Convenient and rapid removal of detergent from glycolipids in detergent-resistant membrane microdomains

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Abstract  Although detergents are often essential in protocols, they are usually incompatible with further biochemical analysis. There are several methods for detergent removal, but the procedures are complicated or suffer from sample loss. Here, we describe a convenient and rapid method for detergent removal from sialic acid-containing glycosphingolipids (gangliosides) and neutral glycolipids in detergent-resistant membrane (DRM) microdomain. It is based on selective detergent extraction, in which the sample is dried on a glass tube, followed by washing with organic solvent. We investigated 18 organic solvents and used high performance thin-layer chromatography (HPTLC) and matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOF MS) to confirm that dichloroethane (DCE) was the most suitable solvent and completely removed the nonionic detergent Triton X-100. Furthermore, DCE extraction effectively removed interference caused by other nonionic, zwitterionic, or ionic detergents in MALDI-QIT-TOF MS analysis.

Gangliosides are sialic acid-containing glycosphingolipids that are abundant in the plasma membrane (1, 2). They are composed of a hydrophilic carbohydrate chain and a hydrophobic ceramide moiety (1, 2). The carbohydrate chain protrudes toward the exterior of the cell, and the ceramide moiety functions to anchor the ganglioside in the membrane. Recent studies have demonstrated that various signal transductions are frequently regulated by changing the ratio of the lipid component in the membrane, especially gangliosides, and that ceramide molecular species of gangliosides contribute to changes in membrane fluidity and various receptor distributions (3, 4). In a state of tumor necrosis factor (TNF)-induced insulin resistance in 3T3-L1 adipocytes, the inhibition of insulin metabolic signaling is attributed to the elimination of the insulin receptor from the immobile caveolae microdomain via increased levels of ganglioside GM3 (5, 6). Destruction of detergent-resistant membrane (DRM) microdomains in mouse cerebellum is caused by the knock-out of ganglioside synthase with a decrease in intensity depending on the genetic defect, inducing inflammation via the complement system and abnormal proliferation of astrocyte and glia (7). Therefore, there has been tremendous interest in ganglioside-rich membrane microdomains over the past two decades; biochemical isolation and detailed structural characterization of gangliosides are required for functional elucidation of the molecules that exist in the DRM (7-10). Nonionic detergents, such as Triton X-100, Brij 58/97, and Nonidet P-40 (NP-40), have commonly been used for the extraction of membrane microdomains from cells. However, residual detergents often complicate further biochemical analysis and interfere with the development of glycobiological research (11-13). There are several detergent-removal methods, such as ethyl acetate extraction, dichloromethane extraction, and commercially available spin columns, but they are designed for proteomic investigations and are either laborious or suffer from sample loss (14-17).

In the present study, we developed a convenient and rapid method based on the extraction of nonionic detergents with organic solvents from ganglioside extracts in glass tubes.

Abbreviations: DCE, dichloroethane; DRM, detergent-resistant membrane; HPTLC, high performance thin-layer chromatography; MALDI-QIT-TOF MS, matrix-assisted laser desorption/ionization quadrupole ion trap time-of-flight mass spectrometry.

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**EXPERIMENTAL PROCEDURES**

**Materials**

GM3 (Neu5Acα2-3Galβ1-4Glcβ1-1′Cer) prepared from bovine brain was purchased from HyTest, Ltd. (Turku, Finland). Neutral lipids prepared from human erythrocyte were donated by Dr. Yasunori Kushi (Department of Materials and Applied Chemistry, Nihon University). Benzene was purchased from Kekusen Chemical Co. (Tokyo, Japan). 1-Butanol, cyclohexane, and 1,2-dichloroethane (DCE) were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Other organic solvents and detergents were purchased from Nacalai Tesque (Kyoto, Japan).

**Cell line and culture conditions**

Murine 3T3-L1 preadipocytes were cultured and maintained as described previously (18). Briefly, cells were seeded and grown in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% calf serum and passaged when the culture reached 70% confluence.

**Sucrose gradient centrifugation**

DRM microdomains were fractionated from 3T3-L1 preadipocytes as described previously (6). Briefly, 3T3-L1 preadipocytes were washed with phosphate buffered saline (PBS) and lysed in 2 ml of TNE buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM ethylenediaminetetra-acetic acid) containing protease inhibitors, 2 mM Na3VO4, and 0.08% Triton X-100. Lysates were centrifuged for 5 min at 1,500 g to remove nuclei and large cellular debris, and the supernatants were diluted with equal volumes of 85% (w/v) sucrose in TNE buffer. The diluted lysates in an ultracentrifuge tube were overlaid with 4 ml of 30% sucrose (w/v) in TNE buffer, followed by 4 ml of 5% sucrose (w/v) in TNE buffer. The samples were centrifuged at 39,000 rpm for 18 h in an SW41 rotor (Beckman Instruments, Palo Alto, CA); 1 ml fractions were collected from the top, desalted by a Sep-Pak C18 cartridge, and analyzed by high performance thin-layer chromatography (HPTLC) and matrix-assisted laser desorption/ionization quadropole ion trap time-of-flight mass spectrometry (MALDI-QIT-TOF MS). All steps were carried out at 4°C.

**Classical preparative column chromatography**

DEAE A-25 sephadex, Iatrobeads, and Florisil column beads were packed into a standard glass Pasteur pipette (60 mm × 6 mm i.d.). To confirm the detergent removal ratio, GM3 (4 μg) and Triton X-100 (4 mg) mixtures were applied and washed with each solvent system as described previously (19).

**Detergent extraction with organic solvent**

To confirm the detergent extraction ability from the ganglioside of the organic solvent, GM3 (4 μg) and Triton X-100 (4 mg for MS, 30 μg for HPTLC) were mixed and dried in Pyrex glass tubes. The GM3-Triton X-100 mixture was washed three times with 2 ml of various organic solvents. The washing fractions were combined and dried by N2 flow, and the washing and residue fractions were applied to HPTLC or MALDI-QIT-TOF MS, respectively. The fractions of 3T3-L1 preadipocyte cells after the sucrose gradient and desalting by the Sep-Pak C18 cartridge were washed three times with 2 ml of DCE. The residues were analyzed by MALDI-QIT-TOF MS.

**Thin-layer chromatography**

Samples dissolved in chloroform/methanol (C/M, 1:1, v/v) were applied as 5-mm spots to high-performance thin-layer chromatography (HPTLC)-silica gel 60 plates with an aluminum...
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backing (Merck, Darmstadt, Germany). The HPTLC plates were developed with a solvent system of C/M/0.2% aqueous CaCl₂ (60:40:9, v/v/v). The plates were dried, and 0.001% primuline in acetone/H₂O (8:2, v/v) was sprayed evenly onto the plate. The plate was dried and visualized by densitometry (Atto Densitograph, Tokyo, Japan). Identities of the stained lipids and Triton X-100 bands were ascertained by referring to standards. Finally, the cholesterol and glycosphingolipids on the plate were visualized by spraying with orcinol/H₂SO₄ reagent followed by heating.

**MALDI-QIT-TOF MS/MS analysis of glycolipids**

MALDI-QIT-TOF MS was performed on an AXIMA MALDI-QIT-TOF mass spectrometer (SHIMADZU, Kyoto, Japan) equipped with a 337 nm nitrogen laser. MS and MSⁿ spectra were calibrated externally using a peptide calibration standard mixture containing bradykinin ([M+H]⁺ 757.40) and human ACTH (fragments 18–39) ([M+H]⁺ 2465.20) as 1 pmol/µl solutions. The matrix was 2,5-dihydroxybenzoic acid (DHB) at a concentration of 10 mg/ml in water. The gangliosides were dissolved in 2 µl of C/M (1:1, v/v), and matrix solutions were mixed and placed on a target plate for crystallization. Crystallization was accelerated by a gentle stream of cold air.

**RESULTS AND DISCUSSION**

### Confirmation of detergent interference for MALDI-QIT-TOF MS analysis of gangliosides

The presence of detergents is known to interfere with many analytical techniques, including mass spectrometry (14–17, 20). To determine the detection limit of Triton X-100 interference, various concentrations of Triton X-100 were evaluated. The experiments were conducted using MALDI-QIT-TOF MS spectra of gangliosides in the presence of different detergent concentrations.

**TABLE 1. Organic solvents used in this study**

| Solvent             | Molecular Formula | Polarity   |
|---------------------|-------------------|------------|
| Acetonitril         | CH₃CN             | Polar      |
| Methanol            | CH₂OH             | Polar      |
| Acetone             | CH₂COCH₃          | Polar      |
| 1-Butanol           | C₄H₉O             | Polar      |
| Pyridine            | C₅H₅N             | Polar      |
| Tetrahydrofuran     | C₄H₈O             | Polar      |
| Methyl acetate      | CH₃COOCH₃         | Bipolar    |
| Diethyl ether       | (C₂H₅)₂O          | Nonpolar   |
| Hexane              | CH₂CH₂CH₂CH₂CH₂CH₂ | Nonpolar  |
| Cyclohexane         | C₆H₁₂            | Nonpolar   |
| Heptane             | H₃C(CH₂)₃CH₃      | Nonpolar   |
| Toluene             | C₆H₅CH₃           | Nonpolar   |
| Benzene             | C₆H₅              | Nonpolar   |
| Xylene              | C₅H₈C₆H₁₀        | Nonpolar   |
| Diisopropylether    | C₄H₉O             | Nonpolar   |
| Chloroform          | CHCl₃             | Nonpolar   |
| Dichloromethane     | CH₂Cl₂            | Nonpolar   |
| 1,2-Dichloroethane  | CH₂Cl₂HCl         | Nonpolar   |
GM3 were detected at m/z 1225.5 and 1253.5, and ions derived from NeuAc-Hex-dissociated ions were detected at m/z 750.6 and 778.7 (Fig. 2A). Triton X-100 was detected in positive ion mode by a characteristic ion pattern at 44 Da intervals (Fig. 2B). After purification by Sep-Pak C18 cartridge, DEAE sephadex A-25, Iatrobeads, and Florisil column chromatography methods, the peaks derived from residual Triton X-100 were detected in all spectra (Fig. 2C–F). The spectrum obtained after purification by the Sep-Pak C18 cartridge showed a similar pattern to the spectrum of the Triton X-100 standard (Fig. 2B, C). The spectra developed after purification by other chromatography methods showed that the +32-shifted peaks derived from residual Triton X-100 and methanol adduct ions increased after purification by other chromatography methods (Fig. 2D–F). These results indicated that classical column chromatography, except for Sep-Pak C18 cartridge, can remove almost all Triton X-100 from the GM3-Triton X-100 mixture but that the residual Triton X-100 is still too great for MALDI-QIT-TOF MS analysis.

(1 mg, 100 µg, 10 µg, 1 µg, and 100 ng) were analyzed by MALDI-QIT-TOF MS in positive ion mode (Fig. 1A–E). In the MS spectra, the lower detection limit of Triton X-100 was 10 µg (Fig. 1C). Furthermore, the GM3 (100 pmol)-derived ions were detected in the presence of less than 10 µg Triton X-100 (data not shown). These results indicated that Triton X-100 needs to be removed at a concentration range of 1–10 µg for ganglioside analysis by MALDI-QIT-TOF MS.

**Detergent removal from a GM3-Triton X-100 mixture by classical preparative column chromatography**

Several classic column chromatography methods have been used for detergent removal (15). To confirm the removal efficiency of Triton X-100, we analyzed MALDI-QIT-TOF MS spectra of a GM3-Triton X-100 mixture after purification by classic column chromatography. First, we confirmed the MALDI-QIT-TOF MS spectrum of GM3 in the positive ion mode. Although the main peaks derived from NeuAc-dissociated ions were detected at m/z 912.7 and 940.8 in the MS spectrum, molecular-related ions of GM3 were detected at m/z 1225.5 and 1253.5, and ions derived from NeuAc-Hex-dissociated ions were detected at m/z 750.6 and 778.7 (Fig. 2A). Triton X-100 was detected in positive ion mode by a characteristic ion pattern at 44 Da intervals (Fig. 2B). After purification by Sep-Pak C18 cartridge, DEAE sephadex A-25, Iatrobeads, and Florisil column chromatography methods, the peaks derived from residual Triton X-100 were detected in all spectra (Fig. 2C–F). The spectrum obtained after purification by the Sep-Pak C18 cartridge showed a similar pattern to the spectrum of the Triton X-100 standard (Fig. 2B, C). The spectra developed after purification by other chromatography methods showed that the +32-shifted peaks derived from residual Triton X-100 and methanol adduct ions increased after purification by other chromatography methods (Fig. 2D–F). These results indicated that classical column chromatography, except for Sep-Pak C18 cartridge, can remove almost all Triton X-100 from the GM3-Triton X-100 mixture but that the residual Triton X-100 is still too great for MALDI-QIT-TOF MS analysis.

**Fig. 3.** Detergent removal from a GM3-Triton X-100 mixture in glass tubes by washing with organic solvents. HPTLC of the GM3-Triton X-100 mixture after washing with various organic solvents. Nos. 1, acetonitril; 2, methanol; 3, acetone; 4, 1-butanol; 5, pyridine; 6, tetrahydrofuran; 7, methylacetate; 8, diethyl ether; 9, hexane; 10, cyclohexane; 11, heptane; 12, toluene; 13, benzene; 14, xylene; 15, diisopropylether; 16, chloroform; 17, dichloromethane; and 18, dichloroethane. R, residue in glass tube; W, wash fraction.
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Various organic solvents are used in column chromatography to remove detergents, salt, and other contaminants, including chloroform, methanol, hexane, and DCE, that are absorbed onto the columns. Furthermore, ethyl acetate extraction or DCE extraction is used for detergent removal from peptides (14–17). Gangliosides have several hydroxy groups in the oligosaccharide moiety, and therefore, the hydrogen bond interactions between the oligosaccharide moiety of gangliosides and the hydroxy group on a glass surface are thought to form stronger bonds compared to the interactions between detergents and the hydroxy groups on a glass surface. Therefore, we attempted to establish a new method based on selective detergent extraction by washing absorbed ganglioside on a glass tube with organic solvents. In this study, we used 18 organic solvents (Table 1). We confirmed the detergent removal efficiency and sample loss by HPTLC using the described method (Fig. 3). Although

HPTLC analysis of detergent removal efficiency after washing with various organic solvents

Fig. 4. Confirmation of detergent removal efficiency using the selected organic solvent. (A) HPTLC of a GM3-Triton X-100 mixture after fractionation by Svennerholm’s partition or washing with organic solvents. Nos. 1 and 6, Svennerholm’s partition; 2 and 7, methanol; 3 and 8, acetone; 4 and 9, hexane; 5 and 10, DCE washing. L, lower phase; R, residue in glass tube; U, upper phase; W, wash fraction. MALDI-QIT-TOF MS spectra of GM3-Triton X-100 after Svennerholm’s partition or extraction with organic solvent: Svennerholm’s upper (B), methanol (C), acetone (D), hexane (E), and DCE (F). Arrowheads indicate GM3-derived ions.
diisopropylether. Almost all the GM3 was retained on the glass tubes by washing with hexane, heptane, benzene, dichloromethane, or DCE. The nonpolar organic solvents, including hexane, cyclohexane, and heptane, which have low permittivity and low solubility to water, showed incomplete removal of Triton X-100 (Fig. 3, nos. 9–11). Therefore, DCE was the most suitable solvent, and it completely removed the nonionic detergent Triton X-100.

MALDI-QIT-TOF MS analysis of detergent removal efficiency after washing with various organic solvents

Although there are neutral lipids as well as gangliosides in the DRM of biomaterials, NeuAc-dissociated ions were mainly detected in the MALDI-QIT TOF MS spectra. Triton X-100 was completely isolated from mixtures of GM3-Triton X-100 by washing with polar and bipolar organic solvents, almost all the GM3 tended to be lost from the glass tubes (Fig. 3, nos. 1–8). GM3 and Triton X-100 were removed together from the glass surface by washing with methanol, 1-butanol, pyridine, or tetrahydrofuran. GM3 remained on the glass tubes to some extent after washing with acetonitrile, acetone, or methyl acetate; GM3 loss was greater than 50% (Fig. 3, nos. 1–8). However, Triton X-100 was removed by washing with nonpolar organic solvents, and the ganglioside tended to be retained on the glass tubes (Fig. 3, nos. 9–18). Almost all the GM3 was removed from the glass surface by washing with diethyl ether, xylene, or chloroform, and equal quantities of GM3 were removed by cyclohexane, toluene, or diisopropylether. Almost all the GM3 was retained on the glass tubes by washing with hexane, heptane, benzene, dichloromethane, or DCE. The nonpolar organic solvents, including hexane, cyclohexane, and heptane, which have low permittivity and low solubility to water, showed incomplete removal of Triton X-100 (Fig. 3, nos. 9–11). Therefore, DCE was the most suitable solvent, and it completely removed the nonionic detergent Triton X-100.

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Spectrum (Fig. 2A). To distinguish between the peaks derived from NeuAc-dissociated or neutral lipids, the residues were divided into gangliosides and neutral lipids by Svennerholm’s partition and analyzed by HPTLC and MALDI-QIT-TOF MS (Fig. 4A, no. 1, and B). Furthermore, we selected methanol, acetone, hexane, and DCE to analyze the removal efficiency of Triton X-100 by HPTLC and MALDI-TOF MS (Fig. 4A, nos. 2–5, and C–F). Using HPTLC, the Triton X-100 was distributed into the upper phase, and almost all the GM3 was distributed into the lower phase of the Svennerholm’s partition (Fig. 4A, no. 1). Extraction with methanol, acetone, hexane, or DCE showed reproducible results for HPTLC (Fig. 3). MALDI-QIT-TOF MS results showed that the peaks derived from GM3 were detected in residues of acetone and DCE. In the lower phase of Svennerholm’s partition, residues of methanol or hexane, only the peaks derived from Triton X-100 were detected. These results indicated that the removal efficiencies of Triton X-100 by washing with acetone or DCE were high enough to use MALDI-QIT-TOF MS as well as HPTLC analysis.

Fig. 6. HPTLC and MALDI-QIT-TOF MS analyses of cholesterol, glycosphingolipids, and residual Triton X-100 in sucrose gradient fractions isolated from 3T3L-1 preadipocytes after extraction with DCE. (A) The sucrose gradient fractions derived from 3T3L-1 preadipocytes were desalted with a Sep-Pak C18 cartridge and then applied to the HPTLC plates. The HPTLC plates were developed with C/M/0.2% CaCl2 aq. (60/40/9, v/v/v) and visualized with primuline reagent and UV light. This was followed by spraying the HPTLC plate with orcinol-H2SO4 reagent. The cholesterol and glycosphingolipids were visualized by heating at 120°C for 5 min. The asterisk indicates contaminated monosaccharides derived from sucrose. (B) MALDI-QIT-TOF MS spectra of sucrose gradient fraction nos. 1–3 after desalting with a Sep-Pak C18 cartridge; (C) nos. 4 and 5; (D) nos. 6–9; and (E) nos. 10–12. Arrowheads indicate GM3-derived ions.
Half of the quantity was applied to HPTLC; the remainder was separated into gangliosides or other lipids via Svennerholm’s partition, and the resulting lower phase was applied to MALDI-QIT-TOF MS for analysis (Fig. 6).

Using HPTLC, Triton X-100, cholesterol, and other simple lipids were completely removed by DCE washing (Fig. 6A). In the MS spectra, the peaks derived from GM3 were detected in fraction nos. 4–6 and 6–9 (Fig. 6B–E). Furthermore, the detailed structural analyses of the detected GM3 were confirmed by MALDI-QIT-TOF MS n and LC-IT-TOF MS n analyses. The ceramide components of GM3 derived from 3T3-L1 preadipocytes were composed of d18:1–C16:0, C18:0, C20:0, C22:0, C22:1, C23:0, C24:0, and C24:1 (data not shown). These results indicated that DCE extraction was also useful for Triton X-100 removal of gangliosides derived from biological sources.

Recovery confirmation of gangliosides and neutral glycolipids after extraction with DCE

To determine whether the DCE washing can be applied to detergent removal from other gangliosides and neutral glycolipids, we analyzed HPTLC of gangliosides or neutral glycolipids-Triton X-100 mixtures after extraction with DCE. The 3T3-L1 preadipocytes were lysed with lysis buffer containing 0.08% Triton X-100 and fractionated into 12 tubes using a sucrose gradient. These were desalted by the Sep-Pak C18 cartridge, and the presence of Triton X-100 and glycolipids was confirmed by HPTLC and MALDI-QIT-TOF MS (see Experimental Procedures). As shown in Fig. 5, Triton X-100 was detected in the top HPTLC phase, and the concentrations gradually increased with increasing sucrose concentrations. Cholesterol and glycosphingolipids containing gangliosides were detected in fraction nos. 4 and 5 by HPTLC (Fig. 5A). MALDI-QIT-TOF MS spectra showed that the peaks derived from GM3 were not detected due to background peaks derived from Triton X-100 (Fig. 5B–E).

Confirmation of detergent interference for ganglioside analysis by MALDI-QIT-TOF MS after fractionation using a sucrose gradient

Fractions of 3T3-L1 preadipocytes after fractionation using a sucrose gradient and desalting with the Sep-Pak C18 cartridge were washed three times with 2 ml of DCE. The residues were dissolved in 100 μl of C:M (1:1, v/v). Half of the quantity was applied to HPTLC; the remainder was separated into gangliosides or other lipids via Svennerholm’s partition, and the resulting lower phase was applied to MALDI-QIT-TOF MS for analysis (Fig. 6). Using HPTLC, Triton X-100, cholesterol, and other simple lipids were completely removed by DCE washing (Fig. 6A). In the MS spectra, the peaks derived from GM3 were detected in fraction nos. 4–6 and 6–9 (Fig. 6B–E). Furthermore, the detailed structural analyses of the detected GM3 were confirmed by MALDI-QIT-TOF MS n and LC-IT-TOF MS n analyses. The ceramide components of GM3 derived from 3T3-L1 preadipocytes were composed of d18:1–C16:0, C18:0, C20:0, C22:0, C22:1, C23:0, C24:0, and C24:1 (data not shown). These results indicated that DCE extraction was also useful for Triton X-100 removal of gangliosides derived from biological sources.

Recovery confirmation of gangliosides and neutral glycolipids after extraction with DCE

To determine whether the DCE washing can be applied to detergent removal from other gangliosides and neutral glycolipids, we analyzed HPTLC of gangliosides or neutral glycolipids-Triton X-100 mixtures after washing with DCE. Although faint bands derived from ganglioside GD1a and GT1b were detected in the wash fraction, almost all gangliosides and neutral glycolipids were recovered in residue
fractions (Fig. 7A). However, the recovery of GM3 was gradually decreased with increasing Triton X-100 amount (Fig. 7B). These results indicated that DCE extraction was useful for Triton X-100 removal from other gangliosides and neutral glycolipids and that some ganglioside loss was observed in the presence of much higher amounts of Triton X-100.

**Removal of other nonionic, zwitterionic, or ionic detergents by DCE extraction**

We applied the established DCE extraction method to the removal other nonionic, zwitterionic, or ionic detergents. We mixed Brij 58/97, Nonidet P-40 (NP-40), CHAPS, taurocholic acid, and deoxycholic acid with GM3 and extracted their detergents with DCE. As shown in Fig. 8, the DCE extraction effectively removed interference of other nonionic, zwitterionic, or ionic detergents in MALDI-QIT-TOF MS analysis. These results indicated that the DCE extraction can be applied not only to detergent removal from DRM fractions using a variety of nonionic detergents but also to other experiments, such as detergent removal from glycolipids after synthesis by glycosyltransferases.

Recently, to evaluate how membrane heterogeneity regulates receptor distributions or signal transductions, hardware and software of fluorescence microscope imaging have been developed. Spectroscope-stimulated emission depletion microscopy (STED), photoactivated localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), and structured illumination microscopy (SIM) were developed as hardware (21, 22). Fluorescence resonance energy transfer (FRET), fluorescence recovery after photobleaching (FRAP), single-particle tracking (SPT), fluorescence correlation spectroscopy (FCS), point scan-FCS, and raster image correlation spectroscopy (RICS) were developed for fluorescence microscopy applied to the dynamics of membrane molecules (21, 22). Although these new approaches are powerful tools for the aforementioned research fields, unresolved problems still remain. For example, fluorescence labeling of the ganglioside is challenging because of steric hindrance, and there are thousands of species in membrane lipids. Furthermore, it is well known that membrane lipids are mobile even after chemical fixation (23). Therefore, other classical approaches, including biochemical isolation and analysis of molecules in the DRM, are still required for functional elucidation of gangliosides. Here, we described the successful application of detergent removal using DCE extraction in the analysis of gangliosides and neutral glycolipids in DRM. The established method does not require expensive instruments or machines and can effectively remove nonionic, ionic, or zwitterionic detergents using a simple procedure.

**CONCLUSIONS**

We presented results that suggest that DCE extraction is a convenient and rapid method for detergent removal
from glycolipids. Although some sample loss was observed in the presence of a much higher amount of Triton X-100, the peaks derived from glycolipids can be detected in the MS spectrum after DCE extraction. This method can also be used in broader detergent removal applications, such as glycolipid biosynthesis. In the future, this method should be refined and shown to be an automatic method.

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