m_C/m_D = 1.3$ and $m_V/m_D = 5.4$. Solving the differential equations (eqs 14 and 15), we have

$$[C_{m_{\rm C}}] = \frac{c}{M_{\rm w,C}} \exp[-(k_{\rm D}^{\infty} + k_{\rm D}^{*})t]$$
(17)

$$[D_{m_{\rm D}}^{\infty}] = \frac{k_{\rm D}^{\infty}}{k_{\rm D}^{\infty} + k_{\rm D}^{\ast}} \left(\frac{c}{M_{\rm w,D}} - [C_{m_{\rm C}}] \right)$$
(18)

where $M_{\rm w,C}$ and $M_{\rm w,D}$ are the weight-average molar mass of the cylindrical micelles (=5.0 × 10⁷ g/mol) and disklike micelles (=4.0 × 10⁷ g/mol), respectively. The differential equations (eqs 16 and 11) can be solved by numerical integration to obtain $[D_{m_{\rm D}}^*]$ and $[V_{m_{\rm V}}]$.

The solid black curves in Figure 10a indicate fitting results for $w_{\rm C}$, $w_{\rm D}$, and $w_{\rm V}$ by eqs 10–13 and 16–18, where $w_{\rm C}$, $w_{\rm D}$, and $w_{\rm V}$ are related to $[C_{m_{\rm C}}]$, $[D_{m_{\rm D}}^{\infty}]$, $[D_{m_{\rm D}}^{\ast}]$, and $[V_{m_{\rm V}}]$ by

$$w_{\rm C} = \frac{M_{\rm w,C}}{c} [C_{m_{\rm C}}], w_{\rm D} = \frac{M_{\rm w,D}}{c} ([D_{m_{\rm D}}^{\infty}] + [D_{m_{\rm D}}^{*}]),$$
$$w_{\rm V} = \frac{M_{\rm w,V}}{c} [V_{m_{\rm V}}]$$
(19)

The rate constants used for fitting are listed in Table 3. The theoretical curves fit to the experimental results satisfactorily, demonstrating the validity of our elementary reaction scheme for the micellar morphology transformation.

Table 3. Rate Constants in the Fitting to W_{cyb} W_{disk} , and W_{ves} in Figure 10a

	k_{D}^{∞}	$k_{\rm D}^{*}$	$k_{ m v}$
$S \rightarrow V$ process	0.16	0.11	10 ¹¹
$C \rightarrow V$ process	0.15	0.09	10 ⁴⁷

Here, we discuss the reason why the two types of disklike micelles are present (the stable disk D^{∞} and unstable disk D^*). D* is transformable to the vesicle, although we treated the disklike micelle as a single component in USAXS/SAXS profile fitting. As shown in Scheme 1b, the vesicles may be formed through the bowl-like micelles, 42,57 but the USAXS/SAXS profiles of the planar disklike micelles and bowl-like micelles are difficult to distinguish each other (see Figure S5). Moreover, the USAXS/SAXS profile of the disklike micelles is insensitive to dispersity of the molar mass, even if the disklike micelles have a bimodal distribution (Supporting Information). Thus, it should be difficult to distinguish the planar disklike and bowl-like morphologies by USAXS/SAXS. However, the slight differences in the molar mass and topology might influence the vesicle formation process. In addition, the kinetically trapped states have been often observed in polyelectrolyte complex micelles,⁵⁶ compared with conventional neutral micelles. In particular, in our previous study concerning the spherical micelles to cylindrical micelles bearing the polyelectrolyte complex core,²⁸ the growth of cylindrical micelles ceased and short cylindrical micelles remained for a long time. Therefore, in the present study, a part of the disklike micelles (D*) could be kinetically trapped at a certain size and remained for a long time.

 $M_{w,V}$ and k_V are different in the S \rightarrow V and C \rightarrow V processes, as seen from Figure 10b and Table 3. These differences imply that D* formed in the two processes should not be identical, although the fitting of the SAXS profiles provided the same $M_{w,D}$ and \mathcal{D}_D in the two processes. It may be difficult to distinguish the differences in M_w of D* in the two processes due to the insensitivity of \mathcal{D}_D in the USAXS/SAXS profiles. (We have to characterize the disklike micelles in more detail by some other techniques than SAXS to distinguish between D[∞] and D*.)

The behavior of the vesicle formation here is different from that found in mixtures of low-molecular-weight zwitterionic and anionic surfactants.³⁶ In those cases, the radius of the disklike micelles formed at an earlier stage gradually evolved following the single exponential function where the disk thickness was ca. 4 nm. The disklike micelles have surface charges, which may have stabilized them for a longer period. Once the disk radius reached the threshold values (~12–15 nm), the instability of the edge caused the closure of the disklike micelles to form the vesicles. In contrast, in the present case, the value of $M_{\rm w,disk} = 4 \times 10^7$ g/mol (the disk radius is ca. 50 nm) should be already at a threshold value for closure.

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According to the literature, the balance of the edge energy and bending energy determines the maximum disk radius (R_{MAX}) as follows^{14,36,37,58,59}

$$R_{MAX} \sim \frac{4(2\kappa + \bar{\kappa})}{\Lambda}$$
(20)

where κ and $\overline{\kappa}$ denote the mean bending modulus and the saddle-splay modulus of the bilayer and Λ is the line tension of the edge (related to the unfavorable edge energy). In lowmolecular-weight surfactants, the $2\kappa + \overline{\kappa}$ value was $3-5 k_{\rm B}T$ (where $k_{\rm B}T$ is the Boltzmann constant multiplied by the temperature). The thickness of the bilayer in the present vesicles is 4 times larger than that in the low-molecular-weight surfactants, and the bending rigidity is proportional to the square of the thickness. Thus, in the present case, the rigidity of the bilayer is approximately 4² times larger,⁶⁰ assuming that the rigidity per unit volume is the same. Meanwhile, Λ almost linearly increases with the bilayer thickness. Consequently, the R_{MAX} values can be 4 times larger than the low-molecularweight surfactant case (i.e., 48-60 nm) following these assumptions. In fact, when the $M_{\rm w,disk}$ is 4 \times 10⁷ g/mol, the disk radius is 50 nm, which may correspond to R_{MAX}. Hence, it is presumed that the size of disklike micelles was already the threshold value (the radius of 50 nm and $M_{\rm w,disk} = 4 \times 10^7 \text{ g/}$ mol) at the nascence and then a part of the disklike micelles could get over the energy barrier to become bowl-like micelles and then vesicles. Here, 2.3 and 5.4 disklike micelles fused into one vesicle in the S \rightarrow V and C \rightarrow V processes, respectively.

In Figure 10b, $M_{w,V}$ slightly increased with time for $t \gtrsim 100$ s and more than 10 times at $t = 65\,000$ s, while w_V stayed constant until the late stage. Similar growth of vesicles at a slow late was observed in low-molecular-weight surfactants.³ Regarding the mechanism of the vesicle growth in the present study, we have three scenarios: the first is the fusion and/or Ostwald ripening between the vesicle and disklike micelle, but $w_{\rm D}$ and $w_{\rm V}$ remained unchanged, and thus, this process would not occur. The second scenario is the fusion between the vesicles,⁴³ but $M_{w,V}/M_{n,V}$ were maintained to be 1.5. If the fusion took place, $M_{\rm w,V}/M_{\rm n,V}$ would become close to 2. The third scenario is the Ostwald ripening among vesicles. Although the experimentally obtained data do not directly demonstrate this Ostwald ripening of the vesicles (the breakup of small vesicles via Ostwald ripening could not be detected), the data are consistent with this process.

CONCLUSIONS

We monitored the vesicle formation in mixtures of cationic– neutral and anionic–neutral block copolymers in aqueous NaCl solutions by time-resolved USAXS/SAXS. In particular, we examined the x_+ -induced transformation from spherical micelles to vesicles (S \rightarrow V) and the C_{NaCl} -induced transformation from cylindrical micelles to vesicles (C \rightarrow V). The analysis elucidated that the transformations of spherical micelles \rightarrow cylindrical micelles \rightarrow disklike micelles \rightarrow vesicles were involved in the S \rightarrow V process, while the C \rightarrow V process followed the transformations sequence cylindrical micelles \rightarrow disklike micelles \rightarrow vesicles.

The polymeric bilayer membrane is thick, and the bending rigidity is ca. 16 times higher than vesicle membranes formed from low-molecular-weight surfactants. For the transformation from the disklike micelles to the vesicles, the bowl-like structure should be formed as an intermediate, but due to the high bending rigidity, the population of the bowl-like structure during the transformation was negligibly low. Further, the size of disklike micelles was already at a threshold value for closure upon nascence (at the transition point from the disklike micelles to the vesicles). That is, the formation of bowl-like structure was the key process; once the bowl-like structure was formed, it quickly converted to vesicles. A part of the disklike micelles could not overcome this energetic barrier and present for a long time ($t = 65\,000\,$ s).

These insights should be helpful in designing the preparation of vesicles *via* the facile manner toward applications, including drug loading into vesicles. More specifically, it is possible that the high edge and bending energy make the vesicle formation difficult; thus, a molecular design to reduce the energies may be effective to prepare the polymer vesicles. The disk closure pathway identified in this study leads to the high loading efficiency of hydrophilic molecules compared with the other pathways, which is of practical advantage. Regarding the growth behavior of the vesicles at the late stage (t = 300- $65\,000$ s), further experiments are needed to elucidate the detailed mechanisms; future work will be focused on the latestage kinetics.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.macromol.1c02210.

SEC data, scattering form factors of each morphology, dispersity effect on the form factor of disklike micelles, and comparison of the form factor between the disklike and bowl-like micelles (PDF)

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Notes

The authors declare no competing financial interest.

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