Synthesis of Temperature-Dependent Elastin-Like Peptide-Modified Dendrimer for Drug Delivery

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ABSTRACT:

Dendrimers are synthetic macromolecules with a unique structure that are potential unimolecular drug carriers and potential scaffolds for peptides. Elastin is one of the main components of the extracellular matrix, as well as a temperature-sensitive biomacromolecule. Val-Pro-Gly-Val-Gly repeats, an elastin-like peptide, have been used for designing artificial elastin molecules. In this study, we have synthesized a novel type of temperature-dependent drug carrier by conjugating Ac-Val-Pro-Gly-Val-Gly to a dendrimer, named elastin-mimetic dendrimer. The elastin-mimetic dendrimer formed β-turn structure by heating. The elastin-mimetic dendrimer exhibited the inverse phase transition, depending on pH and NaCl concentration in addition to temperature. The elastin-mimetic dendrimer could encapsulate a model drug, rose bengal, even though the complex stability was similar to the dendrimer without elastin-like peptide. Therefore, the elastin-mimetic dendrimer is a potential drug carrier with temperature- and pH-dependent properties. (134 words)
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INTRODUCTION

Dendrimers have highly controllable size, topology, and surface properties and are quite different from linear polymers. Many researchers have designed new functional materials that are based on dendrimers for a variety of applications. In the biological applications of drug and gene delivery, bioactive molecules can be conjugated to dendrimers and/or encapsulated into them.[1–4] Dendrimers can be used as a platform of peptides. Dendrimers that conjugate to collagen model peptides, such as $(Pro\text{-}Pro\text{-}Gly)_n$ and $(Pro\text{-}Hyp\text{-}Gly)_n$, form collagen-like triple-helical structures and temperature-dependent hydrogels that promote drug delivery.[5–11] Dendrimers are a potential unimolecular drug carrier as well as a potential scaffold of peptides for the design of artificial proteins.

Elastin, which is elastic, is one of the main components of the extracellular matrix. Elastin has been used as a temperature-sensitive biomaterial. It is composed of repeat sequences of valine-proline-glycine-valine-glycine (Val-Pro-Gly-Val-Gly, VPGVG). This is called an elastin-like peptide (ELP) and is applied to elastin-mimetic materials.[12–14] Previous reports show that ELPs induce the temperature-dependent structure change from random coil into β-turn and/or β-spiral and lead to coacervation due to the hydrophobic interaction.[12–14] Most

elastin materials extracted from animals are in insoluble forms; however, soluble forms with temperature dependency under physiological conditions are desired for applications. Some researchers have studied polypeptides containing ELPs for biomedical applications such as drug delivery systems and temperature-sensitive hydrogel formations.[15–17] On the other hand, short ELPs, less than four repeats, could not be applied to the applications, because they did not exhibit temperature-dependent properties.[18] It was reported that synthetic polymers conjugated to ELPs, such as ELP-grafted poly(methacrylate)s and ELP-grafted poly(norbornene)s, exhibited temperature-sensitive properties.[19–21] However, all of the synthetic ELPs responded only at low pH.[19–21] Therefore, chemically synthesized elastin-like materials responding under the physiological conditions should be prepared.

We have designed elastin-mimetic materials based on ELPs and dendrimers. The advantages of using dendrimers to design artificial elastin materials are a clustering effect of conjugated ELP for the induction of the higher order structure and a unimolecular nanoparticle for a drug delivery system. Recently, Koga et al.[22] reported that ELP-bound dendrimers exhibited temperature-dependent properties. However, possible applications using this kind of ELP-bound dendrimer remain to be investigated. In this study, VPGVG peptide was synthesized and attached to polyamidoamine (PAMAM) dendrimers to produce an elastin-mimetic dendrimer. The synthesized compounds were characterized by nuclear magnetic resonance (NMR) and high-performance liquid chromatography (HPLC). We performed circular dichroism (CD) measurements at various temperatures to evaluate the temperature-dependent higher order structure, which was compared with the free ELP peptide. We examined temperature-dependent coacervation using turbidity measurements under various conditions and investigated the dependency on pH and salt concentration in addition to temperature. The release behavior of a model drug, rose bengal, from the elastin-mimetic dendrimer was also examined.

EXPERIMENTAL

Materials

Amino-terminated and hydroxyl-terminated ethylenediamine core PAMAM dendrimers of generation 4 (G4) were purchased from Sigma-Aldrich (St. Louis, MO). *N*-[(tert-Butoxy)carbonyl]-valine (Boc-Val), *N*-[(tert-butoxy)carbonyl]-proline (Boc-Pro), and glycine benzylester (Gly-OBzl) were obtained from the Peptide Institute (Osaka, Japan). *o*-(7-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *o*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), and Ac-VPGVG were obtained from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). Triethylamine (TEA), trifluoroacetic acid (TFA), acetic anhydride, and celite (545RVS) were obtained from Nacalai Tesque (Kyoto, Japan). Rose bengal was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

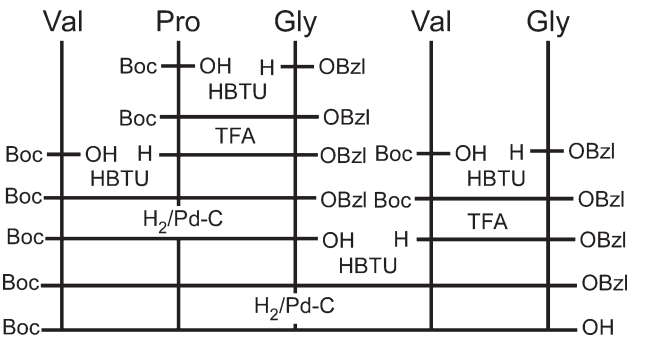

FIGURE 1 Synthetic pathway of Boc-VPGVG.

Synthesis of an Elastin-Like Peptide (Boc-VPGVG)

Boc-VPGVG was synthesized as shown in Figure 1. The detailed procedures are described below.

Synthesis of Boc-Pro-Gly-OBzl. Boc-Pro (9.1 mmol), Gly-OBzl tosylate (9.0 mmol), and TEA (22 mmol) were dissolved in distilled acetonitrile (72 mL). HBTU (9.0 mmol) was added and stirred for 30 h at room temperature. Saturated NaCl solution (210 mL) was added, and the crude compounds were extracted with ethyl acetate. The organic phase was washed with 10% citric acid aqueous solution, water, 4% sodium hydrogen carbonate, and then water. The compound was purified with a silica gel column (elute: ethyl acetate/hexane = 1/1). Yield 78%. ^{1}H NMR [400 MHz, dimethyl sulfoxide (DMSO)] δ 1.32 and 1.39 (s, Boc), 1.75 and 2.05 (br, H_β, and H_γ for Pro), 3.27 (m, H_δ for Pro), 3.87 (m, H_α for Gly), 4.10 (br, H_α for Pro), 5.12 (s, Bzl), 7.37 (s, Phenyl). ^{13}C NMR (400 MHz, DMSO) δ22.9 and 23.6 (C_γ for Pro), 27.9 ((CH_3)$_3$C for Boc), 29.8 and 30.8 (C_β for Pro), 40.6 (C_α for Gly), 46.3 (C_δ for Pro), 59.6 (C_α for Pro), 65.8 (Bzl), 78.5 ((CH_3)$_3C$ for Boc), 127.9, 128.0, 128.3, and 135.8 (phenyl), 153.2, 169.6, and 173.0 (C = O).

Synthesis of TFA·H-Pro-Gly-OBzl. Boc-Pro-Gly-OBzl (7.0 mmol) was dissolved in TFA (5 mL) and incubated at 4°C for 4 h. The reaction mixture was evaporated. After the addition of water, subsequent evaporation was performed four separate times. The crude compound was recrystallized from ethyl acetate/ether/hexane. Yield 96%. ^{1}H NMR (400 MHz, D_2O containing 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium

salt) δ2.01 and 2.41 (br, H_β, and H_γ for Pro), 3.40 (br, H_δ for Pro), 4.05 (m, H_α for Gly), 4.45 (br, H_α for Pro), 5.23 (s, Bzl), 7.46 (s, phenyl). ^{13}C NMR (400 MHz, D_2O containing 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt) δ26.1 (C_γ for Pro), 32.5 (C_β for Pro), 44.5 (C_α for Gly), 49.5 (C_δ for Pro), 62.6 (C_α for Pro), 70.8 (Bzl), 131.5, 131.7, 131.8, and 138.1 (phenyl), 173.2 and 173.9 (*C* = O).

Synthesis of Boc-Val-Pro-Gly-OBzl. TFA·H-Pro-Gly-OBzl (6.2 mmol) was dissolved in distilled acetonitrile (50 mL) and TEA (15 mmol). Boc-Val (6.2 mmol), followed by HBTU (6.2 mmol), were added and stirred for 4 days at room temperature. Saturated NaCl solution (150 mL) was added, and the crude compounds were extracted with ethyl acetate. The organic phase was washed with 10% citric acid aqueous solution, water, 4% sodium hydrogen carbonate, and then water. The compound was purified with a silica gel column (elute: ethyl acetate/hexane = 1/1). Yield 72%. ^{1}H NMR (400 MHz, DMSO) δ0.83 and 0.89 (m, H_γ for Val), 1.34 (s, Boc), 1.80 and 2.02 (br, H_β, and H_γ for Pro), 1.90 (br, H_β for Val), 3.57 and 3.67 (br, H_δ for Pro), 3.78, 3.83, 3.94, and 3.98 (m, H_α for Gly and H_α for Val), 4.36 (br, H_α for Pro), 5.11 (s, Bzl), 7.37 (s, phenyl). ^{13}C NMR (400 MHz, DMSO) δ18.3 and 19.1 (C_γ for Val), 24.3 (C_γ for Pro), 28.1 ((CH_3)$_3$C for Boc), 29.1 and 29.7 (C_β for Pro and Val), 40.6 (C_α for Gly), 46.9 (C_δ for Pro), 57.2 and 59.0 (C_α for Pro and Val), 65.8 (Bzl), 77.9 ((CH$_3$)$_3$*C* for Boc), 127.9, 128.0, 128.4 and 135.8 (phenyl), 155.5, 169.6, 170.3 and 172.1 (*C* = O).

Synthesis of Boc-Val-Pro-Gly-OH. Boc-Val-Pro-Gly-OBzl (4.5 mmol) was dissolved in ethanol (74 mL). Pd-C (1.5 mmol) and 1,4-cyclohexadiene (45 mmol) were added and reduced with H_2 gas. The reaction mixture was filtered in the presence of celite (545RVS) and evaporated. Coevaporation with ethanol was performed four times, and the residual was dried under vacuum. Yield 95%. ^{1}H NMR (400 MHz, DMSO) δ 0.84 and 0.90 (m, H_γ for Val), 1.37 (s, Boc), 1.85 and 2.03 (br, H_β, and H_γ for Pro), 1.91 (br, H_β for Val), 3.57 and 3.67 (br, H_δ for Pro), 3.62, 3.66, 3.80, 3.84, and 3.97 (H_α for Gly and H_α for Val), 4.37 (br, H_α for Pro). ^{13}C NMR (400 MHz, DMSO) δ 18.3 and 19.1 (C_γ for Val), 24.4 (C_γ for Pro), 28.2 ((CH_3)$_3$C for Boc), 29.1 and 29.7 (C_β for Val and Pro), 40.6 (C_α for Gly), 46.9 (C_δ for Pro), 57.2 and 59.0 (C_α for Val and Pro), 77.9 ((CH$_3$)$_3$*C* for Boc), 155.5, 169.6, 170.3, and 172.1 (C = O).

Synthesis of Boc-Val-Gly-OBzl. Boc-Val (9.0 mmol), Gly-OBzl tosylate (9.0 mmol), and TEA (22 mmol) were dissolved in distilled acetonitrile (72 mL). HBTU (9.0 mmol) was added and stirred for 30 h at room temperature. Saturated NaCl solution (210 mL) was added, and the crude compounds were extracted with ethyl acetate. The organic phase was washed with 10% citric acid aqueous solution, water, 4% sodium hydrogen carbonate, and then water. The compound was purified with a silica gel column (elute: ethyl acetate/hexane = 1/2). Yield 83%. ^{1}H NMR (400 MHz, DMSO) δ 0.82 and 0.85 (m, H_γ for Val), 1.38 (s, Boc), 1.92 (br, H_β for Val), 3.83, 3.87, 3.94, and 3.98 (m, H_α for Val and H_α for Gly), 5.12 (s, Bzl), 7.36 (s, C_6H_5). ^{13}C NMR (400 MHz, DMSO) δ 18.0 and 19.1 (C_γ for Val), 28.1 ((CH_3)$_3$C for Boc), 30.4 (C_β for Val), 40.6 (C_α for Gly), 59.4 (C_α for Val), 65.8 (Bzl), 77.9 ((CH$_3$)$_3$*C* for Boc), 127.9, 128.0, 128.3, and 135.8 (phenyl), 155.3, 169.6, and 171.9 (*C* = O).

Synthesis of TFA·H-Val-Gly-OBzl. Boc-Val-Gly-OBzl (7.4 mmol) was dissolved in TFA (6 mL) and incubated at 4°C for 4 h. The reaction mixture was evaporated. After the addition of water, evaporation was performed four separate times. The crude compound was recrystallized from ethyl acetate/hexane. Yield 96%. ^{1}H NMR (400 MHz, D_2O containing 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid, sodium salt) δ 1.00 (br, H_γ for Val), 2.19 (br, H_β for Val), 3.85 (br, H_α for Val), 4.03, 4.08, 4.19, and 4.23 (m, H_α for Gly), 5.23 (s, Bzl), and 7.45 (s, phenyl). ^{13}C NMR (400 MHz, D_2O containing 3-(trimethylsilyl)-propionic-2,2,3,3-d_4 acid, sodium salt) δ 19.7 and 20.2 (C_γ for Val), 32.8 (C_β for Val), 44.2 (C_α for Gly), 61.4 (C_α for Val), 70.7 (Bzl), 131.4, 131.7, and 137.9 (phenyl), 172.7 and 173.7 (*C* = O).

Synthesis of Boc-Val-Pro-Gly-Val-Gly-OBzl. TFA·H-Val-Gly-OBzl (4.2 mmol) was dissolved in distilled acetonitrile (36 mL) and TEA (11 mmol). Boc-Val-Pro-Gly-OH (4.2 mmol), followed by HBTU (4.2 mmol), were added and stirred for 4 days at room temperature. Saturated NaCl solution (120 mL) was added, and the crude compounds were extracted with ethyl acetate. The organic phase was washed with 10% citric acid aqueous solution, water, 4% sodium hydrogen carbonate, and then water. The compound was purified with a silica gel column (elute: ethyl acetate/methanol = 95/5) and a Sephadex LH-20 column (GE Healthcare Life Sciences) (elute: methanol). Yield 28%. ^{1}H NMR (400 MHz, DMSO) δ 0.78 (m, H_γ for Val), 1.35 (s, Boc), 1.82 and 2.05 (br, H_β, and H_γ for Pro), 1.96 (br, H_β for Val), 3.57 and 3.80 (br, H_δ for Pro), 3.73, 3.88, 3.91, 3.93, 3.97, and 4.18 (m, H_α for Val and H_α for Gly), 4.29 (m, H_α for Pro), 5.11 (s, Bzl), and 7.34 (s, phenyl). ^{13}C NMR (400 MHz, DMSO) δ 18.1, 18.5, and 19.1 (C_γ for Val), 24.6 (C_γ for Pro), 28.2 ((CH_3)$_3$C for Boc), 29.1, 29.7, and 30.4 (C_β for Pro and Val), 40.7 and 42.1 (C_α for Gly), 47.1 (C_δ for Pro), 57.6, 57.7, and 59.6 (C_α for Val and Pro), 65.8 (Bzl), 78.0

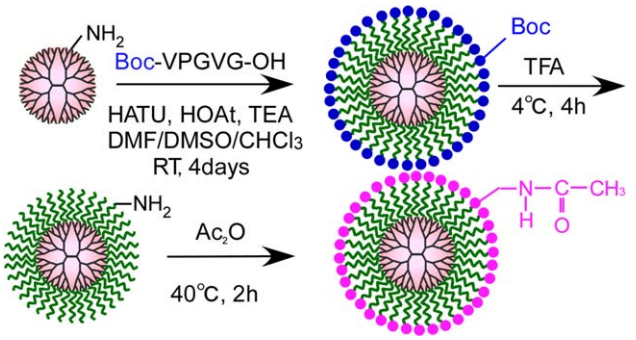

FIGURE 2 Synthetic pathway of elastin-mimetic dendrimer.

($(CH_3)_3C$ for Boc), 128.0, 128.1, 128.4, and 135.8 (phenyl), 155.5, 168.6, 169.6, 170.0, 171.3, and 172.0 (C = O).

Synthesis of Boc-VPGVG. Boc-Val-Pro-Gly-Val-Gly-OBzl (1.2 mmol) was dissolved in ethanol (21 mL). Pd-C (0.40 mmol) and 1,4-cyclohexadiene (12 mmol) were added and reduced with H_2 gas. The reaction mixture was filtered in the presence of celite (545RVS) and evaporated. Coevaporation with ethanol was performed four times, and the residual was dried under vacuum. Yield 97%. Purity 97% (estimated by HPLC). ^{1}H NMR data was shown in the following results section. ^{13}C NMR (400 MHz, DMSO) δ 18.1, 18.5, and 19.1 (C_γ for Val), 24.6 (C_γ for Pro), 28.2 ($(CH_3)_3C$ for Boc), 29.1, 29.7, and 30.6 (C_β for Val and Pro), 40.7 and 42.1 (C_α for Gly), 47.1 (C_δ for Pro), 57.3, 57.7, and 59.6 (C_α for Pro and Val), 78.0 ($(CH_3)_3C$ for Boc), 168.6, 170.6, 171.0, 171.1, and 172.0 (C = O).

Synthesis of an Elastin-Mimetic Dendrimer

The elastin-mimetic dendrimer was synthesized as shown in Figure 2. Three lots of the elastin-mimetic dendrimer were synthesized, and a typical synthetic method is described below.

Synthesis of Boc-VPGVG-Conjugated Dendrimer. Boc-VPGVG conjugation to dendrimer was performed according to previous reports.[7,8] Briefly, PAMAM dendrimer of G4 (3.2 μmol), Boc-VPGVG (0.31 mmol), and HOAt (0.19 mmol) were dissolved in DMSO/*N*,*N*-dimethylformamide/chloroform at the ratio of 3/3/2 (840 $\mu\Lambda$). HATU (0.37 mmol) was then added under nitrogen. TEA (1.6 mmol) was added, followed by stirring at room temperature for 4 days. The mixture was purified using a Sephadex LH-20 column with methanol as the eluent. The final product was obtained by freeze-drying. Yield 82%.

Acetylation of Elastin-Mimetic Dendrimers. Boc-Val-Pro-Gly-Val-Gly-dendrimer (2.9 µmol) was dissolved in TFA (3 mL) and incubated at 4°C for 6 h. The reaction mixture was evaporated. After the addition of water, subsequent evaporation was performed four times. Freeze-dried VPGVG-dendrimer (2.3 µmol) was dissolved in acetic anhydride (11 mL) and stirred at 40°C for 2 h. The reaction mixture was evaporated, then dialyzed (pore: MW1000) in aqueous solution (pH 8) for 1 day. The final product was obtained by freeze-drying. Yield 89%.

Characterization

The obtained products were characterized by ^{1}H-NMR and ^{13}C-NMR (JEOL, 400 MHz). The HPLC system was equipped with a cosmosil 5C18-MS-II column (Nacalai Tesque, Inc., Kyoto, Japan) and a UV detector (220 nm; UV-2075Plus; Jasco, Inc., Tokyo, Japan). Samples (5 μL) were injected with an autosampler (AS-2057Plus; Jasco, Inc.) and eluted with methanol/2% phosphoric acid = 5/95 (solvent A) at 1.0 mL min^{-1}. The solvent B (methanol) was gradually increased to 60% over 30 min.

CD Measurements

CD spectra were measured with a J-820 spectropolarimeter (Jasco) from 5°C to 65°C. Before each measurement, the sample solutions [0.05 mg/mL, 10 m*M* phosphate buffer (pH 7.4)] were incubated at the designated temperature for 10 min. The CD spectra were obtained using a 0.1-cm path length cell, by signal integrating 10 scans from 190 to 260 nm at a scan speed of 50 nm/min. Data were processed by the simple moving average method. The molar ellipticity at 196 and 218 nm was estimated from the spectra, and the averaged data of three different lots of dendrimer were calculated.

Measurement of Phase Transition

Various solutions of elastin-mimetic dendrimers [1 mg/mL, 10 m*M* buffer (pH 4, 5, 6, and 7.4) containing 0.0–1.0*M* NaCl] were prepared by using 100 m*M* phosphate buffer (pH 7.4) or citrate buffer (pH 4, 5, and 6), 4*M* NaCl solution, and 10 mg/mL dendrimer solution. The turbidity was measured at 500 nm using a Jasco Model V-630 spectrophotometer equipped with a Peltier-type thermostatic cell holder coupled with an ETC-717 controller. The heating rate of the sample cell was maintained at 1.0°C min^{-1}. The cloud points were taken as temperature at the transmittance of 50%. The averaged cloud points of the three different lots of dendrimer were calculated.

Release Assay From the Dendrimer

The release of rose bengal from the elastin-mimetic dendrimer and hydroxyl-terminated PAMAM dendrimer of generation 4 was estimated according to previous reports.[7,11] Briefly, the dendrimers encapsulating rose bengal at 10 equivalents per

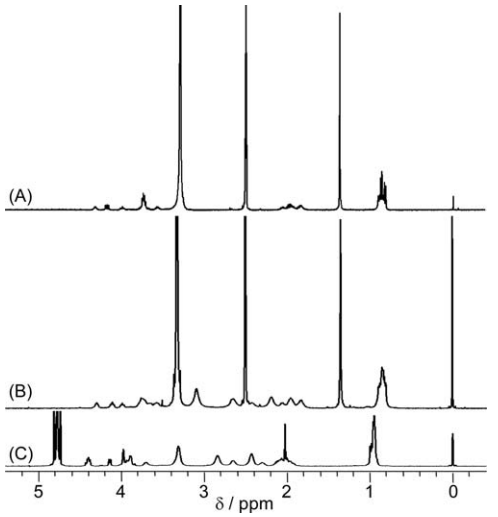

FIGURE 3 ^{1}H-NMR spectra of Boc-VPGVG (A) in DMSO-d_6, dendrimers conjugating ELPs with Boc-termini (B) in DMSO-d_6, and acetyl-termini (C) in D_2O.

dendrimer were dissolved in 1 mL of 10 m*M* phosphate buffer containing 150 m*M* NaCl [den 23 μM (1 mg/mL); RB 232 μM], and dialyzed (pore size: 2000 Da) at 37°C in the same solvent (30 mL). The outer phase from the dialysis bag was monitored by measuring the absorbance at 553 nm. Solutions of free rose bengal (550 nm) was also analyzed. The standard curves of rose bengal in the absence and the presence of the hydroxyl-terminated dendrimer were obtained to calculate percent of released rose bengal. Three independent experiments were performed.

RESULTS AND DISCUSSION

Synthesis of Elastin-Mimetic Dendrimer

Boc-VPGVG was synthesized as ELP by Boc-based liquid phase synthesis, as shown in Figure 1. The elastin-dendrimer was synthesized according to Figure 2. PAMAM dendrimers of generation 4 were reacted with Boc-VPGVG using HATU and HOAt. TFA was treated to remove the Boc group and were reacted with acetic anhydride (Ac_2O). The synthesized compounds were characterized by ^{1}H NMR. The NMR spectra of Boc-VPGVG, Boc-VPGVG-conjugated dendrimer (G4), and Ac-VPGVG-conjugated dendrimer (G4) are shown in Figure 3. The spectrum of Boc-VPGVG-conjugated dendrimer revealed signals derived from PAMAM dendrimer (2.2, 2.4, 2.7, and 3.1 ppm), the peptide unit (0.85, 1.7–2.1, and 3.5–4.4 ppm), and Boc unit (1.3 ppm). The integral ratio of the Boc and peptide unit signals to the PAMAM dendrimer signals (3.1 ppm) indicated that virtually all terminal amino groups of the dendrimer were modified with the peptide. In the spectrum of the Ac-VPGVG-conjugated dendrimer, the signal from the Boc unit (1.3 ppm) disappeared and that from the Ac unit (2.0 ppm) emerged. The integral ratios of the Ac and peptide unit signals to the dendrimer signals indicated that essentially all terminal groups were acetylated. In addition, HPLC analysis indicated that there were no contaminations from free peptides. Therefore, we synthesized fully ELP-modified dendrimers.

Secondary Structure of the Elastin-Mimetic Dendrimer

CD spectrometry is useful for the estimation of protein secondary structure. Elastin-mimetics can form β-turn, whose CD spectrum has a negative cotton effect around 218 nm.[18,23,24] CD spectra of the elastin-mimetic dendrimer were measured, and the Ac-VPGVG peptide was used as a control (Figure 4). The elastin-mimetic dendrimer and the Ac-VPGVG exhibited the negative cotton effect corresponding to β-turn structure (218 nm) and random coil (196 nm) at a similar level. This suggested that the elastin-mimetic dendrimer had an elastin-mimetic structure, and conjugation of the ELP to the dendrimer did not interfere with the higher order structure.

The CD spectra were measured at different temperatures, as shown in Figures 4A and 4B. The negative cotton effect at 196 nm was mainly observed at low temperature, corresponding to random coil structure. The negative cotton effect at 218 nm, corresponding to the β-turn structure, increased at higher temperature in both materials. The molar ellipticities at 218 and 196 nm were plotted against temperature (Figure 4C). The gradual increase of the 218 nm (β-turn) signal and the gradual decrease of the 196 nm (random coil) signal were observed with increasing temperature, indicating that the random coil structure was gradually changed into the β-turn structure by heating. The Ac-VPGVG exhibited similar behavior to the elastin-mimetic dendrimer. Therefore, the elastin-mimetic dendrimer is an artificial elastin-mimetic material with temperature-dependent conformation change.

Phase Transitions of Elastin-Mimetic Dendrimer

The phase transition of aqueous solutions containing the elastin-mimetic dendrimer was examined. The phase transition for the Ac-VPGVG peptide and the acetylated dendrimer in 10 m*M* phosphate buffer containing 150 m*M* sodium chloride (pH 7.4) was not observed. The solution containing elastin-mimetic dendrimer (Ac-VPGVG-conjugated dendrimer) became turbid upon heating, which is a phase transition. The change in the transmittance of the solution was monitored with increasing temperature. It changed drastically at 48°C,

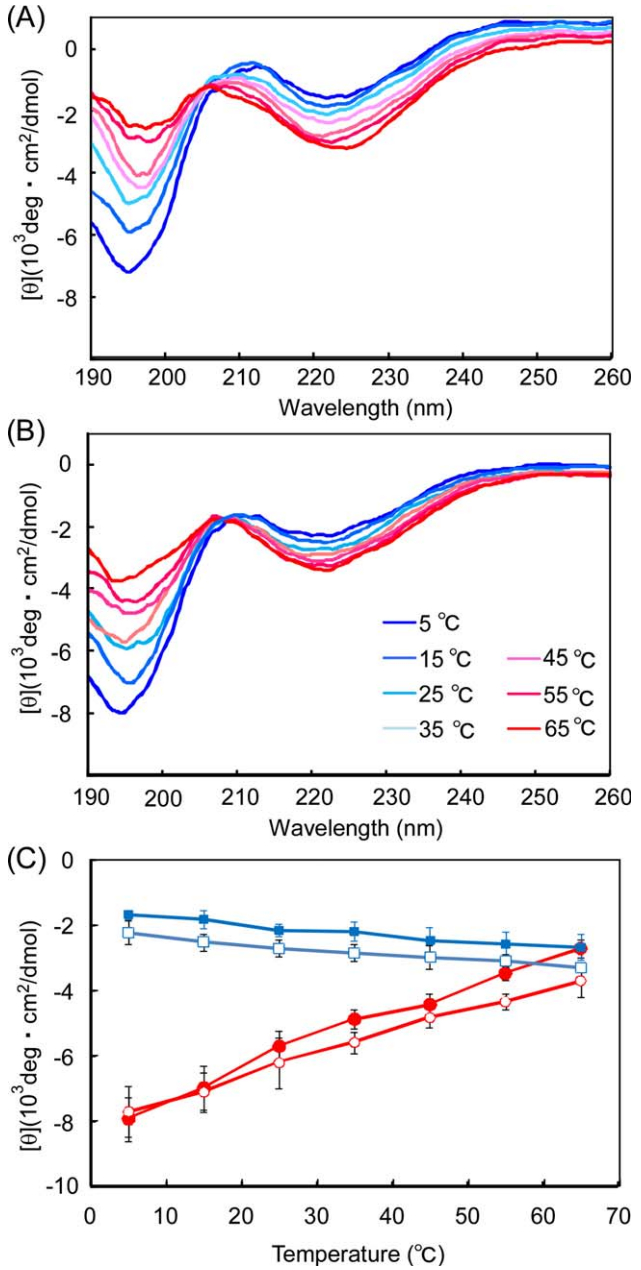

FIGURE 4 CD spectra of elastin-mimetic dendrimer (A) and Ac-VPGVG peptide (B) at different temperatures. (C) Correlation of the molar ellipticity corresponding to random coil (196 nm, red) and β-turn (218 nm, blue) to temperature. Closed and open symbols correspond to elastin-mimetic dendrimer and the Ac-VPGVG peptide, respectively.

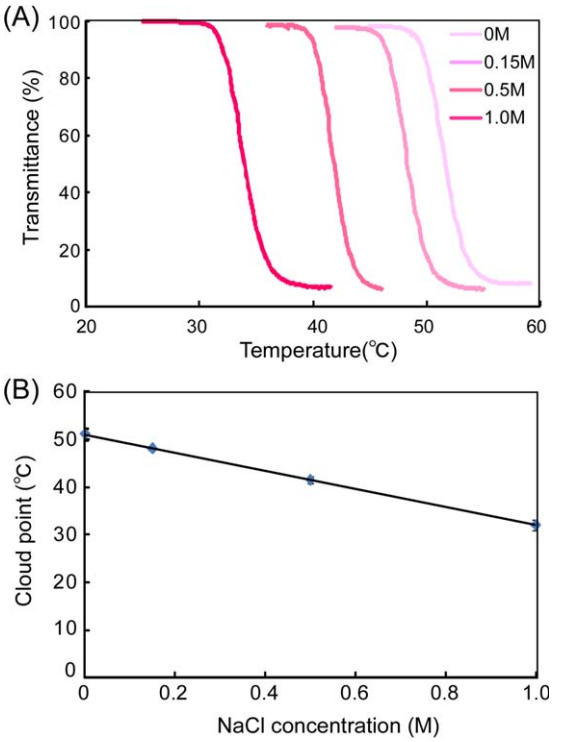

FIGURE 5 Temperature sensitivity of elastin-mimetic dendrimer in different salt concentrations. (A) Temperature dependence of transmittance for solutions of elastin-mimetic dendrimer in phosphate buffer (pH 7.4) containing NaCl (0.0–1.0 *M*). (B) Correlation of the cloud point to NaCl concentration. The results are shown as an average from three different lots of elastin-mimetic dendrimer.

which is called a cloud point (Figure 5). It was reported that elastin-mimetics have the salt-dependent thermosensitive property.[18,24,25] We examined the influence of the salt concentration on the thermosensitivity of the elastin-mimetic dendrimer. Figure 5A shows the temperature-dependent optical transmittance of aqueous solutions (pH 7.4) containing elastin-mimetic dendrimer in different NaCl concentrations (0.0–1.0*M*). The transmittance changed drastically at 51, 48, 41, and 32°C in 0, 0.15, 0.5, and 1.0*M* NaCl. The correlation between salt concentration and cloud point is shown in Figure 5B. A linear relationship was observed, with a slope of −19.0. The linear decrease of cloud point with increasing salt concentration was consistent with previous observations.[18,24,25] It is thought that salt might suppress the hydration on ELP, called the Hofmeister effect.[24,25] The slope of the elastin-mimetic dendrimer was almost similar to ELP polypeptides and less steep than short ELP.[18,24,25] These findings suggest that the elastin-mimetic dendrimer has similar properties to elastin proteins but not to ELPs.

We investigated the influence of pH on the thermosensitivity of the elastin-mimetic dendrimer (Figure 6). The cloud points of the elastin-mimetic dendrimer at pH 5, 6, and 7.4 in 0.15*M* NaCl were 66, 51, and 48°C, respectively, even though the cloud point was not observed at pH 4. The protonation occurred on the inner tertiary amine of the dendrimer at low pH. The protonated amino groups at lower pH may increase hydrophilicity, leading to a higher cloud point. This suggests

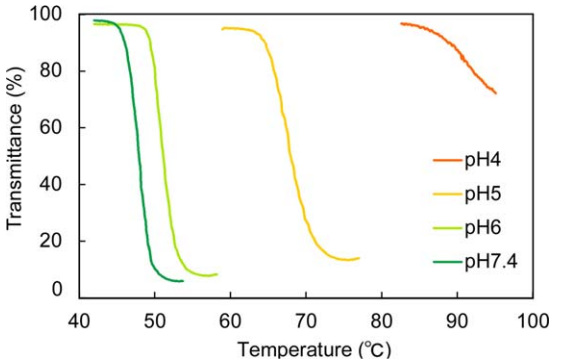

FIGURE 6 pH sensitivity of elastin-mimetic dendrimer in different pH solutions containing NaCl (0.15 *M*).

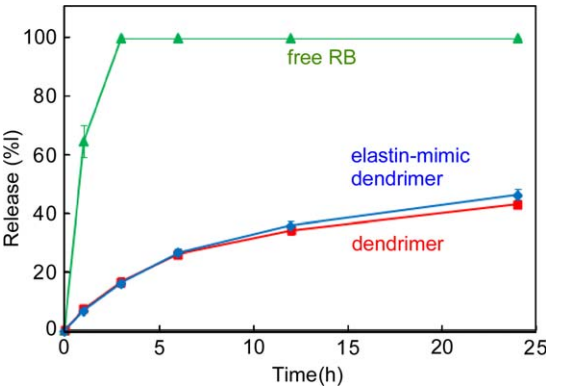

FIGURE 7 Release of a model drug, rose bengal, from elastin-mimetic dendrimer and the PAMAM dendrimer in the phosphate buffer containing 0.15 *M* NaCl (pH 7.4) at 37°C. Free rose bengal was also analyzed in the dialysis assay as a control.

that the sensitivity was influenced by the protonation of the dendrimer. Consequently, the elastin-mimic dendrimer can sense salt concentration and pH in addition to temperature.

The possible mechanism for the temperature dependency of the elastin-mimetic dendrimer is described as follows. Our CD results indicated that the conformation of the ELP at the dendrimer surface was changed from random coil to β-turn by heating. The β-turn peptides were more hydrophobic than random coil peptides, since bound water molecules were dissociated from the peptides.[23] Therefore, the conformation change was a cue for the phase transition. Even though the conformation of the elastin-mimetic dendrimer was changed gradually by heating, the turbidity was changed drastically. This suggests that the phase transition occurred when the hydrophobicity of the dendrimer was beyond the threshold. The peptide (Ac-VPGVG) by itself also exhibited the temperature-dependent conformation change, which did not become turbid by heating. This suggests that the phase transition was not induced only by the conformation change. Ac-VPGVG seemed more hydrophilic than the elastin-mimetic dendrimer. It was reported that the molecular weight of the elastin-mimetics affected the phase transition behaviors.[13,14] The elastin-mimetic dendrimer has a larger molecular weight (43 kDa) than Ac-VPGVG (470 Da), which induced the phase transition efficiently. It is also likely that a clustering effect at the dendrimer surface induced the self-assembly of ELP to induce the phase transition. The detailed temperature-dependent properties of elastin-mimetic dendrimers remain to be investigated.

Controlled Release of a Model Drug From Elastin-Mimetic Dendrimer

Since dendrimers are a potent drug carrier, elastin-mimetic dendrimers can be applicable to drug delivery systems. We investigated the release of a model drug, rose bengal, from the elastin-mimetic dendrimer and an intact dendrimer by dialysis assay (Figure 7). The dialysis membrane allows permeation of small model drug molecules but not large dendrimers. The absorbance in the outer phase was monitored over 24 h. Free rose bengal was rapidly distributed thorough the dialysis membrane. Rose bengal molecules encapsulated in the dendrimer compounds were distributed more slowly, indicating that these dendrimers formed complexes with rose bengal molecules. The complex stability of the elastin-mimetic dendrimer was similar to that of PAMAM dendrimer without ELP. As shown in Figure 5, the phase transition temperature of the elastin-mimetic dendrimer was 48°C in solution (pH 7.4 and 0.15*M* NaCl), which was much higher than 37°C. The elastin-mimetic dendrimer was still in a soluble state at 37°C. Thus, optimization of the phase transition temperature in elastin-mimetic dendrimers is indispensable for biomedical applications. The temperature-dependent properties can be controlled by peptide chain length, peptide sequence, and dendrimer generation,[13–18,22] and work on this is currently underway in our laboratory.

CONCLUSION

We synthesized and characterized the fully Ac-VPGVG-conjugated dendrimer. The structure of elastin-mimetic dendrimer changed from random coil into β-turn by heating, suggesting that this kind of dendrimer is an elastin mimetic. The elastin-mimetic dendrimer underwent temperature-dependent phase transition even at the physiological pH. The phase transition temperature was affected by salt concentration and pH. Since dendrimers are potential unimolecular drug carriers, this type of artificial elastin material has potential as a functional biomaterial for drug delivery systems.

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