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Chaperone-like α-cyclodextrins assisted self-assembly of double hydrophilic block copolymers in aqueous medium

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ABSTRACT

Chaperones are defined as a family of protein that mediates the correct assembly of other polypeptides but that are not components of the functional assembled structures. In this work, we have devised a novel method in which α-cyclodextrins (α-CDs), "artificial chaperones", facilitate block copolymers self-assembling into the expected structure. Poly(ethylene oxide)-*b*-poly(4-vinylpyridine) (PEO_{45}-*b*-$P4VP_{70}$) is first threaded by α-CDs, leading to the formation of metastable micelles. After stabilizing the metastable micelles by shell cross-linking with poly(ethylene oxide)-*b*-poly(acrylic acid) (PEO_{114}-*b*-PAA_{50}), α-CDs are removed and the expected structure, polymeric vesicle, is achieved. On the contrary, only spherical micelles are formed in the similar conditions without the assistance of α-CD. Therefore, α-CDs, which act as chaperones, guide the self-assembly of block copolymers in the expected pathway.

1. Introduction

Self-assembly is a class of spontaneously organizing processes of molecular units, which exists widely in both biology systems and chemistry/material science. In biology system, there are two types of protein self-assembly, strict self-assembly and assisted self-assembly. In the assisted self-assembly, the appropriate molecular chaperone is required in addition to the primary structure to allow correct assembly to predominate over incorrect assembly [1]. Molecular chaperones are defined as a family of protein that mediate the correct assembly of other polypeptides but that are not components of the functional assembled structures [2–4]. Their roles can be characterized as preventing perturbations of the main protein folding and assembling pathways by stabilizing the metastable conformations, thereby increasing the yields of correct substrate proteins. Many proteins are unable to attain their correct structures without such external assistances. For example, the chaperonin 60 of *Escherichia coli* is required for the assembly of bacteriophages lambda, T4 and T5. In the absence of chaperonin 60, non-functional large insoluble aggregates will be formed [5,6]. Therefore, chaperone has become a biotechnology tool to assist the correct folding and assembling of proteins [7–9].

Like proteins, block copolymers can also self-assemble into various three-dimensional structures in specific conditions [10,11]. In the past decade, self-assembly of block copolymers has been extensively studied and several pioneering groups have developed diverse methods to form polymeric micelles with various structures and morphologies [12–21]. However, most of these self-assembling processes are dominated only by the primary structures and the properties of block copolymers, which are much similar to the strict self-assembly of proteins in biology systems. With the development of nanotechnology, self-assembly of more accurate and functional polymeric micelles is highly demanded. Classic methods of self-assembly of block copolymers cannot meet these demands because the misformation, which exists extensively in the self-assembling process of block copolymers, leads to the unexpected structures of final nanoparticles. Chaperones, which assist in the folding and assembling process by stabilizing metastable conformations of protein on the way to its correct conformation but do not exist in final structures [22,23], inspire us a solution to the problem. We believe that the self-assembly of block copolymers can be assisted by some chaperone-like molecules, which may be a feasible pathway to the construction of micelles with specific structure.

Cyclodextrins are cycle oligosaccharides, which have a torus-like conformation with a hydrophilic outer surface and a hydrophobic cavity. The cavity can act as a host for a great variety of molecular guests through non-covalent interactions [24,25]. In biology researches, cyclodextrins have been widely used [26–29] to renature proteins in vitro as part of the artificial chaperone system that was first reported by Rozema and Gellman [30]. Recently, Chen's [17,21] and Tam's [19] groups have performed some novel

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kinds of self-assembly of block copolymers which are induced by α-cyclodextrin (α-CD) due to the abilities to form insoluble inclusion complex between poly(ethylene oxide) and α-CD [31–34]. However, self-assembly of block copolymers which assisted by "artificial chaperone" was seldom reported. In this work, we describe an example of assisted self-assembly of block copolymer, where small molecules α-CDs act as molecular chaperones in the formation of polymeric vesicles composed of poly(ethylene oxide)-*b*-poly(4-vinylpyridine) (PEO_{45}-*b*-$P4VP_{70}$) and poly(ethylene oxide)-*b*-poly(acrylic acid) (PEO_{114}-*b*-PAA_{50}) in aqueous media. Our synthetic strategy is shown in Fig. 1. In the presence of chaperones, vesicles, which use P4VP/PAA as wall and PEO chains as coronas, are achieved. In the absence of chaperones, formation of spherical micelles using P4VP/PAA as core and PEO chains of double hydrophilic block copolymers (DHBCs) as shell is observed, which may be driven by strong interactions between P4VP and PAA chains [35].

2. Experimental section

2.1. Materials

Two kinds of poly(ethylene oxide) end-capped with monomethyl ethers (PEO), $M_w = 5000$ (the polydispersity index PDI = 1.05) and $M_w = 2000$ (PDI = 1.04), were purchased from Fluka. 4-vinyl pyridine (4VP) and *tert*-butyl acrylate (*t*BA) were purchased from Aldrich and purified by vacuum distillation. α-cyclodextrin (α-CD) was purchased from Acros Organics and used as-received. De-ionized water was used in all experiments.

2.2. Synthesis and characterization of block copolymers

Block copolymer PEO-*b*-P4VP was synthesized by atom transfer radical polymerization (ATRP) of 4-vinylprydine with PEO_{45}–Br as the macroinitiator and CuCl/Me_6TREN (tri[2-(dimethylamino)-ethyl]amine) as the catalyst [36]. PEO-*b*-PAA was achieved by hydrolysis of PEO-*b*-PtBA with TFA (trifluoroacetic acid). Block copolymer PEO-*b*-PtBA was synthesized by ATRP of *tert*-butyl acrylate with PEO_{114}–Br as the macroinitiator and CuCl/PMDETA (*N*,*N*,*N*′,*N*′,*N*-pentamethyldiethylenetriamine) as the catalyst [37]. These block copolymers can be denoted as PEO_{45}-*b*-$P4VP_{70}$ and PEO_{114}-*b*-PAA_{50} where the subscripts indicates the number of repeating units which were determined by ^{1}H NMR. The polydispersity indexes (PDIs) of PEO_{45}-*b*-$P4VP_{70}$ and PEO_{114}-*b*-$PtBA_{50}$ were 1.28 and 1.13, respectively. All PDIs were characterized on a Waters 600E gel permeation chromatography (GPC) analysis system, where narrow-polydispersity polystyrene was used as a calibration standard and $CHCl_3$ and THF were used as eluents respectively. Detailed methods of synthesis and characterization of block copolymers can be found in Supporting information.

2.3. Chaperone-assisted self-assemble micellization of block copolymers

The procedure of chaperone assisted self-assembly was achieved in three steps. First, the solution of PEO-*b*-P4VP (pH = 4, 4×10^{-4} g/ml, 5 ml) and α-CD (pH = 4, 1.2×10^{-3} g/ml, 5 ml) were mixed (1:1, v/v) by the addition of α-CD solution dropwise into copolymer solution with vigorous stirring, followed by immersing into ultrasonic bath for 10 min. The mixed solution was kept at room temperature for 24 h to allow the complete formation of metastable micelles. Second, PEO-*b*-PAA (pH = 4, 4×10^{-4} g/ml, 3 ml) was added into the solution of the metastable micelles gradually to give a shell cross-linking degree of ca. 50%. Finally, chaperone molecule α-CDs were removed by dialyzing the SCL micelle solution against acid water (pH = 4) at 50 °C for 3 days. The final micelle solution was collected and stored at room temperature (ca. 20 °C) for further characterizations.

2.4. ^{1}H NMR, 2D NOE characterization and TEM, light scattering measurements

^{1}H NMR and two-dimensional nuclear overhauser effect (2D NOE) spectra were recorded on a Varian UNITY-plus 400 spectrometer. Dynamic light scattering (DLS) measurement was performed on laser light scattering spectrometer (BI-200SM) equipped with a digital correlator (BI-9000AT) at 636 nm at given temperatures. All samples for DLS was prepared by filtering 2 ml of aqueous solutions through 450 nm Millipore filters. Transmission electron microscopy (TEM) measurements were performed with a commercial Philips T20ST electron microscope at an acceleration voltage of 200 kV. To prepare the TEM samples, a small drop of micelle solutions was deposited onto a carbon-coated copper electron microscopy (EM) grid and then dried under room temperature at atmospheric pressure.

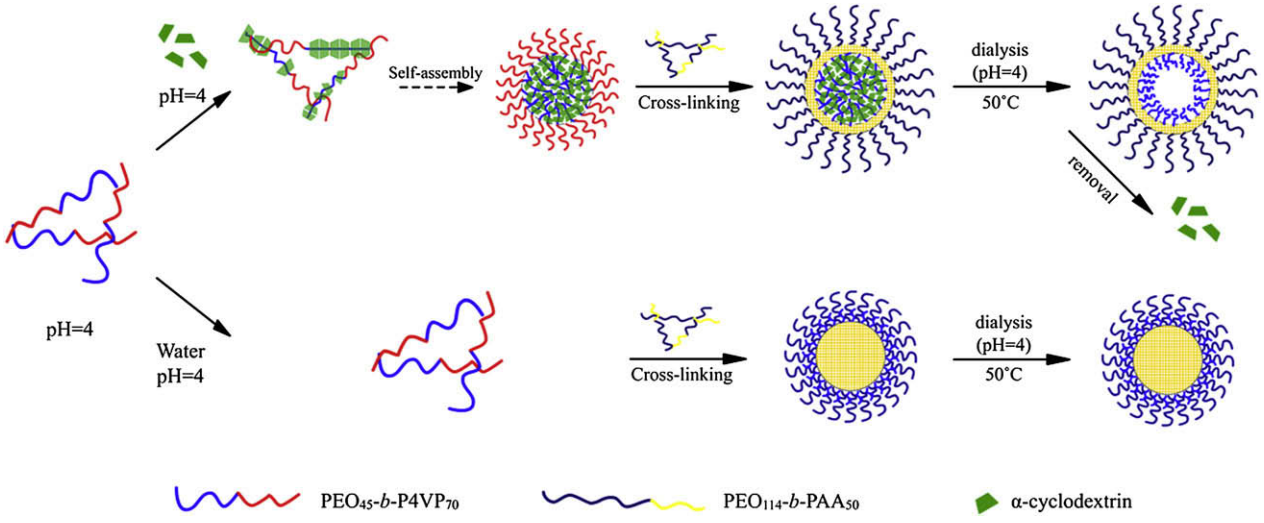

Fig. 1. Schematic presentation of self-assembly process of PEO-*b*-P4VP and PEO-*b*-PAA with/without the assistance of molecular "chaperones" α-cyclodextrins.

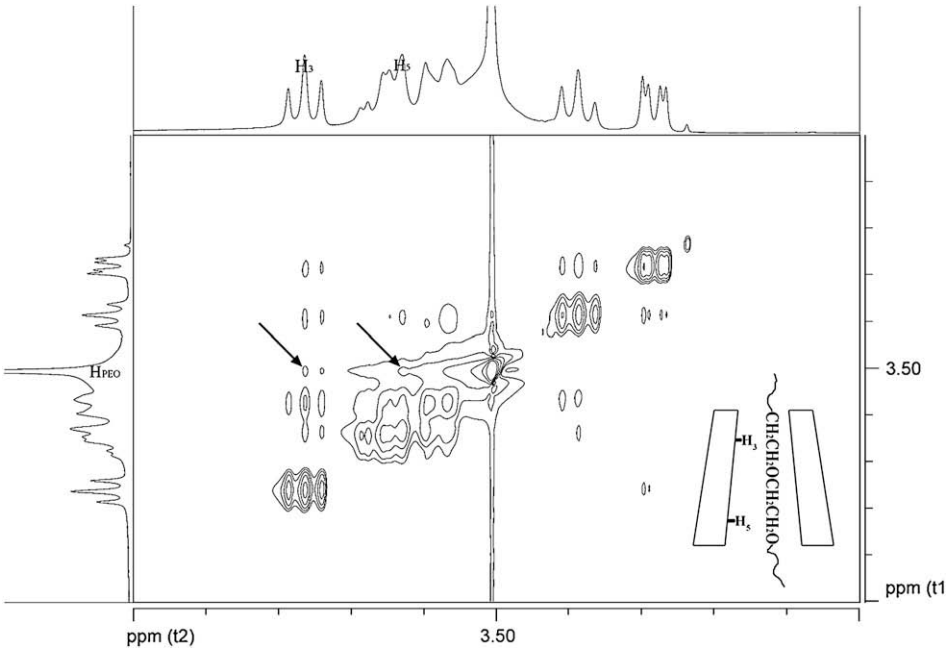

Fig. 2. Portion of 400 MHz NOESY Spectrum of PEO-*b*-P4VP/alpha-cyclodextrin complex micelles in DMSO-d_6, with mixing times of 600 ms.

3. Results and discussion

As described in the experiment part, the PEO_{45}-*b*-$P4VP_{70}$ metastable micelles were prepared by adding the solution of α-CD into diblock copolymer PEO-*b*-P4VP solution. Two solutions were mixed in a final volume ratio of 1:1. In the mixed solution, the concentrations of PEO-*b*-P4VP copolymers and α-CDs changed to 2×10^{-4} g/ml and 6×10^{-4} g/ml while the molar ratio of EO units to α-CDs was ca. 2.0.

According to Harada's literatures [31,34], less hydrophilic inclusion complexes (ICs), pseudopolyrotaxanes, were obtained when the aqueous solution of PEO was added to α-CD solution. Thus, α-CDs were expected to thread onto PEO chains of PEO-*b*-P4VP block copolymer to form pseudopolyrotaxane-*b*-P4VP in the mixed solution. In order to prove the formation of PEO/α-CD complexes, the mixed solution was freeze-dried first and then dissolved in DMSO-d_6 for two-dimensional proton nuclear overhauser effect (2D NOE) experiments. The NOESY Spectrum (Fig. 2) demonstrated correlation peaks between protons inside α-CD's cavities and those of EO units, indicating both the occurrence of α-CDs threading onto PEO chains and the formation of pseudopolyrotaxane-*b*-P4VP.

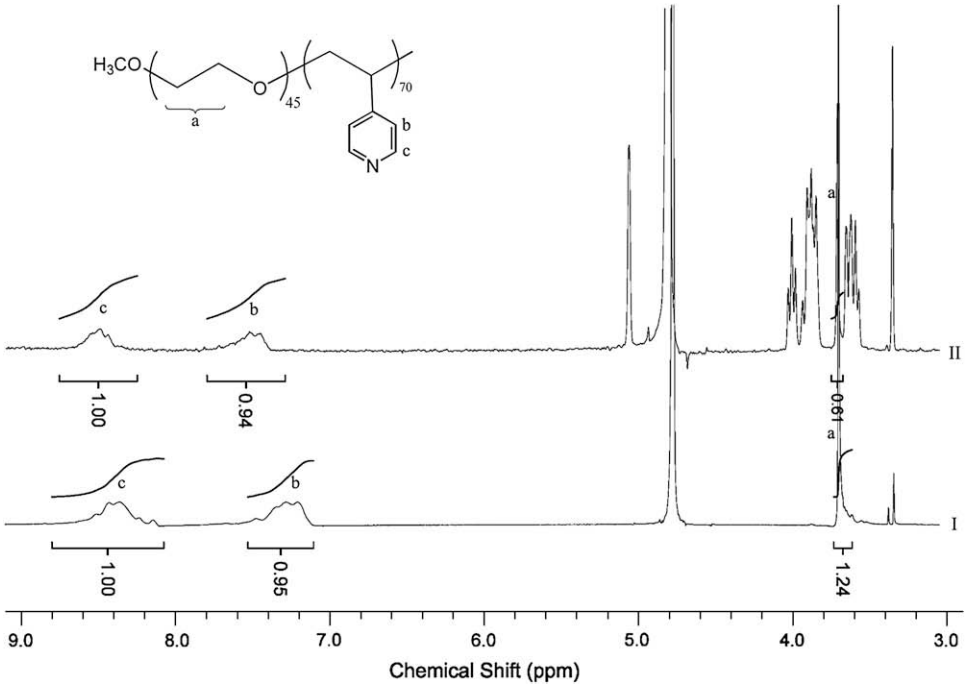

Fig. 3. ^{1}H NMR Spectrum of PEO_{45}-*b*-$P4VP_{70}$ block copolymers (I) and micelles formed in the PEO-*b*-P4VP/α-CD solution (II) recorded in D_2O at pH = 2 and 25 °C.

In aqueous media at pH 4, PEO-*b*-P4VP block copolymers were dissolved molecularly due to the protonation of P4VP. After the formation of pseudopolyrotaxane by adding α-CDs, the PEO/α-CD IC chains became less hydrophilic so that they were no longer dissolved in water. Considering the mixed solution of our experiment, pseudopolyrotaxane-*b*-P4VP should self-assemble into core-shell micelles, which use PEO/α-CD ICs as core and P4VP chains as shell. Dynamic light scattering (DLS) experiments indicated a number-average diameter of 34.8 nm at 25 °C, confirming the formation of micelles. Besides, the transmission electron microscopy (TEM) image (Fig. 4A) also showed a well-defined and spherical morphology for dried micelles with diameters ranging from 25 to 30 nm, which were in good agreement with those obtained by DLS (see Supporting information). Furthermore, the core-shell structure of micelles was determined by ^{1}H NMR analysis as shown in Fig. 3. Spectrum I and Spectrum II were recorded for pure PEO-*b*-P4VP block copolymers and micelles formed in mixed solution in D_2O at pH 2 respectively. The molar composition of PEO and P4VP chains could be determined from the relative intensities at 3.7 ppm, which was attributed to the $-CH_2CH_2O$ protons of PEO, and those around 7.5 ppm and 8.5 ppm, corresponding to the pyridyl protons of P4VP chains. Compared to Spectrum I, the intensity ratio of PEO chains to P4VP chains decreased significantly in Spectrum II, indicating that the mobility of part of PEO chains had been restricted seriously due to the formation of insoluble pseudopolyrotaxane. Besides, this fact also confirmed the formation of the micelle structure with pseudopolyrotaxane core surrounded by P4VP shell.

Additionally, it is worth noting that the threading process of α-CD onto PEO chains was reversible and the dissociate process can be easily achieved by heating [31]. This is a valuable feature for α-CD acting as the molecular chaperone in our system due to the possibility to be removed after assisting the self-assembly. Thus, we believe that micelles formed in the mixed solution are metastable and are expected to dissociate by heating the solution to 50 °C, since pseudopolyrotaxane chains will dissociate and then block copolymers, PEO-*b*-P4VP, become soluble again at high temperature. In order to prove our presumption, a set of simple experiments was performed by heating the mixture to 50 °C for 2 h. DLS study (also performed at 50 °C) could neither give a correlation curve nor determine the value of efficient diameter, indicating that no micelles could be detected in the mixed solution at 50 °C. In other words, metastable micelles dissociated at high temperature, as was expected.

Shell cross-linking (SCL) of metastable micelles was achieved by adding the solution of PEO_{114}-*b*-PAA_{50} block copolymer dropwise. The amount of PEO-*b*-PAA solution was controlled carefully so as to limit the ratio of AA units to pyridyl units of PEO-*b*-P4VP less than 50%, which could both minimize intermicelle fusion and avoid the cross-linked PAA/P4VP layers becoming too dense for internal

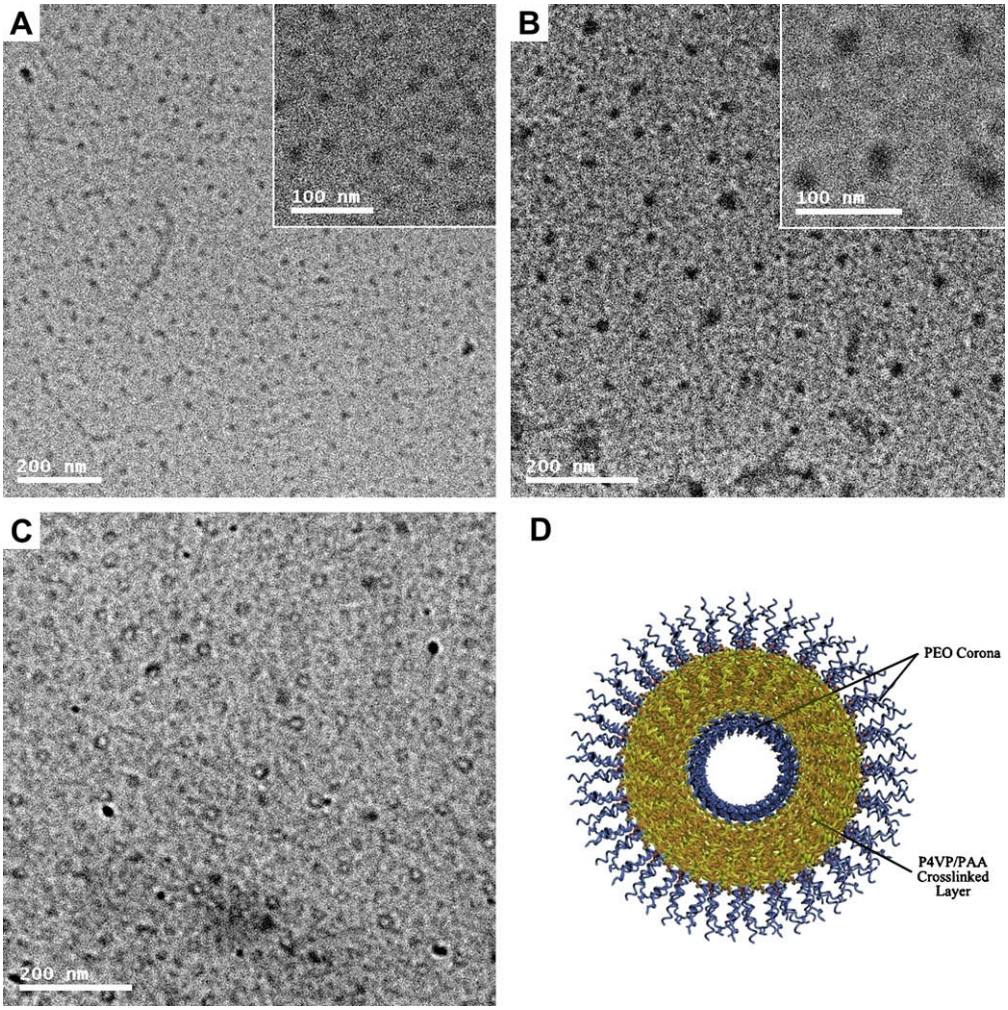

Fig. 4. TEM images of PEO_{45}-*b*-$P4VP_{70}$/α-CD metastable micelles (A), the PEO_{114}-*b*-PAA_{50} SCL micelles (B), final vesicles (C) and proposed structure of final vesicle using cross-linked P4VP/PAA layer as wall and PEO as both inner and outer coronas (D). The number-average diameters were 30 nm (A), 36 nm (B) and 34 nm (C), respectively.

α-CDs to release. Not only could longer PEO ($M_n = 5000$) enhance the stability of final structures, but also minimize the formation of pseudopolyrotaxanes between outer PEO chains of SCL micelles and free α-CDs in the mixed solution due to the slow rate of PEO/α-CD IC formation when the molecular weight of PEO is more than 3000 [38]. After cross-linking, the solution was kept overnight for further characterizations. DLS study indicated a number-average micelle diameter of 43.5 nm as well as TEM image (Fig. 4B) showed a spherical morphology of dried cross-linked micelles with diameters ranging from 30 to 37 nm. Those diameters were somewhat larger than those of uncross-linked micelles, which should be ascribed to the outer coronal PEO chains of PEO-*b*-PAA block copolymers.

After stabilizing the metastable micelles by SCL process, we removed the chaperone-like molecules, α-CDs, from the micelle core to achieve the final vesicle morphology. The method was similar as the dissociation of metastable micelles. The cross-linked micelles were dialysed against acid water (pH = 4) at 50 °C for 3 days. At this temperature, α-CDs in the micelle core should slip away from PEO chains, resulting in the solubilization of the micelle core and then the formation of vesicles with P4VP/PAA cross-linked layer as wall and PEO chains as both inner and outer coronas. TEM images confirmed our presumption, indicating nanoparticle diameters of about 34 nm with well-defined vesicle morphologies (Fig. 4C and D). To our surprise, the diameter of final vesicles is nearly the same as that of the cross-linked micelles, instead of swelling after solubilization of the hydrophobic core. We believed this unusual phenomenon was caused by the rigidity of pseudopolyrotaxane. Before the removal of α-CD, the P4VP/PAA cross-linked layers were fully extended due to the support of relative rigid pseudopolyrotaxanes. Thus, the cross-linked layers will no longer swell after the solubilization of micelle cores. Besides, both inner and outer PEO chains of final micelles are both hydrophilic, therefore, the cross-linked layers will not collapse and the particles could still disperse well and keep their vesicle morphology in aqueous media (pH = 4).

As we mentioned at the beginning of this work, chaperones prevent proteins from misassembling by stabilizing their metastable conformations in biological system. In our experiment, α-CD acts as a chaperone-like molecule in the whole process of self-assembly. At the beginning, α-CDs stabilize metastable micelles by in situ formation of hydrophobic pseudopolyrotaxanes with PEO chains of block copolymer PEO-*b*-P4VP. It is worth emphasizing that the metastable micelles act as templates directing the self-assembly process to follow the expected way in the formation of the final vesicle morphology. After stabilizing the metastable structure by shell cross-linking, α-CDs can be easily removed by dialysis at high temperature and then the final structure, vesicle, was formed. Although the driving forces of the formation of vesicle morphology are still the interactions between P4VP and PAA polyelectrolytes, the whole process of self-assembly is guided by chaperone-like molecules α-CDs, which is much different from other self-assembly process reported previously. Most importantly, some novel structures, which can be easily formed via chaperone assisted way, are difficult to form via self-assembly without chaperones in the same conditions.

In order to demonstrate the significant effects of α-CD in the process of self-assembly, a set of simple comparative experiments was performed in almost the same conditions as the chaperone assisted self-assembly process, except the addition of pure acid water (pH = 4) instead of α-CD solution at the first step (Fig. 1). The final solution was determined by TEM. As expected, only spherical micelles were observed in the TEM image (see Supporting information). We believed that the spherical micelles consisted of P4VP/PAA complex as core and PEO chains as shell due to the strong interactions between P4VP and PAA chains. Clearly, vesicles cannot be formed in this condition without the assistance of α-CDs. In other words, chaperone-like α-CDs suppressed the misformation in the self-assembly process.

4. Conclusion

In summary, we have demonstrated a novel example of assisted self-assembly of block copolymer, where α-CDs act as chaperones in the self-assembly of block copolymers in aqueous media. It is worth emphasizing that the final structure, polyelectrolyte vesicle, is formed by ordinary DHBCs in mild conditions and this structure cannot be achieved in ordinary self-assembly method without assistance. Furthermore, the results described in this article provide a novel principle that potentially widens the scope of self-assembly of block copolymers.

Acknowledgements

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Appendix. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.polymer.2008.12.005.

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