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Encapsulation-Release Property of Amphiphilic Hyperbranched D-Glucan as a Unimolecular Reverse Micelle

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ABSTRACT:

The synthesis of a novel unimolecular reverse micelle, the hyperbranched D-glucan carbamate (**3**), was accomplished through the carbamation reaction of the hyperbranched D-glucan (**1**) with the *N*-carbonyl L-leucine ethyl ester (**2**) in pyridine at 100 ºC. Polymer **3** was soluble in a large variety of organic solvents, such as methanol, acetone, chloroform, and ethyl acetate, and insoluble in water, which remarkably differed from the solubility of **1**. The degree of carbamate substitution (DS) for **3** was controlled by the feed rate of **2**, and the DS values were in the range of 46.0 to 93.7 %. **3** possessed the encapsulation ability for water-soluble molecules, such as rose bengal, thymol blue, and alizarin yellow in chloroform, and the encapsulation ability depended on the hydrophilicity of **3** and the molecular size of the dye. The rose bengal (RB) encapsulated polymer (RB/**3**) showed a slow release from the RB/**3** system

into water at neutral pH, while the release rate was significantly accelerated by the hydrolysis of the hydrophobic polymer shell under basic conditions.

Keywords: amphiphilic hyperbranched polymer, unimolecular reverse micelle, encapsulation.

Introduction

Amphiphilic macromolecules have attracted growing interest with respect to biotechnological and pharmaceutical applications [1-9]. In particular, polymeric micelles, which are spherical aggregates of amphiphilic block copolymers in water, are good candidates for a drug delivery carrier because this leads to improvement in water solubility and a drastic decrease in the toxicity of hydrophobic drugs. However, polymeric micelles become significantly unstable under extremely diluted condition and collapse into a free chain because of the concentration dependency for the micelle formation. On the other hand, amphiphilic dendritic polymers, which are referred to as a "unimolecular micelle", demonstrate a micellar behavior as a single molecule; therefore, they are suitable for the use as a versatile molecular container at various polymer concentrations [10-19].

Amphiphilic dendritic polymers consist of both a dendritic polymer as a core and external substituents having different solubility from the core. Hyperbranched polymers are preferable building blocks as the core of such amphiphiles because they possess spherical three-dimensional architecture and numerous functional groups located on the exterior of the molecules. Furthermore, they can be easily synthesized in a one-pot process; hence, amphiphilic hyperbranched polymers have potential applications with practicality in the field of drug delivery. There are several studies on the synthesis and characterization of amphiphilic hyperbranched polymers, e.g., a water-soluble hyperbranched polyphenylene, synthesized by metallation of hyperbranched polyphenylene with *n*-BuLi followed by a reaction with CO_2, which product exhibited the property of forming a complex with *p*-toluidine in water [20]. In addition, Frey et al. reported that the construction of molecular nanocapsules with hydrophilic interiors has been achieved using narrow polydispersity hyperbranched polyglycerols and a simple esterification with fatty acids [21-25].

Recently, we reported that a novel unimolecular reverse micelle consisting of a poly(L-lactide) shell and a hyperbranched D-mannan core, which was synthesized by the ring-opening polymerization of L-

lactide on a hyperbranched D-mannan, possessed the controlled release property of the encapsulated guest molecules based on the biodegradability of the poly(L-lactide) shell [26]. Thus, of great interest is the design and synthesis of a unimolecular reverse micelle with a hyperbranched polysaccharide core, which is capable of active release by other stimuli, such as pH change, of hydrophilic guest molecules. We now report the reaction of hyperbranched D-glucan (**1**) with *N*-carbonyl L-leucine ethyl ester (**2**) producing a novel amphiphilic polymer consisting of a hyperbranched D-glucan core and an L-leucine ethyl ester shell (**3**), as shown in Scheme 1. In addition, the encapsulation and release properties of **3** toward water-soluble dyes, such as rose bengal, alizarin yellow, thymol blue, methyl blue, and congo red (Chart 1), are discussed from the viewpoint of the unimolecular reverse micelle property of **3**.

Scheme 1

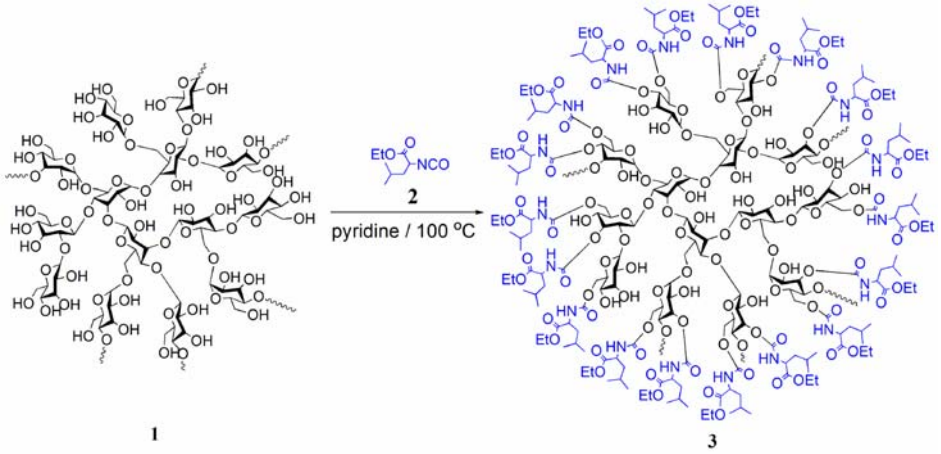
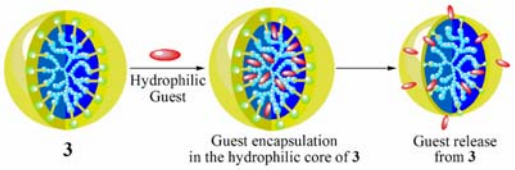
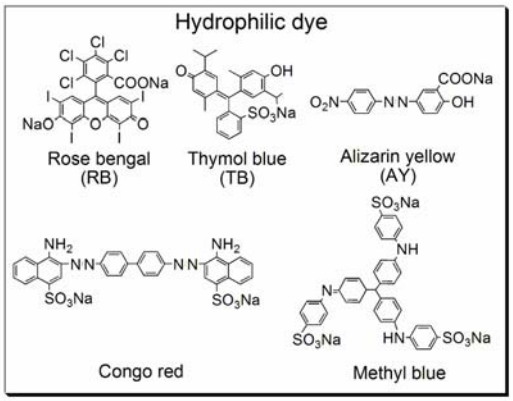


Chart 1





Experimental Section

Materials. Hyperbranched D-glucan (**1**) was synthesized from 1,6-anhydro-β-D-glucopyranose according to previous work [27-29] and dried in vacuo at 40 °C for 2 days. The $M_{w,SEC}$ and M_w/M_n values were 5,200 and 1.47, respectively. The $M_{w,SLS}$ value of **1** was 70,600 (dn/dc = 0.146). The ratio of the terminal units in **1** (r_{ter}) was 0.385, which was determined by methylation analysis [27]. The ratio of the terminal hydroxyl groups in **1**, that is, the ratio of the external hydroxyl groups in **1** (r_{ext}), was calculated by eq. 1:

$$r_{ext} = \frac{(\text{number of terminal OH groups})}{(\text{number of total OH groups})} = \frac{(\text{number of glucose units in } \mathbf{1}) \times r_{ter} \times (\text{number of OH groups per terminal unit})}{(\text{number of glucose units in } \mathbf{1}) \times (\text{average number of OH groups per glucose unit in } \mathbf{1})} \quad \text{(eq.1)}$$

where the number of the OH groups per terminal unit and the average number of OH groups per glucose unit in **1** were 4 and 3, respectively. The number of glucose units in **1** was obtained by eq. 2.

$$\frac{(M_{w,SLS} \text{ of } \mathbf{1})}{(\text{molecular weight of glucose unit})} = \frac{70{,}600}{162} = 436 \quad \text{(eq. 2)}$$

From these data, the r_{ext} value of **1** can be calculated to be 0.513.

N-Carbonyl L-leucine ethyl ester (**2**) was synthesized from L-leucine according to previous work [30]. Pyridine was distilled over CaH_2 just before use. Rose bengal was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), alizarin yellow from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan), and thymol blue, methyl blue, and congo red from Kanto Chemical Co., Ltd. (Tokyo, Japan) and were used without further purification. Chloroform ($CHCl_3$) and dimethyl sulfoxide (DMSO) for the spectroscopy were obtained from Kanto Chemical Co., Ltd. Methanol, toluene, and sodium nitrate ($NaNO_3$) were obtained from Kanto Chemical Co., Ltd. and used as received. Igepal CO-210 (IGP) was purchased from Aldrich (Milwaukee, WI) and used as received. Dialysis membranes (Spectra por®6 regenerated cellulose, MWCO 1,000) were purchased from Spectrum Laboratories, Inc. (Rancho Dominguez, CA).

Instruments. The ^{1}H NMR and ^{13}C NMR spectra were recorded using a JEOL JNM-A400II instrument. A static light scattering (SLS) measurement was performed at 23 °C in an aqueous $NaNO_3$

solution (0.2 mol·L^{-1}) using an Otsuka Electronics CALLS-1000 light scattering spectrometer (λ = 632.8 nm). The refractive index value (d*n*/d*c*) was measured in an aqueous $NaNO_3$ solution (0.2 mol·L^{-1}) using an Otsuka Electronics DRM-1021 double-beam differential refractometer. Size exclusion chromatography (SEC) for the water soluble polymer was performed in aqueous $NaNO_3$ solution (0.2 mol·L^{-1}) at 40 °C (1.0 mL· min^{-1}) using a TOSHO HPLC system (HLC-8020) equipped with two TSK_{gel} $GMPW_{XL}$ columns (7.8 mm × 300 mm; bead size, 13 μm; exclusion limit, 5×10^7) and a refractive index detector. The weight-average molecular weight ($M_{w,SEC}$) and molecular weight distribution (M_w/M_n) of the polymer sample were calculated on the basis of a poly(ethylene glycol) calibration. For the organic solvent-soluble polymer, the SEC measurement was performed at 40 °C in THF (0.8 mL·min^{-1}) using a Jasco GPC-900 system equipped with a set of Waters Ultrastyragel 7-mm columns (linear, 7.8 mm × 300 mm; exclusion limit, 1×10^7) and two Shodex KF-804L columns (linear, 8 mm × 300 mm; bead size, 7 μm; exclusion limit, 4×10^5). The UV-vis spectra were measured at 23 °C in $CHCl_3$, $CHCl_3$/DMSO (1/7, v/v), and water with 10-mm and 5-mm path lengths using a Jasco V-550 spectrometer with a deuterium lamp as the light source for the UV range (190-350 nm) and a halogen lamp for the visible range (330-900 nm). The CD spectra were measured at 23 °C in $CHCl_3$ with a 5-mm path length using a Jasco J-720 spectropolarimeter. The degree of substitution (DS) was determined by elemental analysis using a Yanako MT-6 CHN corder (Center for Instrumental Analysis, Hokkaido University).

Synthesis of Amphiphilic Hyperbranched D-Glucan (3a). A typical procedure for the carbamation reaction of **1** is as follows: **2** (1.3 g, 7.1 mmol) was added to a solution of **1** (0.50 g, 3.1 mmol) in freshly distilled pyridine (23 mL) at 100 °C. After 24 h, methanol (10 mL) was added to quench the remaining isocyanate. The solvent was evaporated and residual pyridine was removed by azeotropic distillation with toluene three times. The residue was purified by dialysis (Spectra por®6 regenerated cellulose, MWCO 1,000) in methanol for 2 days. After dialysis, the methanol-insoluble portion was removed by filtration. The solvent was evaporated and the residue was dried in vacuo to afford polymer **3a**. The yield was 0.67 g and the degree of substitution (DS) was 46.0 %. The $M_{w,SEC}$ and M_w/M_n values were 5,600 and 1.38, respectively. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.54-3.96 (-NH-C<u>H</u>-, -O-CH_2-, br), 3.06 (-NH-, br, 1H), 1.88-1.67 (C-CH-, br, 1H), 1.67-1.37 (-C<u>H</u>$_2$-, br, 2H), 1.26 (-OCH$_2$C<u>H</u>$_3$, br, 3H), 0.92 (-CH_3, br, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 176.16-173.03 (-NHC=O, m), 157.28-153.40 (-COO-, m), 76.12-62.72 (polysaccharide scaffold, m), 64.13 (-OCH_2-), 54.08-51.70 (-NHCH-, m), 42.72-38.98 (-CH_2-, m), 24.69 (-CH-), 22.87, 21.82 (-CH_3 × 2), 14.17 (-CH_2<u>C</u>H$_3$). IR (KBr film, cm^{-1}) 3,372 (-OH), 2,972 (-CH-,

-CH_2-, and -CH_3), 1,742 (-NHC=O), 1,544 (N-H), 1,056 (-C-O-C-). The molecular weight of **3a** ($M_{w,cal}$) was 1.8×10^5, calculated using eq. 3.

$$M_{w,cal} = (M_{w,SLS} \text{ of } \mathbf{1}) + (\text{number of glucose units in } \mathbf{1}) \times (\text{average number of OH groups per glucose unit in } \mathbf{1}) \times \frac{(\text{DS of } \mathbf{3})}{100} \times (\text{molecular weight of } \mathbf{2}) \quad \text{(eq. 3)}$$

Encapsulation of Water-Soluble Dyes. A typical procedure for the solid/liquid phase transfer is as follows: Rose bengal (RB) (21 mg, 21 mmol) was added to a dispersion of **3a** (11 mg) in chloroform (2.0 mL) and then the suspension was placed in a water bath shaker at 37 ^oC. After 24 h, any undissolved dye was removed by filtration using 0.45 μm membrane filters. The colored filtrate was diluted with chloroform and characterized by its UV-vis and CD spectra. UV-vis ($CHCl_3$, 10 mm cell); λ_{max} (abs) = 562 nm (2.49). The RB/**3a** system (0.3 mL) was diluted with DMSO (2.1 mL) and characterized by its UV-vis spectrum. The encapsulated amount of dye per **3a** ([RB]/[**3a**]) was quantitatively determined. UV-vis ($CHCl_3$/DMSO = 1/7, 10 mm cell); λ_{max} (abs) = 565 nm (0.31). $\varepsilon_{max} = 1.47 \times 10^5$ $mol^{-1} \cdot L \cdot cm^{-1}$. [RB]/[**3a**] = 27.9.

Unimolecular Reverse Micelle Property. A suspension of RB in chloroform (4.0 $mg \cdot mL^{-1}$, 2.0 mL) was added to a solution of **3c** (0.004 - 0.625 $mg \cdot mL^{-1}$, 2.0 mL) or Igepal CO-210 (IGP; 1.0 - 6.0 $mg \cdot mL^{-1}$, 2.0 mL) in chloroform. The mixture was placed in a water bath shaker at 37 ^oC for 24 h. After removal of any undissolved dye using a 0.45 μm membrane filter, the colored filtrate was characterized by its UV-vis spectrum.

Release Study of Rose Bengal. A suspension of the RB-encapsulated polymer (5.0 mg) in ion-exchanged water (1 mL) or an aqueous RB solution (22 $\mu mol \cdot L^{-1}$, 1 mL) was dialyzed at 37 ^oC using a dialysis tube (Spectra por®6 regenerated cellulose, MWCO 1,000) in ion-exchanged water (49 mL). At scheduled time intervals, ca. 3 mL of the outer ion-exchanged water was transferred to a UV-vis cuvette, and its UV-vis spectrum was characterized (λ_{max} = 549 nm; $\varepsilon_{max} = 8.85 \times 10^4$ $mol^{-1} \cdot L \cdot cm^{-1}$). After the RB-release experiment was carried out for 120 h, an aqueous NaOH solution (0.1 $mol \cdot L^{-1}$) was added until the release solution reached pH 12.0 (λ_{max} = 549 nm; $\varepsilon_{max} = 8.34 \times 10^4$ $mol^{-1} \cdot L \cdot cm^{-1}$).

Results and Discussion

Syntheses of Carbamate Derivatives of Hyperbranched D-Glucan. In order to prepare the carbamate derivative of the hyperbranched polysaccharide, the reaction of hyperbranched D-glucan (**1**) with *N*-carbonyl L-leucine ethyl ester (**2**) was carried out in pyridine at 100 ºC. The carbamation reaction homogeneously proceeded to afford a light brown solid. In the ^{13}C NMR spectrum of the product, the signals due to ethyl ester moieties appeared at 14.17 and 64.13 ppm along with the characteristic signals at 62.72-76.12 ppm due to polysaccharide core (Figure 1a). The ^{1}H NMR spectrum of the product also showed the proton signals due to the L-leucine ethyl ester and polysaccharide moiety at 0.92-1.88 ppm and 3.20-5.25 ppm, respectively (Figure 1b). In addition, the peak due to the amide group appeared at 1,742 cm^{-1} in the IR spectrum, while no peak due to the isocyanate group was observed at 2,264 cm^{-1} (Figure 2). These results indicated that the product was assignable to the carbamate derivatives of hyperbranched D-glucan, **3**.

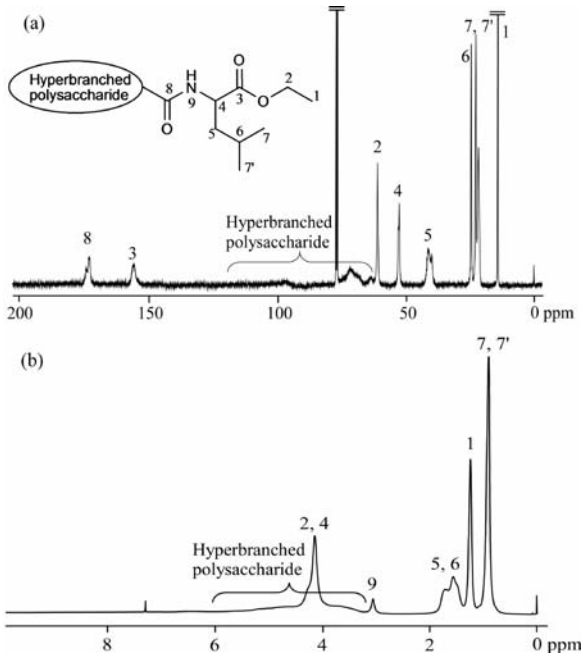


Figure 1. (a) ^{13}C NMR and (b) ^{1}H NMR spectra of **3b** in $CDCl_3$.

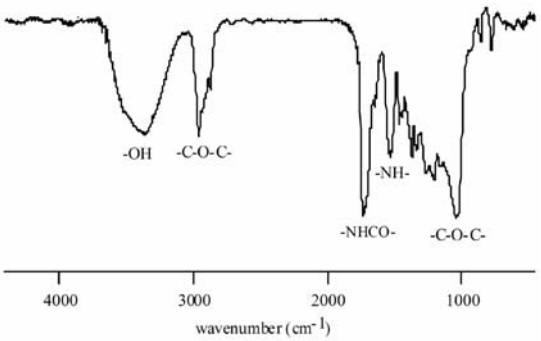


Figure 2. IR spectrum of **3a** (KBr film).

Table 1 shows the results of the carbamation reaction of **1**. The degrees of substitution (DS) determined by elemental analysis could be easily controllable by the feed rate of **2**, and those values gradually increased with the increasing molar ratio of **2** and glucose units in **1** ($[\mathbf{2}]_0$/[glu]); the DS values were 46.0 % for 2.3 of $[\mathbf{2}]_0$/[glu] (**3a**), 68.7 % for 3.6 of $[\mathbf{2}]_0$/[glu] (**3b**), and 93.7 % for 10.0 of $[\mathbf{2}]_0$/[glu] (**3c**). Polymers **3a-c** are soluble in a large variety of organic solvents, such as methanol, acetone, chloroform, and ethyl acetate, and insoluble in water, which remarkably differed from the solubility of **1**.

Table 1. Carbamation Reaction of Hyperbranched D-Glucan (1) with *N*-Carbonyl L-Leucine Ethyl Ester (2) [a]

sample	$[\mathbf{2}]_0$/[glu]	yield (g) [b]	DS (%) [c]	$M_{w,cal} \times 10^{-5}$ [d]
3a	2.3	0.67	46.0	1.8
3b	3.6	1.55	68.7	2.4
3c	10.0	1.54	93.7	3.0

[a] **1**, 0.50 g; temp, 100 °C; time, 24 h. [b] Methanol soluble parts. [c] Degree of substitution determined by elemental analysis. [d] Molecular weight calculated by eq.2 (see Experimental).

Encapsulation Property of 3 toward Various Water-Soluble Dye Molecules. In order to study the unimolecular reverse micelle property of **3**, we examined the encapsulation-release characteristics for the water-soluble dye, rose bengal (RB), using the solid/liquid phase transfer method. For the encapsulation experiment, it is necessary to estimate the molecular weight of **3**. However, it is well-known that the molecular weights for highly branched polymers, which are estimated by size exclusion chromatography, are significantly lower than the absolute molecular weights, because the hydrodynamic volumes of these polymers are smaller than those of the corresponding linear polymers [31]. Actually, the weight average-molecular weight value of **1** estimated by size exclusion chromatography ($M_{w,SEC}$) was 5,200, which was 0.074 of the absolute molecular weight value of **1** estimated by static light scattering ($M_{w,SLS}$, 70,600). Thus, the weight-average molecular weight for **3** ($M_{w,cal}$) is estimated using eq. 1 based on the DS value of

3 and the absolute molecular weight of **1** as a core. The $M_{w,cal}$ values of **3a-c** were calculated to be 1.8×10^5 - 3.0×10^5, as listed in Table 1.

RB as a solid was added to the chloroform solutions containing **3a-c**, and the heterogeneous mixtures were shaken for 24 h at 37 °C. After removal of any undissolved RB using membrane filters, the chloroform phases were apparently colorized. In contrast, the control experiment in the absence of **3** showed no coloration. Figure 3 shows the UV-vis spectra of the chloroform solutions for the RB/**3b** system and control. For the RB/**3b** system, the characteristic absorption due to RB appeared in the visible region from 470 to 600 nm.

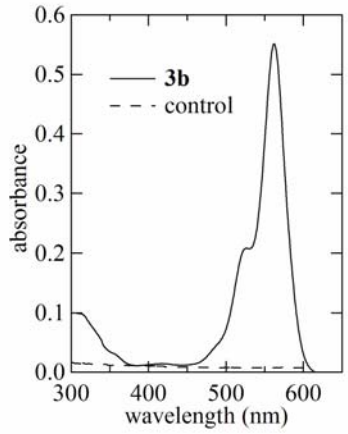


Figure 3. UV-vis spectra of RB/**3b** system ([**3b**] = 2.45×10^{-7} mol·L^{-1}) in $CHCl_3$.

Additionally, the chiroptical property of the RB/**3b** system was estimated using circular dichroism (CD) spectroscopy (Figure 4a). In the CD spectrum, a negative Cotton effect was observed in the range from 550 to 580 nm corresponding to the absorption of RB, indicating that RB existed under chiral hydrophilic circumstances, i.e., the hyperbranched polysaccharide core in **3b**. Furthermore, in the ^{1}H NMR spectra of the RB/**3b** system, the signals due to all protons appeared in $CDCl_3$/dimethyl sulfoxide-d_6 (DMSO-d_6) (3/2, v/v), whereas the signal due to RB did not appear in $CDCl_3$ (Figure 4b). These results showed that the intermolecular mobility of the RB protons was significantly limited because the RB encapsulation in $CDCl_3$ occurred inside the hydrophilic core of **3b**.

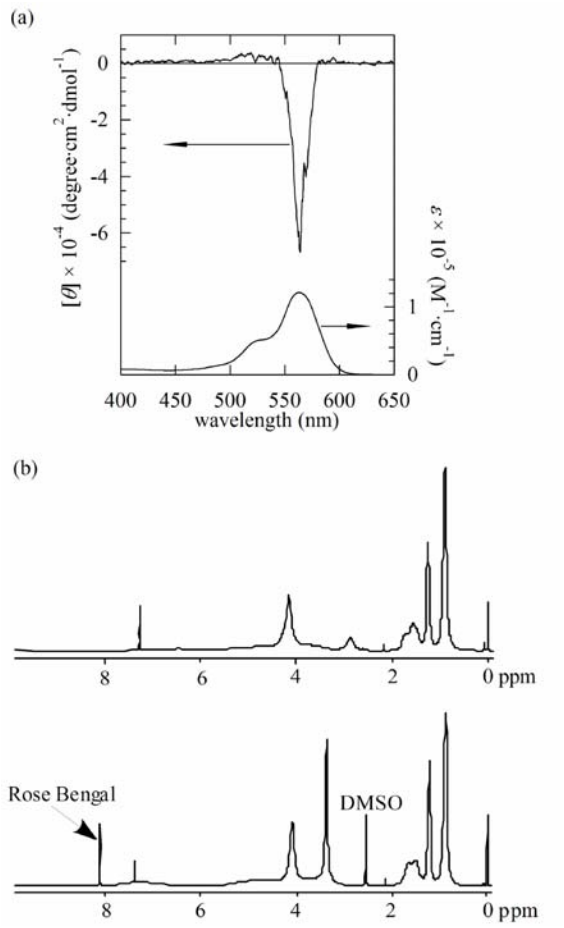


Figure 4. (a) CD (upper) and UV (lower) spectra of RB/**3b** system in $CHCl_3$ using a 5mm path length (RB in **3b**/chloroform = 4.52×10^{-5} mol·L^{-1}). (b) ^{1}H NMR spectra of RB/**3b** system in $CDCl_3$ (upper) and in $CDCl_3$/DMSO-d_6 (3/2, v/v) (lower).

The encapsulation ability of **3** was also examined using other water-soluble dyes, such as thymol blue, alizarin yellow, methyl blue, and congo red. Although thymol blue and alizarin yellow were readily encapsulated by **3** as well as RB (Figure 5), methyl blue and congo red, which had greater extended structures than the other dyes, were not encapsulated. The results indicated that the encapsulation ability of **3** toward these dye molecules should relate to the molecular size of the dye, that is, the space needed for encapsulating methyl blue and congo red was insufficient in **3**. The encapsulation amounts of hydrophilic dyes per **3a-c** ([Dye]/[**3**]) were determined by measuring the UV-vis spectra of the Dye/**3** systems diluted with DMSO. The [Dye]/[**3**] values are summarized in Table 2. **3b** encapsulated the three dyes more efficiently than did **3a** and **3c**. The DS value of **3a** was 46.0 %, which was lower than the

expected value when all the external hydroxyl groups of **1** were reacted (ca. 51 %, see Experimental), indicating that a somewhat denser shell was required to encapsulate many dyes. On the contrary, the lower encapsulation values of **3c** were ascribed to the fact that the higher DS value of **3c** led to a narrower hydrophilic interior than in **3b**. The [Dye]/[**3c**] values, however, were relatively high regardless of the narrow hydrophilic interior. This fact suggests that the driving force for encapsulation of dyes is not only the amphiphilicity of **3** but also the additional interactions between the dyes and the L-leucine residue as the shell of **3**. Thus, the L-leucine residue as the shell of **3** also affected the encapsulation of the hydrophilic dyes.

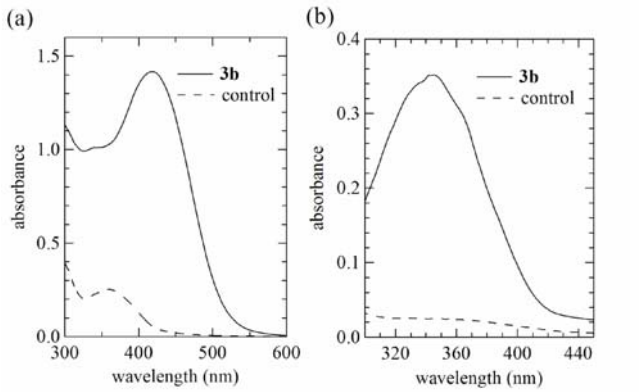


Figure 5. UV-vis spectra of (a) thymol blue/**3b** system ([**3b**] = 2.19 × 10^{-6} mol·L^{-1}) and (b) alizarin yellow/**3b** system ([**3b**] = 1.07 × 10^{-6} mol·L^{-1}) in $CHCl_3$.

Unimolecular Reverse Micelle Property. An interesting feature of unimolecular reverse micelles is that they have no critical micelle concentration (CMC), and thus they can encapsulate hydrophilic guests at extremely low concentrations [18, 19]. In order to clarify the unimolecular reverse micelle characteristics of the amphiphilic hyperbranched D-glucan, the encapsulation property of **3c** toward RB was compared with that of a nonionic surfactant, Igepal CO-210 (IGP). In Figure 6a, the absorbances at λ_{max} of RB/**3c** and RB/IGP systems in chloroform were plotted versus the concentrations of **3c** and IGP. For IGP, a typical S-shaped curve was observed with the deflection point at 2 - 4 mg·mL^{-1}. Because the dye-encapsulation property of IGP depended on the formation of the reverse micelle, the absorbances at IGP concentrations < 2 mg·mL^{-1}, where the micellar structure became unstable, were extremely low. On the contrary, for polymer **3c**, the absorbances at λ_{max} for RB linearly and dramatically increased with the increasing concentration of **3c** with no deflection point. A linear relation between the concentrations of

the encapsulated RB and **3c** is observed as shown in Figure 6b, indicating that **3c** was a covalently linked unimolecular reverse micelle having no CMC in the polymer concentration range of 8.17 - 204 × 10^{-8} mol·L^{-1}.

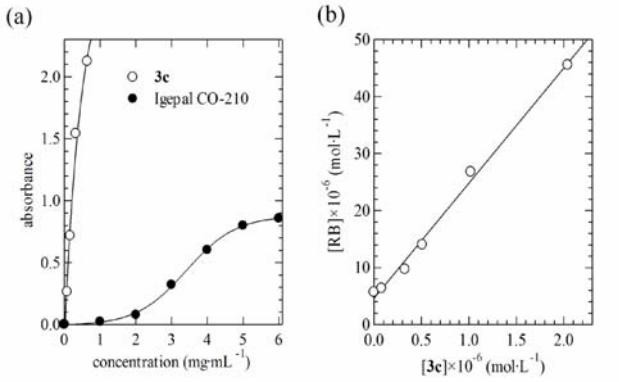


Figure 6. (a) Absorbance at λ_{max} of RB/**3c** system (open circle, λ_{max} = 578 nm) and RB/IGP system (closed circle, λ_{max} = 560 nm) in $CHCl_3$. (b) Concentration of encapsulated RB, [RB] vs [**3c**].

Release Study. The RB-release property for the RB/**3a-c** systems in water was examined using UV-vis spectroscopy. Figure 7a shows the RB-release results. In order to clarify the effect of the unimolecular reverse micelle on the RB-release, the permeation of RB (free RB) through a cellulose membrane was carried out in the absence of **3** as a control. In contrast to the fast permeation of free RB through the cellulose membrane, the release of the RB encapsulated by **3** (RB/**3**) was very slow and no initial release burst was observed. The release rate decreased with the increasing DS value, suggesting that the hydrophobic shells in **3** prevented the diffusion of RB from the hydrophilic core in **3**.

To clarify the influence of the hydrophobic shell on the release rate of RB, an aqueous NaOH solution (0.1 mol·L^{-1}) was added until the release solution reached pH 12.0 after the beginning of the release experiment for 120 h. The release rates for RB/**3b** and RB/**3c** little changed due to the dense shell which could not be adequately destroyed under these conditions, whereas the release rate of RB/**3a** gradually accelerated and the released RB for RB/**3a** reached 100 % for 450 h. After the release experiment, the release solution of RB/**3a** became homogeneous, and the hydrolysis of the ethyl ester group of **3a** was confirmed by the ^{1}H NMR spectrum of the system (Figure 7b). These results clearly indicated that the release rate of encapsulated RB could be accelerated by breaking the ester groups linked with the shell in **3a** and that the pH-sensitivity for the release rate depended on the density of the shell.

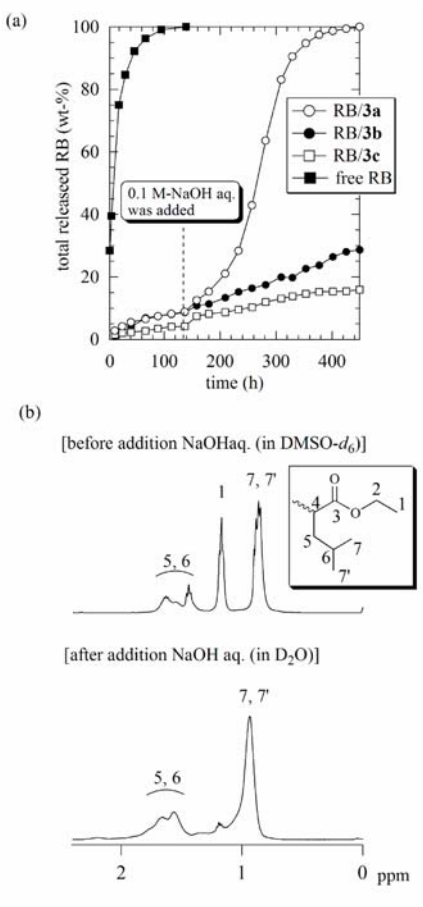


Figure 7. (a) Release of RB from the RB/**3a-c** systems in water at 37 °C. (b) ^{1}H NMR spectra of RB/**3a** after the beginning of the release experiment for 120 h (before addition of 0.1 mol·L^{-1} NaOH aq.) (upper) in DMSO-d_6 and 450 h (after addition of 0.1 mol·L^{-1} NaOH aq.) (lower) in D_2O.

Conclusions

The novel unimolecular reverse micelle consisting of hyperbranched D-glucan (**1**) as a core and L-leucine ethyl ester as the shell was synthesized by the reaction of **1** with the *N*-carbonyl L-leucine ethyl ester. The carbamation reaction was found to be a convenient method for preparing the organic solvent-soluble amphiphilic hyperbranched D-glucan (**3**). The encapsulation ability of **3** toward water-soluble dyes has been investigated by UV-vis and CD measurements in chloroform. These results indicated that **3** was a unimolecular reverse micelle with an encapsulation ability toward hydrophilic dye molecules, such as rose bengal, alizarin yellow, and thymol blue. In addition, the rose bengal encapsulated in **3** showed a

slow release, and its release rate was extremely accelerated under basic conditions caused by the hydrolytic cleavage of the shell of **3**. Polymer **3** build up with the biocompatible and green materials such as sugar and L-amino acid Hence, **3** was a good candidate for pH-sensitive controlled-release systems, and the encapsulation-release property of **3** possibly provides a strategy for designing new drug delivery systems.

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