Functional Capsule Membranes. Part 29.[1] Concanavalin A-induced Permeability Control of Capsule Membranes Corked with Synthetic Glycolipid Bilayers or Grafted with Synthetic Glycopolymers

Yoshio Okahata,* Gen-ichi Nakamura, and Hiroshi Noguchi
Department of Polymer Chemistry, Tokyo Institute of Technology, Ookayama, Meguro-ku, Tokyo 152, Japan.

Large, ultrathin nylon capsule membranes were corked with synthetic glycolipid ($2C_{14}$-glu and $2C_{14}$-gal) or grafted with synthetic polymers having pendant saccharides (Poly-glu and Poly-gal). The permeability of NaCl from the capsule corked with glycolipid bilayers having the α-D-glucopyranosyl head group, but not the β-D-galactopyranosyl head group, was increased by interaction with concanavalin A (Con A) because of the distortion of corking bilayers induced by the specific binding of Con A to the α-D-glucopyranosyl head group on the capsule surface. When the capsule grafted with polymers having pendant α-D-glucopyranosyl units, but not β-D-galactopyranosyl units, was employed, the permeability of water-soluble dyes was reversibly reduced and increased by the alternative addition of Con A and an excess of monosaccharides from outside, respectively. Thus, Con A could specifically form the cross-linked complex with the α-D-glucopyranosyl unit of graft-polymers on the capsule surface and could reduce the permeability. Upon addition of an excess of monosaccharides, Con A was removed from the capsule surface and the permeability reverted to the original fast rate. These permeability changes with Con A largely depend on the molecular size of the permeants. Thus, the lipid bilayers or graft-polymers on the capsule membrane was found to act as a permeation valve responding to specific molecular recognition between lectins and carbohydrates on the membrane surface.

Lectins are carbohydrate-binding proteins of non-immune origin which do not exhibit enzymatic activity.[2] They interact with glycoproteins and glycolipids on the cell surface and can induce a variety of effects such as cell agglutination, cell adhesion to surfaces, mitogenesis, and hormone-like action. These biological effects are of considerable interest because of molecular recognition of hormones and toxins which bind to carbohydrate receptor sites on membranes.[3–5]

Concanavalin A (Con A), isolated from *Canavalia ensiformis*, is a lectin, and a tetrameric protein with four carbohydrate-binding sites which specifically binds α-D-glucopyranosyl or α-D-mannopyranosyl moieties. For model studies of the molecular recognition on the cell surface, Con A has been widely used: (i) liposomes are agglutinated by the addition of Con A if they contain the glycolipid with the α-D-glucopyranosyl moiety,[6–10] and (ii) Con A reacts specifically to form precipitates with the branched polysaccharide[11] or the synthetic polymer[12] containing α-D-glucopyranosyl units at nonreducing ends or in side chains, respectively. These agglutinations and precipitates are reversed by the addition of an excess of low molecular-weight saccharides. Although there is an analogy with the antibody–antigen interaction in biological systems, the specific interaction between lectins and carbohydrates is simply expressed by the formation of precipitates or the increase of turbidity in the aqueous solution.

Recently we developed the lipid bilayer-corked nylon capsule membrane in which multiple lamellae of lipid bilayers are corked in the physically strong, porous nylon capsule wall.[13] Permeation through the capsule membrane can be reversibly controlled by various physical parameters such as temperature,[14] ultrasonic radiation,[15] photoirradiation,[16] and electric field.[17] The signal-receptive permeation control is explained by changes in the molecular orientation of the corking bilayers that act as a permeation valve. When the porous capsule membrane surface-grafted with linear polymers was employed, permeability could be also controlled by various outside effects in which graft-polymers act as a permeation valve by changing their conformation.[18–20]

In this paper, we report that the permeability of the capsule membrane having carbohydrates on the surface can be reversibly controlled by specific molecular recognition with Con A, depending on the molecular size of permeants. The capsule membrane was corked with synthetic glycolipids, such as $2C_{14}$-glu or $2C_{14}$-gal having the α-D-glucopyranosyl or β-D-galactopyranosyl unit as a hydrophilic head group, respectively. The capsule membrane grafted with the synthetic glycopolymers Poly-glu or Poly-gal having pendant α-D-glucopyranosyl or β-D-galactopyranosyl groups, respectively, was also employed. A schematic illustration of the capsule and permeants is shown in Figure 1. This is the first example of specific molecular recognition between lectins and carbohydrates on the membrane surface being amplified by membrane transport as a model of biological antibody–antigen interactions.

Experimental

Materials.—Dialkyl glycolipid, $2C_{14}$-glu, containing α-D-glucopyranosyl-D-gluconamide as a hydrophilic head group was prepared as follows. 4-[α,α-Bis(tetradecyl)acetamido]butylamine was synthesized from α,α-bis(tetradecyl)acetyl chloride, m.p. 34—35 °C, and 1,4-diaminobutane in chloroform in the presence of triethylamine, m.p. 115 °C from ethyl acetate, yield 3.5 g (40%). Maltose [*O*-α-D-glucopyranosyl-(1 ⟶ 4)-D-glucopyranose] was oxidized to a mixture of maltonolactone and maltonoic acid in methanol in the presence of iodine.[21] Maltonolactone containing maltonic acid was allowed to react with 4-[α,α-bis(tetradecyl)acetamido]butylamine in refluxing methanol for 20 h. After cooling the mixture to room temperature, a pale yellow powder precipitated. The powder was filtered, washed with methanol, and recrystallized from ethyl acetate, m.p. 60—190 °C (liquid crystalline behaviour), yield 5.1 g (73%).

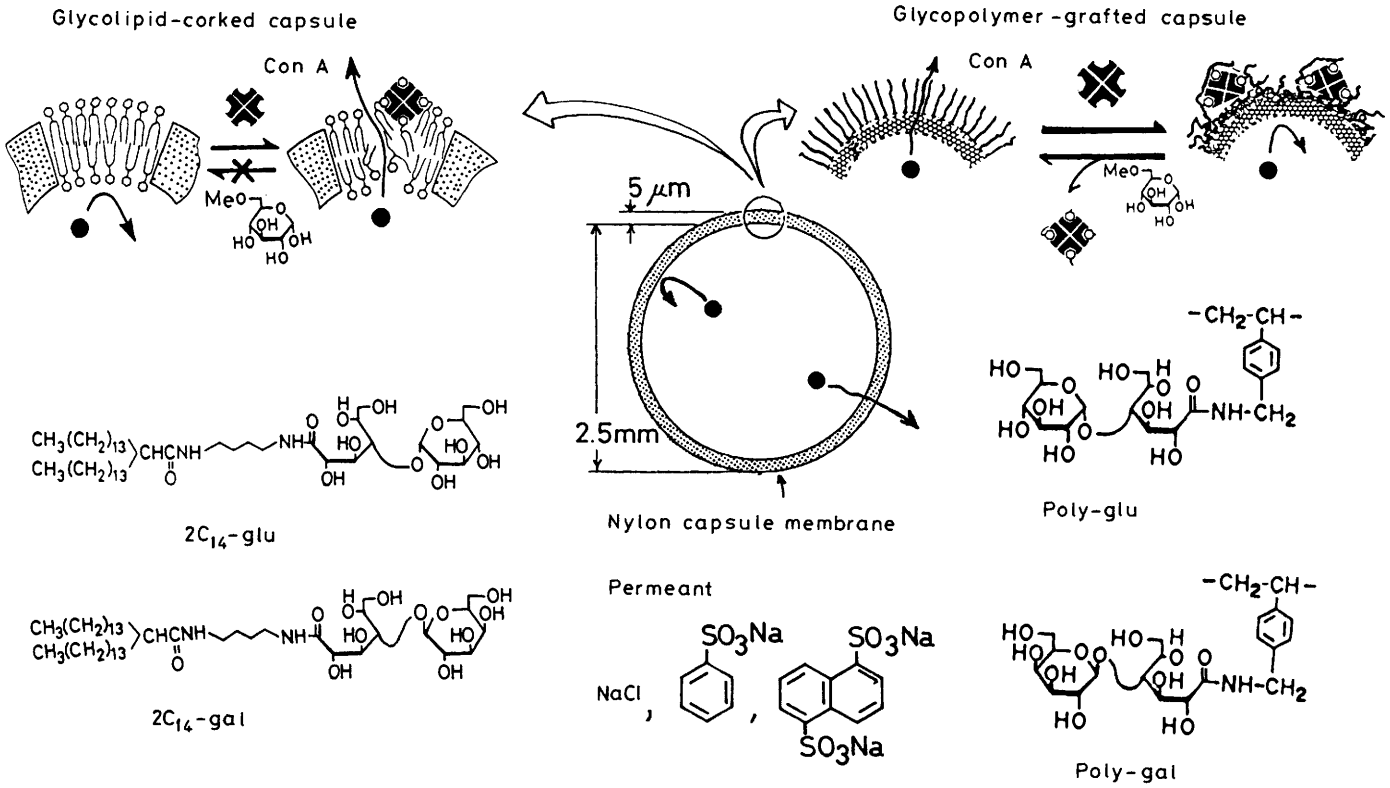

Figure 1. A schematic illustration of glycolipid bilayer-corked capsules and glycopolymer-grafted capsules

Dialkyl glycolipid, $2C_{14}$-gal, containing β-D-galactopyranosyl-D-gluconamide as head group was prepared from the dialkylamine and the oxidized lactose [*O*-β-D-galactopyranosyl-(1 ⟶ 4)-D-glucopyranose] in the same way, pale yellow powder, m.p. 80—190 °C (liquid crystalline behaviour) from ethyl acetate, yield 3.7 g (67%).

Vinyl monomers *N*-4-vinylbenzyl-α-D-glucopyranosyl-D-gluconamide (Mono-glu) and *N*-4-vinylbenzyl-β-D-galactopyranosyl-D-gluconamide (Mono-gal) were prepared from *p*-vinylbenzylamine and the oxidized maltose and lactose, respectively, according to the literature:[12,22] Mono-glu, m.p. 197—198 °C; Mono-gal, m.p. 183—184 °C.

The structure and purity of all synthetic materials were confirmed by n.m.r. spectra, t.l.c. with flame ionization detection (Yatron Laboratories, Tokyo), and elemental analysis (C, H, and N). Concanavalin A (Con A) was purchased from the Sigma Chemical Co. Other reagents were analytical grade used without further purification.

Lipid Bilayer-corked Capsules.—Large, ultrathin, nylon-2,12 capsule membranes (2.5 mm diameter, 5 μm thick) were prepared as previously.[13–17] The semipermeable capsules obtained were dialysed against an aqueous solution of permeants (0.2M-NaCl, 1mM-sodium benzenesulphonate, or 1mM-disodium naphthalene-1,5-disulphonate) to give capsules containing the permeant inside. The porous capsule membrane was corked with synthetic glycolipids, $2C_{14}$-glu and $2C_{14}$-gal, in dodecane by previous methods.[13–17] It was confirmed from *X*-ray diffraction analysis and a transmission electron micrograph that the sponge layer of the nylon capsule membrane was corked with multiple lamellae of lipid bilayers.[14,16] The lipid content of the capsule was estimated to be 20 ± 2 μg per capsule (dry weight of original capsule 25 ± 3 μg), and dodecane was confirmed not to be an impurity in the corking lipid by the weighing method and elemental analysis.

Polymer-grafted Capsules.—The porous nylon-2,12 capsule membranes were first grafted with a small amount of ethylene glycol dimethacrylate in order to introduce vinyl groups onto the membrane surface (2—5 μg per capsule). The capsules were graft-polymerized in a degassed aqueous solution of saccharide-containing styrene monomers (Mono-glu or Mono-gal) with $K_2S_2O_8$ as radical initiator at 70 °C for 4 h under nitrogen.[18–20] The capsules were washed with methanol and aqueous 0.2M-NaCl solution to remove non-grafted polymers and unchanged monomer. After complete hydrolysis of capsule membranes in strong acid conditions, the residual graft-polymer was analysed by gel-permeation chromatography and 70—80 μg of linear polymer (average degree of polymerization 300—800) was confirmed to be surface-grafted per capsule. Polymer-grafted capsules were dialysed against the aqueous solution of the respective permeant in order to obtain the capsule-containing permeants inside (0.2M-NaCl, 1mM-sodium benzenesulphonate, and 1mM-disodium naphthalene-1,5-disulphonate).

Permeation Measurements.—Permeation of NaCl from inside the capsule was followed by detecting increases in the electrical conductance in the outer water phase, after dropping a capsule into 50 ml of distilled water. The effect of Con A or sugars on the permeability was measured as follows. A capsule was picked from the permeation cell, immersed in 1 ml of 0.1M-phosphate buffer (pH 7) containing 0.3—3.0 mg of Con A or 0.03—3.0 g of sugars for 2 min at 30 °C, and then returned to the cell. The dipping buffer solution contains 0.2M-NaCl, 0.1mM-$MnCl_2$, and 0.1mM-$CaCl_2$, in which 0.2M-NaCl is added in order to prevent the release of NaCl during dipping.

Permeation of water-soluble dyes (sodium benzenesulphonate and disodium naphthalene-1,5-disulphonate) was followed by the increase in absorbance at 225 nm in 3 ml of the outer aqueous phase [0.1M-phosphate buffer (pH 7) containing 0.1

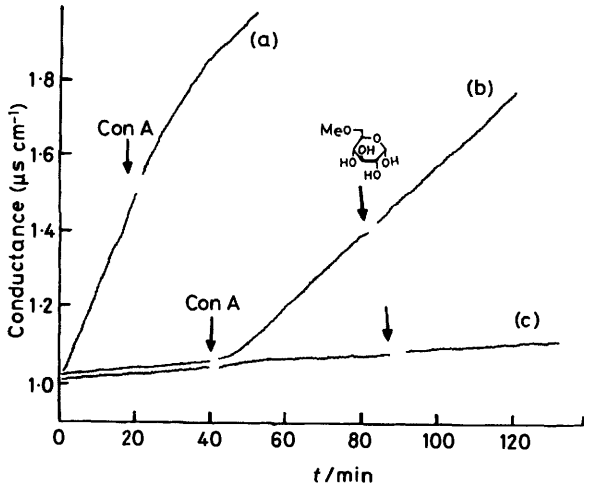

Figure 2. Permeation changes of NaCl from glycolipid bilayer-corked capsule membranes by the interaction with Con A at 30 °C. [NaCl] in capsule 0.2M. A capsule was picked from the cell, immersed in a buffer solution (pH 7) of Con A (1.0 mg ml^{-1}, 1.0 × 10^{-6}M) or methyl α-D-glucopyranoside (1.0 × 10^{-4}M), and returned to the cell at the arrows. (a) Uncorked capsule; (b) $2C_{14}$-glu-corked capsule; (c) $2C_{14}$-gal-corked capsule

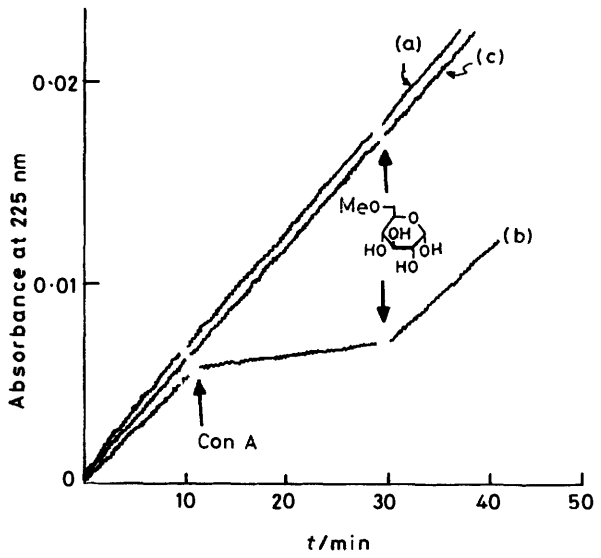

Figure 3. Permeation changes of naphthalenedisulphonate from glycopolymer-grafted capsules by the addition of Con A and monosaccharide at 30 °C (pH 7, phosphate buffer containing 0.1mM-$MnCl_2$ and -$CaCl_2$). Con A (1.0 mg ml^{-1}, 1.0 × 10^{-6}M) and methyl α-D-glucopyranoside (1.0 × 10^{-4}M) were added directly at the arrow. (a) Ungrafted capsule; (b) Poly-glu-grafted capsule; (c) Poly-gal-grafted capsule

mM-$MnCl_2$ and -$CaCl_2$]. Portions of the solution of Con A or methyl α-D-glucopyranoside was directly added to the outer buffer solution ([Con A] 0.3—3 mg ml^{-1}, (0.3—3) × 10^{-6}M; [glucopyranoside] 0.03—3 g ml^{-1}, (0.3—3) × 10^{-4}M).

Apparent permeation rates, P/cm s^{-1}, were calculated from equation (1)[13-20] where k, d, and C_0 are the initial slope of a

$$P = \frac{1}{6} \cdot \frac{kd}{C_0} \quad (1)$$

permeant release, the capsule diameter (2.5 mm), and the concentration of permeants stored in the inner aqueous core, respectively. Permeation measurements were carried out at least in triplicate for individual conditions and P values are the average of these points, containing ±5% deviation for experimental errors.

Results

(1) *Glycolipid Bilayer-corked Capsules.*—Figure 2 shows the typical time course of NaCl leakage from inside the glycolipid-corked capsule membrane, when the capsule was immersed in Con A solution and returned to the cell. In the case of the uncorked, semi-permeable capsule, permeation was very fast and not affected by dipping in an aqueous solution of Con A (0.3—3.0 mg ml^{-1}). In contrast, when the capsules corked with synthetic glycolipid bilayers were employed, the permeation of NaCl was markedly reduced because of the high barrier of the corking bilayer to NaCl permeation as well as the capsule corked with other lipid bilayers.[13-17] In the case of the capsule corked with $2C_{14}$-glu having the α-D-glucopyranosyl unit in the hydrophilic head group, the permeability was significantly increased with an induction period (3—5 min), after being dipped in the Con A solution (1.0 mg ml^{-1}, 1.0 × 10^{-6}M). The permeability was not reduced to the original rate even when the capsule was soaked in buffer solution containing a large excess of methyl α-D-glucopyranoside (1.0 × 10^{-4}M). The extent of rate enhancement by Con A was increased upon increasing the concentration of Con A in the dipping solution in the range of 0.3—3.0 mg ml^{-1} [(0.3—3.0) × 10^{-6}M].

On the other hand, when the capsule corked with $2C_{14}$-gal having the β-D-galactopyranosyl moiety was added alternatively with the aqueous solution of Con A and methyl α-D-glucopyranoside, NaCl leakage from the capsule was hardly increased. When capsule membrane corked with zwitterionic dipalmitoylphosphatidylcholine (DPPC), cationic dioctadecyldimethylammonium ($2C_{18}N^+2C_1$),[14] or anionic didodecylphosphate ($2C_{12}PO_4^-$)[23] was employed, the permeability of NaCl was not affected by Con A. Apparent permeation rates obtained from the slope of Figure 2 and equation (1) in the presence and absence of Con A are summarized in Table 1.

Molecular Size of Permeants.—The increase of permeability may be explained by the change of molecular packing of corking bilayers due to specific binding with Con A. In order to obtain information about the extent of distortion of corking bilayers by the interaction with Con A, relatively large permeants such as benzenesulphonate and naphthalene-1,5-disulphonate were employed as well as the small NaCl. Permeation rates and their ratio in the presence and absence of Con A are summarized in Table 1. The permeability was decreased upon increasing the molecular size of permeants independent of the stereostructure of the head group of the corking glycolipids. The rate enhancement by interaction with Con A (P_{con}/P_0) was much affected by permeants and decreased upon increasing the molecular size of permeants when the $2C_{14}$-glu-corked capsule was employed: P_{con}/P_0 values are 4.0 and 1.4 for the small NaCl and the large naphthalenedisulphonate as a permeant, respectively. Although an excess of methyl α-D-glucopyranoside was added in order to remove Con A from the surface of the capsule membrane, the permeability was not decreased to the original slow rate, independently of the molecular size of permeants.

When the capsule membrane corked with $2C_{14}$-gal having the β-D-galactopyranosyl unit as a head group was employed, the permeability was not affected by the addition of Con A and methyl α-D-glucopyranoside, independent of the molecular size of the permeants.

Table 1. Permeation change of lipid bilayer-corked capsule membranes affected by Con A and methyl α-D-glucopyranoside at 30 °C[a]

Capsule	Permeant	10^7 P/cm s^{-1}			$\frac{P_{con}}{P_0}$
			+ Con A	+ sugar	
Uncorked	NaCl	150	160	170	1.0
DPPC-corked[b]	NaCl	4.8	5.0	4.6	1.0
$2C_{18}N^+2C_1$-corked[c]	NaCl	2.8	3.0	3.1	1.0
$2C_{12}PO_4^-$-corked[d]	NaCl	3.6	3.9	3.5	1.0
$2C_{14}$-glu-corked	NaCl	3.7	15	16	4.0
	Benzenesulphonate	1.4	3.5	3.7	2.5
	Naphthalenedisulphonate	0.86	1.2	1.3	1.4
$2C_{14}$-gal-corked	NaCl	3.2	3.3	3.6	1.0
	Benzenesulphonate	1.8	2.0	2.2	1.1
	Naphthalenedisulphonate	0.76	0.77	0.75	1.0

[a] The capsule was picked from the cell, immersed in a solution of Con A (1.0 mg ml^{-1}, 1.0 × 10^{-6}M) or methyl α-D-glucopyranoside (1.0 × 10^{-4}M) for 2 min, and returned to the cell. [b] Dipalmitoylphosphatidylcholine bilayers. [c] Dioctadecyldimethylammonium bilayers. [d] Didodecylphosphate bilayers.

Table 2. Permeation change of polymer-grafted capsule membranes affected by Con A and methyl α-D-glucopyranoside at 30 °C[a]

Capsule	Permeant	10^6 P/cm s^{-1}			$\frac{P_{con}}{P_0}$
			+ Con A	+ sugar	
Ungrafted	Naphthalenedisulphonate	3.3	3.4	3.6	1.0
Poly-glu-grafted	Naphthalenedisulphonate	3.0	0.06	2.9	0.1
	Benzenesulphonate	14	3.5	13	0.3
	NaCl	83	66	80	0.8
Poly-gal-grafted	Naphthalenedisulphonate	3.1	3.7	3.8	1.0
	Benzenesulphonate	12	11	11	1.0
	NaCl	110	110	120	1.0

[a] Con A (1.0 × 10^{-6}M) and low molecular-weight sugar (methyl α-D-glucopyranoside, 1.0 × 10^{-4}M) was added directly to the permeation cell.

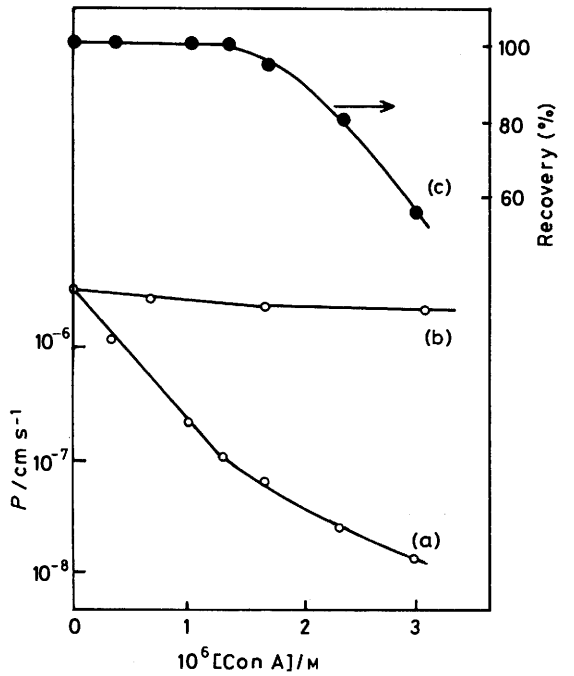

Figure 4. Effect of concentration of added Con A on the permeation of naphthalenedisulphonate at 30 °C. (a) Poly-glu-grafted capsule; (b) Poly-gal-grafted capsule; (c) extent of recovery of permeability when 3 × 10^{-4}M methyl α-D-glucopyranoside was added to the complex of Con A and Poly-glu-grafted capsule

(2) *Glycopolymer-grafted Capsules.*—Permeability could be reversibly controlled by the alternative addition of Con A and monosaccharides, when the capsule grafted with synthetic polymer having a disaccharides unit in the side chain was employed. Figure 3 shows the typical time courses of the permeation of sodium naphthalenedisulphonate from inside glycopolymer-grafted capsules. Con A and methyl α-D-glucopyranoside were directly added into the permeation cell as shown. In the absence of Con A, the permeation of dyes was fast and the permeability is of the same order among the ungrafted, Poly-glu-grafted, and Poly-gal-grafted capsules. In the case of the capsule grafted with Poly-glu having a pendant α-D-glucopyranosyl group, the permeability was immediately decreased by a factor of 10 by the addition of Con A. Upon addition of a large excess of methyl α-D-glucopyranoside, the permeability increased almost to the original fast rate immediately. On the other hand, when the ungrafted capsule or the Poly-gal-grafted capsule was employed, the permeation of dyes was not affected by the addition of Con A and low molecular-weight saccharides.

Effect of Concentration of Con A.—Figure 4 shows the effect of the concentration of Con A and methyl α-D-glucopyranoside on dye permeation. The permeability decreased proportionally with increasing concentration of added Con A in the range [Con A] 0.3—3 mg m^{-1}, (0.3—3) × 10^{-6}M, for the case of Poly-glu-grafted capsule. The permeability reverted to the original fast rate by the addition of a 10^2-fold concentration of methyl α-D-glucopyranoside when (0.3—1.3) × 10^{-6}M-Con A was added. The addition of a higher concentration than 1.5 × 10^{-6}M-Con A did not restore the permeability to the original rate, although a large excess of sugar was added [(3—10) × 10^{-4}M].

When the capsule grafted with Poly-gal with a pendant β-D-galactopyranosyl group was employed, the permeability was not affected by the addition of Con A in the range (0.3—3) × 10^{-6}M.

Effect of Molecular Size of Permeants.—In the polymer-grafted capsule membrane, the reduction of permeability by Con

A may be explained by the conformation change due to the specific binding of a pendant glucopyranosyl group of graft-polymers with Con A. In order to study the effect of the molecular size of permeants on the permeability effect of Con A, small permeants such as NaCl and sodium benzenesulphonate were employed rather than the large naphthalenedisulphonate. Permeation rates and their ratio (P_{con}/P_0) in the presence and absence of Con A are summarized in Table 2. The rate reduction induced by Con A was largely dependent on permeants and the extent was increased with increasing the molecular size of permeants when the Poly-glu-grafted capsule was employed: P_{con}/P_0 values were 0.8 and 0.1 for the small NaCl and the large naphthalenedisulphonate as permeant, respectively. When an excess of low molecular-weight sugar was added in order to remove Con A from the capsule surface, the permeability reverted nearly to the original fast rate independently of the molecular size of the permeants.

When the capsule grafted with Poly-gal having a β-D-galactopyranosyl unit in the side chain which could not bind Con A was employed, the permeability was not affected by the addition of Con A, independently of the permeant size.

Discussion

(1) *Lipid Bilayer-corked Capsules.*—Concanavalin A is known to bind specifically with α-D-glucopyranosyl, but not β-D-galactopyranosyl, in glycolipids and polysaccharides on the cell surface.[2] When Con A is added to an aqueous solution of liposomes containing α-D-glucopyranosyl lipids, vesicles are agglutinated with each other due to the specific binding between Con A and α-D-glucopyranosyl head groups of the bilayer membranes.[6–10] Con A-induced agglutination is inhibited in the presence of an excess of low molecular-weight sugars such as methyl α-D-glucopyranoside and it is reversed by the addition of excess of sugars, because the monosaccharide which has a high binding constant with Con A expels the glucopyranosyl residue of lipids from the binding sites resulting in the dissociation of vesicle agglutination.

When a capsule corked with $2C_{14}$-glu having the α-D-glucopyranosyl head group was employed, the permeability of water-soluble substances stored in the inner aqueous phase was increased by the addition of Con A from outside. On the other hand, the permeability was not affected by the addition of Con A, in the case of a capsule corked with $2C_{14}$-gal bilayers having the β-D-galactopyranosyl head group, zwitterionic DPPC bilayers, cationic $2C_{18}N^+2C_1$ bilayers, and anionic $2C_{12}PO_4^-$ bilayers. This means that the tetrameric Con A specifically recognizes and binds only with the α-D-glucopyranosyl head group of corking bilayers resulting in the distortion of lipid bilayers. Hence the permeability increases. Since Con A cannot bind the β-D-galactosyl unit of glycolipids, the permeability of the $2C_{14}$-gal-corked capsule does not increase upon addition of Con A.

The rate enhancement induced by Con A (P_{con}/P_0) depended largely on the molecular size of the permeants: the permeation of small molecules was much enhanced by the addition of Con A in the order NaCl > benzenesulphonate > naphthalenedisulphonate. Thus the distortion of corking bilayers induced by Con A is not so large and a large permeant cannot easily diffuse through small defects in corking bilayers.

When a large excess of monosaccharides was added in order to remove Con A from the capsule surface, the permeability did not revert to the original slow rate. It was confirmed from turbidity measurements of the bulk solution that most Con A was not detached from the capsule surface by the addition of excess of monosaccharides. When Con A was added to $2C_{14}$-glu-corked capsule, the permeability increased with an induction period of 3—5 min (see Figure 2, curve b). These results indicate that the permeation of a large protein molecule such as Con A into the lamella bilayers is a time-consuming step and Con A in the lamella structures cannot easily leave the capsule surface.

We have reported that the permeability of the capsule membrane corked with phosphatidylethanolamine bilayers was *reversibly* controlled by the alternative addition of Ca^{2+} ions and ethylenediaminetetra-acetic acid (edta) from outside.[23,24] Ca^{2+} ions can interact with the phosphoethanolamine moiety of multiple lamellae of corking bilayers resulting in the distortion of bilayer structures; the permeability is then increased. Upon removing Ca^{2+} ions from corking bilayers by the addition of edta, the permeability can return to the original slow rate due to the reformation of the bilayer structure. The permeability of phospholipid-corked capsule membranes can also be controlled by changing the ambient pH due to structural change in bilayers resulting from the dissociation of head groups.[25] In these cases, the permeability easily reverted to the original slow rate by removal of Ca^{2+} and H^+–OH^-, because small ions such as Ca^{2+} can be easily removed from the lamella structures of corking bilayers and the stable bilayer structure can be reformed. On the other hand, it seems to be difficult to remove large protein molecules such as Con A from the capsule surface without disturbing the corking bilayers and the permeability cannot be controlled reversibly by the addition of Con A and monosaccharide.

(2) *Polymer-grafted Capsule Membranes.*—The permeability of a capsule grafted with Poly-glu having a pendant α-D-glucopyranosyl group could be reversibly controlled by the alternative addition of Con A and monosaccharides: the permeation rate immediately decreased upon addition of Con A and returned to the original fast rate by the addition of an excess of α-D-glucopyranoside. On the other hand, the permeability of the capsule grafted with Poly-gal having a pendant β-D-galactopyranosyl group, which was not recognized by Con A, was not affected by the addition of Con A and monosaccharide. Thus, tetrameric Con A specifically binds to the glucopyranosyl side chains of graft-polymers resulting in the formation of cross-linkage between Con A and graft-polymers. The permeability is decreased by the polymer complexes precipitated on the capsule membrane. Upon addition of an excess of methyl α-D-glucopyranoside, Con A is removed from the capsule because monosaccharide expels the glucosyl side chains of graft-polymers from the binding sites of Con A and the permeability reverts to the original fast rate. Since the graft-polymer can move freely on the capsule membrane, its conformation is easily changed by the interaction and dissociation of large protein molecules. Hence the permeability can be changed reversibly. Thus, the graft-polymer on the capsule membrane acts as a permeation valve responding to specific molecular recognition between carbohydrates and lectins.

When a larger permeant was used, a larger rate decrease was observed by the addition of Con A (see Table 2). Since the cross-linked complex between Con A and graft polymers cannot cover pores in the capsule membrane tightly, the permeation of small molecules such as NaCl is little affected by the addition of Con A.

Conclusions.—By the use of the specific molecular recognition between concanavalin A and glucosyl residues, the permeability of a capsule corked with glycolipid bilayers or one grafted with polymers having pendant glucosyl groups could be changed. In the case of lipid bilayer-corked capsule membrane, the permeability was enhanced by the interaction with Con A due to the distortion of the bilayer structures of the corking lipids. On the other hand, in the case of the glycopolymer-grafted capsule, the permeability was decreased by the addition of Con

A due to the formation of cross-linked precipitates between Con A and graftpolymers on the capsule surface. Thus, lipid bilayers or graft-polymers act as a permeation valve on the capsule membrane responding to the specific molecular recognition between Con A and glucosyl residues on the capsule surface. In the case of glycolipid vesicles and polysaccharides dispersed in aqueous solution, the turbidity of the aqueous solution is increased by the addition of Con A. When the glycolipids or glycopolymers are immobilized on the physically strong capsule membrane, the specific molecular recognition can be converted and amplified in terms of the membrane permeability. These capsules are thus a new and potentially powerful tool in studies of carbohydrate-specific phenomena that occur at cell surfaces.

References

1 Part 28, Y. Okahata and K. Ariga, *J. Chem. Soc., Perkin Trans. 2*, 1987, 1003.
2 I. J. Goldstein and C. E. Hayes, *Adv. Carbohydr. Chem. Metab.*, 1978, **35**, 127.
3 P. H. Fish and R. O. Brady, *Science*, 1976, **194**, 906.
4 R. K. Drapner, D. Chin, and M. I. Simon, *Proc. Natl. Acad. Sci. USA.*, 1978, **75**, 261.
5 R. L. Richards, J. Moss, C. R. Alving, P. H. Fishman, and R. O. Brady, *Proc. Natl. Acad. Sci. USA.*, 1979, **76**, 1673.
6 H. Bader, H. Ringsdorf, and J. Skura, *Angew. Chem., Int. Ed. Engl*, 1981, **20**, 305.
7 T. Williams, N. R. Plessas, and I. J. Goldstein, *Arch. Biochem. Biophys.*, 1979, **19**, 145.
8 J. Slama and R. R. Rando, *Carbohydr. Res.*, 1981, **88**, 213.
9 G. A. Orr, R. R. Rando, and F. W. Bangerster, *J. Biol. Chem.*, 1979, **254**, 4721.
10 R. Y. Hampton, R. W. Holz, and I. J. Goldstein, *J. Biol. Chem.*, 1980, **255**, 6766.
11 L. L. So and I. J. Goldstein, *J. Biol. Chem.*, 1967, **242**, 1617.
12 K. Kobayashi, H. Sumitomo, and Y. Ina, *Polym. J.*, 1985, **17**, 567.
13 For a review see Y. Okahata, *Acc. Chem. Rev.*, 1986, **19**, 57.
14 Y. Okahata, H.-J. Lim, G. Nakamura, and S. Hachiya, *J. Am. Chem. Soc.*, 1983, **105**, 4855; *J. Membr. Sci.*, 1984, **19**, 237; Y. Okahata, N. Iizuka, G. Nakamura, and T. Seki, *J. Chem. Soc., Perkin Trans. 2*, 1985, 1591.
15 Y. Okahata and H. Noguchi, *Chem. Lett.*, 1983, 1517.
16 Y. Okahata, H.-J. Lim, and S. Hachiya, *J. Chem. Soc., Perkin Trans. 2*, 1984; 989; *Makromol. Chem. Rapid Commun.*, 1983, **4**, 303.
17 Y. Okahata, S. Hachiya, K. Ariga, and T. Seki, *J. Am. Chem. Soc.*, 1986, **108**, 2863; Y. Okahata, S. Hachiya, and T. Seki, *J. Chem. Soc., Chem. Commun.*, 1984, 1377.
18 Y. Okahata, K. Ozaki, and T. Seki, *J. Chem. Soc., Chem. Commun.*, 1984, 519; Y. Okahata, H. Noguchi, and T. Seki, *Macromolecules*, 1987, **20**, 15.
19 Y. Okahata, H. Noguchi, and T. Seki, *Macromolecules*, 1986, **19**, 493.
20 Y. Okahata, K. Ariga, and T. Seki, *J. Chem. Soc., Chem. Commun.*, 1986, 73.
21 S. Moor and K. P. Link, *J. Biol. Chem.*, 1940, **133**, 293.
22 K. Kobayashi, H. Sumitomo, and Y. Ina, *Polym. J.*, 1983, **15**, 667.
23 Y. Okahata, H.-J. Lim, and G. Nakamura, *Chem. Lett.*, 1983, 755.
24 Y. Okahata and H.-J. Lim, *J. Am. Chem. Soc.*, 1984, **106**, 4996; T. Seki and Y. Okahata, *J. Microencap.*, 1985, **2**, 13.
25 Y. Okahata and T. Seki, *J. Am. Chem. Soc.*, 1984, **106**, 8065; *Chem. Lett.*, 1984, 1251.

Received 29th September 1986; *Paper* 6/1919