# Change in Membrane Fluidity Induced by Lectin-Mediated Phase Separation of the Membrane and Agglutination of Phospholipid Vesicles Containing Glycopeptides

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ABSTRACT: Changes in membrane fluidity induced by lectin addition to 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) vesicles containing synthetic glycopeptides were measured by depolarization of the fluorescent probes 8-anilino-1-naphthalenesulfonate (ANS) and 1,6-diphenyl-1,3,5-hexatriene (DPH). In the present synthesized glycopeptides, *N*-acetylglucosamine (GlcNAc) and a tripeptide were connected by aliphatic chains of different lengths. A pyrenyl group, which is introduced to the peptide moiety, acted as a probe to characterize the distribution of glycopeptides in the membrane on the basis of its excimer formation. The glycopeptide was shown to be distributed to DPPC vesicles with the peptide moiety buried in the hydrophobic core of the lipid bilayer and the glyco moiety exposed to the outside of the membrane. By the addition of wheat germ agglutinin (WGA) to the vesicles containing the glycopeptides, intravesicular cross-linking of glycopeptides in the membrane and aggregation of vesicles were observed. The intravesicular cross-linking was antagonized by GlcNAc above the phase transition temperature. However, the dissociation of aggregation required the addition of a stronger antagonist, *N,N'*-diacetylchitobiose. The addition of the glycopeptide to DPPC vesicles above the phase transition temperature decreased the membrane fluidity. However, a succeeding addition of WGA caused a large increase of membrane fluidity at either the surface or the hydrophobic core of the lipid bilayer membrane. This increase of membrane fluidity was attributed to two factors by use of two kinds of antagonists having different potencies: one is a WGA-mediated cross-linking of glycopeptides in the membrane, and the other is a close contact of vesicles on aggregation.

The importance of membrane fluidity in cell physiology is well recognized and represented in the fluid mosaic model (Singer et al., 1972) which is widely accepted for the structure of biomembranes. Attempts to correlate membrane fluidity with cell functions have succeeded in explaining the behaviors of carrier-mediated transport through the membrane and the activities of membrane enzymes such as ATPase and adenylate cyclase. However, some points remain controvertial [for review, see Stubbs (1984)].

Factors governing membrane fluidity are classified into two categories. One is different molecular structures of lipids, that is, the length and unsaturation of acyl chains of lipid molecules or the degree of N-methylation of phospholipid (Hirata et al., 1980; Mato et al., 1983). The other is factors physically affecting membrane properties, that is, temperature, dehydration, or chelation with $Ca^{2+}$ of lipid head groups (Hauser et al., 1977; Portis et al., 1979) and cholesterol [Dufourc et al. (1984) and references cited therein].

Ligand binding to a specific receptor macromolecule at the cell surface has been extensively studied over the years. For example, ligand binding has been reported to regulate the cAMP level (Gilman, 1984) or to trigger phosphatidylinositol breakdown. The products from the latter reaction were found to release $Ca^{2+}$ from storage and to activate protein kinase C (Majerus et al., 1985). Accompanying these signal transductions through the membrane, the change in membrane fluidity is observed. For instance, insulin binding to cells has been reported to decrease membrane fluidity (Luly et al., 1979), and the increase of membrane fluidity has been observed to precede the rise in cytosolic calcium concentration in the interaction of interleukin II with T lymphocytes (Utsunomiya et al., 1986).

When the ligand is multivalent, the ligand binding causes redistribution of membrane proteins and induces cell contact, which are supposed to facilitate the cell fusion. For instance, the fusion of phosphatidic acid/phosphatidylethanolamine mixed vesicles in the presence of lectin and glycolipid was brought about by $Ca^{2+}$ at a lower concentration by 1 order of magnitude than that without lectin (Sundler et al., 1983).

These findings suggest that ligand binding at the cell surface, redistribution of membrane proteins, or intercellular contact of membrane should influence the physical properties of the membrane and might be related to the regulation of cell functions.

In the present investigation, we adopted a model system of a cell, which is composed of synthetic glycopeptides and DPPC[1] vesicles, and investigated the relation between the membrane fluidity and the biologically related behaviors of the membrane. By the addition of lectin to this system, intravesicular cross-linking of glycopeptides in the membrane and aggregation of vesicles were realized. The cross-linking and the aggregation were accompanied by a change in membrane fluidity. Their contributions were estimated individually by use of differently devised glycopeptides and lectin inhibitors having different potencies.

## Materials and Methods

The structures of glycopeptides used in the present investigation and their synthetic routes are shown in Figures 1 and

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[1] Abbreviations: DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; ANS, 8-anilino-1-naphthalenesulfonate; DPH, 1,6-diphenyl-1,3,5-hexatriene; GlcNAc, *N*-acetylglucosamine; WGA, wheat germ agglutinin; doxyl, 4,4-dimethyloxazolidine-*N*-oxyl; NMR, nuclear magnetic resonance; IR, infrared; TLC, thin-layer chromatography; CF, 6/5-carboxyfluorescein.

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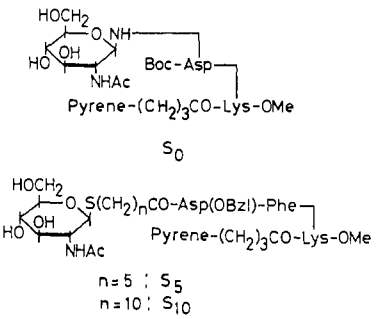


FIGURE 1: Molecular structures of glycopeptides synthesized here.

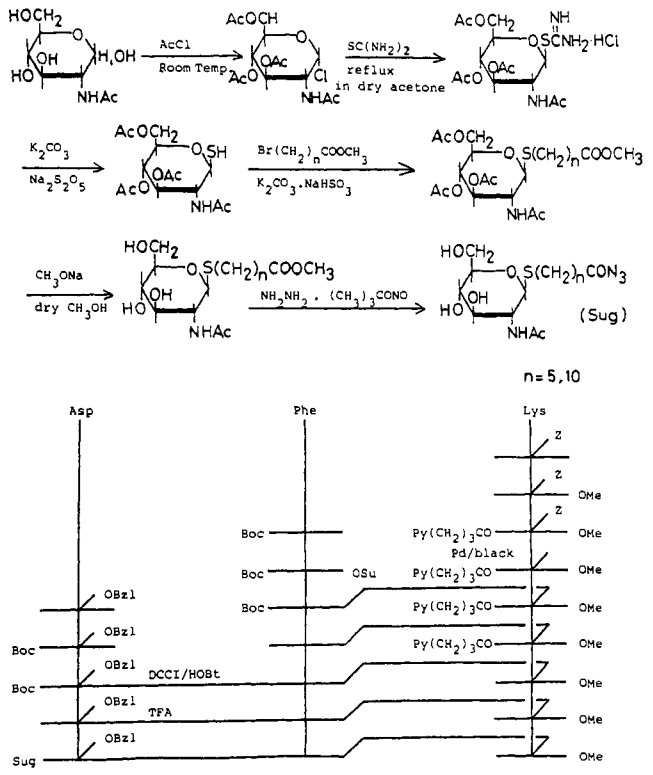


FIGURE 2: Synthetic routes of $S_5$ and $S_{10}$.

2, respectively. The synthetic details are summarized briefly as follows, $S_5$ being taken as an example. The sugar moiety of $S_5$ (Sug) was synthesized according to the methods reported by Horton et al. (1962), Kawaguchi et al. (1981), and Connolly et al. (1980). The tripeptide moiety of $S_5$ was synthesized by a conventional liquid-phase method. The obtained fully protected tripeptide was treated with trifluoroacetic acid to remove the *tert*-butyloxycarbonyl group at the N-terminal. A dimethylformamide solution (1 mL) of tripeptide (0.07 mmol) and triethylamine (0.07 mmol) was added to a dimethylformamide solution (1 mL) of Sug (0.07 mmol) at 0 °C and stirred for 3 days. The solution was condensed under reduced pressure, and methanol was added to the residue. The insoluble part was dissolved in dimethylformamide and purified two times by Sephadex LH-20 with dimethylformamide as an eluant. The coupling yield was 27%. $^1$H NMR and $^{13}$C NMR spectra of $S_5$ are shown in Figure 3. Elemental anal. Calcd for $C_{61}H_{64}O_{13}N_5S\cdot 2H_2O$: C, 63.58; H, 6.39; N, 6.08; S, 2.78. Found: C, 63.75; H, 6.41; N, 6.33; S, 2.86. The purity of all glycopeptides was verified by elemental analyses, IR, $^1$H NMR, $^{13}$C NMR, and TLC.

DPPC (Fluka, Switzerland), WGA, 5-doxylstearic acid, 16-doxylstearic acid, *N*-acetylglucosamine, and *N,N'*-diacetylchitobiose (Sigma) were commercially available.

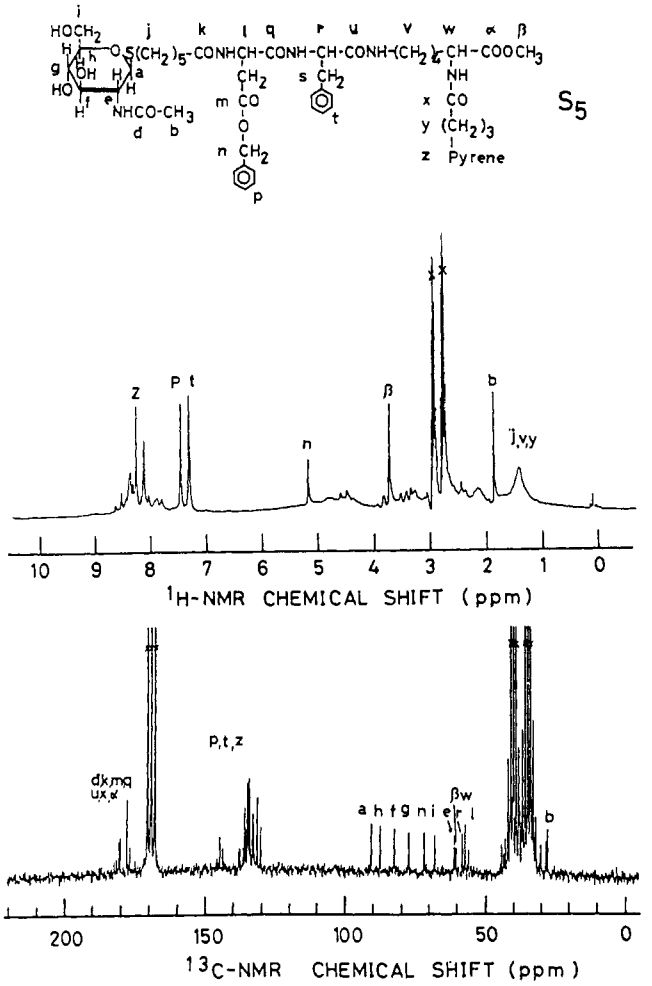


FIGURE 3: $^1$H and $^{13}$C NMR spectra of $S_5$ in dimethylformamide-$d_7$.

Small unilamellar vesicles were prepared by sonication of the lipid dispersion in a buffer solution (10 mM HEPES and 0.1 M NaCl, pH 7.2) and ultracentrifugation at 100000*g*. The stock solutions of glycopeptides were prepared in dimethylformamide solution, and an aliquot of the stock solution was added to the dispersion of small unilamellar vesicles under a limit of organic solvent content to 1% of the total volume.

NMR, fluorescence, and UV measurements were carried out on a JEOL-FX90Q (JEOL, Japan), a MPF-4 fluorescence spectrophotometer (Hitachi, Japan), and a UVIDEC-1 (JASCO, Japan), respectively.

Leakage of CF from vesicles was measured according to the method reported by Barbet et al. (1984). Aggregation of vesicles by the addition of lectin was monitored by the absorption at 360 nm. Other chromophores have no absorption in this region.

Depolarization of fluorescence was measured by equipment installed on a MPF-4 fluorophotometer. One polarizer at the monitor side was fixed in a parallel way. The other polarizer for the incident light was rotated by a stepping motor which is controlled by a microcomputer. A depolarizer was placed in front of the latter polarizer to get fully depolarized light. Four data points of $I_\parallel$ (intensity of parallel polarized component) and $I_\perp$ (intensity of vertically polarized component) were obtained and averaged in 0.5 min by a microcomputer. The sample dispersion was moderately stirred, and the temperature was regulated by a thermostated water jacket.

Excitation wavelengths of ANS and DPH are 395 and 380 nm, respectively. Neither of them overlaps with the absorption of pyrene. It is also confirmed that the emission of ANS or

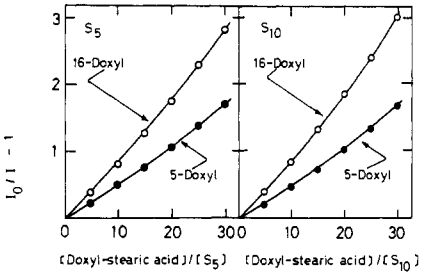


FIGURE 4: Stern–Volmer plot. Quench of monomer emission from $S_5$ and $S_{10}$ with the addition of 5-doxyl- (●) and 16-doxylstearic acid (○) in the presence of DPPC vesicles.

DPH is not interferred with by the pyrenyl group of $S_5$ under these conditions.

## RESULTS

*Distribution of Glycopeptides into the Vesicle.* The peptide moiety of the present glycopeptides comprises hydrophobic amino acid residues, Asp(OBzl), Phe, and pyrenyl-$(CH_2)_3CO$-Lys(OMe). These residues are expected to solubilize the peptide moiety in a hydrophobic core of the lipid bilayer membrane and act as an anchor to the membrane. The pyrenyl group, which is linked to the $\alpha$-amino group of Lys, functions as an indicator of the distribution of glycopeptides in the membrane since the rate of its excimer formation is very sensitive to the concentration. GlcNAc acts as antagonist to lectin because it specifically and reversibly interacts with WGA. The length of the aliphatic spacer between these two moieties was varied so the influence of the spacer length on the interaction between lectin and glycopeptide could be known (Rando et al., 1980; Hoekstra, 1986). The hydrophobic spacer was reported to be inappropriate for lectin binding, because it would take a "ball up" conformation or be buried in the lipid bilayer, which should hinder the interaction with lectin (Slama & Rando, 1980). However, a moderate length of alkyl spacer was found here to enable the glycopeptide in the membrane to interact with lectin.

$S_0$ and $S_5$ are soluble in buffer under the conditions of the fluorescence measurement to show reasonable fluorescence spectra, whereas emission of $S_{10}$ in buffer was not stable because $S_{10}$ should form the aggregate in buffer due to its long alkyl spacer.

Quenching of pyrenyl emission was used extensively to determine the mode of glycopeptide distribution in the membrane. Addition of acrylamide, which is a water-soluble quencher, did not decrease the intensity of $S_5$ emission in the presence of vesicles, though it did so in the absence of vesicles. This means that the pyrenyl group of $S_5$ in the presence of vesicles is buried in a hydrophobic core of lipid bilayer and not exposed to the water phase for quenching.

The radical group, which acts as quencher, of 5-doxyl- or 16-doxylstearic acid is buried to a certain depth of lipid bilayer according to its position of substitution in the stearic acid chain. Figure 4 shows that the emission of $S_5$ and $S_{10}$ in the presence of DPPC vesicles is quenched more efficiently by 16-doxylstearic acid than by 5-doxylstearic acid, which means that the pyrenyl group is buried deeply in the membrane.

On the other hand, the emission of the $S_0$ pyrenyl group was quenched to some extent by acrylamide in the presence of vesicles (data not shown). This indicates that the distribution of $S_0$ to the lipid membrane was lower than that of $S_5$ or $S_{10}$ due to the lack of the alkyl spacer and the hydrophobic amino acid residue Phe in the peptide sequence.

*Lectin-Mediated Aggregation of Glycopeptide-Containing Vesicles.* WGA is a well-known agglutinin which specifically binds to the *N*-acetylglucosamine unit (Matsumoto et al., 1969; Goldstein et al., 1975). The addition of WGA to the $S_5$-containing vesicles actually induced agglutination of vesicles as shown by the increased turbidity of the suspension (Figure 5). In the absence of $S_5$ the change in turbidity was not observed. The aggregation of vesicles through the intervesicular bridge of WGA was dissociated by the addition of *N*,*N*′-diacetylchitobiose reversibly. Since the addition of *N*,*N*′-diacetylchitobiose brought the absorbance to the same level as that in the absence of WGA, fusion of vesicles did not occur under these conditions. It is notable that GlcNAc is too weak to antagonize the aggregation of vesicles.

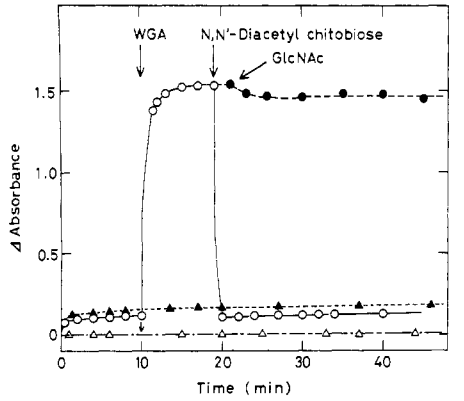


FIGURE 5: Agglutination of $S_5$-containing vesicles ([$S_5$] = $1.89 \times 10^{-2}$ mM, [DPPC] = 1.0 mM) by WGA addition ([WGA] = $1.64 \times 10^{-3}$ mM). (○) Liposome + $S_5$ + WGA + *N*,*N*′-diacetylchitobiose (2.99 mM); (●) liposome + $S_5$ + WGA + GlcNAc (6.34 mM); (△) liposome + WGA + *N*,*N*′-diacetylchitobiose (2.99 mM); (▲) liposome + $S_5$. Additions are shown by arrows.

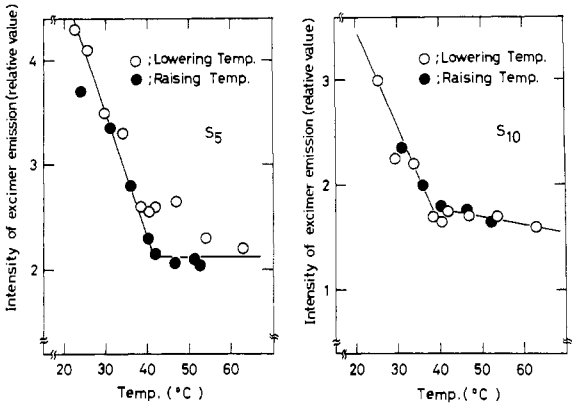


FIGURE 6: Temperature dependence of emission intensity of the pyrenyl excimer from $S_5$ ($1.89 \times 10^{-2}$ mM) and $S_{10}$ ($1.67 \times 10^{-2}$ mM) in the presence of DPPC vesicles (1.0 mM).

WGA addition to $S_0$-containing vesicles also induced aggregation of vesicles. However, the extent of aggregation was less marked than that of $S_5$-containing vesicles and was dissociated reversively by the addition of GlcNAc.

*Temperature Dependence of Glycopeptide Distribution in the Membrane.* Figure 6 shows the temperature dependence of emission intensity from pyrenyl excimer ($I_D$) in the presence of vesicles. $I_D$ remains almost constant above the phase transition temperature. However, as the temperature goes down below the phase transition temperature, $I_D$ increased. This result contrasts with the case of $S_5$ in buffer, where $I_D$ was almost unchanged over the temperature range. The same phenomenon was observed with $S_0$. The increase of $I_D$ below the phase transition temperature is interpreted in terms of segregation of membrane into liquid-crystalline lipid regions and other regions where $S_5$ is condensed, as has been observed with oligopeptides in DPPC vesicles (Uemura et al., 1983).

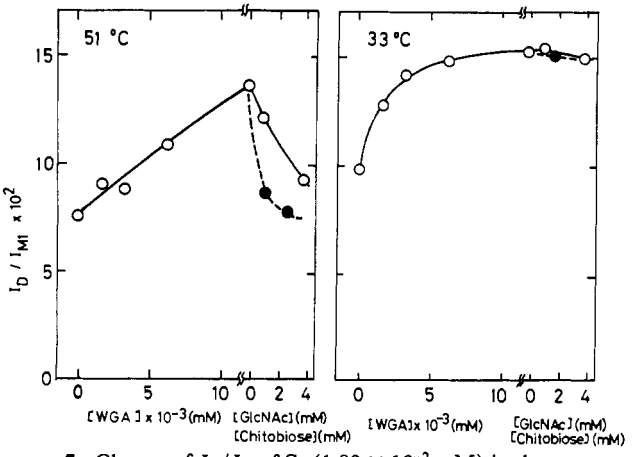


FIGURE 7: Change of $I_D/I_M$ of $S_5$ ($1.89 \times 10^{-2}$ mM) in the presence of DPPC vesicles (1.0 mM) with the addition of WGA. The effects of the addition of GlcNAc (○) or *N,N'*-diacetylchitobiose (●) are shown together.

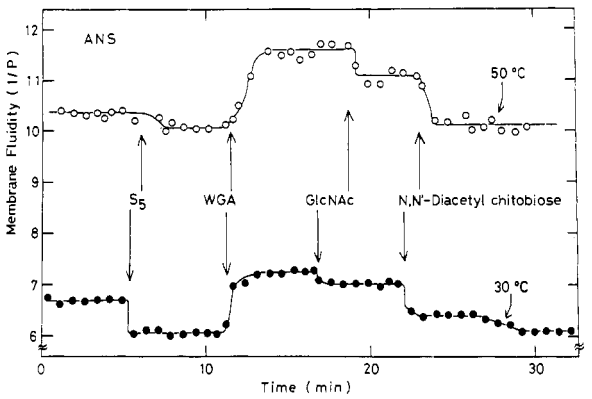


FIGURE 8: Membrane fluidity of DPPC vesicles monitored by ANS with various additives: [DPPC] = 1.0 mM; [$S_5$] = $1.89 \times 10^{-2}$ mM; [WGA] = $9.0 \times 10^{-3}$ mM; [GlcNAc] = 5.9 mM; [*N,N'*-diacetylchitobiose] = 1.36 mM; [ANS] = $1.6 \times 10^{-1}$ mM.

This should be attributed to a more favorable attraction being exerted among lipids or oligopeptides themselves than between lipid and oligopeptide molecules.

*Lectin-Mediated Intravesicular Cross-Linking of $S_5$ in DPPC Vesicles.* Lectin is multivalent and causes the cross-linking of glycopeptides on the cell surface. Figure 7 shows the change in $I_D/I_M$ (intensity of excimer emission/intensity of monomer emission) of $S_5$ by the addition of WGA. As the WGA concentration added to the suspension is increased, the $I_D/I_M$ value increased, indicating intravesicular association of $S_5$ molecules by the cross-linking of the glyco moiety with WGA. The cross-linking of $S_5$ molecules with WGA was enhanced below the phase transition temperature.

The intravesicular cross-linking of $S_5$ induced by WGA above the phase transition temperature was disrupted by the addition of GlcNAc as well as *N,N'*-diacetylchitobiose, and the original distribution of $S_5$ in the membrane was restored reversibly. *N,N'*-Diacetylchitobiose was found to dissociate the association of $S_5$ more potently than GlcNAc. However, below the phase transition temperature the $I_D/I_M$ value was not decreased by the addition of *N,N'*-diacetylchitobiose because of the immobilization of associated $S_5$ molecules in a crystalline state of the membrane.

*Membrane Fluidity.* Fluorescence probes were used to evaluate the membrane fludity of DPPC vesicles. ANS and DPH have been most frequently applied to the membrane for this purpose, and they are known to reflect the membrane fluidity at the membrane surface and the hydrophobic core

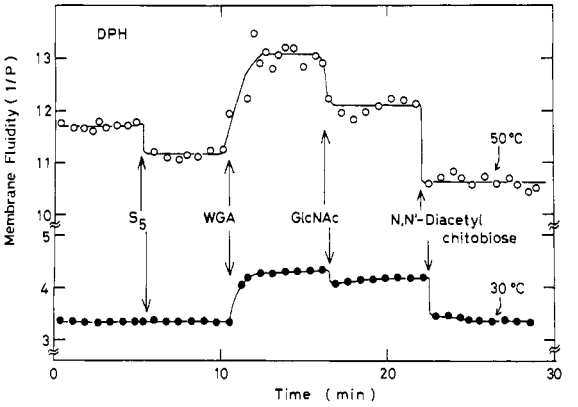


FIGURE 9: Membrane fluidity of DPPC vesicles monitored by DPH with various additives. The concentrations of the additives are same as those in Figure 8, and [DPH] = $1.6 \times 10^{-2}$ mM.

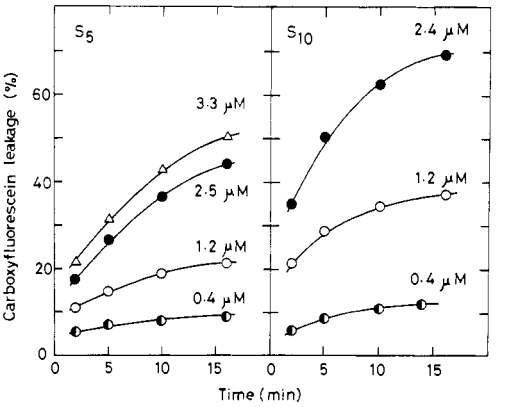


FIGURE 10: CF leakage from DPPC vesicles with the addition of $S_5$ and $S_{10}$ (concentrations are shown in the figure).

of the lipid bilayer, respectively. In the present investigation, the inverse polarization of fluorescence probes ($1/P$) was taken as an indication of membrane fluidity.

The distribution of $S_5$ into DPPC vesicles decreased the membrane fluidity in all cases. However, the fluidity of the hydrophobic core as monitored by DPH at 30 °C did not decrease (Figures 8 and 9). The addition of WGA to $S_5$-containing vesicles strongly increased the membrane fluidity. This action of WGA to the membrane is reversibly antagonized by GlcNAc and *N,N'*-diacetylchitobiose. GlcNAc, which is shown to break the intravesicular cross-linking of $S_5$, restores the membrane rigidity only partly. A complete restoration of membrane rigidity was attained only by the addition of *N,N'*-diacetylchitobiose after the dissociation of intervesicular aggregation.

Changes in membrane fluidity induced by WGA addition to vesicles were observed only in the presence of $S_5$ in the membrane. In the absence of $S_5$, the addition of WGA or GlcNAc did not induce any change in membrane fluidity under these conditions. Therefore, the WGA effects on membrane fluidity were $S_5$ dependent.

It should be noted that in these experiments the side effect of scattered light on the measurement was reduced as much as possible by use of a cutoff filter. If there is a side effect at all, it would increase the polarization apparently, which cannot explain the decreased polarization by the addition of WGA. Furthermore, the emission intensity from fluorescence probes was unchanged throughout these experiments, indicating that the redistribution of probes into the membrane by additives was strictly excluded.

*Membrane Perturbation.* Permeation of CF across the lipid membrane under the experimental conditions was examined

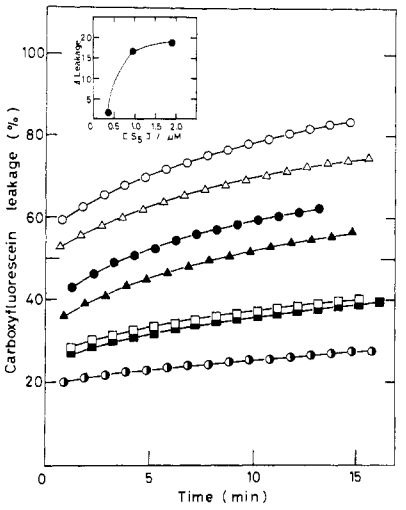


FIGURE 11: CF leakage from DPPC vesicles with the addition of WGA (◑) or $S_5$ [(●) 1.92 μM; (▲) 0.96 μM; (■) 0.38 μM] and with the simultaneous addition of WGA and $S_5$ [(○) 1.92 μM; (△) 0.96 μM; (□) 0.38 μM].

with CF-trapped vesicles. CF leakage would measure the membrane perturbation caused by several additives. CF leakage from vesicles was relatively intensive immediately after the addition of $S_5$ or $S_{10}$ to the suspension of vesicles and became moderate after a certain time lapse (Figure 10). The extent of membrane perturbation induced by the addition of glycopeptide increased in the order $S_0 \ll S_5 < S_{10}$. The CF leakage is linearly related to the concentration of added glycopeptide, excluding the possibility of the disruption of vesicular structure even in the presence of an excess amount of glycopeptide added for one vesicle. These results show that $S_0$, $S_5$, or $S_{10}$ perturbs the membrane structure to some extent at the time of insertion. However, they work differently from detergents which destroy the membrane structure completely.

When WGA was added to DPPC vesicles a few minutes after the $S_5$ addition, there was no change in CF permeation. However, a simultaneous addition of $S_5$ and WGA to CF-trapped vesicles caused more CF leakage as compared to that of vesicles containing $S_5$ only (Figure 11). The addition of WGA alone affected to a negligible extent the CF leakage. This means that the perturbation in membrane structure caused by the $S_5$ distribution is enhanced by the complexation with WGA but that cross-linking of $S_5$ molecules with WGA after the completion of the distribution to the membrane does not induce a defect in membrane structure.

## DISCUSSION

Our aim was to establish the correlation of membrane fluidity or membrane permeability with signal transduction across the membrane through the investigation of the redistribution of glycopeptides in the membrane or aggregation of vesicles induced by lectin. Although DPPC vesicles containing synthetic glycopeptides were chosen for the biomimetic membrane, the experimental results should reproduce the fundamental aspects of the function of biological membrane.

$S_0$, which is composed of dipeptide and GlcNAc, distributed to DPPC membrane, and WGA agglutinated the $S_0$-containing vesicles. In the same process, however, a certain fraction of $S_0$ was pulled out of the membrane by WGA because of insufficient hydrophobicity of the peptide moiety (data not shown). Furthermore, intravesicular cross-linking of $S_0$ by WGA was not clearly detected by the change in excimer formation, probably because the pyrenyl groups of $S_0$ molecules bound by WGA cannot approach close enough for excimer formation. On the other hand, $S_5$, which is a glycotripeptide and possesses a relatively long alkyl chain between the sugar group and the tripeptide unit, possesses hydrophobicity high enough to be captured tightly in the membrane and effective flexibility for two pyrene groups to form an excimer on complexing with WGA intravesicularly. $S_{10}$, in which the alkyl chain between GlcNAc and tripeptide is longer and therefore hydrophobicity increases further, behaved similarly to $S_5$ but perturbed the membrane structure more significantly than $S_5$ when inserted in the membrane. It is, therefore, concluded that $S_5$ is most suitably designed for the present purpose.

The addition of WGA to $S_5$-containing vesicles affected the physical state of the membrane in two different ways. One is intervesicular aggregation, and the other is intravesicular cross-linking of glycopeptides. The former might be referred to as "trans" complex and the latter as a "cis" complex (Portis et al., 1979). In this aspect, $S_5$ is further advantageous over $S_0$ or $S_{10}$; that is, the $S_5$–WGA complex is suitably stable. The experiments on agglutination of $S_5$-containing vesicles by WGA clearly showed that the intervesicular aggregation through the $S_5$–WGA complex is so stable that it is not dissociated by GlcNAc but by a more potential antagonist, *N,N'*-diacetylchitobiose. On the other hand, the intravesicular cross-linking of $S_5$ by WGA is dissociated by GlcNAc, having the excimer in the membrane disappeared. The lower stability of the intravesicular association than the intervesicular counterpart might be ascribed to steric reasons. Therefore, the intervesicular aggregation and the intravesicular cross-linking of $S_5$ can be individually related to membrane fluidity.

Distribution of $S_5$ to DPPC vesicles decreased the membrane fluidity either on the surface or in the hydrophobic core of the lipid bilayer membrane over the phase transition temperature. The decreased fluidity might be explained in terms of dehydration of polar head groups of lipid molecules (Hauser et al., 1977). The dehydration should be caused by replacement of water molecules from the polar head groups by the GlcNAc moiety of $S_5$. Since the GlcNAc moiety exists on the membrane surface, it should be hydrogen-bonded with polar head groups of lipid molecules more favorably than with water (Jarrell et al., 1986). This is an entropy-controlled process. Alternatively, the decreased membrane fluidity might be due to the annular lipids formed around the $S_5$ molecules, in which mobility is supposed to be suppressed.

The fluidity of the hydrophobic core of the lipid bilayer membrane below the phase transition temperature was not changed by $S_5$ addition. At low temperatures, alkyl chains of lipid molecules must be highly ordering.

WGA addition increased the membrane fluidity drastically. This increase is ascribed to two factors, i.e., intravesicular cross-linking of $S_5$ and intervesicular aggregation. The latter was shown to influence the membrane fluidity more strongly than the former (Figures 8 and 9).

When a lipid bilayer approaches the other, a repulsive force acts across the intervening aqueous phase. It is a hydration force based on the hydration of phospholipid bilayers. The hydration force was found to affect membrane structure enormously and dominate over the electrostatic force when the lipid bilayers come closer than 25 Å. It is independent of the conformation of the hydrocarbon chains of the lipid molecules (Rand, 1981). On the other hand, pressure effects on membrane proteins have been investigated with great interest (Heremans, 1982). Increasing pressure was found to decrease nitrogenase activity (Smedt et al., 1979) and to retard the transmembrane mobility of a valinomycin–ion complex (Benz et al., 1986). These phenomena have been explained

in terms of increasing viscosity in the hydrocarbon core of lipid bilayer membrane. This interpretation is consistent with the increase of the phase transition temperature of the membrane with rising pressure (Macdonald, 1978). However, it contradicts the increase of membrane fluidity observed in the present investigation. Presumably, the membrane structure was further modified to relieve pressure caused by a hydration force in the present case. Furthermore, lipid bilayers are not very compressible. Therefore, the thickness of the lipid bilayer and the area occupied by a single lipid molecule do not change significantly by pressure (McIntosh et al., 1986), indicating the insensitivity of membrane fluidity to pressure. On the other hand, it has been reported that lectin-mediated intermembrane contact enhances $Ca^{2+}$-induced fusion of phosphatidate-containing membrane (Sundler, 1983). Therefore, it is reasonable to speculate that the change in the hydration state of the phospholipid molecules, which is brought about by a close contact of the lipid bilayers, modifies the membrane fluidity. It is necessary to investigate this point further.

We cannot exclude the possibility that the WGA–glycopeptide complex formed on the membrane surface distorts the structure of neighboring lipid bilayers and increases the membrane fluidity. However, it is not likely that this distortion induces a notable change in membrane fluidity without affecting CF leakage through the membrane. To investigate this point further, the use of transmembranous glycopeptides is necessary.

The intravesicular cross-linking of $S_5$ also increased the membrane fluidity. This phenomenon can be explained as follows: (i) patch formation of $S_5$ on complexing with WGA increases the domain of free lipid molecules (Taraschi et al., 1982); (ii) in the patching domain, the lipid bilayer structure is distorted because of the formation of a sterically skewed "cis" complex. The latter might explain the restoration of membrane stiffness by the addition of GlcNAc below the phase transition temperature. In relation to the latter explanation (ii), displacement of $S_5$ in a direction normal to the membrane surface may occur. In this process the hydrophobic peptide moiety comes in contact with the hydrophilic membrane surface, and the structure of the lipid bilayer membrane will be disturbed in the neighborhood of the patching domain.

**Registry No.** $S_5$, 115408-85-0; $S_{10}$, 115408-86-1; $S_0$, 115419-86-8; Asp(OBzl)-Phe-Lys($CO(CH_2)_3$Py)-OMe, 115408-92-9; DPPC, 2644-64-6; $Br(CH_2)_5COOCH_3$, 14273-90-6; $Br(CH_2)_{10}COOCH_3$, 6287-90-7; *N*-acetylglucosamine, 7512-17-6; *N*-acetyl-α-D-glucopyranosylamine chloride, 3068-34-6; *S*-aminoiminomethyl *N*-acetyl-β-D-thioglucopyranosylamine triacetate hydrochloride, 51224-18-1; *N*-acetyl-β-D-thioglucopyranosylamine triacetate, 51450-09-0; *S*-5-(carbomethoxy)pentyl *N*-acetyl-β-D-thioglucopyranosylamine triacetate, 77346-22-6; *S*-10-(carbomethoxy)decyl *N*-acetyl-β-D-thioglucopyranosylamine triacetate, 115408-87-2; *S*-5-(carbomethoxy)pentyl *N*-acetyl-β-D-thioglucopyranosylamine, 115408-88-3; *S*-10-(carbomethoxy)decyl *N*-acetyl-β-D-thioglucopyranosylamine, 115408-89-4; *S*-5-(azidocarbonyl)pentyl *N*-acetyl-β-D-thioglucopyranosylamine, 115408-90-7; *S*-10-(azidocarbonyl)decyl *N*-acetyl-β-D-thioglucopyranosylamine, 115408-91-8.

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