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Synthesis of biocompatible dendrimers with a peripheral network formed by linking of polymerizable groups

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Abstract

Toward the production of unimolecular nanocapsules with biocompatible surface, we prepared a fourth generation polyamidoamine dendrimer having both a poly(ethylene glycol) (PEG) with the number average molecular weight of 2000 and a methacryloyl group at every chain end of the dendrimer through an L-lysine residue. The introduced methacryloyl groups of the dendrimer were successfully polymerized by using free radical initiators, such as azobisisobutyronitrile and dibenzoyl peroxide, as judged by ^{1}H NMR. GPC revealed that the molecular weight of the dendrimers having the polymerizable groups did not change after their polymerization, indicating that the polymerization of the methacryloyl groups took place within the single dendrimer. Polymerization of the methacryloyl groups resulted in a significant reduction of the affinity of the dendrimer to a guest molecule, rose bengal, suggesting that the peripheral network formed by the linking of methacryloyl groups hides the dendrimer interior. In addition, when the methacryloyl groups of the dendrimer were polymerized in the presence of rose bengal, the guest molecule was found to be tightly associated with the dendrimer.

Keywords: Dendrimer; Drug delivery system; Nanocapsule

1. Introduction

Dendrimers have attracted much interest because of their unique structures and properties [1,2]. Their size, structure and surface properties are highly controllable. Also, their interiors are capable of encapsulating small molecules [3–5]. Considering these features, dendrimers are highly attractive material for the application in the biomedical field, such as drug delivery and imaging [6–8].

Dendrimers, indeed, do not always have structures and properties which are suitable for the biomedical use. For example, polyamidoamine (PAMAM) and poly(propylene imine) dendrimers, which are the most widely used dendrimers, are shown to be toxic to animal cells [9]. Thus, it is desired to develop dendrimers that are suitable for the biomedical use on the basis of rational design. Several dendrimers have been developed from the aspects of biodegradability and biocompatibility [10–13].

Previously, we have designed PAMAM dendrimers with poly(ethylene glycol) (PEG) chains to obtain dendrimers with biocompatibility [14]. We successfully attached a PEG chain to every chain end of the generation three and four dendrimers. In addition, it was found that anticancer drugs, such as adriamycin and methotrexate, can be loaded into the PEG-modified dendrimers. However, the dendrimers released their contents quickly under physiological conditions. Therefore, considering their use as a carrier for drugs and therapeutic agents, their encapsulation ability is still to be improved.

To elevate the ability of dendrimers to retain small guest molecules in the interior, introduction of shell structures to the dendrimer surface might be an effective strategy. In fact, Meijer and co-workers have constructed a shell of protected amino acid residues at the periphery of poly(propylene imine) dendrimers and shown that the dendrimers could retain rose bengal molecules within the internal cavities of the dendrimer [5]. Also, Zimmerman and co-workers have introduced a shell into dendrimers consisting of three third-generation poly(aryl ether) dendrons with homoallyl ether

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groups on their periphery by their intramolecular linking through a ring-closing metathesis reaction, while they used them to generate a synthetic host which can selectively bind to a target guest molecule [15,16]. Recently, we have introduced a shell of cysteine residues [17] or isobutyramide groups [18] to PAMAM dendrimers to produce functional dendrimers with environment sensitive or temperature sensitive properties.

Toward the production of biocompatible nanocapsule that can retain small molecules in the internal space, we designed PEG-attached dendrimers with a network formed by the linking of polymerizable groups combined to the chain ends of the dendrimer. In this study, we prepared the PAMAM G4 dendrimer having both a PEG chain and a methacryloyl group at every chain end of the dendrimer through an L-lysine residue, and polymerized methacryloyl groups by using free radical initiators. Properties of the PEG-attached dendrimer having a peripheral network as a biocompatible nanocapsule were described.

2. Experimental

2.1. Materials

PAMAM (Starburst) dendrimers of fourth generation and poly(ethylene glycol) monomethyl ether (M-PEG) with the number average molecular weight of 2000 were purchased from Aldrich Chemical (Milwaukee, WI). 4-Nitrophenyl chloroformate, 1,3-dicyclohexylcarbodiimide (DCC), N-hydroxysuccinimide (HONSu), methanesulfonic acid (MSA), thioanisole, methacryloyl chloride, and 4,6-di-tert-butyl-m-cresol were obtained from Tokyo Kasei Kogyo (Tokyo, Japan). Tetrahydrofuran (THF), N, N-dimethyl formamide (DMF), trifluoroacetic acid (TFA), dibenzoyl peroxide (BPO), 2,2-azobisisobutyronitrile (AIBN), triethylamine (TEA), and acetonitrile were purchased from Kishida Chemical (Osaka, Japan). Dimethyl sulfoxide (DMSO) and rose bengal were purchased from Wako Pure Chemical (Osaka, Japan). N^{α}-tert-butoxycarbonyl-N^{ε}-benzocarboxy-L-lysine [Boc-Lys(Z)] was purchased from Peptide Institute (Osaka, Japan).

2.2. Synthesis of dendrimers

Dendrimers were synthesized according to Scheme 1.

2.3. Synthesis of Boc-Lys(Z)-G4 dendrimer

HONSu (1.8 mmol), 1,3-dicyclohexylcarbodiimide (1.9 mmol) and TEA (2.2 mmol) were added to Boc-Lys(Z), (1.5 mmol) dissolved in DMF (4 ml) at 0 °C, stirred for 3 h, and then PAMAM G4 dendrimer (15 μmol) dissolved in DMSO (4 ml) was added to the solution. The mixed solution was stirred for 4 days at room temperature. The end of the reaction was confirmed from the absence of free amino groups as assessed by a ninhydrin test. Boc-Lys(Z)-G4 dendrimer was recovered and purified using a Sephadex LH-20 column with methanol as the eluent. Yield: 81%. ^{1}H NMR (DMSO-d_6); δ 1.13 (br), 1.33 (s), 1.41 (br), 1.54 (br), 2.17 (br), 2.41 (br), 2.64 (br), 2.95 (br), 3.07 (br), 3.80 (br), 4.97 (s), 6.91 (br), 7.30 (s), 7.80 (br), 7.90(br), 7.93 (br).

2.4. Synthesis of PEG-Lys(Z)-G4 dendrimer

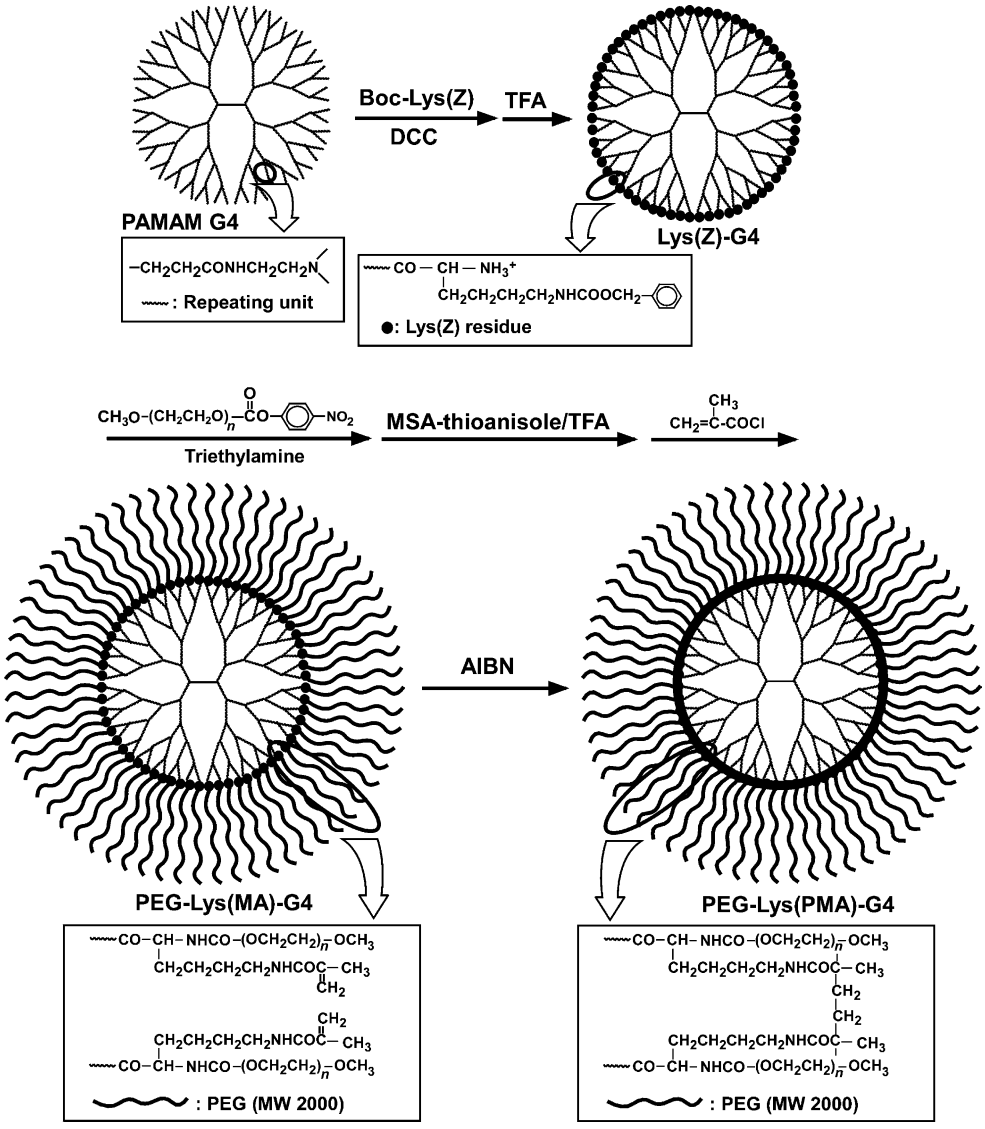
Boc groups of Boc-Lys(Z)-G4 dendrimer (12 μmol) was removed by the treatment with TFA (1 ml) at 0 °C for 3 h. Then TFA was removed by evaporation and subsequent drying under vacuum. M-PEG-4-nitrophenyl carbonate (1.2 mmol), which was synthesized as reported previously [14], was reacted with the deprotected dendrimer in DMSO (12 ml) containing TEA (2.3 mmol) at room temperature for 2 days. The end of the reaction was confirmed from the absence of free amino groups as assessed by a ninhydrin test. The PEG-Lys(Z)-G4 dendrimer was recovered and purified by dialysis against distilled water. The obtained dendrimer was further purified by gel permeation chromatography (GPC) using a Sephadex G-75 column with distilled water and by ultrafiltration using a filter membrane with a molecular cut-off of 10 kDa. Yield: 33%. ^{1}H NMR (D_2O); δ 1.14 (br), 1.26 (br), 1.41 (br), 1.50 (br), 2.21 (br), 2.45 (br), 2.62 (br), 2.90 (br), 3.16 (br), 3.22 (s), 3.59 (m), 3.78 (br), 4.85 (s), 7.24 (s).

2.5. Synthesis of PEG-Lys-G4 dendrimer

Z groups of PEG-Lys(Z)-G4 dendrimer (0.6 μmol) was removed by the treatment with a mixture of MSA, thioanisol, and TFA at the volume ratio of 5.5/9.9/84.6 (477 μl) at 0 °C for 2 h. Then the reaction mixture was diluted by chloroform (10 ml) and TEA (781 μl) was added to the solution to stop the reaction. PEG-Lys-G4 dendrimer dissolved in chloroform was washed with distilled water and recovered by evaporation. The crude product was purified using a Sephadex LH-20 column with methanol as the eluent. Yield: 50%. ^{1}H NMR (D_2O); δ 1.32 (br), 1.54 (br), 1.66 (br), 2.27 (br), 2.48 (br), 2.66 (br), 2.83 (t), 3.16 (br), 3.24 (s), 3.38 (br), 3.59 (m), 3.73 (br), 3.88 (br), 4.04 (br), 4.12 (br).

2.6. Synthesis of PEG-Lys(MA)-G4 dendrimer

TEA (0.16 mmol), 4,6-di-tert-butyl-m-cresol (1 mg) and methacryloyl chloride (41 μmol) were added to PEG-Lys-G4 dendrimer dissolved in DMF (300 μl) and stirred for 1 day at 4 °C. Then TEA (0.16 mmol) and methacryloyl chloride (41 μmol) were added again to the reaction mixture, and stirred at 4 °C for 1 day. The end of the reaction was confirmed from the absence of free amino groups as assessed by a ninhydrin test. PEG-Lys(MA)-G4 dendrimer was recovered and purified by using a Sephadex



Scheme 1. Preparation of PEG-modified dendrimers with polymerizable groups and their linking.

LH-20 column with methanol as the eluent. Yield: 92%. ^{1}H NMR (D_2O); δ 1.24 (br), 1.41 (br), 1.52 (br), 1.63 (br), 1.78 (s), 2.31 (br), 2.60 (br), 2.76 (br), 3.16 (br), 3.24 (br), 3.38 (br), 3.59 (m), 3.74 (br), 3.85 (br), 4.05 (br), 5.30 (s), 5.55 (s).

2.7. Synthesis of PEG-Lys(PMA)-G4

PEG-Lys(MA)-G4 dendrimer (0.10 μmol) and BPO (2.0 μmol) were dissolved in THF (10 ml) and stirred for 14 h at 70 °C in N_2 atmosphere. PEG-Lys(PMA)-G4 dendrimer was recovered and purified using Sephadex LH-20 column with methanol as the eluent. PEG-Lys(PMA)-G4 dendrimer was also synthesized by using AIBN. PEG-Lys(MA)-G4 dendrimer (0.19 μmol) and AIBN (3.8 μmol) were dissolved in THF (19 ml) and stirred for 14 h at 64 °C in N_2 atmosphere. Yield: 94%. ^{1}H NMR (D_2O); δ 1.00–2.00 (br), 2.24 (br), 2.46 (br), 2.66 (br), 3.18 (br), 3.22 (s), 3.59 (m), 3.84 (br).

2.8. Synthesis of PEG-Lys(PMA)-G4 containing rose bengal

Rose bengal of a lactonic form was obtained as precipitate by acidifying an aqueous rose bengal solution with HCl [19]. PEG-Lys(MA)-G4 dendrimer (0.018 μmol) and AIBN (0.7 μmol) were dissolved in THF (4 ml) containing rose bengal of a lactonic form (0.18 μmol) and stirred for 14 h at 64 °C in N_2 atmosphere. The reaction mixture was dried by evaporation and under vacuum overnight. Then, the dried mixture was dissolved in 10 mM phosphate and 0.1 M NaCl solution of pH 7.4 (final concentration of the dendrimer, 0.5 μM) and dialyzed against 10 mM phosphate and 0.1 M NaCl solution of pH 7.4 for 48 h.

2.9. Analytical techniques

GPC was performed using a system equipped with a Shodex SB-2004 column (Showa Denko) with differential refractive index detection (Jasco RI-930) eluting with a mixed solution of aqueous phosphate solution (10 mM, pH 7.4) and acetonitrile (3/1, v/v). PEG was used as the standard for calibration. UV–Vis absorption spectra were recorded on a spectrometer (Jasco V-520) at 25 °C. The binding of rose bengal to the dendrimer was measured as follows: a given volume of the dendrimer dissolved in 10 mM phosphate and 0.1 M NaCl solution of pH 6.0, 7.4 or 9.0 (concentration of dendrimer: 1.3 nM at pH 6.0; 13 nM at pHs 7.4 and 9.0) was added to rose bengal dissolved in the same buffer (final concentration: 1.76×10^{-6} M) and the mixed solution was incubated for 5 min. Concentrations of free and bound rose bengal were determined from absorption spectra of the solutions as previously reported [17].

3. Results and discussion

In a previous study, we have shown that both a PEG chain with the number average molecular weight of 2000 and a cysteine residue were successfully combined to essentially every chain end of the PAMAM G4 dendrimer [17]. Thus, in this study, we used L-lysine (Lys), which is an amino acid with two amino groups, to incorporate both methacryloyl groups and PEG chains to chain ends of the dendrimer.

The ^{1}H NMR was used to evaluate the numbers of Lys residues, PEG chains, and methacryloyl groups of the dendrimers (Table 1). The spectrum of the PEG-Lys(MA)-G4 dendrimer showed peaks corresponding to the PAMAM dendrimer, the Lys residues, the methacryloyl groups and the PEG chain. The signals at 2.31, 3.24, 3.85, and 5.55 ppm, which correspond to the protons of methylenes next to carbonyl groups of the dendrimer, those of the terminal methyl group of PEG, those combined with α-carbon atoms of Lys residues, and those of vinyl groups of methacryloyl group, respectively, were used for the evaluation. From the integral ratios of these protons, the average numbers of the Lys residues, the PEG chains and the methacryloyl groups of the dendrimer were estimated to be 64.4 ± 1.4, 64.6 ± 1.8, and 62.2 ± 0.9, respectively. These numbers agree with the number of chain ends of the parent dendrimer. Therefore, this result indicates that both a PEG chain and a methacryloyl group were combined to essentially every chain end of the dendrimer through a Lys residue.

To examine if the methacryloyl groups of the PEG-Lys(MA)-G4 dendrimer can be linked by using a free radical initiator, BPO was added to the dendrimer dissolved in THF and stirred for 14 h at 70 °C under nitrogen. The ^{1}H NMR spectra of PEG-Lys(MA)-G4 dendrimer before and after the treatment with BPO were depicted in Fig. 1 As is apparent in Fig. 1, signals at 1.78, 5.30, and 5.55, which correspond to methacryloyl groups of the PEG-Lys(MA)-G4 dendrimer, completely disappeared after the treatment with BPO, indicating that these groups were linked each other. In addition, significant broadening of signals corresponding to the PAMAM dendrimer moiety around 2–3 ppm took place after the polymerization of methacryloyl groups, suggesting that mobility of the dendrimer chain was reduced by the network formed on the periphery by the linking of methacryloyl groups. Because no peak corresponding to benzoyl groups was observed around 7.3–8.4 ppm in the ^{1}H NMR spectrum of the resultant dendrimer, it is supposed that only a limited number of benzoyl radicals induced the linking of the methacryloyl groups. We also examined the linking of the methacryloyl groups of the dendrimer by using AIBN. We observed that the polymerizable groups were linked by the treatment with this free radical initiator as judged by ^{1}H NMR.

The molecular weights of the PEG-Lys(MA)-G4 dendrimer before and after the treatment with AIBN were estimated by GPC using a mixed solution of 10 mM phosphate buffer (pH 7.4) and acetonitrile at the volume ratio of 3/1 as an eluent (Fig. 2). The dendrimer after the treatment with AIBN is designated as PEG-Lys(PMA)-G4 dendrimer. As can be seen in Fig. 2, the chromatograms for the PEG-Lys(MA)-G4 and PEG-Lys(PMA)-G4 were approximately the same, indicating that the linking of methacryloyl groups of the dendrimer hardly affected the apparent size of the dendrimer having PEG chains. The number average molecular weight (M_n), the weight average molecular weight (M_w), and the polydispersity index (M_w/M_n) for PEG-Lys(MA)-G4 and PEG-Lys(PMA)-G4 dendrimers were estimated by GPC and listed in Table 2. The estimated molecular weights of these dendrimers were much lower than the calculated molecular weight of the corresponding dendrimers because of the difference in molecular shape between the dendrimers and the PEG used as the standard.

Table 1
Characterization of dendrimers

Dendrimer	Number of chain ends	Number of amino acid residues per dendrimer	Number of PEG chains per dendrimer	Number of methacryloyl groups per dendrimer
Boc-Lys(Z)-G4	64	64.0 ± 1.1	–	–
PEG-Lys(Z)-G4	64	62.9 ± 1.9	63.4 ± 1.2	–
PEG-Lys(MA)-G4	64	64.4 ± 1.4	64.6 ± 1.8	62.2 ± 0.9

Determined by ^{1}H NMR. The means and standard deviations were shown ($n=3$).

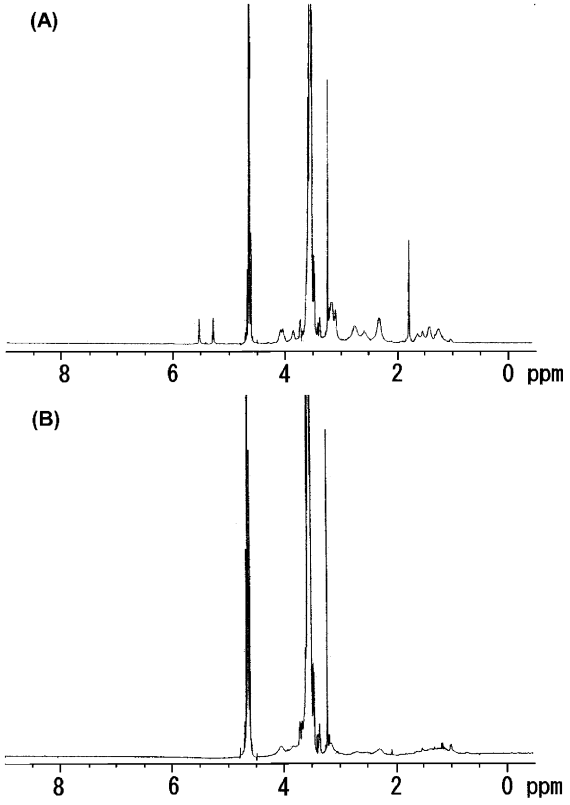


Fig. 1. ^{1}H NMR spectra for PEG-Lys(MA)-G4 (A) and PEG-Lys(PMA)-G4 (B) dendrimers in D_2O. BPO was used for the preparation of PEG-Lys(PMA)-G4 dendrimer.

If crosslinking of the dendrimer molecules takes place during the polymerization of methacryloyl groups, then the dendrimer after the polymerization should exhibit a larger molecular weight than before the polymerization. However, the molecular weight of the dendrimer with the polymerizable groups estimated by GPC did not increase after the treatment with AIBN, indicating that linking of methacryloyl groups took place only within the single dendrimer molecule. In addition, it seems that the dendrimer after the linking showed somewhat lower molecular weight than the dendrimer before the linking, suggesting that the formation of a network on the periphery of the dendrimer through the linking of the methacryloyl groups make its conformation

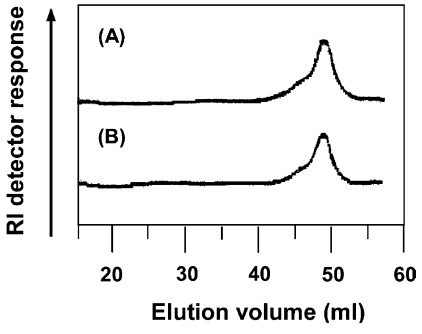


Fig. 2. Typical chromatograms for PEG-Lys(MA)-G4 (A) and PEG-Lys(PMA)-G4 (B).

compact. This is consistent with the result of ^{1}H NMR spectra, which indicates reduced mobility of the dendrimer chain, as mentioned above.

In order to know the effect of the peripheral network of the dendrimer on its properties as a nanocapsule, we examined its binding ability to rose bengal (Fig. 3). Rose bengal has been shown to be a useful guest molecule to investigate dendrimer-small molecule interactions, because this compound yields a bathochromic shift in the maximum absorption wavelength when bound to dendrimers [5]. The absorption spectra of rose bengal in the absence or presence of the dendrimers are shown in Fig. 2A. Rose bengal showed a strong peak at 549 nm in the absence of dendrimers, whereas the peak became smaller and exhibited a red-shift in the presence of the dendrimer, indicating that rose bengal molecules were bound to the dendrimers.

The differential absorption spectra of rose bengal in the presence of the dendrimers were obtained by subtracting the spectra of free rose bengal (Fig. 3B). These spectra showed negative and positive peaks at 545 and 568 nm, respectively. The former corresponds to the decrease in free rose bengal molecules in the solution, whereas the latter is attributable to the increase in the bound molecules [17]. The numbers of rose bengal molecules bound to the dendrimers were estimated from $\Delta_{absorbance}$ at 545 nm [17]. It was found that 20.6 rose bengal molecules were bound per a dendrimer molecule for the PEG-Lys(MA)-G4 dendrimer at pH 6.0, whereas the number of bound dye molecules per a dendrimer molecule was 16.1 for the PEG-Lys(PMA)-G4 dendrimer, indicating that the network formed on the periphery of the dendrimer reduced the binding of rose bengal.

We have previously shown that electrostatic interaction plays an important role in interaction of the polyamidoamine dendrimers with small guest molecules [14]. Because the interior of the polyamidoamine dendrimers contains many tertiary amino groups, microenvironment of the dendrimer interior is known to vary, depending on pH [20]. Thus, we investigated the influence of pH on the association between the dendrimers and rose bengal. We performed titration experiments by adding increasing amounts of dendrimer to rose bengal at varying pHs and the results was analyzed by the Klotz plot, which is widely used to study host–guest interactions [21,22]. Fig. 4 represents the numbers of binding sites and the intrinsic binding constants for the PEG-Lys(MA)-G4 dendrimer and the PEG-Lys(PMA)-G4 dendrimer evaluated by the Klotz plot as a function of pH. As can be seen in Fig. 4A, both dendrimers exhibit essentially the same number of binding sites, when the pH was the same, indicating that the network on the periphery of the dendrimer did not affect the number of binding sites. However, as shown in Fig. 4B, the intrinsic binding constant increases greatly with decreasing pH for the PEG-Lys(MA)-G4 dendrimer, in contrast to the case of the PEG-Lys(PMA)-G4 dendrimer, which exhibited only

Table 2
Molecular weights of dendrimers

Dendrimer	Molecular weight			
		Estimated by GPC		
	Calculated[a]	M_n	M_w	M_w/M_n
PEG-Lys(MA)-G4	154,619	30,100	38,460	1.28
PEG-Lys(PMA)-G4	154,619	28,680	36,070	1.26

[a] Calculated using 2000 for the molecular weight of PEG.

gradual increase in the intrinsic binding constant upon the same pH decrease.

Because protonation of the tertiary amino groups of the dendrimer interior is enhanced with decreasing pH [23,24], the negatively charged rose bengal molecule interacts more strongly with the dendrimer interior with more positive charges. The fact that both dendrimers showed the same number of the binding sites (Fig. 4A) implies that a major factor, which controls the affinity of the dendrimers against rose bengal, would be the same and presumably positive charges of the dendrimer [14]. However, the affinity of the network-bearing dendrimer is lower than that of the network-free dendrimer. As described above, it is likely that the dendrimer with the peripheral network takes on a compact conformation, which might cause generation of a shell consisting of densely packed chain ends around the periphery of the dendrimer. The shell might hide the charged sites in the interior of the dendrimer and decrease its affinity against the dye molecule.

In order to examine whether the dendrimer having the peripheral network can trap rose bengal molecule in its interior space, we carried out polymerization of methacryloyl groups of the PEG-Lys(MA)-G4 dendrimer in the presence of rose bengal by using AIBN. We also treated the dendrimer under the same condition without AIBN as a control experiment. After the treatment, these dendrimers were dialyzed against 10 mM phosphate and 0.1 M NaCl buffer of pH 7.4 for 48 h and their absorption spectra were measured (Fig. 5). The dendrimers without the peripheral network did not show any peak corresponding to rose bengal. Thus, this dendrimer without the peripheral network could not retain this guest molecule after the 48 h dialysis. In contrast, the dendrimer with the peripheral network exhibited a peak around 562 nm, which corresponds to rose bengal bound to the dendrimer [17], indicating that this guest molecule is retained tightly in the dendrimer having the peripheral network. We performed this experiment 2 times and the number of rose bengal molecules associated with a dendrimer molecule was estimated to be 0.4 ± 0.1 from the absorbance at 562 nm [17]. Although rose bengal of 10 equivalent amount was added to the dendrimer during the polymerization and this molecule has a high affinity to the interior of the dendrimer interior, the encapsulation efficiency was low. This result implies that for a large fraction of the dendrimers, the linking of the polymerizable groups could not produce a dense shell in the periphery, which suppresses diffusion of the guest molecule. At present, the reason is not clear. However, it seems possible that binding of rose bengal affected the conformation of the dendrimer. For example, the bound guest molecules may induce deformation of the globular shape of the dendrimer. In that case, the linking of methacryloyl groups attached to

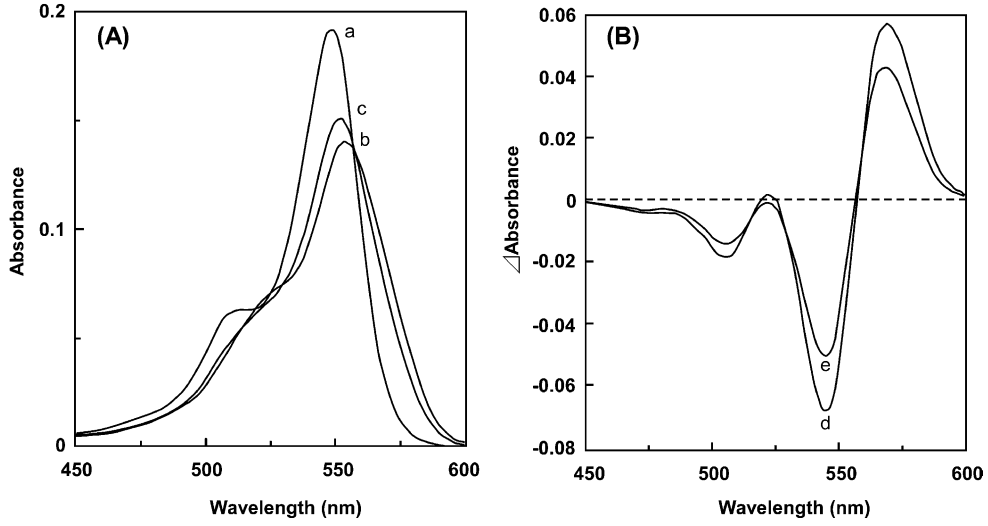


Fig. 3. (A) Absorption spectra of rose bengal in the absence (a) and presence of PEG-Lys(MA)-G4 (b) or PEG-Lys(PMA)-G4 (c) dendrimer in 10 mM phosphate and 0.1 M NaCl buffer (pH 6.0). The concentrations of rose bengal and the dendrimer were 1.9 and 0.076 μM, respectively. (B) Differential absorption spectra of rose bengal in the presence of PEG-Lys(MA)-G4 (d) and PEG-Lys(PMA)-G4 (e) dendrimers.

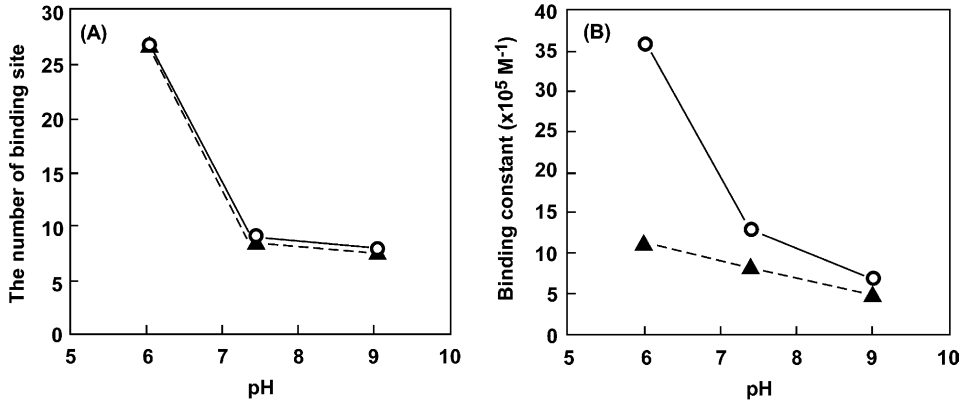


Fig. 4. The number of binding sites (A) and the intrinsic binding constant (B) of PEG-Lys(MA)-G4 (circles) and PEG-Lys(PMA)-G4 (triangles) dendrimers for rose bengal as a function of pH.

the chain terminals might not generate a dense shell formed by closely packed chains of the dendrimer. In addition, some conformational change, such as shrinkage of the dendrimer chain, could be induced during the polymerization process. Such a change in the dendrimer conformation may cause dissociation of the guest molecules from the dendrimer interior, resulting in the low encapsulation efficiency.

At present, the number of the guest molecules associated with the dendrimer is small. However, the encapsulation of the guest molecules is expected to be enhanced by increasing the dendrimer size. Also, the incorporation of network with more balky structure to the dendrimer surface may improve the retention of the guest molecules in the dendrimer interior.

4. Conclusions

We prepared the PEG-modified PAMAM G4 dendrimer having methacryloyl groups on the chain end moieties of the dendrimer and demonstrated that these polymeraizable groups can be linked within a single dendrimer molecule by using free radical initiators, such as AIBN and BPO. We found that after the linking of methacryloyl groups, the dendrimer decreased its affinity against rose bengal, probably because a network formed on the periphery of the dendrimer hide the dendrimer interior. In addition, it was suggested that the dendrimer having the peripheral network retain rose bengal molecules tightly. In the present study, we demonstrated that the linking of polymerizable groups attached to the periphery of the dendrimer could be an efficient approach to the generation of nanocapsules that retain guest molecules in their interior. Some pharmaceutical agents, such as photo-sensitizers for photodynamic therapy and boron10-containing compounds for neutron capture therapy; can exhibit their therapeutic effects, even if these molecules are not released from their carriers. We are currently trying to improve efficiency of encapsulation of guest molecules by increasing dendrimer generation and selecting chemical structure of network introduced into the dendrimers.

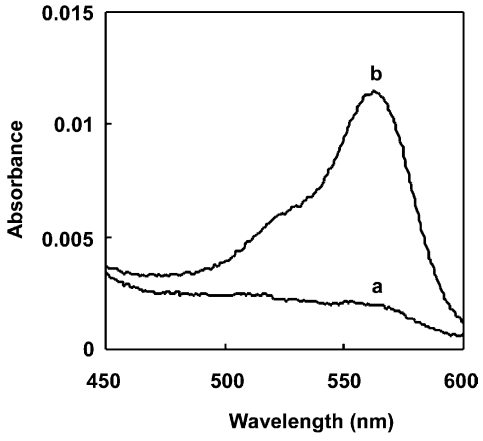


Fig. 5. Absorption spectra of PEG-Lys(MA)-G4 (a) and PEG-Lys(PMA)-G4 (b) dendrimers prepared in the presence of rose bengal. The spectra of the dendrimers were measured in 10 mM phosphate and 0.1 M NaCl buffer at pH 7.4. The concentration of PEG-Lys(MA)-G4 and PEG-Lys(PMA)-G4 were 0.54 and 0.52 μM, respectively.

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