Naturally Occurring Polysaccharide Derivatives Which Behave as an Artificial Cell Wall on an Artificial Cell Liposome

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ABSTRACT: In order to make a liposome more mechanically stable, the liposomal surface was coated with a naturally occurring polysaccharide which bears a hydrophobic anchor such as a cholesterol or palmitoyl residue. The effect of a hydrophobic anchor on the coating efficiency of the liposomal membrane was studied from the viewpoints of the permeability of a polysaccharide-coated liposome and the membrane fluidity. Through these investigations, it was found that coating of the liposomal surface with cholesterol derivatives of the polysaccharides was much better at decreasing the membrane permeability of a water-soluble fluorescent probe (6-carboxyfluorescein) than coating with *O*-palmitoylpolysaccharide.

Introduction

Plant and bacterial cell membranes are covered with a cell wall of polysaccharides.[1-3] The cell wall maintains the shape and stiffness of cytoplasm and protects the plasma membranes against chemical and physicochemical stimuli such as change of osmotic pressure, ionic strength, hydrogen ion concentration, temperature, and so forth. Polysaccharides and other carbohydrate-containing substances exist widely in biomembranes and participate in intercellular communication, recognition, and/or specific binding of cell regulatory molecules.[4] Similar to the cell wall of plant cells or bacteria and to the extracellular matrix of animal cells, several naturally occurring polysaccharides such as dextran, amylopectin, or pullulan significantly interact with biomembranes[5-7] or liposomal membranes[8-10] and may cause cell–cell aggregation[7] or liposome–liposome fusion.[8]

In order to mimic the morphology and function of the cell walls of plant and bacterial cell membranes, several water-soluble and naturally occurring polysaccharides have been hydrophobized by fatty acids. For example, Tsumita and Ohashi first demonstrated that a polysaccharide hydrophobized with acyl groups easily adsorbs onto the erythrocyte surface.[11] Thereafter, it has been reported that such acylated polysaccharides adhere to the surfaces of cytoplasm membranes,[12,13] planar lipid bilayer membranes,[14] and liposomes.[15-17] Sunamoto and his co-workers have substituted the palmitoyl group as a hydrophobic anchor to several naturally occurring polysaccharides.[15,18] They also confirmed the improved stability of the liposome upon coating with *O*-palmitoyl-substituted polysaccharide from two aspects: (1) a decrease in the permeability to a water-soluble probe, 6-carboxyfluorescein (CF), which is encapsulated in the interior water pool of the liposome, and (2) an increase in the resistance of the liposome against enzymatic lyses with phospholipase D and lipid peroxidase.[15,18] Furthermore, they have first employed such polysaccharide-coated liposomes as a receptor-mediated drug carrier.[18,19]

Other water-soluble polymers such as polypeptides[20] also have been utilized to coat the surface of vesicles besides naturally occurring polysaccharides. Tirrell and his co-workers assembled synthesized polyelectrolytes to phospholipid bilayer membranes[21] and studied the pH-induced permeability change of the lipid membranes.[22] Ringsdorf and his co-workers also coated the lipid bilayer membranes with hydrophobized polymers[17] and studied the thermoreversible expansion and construction of the polymer on the liposomal surface.[23] Compared with these synthetic polymers, however, naturally occurring polysaccharides are expected to be less toxic and immunogenic and, subsequently, to be better cell-compatible and biocompatible materials even if they were employed in vitro and in vivo experiments.

Surfactants and lysolecithins, which bear single long alkyl chains, are known to make the membrane more leaky and sometimes destroy the integrity of lipid bilayer membranes.[24-26] On the other hand, the addition of cholesterol into the lipid bilayer membrane above its phase transition temperature makes the membrane more stable against internal and external stimuli.[27,28] In this work, in order to improve further the coating efficiency of the polysaccharide to the liposome, therefore, several naturally occurring polysaccharides were modified with cholesterol groups instead of palmitoyl groups, and the difference in the coating efficiency for liposomes between the two hydrophobic groups was investigated with respect to barrier function and fluidity of the liposomal membrane.

Materials and Methods

Materials. Egg phosphatidylcholine (egg PC) was isolated and purified according to the method described by Singleton et al.[29] Naturally occurring polysaccharides employed in this work are pullulan, amylopectin, mannan, dextran, amylose, inulin, and levan. Structures of the polysaccharides are shown in Figure 1. Pullulan and amylopectin were purchased from Hayashibaru Laboratories (Okayama, Japan). Dextran and mannan were from Nakarai Chemicals Ltd. (Kyoto, Japan), 3′,6′-dihydroxyisothiocyanatospiro[isobenzofuran-1(3*H*),9′(9*H*)-xanthen-3-one] (FITC; isomer I) was from Dojindo Laboratories (Kumamoto, Japan), and inulin and amylose were from Wako Pure Chemicals Ind. Ltd. (Osaka, Japan). Levan was a kind gift from Professor M. Iizuka (Faculty of Science, Department of Biology, Osaka City University, Osaka, Japan). *O*-Palmitoylpullulan and *O*-palmitoylamylopectin were prepared according to the method adopted for preparation of *O*-palmitoyldextran[30,31] with minor modification. For example, 5.0 g of pullulan-50 (weight-average molecular weight 50 000) was dissolved in 55 mL of dry

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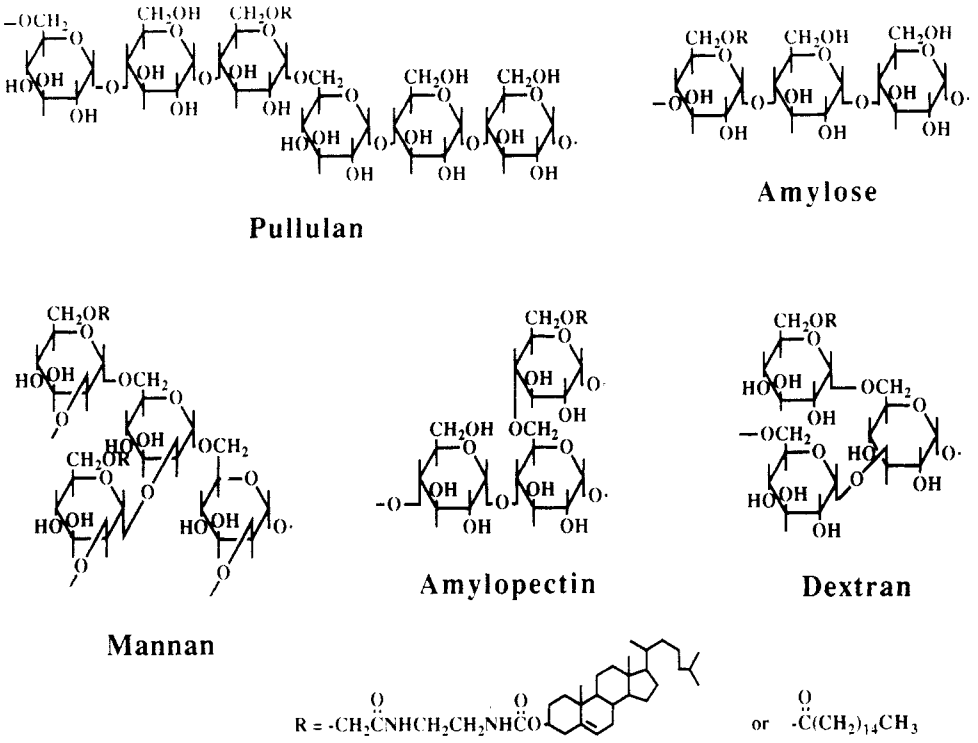

Figure 1. Structures of polysaccharide derivatives employed in this work.

dimethylformamide (DMF) at 60.0 °C. To the resulting solution were added 5.0 mL of dry pyridine and 1.2 mL of dry DMF containing 0.5 g of palmitoyl chloride (Wako Pure Chemicals Ind. Ltd.). The reaction mixture was stirred at 60.0 °C for 2 h and for another 1 h at room temperature. The mixture was poured onto 350 mL of ethanol. Precipitates were collected and washed with 400 mL of ethanol and, then, with 300 mL of diethyl ether. White solid materials obtained were dried in vacuo at 50.0 °C for 2 h; yield 5.0 g. The degree of substitution of palmitoyl residues (per 100 glucose units) of the two polysaccharides was determined by ^{1}H NMR and elemental analysis. When the substitution degree of the palmitoyl residue was 3.4 for pullulan-50, it was coded as OPP-50-3.4. Similarly, OPA-112-4.9 stands for amylopectine (MW 112 000) as substituted by 4.9 palmitoyl groups per 100 glucose units.

The cholesterol moiety was introduced to polysaccharide by the method described elsewhere.[32] An [(aminoethyl)carbamoyl]-methyl (AECM) group was first substituted as the spacer. Then, AECM-substituted polysaccharide was reacted with cholesteryl chloroformate in dry DMSO.[32] The degree of substitution of the cholesterol moiety was determined by both ^{1}H NMR and elemental analysis. For example, when pullulan-50 was substituted by 1.3 cholesterol moieties per 100 glucose units, the product so obtained was coded as CHP-50-1.3. All other polysaccharide derivatives were also coded by the same way, such as CHAp-112-1.2 for amylopectin (MW 11 200), CHM-200-2.4 for mannan (MW 20 000), CHL-2000-0.8 for levan (MW 200 000), CHAs-85-2.0 for amylose (MW 85 000), and CHD-176-1.4 for dextran (MW 176 000).

Introduction of the FITC group to polysaccharide derivatives was carried out by the same method as that adopted for the preparation of FITC-labeled dextran.[7] The substitution degree of the FITC group per 100 glucose units was fluorometrically determined at 520 nm (excitation at 470 nm) in 20 mM Tris-HCl buffer solution (pH 8.6) containing 200 mM NaCl. Their substitution degrees were 0.42 for CHP-50-1.3, 0.50 for CHAp-112-1.2, 1.46 for CHAp-112-2.1, 1.74 for OPAp-112-1.4, and 0.95 for OPAp-112-4.9.

Evaluation of the Coating Efficiency of the Polysaccharide Derivative to the Liposome. Polysaccharide-coated liposomes were prepared by essentially the same procedure as that adopted in previous papers.[15,18,19,32] Conventional egg PC liposomes were prepared by swelling the thin film of egg PC (30.0 mg) in a 20 mM Tris-HCl buffer (4.0 mL) (pH 8.6) containing 200 mM NaCl, and the resulting suspension was further sonicated using a probe-type sonifier (UR-200P, Tomy Seiko Co. Ltd.) at 25 W for 10 min with 1-min intervals. To the liposomal suspension so obtained was added a given amount of the FITC-labeled polysaccharide derivative solution. After stirring for 30 min at 20.0 °C, the suspension was submitted to gel filtration on a Sepharose 4B (Pharmacia Fine Chemicals) column (diameter 16 × 500 mm) preequilibrated with the same buffer in order to separate the FITC-labeled polysaccharide-coated liposomes (multilamellar vesicles (MLV) and small unilamellar vesicles (SUV)). Elution of the polysaccharide-coated liposome from the Sepharose 4B column was monitored by both the turbidity of the liposomal suspension at 360 nm on a Hitachi 220A spectrophotometer and the fluorescence intensity of the fluorophore of the FITC-labeled polysaccharide on a Hitachi 650-10S fluorescence spectrophotometer. For all the liposomal fractions which were gel-filtrated, the concentration of egg PC was determined by using a kit reagent, Wako Phospholipid Test (Wako Pure Chemicals Ind. Ltd., Osaka).

Measurement of CF Leakage. A carboxyfluorescein (CF) release technique is the most popular and convenient method for examining the barrier function of liposomal membranes.[15,33,34] A 2.0-mL suspension of CF-loaded liposomes was mixed with a small amount of an aqueous polysaccharide derivative solution at a given ratio of egg PC to polysaccharide and incubated at 20.0 °C for 30 min. The final concentration of egg PC was 1×10^{-4} M in a cuvette cell. The total amount of CF encapsulated in a liposome was determined after complete destruction of the liposome by adding 30.0 μL of 10% Triton X-100 per 1.0 mL of a liposome suspension. It was, of course, ascertained beforehand that perturbation of polysaccharide to the lipid bilayer was almost negligible during the coating procedures.

Similar to the spontaneous CF release, the CF release in the presence of human plasma and serum also was measured. Plasma and serum were isolated from the blood of a human adult volunteer. A polysaccharide-coated liposome suspension preincubated at 20.0 °C for 30 min was mixed with 18.0% (v/v) human plasma or serum, and then an increase in the fluorescence intensity of CF was monitored as described above. In this case, the complete destruction of liposomes was made by adding 60.0 μL of a 1.3% aqueous sodium deoxycholate solution per 1.0 mL of a liposomal suspension.

Measurement of the Membrane Fluidity. In order to measure the membrane fluidity change upon polysaccharide coating, the fluorescence depolarization (p) was measured by exactly the same method as those described elsewhere.[35,36]

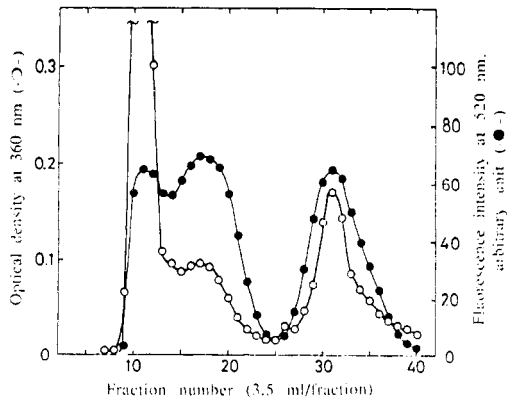

Figure 2. Example of gel chromatographic separation of the liposome coated with FITC-CHP-50-1.3 and free polysaccharide as developed with a 20 mM Tris-HCl buffer containing 200 mM NaCl (pH 8.6) at room temperature. Liposomal fractions were monitored by turbidity at 360 nm, while the polysaccharide fractions were monitored by fluorescence intensity of FITC at 520 nm with excitation at 470 nm.

Labeling of the liposomal membrane with 1,6-diphenyl-1,3,5-hexatriene (DPH; Sigma) or dansylhexadecylamine (DSHA)[35,36] was carried out by mixing a preformed liposomal suspension with a given amount of the fluorescent probe dissolved in terahydrofuran and by sonicating it for 1 min using a probe-type sonifier. Depolarization measurements were carried out on a Union Giken FS-501S fluorescence depolarization spectrophotometer, whose cell compartment was connected to a Komatsu-Yamato Coolnics Model CTR-120. A Sord Microcomputer M 200 Mark II system was employed to control the measurement conditions and to collect the data.

The fluorophore, FITC, was excited at 470 nm, and the emission was collected using a sharp cutoff filter Y-46 (Hoya Glass Works, Tokyo) to completely eliminate any wavelength below 460 nm. DPH was excited at 360 nm, and the fluorescence was measured by using a Y-42 cutoff filter. Similarly, for DSHA, excitation was made at 340 nm and the emission was collected by using a Y-49 cutoff filter.

Measurement of the Size of the Liposomes. The hydrodynamic diameter of the liposomes before and after coating with a polysaccharide was measured by a quasi-elastic light scattering method on a Photal DLS-7000 (Otsuka Electronics Co., Osaka, Japan).

Results and Discussion

Coating Efficiency. In order to investigate the coating efficiency of the polymer to the liposomes, both the amount of the polymer adsorbed and the strength of the interaction between the polymer and the liposome were determined by gel chromatographic separation of the polysaccharide-coated liposomes on a Sepharose 4B column. Under the conditions employed, polysaccharides were found not to be incorporated into the interior of the liposome, similar to the case reported by Oku et al.[37] Figure 2 shows an example of a gel chromatogram of MLV and SUV as coated with FITC-labeled CHP-50-1.3 on a Sepharose 4B column. The fractionation was monitored by both the turbidity and fluorescence intensity of FITC. Fractions 9–13 correspond to MLV and fractions 15–23 contain SUV. In order to determine the amount of the polymer adsorbed onto the liposomal surface, the concentrations of both egg PC and FITC-labeled polysaccharide were determined for both MLV and SUV fractions. Ratios of polymer to lipid before and after gel filtration are given in Figure 3. The pullulan derivative was better at coating the liposomal surface than the amylopectin derivative. The difference between the two polysaccharides might come from the difference in the structure, or exclusion volume, in solution.[38] The amount of the polysaccharides adsorbed

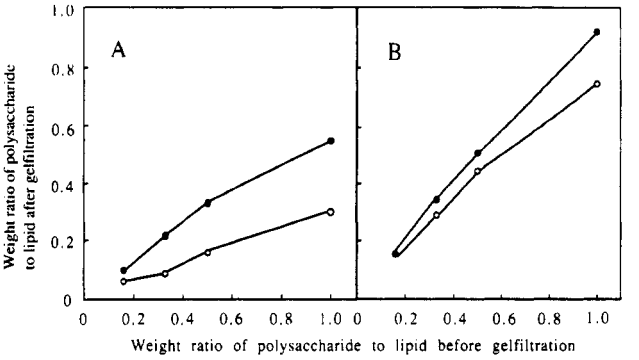

Figure 3. Coating efficiency of the liposome with polysaccharide derivatives which was investigated by the relationship between the amounts of the polysaccharide added before and those recovered after gel filtration of egg PC MLV (A) and egg PC SUV (B) as coated by FITC-labeled CHP-50-1,3 (●) and FITC-labeled CHAp-112-1.0 (○).

Table I
Difference in the Coating Efficiency of MLV between the Two Hydrophobic Anchors, Palmitoyl and Cholesterol Residues

polysaccharide	[FITC-PS]/[egg PC] (by weight)[a]	% area of liposomal surface covered by FITC-PS[b]
OPAp-112-1.4	0.01	16
OPAp-112-4.9	0.44	663
CHAp-112-1.2	0.14	203
CHAp-112-2.1	0.38	572

[a] FITC-PS stands for a fluoresceinylisothiocyanate-labeled polysaccharide. [b] This was estimated as follows: 100 × (area of the MLV surface covered by a polysaccharide derivative)/(area of the outermost surface of MLV). Details are given in the text.

on a given surface area of MLV was more than that on a comparable area of SUV. To explain this, it is necessary to consider the difference in the curvature, the size of the vesicles, between MLV and SUV.

Furthermore, the difference in the coating efficiency between the two hydrophobic anchors, cholesterol and palmitoyl groups, was investigated. A liposomal suspension was mixed with an aqueous solution of polysaccharide derivatives at a given weight ratio (0.5) of polysaccharide to egg PC. The mixture was incubated at 20 °C for 30 min and then submitted to a column of Sepharose 4B in order to remove free polysaccharide. The coating efficiency was calculated from the concentrations of both FITC-labeled polysaccharide and egg PC by assuming that the diameter of the liposome employed is 97 nm (surface area 295.6 nm^2) and the covering area of a glucose unit on the liposomal surface is approximately 0.49 nm^2. Results are given in Table I. For OPAp-112-1.4, no effective coating was observed; namely, only 16% of the surface area of the liposome was coated by the polymer. When the substitution degree of the palmitoyl group increased, a slight increase in the coating efficiency was observed. On the other hand, when the polymer was conjugated with a cholesterol moiety, such as CHAp-112-1.2, a significantly higher coating efficiency was observed: 200% of the surface of MLV was coated by the polysaccharide. When the polysaccharide was substituted by more cholesterol groups such as CHAp-112-2.1, the coating efficiency increased further. Judging from these results, in any event, cholesterol was considered a much better hydrophobic anchor for coating the liposome surface than the simple fatty acid. The fact that more than 100% of the surface area of the liposome was coated by the polysaccharide suggests that

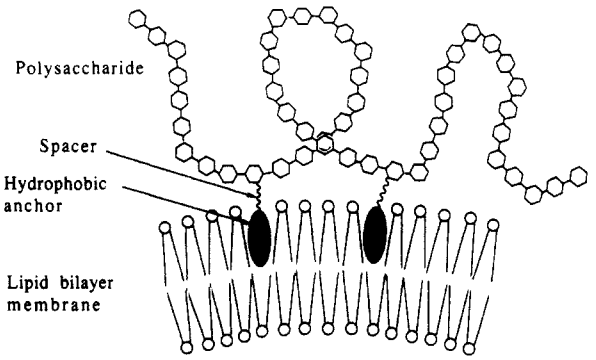

Figure 4. Schematic representation of the coating of the liposomal surface with hydrophobized polysaccharide.

Table II
Average Hydrodynamic Radius of Polysaccharide-Coated Egg PC MLV[a]

coating	mean diameter ±SD (nm)
none	183.3 ± 4.3
with pullulan-50	188.1 ± 3.6
with CHP-50-1.0	208.7 ± 9.5

[a] [Egg PC] = 3.0×10^{-4} M in 20 mM Tris-HCl containing 200 mM NaCl (pH 7.4). [Polysaccharide]/[egg PC] = 0.5 by weight. Polysaccharide coating was carried out at 47.0 °C.

the polysaccharide molecules adsorbed form loops on the liposomal surface as schematically represented in Figure 4.

The coating of the liposomal surface with the polysaccharide derivatives is confirmed also by an increase in the hydrodynamic diameter of the vesicle which is measured by the dynamic light scattering method before and after the polysaccharide coating. Table II shows the result of egg PC SUV coated by CHP-50-1.0 and parent pullulan-50. In the case of coating with parent pullulan-50, no significant increase in the diameter of the liposome was observed, while in the case of CHP-50-1.0 the size of the liposome increased by approximately 20 nm. Kobayashi et al. also proposed such a "loop–train–tail" model in the absorption state of *O*-octadecyldextran to the liposomal surface.[16] Ringsdorf and his co-workers coated the liposome with a hydrophobized water-soluble polymer and showed that such a polymer thermoreversibly anchors to the lipid bilayer.[17,23] Penetration of the hydrophobic anchor into the lipid bilayer membrane is already confirmed by several methods.[39-41]

Mobility of the FITC-Labeled Polysaccharide. In order to ascertain the restriction of the molecular motion of the polysaccharides upon absorption on the liposomal surface, steady-state fluorescence depolarization was measured using FITC-labeled polysaccharide derivatives. Figure 5 shows the *p* value of the FITC-labeled polymers as a function of the amount of the polymer adsorbed onto MLV. We have previously clarified that *O*-palmitoylpolysaccharide adsorbs more tightly to MLV than to SUV and the mobility of the polysaccharide bound to the MLV surface is more largely restricted than that bound to the SUV surface.[18] Clearly from Figure 5, the *p* value of FITC-labeled CHP adsorbed onto MLV was obviously larger than that of FITC-labeled CHAp. This means that the pullulan derivative more tightly adsorbs to the liposomal surface than the amylopectin derivative. For FITC-labeled polysaccharide without any hydrophobic anchor, the *p* value of the polymer was not changed at all irrespective of the presence or the absence of liposome.

Fluidity of the Liposomal Membrane As Affected by Polysaccharide-Coating. In order to obtain additional evidence of the coating of the liposome with polysaccharide derivatives, the membrane fluidity of the liposomes was studied from the *p* value change of two fluorescent probes, DPH and DSHA, embedded in the liposomal membrane. Results are shown in Table III. The fluidity of the region close to the membrane surface which was reported by the *p* value of DSHA did not change at all even if the liposomal surface was coated with polysaccharide derivatives and the incubation temperature was changed. However, the fluidity of the deep hydrophobic region of the membrane, which was reported by the *p* value of DPH, slightly, but obviously, decreased by coating with the cholesterol-bearing polysaccharide. The present results reveal again that the cholesterol moiety certainly is intercalated in the lipid bilayer.[39-41] The extent of the decrease in fluidity was larger when the membrane was coated by CHP compared with OPP. In addition, the effect was larger when the liposome was incubated at the lower temperature (37.0 °C) than when it was incubated at the higher temperature (50.0 °C). These results are not inconsistent with previous facts; for example, the addition of cholesterol to the lipid bilayer makes the membrane less fluid above the phase transition temperature.[42]

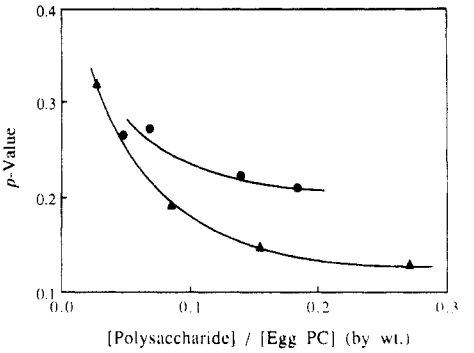

Figure 5. Fluorescence depolarization (*p* value) of FITC-labeled polysaccharide derivatives as a function of the amount of polysaccharide bound to the liposomal surface in 20 mM Tris-HCl containing 200 mM NaCl (pH 7.4) at 25.0 °C: (●) FITC-(0.42)-CHP-50-1.3-coated egg PC MLV; (▲) FITC(1.16)-CHAp-112-1.2-coated egg PC MLV.

Table III
Fluidity of the Liposomal Membrane Coated with a Hydrophobized Polysaccharide[a]

	p value			
	with DPH		with DSHA	
polysaccharide	37.0 °C	50.0 °C	37.0 °C	50.0 °C
none	0.09	0.07	0.05	0.04
OPP-50-1.8(1)	0.09	0.06	0.05	0.03
OPP-50-1.8(2)	0.10	0.07	0.05	0.04
CHP-50-1.3(1)	0.11	ND	0.05	0.04
CHP-50-1.3(2)	0.12	0.09	0.06	0.04

[a] [Egg PC] = 1.0×10^{-4} M and [DPH or DSHA] = 5.0×10^{-7} M. ND means not determined. (1) is [polysaccharide]/[egg PC] = 1.0 by weight. (2) is [polysaccharide]/[egg PC] = 2.0 by weight.

Barrier Function of the Liposomal Membrane. The effect of coating the liposomal surface with polysaccharide derivatives on the barrier function of the liposomal membrane was investigated by means of CF leakage. The spontaneous release of CF from SUV coated with OPA_p or CHA_p was followed at 50.0 °C. Substitution of the cholesterol moiety as the hydrophobic anchor more largely depressed the CF leakage from the liposome compared with that of the palmitoyl substituent (Figure 6). As the substitution degree of the cholesterol moiety increased from 0.7 to 2.1, the membrane permeability decreased

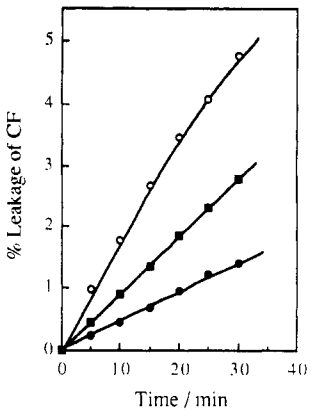

Figure 6. Effect of polysaccharide coating on the barrier function of egg PC SUV in 20 mM Tris-HCl containing 200 mM NaCl (pH 8.6) at 50.0 °C: (○) without a polysaccharide coat; (■) coated with OPAp-112-1.4; (●) coated with CHAp-112-1.0.

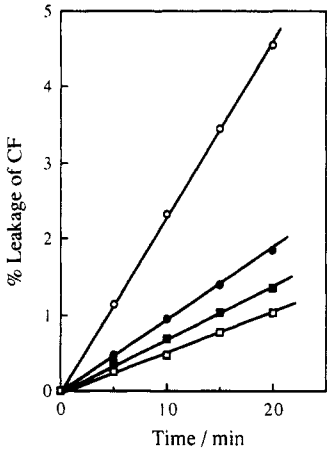

Figure 7. Effect of the substitution degree of the cholesterol moiety on the CF release from CHAp-112-coated egg PC SUV at 50.0 °C in 20 mM Tris-HCl containing 200 mM NaCl (pH 8.6): (○) without a polysaccharide coat; (●) coated with CHAp-112-0.7; (■) coated with CHAp-112-1.2; (□) CHAp-112-2.1. [Polysaccharide]/[egg PC] = 1.0 (by weight).

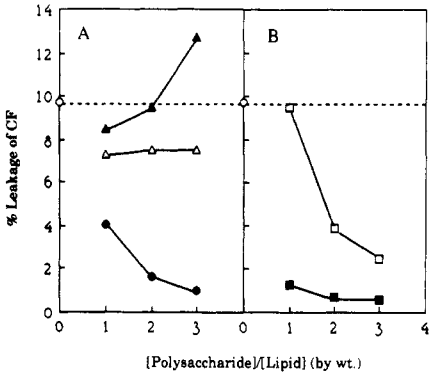

Figure 8. Effect of the coating efficiency on the barrier function of egg PC SUV in 20 mM Tris-HCl containing 200 mM NaCl (pH 8.6) at 50.0 °C. The liposome was coated by amylopectin (A) or pullulan (B): (○) without a polysaccharide coat; (▲) coated with OPAp-112-4.9; (△) OPAp-112-1.4; (●) coated with CHAp-112-1.0; (□) coated with OPP-50-1.8; (■) CHP-50-1.3.

(Figure 7). The percent leakage of CF from SUV coated with various polysaccharides was measured after incubation for 30 min at 50.0 °C as a function of the ratio of [polysaccharide]/[egg PC]. The suppression of membrane permeability was more remarkable when the liposomal surface was coated with a cholesterol-bearing polysaccharide than when it was coated with *O*-palmitoylpolysaccharide (Figure 8). In the case of OPAp-112-4.9, when the liposome was coated with the larger amount of the polymer,

Table IV
CF Leakage from Polysaccharide-Coated and Uncoated SUV with or without Free Cholesterol after Incubation for 30 min at 50.0 °C in 20 mM Tris-HCl (pH 8.6) Containing 200 mM NaCl[a]

conventional SUV		CHP-50-1.3-coated SUV	
amount of free cholesterol (mol %)	% CF leakage	amount of total cholesterol[b] (mol %)	% CF leakage
0	5.1	0 + 5.6	1.1
10	2.7	10 + 4.5	1.1
20	1.3	20 + 3.6	0.4

[a] [Egg PC] = 1.0×10^{-4} M and [CHP-50-1.3]/[egg PC] = 1.0 by weight. [b] Amount of cholesterol (mol %) additionally brought about by CHP-50-1.3.

the membrane permeability rather increased. This means that the penetration of the larger amount of single long alkyl chains into the lipid bilayer membrane causes an increase in the membrane permeability.[24-26] In order to know whether the decreased permeability of the liposome by coating with polysaccharide derivatives is due to the polymer effect of the polysaccharide skeleton or simply due to the effect of addition of cholesterol, the barrier function of the polysaccharide-coated liposome was investigated by comparing it with the case when free cholesterol was simply added. Liposomes, in which 10 and 20 mol % cholesterol were added, were first prepared, and then they were further coated by an equal amount of CHP-50-1.3 to the lipid. CF leakages from these liposomes were monitored. Results are given in Table IV. As expected, the addition of free cholesterol above the phase transition temperature of the liposome employed (at 50.0 °C) effectively decreased the membrane permeability (the left-hand column of Table IV). When the liposome was coated by the cholesterol-bearing polysaccharide (in this case, the cholesterol content of the liposome corresponds to 5.6 mol % of the total lipids), however, the effect of the decrease in the membrane permeability was larger than the case when 20 mol % of the free cholesterol was added (top row, right-hand column of Table IV). Of course, more addition of the free cholesterol brought about more of a decrease in the membrane permeability. As a result, in any event, the coating of the liposomal surface with a cholesterol-bearing polysaccharide results in a more effective increase in the barrier function of the liposomal membrane compared with the simple addition of the same amount of cholesterol. The difference in the barrier function between the liposomes coated by the two different polysaccharide derivatives, CHP and OPP, was consistent with that observed in the difference in the binding strength between the two polysaccharide derivatives.

One of problems in the liposomal drug delivery system (DDS) is the limited colloidal stability of the liposome in blood after in vivo administration. Liposomes are easily destroyed, for example, by interaction with serum proteins. The membrane permeability of the polysaccharide-coated liposomes was, therefore, investigated in the presence of human serum and plasma. Results are shown in Figure 9. Even in the presence of serum and plasma, CF leakage from SUV coated with cholesterol-modified polysaccharide was significantly depressed compared with a conventional liposome without a polysaccharide coat.

In conclusion, the cholesterol moiety is much better as a hydrophobic anchor of polysaccharides than the palmitoyl moiety in terms of the coating efficiency and the colloidal stability of the polysaccharide-coated liposome. Polysaccharide-coated liposomes were more stable even in the presence of blood serum and plasma than simple liposomes without any polysaccharide coat. These results

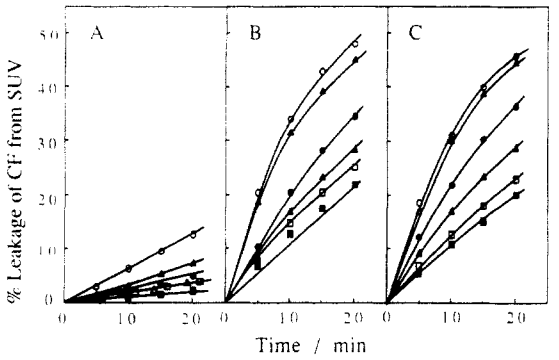

Figure 9. Effect of human plasma and serum on the barrier function of egg PC SUV as coated with CHAs-85-2.0 (△), CHAp-112-1.0 (●), CHM-200-2.4 (▲), CHP-50-1.3 (□), and CHD-176-1.4 (■) or without a polysaccharide coat (○) in 20 mM Tris-HCl containing 200 mM NaCl (pH 7.4) at 37.0 °C: (A) in the absence of blend fluid; (B) in the presence of 18.0% (v/v) human serum; (C) in the presence of 18.0% (v/v) human plasma.

suggest that the hydrophobized polysaccharide-coated liposome is promising as a drug carrier. In fact, several successful results by using such polysaccharide-coated liposomal DDS have been reported.[32,43-45]

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References and Notes

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