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Application of Synthetic Alkyl Glycoside Vesicles as Drug Carriers. I. Preparation and Physical Properties[1)]

HIROSHI KIWADA,* HITOSHI NIIMURA, YUICHI FUJISAKI, SUNJU YAMADA, and YURIKO KATO

Faculty of Pharmaceutical Sciences, Science University of Tokyo, 12, Ichigaya Funagawaramachi, Shinjuku-ku, Tokyo 162, Japan

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It was observed that alkyl glycosides formed lamellar vesicles like phosphatidylcholine vesicles (liposomes), and the application of these vesicles as drug carriers was attempted. Various alkyl glycosides were synthesized and vesicles were prepared with these glycosides. The encapsulation capacity of the vesicles was examined in relation to alkyl chain length, sugar moiety, and lipid composition. The glucosides of myristyl, cetyl, and stearyl alcohols formed vesicles, but those of lauryl, decyl, and octyl alcohols did not. The vesicles of glucoside, galactoside, and mannoside showed fairly good encapsulation capacity but those of lactoside showed low capacity. An appropriate ratio of glycoside, cholesterol, and dicetylphosphate is an important factor for the formation of these vesicles, especially with regard to dicetylphosphate.

The alkyl glycoside vesicles show longer lives on stage in an ampule at 20 °C than phosphatidylcholine vesicles. The stability in plasma was also examined. The glycoside vesicles showed rapid release of about 40% of the aqueous contents, but after that, they showed outstanding stability for 48 h in plasma at 37 °C. On the other hand, phosphatidylcholine vesicles showed rapid release of only about 30%, but they disintegrated gradually and showed low encapsulation capacity (about 20%) after 48 h. The present results suggest that the application of alkyl glycoside vesicles as drug carriers may be feasible.

Keywords——alkyl glycoside; alkyl glycoside vesicle; cetyl glucoside; phosphatidylcholine vesicle; liposome; drug carrier; stability

Introduction

Phospholipid vesicles (liposomes) have received much attention as potential drug carriers for encapsulating water-soluble drugs.[2)] However, the phospholipids are mainly obtained from biological sources, and their extraction and purification are complicated. These phospholipids contain unsaturated fatty acid moieties, and are susceptible to atmospheric oxidation. Liposomes composed of oxidized phospholipids lose their encapsulation capacity, and this may be one of the reasons why the use of natural phospholipids to prepare drug carriers has been unsuccessful. On the other hand, stable synthetic phospholipids based on saturated fatty acids are available commercially, but are very expensive.

It is known that some synthetic amphiphilic substances form bilayered vesicles like liposomes. Kunitake *et al.*[3)] reported that didodecyldimethylammonium bromide forms liposome-like bilayer structures. Recently, Riberio and Chaimovich studied these vesicles,[4)] but it remains uncertain whether this compound is safe *in vivo* after administration in large amounts administration as a drug carrier. Endo *et al.*[5)] reported the physical properties and barrier functions of vesicles formed by synthetic glyceroglycolipids. The structures of these compounds are very similar to those of the biological glycolipids, so it is expected that vesicles composed with these lipids will be nontoxic. However, the synthesis of these glycolipids is not easy.

We observed that some alkyl glycosides form liposome-like vesicles (AGV). The structures of the glycosides are very simple and it is easy to synthesize them. In this study, therefore, we attempted to design drug carriers whose base is not natural phospholipids but synthetic alkyl glycosides, with the aim of developing stable drug carriers. The encapsulation capacity and stability of the vesicles were also examined.

Experimental

Materials——Egg L-α-phosphatidylcholine (PC) was purchased from Sigma Chem. Co. (St. Louis, MO). Dicetylphosphate (DCP) was from Nakarai Chem. Ltd. (Kyoto). Cholesterol (CH) was from Kanto Chem. Co. (Tokyo), and was recrystallyzed from ethanol before use. Reagent-grade higher alcohols and carbohydrates were purchased from Tokyo Kasei Co. (Tokyo) or Nakarai Chem. Ltd. and used without further purification. ^{14}C-Sucrose was from New England Nuclear (Boston, MA). All other chemicals were of reagent grade or better.

Syntheses of Alkyl Glycosides——The following alkyl glycosides were synthesized by the method of Hori *et al.*[6] and Weigel *et al.*[7] and their identities were confirmed by elemental analysis, nuclear magnetic resonance (NMR) spectroscopy, and mass spectrometry: octyl glucoside (Glu-OC), decyl glucoside (Glu-DC), lauryl glucoside (Glu-LU), myristyl glucoside (Glu-MR), cetyl glucoside (Glu-CT), stearyl glucoside (Glu-ST), cetyl galactoside (Gal-CT), stearyl galactoside (Gal-ST), cetyl mannoside (Man-CT), and cetyl lactoside (Lac-CT).

Calorimetric Study——A differential scanning calorimeter (Rigaku Denki Co., CN-8085-DI, Tokyo) was used for examination of the thermotropic properties of alkyl glycosides with a heating rate of 10 °K/min. About 7 mg of powdered sample was used.

Preparation and Characterization of Vesicles——Reverse-phase evaporation vesicles (REV) were prepared by the method of Szoka and Papahadjopoulos[8] with some modifications as follows; lipids were dissolved in chloroform and about one-fourth volume of phosphate-buffered saline (PBS, pH 7.4, 280 mOs/kg) containing ^{14}C-sucrose as an aqueous space marker. The mixture was sonicated for 10 min in a bath type sonicator (Tocho ultrasonic cleaner, IUC-2811, Ueno Seisakusho, Tokyo) and evaporated under reduced pressure (about 300 mmHg) at 40 °C. The lipid formed a gel and then the gel started to hydrate. The evaporation was continued at about 700 mmHg until the hydration was completed. Multi-lamellar vesicles (MLV) were prepared as follows; dried lipid film in a round-bottomed flask was hydrated with PBS containing labeled marker by shaking with a mechanical shaker (Iwaki KM shaker V-S, Iwaki Co., Ltd., Tokyo). Small unilamellar vesicles (SUV) were prepared by the sonication method from MLV. The MLV were sonicated in a bath-type sonicator until the suspension became clear (about 1 h).

Prepared vesicles were dialyzed in cellulose dialyzing tubing (Visking Co.) against 2 l of PBS for 48 h at 4 °C to remove unencapsulated labeled marker. The PBS was changed 3 or 4 times. Before and after the dialysis, the radioactivity of the vesicle suspension was counted with a liquid scintillation counter (Aloka LSC-673, Tokyo) in emulsifier scintillation cocktail (Scintisol EX-H, Wako Pure Chem., Osaka). Encapsulation capacity was calculated from the radioactivities of the suspension before and after the dialysis.

The vesicles were characterized by optical microscopy and the mean diameter of the vesicles was measured with a sub-micron particle analyzer (Coulter model N4).

Long-term Stability Study——One ml aliquots of the vesicle suspension prepared as above were transferred into ampules, which were sealed and kept at 20 °C in the dark. Every 2 weeks or so, an ampule was opened and the sample was pipetted out. The suspension was dialyzed and the radioactivities were counted before and after the dialysis as above.

Stability Study in Plasma——An equilibrium dialysis cell with two compartments separated by a polycarbonate membrane having a pore size of 0.1 μm (Nucleopore Co., CA) was used. Fresh plasma (0.5 ml) from a Wistar male rat and 0.5 ml of vesicle suspension (14 μmol total lipid/ml) were placed in one compartment, and 1 ml of PBS was placed in the other compartment. The cell was shaken in an incubator at 37 °C. Fifty μl aliquots were taken from the PBS compartment at appropriate times during the incubation, and the radioactivity was counted. After each sampling, 50 μl of fresh PBS was added to the PBS compartment of the cell.

Results and Discussion

Thermotropic Properties of Alkyl Glycosides

Gel-to-liquid crystalline phase transition of the alkyl glycosides was observed by differential scanning calorimetry (DSC) as shown in Fig. 1. Phase transition temperature (Tc) of these glycosides were higher than those of dipalmitoyl phosphatidylcholine and synthetic glyceroglycolipids, reported by Endo *et al.*[5] The transition temperatures and melting points

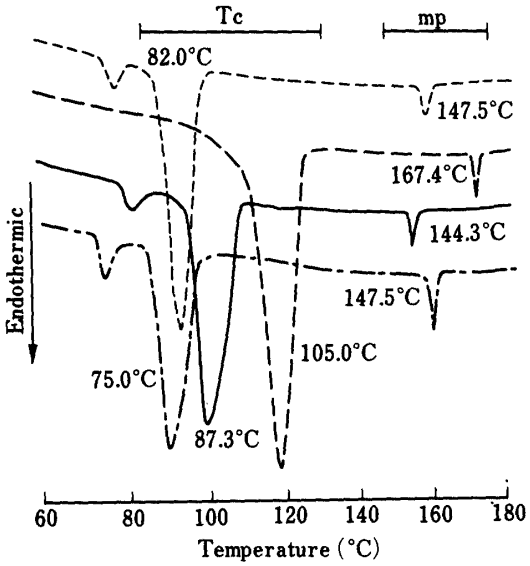

Fig. 1. Differential Scanning Calorimetry Heating Scans of Synthetic Alkyl Glycosides

—, stearyl glucoside; —·—, cetyl glucoside; ------, myristyl glucoside; – – –, stearyl galactoside. Tc, transition temperature; mp, melting point.

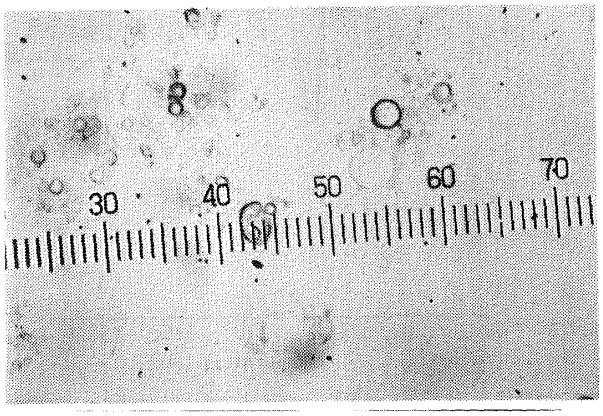

Fig. 2. Optical Micrographs of Alkyl Glycoside Vesicles (REV) Composed of Stearyl Glucoside, Dicetylphosphate, and Cholesterol in a Molar Ratio of 4:1:2 (×600)

TABLE I. Encapsulation Capacity for Aqueous ^{14}C-Sucrose Solution of Alkyl Glycoside Vesicles of Various Lipid Compositions

Lipid composition Glu-CT:DCP:CH (molar ratio)	Encapsulation capacity (%)
4:0:0	0.05±0.01
4:1:0	2.58±0.24
4:0:2	0.18±0.03
4:1:2	7.74±1.57
4:1:4	8.63±1.32

Type of vesicles: REV. Values represent mean±S.D. of three experiments.

(mp) of the alkyl glycosides did not show a clear dependence on the length of alkyl chain. They seemed to be influenced by the sugar moiety and an interaction between the sugar moiety and alkyl chain.

Formation of Vesicle Structures and Lipid Composition

An optical micrograph of alkyl glycoside vesicles (AGV) composed of Glu-CT, DCP, and CH (molar ratio 4:1:2), prepared by the REV procedure, is shown in Fig. 2. Spherical vesicle structures were observed. Their mean diameter was 1.03 μm.

Encapsulation capacities for aqueous ^{14}C-sucrose solution of the AGV of various lipid compositions are shown in Table I. It is apparent that an appropriate molar ratio of glycoside, CH, and DCP is important for the formation of the vesicle structures, especially as regards DCP.

The thermotropic properties of the components of the vesicles obtained by DSC are shown in Fig. 3. The mixture of the components (Glu-ST:DCP:CH=4:1:2) showed a broad endothermic peak. This broad transition or melting property shows that these

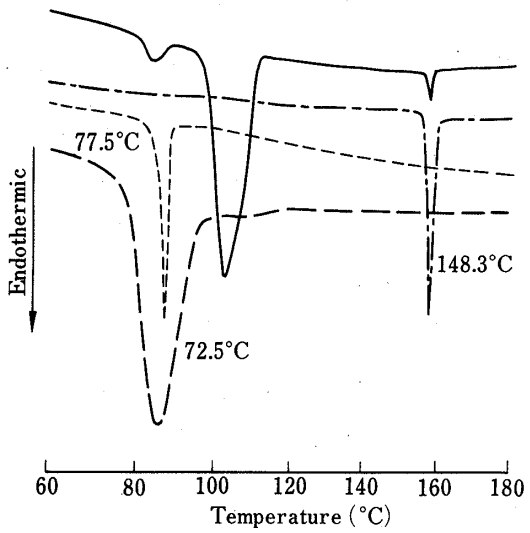

Fig. 3. Differential Scanning Calorimetry Heating Scans of Components of Alkyl Glycoside Vesicles

——, stearyl glucoside; —·—, cholesterol; ------, dicetylphosphate; – – – –, mixture of stearyl glucoside, dicetylphosphate, and cholesterol in a molar ratio of 4:1:2.

TABLE II. Encapsulation Capacity of Various Types of Alkyl Glycoside Vesicles and Effects of Lipid Concentration

Type of vesicles	Total lipid concentration (μmol/ml)	Encapsulation capacity (%)
REV	1.4	1.77±0.26
SUV	14.0	1.29±0.13
REV	14.0	7.74±1.57
MLV	14.0	5.52±1.50
REV	70.0	27.46±5.84

Lipid composition: Glu-CT:DCP:CH=4:1:2. Values represent mean±S.D. of three experiments.

components form a homogeneous melting phase at this molar ratio, and it is assumed that this plays an important role in the formation of the vesicle structure. If the components do not form a homogeneous phase, it seems likely that the vesicle formation will not occur completely. The thermotropic properties of the hydrated mixture were not clarified in the present study.

Incorporation of the glycoside into the vesicle structure was confirmed by gas-liquid chromatography (GLC) (OV-17/N_2, Shimadzu GC-4CM, Kyoto), after acetylation or trimethylsilylation following extraction with chloroform–methanol. Ninety-three to ninety-seven percent of the glycoside used in the preparation was detected in the vesicles.

Lipid Concentration and Type of Vesicles

The effect of lipid concentration in the preparation of the Glu-CT vesicles and that of type of vesicle on the encapsulation capacity were examined and the results are listed in Table II. Encapsulation capacity depends on the concentration of lipids, and high encapsulation efficiency (27.5%) was observed when the vesicles were prepared with a high lipid concentration (70.0 μmol total lipid/ml PBS). The encapsulation capacities of SUV and MLV (14.0 μmol/ml) were 1.3 and 5.5%, respectively. The low capacity of SUV seems to be due to the small particle size.

Effect of Alkyl Chain Length

Encapsulation capacities of alkyl glucosides with various lengths of alkyl chain in REV

TABLE III. Effects of Alkyl Chain Length on the Encapsulation Capacity of Alkyl Glycoside Vesicles

Base	Encapsulation capacity (%)
Glu-OC	2.28±0.57
Glu-DC	1.11±0.20
Glu-LU	1.27±0.73
Glu-MR	5.52±0.35
Glu-CT	7.74±1.57
Glu-ST	5.76±0.67
PC	5.54±1.56

Lipid composition: Base:DCP:CH=4:1:2. Type of vesicles: REV. Values represent mean±S.D. of three experiments.

TABLE IV. Effects of Carbohydrate Moiety on the Encapsulation Capacity of Alkyl Glycoside Vesicles

Base	Encapsulation capacity (%)
Glu-CT	7.74±1.57
Gal-CT	5.19±0.91
Man-CT	7.52±0.89
Lac-CT	3.14±0.75
PC	5.54±1.56

Lipid composition: Base:DCP:CH=4:1:2. Type of vesicles: REV. Values represent mean±S.D. of three experiments.

preparations were examined, and the results are listed in Table III. The vesicles of Glu-OC, -DC, and -LU showed low encapsulation capacities. Encapsulation capacity comparable to that of PC liposomes was observed in Glu-MR, -CT, and -ST vesicles. The differences of encapsulation capacity between shorter and longer alkyl chain are clear. This result shows that an alkyl chain length of not less than myristyl is needed for the formation of the liposome-like stable vesicles. Schwendener *et al.*[9] reported on liposome formation by the detergent dialysis method with *n*-alkyl glucosides as detergents. They used shorter alkyl glucosides, hexyl- heptyl- and octyl-glucosides, and showed that these glucosides were removed by dialysis. In the present study, shorter chain glucosides were not detected by GLC after dialysis and the vesicle structure was not observed by optical microscopy. It is suggested that alkyl glycosides shorter than myristyl- could not form the bilayered structure and might therefore be removed by dialysis.

Effect of Carbohydrate Moiety

The encapsulation capacities of the AGV (REV) with various sugars were examined and the results are shown in Table IV. Cetyl glycoside vesicles with glucose, galactose, and mannose showed encapsulation capacity comparable to that of PC liposomes. On the other hand, those with lactoside showed lower capacity as shown in Table IV. Hydration of galactoside vesicles is slow, possibly due to the high transition temperature of the galactoside as shown in Fig. 1. Lactoside vesicles were very hard to hydrate and formed aggregates, so it is possible that lactoside is not incorporated into the bilayer structure completely. These results

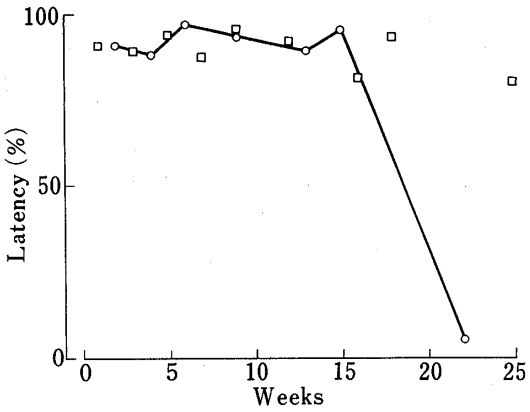

Fig. 4. Long-term Stability of Alkyl Glycoside Vesicles and Phosphatidylcholine Vesicles

□, stearyl glucoside vesicles; ○, phosphatidylcholine vesicles.
Lipid composition: Base: DCP: CH = 4: 1: 2 (REV).

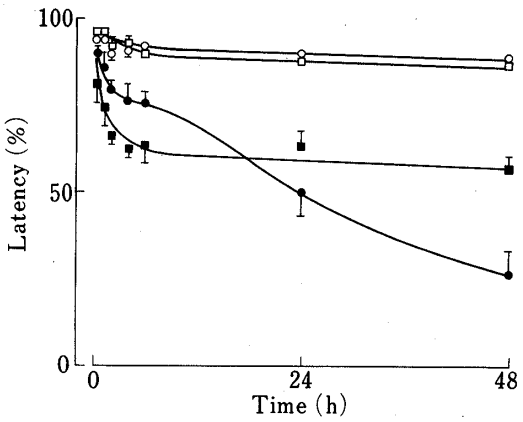

Fig. 5. Stability of Alkyl Glycoside Vesicles and Phosphatidylcholine Vesicles in Plasma at 37 °C

□, cetyl glucoside vesicles in PBS; ■, cetyl glucoside vesicles in plasma; ○, phosphatidylcholine vesicles in PBS; ●, phosphatidylcholine vesicles in plasma.
Lipid composition: Base: DCP: CH = 4: 1: 2 (REV).
Points are means ± S.D. of three experiments.

indicate that the balance of lipophilicity and hydrophilicity of the glycoside is a very important factor for the formation of the vesicles.

Long-term Stability

Long-term stability of the vesicles is shown in Fig. 4. The PC vesicles disintegrated within 22 weeks, but Glu-CT vesicles did not for at least 25 weeks. Alkyl glycoside, dicetylphosphate, and cholesterol are expected to be stable under ordinary conditions. Therefore, vesicles composed of these compounds are expected to be more stable than vesicles of naturally occurring PC.

Stability in Plasma

For the application of the AGV as drug carriers *in vivo*, they must be stable in plasma. In the case of PC vesicles, it has been reported[10] that phospholipid molecules are removed from the vesicles by plasma high density lipoproteins, and this leads to the formation of pores in the bilayer structures and the release of entrapped solutes. The stability of the AGV in plasma was examined and the results are presented in Fig. 5. The Glu-CT vesicles released about 40% of their encapsulated contents rapidly, and then they retained the residual contents for a long time. After incubation in plasma for 48 h, they retained more than 60% of the initial contents. On the other hand, PC vesicles showed less release of their contents at the early phase, but they showed gradual release in the subsequent phase, as shown in Fig. 5. It is suspected that the rapid release in the early phase is due to the degradation of the vesicles by blood components in a way that is related to their lamellar structure, and the following phase is due to the degradation of the vesicle components. It is considered that the AGV are less rigid in lamellar structure, because of their single hydrocarbon chain anchor, but they are more resistant to degradation in plasma compared with PC liposomes, because the alkyl glycosides are not natural biological components and thus may not interact with the plasma components.

In this study, we investigated the feasibility of the application of alkyl glycoside vesicles (AGV) as drug carriers *in vivo*. However, they are less stable than PC vesicles in plasma. If a greater rigidity of the lamellar structure can be introduced, alkyl glycoside vesicles might have

potential for use as drug carriers *in vivo*.

References and Notes

1) A part of this work was presented at the 103rd Annual Meeting of the Pharmaceutical Society of Japan, Tokyo, April 1983.
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