Self-Assembly of Polypeptide-Containing ABC-Type Triblock Copolymers in Aqueous Solution and Its pH Dependence

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Self-assembling of novel biodegradable ABC-type triblock copolymer poly(ethylene glycol)-poly(L-lactide)-poly(L-glutamic acid) (PEG-PLLA-PLGA) is studied. In aqueous media, it self-assembles into a spherical micelle with the hydrophobic PLLA segment in the core and the two hydrophilic segments PEG and PLGA in the shell. With the lengths of PEG and PLLA blocks fixed, the diameter of the micelles depends on the length of the PLGA block and on the volume ratio of H_2O/dimethylformamide (DMF) in the media. When the PLGA block is long enough, morphology of the self-assembly is pH-dependent. It assembles into the spherical micelle in aqueous media at pH 4.5 and into the connected rod at or below pH 3.2. The critical micelle concentration (cmc) of the copolymer changes accordingly with decreasing solution pH. Both aggregation states can convert to each other at the proper pH value. This reversibility is ascribed to the dissociation and neutralization of the COOH groups in the LGA residues. When the PLGA block is short compared to the PEG or PLLA block, it assembles only into the spherical micelle at various pH values.

Introduction

In the past few years, increasing interest has been given to the self-assembly of block copolymers in aqueous solution, because of their potential applications in nanoscience and nanotechnology, such as carriers for drug and gene delivery, diagnostic imaging, and nanoreactors.[1−4] Compared to low molecular weight surfactants, polymer micelles are more stabilized because of their macromolecular nature. A great number of aggregate morphologies has been found for diblock and ABA triblock copolymers.[5−8] ABC-type triblock copolymers have more complicated structure. Thus, they can form new aggregates of various morphologies.[9,10] For example, poly(ethylene glycol)-*b*-poly(glycerol monomethacrylate)-*b*-poly(2-(diethylamino)ethyl methacrylate) (PEG-PGMA-PDEA) tripolymers can aggregate into three-layer "onion" micelles.[11] At pH 1, poly(acrylic acid)-*b*-polystyrene-*b*-poly(4-vinylpyridine) (PAA-PS-P4VP) can spontaneously form vesicles in water through electrostatic interactions.[9] With a similar structure, poly(2-vinylpyridine)-*b*-poly(methyl methacrylate)-*b*-poly(acrylic acid) (P2VP-PMMA-PAA) also can aggregate into vesicles, but the vesicles formed are not stable when the lateral hydrophilic segments are much longer than the middle hydrophobic segment.[12] In toluene, polystyrene-*b*-poly(2-vinylpyridine)-*b*-poly(methyl methacrylate) (PS-P2VP-PMMA) ABC triblock copolymers can form spherical micelles.[13]

Recently, great efforts have been made to incorporate proteins or polypeptides into some synthetic materials to improve their biological properties and to enlarge their applications in self-assembly, biological sensors, drug delivery, and tissue engineering.[14−19] Proteins are made up of amino acids through amide bonds, which are their primary structures. Their higher order structures (secondary, tertiary, and quaternary) are formed via intra- and intermolecular interactions between the functional groups of residual amino acids. So incorporation of polypeptide into a synthetic polymer often affects its nature of self-assembly. Compared to the former material, this kind of material has not been researched so much. To date, most of the polypeptide-based amphiphilic copolymers studied are AB-type diblock copolymers consisting of a hydrophilic polypeptide segment and another hydrophobic segment. Deming et al. demonstrated the charged polypeptide vesicles with promising biomimetic encapsulants.[20] Rodriguez-Hernández and Lecommandoux reported reversible inside-out vesicles caused by pH changing.[21]

In this paper, we report on the self-assembly of a novel biodegradable ABC triblock copolymer, poly(ethylene glycol)-poly(L-lactide)-poly(L-glutamic acid) (PEG-PLLA-PLGA). This ABC-type triblock copolymer is composed of a central hydrophobic PLLA block, a neutral hydrophilic PEG block, and a PLGA block whose charge state and solubility are pH-dependent. Therefore, its self-assembly is expected to rely on the solvent and pH value.

Experimental Section

(1) Materials. PEG-PLLA-PLGA copolymers were reduction products of PEG-*b*-PLLA-*b*-PBLG (BLG = γ-benzyl-L-glutamate), which were synthesized via the ring-opening polymerization (ROP) of BLG-NCA initiated by PEG-*b*-PLLA-NH_2 as reported by Deng et al.[22]

(2) Preparation of Polymeric Micelles. The triblock copolymer was first dissolved in *N*,*N*-dimethylformamide (DMF), which was a common solvent for the three blocks, with the initial concentration of 2.0 wt %. Then the given amount of deionized water was added to the copolymer solution under gentle stirring. To reach an equilibrium, the mixture was stirred overnight. After that, the mixture was further diluted with a large amount of water and was dialyzed against deionized water to remove DMF from the solution.

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(3) Fluorescence Measurement. A pyrene probe is used to prove the formation of micelles. Steady-state fluorescence spectra were obtained by Perkin-Elmer LS50B luminescence spectrometer. The copolymer solution and distilled water were added consecutively into the volumetric flasks containing pyrene, and the copolymer concentration was from 10^{-4} to 0.4 g/L. The pyrene concentration in each final solution was 6×10^{-7} mol/L (the saturation solubility of pyrene in water at 22 °C). The emission wavelength was 391 nm for fluorescence excitation spectra. The spectra were recorded at a scan rate of 240 nm/min. For the pH = 3.2 micelle system, after the final dilution with doubly distilled water, 0.1 mol/L HCl solution was added to the copolymer/pyrene solution to give the final pH value (pH = 3.2).

(4) ^{1}H NMR Spectroscopy. ^{1}H NMR spectra were measured in D_2O and in a mixture solvent of $CDCl_3$ and CF_3COOD (TFA-*d*; 1/1, v/v) at room temperature (20 ± 1 °C) by an AV-400 NMR spectrometer.

(5) Dynamic Light Scattering Measurements. Dynamic light scattering (DLS) measurements were carried out with a DAMN EOS instrument equipped with a He−Ne laser at the scattering angle of 90°. The micelle solution of about 0.4 mg/mL was passed through a 0.45 μm filter before measurement.

(6) Environmental Scanning Electron Microscopy Measurements. The environmental scanning electron microscopy (ESEM) images were recorded with model XL 30 ESEM FEG from Micro FEI Philips. The dilute micelle solution was deposited on a silicon wafer to a very thin layer and dried at room temperature. A thin layer of Au was coated on the sample surface before measurement.

(7) Transmission Electron Microscopy Measurements. Transmission electron microscopy (TEM) measurements were performed on a JEOL JEM-1011 electron microscope operating at an acceleration voltage of 100 kV. A drop of the dilute aqueous solution was deposited onto a copper grid for about 5 min and then was blotted up with a piece of filter paper. At last, the sample was kept and measured at room temperature.

Results and Discussion

(1) Synthesis and Characterization of the Triblock Copolymers. Recently, Deng et al. reported on the synthesis of a novel triblock copolymers PEG-PLLA-PLGA. First, MPEG-*b*-PLLA-OH was prepared by ROP of L-lactide with monomethoxypoly(ethylene glycol) as the initiator and stannous octoate as catalyst. Then it was converted to PEG-*b*-PLLA-NH_2 by reacting with *tert*-butoxycarbonyl-L-phenylalanine (Phe-^{N}BOC), and by subsequent deprotection. The third block was synthesized by ROP of *N*-carboxyanhydride of γ-benzyl L-glutamate with PEG-*b*-PLLA-NH_2 as a macroinitiator and deprotected with Pd/C. The sample was purified by dissolution/precipitation in tetrahydrofuran (THF)/petroleum ether. The ^{1}H NMR spectrum is shown in Figure 3a(I).

(2) Formation of the Heteroarm Micelles. Two kinds of PEG-PLLA-PLGA samples are employed for micelle preparation. They are PEG(17)-PLLA(23)-PLGA(10) and PEG(17)-PLLA(23)-PLGA(60) (the numbers in the parentheses designate the degree of polymerization (DP)). Both samples have identical PEG and PLLA blocks but are different in the length of the PLGA block. Their micelle solutions are prepared by a process of solvent replacement. The formation of micelles is confirmed by a fluorescence technique using pyrene as a probe. The cmc values for the two samples PEG(17)-PLLA(23)-PLGA(10) and PEG(17)-PLLA(23)-PLGA(60) are 2.82×10^{-3} and 5.01×10^{-3} g/L, respectively. The method of cmc measurement is referred to in ref 23. The size and morphology of the micelles are studied by ESEM and DLS (Figure 1 and Figure 2). ESEM studies show that the micelles of PEG(17)-PLLA(23)-PLGA(10) and PEG(17)-PLLA(23)-PLGA(60) are spherical (Figure 1a,b), and mean diameters are about 35.6 and 57.7 nm, respectively. It seems that the longer PLGA block leads to the bigger particle size. As can be seen, all the particles are dispersed very well, and almost no cohesion happens during drying. This can be confirmed by DLS result. The hydrodynamic radius (R_H) is calculated from the DLS data by the Stokes−Einstein equation, assuming that the micelles are of sphere shape.[24] The average hydrodynamic radii measured by DLS for PEG(17)-PLLA(23)-PLGA(10) and PEG(17)-PLLA(23)-PLGA(60) are 30.7 and 34.5 nm, respectively. So assuming sphere shape is consistent with the micrographs of ESEM. However, the particle diameters measured by ESEM are a little smaller than hydrodynamic diameters measured by DLS. This may be because of the volume shrinkage during sample drying. Volume change during drying can be different for different regions of the micelle structure, while the basic morphology is still spherical both by ESEM and DLS measurement. Because of polymer polydispersity, the size distributions of micelles are broad for both samples by DLS measurement. However, the size of most micelles is still close to the mean radii, consistent with that in ESEM micrographs. To reveal the locations of the three blocks in the micelles, ^{1}H NMR analyses are performed in different solvents. As shown in Figure 3a(I), all chemical shifts of the three blocks are observed in the ^{1}H NMR spectrum measured in $CDCl_3$/TFA-*d*, because the mixture solvent is a good solvent for the three blocks. The signals of the PLLA block at 1.7 and 5.3 ppm almost disappear in Figure 3a(II) measured in D_2O, while those of PEG methylene protons (~3.7 ppm) and PLGA

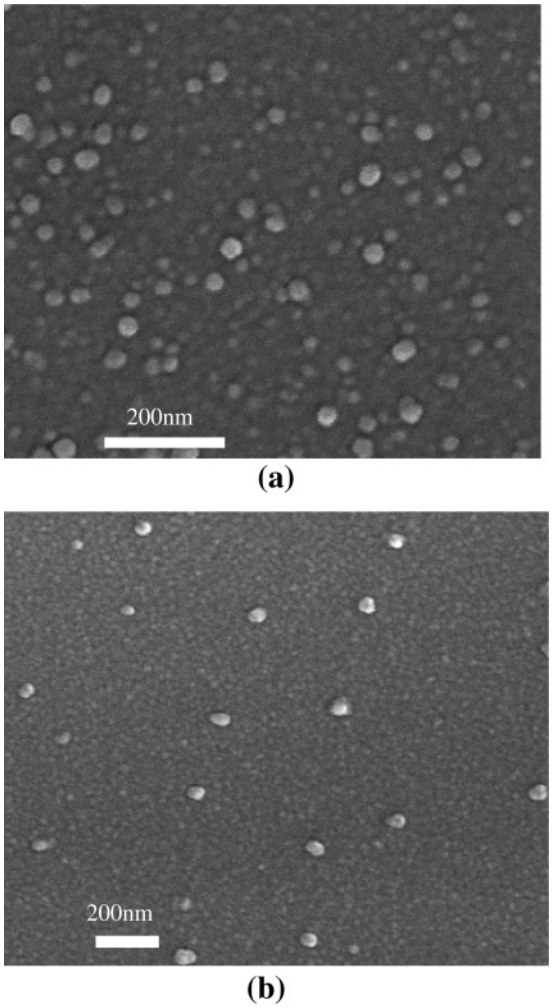


Figure 1. ESEM micrographs of micelles prepared in H_2O: spherical micelles from (a) PEG(17)-PLLA(23)-PLGA(10) and (b) PEG(17)-PLLA(23)-PLGA(60).

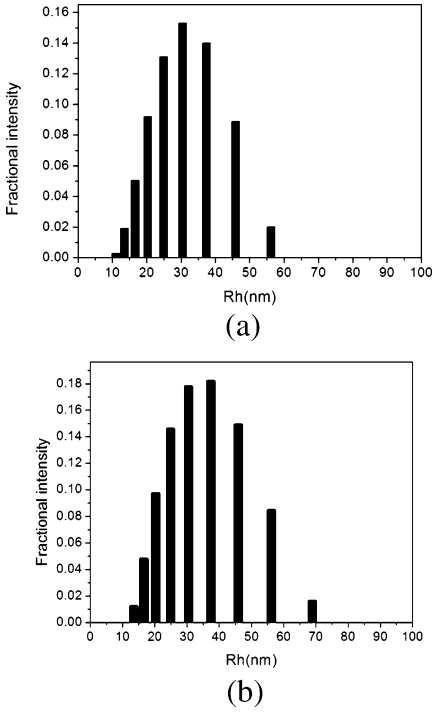


Figure 2. DLS graphs of the micelle size distribution of (a) PEG-(17)-PLLA(23)-PLGA(10) and (b) PEG(17)-PLLA(23)-PLGA(60).

protons (2.0−2.4 ppm, 4.6 ppm) are still observed, although the resonance peaks measured in D_2O have different positions compared to those measured in $CDCl_3$/TFA-*d*. It indicates that the PLLA block has left the water phase and the PEG and PLGA segments remain solvated in water. From the calculation of the intensity between peaks a and d in Figure 3a(II), it implies that PLGA is completely solvated in deionized water as PEG does. That is to say, insoluble PLLA block constitutes the core of the micelle, while hydrophilic PEG and PLGA blocks together form the shell of the micelle. It is a heteroarm micelle.

(3) Influence of pH. The above experiments verify the formation of spherical micelles from PEG(17)-PLLA(23)-PLGA(60) and its heteroarm structure with both PEG and PLGA as hydrophilic segments. It is well-known that there is a pendent carboxyl group on each LGA unit. These carboxyl groups can exist in acidic form or in salt form under proper conditions. Their charging status may influence their aggregation. In fact, the value of the micellar system is pH 4.5 when the solvent DMF is dialyzed completely. Under this condition, the carboxyl groups reach their dissociation equilibrium and the micelles are of spherical shape, as shown in Figure 1a. When aqueous HCl of 0.1 mol/L is added to the micelle solution, it will become bluish and milky. Figure 4a shows the morphology of PEG-(17)-PLLA(23)-PLGA(60) obtained at pH 3.9. They are still spherical micelles but with smaller size, 43.2 nm in diameter. Further decreasing the solution to pH 3.2, the morphology of the micelle will change significantly. In addition to a few spherical micelles, many connected rods are observed (Figure 4b). The diameter of the rods is ca. 28 nm, which is approximately equal to the diameter of the spheres. It can be speculated that the spheres fuse into the rods and both of them constitute the connected rods. In other words, it is the result of adhesive collision of micelles. In Figure 4c,d, we can see some fragments which constitute the connected rods. It is reported that the poly(L-glutamic acid) can be protonated in acidic media, and its secondary conformation changes from coil to compact α-helix.[25] In our case, when the solution is adjusted to pH ≤ 3.2, the PLGA blocks assume the acid form and become

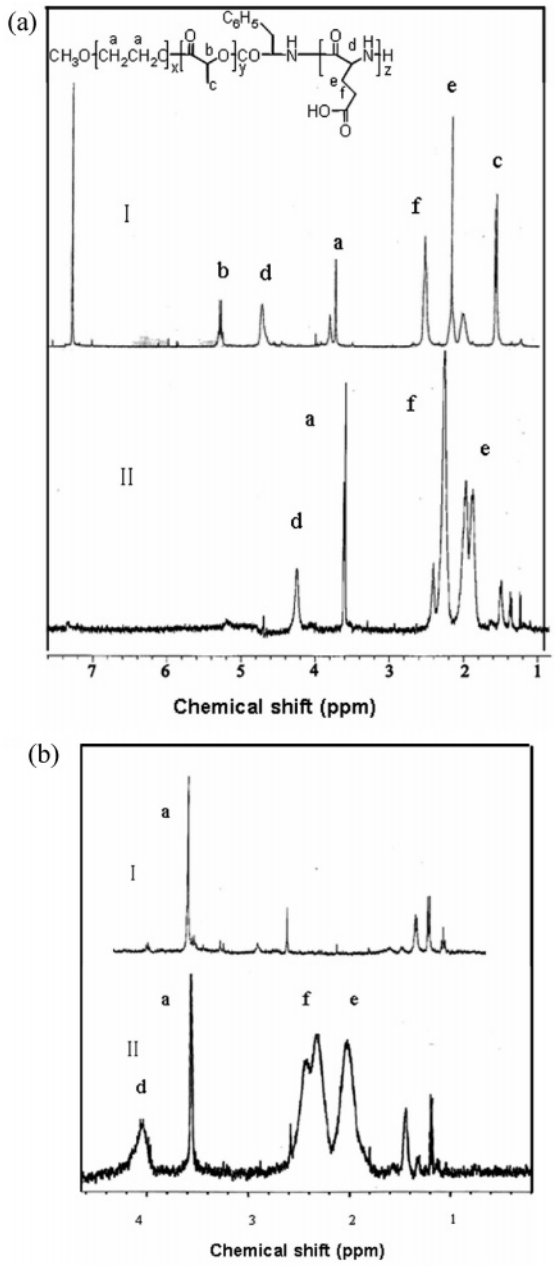


Figure 3. ^{1}H NMR spectra of PEG(17)-PLLA(23)-PLGA(60), measured in $CDCl_3$/TFA-*d* mixture (1:1, v/v) (a, I), in D_2O (a, II) at pH 4.5, in D_2O at pH 3.2 (b, I), and in D_2O at pH 4.5 back from pH 3.2 (b, II).

insoluble in water. Therefore, they may get aggregated and become a part of the core. The aggregation of PLGA blocks is confirmed by ^{1}H NMR measurement and fluorescence measurement. As shown in Figure 3b(I), when the ^{1}H NMR spectrum of PEG(17)-PLLA(23)-PLGA(60) is recorded in D_2O at pH 3.2, all ^{1}H NMR signals of the PLGA segments at 2.0−2.4 and 4.2 ppm disappear almost completely. In other words, the change from pH 4.5 to 3.2 of the medium leads to the change in the aggregation state and function of the PLGA block: it undergoes a process of desolvation and aggregation and joins the PLLA block to become a part of the core. Under this circumstance, the core is composed of both PLLA and PLGA blocks while only PEG block is left in the water phase. The fluorescence measurement proves this viewpoint. When the values of solution are pH 3.2, the cmc values of PEG(17)-PLLA(23)-PLGA(10) and PEG(17)-PLLA(23)-PLGA(60) change to 2.14×10^{-3} and 4.26×10^{-3} g/L, both getting smaller than those at pH 4.5.

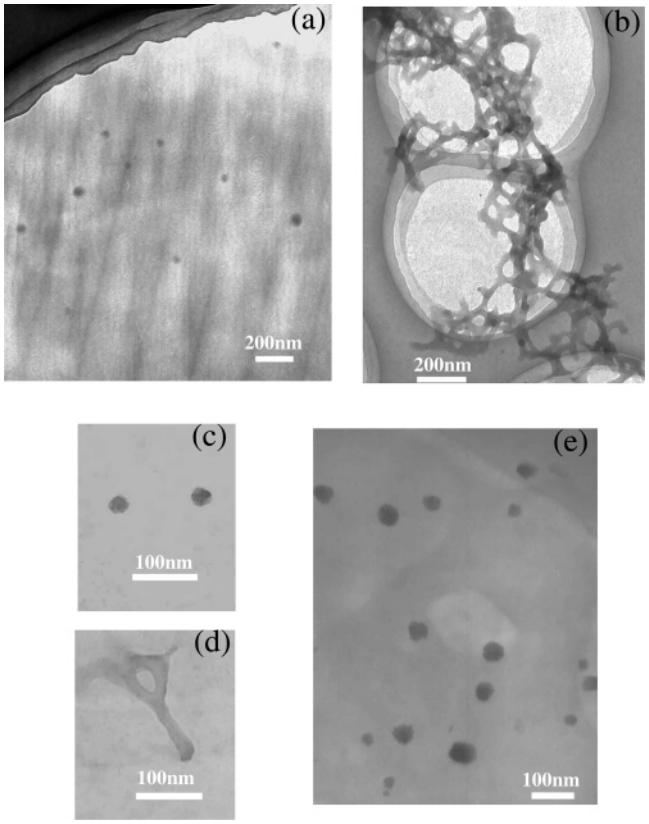


Figure 4. TEM micrographs of the micelles from PEG(17)-PLLA-(23)-PLGA(60) at different pH: (a) spheres at pH 3.9; (b−d) morphologies at pH 3.2 (connected rods and spheres); (e) spherical micelles after adding NaOH back to pH 4.5.

This indicates that the hydrophobic property of the micellar core is increasing and the composition is changing, due to the PLGA block joining the core part. At this moment if the aggregate assumes a spherical micelle, it would be unstable because the PEG block is not long enough to stabilize the micelle, compared to the hydrophobic segments. Therefore, the aggregate assumes a rod shape, because a rod has much smaller specific surface area compared to spherical micelles of the same volume. Furthermore, two additional factors may help the formation of the rod-shaped aggregates. One is the charging status of the micelles. At pH ≥ 4.5, the LGA residues are solvated. The electric repulsion between the LGA residues helps enlarge the volume occupied by the PLGA segments and, thus, helps stabilize the micelle system. When the solution decreases to pH 3.9 and to 3.2 step by step, this leads to the decrease in repulsion between the LGA residues and helps the PLGA segments to aggregate. Another possibility is the change of the secondary conformation of PLGA segments. Because the PLLA core is formed at pH 4.5, it cannot change during the pH variation. When the PLGA segments aggregate from the water phase, they trend to retain their own phases, i.e., α-helixes, because of the formation of hydrogen-bonding in PLGA. Therefore, it is deduced that the rods formed might be composed of three layers: PLLA core plus PLGA middle layer plus PEG surface (Scheme 1). As seen in Figure 4, with pH decreasing, the diameter of the micelle is getting smaller and smaller. This may result from the increasing of α-helix ratios.

Up to now, we can describe the pH dependence as follows. At a high-pH value, both PEG and PLGA blocks are fully solvated. There may be hydrogen-bonding interactions between carboxylic acid groups in PLGA and the ether oxygen in PEG as in many systems.[26−28] With the pH decreasing, the PLGA block is becoming insoluble and leaving the micelle corona gradually. Correspondingly, the stabilizing ability of the micelle is weakened. As a result, the aggregation number may change, which results in the smaller diameters under the drying condition.[29] When pH is less than 3.2, all PLGA segments leave the corona and become a part of the core in the form of α-helixes, the aggregates assume the rod shape with a smaller diameter. Therefore, α-helix formation of the PLGA segments may be the direct driving force for the rod formation, as in many biological systems.

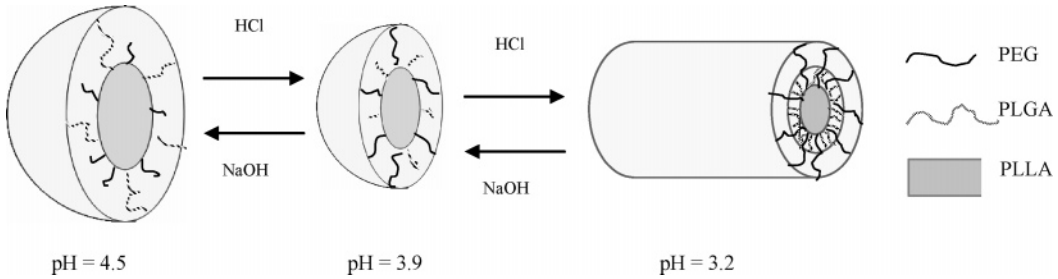
If pH is the governing factor for the formation of the rod-shaped aggregates, the process should be reversible. In fact, when the system is adjusted back to pH 4.5 by adding aqueous NaOH solution of 0.1mol/L, the system turns clear immediately. The signals of PLGA in the ^{1}H NMR spectrum appear again (Figure 3b, II)). The aggregate morphology changes from connected rods to spheres, as shown in Figure 4e. This reversibility provides powerful evidence for the above formation mechanisms of the spherical micelles and connected rod aggregates.

It is noticed that the particle diameter of the spheres measured by TEM after NaOH addition becomes 49 nm (Figure 4e), slightly smaller than the spheres in the original aqueous media. This may be attributed to the salt effect. During the course of base addition, NaCl salt is formed. It can screen the repulsion between PLGA segments, leading to contraction of the micelles.

It should be mentioned here that the pH variation does not cause the formation of the rod-shaped aggregates of PEG(17)-PLLA(23)-PLGA(10). The spherical micelles remain as the main morphology until pH < 3.2. This is because its PLGA block is relatively short. Even if the PLGA segments aggregate from the water phase into the core phase, the relative length of the PEG segments is still long enough to stabilize the spherical micelles without fusing.

(4) Effect of the DMF/H_2O Ratios. To examine the effect of the solvent, the micellization was carried out in two steps.

Scheme 1. Schematic Representation of Reversible Conversion of Spherical Micelles and Rod-Shaped Aggregates of PEG(17)-PLLA(23)-PLGA(60)



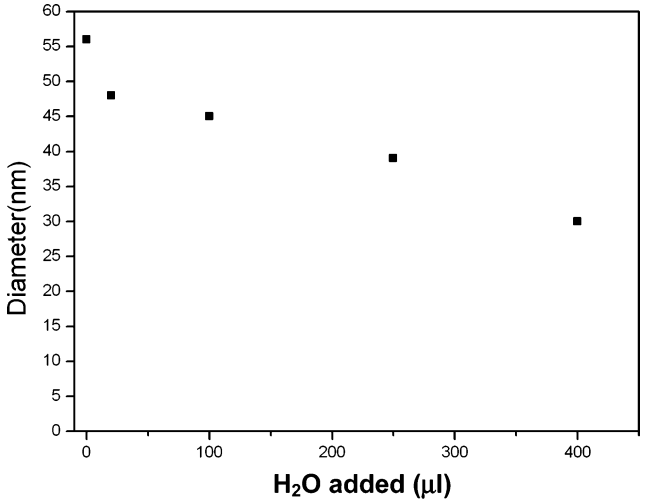


Figure 5. Plot of micelle diameter vs H_2O volume added to 1 mL of DMF solution (2 wt %) of PEG(17)-PLLA(23)-PLGA(60). The micelle diameter was averaged over 100 spheres in the TEM images.

In the first step, 1 mL of DMF solution (2 wt %) of PEG(17)-PLLA(23)-PLGA(60) was mixed with a given amount of deionized water and stirred for one night. In the second step, the micelle solution was further diluted to solidify the micelles. When the DMF was removed completely via dialysis, the micelle size was measured by TEM. Figure 5 shows the measured particle diameter as a function of H_2O/DMF ratio in the first dilution. It is seen that the particle diameter decreases with increasing H_2O/DMF ratio. This dependence can be attributed to the presence of the PLGA blocks. Because the COOH groups in the LGA residues are dissociable, they can be ionized under basic condition. In the presence of DMF (ϵ = 38.2),[30] this dissociation is enhanced. The PLGA segments interact strongly with polar solvent molecules and get more solvated and stretched. In other words, more water molecules are fastened in the neighborhood of PLGA segments. Because the PLLA block is soluble in DMF, the presence of more DMF molecules enhances the swelling of the PLLA core and strengthens the interactions between the PLLA core and the PLGA arms in the corona, resulting in larger micelle spheres.

Conclusions

The poly(L-glutamic acid)-containing triblock copolymer PEG-PLLA-PLGA can self-assemble into spherical micelles in aqueous media. The hydrophobic PLLA segments segregate into the core, while the two hydrophilic segments PEG and PLGA form the shell. The diameter of the micelles depends on the block length of PLGA and on the volume ratio of H_2O/DMF in the first dilution. The micellization of PEG(17)-PLLA(23)-PLGA(60) is pH-dependent. It assembles into spherical micelles in aqueous media of pH 4.5, and the size of the spheres becomes smaller with decreasing solution pH. At or below pH 3.2, it assembles into connected rods. These two aggregation states can convert to each other at proper pH values. This reversibility is ascribed to the dissociation and neutralization of the COOH groups in the LGA residues. PEG(17)-PLLA(23)-PLGA(10) mainly assembles into the spherical micelle at different pH values, because its PLGA segments are relatively short and cannot interfere with the stabilizing capacity of PEG segments.

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References and Notes

(1) Krämer, M.; Stumbé, J. F.; Türk, H.; Krause, S.; Komp, A.; Delineau, L.; Prokhorova, S.; Kautz, H.; Haag, R. *Angew. Chem., Int. Ed.* **2002**, *22*, 41.
(2) Kwon, G. S.; Okano, T. *Adv. Drug Delivery Rev.* **1996**, *21*, 107. Sheihet, L.; Dubin, R. A.; Devore, D.; Kohn, J. *Biomacromolecules* **2005**, *6*, 2726.
(3) Finne, A.; Andronova, N.; Albertsson, A. C. *Biomacromolecules* **2003**, *4*, 1451.
(4) Lei, L. C.; Gohy, J. F.; Willet, N.; Zhang, J. X.; Varshney, S.; Jérôme, R. *Macromolecules* **2004**, *37*, 1089.
(5) Zhang, L.; Eisenberg, A. *Science* **1995**, *268*, 1728.
(6) Geng, Y.; Ahmed, F.; Bhasin, N.; Discher, D. E. *J. Phys. Chem. B* **2005**, *109*, 3772.
(7) Pochan, D. J.; Chen, Z. Y.; Cui, H. G.; Hales, K.; Qi, K.; Wooley, K. L. *Science* **2004**, *306*, 94.
(8) Li, Z. B.; Kesselman, E.; Talmon, Y.; Hillmyer, M. A.; Lodge, T. P. *Science* **2004**, *306*, 98.
(9) Liu, F.; Eisenberg, A. *J. Am. Chem. Soc.* **2003**, *125*, 15059.
(10) Zheng, W.; Wang, Z. G. *Macromolecules* **1995**, *28*, 7215.
(11) Liu, S. Y.; Weaver, J. V. M.; Save, M.; Armes, S. P. *Langmuir* **2002**, *18*, 8350.
(12) Sfika, V.; Tsitsilianis, C.; Kiriy, A.; Gorodyska, G.; Stamm, M. *Macromolecules* **2004**, *37*, 9551.
(13) Tsitsilianis, C.; Sfika, V. Macromol. *Rapid. Commun.* **2001**, *22*, 647.
(14) Kros, A.; Jesse, W.; Metselaar, G. A.; Cornelissen, J. J. L. M. *Angew. Chem., Int. Ed.* **2002**, *8*, 41.
(15) Chécot, F.; Lecommandoux, S.; Gnanou, Y.; Klok, H. A. *Angew. Chem. Int. Ed.* **2005**, *44*, 4349.
(16) Mao, C. B.; Solis, D. J.; Reiss, B. D.; Kottmann, S. T.; Sweeney, R. Y.; Haryhurst, A.; Georgiou, G.; Iverson, B.; Belcher, A. M. *Science* **2004**, *303*, 213.
(17) Huh, K. M.; Lee, S. C.; Cho, Y. W.; Lee, J.; Jeong, J. H.; Park, K. *J. Controlled Release* **2005**, *101*, 59.
(18) Nowak, A. P.; Breedveld, V.; Pakstis, L.; Ozbas, B.; Pine, D. J.; Pochan, D.; Deming, T. J. *Nature* **2002**, *417*, 424.
(19) Lübbert, A.; Castelletto, V.; Harmley, I. W.; Nuhn, H.; Scholl, M.; Bourdillon, L.; Wandrey, C.; Klok, H. A. *Langmuir* **2005**, *21*, 6582.
(20) Holowka, E. P.; Pochan, D. J.; Deming, T. J. *J. Am. Chem. Soc.* **2005**, *127*, 12423.
(21) Rodriguez-Hernández, J.; L Lecommandoux, S. *J. Am. Chem. Soc.* **2005**, *127*, 2026.
(22) Deng, C.; Rong, G. Z.; Tian, H. Y.; Tang, Z. H.; Chen, X. S.; Jing, X. B. *Polymer* **2005**, *46*, 653.
(23) Guan, H. L.; Xie, Z. G.; Zhang, P. B.; Deng, C.; Chen, X. S.; Jing, X. B. *Biomacromolecules* **2005**, *6*, 1954.
(24) Wu, C.; Chu, B. *Macromolecules* **1994**, *27*, 1766.
(25) Johnson, W. C.; Tonico, I. *J. Am. Chem. Soc.* **1972**, *94*, 4389.
(26) Holappa, S.; Kantonen, L.; Winnik, F. M.; Tenhu, H. *Macromolecules* **2004**, *37*, 7008.
(27) Song, T.; Goh, S. H.; Lee, S. Y. *Macromolecules* **2002**, *35*, 4133.
(28) Smith, G. D.; Bedrov, D. *J. Phys. Chem. B* **2003**, *107*, 3095.
(29) Arimura, H.; Ohya, Y.; Ouchi, T. *Biomacromolecules* **2005**, *6*, 720.
(30) Yu, Y.; Eisenberg, A. *J. Am. Chem. Soc.* **1997**, *119*, 8383.

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