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Regioselectively Modified Stereoregular Polysaccharides. 8. Synthesis and Functions of Partially 3-O-Octadecylated (1→6)-α-D-Glucopyranans

Kazukiyo Kobayashi,* Hiroshi Sumitomo, and Haruo Ichikawa

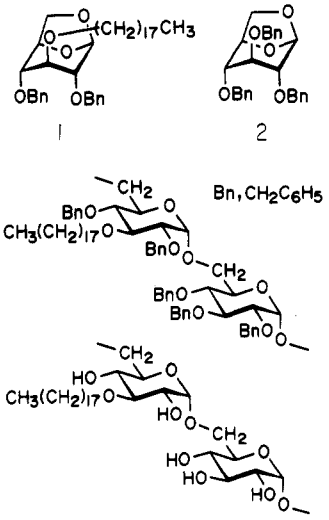
Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464, Japan.
Received August 6, 1985

ABSTRACT: Ring-opening copolymerization between 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-octadecyl-β-D-glucopyranose (1) and 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (2) was carried out in the presence of PF_5 initiator at −60 °C. The reactivity of monomer 1 was high, and stereoregular homo- and copolymers of high molecular weight were obtained. Debenzylation of the copolymers with sodium metal in liquid ammonia produced (1→6)-α-D-glucopyranans having a 3-*O*-octadecyl group, the degree of substitution (DS) of which was up to 0.22. The following findings on properties of the polysaccharides were obtained. (1) The polysaccharides were hydrolyzed by a dextranase. (2) An organic solute magnesium 1-anilino-8-naphthalenesulfonate was bound to a hydrophobic region of the polysaccharides in water, indicating that the polysaccharides formed a micellar conformation. (3) Flexible membranes of the polysaccharides (DS 0.07–0.22) could be cast from dimethyl sulfoxide solutions. (4) The polysaccharides (DS 0.01–0.05) could be incorporated into liposomes by anchoring the long hydrocarbon chains into the lipid layer of the liposomes, and the resulting polysaccharide-coated liposomes could be isolated. The anchoring effect of the hydrocarbons and the conformation of the polysaccharides on the liposomes are discussed.

Well-defined polysaccharides having both membrane-forming abilities and recognition functions are of interest as a polymeric model of glycolipids. Glycolipids occurring in cell membranes are composed of oligosaccharides and lipids and play an important role in cellular recognition.[1] Glycolipids and their analogues were synthesized and incorporated into cell membranes, and the resulting artificial assemblies proved useful for investigations of various cellular phenomena.[2,3] Incorporation of synthetic glycolipids into a cell-membrane model, liposome, is also extensively investigated, especially for the application of a drug delivery system that employs carbohydrate moieties as recognition markers to target specific organs.[4–8] For this purpose, a strengthening of the liposomal structure is required, and synthesis of polymerized glycolipid models[9–11] and surface coating of liposomes with polysaccharides[12–15] have been attempted. Polysaccharide-coated liposomes were reported by Sunamoto:[14,15] naturally occuring polysaccharides such as dextrans, pullulans, and amylopectins were modified with long-chain fatty acids and assembled into liposomes. It was found that these polysaccharide-coated liposomes were effective for stabilization of liposomes and also for targeting.

In this paper, 3-O-octadecylated (1→6)-α-D-glucopyranans capable of forming polysaccharide-coated liposomes and polymeric membranes have been prepared according to Scheme I: ring-opening copolymerization between 1,6-anhydro-2,4-di-*O*-benzyl-3-*O*-octadecyl-β-D-glucopyranose (1) and 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (2) followed by debenzylation of the resulting copolymers. Polysaccharide synthesis via ring-opening polymerization of anhydro sugar derivatives is a useful method of preparing various polysaccharides of well-defined structures.[16–18] The required amount of long hydrocarbon chains could be introduced into (1→6)-α-D-glucopyanans regioselectively and at relatively uniform intervals. 3-*O*-Octadecyldextrans thus obtained are a biodegradable and hydrophilic–hydrophobic (amphiphilic)

Scheme I



material. This paper deals with their enzymatic hydrolysis, binding of organic solute in water, membrane formation, and interaction with liposomes and compares some of the properties to those of 3-O-methylated and 3-O-dodecylated glucopyanans previously synthesized.[19-21]

The ring-opening polymerization method is also effective for the insertion of desired carbohydrate branches as recognition markers into polysaccharides.[22-24] This synthetic approach is a promising means of preparing the polysaccharides having membrane-forming anchors and recognition markers.

Experimental Section

1,6-Anhydro-2,4-di-*O*-benzyl-3-*O*-octadecyl-β-D-glucopyranose (1). 1,6-Anhydro-2,4-di-*O*-benzyl-β-D-glucopyranose (10.3 g) was treated with sodium hydride (2.0 g) in dimethylformamide (DMF) (100 mL) at 50 °C for 90 min. 1-Bromooctadecane (13.3 g) in DMF (30 mL) was added, and the mixture was heated at 70 °C for 60 min. Another portion of sodium hydride (2.0 g) and 1-bromooctadecane (13.3 g) was added, and the reaction was continued at 85 °C for a further 150 min. The solution was treated with chloroform (200 mL) and water (100 mL), and the chloroform layer was washed with water (3 × 100 mL) and concentrated. The product was purified by silica gel chromatography with benzene–ether (8:1 (v/v)) as eluent. A colorless crystalline material was obtained in a 73% yield: mp 36.5–37.0 °C; $[\alpha]^{25}_D$ −20.3° (in chloroform, *c* 1.0). Anal. Calcd for $C_{38}H_{58}O_5$: C, 76.73; H, 9.83. Found: C, 76.59; H, 9.67. ^{13}C NMR ($CDCl_3$) δ 137.75, 128.21, 127.73, 127.58 (aromatic), 100.49 (C-1), 77.09 (C-3), 76.90 (C-2), 76.32 (C-4), 74.32 (C-5), 71.79, 71.16 (benzyl CH_2), 70.38 (octadecyl OCH_2), 65.27 (C-6), 31.91, 29.72, 29.38, 26.12, 22.71 (octadecyl CH_2), 14.15 (octadecyl CH_3).

Polymerization. 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (2) was synthesized and purified according to the previously described method,[19-21] and *p*-chlorobenzenediazonium hexafluorophosphate and dichloromethane were purified in the usual manner. A mixture of monomers 1 and 2 was thoroughly dried in an improved polymerization vessel, and copolymerization was carried out under high vacuum in anhydrous dichloromethane with phosphorus pentafluoride (PF_5).[20] The polymerization was terminated by adding a cold mixture of methanol and petroleum ether. A higher ratio of methanol–petroleum ether was used to precipitate the copolymers of higher content of 1. The copolymers were dissolved in chloroform, precipitated into the methanol–petroleum ether mixture 4 times, and isolated by freeze-drying from benzene.

Debenzylation. A solution of the copolymer (1 g) in toluene–1,2-dimethoxyethane (30 mL/15 mL) was added to liquid ammonia (100 mL) at −33 °C, and small pieces of freshly cut sodium metal were added in several portions until the dark blue of the solution persisted. Anhydrous ammonium chloride and water (60 mL) were added, and ammonia was evaporated. The product was obtained from both the aqueous solution and the precipitate. the aqueous solution was dialyzed, concentrated with a rotary evaporator, and freeze-dried to yield a water-soluble fraction. The precipitate was collected on a glass filter and dissolved in dimethyl sulfoxide (Me_2SO). A Me_2SO-isoluble impurity was removed by filtration through Celite, the filtrate was reprecipitated into methanol, and the water-insoluble product was dried in vacuo.

Characterization. ^1H and ^{13}C NMR spectra were recorded on Japan Electron Optics Laboratory JNM-FX-100 and FX-200 Fourier transform NMR spectrometers. Tetramethylsilane was employed as internal standard. Optical rotation measurements were carried out on polymer solutions in chloroform at 25 °C by a Japan Spectroscopic Co. (JASCO) DIP-181 digital polarimeter. Viscosities were measured in chloroform and Me_2SO in Ubbelohde viscometers at 25 °C. Melting points of copolymers were measured on a Perkin-Elmer DSC-2 differential scanning calorimeter. Gel permeation chromatography was performed by using a Shodex GPCA-80M column (8 mm i.d. × 1000 mm) on a Hitachi 634A high performance liquid chromatograph (eluent, chloroform; polystyrene standard). Number-average molecular weights were determined by membrane osmometry with a Hewlett Packard 502 high-speed membrane osmometer in toluene at 37 °C. UV spectra were recorded on a JASCO UVIDEC-1 digital double-beam spectrophotometer. Fluorescence spectra were recorded on a JASCO FP-550 spectrofluorometer at room temperature. Fluorescence depolarization measurements were performed on a Hitachi MPF-2A spectrofluorometer at 20 °C.

Enzymatic Hydrolysis. Dextranase (1,6-α-D-glucan-6-glucanohydrolase; EC 3. 2. 1. 11) from a *Penicillium* species was obtained from Sigma Chemical Co. (product number, D-5884; activity, 15–40 unit per milligram of solid) and used without further purification. The dextranase was dissolved in acetate buffer (0.48 mg/L, pH 5.2), an aliquot (3 mL) was added to a polysaccharide (16 mg) solution in 7 mL of acetate buffer, and the mixture was incubated at 40 °C. At appropriate intervals, samples (1 mL) were withdrawn and the content of reducing sugar was determined by the Somogyi–Nelson method.[25] The relation between the concentration of the reducing sugar and the absorbance at 660 nm was obtained by calibration with maltose as a reference.

Binding of Organic Solute. Magnesium 1-anilino-8-naphthalenesulfonate (ANS) obtained from Nakarai Chemicals Ltd. was recrystallized twice from hot water. The polysaccharide (25 mg) was sonicated (20 kHz, 50 W) in phsophate buffer (5 mL, pH 7.2); 1 mL of ANS solution in phosphate buffer ([ANS] = 5 × 10^{-4} M) was added to an aliquot (1 mL) of the resulting solution or white suspension. Fluorescence emission spectra were measured at an excitation wavelength of 380 nm and a slit width of 10 nm at room temperature.

Preparation of Membranes. A polysaccharide solution (2 wt %) in Me_2SO was placed on a clean glass plate and heated at 55 °C with an infrared lamp to evaporate the solvent. The resulting transparent membrane of 5–10-μm thickness was removed by immersing the glass plate in water and dried in a vacuum desiccator. Advancing-contact angles of water droplets on a membrane were measured with a goniometer at room temperature. Twice-distilled water was used.

Preparation of Polysaccharide-Coated Liposomes.[26] DL-α-Dipalmitoylphosphatidylcholine (DPPC, Sigma Chemical Co.), cholesterol (Kishida Chemical Co.), tris(hydroxymethyl)aminomethane (Nakarai Chemical Co.), 1,6-diphenyl-1,3,5-hexatriene (DPH, Tokyo Chemical Industry Co.), fluorescein isothiocyanate (FITC, Tokyo Chemical Industry Co.), and dibutylbis(dodecanoyloxy)stannane (Kishida Chemical Co.) were used without further purification.

DPPC (6.5 mg) and cholesterol (1.2 mg) were dissolved in chloroform (3 mL) in a round-bottomed flask, and the solvent was removed to dryness with a rotary evaporator. The remaining thin film was dispersed in 6.5 mL of Tris buffer (0.2 M, pH 7.2) containing 0.1 M sodium chloride under magnetic stirring. The milky suspension was sonicated (20 kHz, 15 W, 10 min) at 50 °C with an Ohtake Works 5203 PZT sonicator. A DPH solution in tetrahydrofuran (THF) (0.1 M, 100 μL) was placed in a vial, the solvent was evaporated, and a liposome suspension was added and sonicated (15 W, 3 min). The prescribed amount of polysaccharide was added to a 1.6-mL aliquot of the liposome suspension, and the mixture was sonicated again (15 W, 2 min). The suspension was aged for 30 min at 20 °C and gel-chromatographed through a Sephadex G-50 column (25 mm i.d. × 100 mm). The DPH content of each fraction (3.5 mL) was determined by fluorescence intensity at 430 nm (exitation, 390 nm), and sugar content of an aliquot (1 mL) by the phenol–sulfuric acid method.[27] Formation of multilamellar liposomes (50–200-nm diameter) was observed for negatively stained samples with a Hitachi HU12A transmission electron microscope.

Fluorescein-labeled dextran (FITC–dextran) was prepared according to DeBelder's procedure[13,28] and purified by gel-chromatography on a Sephadex G-50 column.

Results and Discussion

Polymerization. Homo- and copolymerization of 1 and 2 were carried out in anhydrous dichloromethane with phosphorus pentafluoride as an initiator. The polymerization conditions and characterizations of the resulting copolymers are summarized in Table I. The polymerization temperature was −60 °C except for experiment I–9 (0 °C), and the amount of initiator used was 5 mol % to

Table I
Copolymerization between 1,6-Anhydro-2,4-di-*O*-benzyl-3-*O*-octadecyl-β-D-glucopyranose (1) and 1,6-Anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (2)[a]

expt. no.	1, g	2, g	mol fract of 1 in feed	time, min	yield, %	mol fract of 1 in copolym	mp,[b] °C	$[\alpha]^{25}_{D}$,[c] deg	$[\eta]$,[d] dL/g	$10^{-5}M_n$[e]	$10^{-5}M_n$[f]
I-1	1.19	0	1.00	7	75	1.00[i]		+83.1	0.85	3.8	2.3
I-2	1.19	0.865	0.50	8	90	0.50[i]		+96.2	0.75	2.2	1.3
I-3	1.19	3.46	0.20	15	87	0.22[i]		+106.6	0.93	2.5	2.7
I-10	0.892	3.68	0.15	17	89	0.16[i]		+108.7	0.82	1.9	
I-4	0.594	3.89	0.10	19	84	0.07[i]		+110.5	0.83	2.3	2.6
I-5	0.297	4.11	0.05	16	61	0.05[j]	40–44	+111.9	0.75	1.6	
I-6	0.197	4.20	0.03	16	49	0.03[j]	37–40	+111.7	0.94	3.1	
I-7	0.0595	4.28	0.01	12	38	0.01[j]	39–41	+113.0	0.92	3.0	
I-8	0	4.33	0	20	78	0[j]	41–45	+114.3	0.83	2.3	2.3
I-9[g]	1.19	0	1.00	10	78	1.00[i]		+74.8	0.11	0.22	
I-11[h]	1.19	0.865	0.50	6	12	0.45[i]		+96.5		1.0	

[a] Initiator, PF_5, 5 mol % to monomers; solvent, dichloromethane; concentration of monomers, 0.67 mol/L; temperature, −60 °C. [b] Determined by DSC. [c] In chloroform; c 1g/100mL. [d] In chloroform at 25 °C. Calculation was made by using the concentration expressed in g/100mL. [e] Determined by GPC by using polystyrene standards in chloroform. [f] Determined by membrane osmometry in toluene at 37 °C. [g] Temperature, 0 °C. [h] Initiator concentration, 2.5 mol % to monomers. [i] Determined by ^1H NMR spectrum of the copolymer. [j] Mole fraction of 1 in feed.

monomers except for experiment I-11 (2.5 mol %). As a relatively large amount of the initiator was used, the homo- and copolymerization proceeded rapidly to reach a high conversion in a short time. The apparent polymerization rate tended to increase with an increase of feed composition of **1**.

When the mole fraction of **1** in the feed was higher than 0.05, the copolymer composition was estimated from the area ratio of the alkyl signals (δ 1.25 and 0.86) and the phenyl signal (δ 7.20) of the ^1H NMR spectra. The amount of the **1** unit incorporated into the copolymer was similar to that in the feed. Therefore, for the copolymers of the lower octadecyl content (<0.05), the feed composition was taken as the copolymer composition. To detect small differences in the monomer reactivity between **1** and **2**, the copolymerization (I-11) of 0.5:0.5 molar feed composition was carried out with a smaller amount of the initiator (2.5 mol %) and terminated in a shorter time, and the low-conversion product was analyzed. The copolymer composition of **1** was 0.45, suggesting that monomer **1** had slightly less reactivity than **2**.

The polymers were grouped into two classes by their solubility. The polymers of the octadecyl content (DS) above 0.5 were soluble in hexane and petroleum ether and those below 0.2 were soluble in acetone and DMF. Common solvents for both groups were benzene, toluene, carbon tetrachloride, chloroform, dichloromethane, THF, and pyridine.

The copolymers of octadecyl content of 0–0.2 were powdery at room temperature, those of ~0.5 were waxy, and the octadecyl homopolymers were powdery again. The crystalline melting point of some copolymers could be determined by differential scanning calorimetry (DSC) as listed in Table I; those of the other copolymers were lower than room temperature. We previously pointed out[21] that the crystalline melting point determined by DSC analysis is a measure of some irregularity of the crystalline arrangement of tri-*O*-benzyl-(1→6)-α-D-glucopyranan chain. The melting points of 3-O-octadecylated copolymers were lower than those of the analogous 3-O-dodecylated copolymers, suggesting that the crystalline structure was disordered more effectively by the longer octadecyl chain.

High α-anomer stereoregularity of the homo- and copolymers was suggested by the ^{13}C NMR spectra and specific rotations. No signal due to β-anomeric structure was detected and all peaks of the copolymers were superimposable on peaks of one or the other homopolymers. Specific rotations were positive and high and their plots against the copolymer weight fraction gave a linear relationship.

The molecular weight of the polymers obtained was high in spite of the relatively large amount of the initiator used. Most of 3-*O*-octadecyl polymers possessed intrinsic viscosities of 0.75 dL/g or higher, and molecular weights ranging from 1×10^5 to 3.8×10^5. The degrees of polymerization corresponded to 200–600, which were lower than those (800–1200) of 3-*O*-dodecyl copolymers obtained under similar polymerization conditions.

When the polymerization temperature was increased to 0 °C (experiment I-9), the optical rotation, intrinsic viscosity, and molecular weight of the resulting homopolymer became lower than those of the corresponding homopolymer obtained at −60 °C (experiment I-1). Chain transfer and termination at the elevated temperature could be responsible for the lower degree of polymerization. Although no β-anomeric signal was detected by the conventional ^{13}C NMR technique, the lowering of the optical rotation suggested that a small amount of β-structure (<5%) was introduced in the chains.

We have carried out homo- and copolymerizations of 1,6-anhydro-2,3,4-tri-*O*-benzyl-β-D-glucopyranose (**2**) and its 3-*O*-methyl, 3-*O*-dodecyl, and 3-*O*-octadecyl homologues. The polymers obtained with PF_5 initiator at −60 °C were of complete α-anomeric configuration, suggesting that the copolymerizations proceeded by an oxonium ion mechanism. All of these 3-O-alkylated monomers showed high reactivity in terms of apparent polymerization rate, copolymerization reactivity, and degree of polymerization, although octadecyl and dodecyl ether chains tended to increase the apparent polymerization rate and decrease slightly both copolymerization reactivity and degree of polymerization compared with benzyl ether chain. According to the mechanistic consideration as previously reported,[29] the reactivity of 1,6-anhydro sugar derivatives decreased with an incorporation of a bulky substituent. It is reasonable to assume that dodecyl and octadecyl chains are long but flexible and hence have similar reactivities to **2**.

Partially 3-O-Octadecylated (1→6)-α-D-Glucopyranans. The copolymers of less octadecyl content could be debenzylated successfully by the Birch reduction using sodium in liquid ammonia (Table II). The water-soluble product was purified by dialysis and then freeze-dried; the water-insoluble product was isolated by reprecipitation from a Me_2SO solution into methanol and dried in a desiccator. The content of the water-soluble fraction was

Table II
Synthesis of 3-O-Octadecylated (1→6)-α-D-Glucopyranan[a]

expt. no.	DS[b]	sodium, g	time, min	yield, % sol[c]	yield, % insol[d]	[η],[e] dL/g sol[c]	[η],[e] dL/g insol[d]	$10^{-4}M_n$[f]
ID-3	0.22	0.73	87	6.9	93[g]		0.40	0.9[d]
ID-10	0.16	1.40	85	4.4	88[g]		0.65	2.1[d]
ID-4	0.07	0.51	100	11	87[g]		0.28	1.2[d]
ID-5	0.05	0.67	80	19	78[g]	0.26	0.38	2.4[d]
ID-6	0.03	0.48	80	48[g]	21	0.23	0.30	1.8[c]
ID-7	0.01	0.86	90	45[g]	11			3.2[c]
ID-8	0	1.10	80	100[g]	0	0.45		2.1[c]

[a] Polymer, 1 g; solvent, 1,2-dimethoxyethane–toluene, 15 mL/30 mL; liquid ammonia, 100 mL. [b] Mole fraction of the 1 unit in copolymer listed in Table I was employed as degree of substitution (DS). [c] Water-soluble fraction. [d] Water-insoluble fraction. [e] In Me_2SO at 25 °C. Calculation was made by using the concentration expressed in g/100 mL. [f] Determined by reducing-end analysis according to the Somogyi–Nelson procedure. [g] The fraction was used for the experiments of the following sections.

lowered with an increasing amount of 3-*O*-octadecyl substitutent. Me_2SO, DMF, and pyridine were solvents for both fractions.

Complete debenzylation proceeded without anomeric change, which was demonstrated by ^{13}C NMR spectroscopy. The molecular weight estimated by determination of the reducing end indicated that a partial cleavage of the main chain occurred during the debenzylation. The major difference between the water-soluble and water-insoluble fractions was intrinsic viscosities. Other structural properties such as optical rotation and octadecyl content, the latter of which were determined from the intensity of alkyl resonances in the ^1H NMR spectra, were almost the same.

Thus 3-O-octadecylated stereoregular linear dextrans, the degree of substitution (DS) of which was up to 0.22, were obtained. It is worthy of remark that the long hydrocarbon chains could be inserted into the polymer chain regioselectively and at random intervals. The larger fraction of either water-soluble or insoluble polymer was used for the following experiments.

Debenzylation of the polymers with higher octadecyl content was unsuccessful and the starting polymers were recovered. We assumed that the compatibility between the octadecyl substituent and liquid ammonia was poor and hence the starting polymer could not come into contact with the active reductant, solvated electrons, in the inorganic medium.

Enzymatic Hydrolysis. The copolymers were degraded with use of an endo-acting dextranase. The percent of glycoside linkage cleaved was determined from the concentration of reducing sugars and expressed as the degree of hydrolysis. The maximum degree of hydrolysis of a nonsubstituted dextran was 53%, and the main product was a disaccharide isomaltose. The octadecylated dextrans of DS < 0.05 were hydrolyzed in almost the same manner as the nonsubstituted dextran. However, the dextrans of DS > 0.07 showed slower hydrolysis, and the maximum degree of hydrolysis of the sample of DS 0.22 was only 14%. For comparison, enzymatic hydrolysis of water-soluble 3-O-methylated dextrans was attempted. Completely 3-O-methylated dextran (DS 1.0) was not hydrolyzed, but that of DS 0.57 gave the maximum degree of hydrolysis of 16%. It was indicated that an octadecyl substituent depressed hydrolysis of dextrans more efficiently than a methyl substituent. We assumed that the bulkiness of the octadecyl chains interfered with the hydrolysis of not only the octadecylated units but also several of their neighboring units.

Binding of Organic Solute in Water. Solute binding properties of the polysaccharides in water were investigated by using magnesium 1-anilino-8-naphthalenesulfonate (ANS) as a hydrophobic fluorescence probe.[30] Figure 1 depicts fluorescence emission spectra of ANS in the

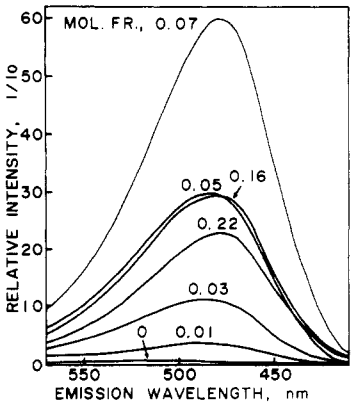


Figure 1. Fluorescence emission spectra of ANS in the presence of octadecylated dextrans: [ANS] = 2.5×10^{-4} M; [polym] = 2.5 g/L; in phosphate buffer.

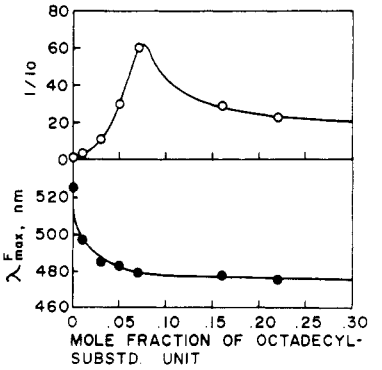


Figure 2. Relative intensity (I/I_0) and maximum emission wavelength (λ^F_{max}) of ANS in the presence of octadecylated dextrans: [ANS] = 2.5×10^{-4} M; [polym] = 2.5 g/L; in phosphate buffer.

presence of the polysaccharides of different octadecyl content. ANS in aqueous solution had a weak fluorescence at 525 nm and dextran homopolymer exerted little effect on its fluorescence. However, the copolymers induced a striking enhancement of the fluorescence and a blue-shift of the emission maximum. In Figure 2 the relative fluorescence intensity (I/I_0) and maximum wavelength (λ^F_{max}) of ANS were plotted against mole fraction of octadecyl-substituted units. The relative fluorescence intensity increased with an increase of the DS and reached 60 times that of free ANS in a copolymer of DS 0.07, but additional increase of the octadecyl content resulted in a decrease of the intensity. On the other hand, the emission maximum decreased and took on a constant value (480 nm) at copolymers of DS higher than 0.07. The large blue shift of 45 nm indicated that ANS remained in a hydrophobic microenvironment.

A strong affinity of ANS to the (1→6)-α-D-glucans having octadecyl substitutents has been thus demonstrated. We assumed that the polysaccharides formed a polymeric micellar conformation and the organic solute was bound to nonpolar regions surrounded by the long hydrocarbon chains. In contrast to octadecylated polysaccharides, little interaction was observed between dodecylated polysaccharides (DS 0.02–0.19) and ANS. It is probable that dodecyl chains in the polysaccharides were too short to form hydrophobic aggregates.

The relative fluorescence intensity (I/I_0) was treated with Benesi–Hildebrand[31] and Klotz[32] equations (eq 1 and 2, respectively), where I_∞/I_0 is the assumed fluorescence

$$\frac{1}{I/I_0} = \frac{1}{[\text{polym}]}\frac{1}{K'(I_\infty/I_0)} + \frac{1}{I_\infty/I_0} \quad (1)$$

$$\frac{[\text{polym}]}{[\text{ANS}]_B} = \frac{1}{[\text{ANS}]_F}\frac{1}{Kn} + \frac{1}{n} \quad (2)$$

of the bound ANS and $[\text{ANS}]_B$ and $[\text{ANS}]_F$ are the concentrations of the bound and free ANS, respectively. The treatment was made for the polysaccharide of DS 0.07, which induced the strongest fluorescence among the polysaccharides, and linear relationships were obtained. The Benesi–Hildebrand (K') and Klotz (Kn) binding constants were estimated from the slope to be 34 and 41 M^{-1}, respectively. The term $1/n$, the minimum number of the structural units required to bind a solute molecule,[32] was 170. It corresponded to the molecular weight of the copolymer of 2.8×10^4. This means that 2.3 polymeric chains were required to bind one molecule of ANS.

Formation of Polymeric Membranes. Transparent, flexible membranes were obtained by casting a polysaccharide solution in Me_2SO on a glass plate. Naturally occurring dextrans have no ability to form a stable membrane, but incorporation of a suitable amount of octadecyl (DS 0.07–0.22) or dodecyl (DS 0.19) group was found to develop membrane-forming ability.[21]

A membrane obtained had two different surfaces, each of which had been kept in contact with the glass and the atmosphere (air) during its preparation. The surface of the atmosphere side had a larger contact angle of a water droplet than that of the glass side. For instance, the copolymer membrane of DS 0.07 had contact angles of 93° (atmosphere side) and 80° (glass side). We assumed that a local microphase separation occurred and long hydrocarbons were somewhat concentrated on the atmosphere side and the hydrophilic polysaccharides on the glass side.[33]

Polysaccharide-Coated Liposomes. Polysaccharide-coated liposomes were prepared by mixing the polysaccharides with multilamellar liposomes of DL-α-dipalmitoylphosphatidylcholine (DPPC) in aqueous solution and then isolated by chromatography on a Sephadex G-50 column. Liposomes were monitored by fluorescence intensity at 430 nm (F_{430}) of 1,6-diphenyl-1,3,5-hexatriene (DPH), which was incorporated as a probe into the hydrocarbon region of the liposomes. Polysaccharide was detected by a phenol–sulfuric acid colorimetric method,[27] and its amount was expressed in terms of A_{490}.

Figure 3 demonstrates that there occurred strong interaction between the octadecylated dextran and DPPC liposomes. When each component was chromatographed, the polysaccharide was eluted at the fraction number 9–15, and the DPPC liposomes were adsorbed onto the gels and not eluted under these conditions (Figure 3, parts A and B).[26] In contrast, when both substances were mixed together by sonication prior to the gel chromatography, the polysaccharide and the liposomes were eluted at the void

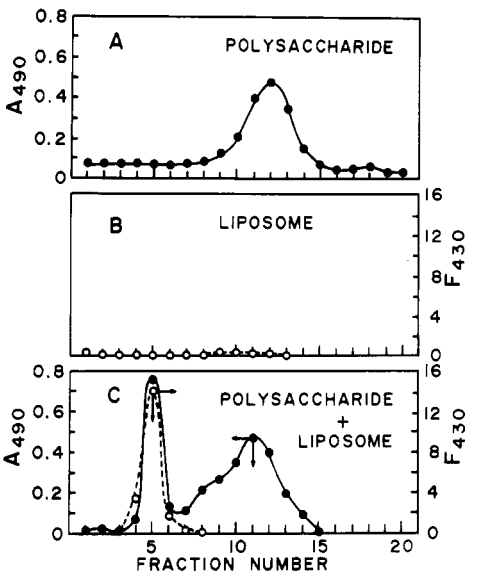


Figure 3. Isolation of polysaccharide-coated liposomes by gel chromatography: Octadecylated dextran (DS 0.01), 1.6 mg; DPPC, 1.6 mg; cholesterol, 0.3 mg. (●) The polysaccharide determined by the colorimetric method and represented in terms of A_{490}. (○) The liposome determined by the fluorometry of 1,6-diphenyl-1,3,5-hexatriene and represented in terms of F_{430}.

volume (Figure 3C, fraction number 4–6). It is reasonable to assume that the surface of the liposomes was modified by the octadecylated dextran, and polysaccharide-coated liposomes were isolated. Twenty-five percent of the polysaccharide was incorporated into the assemblies, and the remainder was separated as free polysaccharides (75%).

The interaction between liposomes and the dextrans of octadecyl content 0.01–0.05 was strong enough, and the octadecyl dextran of DS 0.03 yielded the largest amount of polysaccharide-coated liposomes. However, no polysaccharide-coated liposomes were isolated from those of DS 0.07 and higher, only a minor amount was isolated from the dextrans with dodecyl substituent (DS 0.02 and 0.04), and none was isolated from clinical dextrans with no alkyl substituents.

The above findings may be interpreted as follows. Dextrans are known to be adsorbed on the surface of liposomes,[12] but the adsorption is an equilibrium process and their dissociation into each component easily occurred during the gel chromatography. The dissociation could be suppressed by introducing long hydrocarbon chains into dextran and anchoring them deeply into the lipid layer of liposomes. Octadecyl chains exhibited more anchoring effect than dodecyl chains, but incorporation of too many octadecyl anchors (DS > 0.07) disordered the structure of the liposomes and, as the result, made liposomes unstable.

The anchoring effect was also investigated by fluorescence polarization measurements with fluoresceinyl(thiocarbamoyl)dextran (FITC–dextran) as the probe. The fluorescence polarization (p) was obtained by a simultaneous measurement of the fluorescence intensities $F_\parallel$ and $F_\perp$, which were detected through a polarizer oriented parallel and perpendicular to the direction of polarization to the excitation beam.

$$p = \frac{F_\parallel - F_\perp}{F_\parallel + F_\perp}$$

The free FITC–dextran showed a relatively small fluorescence polarization, but the polarization was increased by assembling the dextran into liposomes (Table III). The mobility of the probe was restricted due to anchoring of the hydrocarbon chains of the polysaccharide

Table III
Fluorescence Polarization (p) of Fluorescein-Labeled Dextran in the Polysaccharide-Coated Liposomes at 20 °C

FITC–dextran			DPPC,		
alkyl	DS[a]	mg[b]	mg[b]	cholesterol, mg[b]	p[c]
octadecyl	0.03	1.6	0	0	0.13
	0.03	1.6	1.6	0.3	0.19
	0.03	0.4	1.6	0.3	0.18
	0.03	0.2	1.6	0.3	0.20
dodecyl	0.02	1.6	0	0	0.12
	0.02	1.6	1.6	0.3	0.16

[a] Degree of substitution. [b] Weight in the feed. [c] Excitation at 485 nm and fluorescence emission at 520 nm.

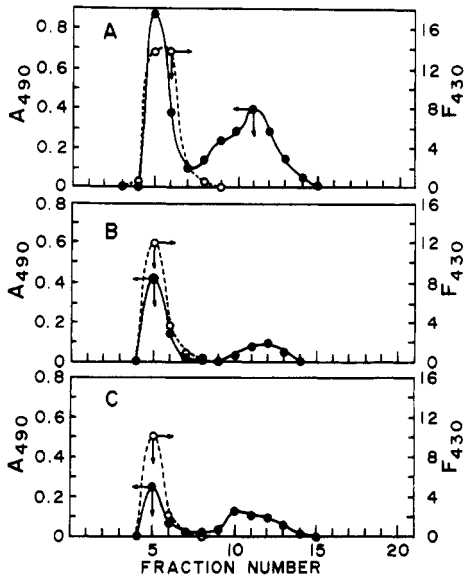


Figure 4. Effect of feed ratio on binding of polysaccharide to liposomes: DPPC, 1.6 mg; cholesterol, 0.3 mg; octadecylated dextran (DS 0.03) (A) 1.6 mg, (B) 0.4 mg, (C) 0.2 mg. The symbols are explained in the caption to Figure 3.

into liposomes. It was suggested again that the dodecyl chain produced less anchoring effect than the octadecyl chain.

It is important to note that the liposomes obtained were surrounded by a larger amount of the polysaccharides than the amount calculated by assuming that the polysaccharide chains were spread flat in single layers over the outer surface of liposomes. An example showing that excess polysaccharide is given in Figure 4A. When 1.6 mg of polysaccharide of DS 0.03 was interacted with multilamellar liposomes consisting of 1.6 mg of DPPC and 0.30 mg of cholesterol, the resulting polysaccharide-coated liposomes were found to contain 0.50 mg of the polysaccharide. If the liposomes had been single lamellar, 0.32 mg of the polysaccharide would be required.[34] Actually, the liposomes were multilamellar, and hence a smaller amount of the polysaccharide would be sufficient.

We propose a binding mode between liposomes and polysaccharides as illustrated in Figure 5.[38] This conformational model can be regarded as a modification of the "loop–train–tail" model that has been widely accepted from both theoretical and experimental viewpoints as the conformational model for macromolecules adsorbed on an interface.[39-41] A part of the polysaccharide chains (trains) is in contact with the surface of the liposomes through octadecyl anchors, while the remaining segments extend into the solution to form loops and tails that are adsorbed at both end groups or one end group, respectively. In contrast to the general loop–train–tail model in which all segments have an equal chance to become trains (no anchor segments), the anchor segments including the neighboring glucose units are preferentially adsorbed on the liposomes to become trains. We assume that the presence of the loops and tails bring about the adsorption of excess polysaccharides to the liposomes.

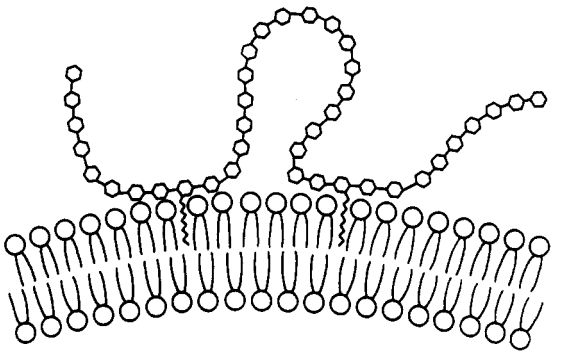


Figure 5. A proposed conformational model of the adsorption of polysaccharide onto liposome.

When the feed amount of the polysaccharides was decreased to 0.4 and 0.2 mg while keeping the amount of liposomes constant (Figure 4, part B and C), there still remained free polysaccharide, and the eluted amount of the polysaccharides on liposomes was decreased to 0.27 and 0.09 mg, respectively. However, the p values of the FITC–dextran probe in these polysaccharide-coated liposomes were almost independent of the feed amount of polysaccharides as listed in Table III, suggesting that the mobility of the polysaccharide was not changed. We assumed that the loop–train–tail model was valid even when the liposomes were adsorbed by a smaller amount of polysaccharides.

Summary of Properties. We can summarize the properties of the octadecylated dextrans and their dependence on the degree of substitution (DS) as follows.

(1) The polysaccharides of lower octadecyl content (DS 0.01–0.05) were subject to enzymatic hydrolysis in a manner similar to nonsubstituted dextran. They could be incorporated into liposomes, and polysaccharide-coated liposomes could be isolated. The polysaccharides of DS 0.03 interacted with liposomes most strongly.

(2) The polysaccharides of higher octadecyl content (DS 0.07–0.22) were also biodegradable but their hydrolysis rate became slow. No stable polysaccharide-coated liposomes were obtained, but flexible polymeric membranes could be cast from their Me_2SO solutions.

(3) A polymeric micellar conformation was formed by the polysaccharides (DS 0.01–0.22) in water, and an organic solute ANS was bound to the nonpolar hydrocarbon regions. The strongest interaction was observed for the copolymer of DS 0.07.

(4) The dodecylated polysaccharides of DS 0.02–0.19 formed little polysaccharide-coated liposomes or micellar conformation but formed polymeric membranes (DS 0.19).

In conclusion, the ability of the polysaccharides to form these assemblies was determined by a hydrophobic–hydrophilic balance that depended on the length of the hydrocarbon chains and the degree of substitution.

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Block Copolymers from Cyclic Imino Ethers: A New Class of Nonionic Polymer Surfactant

Shiro Kobayashi,* Toshio Igarashi, Yasuhiro Moriuchi, and Takeo Saegusa

Department of Synthetic Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan. *Received July 8, 1985*

ABSTRACT: Various types of block copolymers from cyclic imino ether monomers have been designed and prepared by utilizing the living nature of the cationic ring-opening polymerization of the monomers. Block copolymers prepared are AB and BA type diblock (**3**) and ABA and BAB type triblock copolymers (**5** and **9**). All copolymers consist of a hydrophilic chain (A block) and a lipophilic chain (B block). The chains are of an amide type structure. The monomers used are seven 2-substituted 2-oxazolines (OZO) and three (unsubstituted and two 2-substituted) 5,6-dihydro-4*H*-1,3-oxazines (OZI). 2-Methyl-2-oxazoline (MeOZO) was used most often for construction of an effective hydrophilic block of an *N*-acetylethylenimine unit. 2-*n*-Butyl- (BuOZO), 2-*n*-octyl- (OcOZO), 2-*n*-dodecyl- (DoOZO), and 2-phenyl-2-oxazolines (PhOZO) produce a lipophilic block. These block copolymers exhibit excellent surface activities and, hence, are a group of nonionic polymer surfactants. The surface activities reflected by the surface tension (γ) in water are very high: a γ value as low as 27.5 dyn/cm for an AB type diblock copolymer from MeOZO/BuOZO and 23.7 dyn/cm for a BAB type triblock copolymer from MeOZO/OcOZO. One characteristic of the present polyamide type surfactants is that they do not show a clouding point.

Cationic ring-opening polymerization of cyclic imino ethers is a versatile, convenient method to prepare linear poly(*N*-acylalkylenimines).[1] During the course of our studies on the polymerization of cyclic imino ethers, we have found that poly(*N*-acetylethylenimine) is highly hygroscopic and possesses a good hydrophilic property. These findings prompted us to prepare block copolymers from cyclic imino ethers, which contain both hydrophilic and lipophilic chains in the same molecule and, hence, are expected to exhibit surfactant properties. The present paper reports the synthesis and surfactant properties of AB and BA type diblock and ABA and BAB type triblock copolymers derived from cyclic imino ethers. Throughout this paper A and B blocks represent hydrophilic and lipophilic blocks, respectively.

Results and Discussion

I. Diblock Copolymers. The synthesis of AB type block copolymers **3** utilizes a "one-pot two-stage copolymerization" technique on the basis of the mechanistic living nature of the cationic polymerization; i.e., one of cyclic imino ethers **1**, such as 2-methyl-2-oxazoline (MeOZO), is first polymerized (the first stage) to give A block, and, then, the other monomer **2** (e.g., 2-*n*-butyl-2-oxazoline (BuOZO), is polymerized by the living ends of the first polymerization system (the second stage) to give B block.

0024-9297/86/2219-0535$01.50/0