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A unimolecular nanocapsule: Encapsulation property of amphiphilic polymer based on hyperbranched polythreitol

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Abstract

Hyperbranched polythreitol (**1**) with different molecular weights ($M_{w,SLS}$: 1.18×10^4 and 4.79×10^4) was reacted with trityl chloride in DMF to afford a novel amphiphilic polymer (**2**) consisting of **1** as the hydrophilic core and the trityl groups as the hydrophobic shell. Compound **2** was tested for its ability to act as a unimolecular nanocapsule toward the water-soluble dye, rose bengal (RB). Their encapsulation and release properties were also evaluated by comparison with the degree of substitution (DS) of the trityl groups, i.e., the hydrophobic shell density. The polymers were found to have very good unimolecular nanocapsule characteristics even at extremely low concentrations. The average number of RBs per polymer molecule depended on the hydrophilic core size and the hydrophobic shell density. The increasing DS value led to a decrease in the encapsulated amount due to the decrease in the hydrophilic core space, while the low DS value (less than ca. 20 mol%) led to a destabilization as a unimolecular nanocapsule and a lower encapsulation ability. In particular, **2** with ca. 23% DS value showed an efficient encapsulation. Based on a release test of the RB-loaded unimolecular nanocapsules, the polymers showed a high RB-holding ability in water.

Keywords: Amphiphiles; Core–shell polymers; Micelles

1. Introduction

A spherical amphiphile with a core–shell morphology is, due to its three-dimensional structure, an attractive nanoscale material. An amphiphilic structure consisting of a covalently linked dendritic core and shell parts promised to be a unimolecular micelle in solution, which could stably exist under various conditions, such as when the concentration, temperature, and pH were significantly changed [1–4]. The high stability as a unimolecular micelle is produced in a certain cavity in the interior of the spherical amphiphile, and this space could encapsulate suitable guest molecules. Therefore, there have been many studies on the design and synthesis of amphiphilic dendritic polymers as nanomaterials, such as a drug delivery agent [5–10], nanoreactors [11–14], and nanocapsules [15–23]. For example, Fréchet et al. reported the synthesis and enhanced catalytic activity of an amphiphilic dendrimer bearing a benzophenone derivative core as a light-driven catalytic site [24,25]. Okada et al. also reported the synthesis and characterization of a fluorescent-probe encapsulated glycodendrimer, the so-called ''sugar ball'' [26].

Hyperbranched polymers have recently received much attention as one class of spherical compounds even with their imperfectly branched structures when compared to a dendrimer, because their preparations were quite convenient versus the dendrimer synthesis [27–29]. Thus, it is interesting to use hyperbranched polymers as the key starting materials

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for the preparation of a spherical amphiphile with a unimolecular micellar property. For example, Frey et al. reported the synthesis and encapsulation properties of an amphiphilic ''molecular nanocapsule'' consisting of a hyperbranched polyglycerol core [30–32]. Brooks et al. also reported the effect of amphiphilic hyperbranched polyglycerols as a nanoreactor for unimolecular elimination reactions [33]. In addition, we reported the synthesis of novel unimolecular reversed micelles consisting of a hyperbranched polysaccharide core and a polylactide [34] or L-leucine ethyl ester [35] shell, which had encapsulation and slow-release abilities for hydrophilic molecules.

However, recent research has indicated that some of the amphiphilic dendritic polymers do not become a unimolecular micelle and form a large micellar structure in solution. For example, Richtering et al. reported that the amphiphilic hyperbranched polyglycerol with a low alkyl group functionality underwent strong aggregation in a hydrophobic solvent [36], indicating that the molecular form of the amphiphilic hyperbranched polymer can differ according to the outer functionality, i.e., the shell density of the polymer. The structural transition from a unimolecular form to an aggregate form should have an obvious effect on its molecular encapsulation property and release property. Therefore, it is very important to elucidate the effect of the shell density on the encapsulation properties for amphiphilic hyperbranched nanocapsules, but it has not yet been reported. In order to discuss the relation between the shell density of the amphiphilic hyperbranched nanocapsule and its encapsulation property for a guest molecule, we now report the preparation and unimolecular reversed micellar property of a novel unimolecular nanocapsule (**2**) consisting of a hydrophilic hyperbranched polythreitol (**1**) [37] core and a hydrophobic trityl shell.

2. Experimental

2.1. Materials

Hyperbranched polythreitol (**1**), which is a hyperbranched carbohydrate polymer with a high water solubility, was synthesized from 2,3-anhydro-erythritol according to our previous paper [37]. In this study, two samples of **1** with the weight average-molecular weight value estimated by static light scattering ($M_{w,SLS}$) of 1.18×10^4 and 4.79×10^4 were used as the core molecule. The weight average-molecular weight value estimated by size exclusion chromatography ($M_{w,SEC}$) of these samples were 1.43×10^3 ($M_{w,SEC}/M_{n,SEC}$: 1.66) and 1.85×10^3 ($M_{w,SEC}/M_{n,SEC}$: 1.58), respectively. Polymer **1** was dried in vacuo at 40 °C for 2 days, followed by azeotropic distillation with dry toluene just before use. The degree of branching (DB) value of **1** was 0.47, which was calculated using the ratio of the terminal units (T, 0.31) in **1** [37]. The ratio of the primary hydroxyl groups on the polymer **1** surface was 31 mol% of the total hydroxyl groups, which was calculated using Eq. (1).

$$\begin{aligned}&\frac{\text{number of primary hydroxyl groups of terminal units in } \mathbf{1}}{\text{number of total hydroxyl groups in } \mathbf{1}} \times 100 \\ &= \frac{\left(M_{w,SLS} \text{ of } \mathbf{1}/M_{w,ery}\right) \times T \times \left(\text{number of primary hydroxyl groups per terminal unit}\right)}{\left(M_{w,SLS} \text{ of } \mathbf{1}/M_{w,ery}\right) \times \left(\text{number of hydroxyl groups per monomer unit}\right)} \times 100 \\ &= \frac{\left(M_{w,SLS} \text{ of } \mathbf{1}/M_{w,ery}\right) \times T \times 2}{\left(M_{w,SLS} \text{ of } \mathbf{1}/M_{w,ery}\right) \times 2} \times 100(\%)\end{aligned} \quad (1)$$

$M_{w,ery}$ – molecular weight of erythritol repeating unit = 104.1.

Trityl chloride (99%) was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan) and used as received. 4-(Dimethylamino)pyridine (DMAP, >99%) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and recrystallized from dry toluene. *N*,*N*-Dimethylformamide (DMF, Kanto Chemical Co., Ltd. >99.0%) was dried over CaH_2 and distilled prior to use under reduced pressure. Triethylamine (Kanto Chemical Co., Ltd. >99%) was distilled over CaH_2. Dry toluene (>99.5%, water content < 0.001%) was purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan) and used without further purification. Rose bengal (RB) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methanol (>99.5%), acetone (>99.0%), ethyl acetate (>99.0%), chloroform (>99.0%, spectroscopic grade), and dimethyl sulfoxide (DMSO, >99.7%, spectroscopic grade) were purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan) and used without further purification. The dialysis membrane (Spectra/Por 6 regenerated cellulose, MWCO 1000) was purchased from Spectrum Laboratories, Inc.

2.2. Instrumentation

The ^{1}H NMR spectra were recorded using a JEOL JNM-A400II instrument. The IR spectra were recorded using a Perkin–Elmer Paragon 1000. Size exclusion chromatography (SEC) was performed in an aqueous sodium nitrate ($NaNO_3$) solution (0.2 mol L^{-1}, 1.0 mL min^{-1}) at 40 °C using a Tosoh HPLC system (HLC-8020) equipped with two TSKgel $GMPW_{xL}$ columns (pore size, 12.5–100 nm; bead size, 13 μm; exclusion limit, 5×10^7) and a refractive index detector. The $M_{w,SEC}$ and the $M_{w,SEC}/M_{n,SEC}$ values were calculated on the basis of a poly(ethylene glycol) calibration. The preparative SEC was performed in chloroform (3.8 mL min^{-1}) at 23 °C using a JAI LC-908 equipped with a JAI JAIGEL-2H

polystyrene column (pore size, 40–50 Å; bead size, 15 μm; exclusion limit, 5×10^3), a JAIGEL-2.5H polystyrene column (pore size, 60–70 Å; bead size, 16.5 μm; exclusion limit, 2×10^4), and JAI UV-310 and JAI RI-5HC detectors. The ultraviolet–visible (UV–vis) spectra were measured in $CHCl_3$ and $CHCl_3$/DMSO (1/7, v/v) with a 10-mm path length at 23 °C using a JASCO V-550 spectrometer. Static light scattering (SLS) measurements of **1** were performed in an aqueous $NaNO_3$ solution (0.2 mol L^{-1}) at 40 °C using an Otsuka Electronics CALLS-1000 light scattering spectrophotometer ($\lambda = 632.8$ nm). The refractive index increments (d*n*/d*c*) of **1** were 0.140 ($M_{w,SLS}$ of 1.18×10^4) and 0.135 ($M_{w,SLS}$ of 4.79×10^4) which were measured in an aqueous $NaNO_3$ solution (0.2 mol L^{-1}) at 40 °C using an Otsuka Electronics DRM-1021 double-beam differential refractometer ($\lambda = 632.8$ nm).

2.3. Synthesis of tritylated hyperbranched polythreitol (*2b*)

All procedures were performed under an argon atmosphere. A solution of trityl chloride (0.82 g, 2.94 mmol) in freshly distilled DMF (10 mL) was added dropwise into a solution of **1** (0.30 g, 5.77 mmol hydroxyl group; $M_{w,SLS}$, 1.18×10^4), DMAP (0.057 g, 0.47 mmol), and triethylamine (1.2 mL, 8.6 mmol) in freshly distilled DMF (2.5 mL) at 23 °C. After 24 h, methanol was added to quench the reaction. The organic solvent was evaporated and the residue was purified by dialysis (MWCO 1000) in acetone for 2 days. After acetone was evaporated to dryness, the residue was subjected to preparative SEC to remove any unreacted chemicals. The solvent was evaporated and the residue was dried in vacuo to afford **2b**. The yield was 0.46 g. ^{1}H NMR (400 MHz, CD_2Cl_2): δ (ppm) 5.12–2.55 (br, polythreitol), 7.61–6.69 (br, trityl group). IR (KBr): ν (cm^{-1}) 704, 751 (Ar, bend), 1077 (–C–O–C–, stretch), 1216 (ArC–O, stretch), 1447, 1491 (Ar, stretch), 2877, 2924 (C–H, stretch), 3025, 3055 (Ar, stretch), 3399 (OH, stretch). The degree of substitution (DS) was 23.8%, which was calculated using Eq. (2).

$$\frac{(\text{the peak area due to trityl group})/n_{tr}}{n_{OH} \times (\text{the peak area due to } \mathbf{1} \text{ moiety})/n_{\mathbf{1}}} \times 100(\%) \quad (2)$$

n_{tr} – number of protons per trityl group = 15; n_{OH} – number of hydroxyl groups per monomer unit = 2; $n_{\mathbf{1}}$ – number of protons per monomer unit = 6.

The calculated molecular weights (M_{cal}) of **2a–f** were estimated using Eq. (3) based on the $M_{w,SLS}$ of **1** and DS value.

$$M_{w,SLS} \text{ of } \mathbf{1} + \frac{M_{w,SLS} \text{ of } \mathbf{1}}{M_{w,ery}} \times \frac{DS}{100}(M_{w,Tr} - M_{w,H}) \quad (3)$$

$M_{w,Tr}$ – molecular weight of trityl group = 243.3; $M_{w,H}$ – molecular weight of proton = 1.0.

2.4. Encapsulation property of rose bengal

A typical procedure for the solid/liquid phase transfer is as follows: RB (20 mg, 20 mmol) was added to a solution of **2** in chloroform (3.85 mg mL^{-1}) and the suspension was then placed in a water bath shaker at 37 °C. After 24 h, any undissolved dye was removed by filtration using 0.45 μm PTFE membrane filters. The colored filtrate was diluted with chloroform for characterization by UV–vis spectroscopy (UV–vis ($CHCl_3$, 10 mm cell); λ_{max} (abs) = 562 nm (1.15)). The RB-loaded **2** (RB/**2**, 0.3 mL) system was diluted with DMSO (2.1 mL) and the encapsulated RB per **2** (N_{RB}) was quantitatively determined by its UV–vis measurement. UV–vis ($CHCl_3$/DMSO = 1/7, v/v, 10 mm cell); λ_{max} (abs) = 565 nm (0.207). $\varepsilon_{max} = 1.47 \times 10^5$ mol^{-1} L cm^{-1}. $N_{RB} = 1.4$.

2.5. Dynamic light scattering (DLS) measurements

DLS measurements of obtained polymers were performed in chloroform at 20 °C using an Otsuka Electronics FDLS-3000 light scattering spectrophotometer ($\lambda = 532$ nm, scattering angle = 90°). Before the measurement, the samples were filtered using 0.45 μm PTFE membrane filters to eliminate any dust particles. Data analysis was carried out using the histogram methods including the Marquadt analysis.

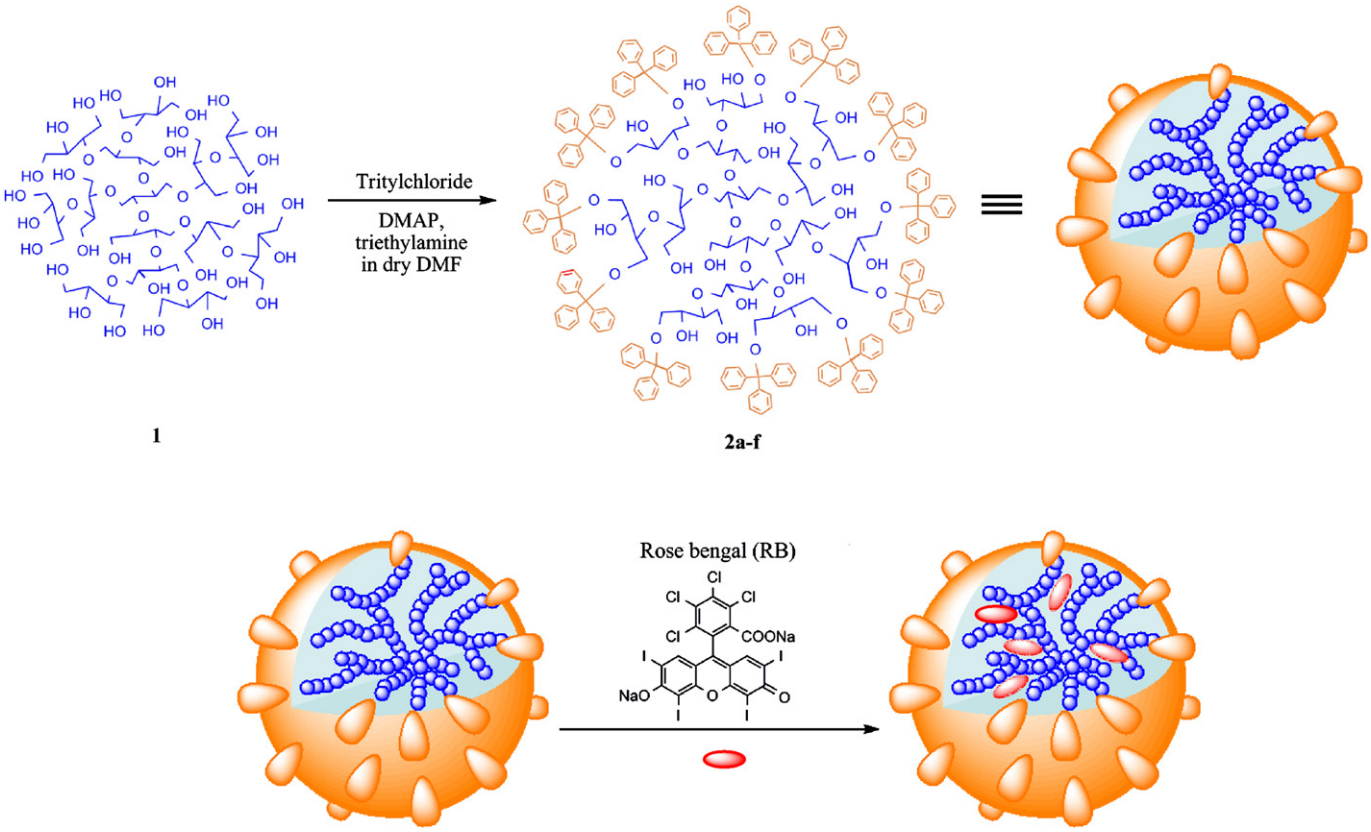
2.6. Release property of encapsulated guest

The particulate of the RB-encapsulating polymer (RB/**2**, 5.00 mg), which was prepared by evaporation of the RB/**2** system in chloroform, was placed in a vial with a 0.067 mol L^{-1} phosphate buffer solution (pH 7.4, 50 mL), and the suspension was then agitated in a bath shaker at 37 °C. The phosphate buffer of pH 7.4 was used to maintain near neutral conditions during the release experiment. A sample of the solution was taken and the RB concentration was determined by a UV–vis measurement ($\lambda_{max} = 549$ nm, $\varepsilon_{max} = 9.08 \times 10^4$ mol^{-1} L cm^{-1}).

3. Results and discussion

3.1. Preparation of amphiphilic tritylated hyperbranched polythreitol

The reaction of the hyperbranched polythreitol with trityl chloride is shown in Scheme 1. Two hyperbranched polythreitols (**1**) with different molecular weights ($M_{w,SLS}$: 1.18×10^4 and 4.79×10^4) were partially reacted with trityl chloride in dry *N*,*N*-dimethylformamide (DMF) and 4-(dimethylamino)-pyridine (DMAP), affording the organic solvent-soluble products. The reaction homogeneously proceeded to produce a brown solid after purification by dialysis and preparative size exclusion chromatography (preparative SEC). The ^{1}H NMR spectrum of the product clearly showed the presence of signals due to the trityl groups that appeared at 6.9–7.6 ppm along with the characteristic signals at 2.6–4.8 ppm due to **1** as the core (Fig. 1a). In addition, the peaks due to



Scheme 1.

the trityl group appeared at 700–750, 1400–1500, and 3000–3100 cm^{-1} in the IR spectrum, as shown in Fig. 1b. These results indicated that the product was assignable to the tritylated hyperbranched polythreitol (**2a–f**). Table 1 summarizes the reaction conditions and results. The degree of substitution (DS), which was estimated by the ^1H NMR analysis (Eq. (2)), could be controlled by the feed molar ratio of the trityl chloride and hydroxyl groups in **1** ([Tr]/[OH]); that is, the increasing [Tr]/[OH] values lead to increasing DS values. The DS value was 15.7 mol% under the condition of [Tr]/[OH] of 0.25 at 23 °C for 24 h (**2a**), 23.8 mol% for [Tr]/[OH] of 0.50 (**2b**), and 38.7 mol% for [Tr]/[OH] of 1.50 (**2c**). The disagreement between the [Tr]/[OH] and DS values, i.e., the DS values were always lower than the [Tr]/[OH] values, could be caused by the lower reactivity of the sterically hindered inner hydroxyl groups in **1** than that of the outer one.

The solubility characteristics of **2a–f** with certain DS values were examined using various solvents and are summarized along with that of **1** in Table 2. Compound **1** was soluble in water and methanol, but insoluble in other organic solvents, while **2a–f** was soluble in chloroform and acetone, but insoluble in water. The solubilities of **2a–f** were also changed by the DS values, i.e., **2c**, **2e**, and **2f** with high DS values were soluble in toluene and ethyl acetate, but insoluble in methanol, while **2a**, **2b**, and **2d** with low DS values were soluble in chloroform, but insoluble or swelling in toluene and ethyl acetate.

*3.2. Encapsulation properties of **2a–f** toward rose bengal*

To investigate the encapsulation properties of **2a–f**, the encapsulation characteristics of the water-soluble dye, rose bengal (RB), were examined using the solid/liquid phase transfer method. RB as a solid was added to a chloroform solution of **2a–f** and the heterogeneous mixture was then shaken for 24 h at 37 °C. After removal of any undissolved RB, the chloroform phases were apparently colorized (Fig. 2a). In contrast, the control experiment without **2a–f** showed no coloration, meaning that RB is almost insoluble in chloroform. Fig. 2b shows the UV–vis spectra of the chloroform solutions used in these experiments. For the RB-loaded **2a–f** (RB/**2a–f**) systems, the characteristic absorption due to RB appeared in the visible region from 470 to 600 nm even though the RB absorption did not appear for the control, indicating that **2a–f** possessed an encapsulation property toward RB. In addition, the results also suggested that RB existed under a more compatible environment than in chloroform, i.e., the hydrophilic hyperbranched polythreitol core in **2a–f**, as shown in Scheme 1.

The quantification of RB in the RB/**2a–f** systems was carried out by measuring the absorbance at 565 nm due to RB in the $CHCl_3$/DMSO (1/7, v/v) mixed solvent. The average number of RB molecules per **2a–f** molecule is shown as N_{RB} in Table 1. Two tendencies between N_{RB} and the polymer structure were recognized in the table. One is that N_{RB} increased

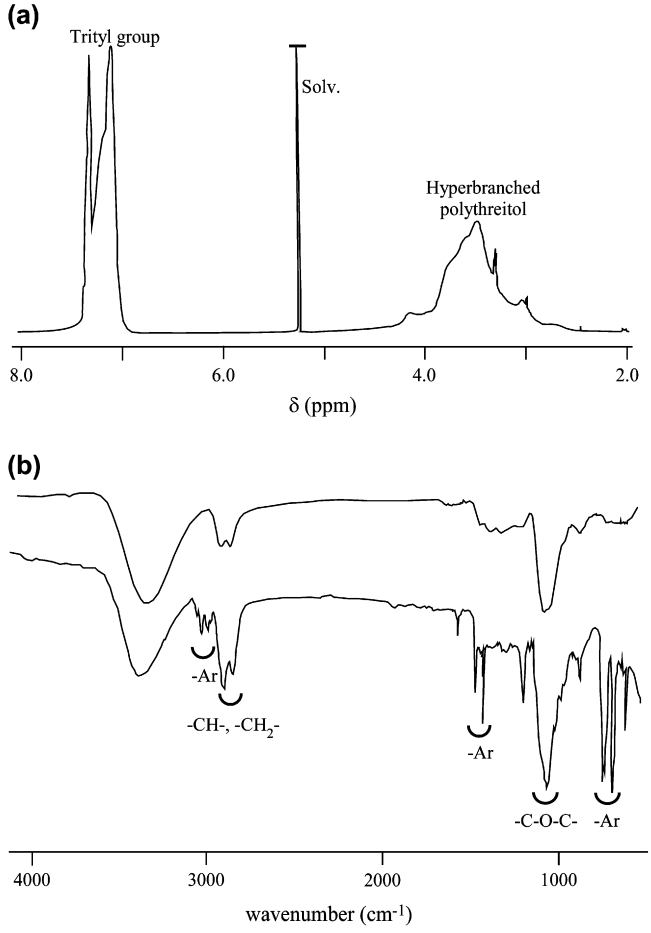


Fig. 1. (a) ^{1}H NMR spectrum of **2d** in CD_2Cl_2. (b) IR spectra of **1** (upper) and **2d** (lower) on KBr film.

with the increasing hydrophilic core size, i.e., 0.5–1.4 for **2a–c** and 4.3–4.9 for **2d–f**. The other is that N_{RB} depends on the DS value, i.e., the shell density of the trityl groups. Based on the results in Table 1, it seems that polymer **2** with ca. 23% DS value showed the maximum efficiency. The decrease in the N_{RB} value with the increasing DS value (e.g., **2b** > **2c** and **2d** > **2e** > **2f**) should be due to a decrease in the hydrophilic

Table 1
Synthesis of tritylated hyperbranched polythreitol (**2a–f**)[a]

Polymer	[Tr]/[OH][d]	DS[e] (mol%)	M_{cal}[f] ($\times 10^{-4}$)	N_{RB}[g]
2a[b]	0.25	15.7	1.61	0.5
2b[b]	0.50	23.8	1.83	1.4
2c[b]	1.50	38.7	2.24	1.3
2d[c]	0.50	23.2	7.37	4.9
2e[c]	1.00	43.2	9.60	4.4
2f[c]	2.00	61.0	11.60	4.3

[a] Ar atmosphere; DMAP, 0.08 equiv; temp, 23 °C; time, 24 h.
[b] Prepared from **1** with $M_{w,SLS} = 1.18 \times 10^4$.
[c] Prepared from **1** with $M_{w,SLS} = 4.79 \times 10^4$.
[d] Feed molar ratio of trityl chloride to the hydroxyl group in **1**.
[e] Degree of substitution determined by ^{1}H NMR spectra in CD_2Cl_2.
[f] Calculated molecular weight values estimated by Eq. (3) (see Section 2).
[g] Number of encapsulated rose bengal per **2**.

Table 2
Solubility of hyperbranched polythreitol (**1**) and tritylated hyperbranched polythreitol (**2a–f**)[a]

Polymer	DS (mol%)	H_2O	MeOH	Acetone	$CHCl_3$	AcOEt	Toluene
1	0	++	++	–	–	–	–
2a	15.7	–	+	+	++	–	–
2b	23.8	–	–	++	++	+	+
2c	38.7	–	–	++	++	++	++
2d	23.2	–	–	++	++	+	+
2e	43.2	–	–	++	++	++	++
2f	61.0	–	–	++	++	++	++

[a] Examined by using 5 mg of polymer in 0.5 mL of solvent at room temperature (++: soluble; +: swelling; –: insoluble).

core space. Fig. 3a shows the plot of the absorbance at the λ_{max} (562 nm) of RB versus the polymer concentration in chloroform. In general, the encapsulation capacities of the unimolecular micelles are invariable at any polymer concentration. Thus, if these polymers act as unimolecular reversed micelles, the absorbance values are expected to proportionally increase with the polymer concentration. RB/**2d** with a DS of 23.2% shows a proportional and linear relation between the absorbance value and polymer concentration even at extremely low concentrations, indicating that **2d** existed as a unimolecular reversed micelle in chloroform. However, a non-linear relationship with a deflection point at ca. 1.2×10^{-5} mol L^{-1}

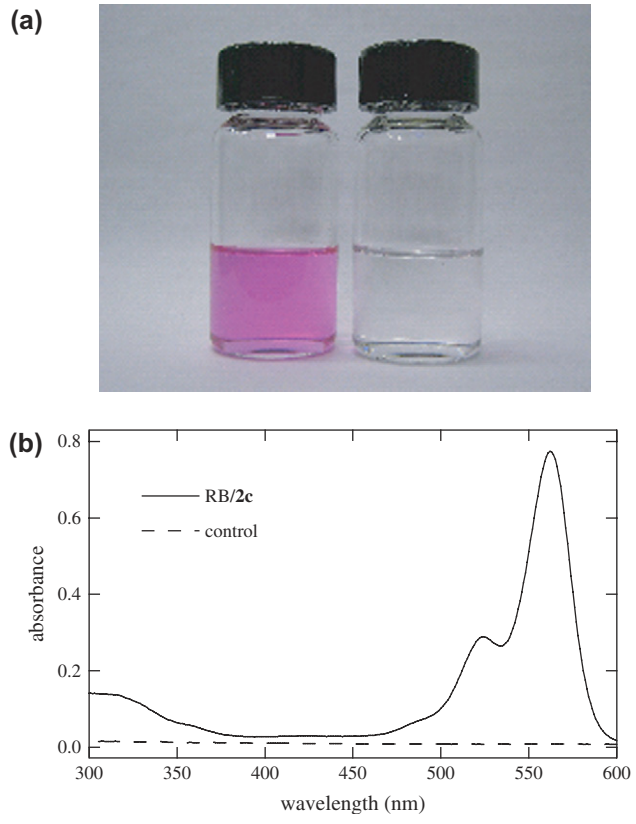


Fig. 2. (a) Demonstration of the encapsulation property of **2c** toward RB in chloroform (left vial: RB/**2c**; right vial: control). (b) UV–vis spectra of RB/**2c** and control in chloroform.

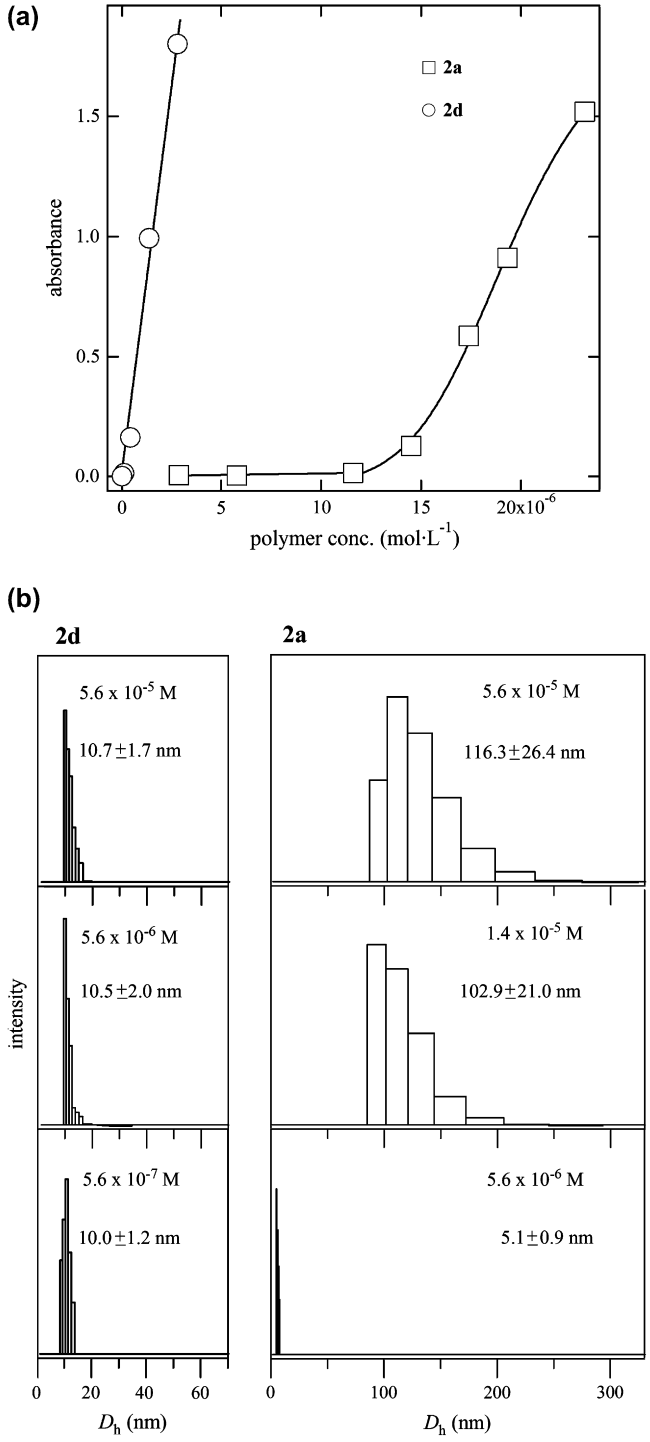


Fig. 3. (a) Absorbance at λ_{max} (562 nm) of RB/**2a** and RB/**2d** in chloroform. (b) Particle size distribution and number average hydrodynamic diameter ($\langle D_h \rangle$) estimated by dynamic light scattering (DLS) measurements of **2d** (left side) and **2a** (right side) in chloroform.

is obtained for RB/**2a** with the DS of 15.7%. This result suggested that the RB-encapsulation state of **2a** was quite different from that of the unimolecular reversed micelle.

To compare the solution structure of **2a** with that of **2d**, we performed dynamic light scattering (DLS) measurements at various concentrations in chloroform (Fig. 3b). The number average hydrodynamic diameter ($\langle D_h \rangle$) of **2d** was ca. 10 nm, which has no influence on the polymer concentration in the range from 5.6×10^{-7} to 5.6×10^{-5} mol L^{-1}. This result reveals that no self-aggregation occurred at those concentrations. On the other hand, the $\langle D_h \rangle$ value of **2a** drastically changed with the changing polymer concentration, i.e., the $\langle D_h \rangle$ value (ca. 103 nm) of 1.4×10^{-5} mol L^{-1} was ca. 20 times greater than that (ca. 5 nm) of 5.6×10^{-6} mol L^{-1}, indicating that **2a** formed a large aggregate above 1.4×10^{-5} mol L^{-1} and its aggregate dissociated into a unimolecular form at 5.6×10^{-6} mol L^{-1}. In Fig. 3a, no absorbance of RB was observed at 5.6×10^{-6} mol L^{-1} of **2a**, thus, it is clear that **2a** was incapable of encapsulating RB as a unimolecular form.

The primary hydroxyl groups of **1**, which can react with trityl chloride, on the polymer surface are present at 31 mol% of the total hydroxyl groups, as calculated by Eq. (1) in Section 2. Therefore, when all of the primary hydroxyl groups on the polymer surface had reacted, the DS value is also 31 mol%, which should be the ideal DS value for the formation of a unimolecular nanocapsule. This ideal DS value was close to those (ca. 23 mol%) of **2b** and **2d** possessing a high encapsulation efficiency in the range of the polymer concentrations. On the other hand, **2a** with a low DS value could not form a stable unimolecular reversed micelle and could not encapsulate RB at a low polymer concentration, because **2a** had an incomplete shell to confine RB to a polymer core. But **2a** at a high polymer concentration became to show an encapsulation property by the formation of an aggregate, as shown in Chart 1.

3.3. Release study on RB/*2a–f*

Because **2a–f** are able to encapsulate a hydrophilic compound, release tests were performed. The particulates of the RB-encapsulating polymers (RB/**2a–c**) were suspended in a 0.067 mol L^{-1} phosphate buffer solution (pH 7.4) and the resulting solutions were shaken at 37 °C. Fig. 4 shows the RB release results. The amount of RB released from the RB/**2a–c** was measured by UV–vis spectroscopy at 549 nm.

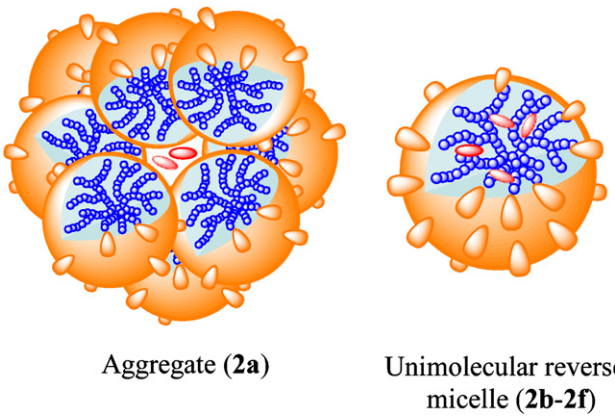


Chart 1.

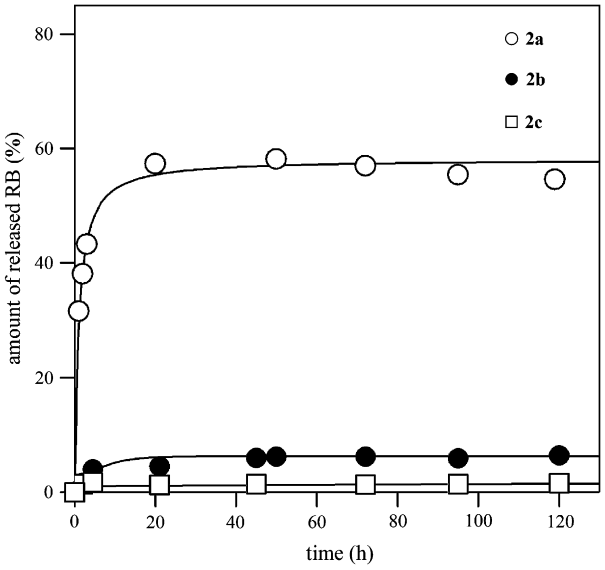


Fig. 4. Release test of RB from RB/**2a–c** in phosphate buffer solution (pH 7.4).

For the RB/**2a** aggregate capsule, the encapsulated RB molecules were rapidly released from the polymer and the amount of the released RB remained constant at ca. 55% after 20 h. On the contrary, a slight release of less than 10% was observed for the RB/**2b–2c** unimolecular capsules even after more than 120 h. These results indicated that the release rate of the encapsulated RB molecules mostly depended on the type of the molecular form of **2** when RB was entrapped. A slight release was also observed for RB/**2d–f**, meaning that the unimolecular reversed micelles **2b–f** have a high RB-holding ability in water.

4. Conclusions

A novel amphiphilic polymer having a hydrophilic hyperbranched polythreitol core and a hydrophobic trityl shell has been prepared by the reaction of the hyperbranched polythreitol with trityl chloride. The obtained amphiphile (**2**) exhibited an encapsulation ability toward rose bengal (RB) as the hydrophilic molecule, and the encapsulation ability mainly depended on the degree of substitution (DS) value of the trityl groups, i.e., the hydrophobic shell density. The low DS value (less than ca. 20 mol%) of **2** led to destabilization as a molecular capsule due to the lower hydrophobicity and a decrease in the encapsulated amount. On the contrary, **2** with a high DS value (greater than ca. 20 mol%) behaved as a unimolecular reversed micelle and its performance was retained at any polymer concentration. Especially, **2** with ca. 23% DS value showed an excellent encapsulation capacity. Based on a release test of the RB-loaded nanocapsules, **2** with a high DS value exhibited a high RB-holding ability in water.

In this study, we demonstrated that the unimolecular reversed micellar properties of **2**, such as the molecular form, encapsulation property, and release property, were dramatically changed with the tuning of the hydrophobic shell density of **2**. Hence, our study possibly provides a strategy for the development of novel molecular nanocapsules, and the further molecular design of amphiphilic hyperbranched polymers will be used for medical applications such as drug delivery.

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