

SYNTHESIS AND pH-SENSITIVE SELF-ASSEMBLY OF DENDRITIC POLY(AMIDOAMINE)-*b*-POLY(L-GLUTAMATE) BIOHYBRIDS*

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Abstract Dendritic poly(amidoamine)-*b*-poly(L-glutamate) (PAMAM-*b*-PLG) biohybrids were synthesized by the ring-opening polymerization of γ -benzyl-L-glutamate *N*-carboxyanhydride monomer, followed by the deprotection of benzyl groups on poly(benzyl-L-glutamate), and were characterized by ¹H-NMR, FT-IR and gel permeation chromatography. The self-assembly behavior of the PAMAM-*b*-PLG biohybrid was investigated by means of UV-Vis, dynamic light scattering (DLS), transmission electronic microscopy (TEM) and ¹H-NMR. UV-Vis analysis demonstrated that the critical aggregation concentration of PAMAM-*b*-PLG was dependent on the pH value of aqueous solutions. DLS results further evidenced that the PAMAM-*b*-PLG biohybrid exhibited a pH-sensitive self-assembly behavior, and the average size of the self-assembled nanoparticles decreased gradually with the increasing pH value of the PAMAM-*b*-PLG solution. The self-assembled nanoparticles gave a nearly spherical morphology, and the pH-induced self-assembly had no obvious effect on the morphology of nanoparticles.

Keywords: Dendritic PAMAM-*b*-PLG; pH-sensitive self-assembly; Nanoparticles; Drug/gene delivery.

INTRODUCTION

Natural proteins, polypeptides, and artificial peptide mimetics are increasingly receiving much attention and proved to be versatile building blocks for constructing biological nanostructures such as micelles, bilayered vesicles and fibers, which provide useful platforms for studying naturally disease-related self-assembly processes in living systems and for biomedical applications such as drug/gene delivery and tissue engineering^[1–4]. For example, Stupp *et al.* reported the preprogrammed nanofibers from peptide-based amphiphiles, which were adjusted by pH value, divalent cation induction, and concentration^[1]. As an extension study, the macromolecular conjugates of polypeptides with synthetic polymers have been intensively studied because they can combine the respective advantages of synthetic polymers with biological systems. For examples, a series of biocompatible poly(ethylene glycol)-*b*-polypeptides was synthesized and thoroughly investigated for drug delivery and gene therapy^[5]. Hyperbranched polypeptide-based copolymers and their self-assembly properties were also studied^[6].

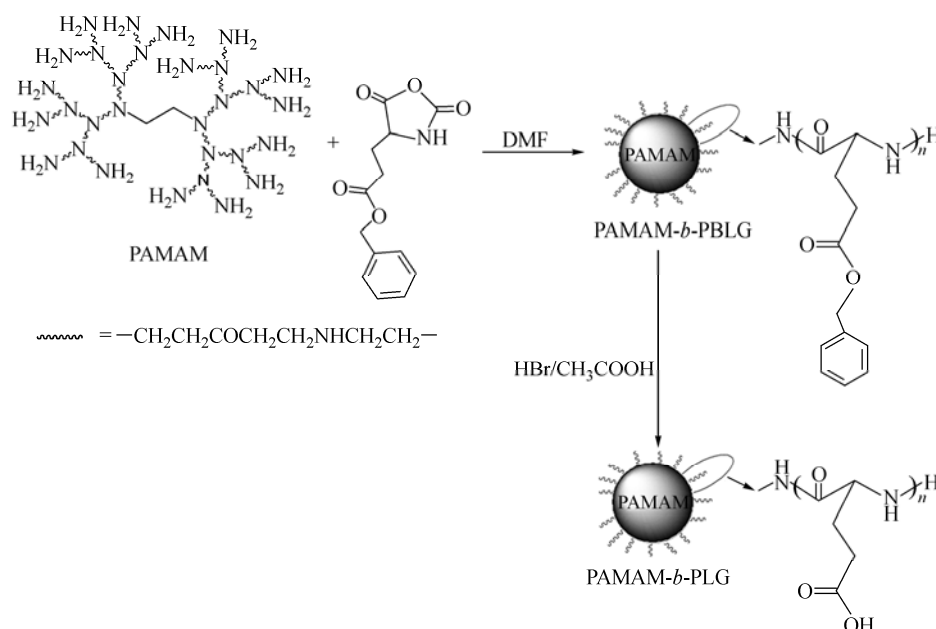
Recently, biodegradable and biocompatible polymeric nanoparticles have been intensively investigated for drug/gene delivery and biological diagnosis^[7–10]. In this case, supramolecular self-assembly of amphiphilic biocompatible copolymers provides a versatile method to fabricate a variety of hierarchical nanostructures^[8]. Moreover, with the advent of “living”/controlled radical polymerization techniques, the tunable macromolecular architecture (*e.g.*, star-shaped polymers, hyperbranched polymers, dendrimers and dendrons) of synthetic polymers provides a convenient method to adjust both the morphology and the size of self-assembled nanostructures^[11]. As a commercial dendrimer, hydrophilic poly(amidoamine) (PAMAM) and its derivatives

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have been widely investigated for biomedical applications such as magnetic resonance imaging and drug/gene delivery. Thus, the conjugates of PAMAM with polypeptides will provide a simple method to generate a class of biodegradable and biocompatible biohybrids^[12–14]. For example, Li *et al.* reported the synthesis of folic acid conjugated poly(L-glutamate) with branched architecture potential for targeted drug delivery^[15]. Jia *et al.* studied the interactions of PAMAM with cytochrome C^[16]. However, it seems to us that no investigation has been done for the self-assembly of dendritic PAMAM-*b*-poly(L-glutamate) (PAMAM-*b*-PLG) in aqueous solutions. In this work, the dendritic PAMAM-*b*-PLG biohybrids were synthesized by the ring-opening polymerization (ROP) of γ -benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) using primary amine-terminated PAMAM dendrimer as the macroinitiator, followed by the deprotection of benzyl groups on poly(benzyl-L-glutamate) (PBLG), as shown in Scheme 1. The pH-sensitive self-assembly of the PAMAM-*b*-PLG biohybrid was investigated by means of UV-Vis, DLS, TEM and ¹H-NMR. It is demonstrated that the PAMAM-*b*-PLG biohybrids self-assembled into biodegradable and biocompatible nanoparticles, in which the size of nanoparticles might be adjusted by the pH-sensitive secondary structures of PLG (*i.e.*, α -helix and coil conformations)^[17–19].



Scheme 1 Synthesis of dendritic PAMAM-*b*-PLG biohybrid

EXPERIMENTAL

Materials

Phosphorous trichloride was distilled and diluted in CH_2Cl_2 ($\text{PCl}_3:\text{CH}_2\text{Cl}_2 = 1:2$, V/V) before use. *N,N*-dimethylformamide (DMF) was distilled from calcium hydride under reduced pressure and stored over molecular sieves. Methyl acrylate was distilled directly from calcium hydride and stored at 4°C. Chloroform and ethylenediamine were distilled directly from calcium hydride, respectively. γ -Benzyl-L-glutamate *N*-carboxyanhydride (BLG-NCA) monomer was synthesized from *N*-*tert*-butoxycarbonyl- γ -benzyl L-glutamic acid and phosphorous trichloride, as detailed elsewhere^[20]. ¹H-NMR of BLG-NCA (CDCl_3 , δ): 2.05–2.25 (m, 2H, $-\text{CH}_2\text{CH}_2-\text{COO}-$), 2.58–2.63 (m, 2H, $-\text{CH}_2\text{CH}_2-\text{COO}-$), 4.34–4.39 (t, 1H, $-\text{CH}-$), 5.13–5.15 (s, 2H, $\text{PhCH}_2\text{O}-$), 6.17–6.20 (s, 1H, NH), 7.32–7.40 (m, 5H, Ph). The PAMAM dendrimer with 16 terminal amines was synthesized by an iterative two-step reaction consisting of Michael addition of primary amine with methyl acrylate and amidation of methyl ester groups with a large excess of ethylenediamine according to the well-known procedure^[12], and analyzed by means of FT-IR and ¹H-NMR. ¹H-NMR of PAMAM (CDCl_3 , δ):

7.50–8.12 (28H, bm), 3.18–3.36 (56H, bm), 2.60–2.94 (116H, bm), 2.47–2.56 (24H, t), 2.28–2.43 (32H, bt), 1.98–2.10 (32H, bs). 1,6-Diphenyl-1,3,5-hexatriene (DPH) was purchased from Aldrich and used as a probe molecule. The other reagents and solvents were local commercial products and used without further purification.

Measurements

Fourier transform infrared (FT-IR) spectra were recorded on a Perkin Elmer Paragon 1000 spectrometer at frequencies ranging from 400 cm^{-1} to 4000 cm^{-1} . Samples were thoroughly mixed with KBr and pressed into pellet form. ^1H -NMR spectrum was performed at room temperature on a Varian Mercury-400 spectrometer. Dendritic PAMAM-*b*-PBLG and PAMAM-*b*-PLG were dissolved in mixed solvents of CF_3COOD and CDCl_3 ($\text{CF}_3\text{COOD}:\text{CDCl}_3 = 1:1$, V/V). The polydispersity indices (M_w/M_n) of polymers were determined on a gel permeation chromatograph (GPC, Perkin-Elmer Series 200) equipped with a refractive index detector at 30°C. The elution phase was DMF (elution rate: 1.0 mL/min), and polystyrene was used as the calibration standard. UV-Vis spectra of samples were recorded at room temperature using a Spectrumlab54 UV-Visible spectrophotometer. The mean size of nanoparticles was determined by dynamic light scattering (DLS) using a Malvern Nano_S instrument (Malvern, UK) at 25°C. All the measurements were repeated three times, and the average values reported were the mean diameter $\pm$ standard deviation. Transmission electron microscopy (TEM) was performed using a JEM-2010/INCA OXFORD TEM (JEOL/OXFORD) at a 200 kV accelerating voltage. Samples were deposited onto the surface of 300 mesh Formvar-carbon film-coated copper grids. Excess solution was quickly wicked away with a filter paper. The image contrast was enhanced by negative staining with phosphotungstic acid (0.5 wt%).

Preparation of Dendritic PAMAM-*b*-PLG Biohybrids

Dendritic PAMAM-*b*-PLG was synthesized from the ROP of BLG-NCA using primary amine-terminated PAMAM as the macroinitiator at room temperature, followed by the deprotection of benzyl groups on poly(benzyl-L-glutamate). In a representative reaction, the PAMAM with 16 terminal amines (6.7 mg, 0.0021 mmol) was added to a 25 mL round-bottom flask equipped with a stirring bar. The flask was sealed with a rubber septum, and 0.5 mL of DMF was introduced *via* a syringe. The mixture was stirred moderately until the PAMAM macroinitiator dissolved completely. The solution was then degassed three times by freeze-pump-thaw cycles, after which a degassed solution of BLG-NCA (0.35 g, 1.33 mmol) in 3.0 mL of DMF was added *via* a syringe under nitrogen atmosphere. The resulting solution was allowed to stir vigorously at room temperature for 24 h. After the solvent was removed under reduced pressure, the mixture was diluted with THF (5 mL) and then dropwise precipitated into a large excess of diethyl ether (50 mL). The white precipitate was filtered and dried *in vacuo* at 40°C to give 0.25 g of PAMAM-*b*-PBLG₃₀ precursor (85.9% yield).

In the second step, the deprotection of benzyl groups on PBLG was performed using HBr/ CH_3COOH mixed solvents. The above obtained PAMAM-*b*-PBLG precursor (181 mg) was completely dissolved in the mixed solvents of HBr (0.7 mL, 40 wt%) with acetic acid (14 mL), and stirred vigorously for 15 h at 45°C. The reaction mixture was then poured into a large amount of diethyl ether (150 mL), and the precipitate was washed repeatedly with diethyl ether for three times. The resulting solid powder was dried *in vacuo* at 40°C to give 141.5 mg of PAMAM-*b*-PLG₃₀ (86.5% yield).

Fabrication of Nanoparticles from Dendritic PAMAM-*b*-PLG Biohybrids

Briefly, 3 mg of the PAMAM-*b*-PLG biohybrid was dissolved directly into 6 mL of distilled water overnight under vigorous stirring at room temperature. The resulting nanoparticle solution was further diluted into 0.1 mg/mL solution, and the mean size of nanoparticles was directly determined by DLS at 25°C.

Measurements of Critical Aggregation Concentration of Dendritic PAMAM-*b*-PLG Biohybrids

The critical aggregation concentration (CAC) of dendritic PAMAM-*b*-PLG biohybrids was determined employing a hydrophobic dye solubilization method using DPH as a probe molecule^[10]. DPH was dissolved in methanol to produce 0.5 mmol/L DPH methanol solution. In a quartz cell, 0.5 mL of PAMAM-*b*-PLG nanoparticle solution and 5 μL of DPH solution were added and capped with Teflon film, and then to

homogenize about 2 min at room temperature. To prevent the evaporation of water during the measurement, the sample cell was sealed with Teflon film. UV-Vis spectra of samples were recorded in the range of 200–500 nm at room temperature.

RESULTS AND DISCUSSION

Synthesis of Dendritic PAMAM-*b*-PLG Biohybrids

It is known that artificial polypeptides and polypeptide-based conjugates can be easily prepared by the ROP of α -amino acid NCA monomers using primary amine-containing compound, where the amine initiator usually undergoes a nucleophilic addition to the C-5 carbonyl group of NCA monomers^[17–20]. Thus, the dendritic PAMAM-*b*-PLG biohybrid was synthesized by the ROP of BLG-NCA monomer using primary amine-terminated PAMAM dendrimer as the macroinitiator to produce the PAMAM-*b*-PBLG precursor, followed by the deprotection of benzyl groups on PBLG segments, as shown in Scheme 1. The results of the dendritic PAMAM-*b*-PLG biohybrid were summarized in Table 1. The actual molecular weights ($M_{n,NMR}$) of PAMAM-*b*-PLG could be easily determined by ¹H-NMR (Fig. 1), which was nearly in agreement with the theoretical value ($M_{n,th} = \{[(M_{NCA} - 134) \times [NCA]/[NH_2] \times \text{arm number} \times \text{yield}] + M_{PAMAM}\}$).

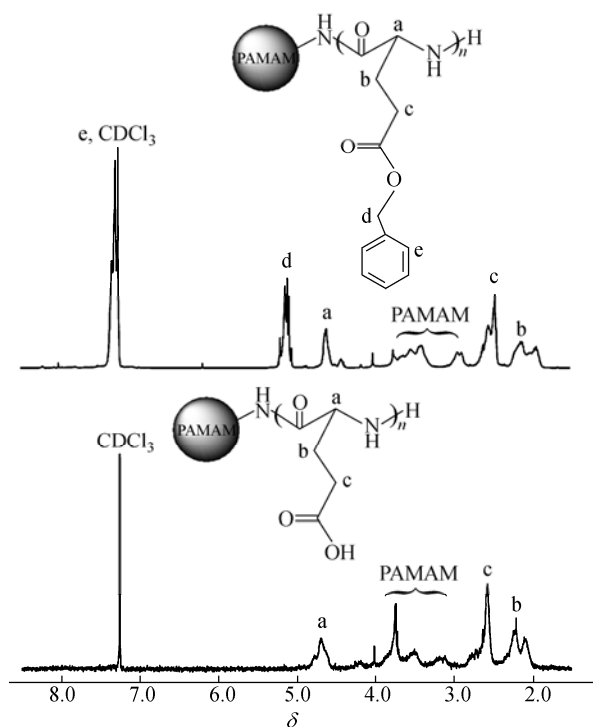


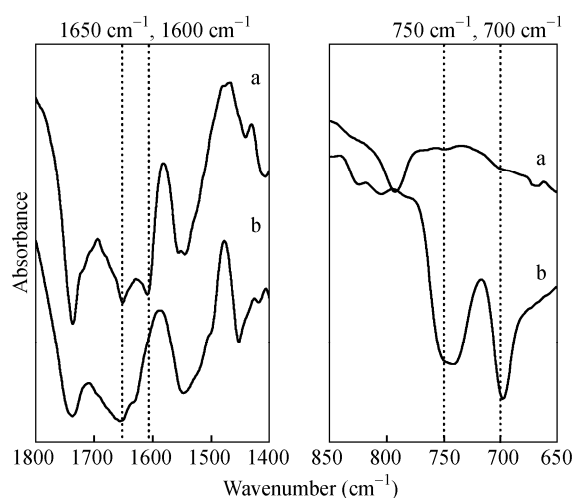
Fig. 1 ¹H-NMR spectra of PAMAM-*b*-PBLG₄ precursor and PAMAM-*b*-PLG₄

After removing benzyl groups from PBLG segments (*i.e.*, deprotection procedure), the proton signals at $\delta = 7.40$ – 7.25 and $\delta = 5.25$ – 5.05 assignable to the benzyl group of PBLG disappeared completely, and the signals of both PAMAM and PLG backbone were retained within the ¹H-NMR of PAMAM-*b*-PLG hybrid (Fig. 1). Moreover, the FT-IR results showed that the vibrational bands of 750 cm^{-1} and 700 cm^{-1} assignable to benzyl groups disappeared completely, and the band of 1600 cm^{-1} assignable to carboxyl group ($-\text{COOH}$) newly appeared (Fig. 2). In all, the above results indicate that the dendritic PAMAM-*b*-PLG biohybrid was successfully synthesized by the ROP of BLG-NCA monomer using primary amine-terminated PAMAM dendrimer as the macroinitiator, followed by the deprotection of benzyl groups on PBLG segments (Scheme 1).

Table 1. The synthesis of dendritic PAMAM-*b*-PLG biohybrids

Entry ^a	[NCA]/[NH ₂]	Yield ^b (%)	M_w/M_n ^c	$M_{n,NMR}$ ^d	$M_{n,th}$ ^e
PAMAM- <i>b</i> -PBLG ₄ ^f	5:1	89.7	1.38	17270	17860
PAMAM- <i>b</i> -PBLG ₃₀ ^f	40:1	85.9	1.13	108380	115380
PAMAM- <i>b</i> -PLG ₄	5:1	86.5	—	11510	11920
PAMAM- <i>b</i> -PLG ₃₀	40:1	81.2	—	65180	72180

^aThe subscript numbers represent the degree of polymerization of PBLG and/or PLG segments, which was determined by ¹H-NMR; ^bThe yield was determined gravimetrically; ^c M_w/M_n denotes the polydispersity index of polymer, where weight-average molecular weight (M_w) and number-average molecular weight (M_n) were determined by GPC in DMF solution; ^d $M_{n,NMR}$ was determined from the integral ratio of the signal on the main chain of PBLG or PLG ($-\text{CH}-$, $\delta = 4.70-4.55$) and the signal on PAMAM core from the ¹H-NMR; ^e $M_{n,th}$ denotes the theoretical molecular weight of the PAMAM-*b*-PBLG precursor, where $M_{PBLG} = \{[(M_{NCA} - 44) \times [\text{NCA}]/[\text{NH}_2] \times \text{arm number} \times \text{yield}] + M_{\text{PAMAM}}\}$ and $M_{PLG} = \{[(M_{NCA} - 134) \times [\text{NCA}]/[\text{NH}_2] \times \text{arm number} \times \text{yield}] + M_{\text{PAMAM}}\}$; ^fThese data were cited from the reference, where the monomer conversion has been transformed into the yield of polymer^[14].

**Fig. 2** FT-IR spectra of PAMAM-*b*-PLG₄ (a) and PAMAM-*b*-PBLG₄ precursor (b)

pH-Sensitive Self-Assembly Properties of Dendritic PAMAM-*b*-PLG Biohybrids

It is reported that PLG ($\text{pK}_a = 4.32$) can molecularly dissolve in aqueous solution and exhibits a coil conformation when the aqueous pH is higher than 6, and its coil-helix transition usually occurs at $\text{pH} = 5.2$ ^[17]. On the contrary, PLG will precipitate from aqueous solution when the aqueous pH is lower than 4.3, exhibiting a helix conformation. As for the PAMAM dendrimer, it is water-soluble at room temperature. Because PAMAM core and PLG segments of the PAMAM-*b*-PLG biohybrid have different solubility in aqueous solution under different pH conditions, the PAMAM-*b*-PLG biohybrid is expected to aggregate into biocompatible nanoparticles. This postulation was confirmed preliminarily by the examination of critical aggregation concentration (CAC) of PAMAM-*b*-PLG in aqueous solutions under different pH conditions (*i.e.*, $\text{pH} = 4$ and $\text{pH} = 10$), which was an important parameter for the thermodynamic stability of self-assembled aggregates in aqueous solutions^[8]. Using a dye solubilization method^[10], 1,6-diphenyl-1,3,5-hexatriene (DPH) as a probe molecule was added to the PAMAM-*b*-PLG aqueous solution, and the absorbance of DPH was measured by a UV-Vis spectrophotometer. The relationship of the absorbance intensity of DPH molecules as a function of the PAMAM-*b*-PLG concentration at room temperature is shown in Fig. 3. It can be seen that the absorbance intensity values of DPH remained nearly constant below a certain concentration. Above that concentration, the intensity increased substantially, reflecting the incorporation of DPH in the hydrophobic region of self-assembled aggregates. The CAC value of PAMAM-*b*-PLG was in the range of $4.6 \times 10^{-4} - 6.6 \times 10^{-3} \text{ mmol} \cdot \text{L}^{-1}$. Moreover, the CAC value apparently decreased with the increasing pH value of aqueous solutions, which suggested that the self-assembly of PAMAM-*b*-PLG was dependent on the pH value of aqueous solution. This phenomenon was further clarified by the following dynamic light scattering (DLS) technique.

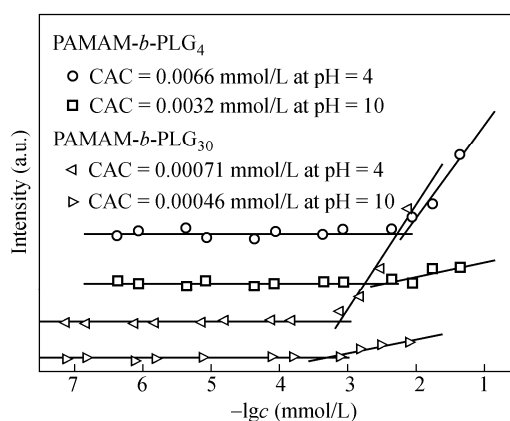


Fig. 3 The relationship of the absorbance intensity of DPH as a function of the PAMAM-*b*-PLG concentration at different pH values of aqueous solutions

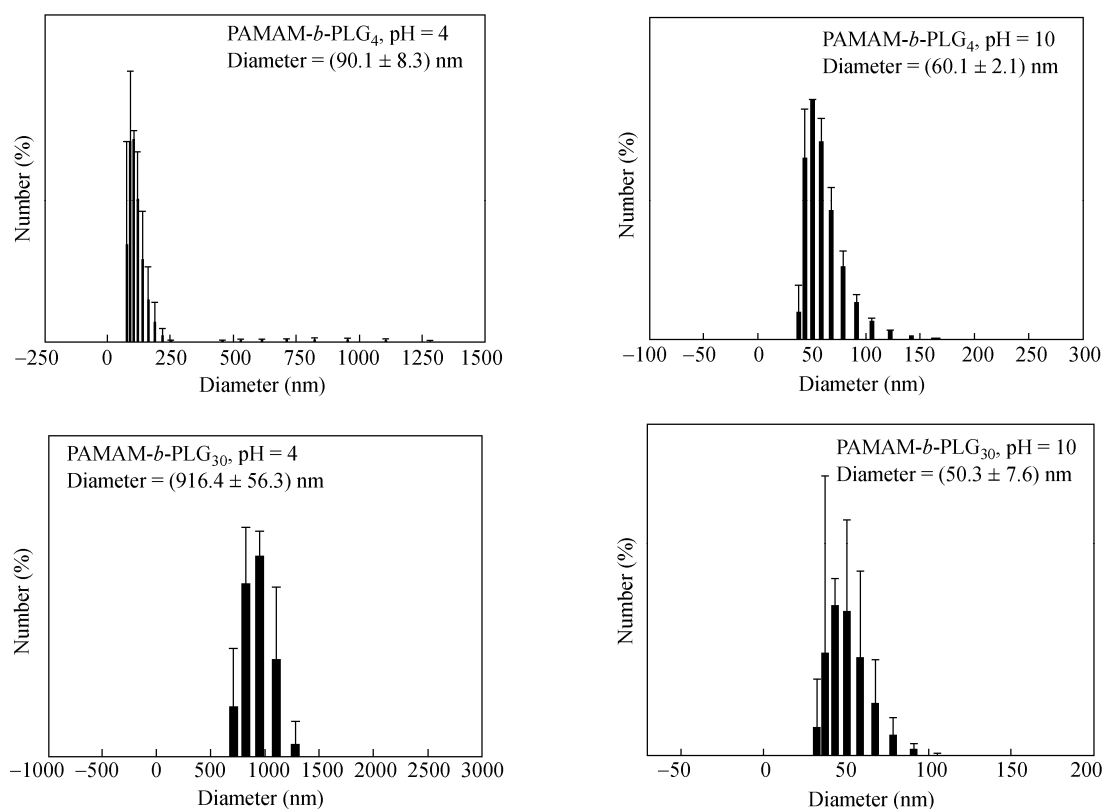


Fig. 4 Nanoparticle size distribution of PAMAM-*b*-PLG₄ and PAMAM-*b*-PLG₃₀ at different pH values of aqueous solutions

The DLS analysis of the self-assembled nanoparticles solution is shown in Fig. 4 and Fig. 5. The average diameter of nanoparticles was in the range of 57.7–112.1 nm for the dendritic PAMAM-*b*-PLG₄ with shorter PLG segments, which was largely more than the theoretical size (about 2.5–3 nm) of unimolecular PAMAM-*b*-PLG₄. This suggests that the nanoparticles were probably formed by multi-molecular self-association in aqueous solution, which was induced by the strong intermolecular hydrogen-bonding interactions^[21]. The tentative mechanism for the multi-molecular self-assembly mode of dendritic PAMAM-*b*-PLG biohybrid is given in

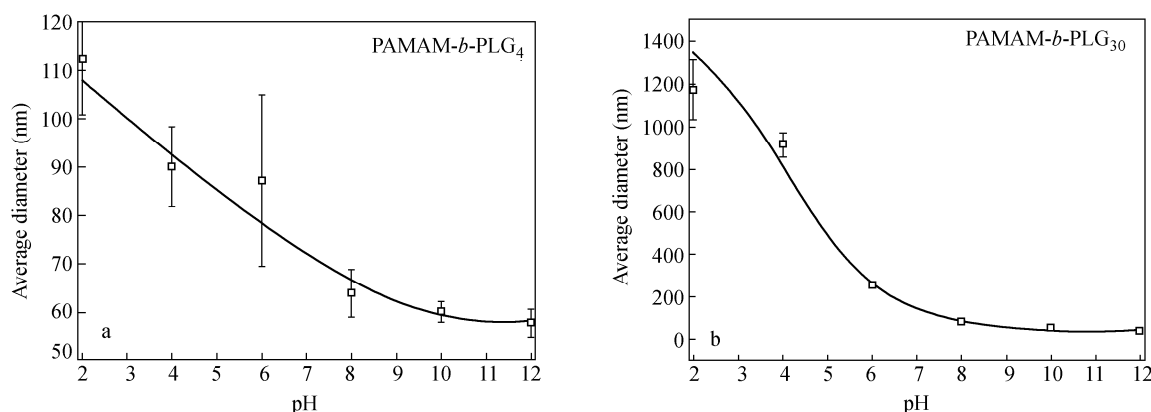
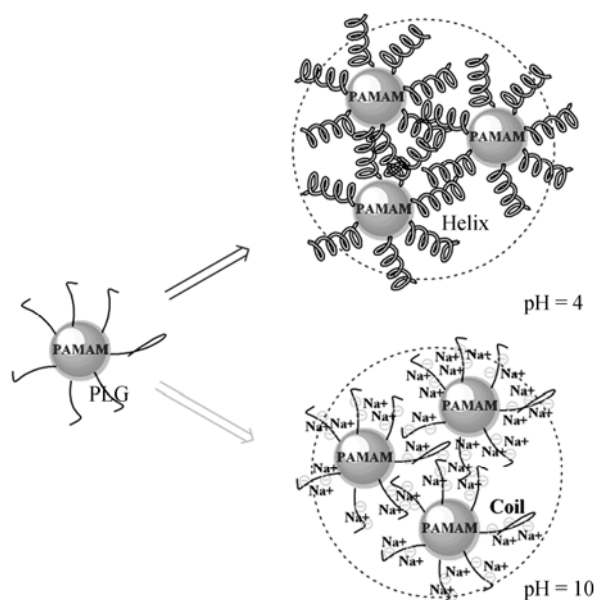


Fig. 5 The average size of PAMAM-*b*-PLG₄ (a) and PAMAM-*b*-PLG₃₀ (b) nanoparticles as a function of pH at room temperature



Scheme 2 The tentative mechanism for the multi-molecular self-assembly mode of PAMAM-*b*-PLG biohybrid in aqueous solutions

Scheme 2, which will be further clarified by the following TEM and ^1H -NMR analyses. As shown in Fig. 5(a), the average size of the PAMAM-*b*-PLG₄ nanoparticles gradually decreased with the increasing pH value of aqueous solutions, which evidences that the dendritic PAMAM-*b*-PLG biohybrid exhibited a pH-sensitive self-assembly behavior in aqueous solutions. This can be attributed to the fact that the intermolecular hydrogen-bonding within the PLG segments (helix conformation) increased at low pH (*e.g.*, pH = 2, 4, and 6) while the electrostatic repulsion within the PLG segments (coil conformation) increased at high pH (*e.g.*, pH = 8, 10, and 12). This result is in agreement with the above CAC analysis. Note that the pH-mediated self-assembly mechanism of dendritic PAMAM-*b*-PLG hybrid needs further investigation. Similarly, the PAMAM-*b*-PLG₃₀ with long PLG segments also presented a pH-sensitive self-assembly behavior, as demonstrated by Fig. 5(b). As a note, the nanoparticle self-assembled from PAMAM-*b*-PLG₃₀ was largely bigger than that from PAMAM-*b*-PLG₄, especially when the pH value of aqueous solution is low (*e.g.*, pH = 2, 4, and 6). This was probably attributed to the increased aggregation number for self-assembled nanoparticles, which was induced by the stronger intermolecular interactions within PAMAM-*b*-PLG₃₀ than those within PAMAM-*b*-PLG₄^[17–21].

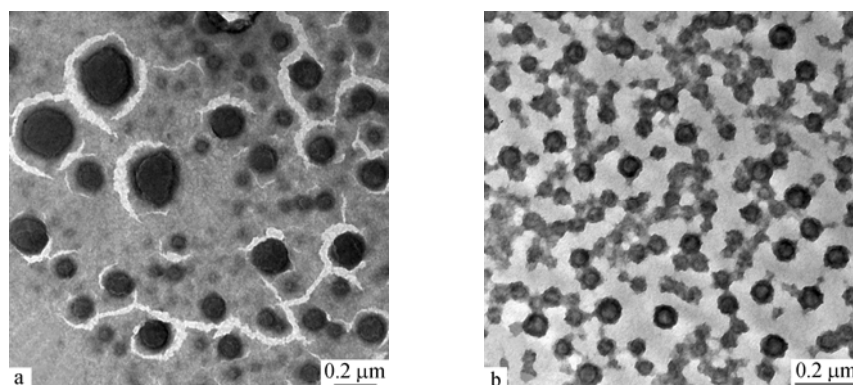


Fig. 6 TEM photographs of PAMAM-*b*-PLG₃₀ nanoparticles at pH = 4 (a) and pH = 10 (b) in aqueous solutions

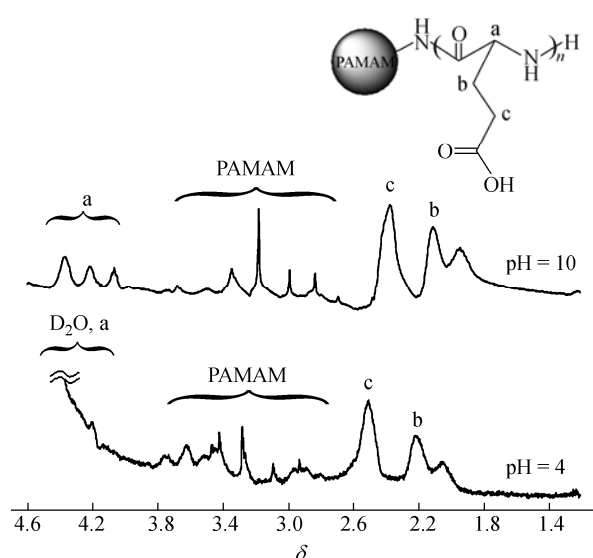


Fig. 7 ^1H -NMR spectra (D_2O) of the PAMAM-*b*-PLG₄ nanoparticles in aqueous solutions at different pH (*i.e.*, pH = 4 and 10)

Does the pH-sensitive self-assembly generate the nanostructures with different morphology? This question was answered by TEM analysis, as shown in Fig. 6. Nearly spherical morphology was presented for the PAMAM-*b*-PLG₃₀ nanoparticles fabricated at pH = 4 and/or pH = 10, although the nanoparticles fabricated at pH = 4 looked more polydisperse than those fabricated at pH = 10. This indicates that the pH-sensitive self-assembly had no obvious effect on the morphology of nanoparticles. Moreover, this result implies that the multi-molecular self-assembly mode is similar for the PAMAM-*b*-PLG at both lower and higher pH, which was indirectly proved by the ^1H -NMR of PAMAM-*b*-PLG (Fig. 7). It can be seen that the proton signals of PLG segments and PAMAM cores appeared clearly, and no signal enhancement for each component was found at lower and higher pH (*i.e.*, pH = 4 and 10). This suggests that the self-assembled nanoparticles had no obvious core-shell structure often generated from amphiphilic block copolymers^[5–8]. This is to say that the self-assembly mode of PAMAM-*b*-PLG in aqueous solution was multi-molecular self-association induced by the strong intermolecular hydrogen-bonding interactions, as shown in Scheme 2. As a note, the size of nanoparticles determined from TEM analysis is smaller than that determined by DLS, which can be attributed to the nanoparticle dehydration induced by water evaporation under the high vacuum condition during TEM measurements^[22]. In all, these results demonstrate that the biodegradable and biocompatible nanoparticles with

adjustable size can be easily fabricated from dissolving dendritic PAMAM-*b*-PLG biohybrid into aqueous solution under different pH conditions.

CONCLUSIONS

Dendritic PAMAM-*b*-PLG biohybrids were successfully synthesized by the ROP of BLG-NCA using primary amine-terminated PAMAM dendrimer as the macroinitiator, followed by the deprotection of benzyl groups on PBLG. The critical aggregation concentration of PAMAM-*b*-PLG was dependent on the pH value of aqueous solutions, and the average size of the self-assembled nanoparticles decreased gradually with the increasing pH value of aqueous solutions. These results confirmed that the PAMAM-*b*-PLG biohybrid exhibited a pH-sensitive self-assembly behavior in aqueous solution. Moreover, the self-assembled nanoparticles presented a nearly spherical morphology, and the pH-induced self-assembly had no obvious effect on the morphology of nanoparticles. Consequently, the biodegradable and biocompatible nanoparticles with adjustable size can be easily fabricated from dissolving dendritic PAMAM-*b*-PLG biohybrids into aqueous solutions under different pH conditions.

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REFERENCES

- 1 Hartgerink, J.D., Beniash, E. and Stupp, S.I., *PNAS*, 2002, 99: 5133
- 2 Rele, S., Song, Y., Apkarian, R.P., Qu, Z., Conticello, V.P. and Chaikof, E.L., *J. Am. Chem. Soc.*, 2007, 129: 14780
- 3 Sallach, R.E., Wei, M., Biswas, N., Conticello, V.P., Lecommandoux, S., Dluhy, R.A. and Chaikof, E.L., *J. Am. Chem. Soc.*, 2006, 128: 12014
- 4 Zhao, X. and Zhang, S., *Adv. Polym. Sci.*, 2006, 203: 145
- 5 Osada, K. and Kataoka, K., *Adv. Polym. Sci.*, 2006, 202: 113
- 6 Tian, H.Y., Chen, X.S., Lin, H., Deng, C., Zhang, P.B., Wei, Y. and Jing, X.B., *Chem. Eur. J.*, 2006, 12: 4305
- 7 Nasongkla, N., Bey, E., Ren, J., Ai, H., Khemtong, C., Guthi, J.S., Chin, S.F., Sherry, A.D., Boothman, D.A. and Gao, J., *Nano. Lett.*, 2006, 6: 2427
- 8 Discher, D.E. and Ahmed, F., *Annu. Rev. Biomed. Eng.*, 2006, 8: 323
- 9 Dong, C.M., Guo, Y.Z., Qiu, K.Y., Gu, Z.W. and Feng, X.D., *J. Control. Release*, 2005, 107: 53
- 10 Feng, H. and Dong, C.M., *Biomacromolecules*, 2006, 7: 3069
- 11 Svenson, S. and Tomalia, D.A., *Adv. Drug. Deliv. Rev.*, 2005, 57: 2106
- 12 Tomalia, D.A., *Prog. Polym. Sci.*, 2005, 30: 294
- 13 Kobayashi, H. and Brechbiel, M.W., *Adv. Drug. Deliv. Rev.*, 2005, 57: 2271
- 14 Huang, H., Dong, C.M. and Wei, Y., *Combinatorial Chem. & High Throughput Screening*, 2007, 10: 368
- 15 Tansey, W., Ke, S., Cao, X.Y., Pasuelo, M.J., Wallace, S. and Li, C., *J. Control. Release*, 2004, 94: 39
- 16 Chen, L.R., Zhong, H., Jia, X.R., Qin, Y., Liao, Q., Li, M.Q. and Wei, Y., *Acta Polymerica Sinica*(in Chinese), 2001, (4): 553
- 17 Kukula, H., Schlaad, H., Antonietti, M. and Förster, S., *J. Am. Chem. Soc.*, 2002, 124: 1658
- 18 Dong, C.M., Sun, X.L., Faucher, K.M., Apkarian, R.P. and Chaikof, E.L., *Biomacromolecules*, 2004, 5: 224
- 19 Dong, C.M. and Chaikof, E.L., *Colloid Polym. Sci.*, 2005, 283: 1366
- 20 Dong, C.M., Faucher, K.M. and Chaikof, E.L., *J. Polym. Sci. Polym. Chem.*, 2004, 42: 5754
- 21 Kim, K.T., Park, C., Vandermeulen, G.W.M., Rider, D.A., Kim, C., Winnik, M.A. and Manners, I., *Angew. Chem. Int. Ed.*, 2005, 44: 7964
- 22 Dai, X.H., Dong, C.M. and Yan, D.Y., *J. Phys. Chem. B*, 2008, 112: 3644