

Fluorescent Glyconanoparticles as a Sensitive Device to Monitor Sugar-Involving Molecular Events

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ABSTRACT: We prepared sugar-displayed fluorescent nanoparticles using photopolymerization of the vesicles of diacetylene-containing glycolipids. After photopolymerization, the nanoparticles exhibited fluorescent emission at 400–600 nm upon excitation at 380 nm. The binding of sugar-recognizing proteins (lectins) onto the fluorescent nanoparticles induced changes in fluorescence. The fluorescent intensity of the nanoparticles decreased with an increase in lectin concentration. On the contrary, the enzymatic release of oligosaccharides from the glyconanoparticles gave an increase in fluorescence. Using a phosphatidylcholine-type lipid as the matrix lipid of the nanoparticles, the non-specific adsorption of lectins on the surface was drastically restricted, leading to the generation of a specific response to the target proteins.

KEY WORDS Glyconanoparticle / Lectin / sLeX / Carbohydrate-Protein Interaction /

Living cells and almost all proteins are covered with carbohydrates. Carbohydrates on the cell surface often act as ligands for a variety of extracellular proteins. Therefore, a sensing technique for carbohydrate-involving events has been an important theme in the fields of biology and biotechnology. Surface plasmon resonance (SPR)¹ and quartz crystal microbalance (QCM)² have been found to be reliable methods for detecting these molecular recognitions. However, such methods require special and expensive apparatus for the sensing. A simple colorimetric sensing system, inspired by molecular recognitions on the cell surface, has been firstly developed by Charych *et al.* using polydiacetylene vesicles incorporating naturally occurring glycolipids.^{3,4} The conjugated ene-yne backbone of 10,12-pentacosadiynoic acid (PDA **1**)-based polydiacetylene vesicles results in a colored solution (Scheme 1). They reported that the color of the vesicles was changed before and after the binding of relatively large ligands, such as cholera toxin and influenza virus, to the carbohydrate on the vesicular surface. This method was also applied to the detection of bacteria.⁵

In this paper, we report glycolipid-based polymerized vesicles (hereafter referred to as nanoparticles) as fluorescent-sensing material for the detection of sugar-protein interactions (Figure 1). We expected sugar-displayed fluorescent nanoparticles to sense the binding of ligands too small to be detected using the conventional colorimetric method since fluores-

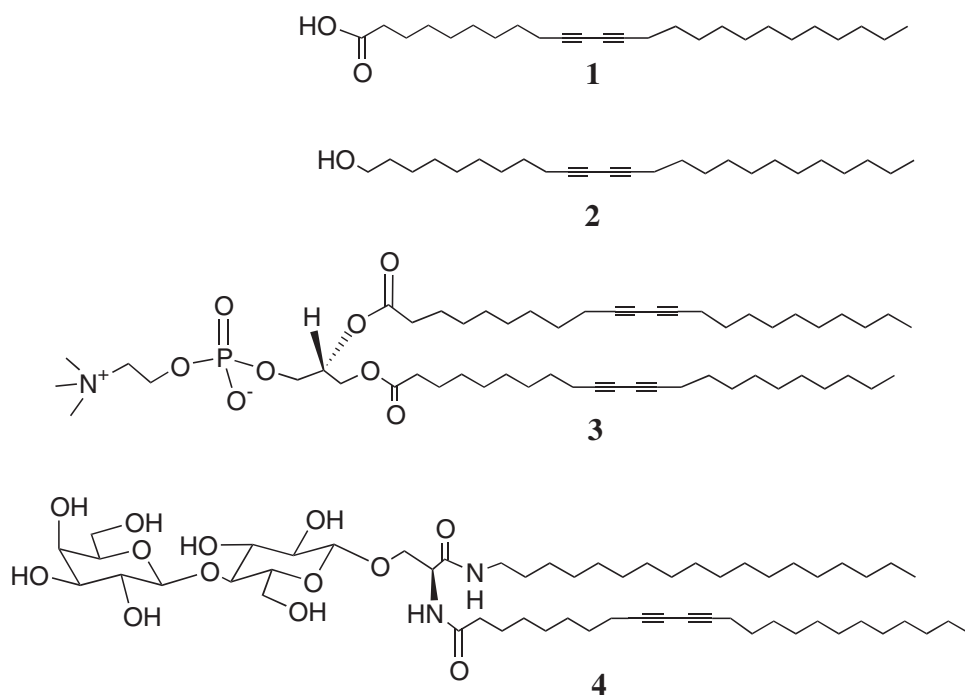
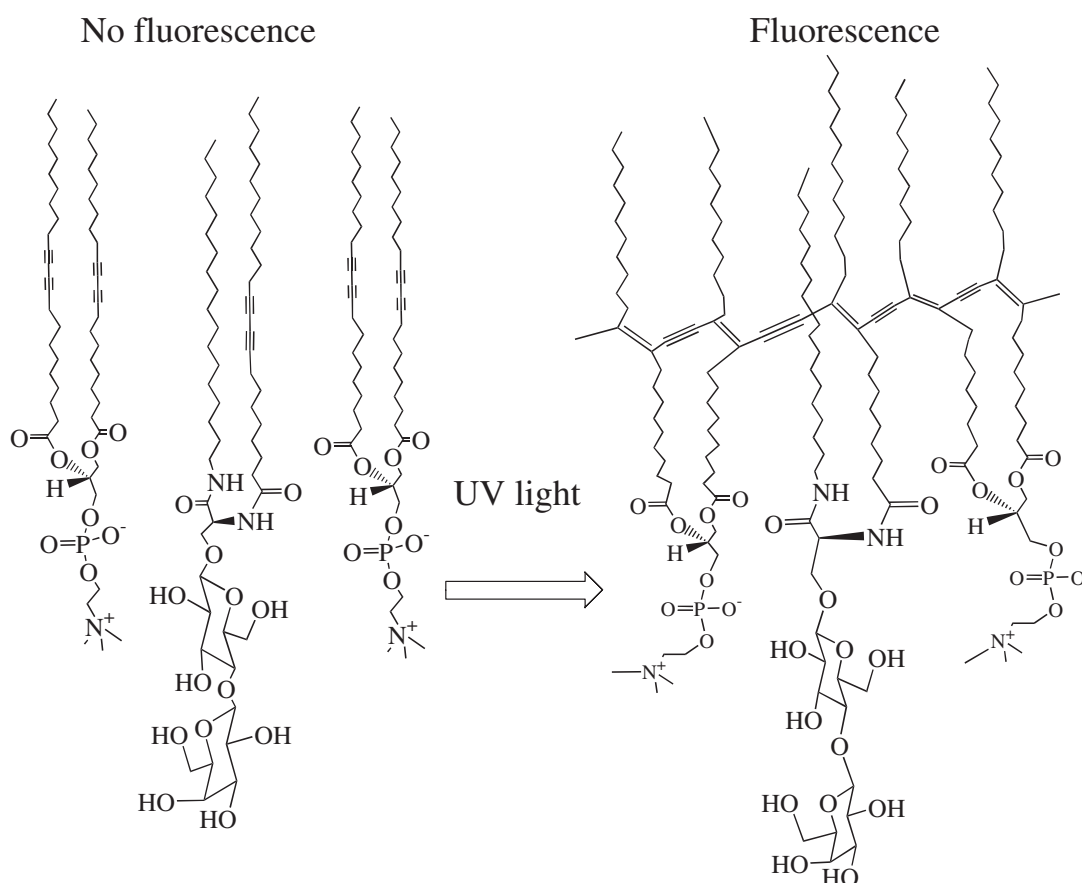
cent detection is generally more sensitive than colorimetric detection. Instead of PDA as a matrix lipid as in Charych's system, when diacetylene-containing phosphatidylcholine derivative **3** was used for the copolymerization as a matrix lipid with glycolipid monomers, the nanoparticles generated relatively strong fluorescence after UV irradiation. The degree of the cross-linkage of the nanoparticle surface was controlled by alternating the photo-irradiation period or adding non-polymerizable lipids to the nanoparticle. The fluorescent response upon binding lectins onto the nanoparticles was evaluated. We also tested the effect of the enzymatic release of oligosaccharides on fluorescent intensities. Here, we report a facile and effective synthetic strategy to construct diacetylene-containing glycolipids by a simple chemical ligation of free carbohydrates with hydroxylamine-attached lipids.

EXPERIMENTAL

10,12-tricosadiynoyl containing phospholipid **3** was purchased from Avanti Polar Lipid, Inc. USA. Winged bean lectin was purchased from Sigma. Fluorescence spectra were measured with an F-2500 spectrophotometer (Hitachi, Ltd., Japan).

Synthesis of Diacetylene-containing Lactose Lipid 4
L-Serineoctadecylamide 7. 10% Pd-C (200 mg) was added to a solution of *N*-Benzyloxycarbonyl-L-

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**Scheme 1.** Chemical structure of photopolymerizable lipids.**Figure 1.** Photopolymerization of diacetylene-containing glycolipids at the surface of vesicle by UV irradiation.

serineoctadecylamide **6** (6.7 g, 13.7 mmol) in 1:1 methanol/chloroform (140 mL). The reaction mixture was stirred for 45 h at 25 °C under a hydrogen atmosphere (0.32 Mpa). The solvent was filtrated and evapo-

rated under reduced pressure: $^1\text{H NMR}$ (CDCl_3): δ 5.12 (s, 1H, NH), 3.92 (m, 1H, NH_2CHCO), 3.24 (m, 2H, NH_2), 1.53 (m, 2H, NHCH_2), 1.27 (m, 30H, CH_2), 0.89 (t, 3H, CH_3). TOF MS m/z 357.85 [$\text{M} +$

H]⁺ (Calcd. for C₂₁H₄₅N₂O₂⁺: 357.35).

N-(Benzophenone)-*L*-serineoctadecylamide **8**.

L-Serineoctadecylamide **7** was added to a solution of benzophenone imine (5.62 g, 31.0 mmol) and camphor-10-sulfonic acid (771.40 mg, 3.32 mmol) in 1:1 methanol/dichloromethane (300 mL) at 0 °C. The reaction mixture was warmed to room temperature and stirred for 40 h. The solvent was evaporated under reduced pressure, and washed with saturated sodium hydrogen carbonate and brine, and dried over magnesium sulfate. The mixture was filtrated and concentrated. Then the residue was chromatographed on silica gel with 2:1 0.5% triethylamine hexane/ethylacetate (0.5% triethylamine) as eluent to give **8** (4.42 g, 62%): ¹H NMR (CDCl₃): δ 7.66–7.13 (m, 10H, Ph₂), 4.11 (m, 1H), 3.98 (m, 1H), 3.86 (m, 1H), 1.35–0.98 (m, 30H, CH₂), 0.88 (t, 3H, CH₃). TOF MS *m/z* 521.7 [M + H]⁺ (Calcd. for C₃₄H₅₂N₂O₂: 521.40).

O-(4-*O*-2,3,4,6-Tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1-4)-*O*-[2,3,4,6-tetra-*O*-acetyl-*D*-glucopyranosyl]-*N*-(benzophenone)-*L*-serineoctadecylamide **10**.

Trifluoromethanesulfonic acid (75 μL, 0.845 mmol) was added to a suspension of *O*-(4-*O*-2,3,4,6-tetra-*O*-acetyl-β-*D*-galactopyranosyl)-(1-4)-*O*-2,3,4,6-tetra-*O*-acetyl-*D*-glucopyranosyl-thiophenol **9** (501.36 mg, 0.69 mmol) and **8** (295.53 mg, 0.83 mmol), and molecular sieves 4 Å in dichloromethane at 0 °C under a nitrogen atmosphere. The reaction mixture was warmed to room temperature and stirred for 15 h. The mixture was filtrated through celite. The filtrate was washed with 10% sodium thiosulfate and saturated sodium hydrogen carbonate and brine, and dried over magnesium sulfate. The mixture was filtrated and concentrated. Then the residue was chromatographed on silica gel with 1:1 hexane/ethylacetate as an eluent to give **10** (743.5 mg, 94.8%): ¹H NMR (CDCl₃): δ 7.66 (d, 2H, Ph), 7.45–7.36 (m, 6H, Ph), 7.12 (d, 2H, Ph), 6.73 (br s, 1H, NH_{ser}), 5.32 (d, 1H, H-4), 5.12 (t, J 9.00, 9.06 Hz, 1H, H-3), 5.08 (t, J 9.00, 9.12 Hz, 1H, H-2), 4.93 (dd, 1H, H-3), 4.87 (t, J 8.28, 8.34 Hz, 1H, H-2), 4.45 (d, J 7.44 Hz, 1H, H-1), 4.41 (d, J 7.92 Hz, 1H, H-1), 4.37 (d, J 11.82 Hz, 1H, H-6b), 4.08–4.03 (m, 3H, H-6a, H-6ab), 3.81–3.76 (m, J 6.72, 6.54, 9.36 Hz, 2H, H-4 and H-5), 3.51 (m, 1H, H-5), 3.27 (m, 2H, –OCH₂), 2.17–1.93 (6s, 21H, 7OAc), 1.26 (m, 34H, 17CH₂), 0.88 (t, 3H, CH₃). TOF MS *m/z* 1138.58 [M] (Calcd. for C₆₀H₈₆N₂O₁₉: 1138.58).

β-*D*-Lactopyranosyl-*N*-(benzophenone)-*L*-serineoctadecylamide **11**. Sodium methoxide (3.36 mg, 0.06 mmol) was added to a solution of compound **10** (235.5 mg, 0.21 mmol) in dry methanol (50 mL) and the mixture was stirred for 4 h at room temperature. The solution was neutralized with Amberlite IR-120 resin, filtered and concentrated: TOF MS *m/z*

868.19 [M+Na]⁺ (Calcd. for C₄₆H₇₂N₂O₁₂Na⁺: 867.50).

β-*D*-Lactopyranosyl-*L*-serineoctadecylamide **12**.

Pd–C (48 mg) was added to the residue in methanol (80 mL) and stirred for 15 h at room temperature under a hydrogen atmosphere. The mixture was filtrated through celite and concentrated to generate **4**: TOF MS *m/z* 681.4 [M + H]⁺ (Calcd. for C₃₃H₆₅N₂O₁₂⁺: 681.45).

N-(10,12-Pentacosadiynoyl)-*O*-β-*D*-lactopyranosyl-*L*-serineoctadecylamide **4**. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) (140 mg, 0.73 mmol) and 10,12-pentacosadiynoic acid (165 mg, 0.44 mmol) were added to a solution of **12** (140.7 mg, 0.21 mmol) and *N*-ethyl-diisopropylamine (12.8 mg, 0.10 mmol) in dry methanol (50 mL) and chloroform (10 mL). The mixture was stirred for 18 h at room temperature. The solvent was evaporated and chromatographed on silica gel, eluted with 10:1–6:1 v/v chloroform/methanol to afford pure **4** (90.2 mg, 42%) as a white solid; ¹H NMR (CD₃OD/CDCl₃ = 1:1): δ 4.34 (d, 1H, H-1, *J* = 7.8 Hz), 4.29 (d, 1H, H-1, *J* = 7.75 Hz), 4.15 (dd, 1H, –OCH₂CHNH), 3.30 (t, 1H, H-2, *J* = 8.05, 8.8 Hz), 3.20 (t, 2H, CH₂NH), 2.26–2.24 (m, 6H), 0.88 (t, 6H, CH₃). ¹³C NMR (CDCl₃): δ 177.6 (NHCO), 173.2 (NHCO), 106.7 (C-1), 106.0 (C-1), 82.7, 80.5, 80.3, 80.0, 78.5, 74.1, 52.0, 51.8, 51.6, 51.5, 51.3, 51.1, 50.9, 34.7 (CH₂), 32.5 (CH₂), 32.4 (CH₂), 32.3 (CH₂), 32.1 (CH₂), 32.0 (CH₂), 31.9 (CH₂), 31.6 (CH₂), 31.1 (CH₂), 25.5 (CH₂CH₃), 21.9, 16.7 (CH₃). ESI-MS *m/z* 1035.9 [M–H][–] (Calcd. for C₅₈H₁₀₃N₂O₁₃: 1035.75).

General Procedure for Chemoselective Ligation

Hydroxylamine-attached lipid **3** (5 mg, 9.1 μmol) and carbohydrates, **a** or **b** (1–5 mg), were mixed in methanol (0.7 mL) at room temperature. The mixture was stirred for 24 h and the solvent was evaporated. The residue was purified by silica gel chromatography, eluted with 6:4 (v/v) chloroform/methanol to afford glycolipids (**5a** and **5b**) in 60–80% yield. The ratios of *trans*- to *cis*-isomers for **5a** and **5b** were 20:1 and 3:1, respectively, as judged from the area of signals corresponding to oxime protons [*trans*-isomer: δ 7.73 (d, *J* = 5.65 Hz); *cis*-isomer: δ 6.98 (d, *J* = 5.00 Hz)] in the NMR spectra in a mixed solvent of CDCl₃ and CD₃OD.

N,*O*-(10,12-Pentacosadiynoyl)-*N*-(α-*D*-mannopyranosyl)hydroxylamine **5a**. ¹H NMR (CD₃OD/CDCl₃ = 1:1): [*trans*-isomer: δ 7.73 (d, *J* = 7.55 Hz); *cis*-isomer: δ 7.00 (brs)], 7.62 (d, 5.13 (1H, H-1), 5.11 (1H, H-1), 4.85 (1H, H-1), 4.79 (1H, H-1), 2.25 (t, 3H, *J* = 6.05, 6.60 Hz), 0.89 (t, 3H, CH₃). TOF MS *m/z* 1267.57 [M + Na]⁺ (Calcd. for C₅₈H₉₇N₃-

O₂₅Na⁺: 1266.61).

N,O-(10,12-Pentacosadiynoyl)-*N*-(sialyl lewis X)-hydroxylamine **5b**. ¹H NMR (CD₃OD/CDCl₃=1:1): [*trans*-isomer: δ 7.73 (d, *J* = 5.65 Hz); *cis*-isomer: δ 6.98 (d, *J* = 5.00 Hz)], 5.06 (d, 1H, H-1, *J* = 5.45 Hz), 5.02 (d, 1H, H-1, *J* = 3.5 Hz), 4.51 (d, 1H, H-1, *J* = 7.8 Hz), 2.83 (d, 1H, *J* = 9.16 Hz), 2.25 (t, 4H, *J* = 6.85, 6.90 Hz), 0.90 (t, 3H, CH₃). TOF MS *m/z* 1258.13 [M+Na]⁺ (Calcd. for C₅₈H₉₇N₃-O₂₅Na⁺: 1258.63).

Preparation of Polymerized Liposomes

Glycolipid **5a** (6.47 mg, 5.20 μmol) and 10,12-tricosadiynoyl containing phospholipid **3** (41.39 mg, 45.2 μmol) were dissolved in CHCl₃/MeOH (9/1) and the solvent was evaporated to yield a thin lipid film on the inner surface of the flask. Deionized water was added to the flask to adjust the concentration of the lipid to 0.3 mM. The aqueous suspension was heated to 70 °C and sonicated using a probe-type sonicator (Sonifier II, Branson Ultrasonics Corp.) for 10 min. The warm, clear solution containing the liposome was then cooled to 4 °C and poured into a quartz cell. The solution was irradiated using a UV lamp (254 nm, 8 W, 100 V) under an argon atmosphere. The distance of the lamp from the reaction cell was 10 cm.

SEM Observation of Photopolymerized Liposomes

The morphological features of the fully photopolymerized liposomes (**5a/3**) were examined by Scanning Electron Microscope (SEM). The liposome solution in water was placed on aluminum foil and then desiccated using vacuum drying, and the residue was coated with Pt/Pb (300 Å in thickness) with an E-102 Hitachi Ion Sputter. SEM pictures were taken using an S-2250N Hitachi Scanning Electron Microscope, 15–25 kV/90–120 mA.

Fluorescence Sensing of the Binding of Lectins onto Nanoparticles

Six samples of the solution (2.5 mL) of polymerized liposomes in 10 mM Tris buffer (pH 7.6) were prepared in quartz cells. Concentrated lectin (1 mg/100 μL) was added to each cell to be the desired concentrations. After equilibrium for 10 min at 25 °C, the fluorescence spectrum of each sample from 400 to 700 nm was recorded by excitation at 380 nm.

RESULTS AND DISCUSSION

Synthesis of Photopolymerizable Glycolipids

We previously reported the synthetic route to diacetylene-containing glycolipids.⁶ Using a similar synthetic route, lactose-appending lipid **4** was synthe-

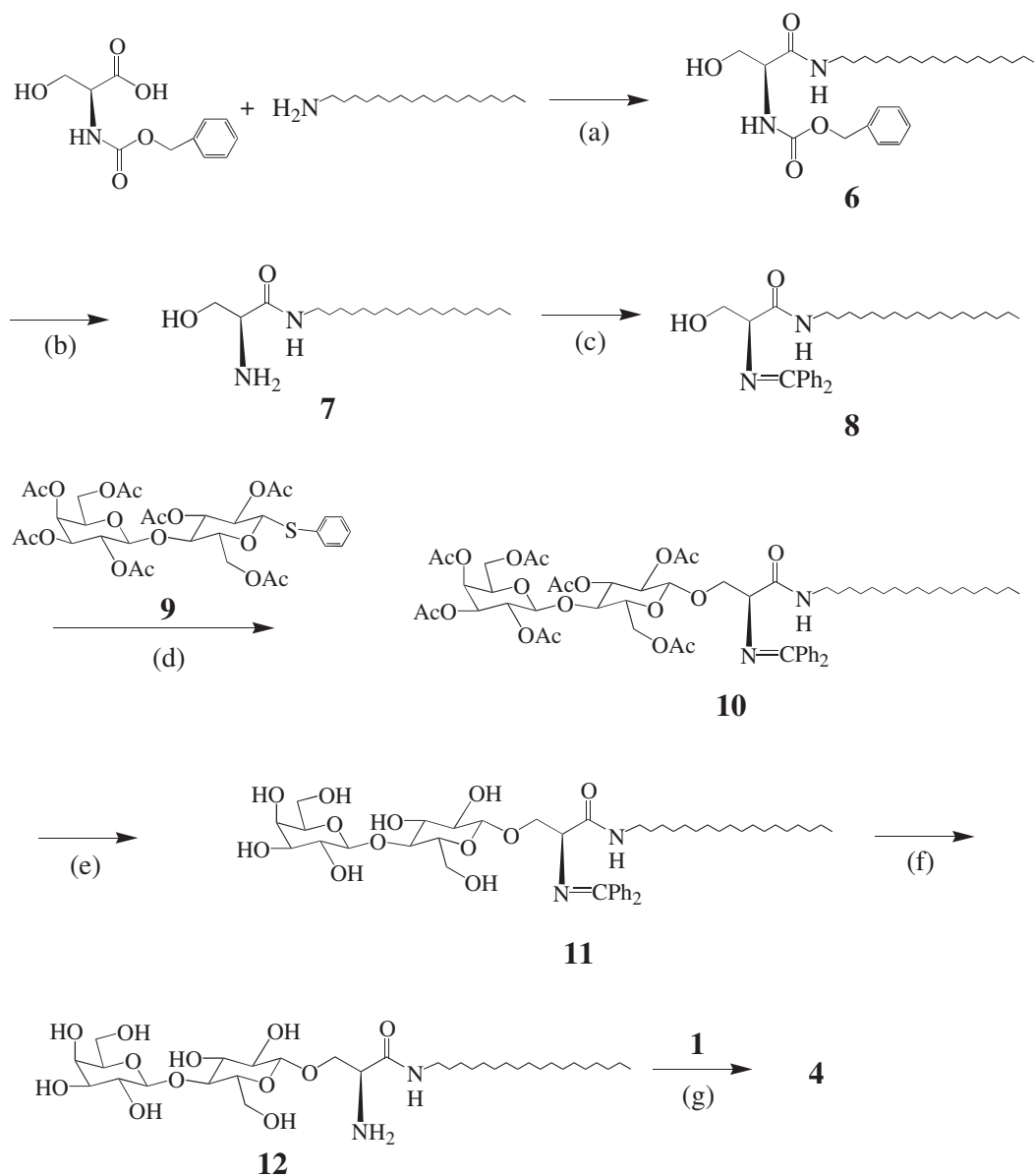
sized from L-serine (Scheme 2). A glycosylation of **8** proceeded smoothly to give **10** in 95% yield when an imine-type protection using *N*-diphenylmethylenamine was employed. However, Boc-protection of the amino group of **7** generated only trace amounts of glycosylated compounds, indicating nucleophilicity of the OH group was reduced by hydrogen-bonding with the hydrogen on the amine. When the structure of the sugar moiety is more complicated, the synthesis of sugar moieties using stepwise protection–deprotection chemistry is a time-consuming process. Thus, we chose the synthetic strategy using a chemical ligation between free sugars and hydroxylamine-attached lipids. The specific feature of free carbohydrates is the hemiacetal terminus, which can readily react with hydroxylamine groups to form a stable oxime linkage.^{7,8} For the effective displaying of a variety of sugars on the surface of the nanoparticles, diacetylene-containing glycolipids were synthesized by single-step coupling with free carbohydrates as shown in Scheme 3. The coupling of lipid **5** and relatively large oligosaccharide molecules, such as mannopentaose and sialyl Lewis X, proceeded smoothly in methanol to give new glycolipids **5a** and **5b** in good yield. Although NMR spectroscopy shows that this method generated a mixture of *cis*- and *trans*-isomers in the oxime bond, it nevertheless has the great advantage of simplicity in that unprotected sugars can be used.

Preparation of Photopolymerized Glyconanoparticles

According to a conventional method of vesicle preparation, a suspension of a mixture of lipid **3** and glycolipid (1/9 mol/mol) in pure water was heated to 70 °C and sonicated (see experimental section). The diacetylene group was polymerized by UV irradiation at 254 nm under an argon atmosphere, and then the color of the vesicular solution turned pale yellow. The fluorescence intensity increased with UV irradiation and became nearly saturated after 1 h, generating spectra with a maximum fluorescence at 460 nm upon excitation at 380 nm. The polymerized vesicles were visualized by scanning electron microscope (SEM), which showed that the vesicles formed nanoparticles with a diameter of around 250 nm (Figure 2).

Fluorescent Detection of the Binding of Lectin onto Glyconanoparticles

Binding of RCA120, which has four binding sites for lactose, was tested using lactose-displayed nanoparticles that were synthesized by photopolymerization of **3** and **4** (**4/3** = 1/9 mol/mol). The change in fluorescence was recorded upon the addition of RCA120 in Tris buffer (pH 7.4, 10 mM). Fluorescence intensity decreased with the increase in the lectin concentration (Figure 3a). Significantly, there was no



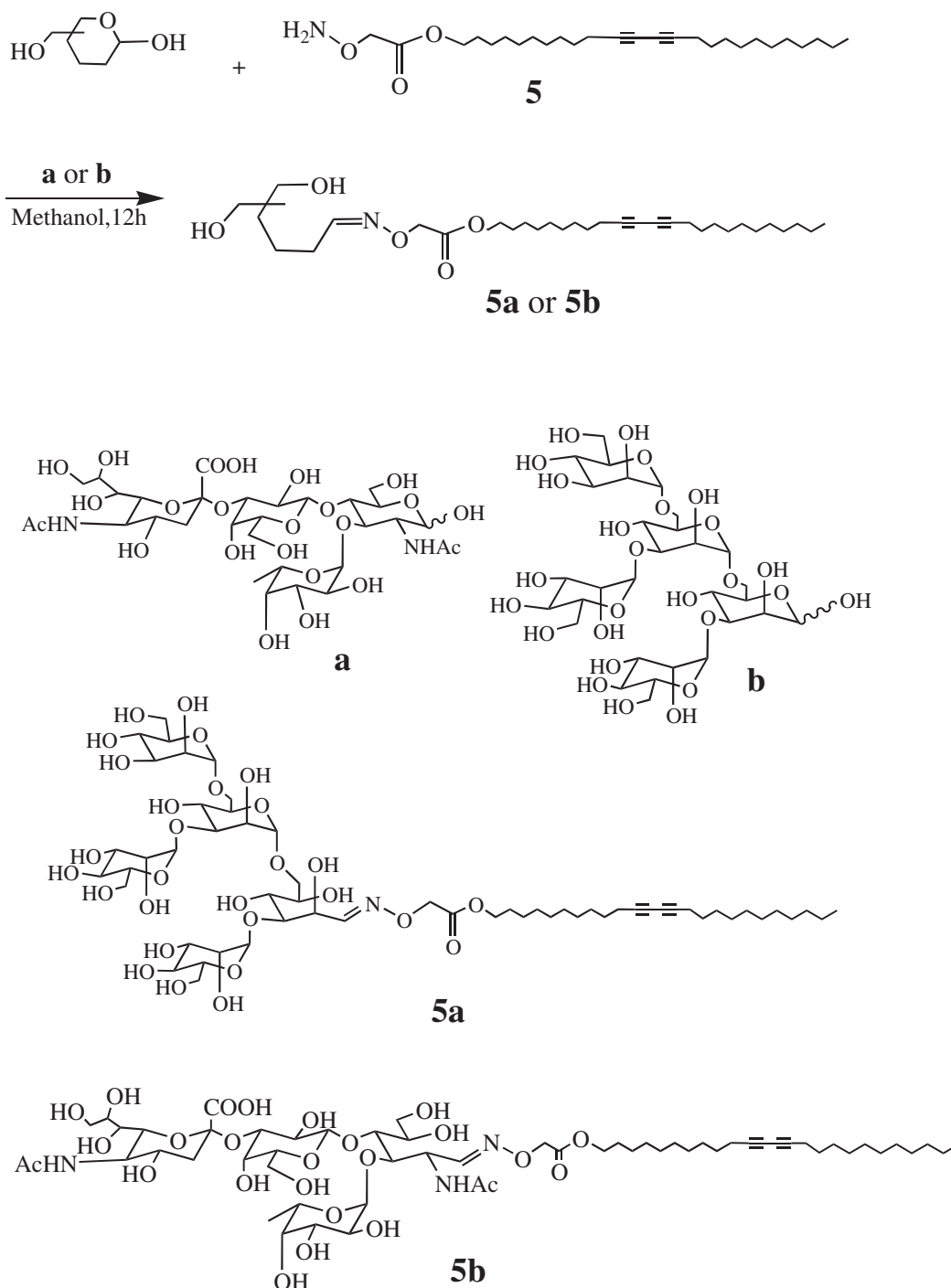
Scheme 2. Chemical synthesis of glycolipid **4**.

(a) EEDQ, benzene, EtOH, 25 °C, 48 h. (b) Pd–C, H₂, 0.32 MPa, CH₃OH, CHCl₃, 25 °C, 45 h. (c) Benzophenone imine, CH₂Cl₂, 25 °C, 40 h. (d) Trifluoromethanesulfonic acid, *N*-iodosuccinimide, molecular sieves 4 Å, CH₂Cl₂, 25 °C, 15 h. (e) Sodium methoxide, CH₃OH, 25 °C, 4 h. (f) Pd–C, H₂, CH₃OH, CHCl₃, 25 °C, 15 h. (g) *N*-Ethyl-diisopropylamine, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, CH₃OH, CHCl₃, 25 °C, 18 h.

change in UV spectra upon the addition of the proteins, showing that there was no aggregation and/or precipitation of the protein–nanoparticle complex by bridging the nanoparticles. As a control experiment, when concanavalinA (ConA), which has a specific affinity to mannose, was added to the solution of lactose-displayed nanoparticle, no change in fluorescence intensity was observed (Figure 3b). In addition, when RCA120 was added to the solution of nanoparticles prepared only from the matrix lipid **3**, no change in fluorescence intensity was observed (Figure 3c). These two control experiments clearly indicate that the fluorescent change in Figure 3a is based on the specific interaction between RCA120 and lactose res-

idues. The concentration-dependency is summarized in Figure 3d. We attempted similar experiments using carboxylic acid-type **1** or neutral-type **2** as the matrix lipid of glyconanoparticles instead of **3**; however, not negligible change in fluorescence by non-specific binding was observed. This suggests that the use of a phosphatidylcholine-type matrix is of key importance in gaining the signals due to specific recognitions.

Binding of E-selectin, which has a single binding site for the tetrasaccharide sialyl-Lewis X (sLe^X), was tested using the sLe^X-displayed nanoparticle. E-selectin is a vascular cell surface protein which mediates leukocyte rolling on the blood vessel wall and is



Scheme 3. Chemical ligation between free carbohydrates and hydroxylamine-attached lipid **5**.

responsible for an early stage of inflammatory response.⁹ Thus, the sensing system of E-selectin binding to sLe^X would be useful in evaluating the inhibitory effects of new blockers against E-selectin. The fluorescence of the solution of sLe^X-displayed nanoparticles also decreased with the increase in E-selectin concentration, whereas no change in fluorescent intensity was induced for the control nanoparticles that were synthesized from the matrix lipid alone (Figure 4a and 4b).

For the binding of concanavalinA (ConA), a lectin

with a high affinity for mannose, similar results with fluorescent changes were obtained. When a lectin solution was added to the mannopentaose-displayed nanoparticles, the fluorescence intensity decreased depending upon the lectin concentration (Figure 4c and 4d). The control nanoparticles constructed using the matrix lipid **3** alone induced no change in fluorescence upon the addition of ConA, indicating there was no non-specific adsorption. In this case also, there was no change in UV spectra upon the addition of ConA at an nM range.

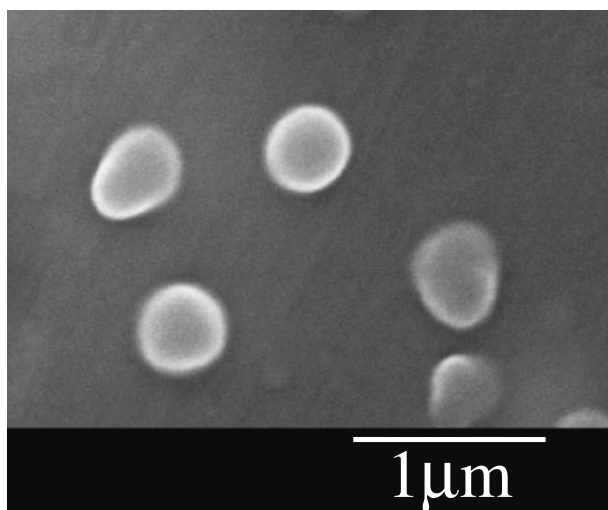


Figure 2. SEM image of the polymerized glyconanoparticles.

The reason for the quenching of fluorescence upon protein binding is not clear at this stage. Since the colorimetric change upon the binding of toxins seems to be caused by the reorientation of lipids and a reduction in the length of the conjugated lipid backbone due to distribution of the polymer network.¹⁰ The reason for the change in fluorescence can be similarly speculated. Therefore, we consider that a reduction in the flexibility of the conjugated lipid backbone after the binding of proteins on the surface of the nanoparticles may lead to a decrease in fluorescence.

To prove our speculation, we modulated the UV-irradiation period to control the degree of cross-linkage in the membrane consisting of the lactose lipid **4** and matrix lipid **3**. When the irradiation period was shortened from 90 to 5 min, the fluorescent response upon the binding of RCA120 was increased [compare Fig-

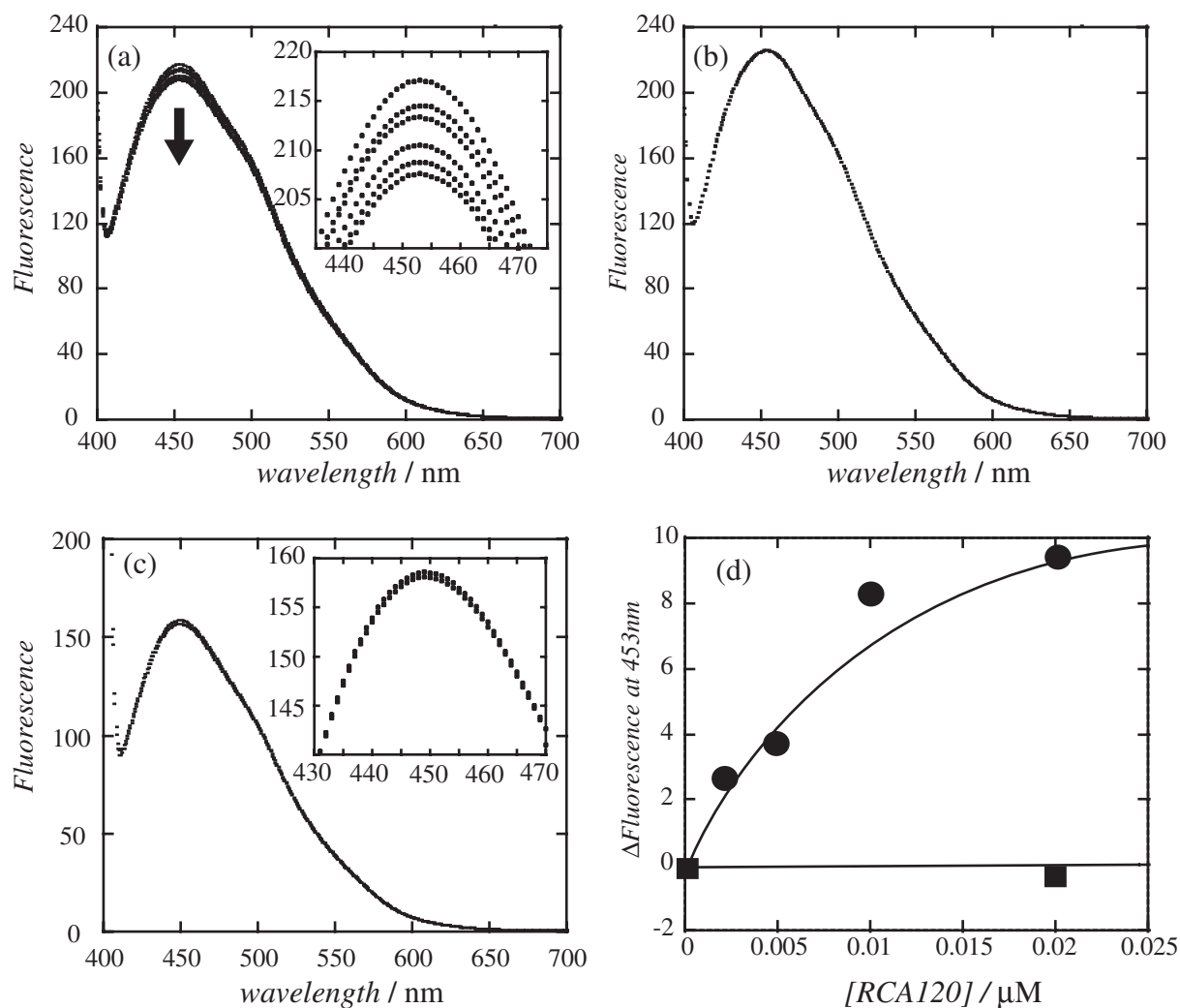


Figure 3. Fluorescent detection of RCA120 using nanoparticle.

(a) Change in fluorescent spectra upon the addition of RCA120 (0, 0.002, 0.005, 0.01, 0.015, 0.02 μ M from top to bottom) to polymerized nanoparticle (**4/3**) in Tris buffer (10 mM, pH 7.4). (b) Change in fluorescent spectra upon the addition of ConA (0, 0.2 μ M from top to bottom) to nanoparticle (**4/3**) in Tris buffer (10 mM, pH 7.4). (c) Change in fluorescent spectra upon the addition of RCA120 (0 and 0.2 μ M) to nanoparticle (**3** alone) in Tris buffer (10 mM, pH 7.4). (d) Change in fluorescence as a function of lectin concentrations for figure (a): (closed circle) and figure (b): (closed square).

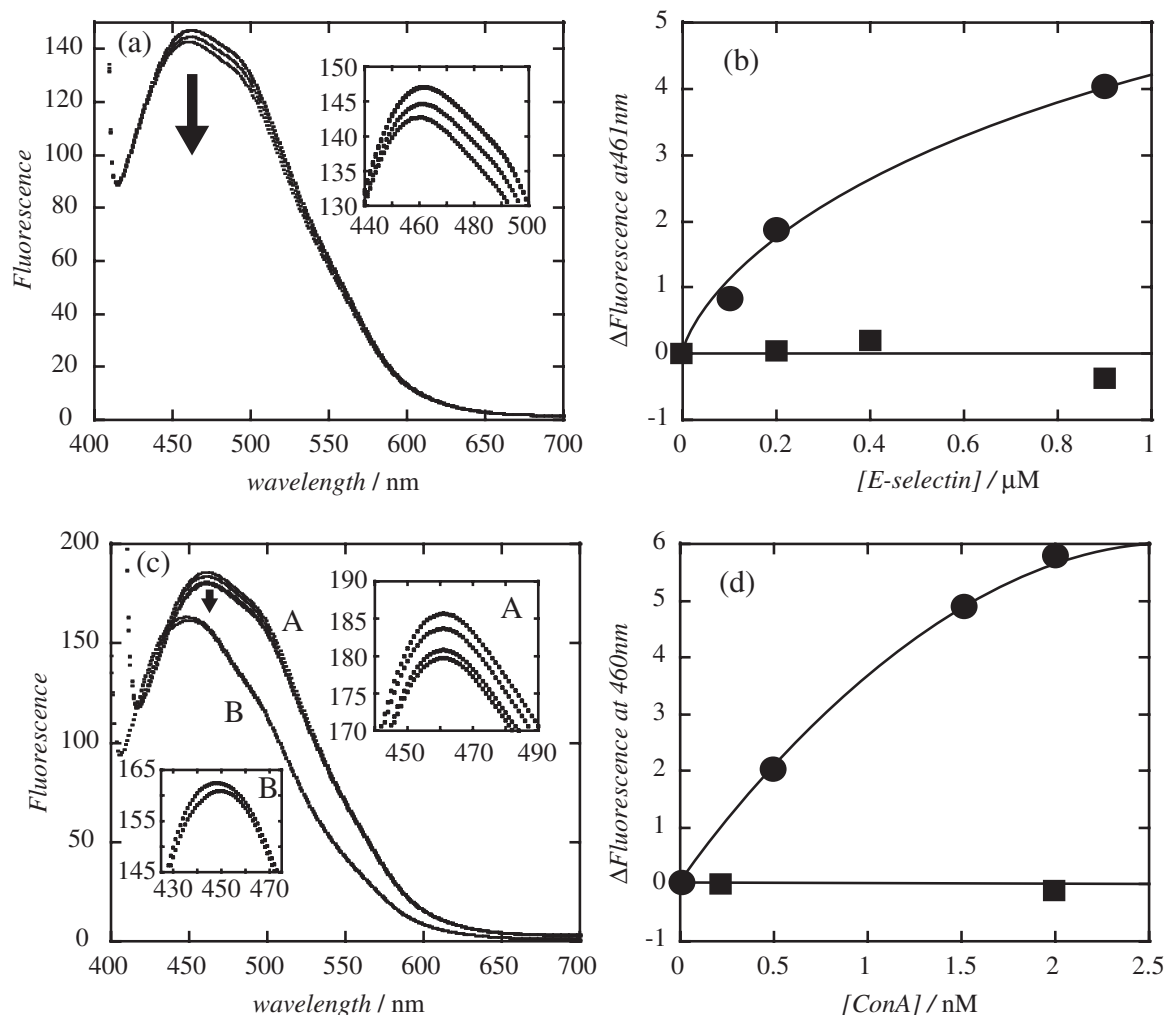


Figure 4. Fluorescent detection of E-selectin and ConA using glyconanoparticles.

(a) Change in fluorescent spectra upon the addition of E-selectin (0, 0.2, 0.9 μM from top to bottom) to polymerized nanoparticle (**5b/3**) in Tris buffer (10 mM, pH 7.4). (b) Change in fluorescence intensity as a function of E-selectin concentration. (closed circle) polymerized nanoparticle of **5b/3**. (closed square) polymerized nanoparticle of **3** only. (c) A: Change in fluorescent spectra upon the addition of ConA (0, 0.5, 1.5, 2 nM from top to bottom) to polymerized nanoparticle (**5a/3**) in acetate buffer (10 mM, pH 5.2). B: Change in fluorescent spectra upon the addition of ConA (0 and 2 nM) to polymerized nanoparticle (**3** only). (b) Change in fluorescence as a function of lectin concentration for A (closed circle) and B (closed square).

ure 3a for 90 min and Figure 5a for 5 min]. This data suggests that the enhanced response of the nanoparticles upon binding is due to the greater mobility of the loosely cross-linked diacetylene network. The effect of irradiation period on fluorescent response is summarized in Table I. When the irradiation period was increased to more than 30 min, only a low response (4%) was observed. This means that the conformation change in the ene-yne backbone of the rigid fully cross-linked membrane cannot be effectively induced during the protein binding.

As another approach to introduce flexibility to the nanoparticles, *L*- α -phosphatidylcholine dioleoyl (DOPC), which has a low phase-transition temperature ($T_c = 20^\circ\text{C}$), was added. The fluorescence spectra after the addition of RCA120 were shown in Figure 5b and the effect of DOPC on the response is

summarized in Table II. The fluorescent response of the DOPC-containing nanoparticles is two times larger than that of nanoparticles without DOPC. As the diacetylene-containing lipids are diluted in the non-polymerizable DOPC liquid phase (binding assay was carried out at 25°C), long-ranged cross-linkage would be inhibited. Therefore, we consider that these nanoparticles have great flexibility due to the loose cross-linkage, resulting in extensive conformation change upon surface protein binding.

We next directed our attention to the monitoring of enzymatic reactions occurring on the surface of the nanoparticles. We monitored the reaction of hydrolysis of manno-oligosaccharides on the nanoparticles catalyzed by mannosidase (*Jack Bean*, Seikagaku Corporation) in acetate buffer (Figure 6). When mannosidase was added to a suspension of the mannopen-

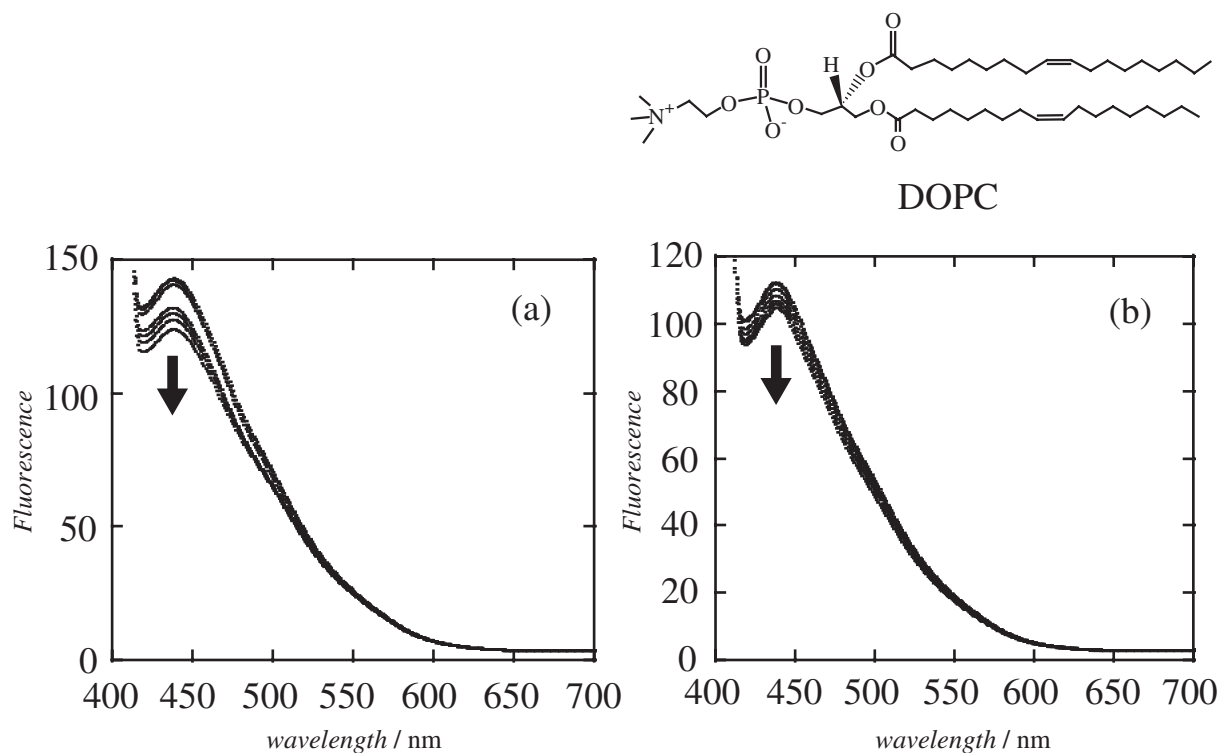


Figure 5. Fluorescent detection of RCA120 using nanoparticles.

(a) Nanoparticle that was prepared by UV irradiation for 5 min. (b) DOPC-containing nanoparticle (3:4:DOPC=80:10:10 mol ratio). [RCA120] = 0 to 20 nM from top to bottom.

Table I. Effect of UV irradiation period on the fluorescence response after the addition of RCA 120 (20 nM) to the nanoparticles (3/4)

UV irradiation time (min)	Fluorescence response (%)
1	8.5
5	12.9
30	4.0
90	4.4

Table II. Effect of DOPC on the fluorescence response after the addition of RCA 120 (20 nM) to the nanoparticles (3/4)

Molecular ratio of the nanoparticles (%)			Fluorescence response (%)
4	3	DOPC	(%)
10	90	0	4.4
10	80	10	3.0
10	40	50	4.2
10	10	80	6.8

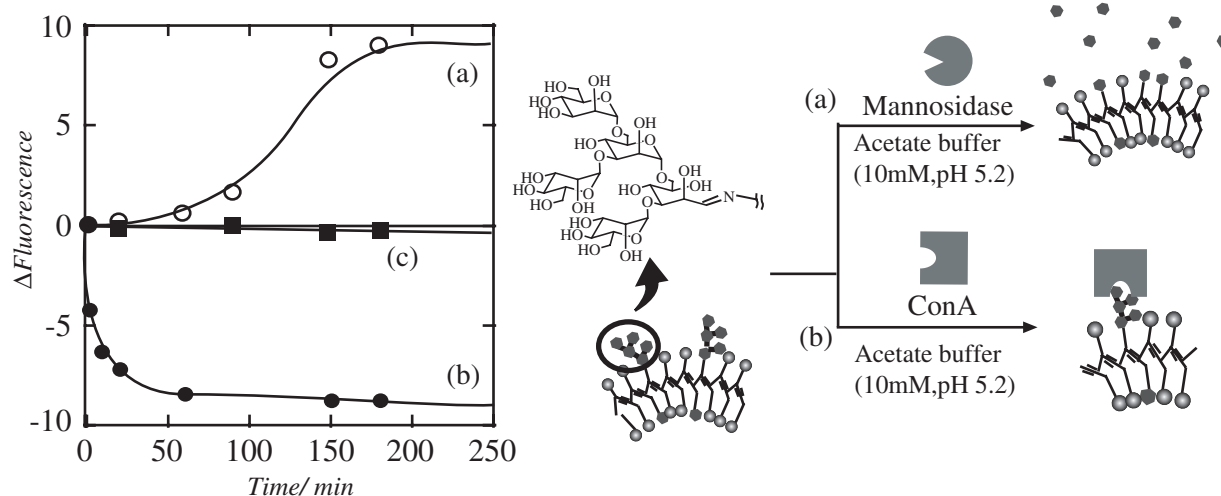


Figure 6. Timecourse of fluorescence intensities from mannopentaose-displayed nanoparticle. (a) After addition of mannosidase from jack bean to the nanoparticle made of 3/4. (b) After addition of RCA120 to the same nanoparticle (3/4). (c) After addition of mannosidase to the nanoparticle made of 3 alone (control).

taose-displayed nanoparticles, fluorescence increased with time. When the nanoparticles made of only matrix lipids were used, no response was observed. However, on the addition of lectins, the change in fluorescence was negative. Therefore, change in fluorescence is speculated to result from changes in stress on the diacetylene network, where the stress is increased or decreased with the binding of ConA or released of mannanpentaose, resulting in respective negative or positive changes in fluorescence (see Figure 6).

SUMMARY

The present method employing fluorescent nanoparticles allowed us to detect the binding of protein receptors too small to change common absorption spectra. Using a phosphatidylcholine-type lipid as a matrix in copolymerization reactions, the non-specific binding of proteins was excluded. The fluorescence transition depends on the quantity of proteins titrated into the solutions containing glyconanoparticles. The nanoparticles having great flexibility due to the loose cross-linkage produced large change in fluorescence. Therefore, the conformation change of the polydiacetylenic moiety induced by surface protein binding would cause the fluorescence change. It is demonstrated that the sensing system using the nanoparticles is applicable to monitoring the time-course of enzymatic reactions occurring on the nanoparticle surface. The fluorescent glyconanoparticles will be useful in the construction of sugar-arrays for the detection of unknown lectins and sugar-related enzymes.

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