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Orientation change of glycopeptide in lipid bilayer membrane induced by lectin binding

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A lectin-induced orientation change of a helical glycopeptide in lipid bilayer membranes was studied. Glycopeptides composed of hydrophobic nona-(G8) and pentapeptide (G4) with a fluorescent probe at the N-terminal and a lactose unit at the C-terminal were synthesized. The glycopeptides were incorporated into lipid bilayer membranes with the lactose unit exposed to the aqueous phase and the peptide chain buried in the membrane. G8 takes a partially helical structure in the membrane, while G4 an irregular structure. Upon binding of lectin to G8 held in the membrane of DPPC liposome, enhancement of fluorescence intensity of the N-terminal anthryl group, reduction of fluorescence quenching of the anthryl group with acrylamide, and increase of CF-leakage from the DPPC liposome were observed. G8′, which lacks the *O*-anthryrlmethylserine residue from G8, formed a voltage-dependent ion channel in BLM experiments. The frequency of single current fluctuations induced by G8′ incorporation increased with addition of lectin. These results indicate that the peptide segment of G8 prefers taking a more perpendicular orientation to the membrane upon association with lectin.

Introduction

Biological roles of carbohydrate fragment of glycoproteins have been discussed from various aspects [1]. For example, modification of secretion proteins with carbohydrates stabilizes the protein conformation [2–6], and protects the protein part from degradation by proteinases [7–9]. In addition, carbohydrates have been shown to act as a recognition site for ABH Ii antigen [10], tumor-associated antibody [11], virus [12], mycoplasma [1], and lectin [1], which are all involved in the signal transduction at the cell membrane. Cell–cell interactions are also modulated by carbohydrate groups of glycoproteins such as adhesion factors N-CAM [13] and ZP3 [14].

Although cell responses induced by ligand binding to the carbohydrate groups existing on the membrane have been studied in detail, the molecular mechanism for cell activation remains to be clarified in many cases. We have adopted a model system of a cell, which is composed of synthetic glycopeptides and dipalmitoylphosphatidylcholine (DPPC) vesicles, and investigated on interactions of glycopeptides with phospholipid membrane especially in the presence of lectin. Our main interests are focussed on the modulation of physical state of phospholipid membrane associated with the action of lectin on glycopeptides. For example, a glycopeptide composed of *N*-acetylglucosamine and a tripeptide was shown to be crosslinked intravesicularly and intervesicularly by addition of wheat germ agglutinin, and thereby increased the membrane fluidity [15]. In other case, α-helical polypeptides having a maltose unit at one end of the chain were shown to be incorporated into DPPC vesicles taking a transmembrane orientation [16]. However, the polypeptides tended to form aggregates in the membrane, which made analysis of lectin addition very difficult. In the present investigation, an octapeptide having an alternate sequence of Ala and α-aminoisobutyric acid (Aib) was chosen and connected to lactose (Fig. 1). Aib is known as a helicogenic residue, and Aib-containing peptides are difficult to take a β-sheet structure [17], thus preventing aggregation. It is shown here that lectin addition to vesicles having the glycopeptide incorporated induces changes of peptide orientation to the membrane and the membrane permeability.

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Materials and Methods

Materials

9-Chloromethylanthracene (9-$ClCH_2$-Ant, Tokyo-Kasei), *N*-acetylgalactosamine (GalNAc, Sigma), lacto-

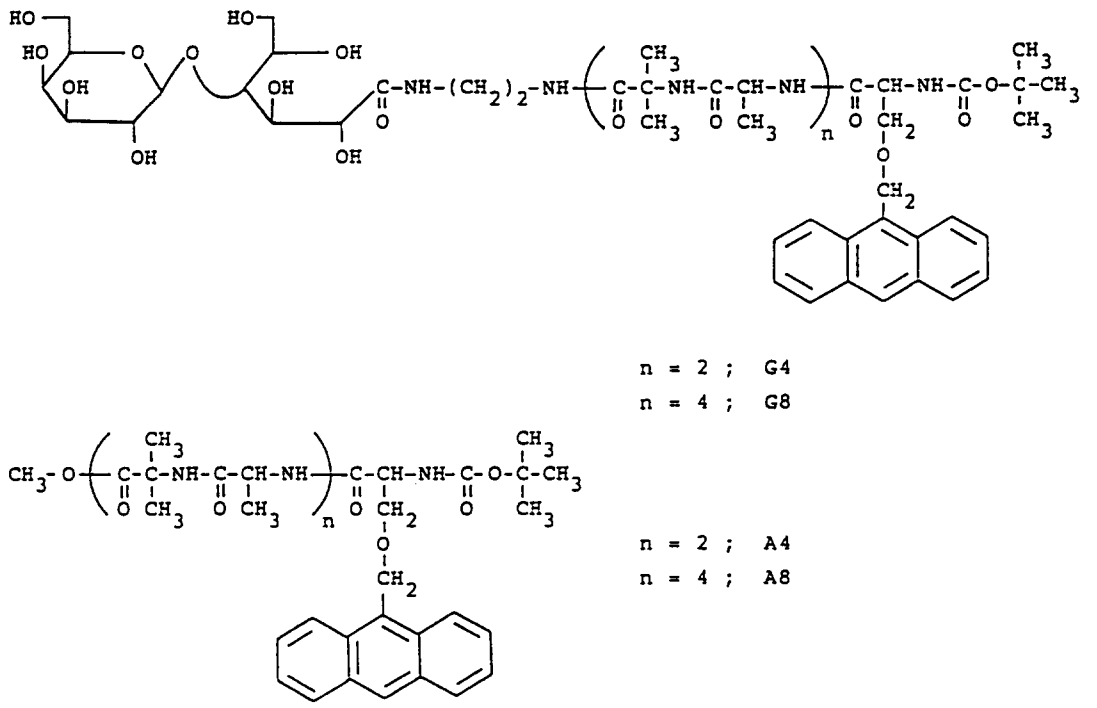

Fig. 1. Molecular structure of glycopeptides and hydrophobic helical peptides.

bionic acid (Aldrich), dipalmitoylphosphatidylcholine (DPPC, Sigma), dimyristoylphosphatidylcholine (DMPC, Sigma), 5/6-carboxyfluorescein (CF, Sigma), and *Ricinus communis* agglutinin II (RCA_{60}, Seikagaku Kogyo) were commercially available and used without further purification.

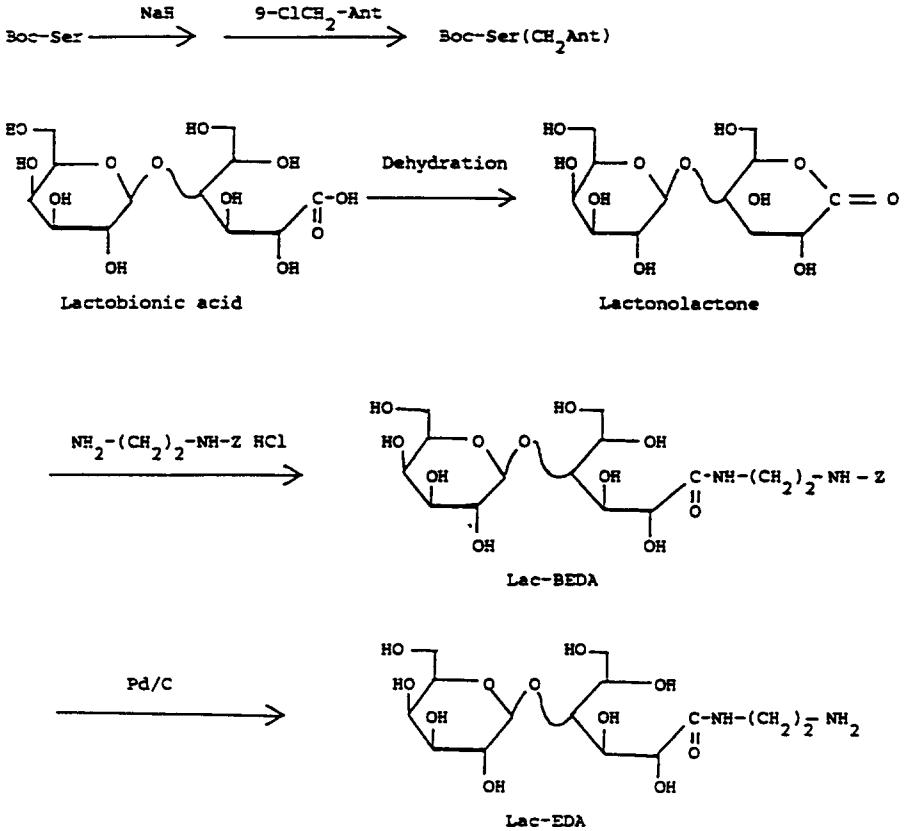

Fig. 2. Synthetic scheme of glycopeptides.

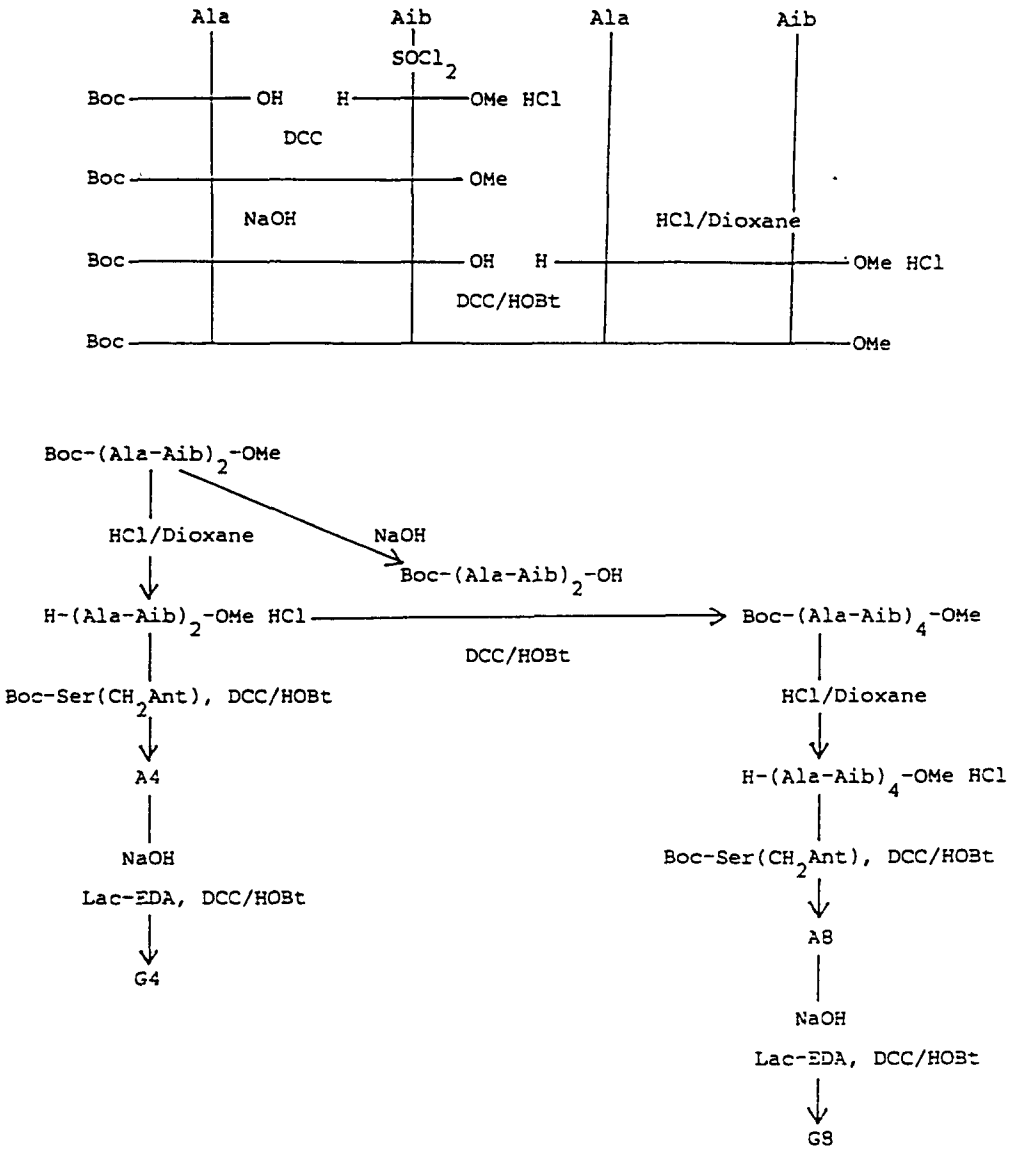

Fig. 2. (continued).

Peptide synthesis

The synthetic route of the glycopeptides is shown in Fig. 2. Each intermediate was identifed by IR and ^{1}H-NMR measurements. The purity was checked by thin layer chromatography using aluminium sheets silica gel 60 (Sigma). The developing solvent system is chloroform/methanol/acetic acid (95:5:3, v/v/v).

Boc-Ser(CH$_2$Ant). A dimethylformamide (DMF) solution of Boc-Ser (0.78 g) was added in a drop wise to a DMF suspension of NaH (0.38 g) at room temperature. Subsequently, a DMF solution of 9-chloromethylanthracene was added. After stirring the solution overnight, a small amount of 10% citric acid was added, and the solution was condensed. The solution was diluted with chloroform, and washed with 4% $NaHCO_3$, 10% citric acid, and water. After drying over $MgSO_4$, the organic phase was condensed under reduced pressure. The residual solid was purified by a column chromatography of Sephadex LH-20 using methanol as eluant. Further purification was carried out by a silica gel column changing the eluant in an order of benzene, chloroform, and methanol. $R_f = 0.51$. Yield; 25%.

Lac-BEDA. A DMF solution of lactonolactone (prepared from lactobionic acid by the method reported by Williams et al. [18], 1.1 g), *N*-benzyloxycarbonylethylenediamine hydrochloride (BEDA · HCl, prepared by the method reported by Lawson et al. [19], 0.78 g), and triethylamine (476 μl) was stirred overnight at 60°C. After evaporation, methanol was added, and an insoluble part was filtered off. The filtrate was purified by a silica gel column using methanol/H_2O (2:1, v/v) as eluant. $R_f = 0.47$. Yield; 30%.

H-(Ala-Aib)$_2$-OMe · HCl, H-(Ala-Aib)$_4$-OMe · HCl. The peptides were synthesized by a conventional liquid phase method using dicyclohexylcarbodiimide (DCCI) and *N*-hydroxybenzotriazole (HOBt) or *N*-hydroxy-

succinimide as coupling reagents according to the synthetic scheme shown in Fig. 2.

Elemental analysis:

Boc-(Ala-Aib)$_2$-OMe. Calcd. for $C_{20}H_{36}N_4O_7$: C, 54.03; H, 8.16; N, 12.60. Found: C, 54.21; H, 8.36; N, 12.60.

Boc-(Ala-Aib)$_4$-OMe · 2H$_2$O. Calcd. for $C_{34}H_{64}N_8O_{13}$: C, 51.50; H, 8.14; N, 14.13. Found: C, 51.83; H, 7.99; N, 14.22. The Boc group of the peptides was removed by 4 M HCl/dioxane treatment.

A4 and A8. To a dichloromethane solution of Boc-Ser(CH$_2$Ant) (0.20 g), H-(Ala-Aib)$_2$-OMe · HCl (0.19 g) and HOBt (0.10 g) were added DCCI (0.14 g) and triethylamine (70 μl). After stirring overnight, the solvent was evaporated. The residue was purified by a column chromatography of Sephadex LH-20 using methanol as eluant. $R_f = 0.64$. Yield, 55%. A8 was prepared by the method similar to that employed for A4. $R_f = 0.42$. Yield, 57%.

A4-OH and A8-OH. A4 (0.15 g) was dissolved in a solution of methanol/dioxane (1:1, v/v) and 1 M NaOH (0.31 ml). After standing for 1.5 h, the solution was diluted with chloroform, and washed with 10% citric acid aqueous solution. The product (A4-OH) was purified by a silica gel column using ethyl acetate/methanol/chloroform (8:1:2, v/v/v) as eluant. $R_f = 0.11$. Yield, 84%. A8-OH was prepared by the method similar to that employed for A4-OH except the elution condition of a silica gel column: the column was washed with ethyl acetate/methanol/chloroform (8:2:2, v/v/v), and the product was eluted by using methanol. $R_f = 0.1$. Yield, 92%.

G4 and G8. Lac-BEDA (0.10 g) in a DMF solution was subjected to hydrogenation using Pd/C as catalyst. After stirring the DMF dispersion for 4 h, A4-OH (0.11 g), HOSu (0.015 g), and DCCI (0.024 g) were added. The product was purified twice by column chromatography of Sephadex LH-20 using methanol as eluant. Yield, 32% for both of G4 and G8.

Elemental analysis:

G4. Calcd. for $C_{51}H_{75}N_7O_{19} \cdot 4H_2O$: C, 52.86; H, 7.19; N, 8.43. Found: C, 52.45; H, 6.54; N, 7.80.

G8. Calcd. for $C_{65}H_{99}N_{11}O_{23} \cdot 3H_2O$: C, 53.60; H, 7.26; N, 10.57. Found: C, 54.03; H, 7.44; N, 10.02.

G8′ was synthesized by coupling Lac-BEDA with HCl · H-(Ala-Aib)$_4$-OMe similarly to the synthesis of G8.

Measurements

CD and fluorescence spectra were measured on a JASCO J-20 spectropolarimeter (optical path length 0.1 cm) and a Hitachi MPF-4 fluorophotometer, respectively.

Fluorescence depolarization. Small unilamellar vesicles were prepared by sonication of DMPC or DPPC dispersion in a buffer solution (10 mM Hepes, 0.1 M NaCl, and 0.1 mM EDTA, pH 7.4) and ultracentrifugation. Lipid concentration was determined by the colorimetric method using phospholipase D (Diacolor, Toyobo). Fluorescence depolarization of the anthryl group of peptides was measured by equipment installed on the fluorophotometer reported previously [15]. Excitation and monitor wavelengths were 367 and 414 nm, respectively.

CF leakage. CF-encapsulated DPPC vesicles were prepared by the method reported by Barbet et al. [20]. The excitation and monitor wavelengths of CF were 470 and 515 nm, respectively. Complete release of CF was attained by treating with Triton X-100 (0.3 wt%).

Bilayer experiments. A thin Teflon film (0.25 mm) with an aperture of 0.1 mm diameter was clamped at the midway of a Teflon trough. The hole was precoated with hexadecane/hexane (6:4, v/v). The azolectin membrane was formed by the method reported by Montal et al. [21]. The electrolyte solution was unbuffered aqueous solution of 1 M KCl. Before the measurement, an AC voltage of 200 mV (peak-to-peak, 1 kHz) was applied to the membrane for 30 min to exclude organic solvent contaminated in lipid bilayer membranes. The peptides were added to both aqueous phases.

Results and Discussion

Binding to DMPC liposome

Distribution of G4 or G8 to DMPC liposome was investigated by an equilibrium dialysis method. When the glycopeptides (1 μM) were incubated with 70-fold amount of DMPC in a molar excess at 15°C, the equilibrium amounts of G4 and G8 distributed in the membrane phase were 85% and 82%, respectively, indicating that the membrane affinities of G4 and G8 are nearly the same. The affinity to DMPC liposome was slightly less in a liquid-crystalline state than in a gel state.

Conformation

Aib residue has been demonstrated to be helicogenic due to the presence of the α,α-dimethyl group, which severely restricts the backbone conformation of Aib-containing polypeptides. G8 in ethanol shows negative Cotton effects at 208 and 222 nm in CD spectrum, indicating a helical conformation (Fig. 3). On the other hand, G4 does not show any indication of a regular structure. Similar results were obtained by CD measurement in trifluoroethanol. In the presence of DMPC lipsome, G8 showed slightly higher helix content than in a buffer solution, while G4 in the membrane took an irregular conformation as in a buffer solution.

It is known that Aib-containing peptides take either 3_{10}- or α-helical conformation depending on composi-

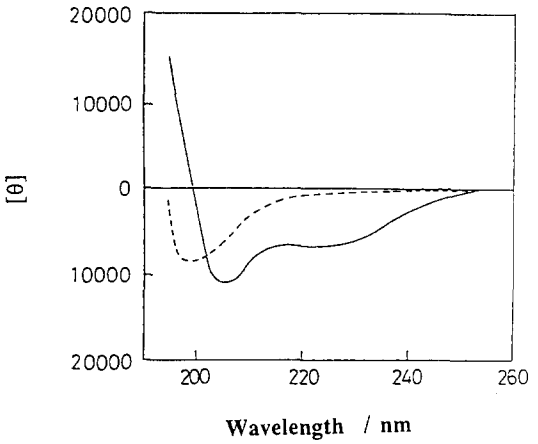

Fig. 3. CD spectra of G8 (———) and G4 (- - - - -) in ethanol. [G4] $3.3 \cdot 10^{-4}$ M, [G8] = $1.4 \cdot 10^{-4}$ M. The ordinate is expressed as the mean residue molar ellipticity.

tion [22], sequence[23], and chain length [24]. Boc-(Ala-Aib)$_4$-OMe in a solid state was shown by X-ray analysis to take 3_{10}-helix conformation over the whole molecule. However, Boc-(Ala-Aib)$_4$-OMe took an α-helical structure in CD_3CN, suggesting that G8 in solution also takes an α-helical conformation.

Location of anthryl group in the membrane

Fluorescence depolarization of the N-terminal anthryl group of G4 and G8 was measured at varying temperatures (Fig. 4). It decreased in the presence of DMPC liposome, indicating that the mobility of anthryl group was restricted upon distribution to the membrane. The temperature dependence of the fluorescence depolarization was discontinuous at the phase-transition temperature of the membrane, reflecting the change of membrane fluidity.

The fluorescence depolarization of G8 in the membrane was lower than G4. Since the membrane affinities of G4 and G8 are similar, the difference should be

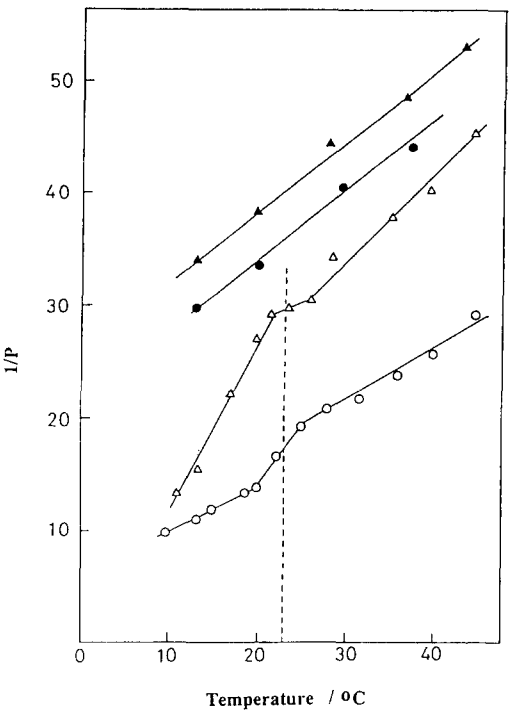

Fig. 4. Temperature dependence of fluorescence depolarization of anthryl group of G4 in buffer (▲), G4 in the presence of DMPC liposome (△), G8 in buffer (●), and G8 in the presence of DMPC liposome (○). Excitation and monitor wavelengths are 367 and 414 nm, respectively. [G4] $2.3 \cdot 10^{-6}$ M, [G8] = $1.9 \cdot 10^{-6}$ M, [DMPC] = $2.4 \cdot 10^{-4}$ M. Dotted line represents the phase-transition temperature of DMPC membrane.

ascribed to different locations in the membrane. Spin-lattice relaxation times of DPPC liposome, which were reported by Seelig and Seelig [25], demonstrated that carbon atoms at the glycerol skeleton and C_2 of palmitoyl chain are most restricted in motion, and the relaxation time gradually increases on going to the terminals. Therefore, G4 should loosely bind to the phosphorylcholine portion of the membrane, while G8 is

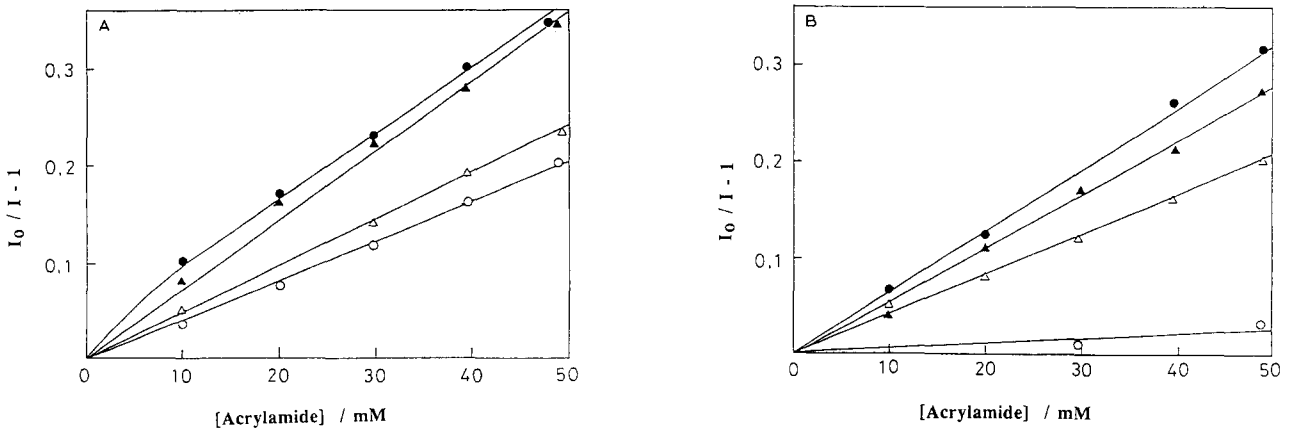

Fig. 5. Stern-Volmer plot for quenching of anthryl emission with acrylamide. ▲, G4 and ●, G8 in a buffer solution. △, G4 and ○, G8 in the presence of DMPC liposome. (A) At 34°C, [G4] = $1.3 \cdot 10^{-6}$ M, [G8] = $1.4 \cdot 10^{-6}$ M, [DMPC] = $1.4 \cdot 10^{-4}$ M. (B) At 15°C, [G4] = $2.3 \cdot 10^{-6}$ M, [G8] = $1.9 \cdot 10^{-6}$ M, [DMPC] = $2.4 \cdot 10^{-4}$ M.

incorporated in the depth of the membrane, probably immediately below the glycerol layer.

The anthryl group of G8 in membrane is, therefore, more restricted in motion than that of G4. This is also deduced from CD measurement. A positive Cotton effect appeared at ^1B_b band of anthryl group below the phase-transition temperature. G8 exhibited more intensive CD signals than G4, indicating that lower moleclar motion of G8 than G4 in the membrane.

In addition, the consideration that the anthryl group of G8 is located in more depth of the membrane than G4 receives support from the following observations. First, addition of acrylamide, which is a water-soluble fluorescence quencher, decreased the emission from the anthryl group more intensively in a buffer solution than in the presence of liposome (Fig. 5). In the latter case, the rate of fluorescence quenching of G8 is smaller than G4, indicating that the anthryl group of G8 is better shielded from the aqueous phase than that of G4. Secondly, as shown in Fig. 6, leakage of CF entrapped in the vesicle was enhanced upon addition of G8, while G4 up to 4 μM did not influence the CF leakage.

Considering the structural situation, G4 takes an irregular conformation in which amide protons and carbonyl oxygens might interact with phosphate and ammonium groups of the membrane. On the other hand, G8 takes a partially helical conformation, which is stabilized by intramolecular hydrogen bonds. The formation of intramolecular hydrogen bonds shifts the peptide chain to the more depth of the membrane.

Temperature effect on G8 orientation in membrane

The rates of fluorescence quenching of G4 and G8 were lower in the gel-state membrane than in the liquid-crystalline membrane (Fig. 5). Especially, the rate of G8 quenching notably decreased in the gel-state membrane, suggesting that the *N*-terminal of G8 moves to more depth in the membrane below the phase-transition temperature. We have reached the same conclusion from the temperature effect on the CF leakage by G8 addition. In this study, A4 was used as a reference compound. A4 does not take a regular conformation and excludes specific interactions with phospholipid membrane. Above the phase-transition temperature, CF leakage induced by G8 addition was significantly lower than by A4 addition. However, below the phase-transition temperature, CF leakages by G8 and A4 were similar, suggesting that G8 changes the structure of the hydrophobic core of bilayers as well as the membrane surface upon incorporation into the depth of the gel-state membrane.

Interaction with lectin

Interactions of RCA_{60}, which specifically associates with β-D-galactose, with G4 and G8 were studied by measuring fluorescence depolarization of the anthryl group. Lectin addition suppressed the fluorescence depolarization of G4 and G8 either in a buffer solution or in the presence of DMPC liposome (Table I), indicating a decreasing molecular motion upon association with the lectin. The glycopeptides restored the original fluorescence depolarization by GalNAc addition to the

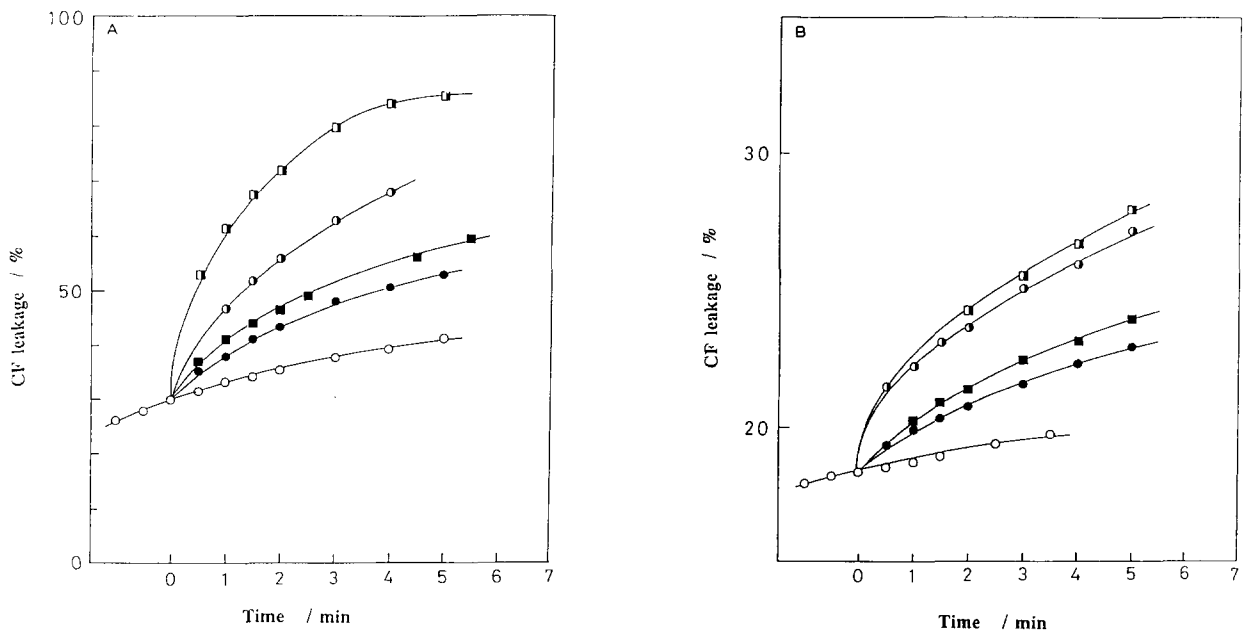

Fig. 6. CF leakage from DPPC liposome with the addition of G4 (■, $1.5 \cdot 10^{-6}$ M; ◨, $3.7 \cdot 10^{-6}$ M),G8 (●, $1.3 \cdot 10^{-6}$ M; ◑, $3.3 \cdot 10^{-6}$ M), and without additives (○). [DPPC] = $2.0 \cdot 10^{-5}$ M. (A) At 48°C, (B) at 27°C.

TABLE I

Fluorescence depolarization (1/P) of anthryl group of G4 and G8, under different environments at 14°C

[G4] = $2.4 \cdot 10^{-6}$ M, [G8] = $2.0 \cdot 10^{-6}$ M, [RCA_{60}] = $7.4 \cdot 10^{-6}$ M, [GalNAc] = $8.5 \cdot 10^{-5}$ M, [DMPC] = $2.9 \cdot 10^{-4}$ M. The average of 8–15 data is shown. The number in parenthesis represents the experimental variability from the average.

	1/P	
	G4	G8
In a buffer solution		
Glycopeptide	32.7 (2.6)	27.3 (2.1)
Glycopeptide + RCA_{60}	12.4 (0.2)	15.7 (0.8)
Glycopeptide + RCA_{60} + GalNAc	26.5 (2.2)	21.5 (1.0)
In the presence of DMPC liposome		
Glycopeptide	13.3 (0.7)	13.7 (0.4)
Glycopeptide + RCA_{60}	10.5 (0.5)	11.6 (0.3)
Glycopeptide + RCA_{60} + GalNAc	14.5 (0.9)	13.9 (0.6)

RCA_{60} complex, indicating the reversible association of the lectin with G4 and G8. On the other hand, the lectin did not influence the fluorescence depolarization of A4.

Interestingly, the location of the N-terminal group of G8 in the membrane changed upon binding of RCA_{60}. This conclusion was obtained from the following observations. First, the fluorescence intensity of G8 in DMPC membranes increased with the addition of RCA_{60} (Fig. 7), while it did not in the absence of liposome. The possibility of extraction of G8 from the membrane by lectin is denied by the fact that the fluorescence increase upon lectin addition was observed in the presence of acrylamide, too. Secondly, quenching of anthryl fluorescence of G8 by acrylamide in the presence of DMPC liposme was obviously suppressed by association with RCA_{60} (Fig. 8). Thirdly, CF leakage from DPPC liposme was enhanced by lectin addition (Fig. 9). Lectin addition in the absence of G8 did not show any increase in fluorescence intensity. These observations indicate that association of RCA_{60} with G8 shifts the N-terminal fluorescent group into more depth in the membrane.

It has been proposed by Schwyzer [26] that the amphiphilicity of the primary sequence of a peptide is one of the factors determining orientation of the peptide to the membrane. For example, a peptide molecule with a large amphiphilic moment tends to take a perpendicular orientation to the membrane. The association of lectin to the C-terminal group of G8 may

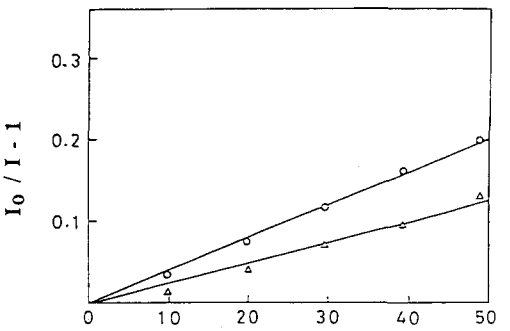

Fig. 8. Change of the rate of fluorescence quenching of G8 by acrylamide upon RCA_{60} addition in the presence of DMPC liposome at 35°C. ○, without RCA_{60}; △, with RCA_{60}. [DMPC] = $1.3 \cdot 10^{-4}$ M, [G8] = $1.4 \cdot 10^{-6}$ M, [RCA_{60}] = $4.1 \cdot 10^{-6}$ M. Excitation wavelength is 345 nm.

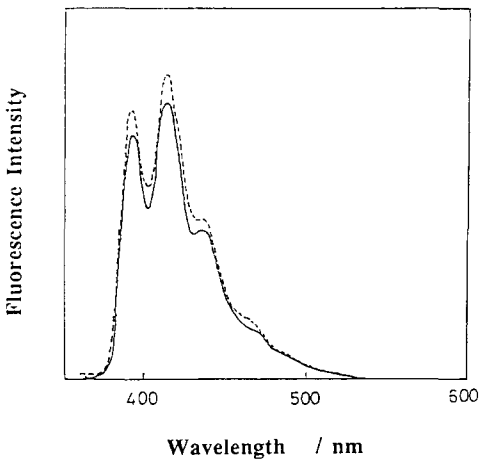

Fig. 7. Change of emission spectra of G8 induced by RCA_{60} addition in the presence of DMPC liposome at 35°C. Before (———) and after (- - - - -) the lectin addition. [G8] = $1.4 \cdot 10^{-6}$ M, [RCA_{60}] = $4.7 \cdot 10^{-6}$ M, [DMPC] = $1.3 \cdot 10^{-4}$ M. The excitation wavelength is 345 nm.

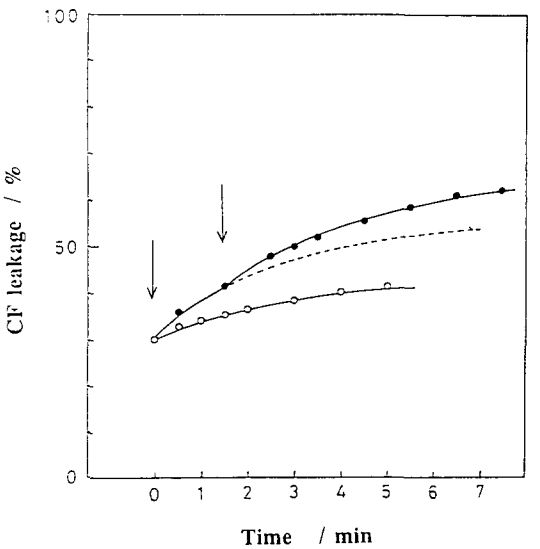

Fig. 9. CF leakage from DPPC liposome at 48°C. ○, without additives; - - - - - -, G8 addition ($1.3 \cdot 10^{-6}$ M) at the first arrow point; ●, G8 addition ($1.3 \cdot 10^{-6}$ M) at the first arrow point followed by RCA_{60} addition ($4.1 \cdot 10^{-6}$ M) at the second arrow point. [DPPC] = $2.0 \cdot 10^{-5}$ M. Excitation and monitor wavelengths were 470 and 515 nm, respectively.

enhance the primary amphiphilicity facilitating the perpendicular orientation.

The effect of lectin addition on CF leakage was observed only above the phase-transition temperature. This is consistent with the result described in the previous section that G8 takes a perpendicular orientation below the phase-transition temperature and association of lectin does not change the orientation of G8 in the membrane.

Ion channel formation

It has been reported that Boc-(Ala-Aib-Ala-Aib-Ala)$_n$-OMe ($n = 1$–4) forms a voltage-dependent ion channel in a planar bilayer membrane [27]. The hydrophobic helical peptides are considered to take a perpendicular orientation to the membrane surface. Thereby, helical rods associate with each other to form a bundle structure, which acts as an ion channel.

Current–voltage (I–V) curves in the planar bilayer membrane were obtained in the presence of Boc-(Ala-Aib)$_4$-OMe or G8′, which has the same molecular structure as G8 but without the N-terminal O-anthrylmethylserine residue (Fig. 10). The current increased dramatically by applying an electric potential beyond a certain voltage (switch-on voltage, V_c), which is characteristic to a voltage-dependent channel in planar bilayers. Interestingly, V_c of G8′ was significantly lower than that of Boc-(Ala-Aib)$_4$-OMe. Although G8′ and Boc-(Ala-Aib)$_4$-OMe are composed of the same octapeptide, the former showed much higher ability for ion-channel formation. The higher ability of G8′ can be explained in terms of two factors. First, G8′ possesses an amphiphilic property in the primary sequence, which facilitates taking a perpendicular orientation of the peptide in the membrane. The perpendicular orientation should be essential for ion-channel formation across the bilayer membrane. Secondly, G8′ is incorporated into the planar bilayer with the lactose

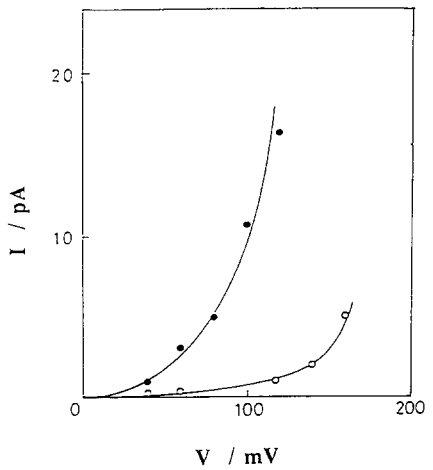

Fig. 10. Current–voltage (I–V) characteristics of G8′ ($3 \cdot 10^{-6}$ g/ml) (●) and Boc-(Ala-Aib)$_4$-OMe ($3 \cdot 10^{-6}$ g/ml) (○) in planar lipid bilayer membranes.

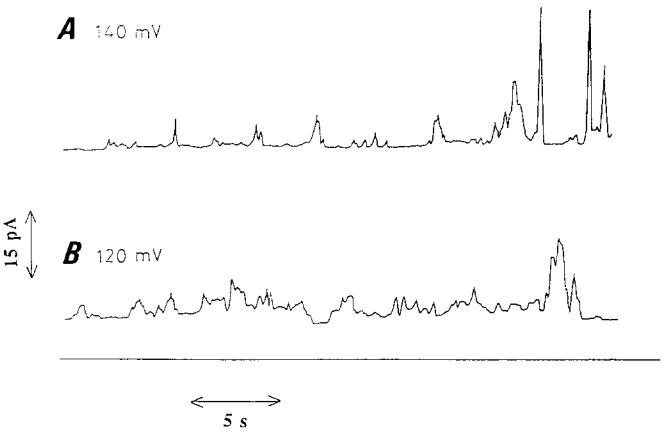

Fig. 11. Current fluctuations of single channel at constant applied voltage in planar bilayer membranes modifed by (A) G8′ ($3 \cdot 10^{-6}$ g/ml) and (B) G8′ ($3 \cdot 10^{-6}$ M) and RCA$_{60}$ ($1.3 \cdot 10^{-5}$ g/ml). Applied voltage: (A) 140 mV, (B) 120 mV.

fragment exposed to an aqueous phase. As a result, the C-terminal enviroment of the peptide in the membrane is so hydrophilic that charged species are easily accessible to the channel to permeate.

Current fluctuations by incorporation of G8′ into the membrane were observed only when an electric potential of slightly higher than 140 mV was applied across the membrane (Fig. 11). The stepwise change of electric current supports the formation of single ion channels within the bilayer membranes. The membrane voltage was turned off, and then RCA$_{60}$ was added. Applying the membrane voltage again, the current fluctuation of single channel was observed at 120 mV, which is lower by 20 mV than that in the absence of RCA$_{60}$. Furthermore, the frequency opening channel increased significantly (Fig. 11). Presumably, RCA$_{60}$ crosslinks G8′ molecules in the lipid bilayer membrane to promote the association of peptide molecules, resulting in stabilization of the single channels.

In the present study, the glycopeptide composed of a hydrophobic α-helical octapeptide was shown to change the peptide orientation in lipid bilayer membrane upon association with lectin. Presumably, hydrophobic α-helical peptides with a medium chain length ranging from octapeptide to dodecapeptide can take various orientations including perpendicular one to the membrane. Lectin binding should shift the equilibrium favorably to perpendicular orientation. Longer peptides, e.g., hydrophobic α-helical eicosapeptide take predominantly a perpendicular orientation to the membrane without assistance from lectin association, which will be reported in a forthcoming paper.

References

1 Sharon, N. and Lis, H. (1975) in The Proteins, Vol. 5, 3rd Edn. (Neurath, H. and Hill, R.L. eds.), pp. 1–144, Academic Press.
2 Bertoli, E. (1981) Biochim. Biophys. Acta 467, 196–202.

3 Wang, F.C. and Hirs, C.H.W. (1977) J. Biol. Chem. 252, 8358–8364.
4 Barr, W.A. and Collee, J.Z. (1967) J. Endocrinol. 28, 395–399.
5 Vaitukaitis, J.L. and Ross, G.T. (1971) J. Clin. Endocrinol. Metab. 33, 308–311.
6 Schooley, J.E. and Garcia, J.F. (1971) Proc. Soc. Exp. Biol. Med. 138, 66–68.
7 Vegarud, G. and Christensen, T.B. (1975) Acta Chem. Scand. B29, 887–888.
8 Gottschalk, A., Fazekas, D.S. and Groth, S. (1960) Biochim. Biophys. Acta 43, 513–519.
9 Barbaric, S., Mrsa, V., Ries, B. and Mildner, P. (1984) Arch. Biochem. Biophys. 234, 567–575.
10 Hakomori, S. (1981) Semin. Hematol. 18, 39–62.
11 Hakomori, S. (1984) Annu. Rev. Immunol. 2, 103–126.
12 Kathan, R.H. and Riff, L.J.M. (1963) Proc. Soc. Exp. Biol. Med. 114, 90–92.
13 Rothbard, J.B., Brackenbury, R., Cunningham, B.A. and Edelman, G.M. (1982) J. Biol. Chem. 257, 11064–11069.
14 Wassermann, P.M. (1987) Annu. Rev. Cell. Biol. 3, 109–142.
15 Ohtoyo, T., Shimagaki, M., Otoda, K., Kimura, S. and Imanishi, Y. (1988) Biochemistry 27, 6458–6463.
16 Otoda, K., Kimura, S. and Imanishi, Y. (1990) Bull. Chem. Soc. Jpn. 63, 489–496.
17 Narita, M., Doi, M., Sugasawa, H. and Ishikawa, K. (1985) Bull. Chem. Soc. Jpn 58, 1473–1479.
18 Williams, T.J., Plessas, N.R. and Goldstein, I.J. (1979) Arch. Biochem. Biophys. 195, 145–151.
19 Lawson, W.B., Leafer, M.D., Jr., Tewes, A. and Rao, G.J.S. (1968) Hoppe-Seylers Z. Physiol. Chem. 349, 251–262.
20 Barbet, J., Machy, P., Truneh, A. and Leserman, L.D. (1984) Biochim. Biophys. Acta 772, 347–356.
21 Montal, M. and Muller, P. (1972) Proc. Natl. Acad. Sci. USA 69, 3561–3566.
22 Karle, I.L., Sukumar, M. and Balaram, P. (1986) Proc. Natl. Acad. Sci. USA 83, 9284–9288.
23 Basu, G., Bagchi, K. and Kuki, A. (1991) Biopolymers 31, 1763–1774.
24 Pavone, V., Benedetti, E., Di Blasio, B., Pedone, C., Santini, A., Bavoxo, A., Toniolo, C., Crisma, M. and Sartore, L. (1990) J. Biomol. Struct. Dynam. 7, 1321–1331.
25 Seelig, J. and Seelig, A. (1980) Q. Rev. Biophys. 13, 19–61.
26 Schwyzer, R. (1986) Helv. Chim. Acta 69, 1685–1698.
27 Menestrina, G., Voges, K.-P., Jung, G. and Boheim, G. (1986) J. Membr. Biol. 93, 111–132.