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The ratio of unsaturated fatty acids in biosurfactants affects the efficiency of gene transfection

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ABSTRACT

An unsaturated hydrocarbon chain in phospholipid was reported to affect a phase transition and a fusogenic activity after mixing membranes, and consequently to achieve a high DNA transfection efficiency. We previously showed that a biosurfactant mannosylerythritol lipid-A (MEL-A) enhances the gene transfection efficiency of cationic liposomes. Here, we have studied the effects of unsaturated fatty acid ratio of MEL-A on the physicochemical properties and gene delivery into cells of cationic liposomes using MEL-A with three different unsaturated fatty acid ratios (9.1%, 21.5%, and 46.3%). The gene transfer efficiency of cationic liposomes containing MEL-A (21.5%) was much higher than that of those containing MEL-A (9.1%) and MEL-A (46.3%). MEL-A (21.5%)-containing cationic liposomes induced highly efficient membrane fusion after addition of anionic liposomes and led to subsequent DNA release. Imaging analysis revealed that MEL-A (21.5%)-containing liposomes fused with the plasma membrane and delivered DNA into the nucleus of NIH-3T3 cells, MEL-A (46.3%)-containing liposomes fused with the plasma membrane did not deliver DNA into the nucleus, and MEL-A (9.1%)-containing liposomes neither fused with the plasma membrane nor delivered DNA into the nucleus. Thus, it is understandable that the unsaturated fatty acid ratio of MEL-A strongly influences the gene transfection efficiency of cationic liposomes.

1. Introduction

There is an interesting report to show that the carrier lipid composed of unsaturated fatty acid is more effective as a gene transfection agent than that of saturated fatty acid using synthesized two cationic phospholipid derivatives (Koynova et al., 2006). In that paper, it is described that the saturated carrier lipid remained lamellar in mixtures with biomembrane-mimicking lipid formation, while the unsaturated lipid exhibited a lamellar–nonlamellar phase transition. Their results suggested that the saturation level of hydrocarbon chain in carrier lipids is strongly involved with gene transfection efficiency by affecting the intracellular phase transition properties.

We previously found that a biosurfactant mannosylerythritol lipid-A (MEL-A), which has two hydrocarbon chains, enhanced DNA transfection efficiency mediated by cationic liposomes (Inoh et al., 2001). MEL-A, a major component of MELs produced by the yeast strain *Candida antarctica* T-34, has surface active properties as well as cell differentiation activities against human leukemia cells, mouse melanoma, and PC12 cells (Kitamoto et al., 1993; Isoda et al., 1997; Wakamatsu et al., 2001). In addition, we have shown that monolayers composed of L-α-dipalmitoylphosphatidylcholine (DPPC) containing MEL-A have greater membrane fluidity than those containing DPPC alone (Kitamoto et al., 2009) and that the unsaturated fatty acids in MEL-A significantly influence surface pressure and packing density in the monolayer (Imura et al., 2008). These results suggest that the fatty acid content, unsaturated fatty acids in particular, of MEL-A affects the physicochemical properties of MEL-A and MEL-A/lipids.

In gene delivery, cationic liposomes are considered as a promising means of introducing foreign genes into target cells due to their high transfection efficiency, low toxicity, ease of preparation, and targeted application (Felgner et al., 1989; Farhood et al., 1992; Lasic, 1998; Nakanishi, 2003; Nishiyama et al., 2005; Kogure et al., 2007) and the physicochemical properties of cationic liposomes, such as lipid packing density, shape, and zeta-potential, have a significant effect on gene transfection efficiency (Takeuchi et al., 1996; Xu et al., 1999; Lin et al., 2003; Wittenberg et al., 2008). Aiming to develop a safe and efficient non-viral vector, in this paper, we have studied the effect of MEL-A unsaturated fatty acids on gene transfection efficiency to target cells. Here we prepared three types of MEL-A with different unsaturated fatty acid ratios (9.1%, 21.5%, and 46.3%), which are known to show the different monolayer packing densities (Kitamoto et al., 2009), and tried to determine the effect

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of unsaturated fatty acid ratios of MEL-A on the physicochemical properties of cationic liposomes and gene delivery into target cells.

2. Materials and methods

2.1. Materials

The synthesis of a cationic cholesterol derivative, cholesteryl-3β-carboxyamido ethylene-*N*-hydroxyethylamine (OH-Chol), has been described in our previous study (Okayama et al., 1997). 1,2-Dioleoyl-*sn*-glycero-3-phosphatidylethanolamine (DOPE) was purchased from Sigma (St. Louis, MO, USA). Rhodamine-conjugated phosphatidylethanolamine (PE) was obtained from Molecular Probes (Eugene, OR, USA) and FITC-conjugated c-myc antisense DNAs (5′-CACGTTGAGGGGCAT-3′; phosphorothioate) from Nihon Gene Research Laboratories (Sendai, Japan). Luciferase plasmid (pGL-3) was purchased from Promega (Madison, MI, USA) and DNase I, ethidium bromide, and EDTA-2Na were purchased from Wako (Osaka, Japan). Triton X-100 was purchased from Sigma (St. Louis, MO, USA).

The MEL-producing yeast strain *Candida antarctica* T-34 was isolated from the exudate of a tree from Mt. Tsukuba, Japan. MEL-A (4-*O*-[(4′,6′-di-*O*-acetyl-2′,3′-di-*O*-alkanoyl)-β-D-mannopyranosyl] meso-erythritol) was synthesized by growing the yeast on a fermentation medium consisting of 8% (v/v) soybean oil, 0.2% $NaNO_3$, 0.02% KH_2PO_4, 0.02% $MgSO_4(7H_2O)$, 0.1% yeast extract, and tap water. MEL-A was extracted from the reaction medium with ethyl acetate. Crude MEL-A was purified by silica gel column chromatography (Wako-gel C-200) using a chloroform–acetate (9:1 to 5:5) mixture as the solvent system.

2.2. Preparation of liposomes

DOPE (20 nmol) was combined with OH-Chol (30 nmol) and the three types of MEL-A (10 nmol) in chloroform and dried with N_2 gas under reduced pressure to remove the chloroform solvent. The lipid film was hydrated with 400 μL of phosphate-buffered saline (PBS) for 1 h. The samples were sonicated in a bath-type sonicator (Branson model B 1200) to generate small unilamellar vesicles according to previously described procedures (Inoh et al., 2001, 2004; Ueno et al., 2007a).

2.3. Cell culture and luciferase assay

NIH-3T3 cells were cultured in DMEM (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Trace, Melbourne, Australia). The target cells were plated on a 60-mm culture dish at a density of 1×10^6 cells/dish. Plasmid pGL-3 DNAs (5 μg) were complexed with the cationic liposomes (400 μL) in the serum-free medium SFM101 (Nissui, Tokyo, Japan) for 15 min and then the complexes were incubated with the target cells for 1 h at 37 °C. The cells were then washed and cultured for 40 h in DMEM at 37 °C. The luciferase assay was performed using the Picagene luciferase assay kit (Toyo Ink, Tokyo, Japan). The transfected cells were washed 3 times with PBS and lysed in a cell lysis buffer. The lysate was centrifuged at 12,000 × *g* at 4 °C for 3 min and the supernatant was subjected to a luciferase assay. The relative light unit (RLU) of chemiluminescence was measured using a luminometer (TD-20/20; Turner Designs; Sunnyvale, CA, USA) and the luminescent RLU values were normalized to the amount of protein determined by BCA assay. The value 1×10^8 RLU represents the activity of approximately 0.1 μM of the luciferase standard. To assess cytotoxicity, MTT assay (Cell proliferation kit I; Roche, Mannheim, Germany) was performed. MTT assay is based on the cleavage of the yellow tetrazolium salt MTT to purple formazan crystals by viable cells. After transfection with MEL-A, 10 μL of MTT labeling reagent was added to each well and incubated for 4 h at 37 °C. After addition of 100 μL of the solubilization solution, incubated overnight at 37 °C. The absorbance at 570 nm was measured by using a plate reader (Model 680; Bio-RAD, Tokyo, Japan).

2.4. Fluorescence resonance energy transfer (FRET) assay for membrane fusion

To study the effects of the cationic liposome containing MEL-A with different unsaturated fatty acids ratios on membrane fusion, we measured the rate of membrane fusion of cationic liposomes complexed with the three types of MEL-As and anionic liposomes (DOPG:DOPE:DOPC = 1:2:1). Anionic liposomes containing 0.5% (mol) NBD-PE and 0.5% (mol) rhodamine-PE were mixed with a 4 times molar concentration of cationic liposomes. Samples were excited at 488 nm and fluorescence spectra (510–600 nm) were obtained using spectrofluorometer (RF5300PC; Shimadzu, Japan). Membrane fusion decreases the efficiency of FRET from NBD to rhodamine, resulting in an increase in the fluorescence intensity of NBD and a decrease in that of rhodamine (Bailey and Cullis, 1997; Struck et al., 1981). The level of membrane fusion was calculated from the NBD fluorescence intensity relative to the maximum NBD fluorescence intensity when the anionic liposomes were completely disrupted by 0.1% Triton X-100.

2.5. Ethidium intercalation assay for cationic liposome plasmid DNA encapsulation efficiency

When ethidium ions are added to a solution of DNA, they intercalate between the base pairs of the DNA double helix and exhibit fluorescence. Ethidium bromide was added (final concentration: 0.5 μg/mL) to cationic liposome–DNA complexes and the fluorescence of ethidium at 595 nm (excited at 520 nm) was monitored continuously using a spectrofluorometer. The amount of DNA protected from ethidium intercalation was calculated from the intensity relative to the maximum intensity obtained when ethidium was added to free plasmid DNA without liposomes.

2.6. DNaseI treatment

To investigate DNA encapsulation by cationic liposomes, DNaseI-mediated digestion of plasmid DNA was evaluated using agarose gel electrophoresis. Cationic liposomes with 100 μL of MEL-A (21.5%) or MEL-A (46.3%) and 1 μg of pGL-3 plasmid DNA formed cationic liposome–DNA complexes in 1 mL of 50 mM Tris buffer (pH 7.4) and were treated with 25 U of DNaseI solution containing 0.9 mM $MnCl_2$ at room temperature for 1 h. Nuclease activity was stopped by addition of 25 mM EDTA and 0.1% Triton X-100. The samples were loaded onto a 1% agarose gel and electrophoresed at 100 V for 40 min. Plasmid DNA was stained with ethidium bromide and visualized with a Digital Image Stocker (Toyobo, Osaka, Japan).

2.7. Ethidium intercalation assay for DNA release from cationic liposomes

After cationic liposome–DNA complexes were formed, ethidium bromide (final concentration: 0.5 μg/mL) and anionic liposomes were added to the complexes, and the fluorescence of ethidium at 595 nm (excited at 520 nm) was measured using a spectrofluorometer. The efficiency of DNA release from the cationic liposomes was calculated from the intensity relative to the maximum intensity obtained when ethidium was added to free plasmid DNA without liposomes.

2.8. FRET assay of cationic liposome–DNA complex formation

To investigate the differences in cationic liposome–DNA complex formation between liposomes composed of MEL-A (21.5%) and MEL-A (46.3%), cationic liposomes including NBD-PE and rhodamine-conjugated oligonucleotide DNA were used as a donor and an acceptor, respectively. Fifteen minutes after oligonucleotide DNA was added to the cationic liposomes, the samples were excited at 488 nm and their fluorescence spectra (510–600 nm) were monitored using a spectrofluorometer.

2.9. Confocal laser scanning microscopy

One hour after the liposome–DNA complexes were transfected into the NIH-3T3 cells, the intracellular distribution of oligonucleotide DNA and lipids in liposomes were observed in an observation chamber (Elecon, Chiba, Japan) using a confocal laser scanning microscope (LSM 510; Zeiss, Oberkochen, Germany) at 37 °C. The DNA and lipids were stained with FITC-conjugated oligonucleotide DNA and rhodamine-conjugated PE (3% in lipids composed of liposomes) were excited by an Ar ion laser (488 nm) and a He–Ne laser (543 nm), respectively. The fluorescence of FITC and rhodamine was detected through a band-pass filter at 505–530 nm and 560–580 nm, respectively.

3. Results

3.1. The effects of the MEL-A unsaturated fatty acid ratio on the transfection efficiency of cationic liposomes

To determine whether the MEL-A unsaturated fatty acid ratio affects gene transfection by MEL-A-containing cationic liposomes, liposomes containing three types of MEL-A (9.1%, 21.5%, or 46.3%) were complexed with pGL-3 and incubated with NIH-3T3 cells for 1 h in a serum-free medium. We performed a luciferase assay 40 h after changing the serum-free medium to a serum-based medium (Inoh et al., 2001). The gene transfer efficiency of MEL-A (21.5%)-containing liposomes was much higher than that of MEL-A (9.1%) and MEL-A (46.3%)-containing liposomes, as shown in Fig. 1, indicating that MEL-A (21.5%) has an optimum ratio of unsaturated fatty acids for achieving high transfection efficiency with cationic liposomes. Here we compared the gene transfection efficiency in the case of content of 10 nmol MEL-A to 20 nmol DOPE and 30 nmol OH-Chol. Furthermore, when we investigated the efficiency in the content of 5, 15, and 20 nmol MEL-A, the luciferase activity was quite low (data not shown), as described in our previous paper (Inoh et al., 2001). In addition, no significant cytotoxicity was observed under any transfection conditions used in this study (data not shown).

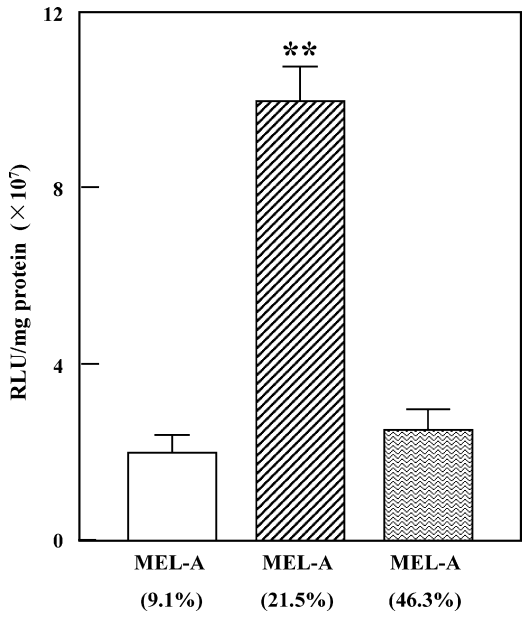

Fig. 1. Effect of different MEL-A unsaturated fatty acid ratios on cationic liposome transfection efficiency. After NIH-3T3 cells were incubated with the liposome–DNA complexes for 1 h, washed, and cultured for 40 h, luciferase expression was measured. The luciferase assay measured the transfection efficiency of cationic liposomes containing MEL-A with three different unsaturated fatty acid ratios, i.e., MEL-A (9.1%), MEL-A (21.5%), and MEL-A (46.3%). All data represent the mean ± S.D. ($n = 3$). $^{**}P < 0.01$ compared with MEL-A (9.1%) and (46.3%) by t-test.

3.2. The effects of the MEL-A unsaturated fatty acid ratio on membrane fusion by cationic liposomes

In our previous study, we showed that membrane fusion between target cells and cationic liposomes plays an important role in achieving high transfection efficiency with MEL-A-containing liposomes (Inoh et al., 2001). We therefore investigated whether the MEL-A unsaturated fatty acid ratio affects membrane fusion with anionic liposomes using fluorescence spectroscopy. Membrane fusion was measured as the disappearance of FRET between two fluorescence-labeled lipids, NBD-PE (donor) and rhodamine-PE (acceptor), in the anionic liposomes by increasing their distance after membrane fusion with cationic liposomes. Therefore, membrane fusion between anionic liposomes and cationic liposomes increased the fluorescence intensity of NBD and decreased the fluorescence intensity of rhodamine. Fig. 2 shows the membrane fusion activity based on the increase in NBD fluorescence intensity at 530 nm at 480 s after the addition of MEL-A containing cationic liposomes. Membrane fusion after the addition of MEL-A (21.5%)- or MEL-A (46.3%)-containing liposomes increased approximately twice as much as that after the addition of MEL-A (9.1%)-containing liposomes. This data suggests that a low level of unsaturated fatty acids in MEL-A results in low membrane fusion activity, and thus, leads to low gene transfection efficiency.

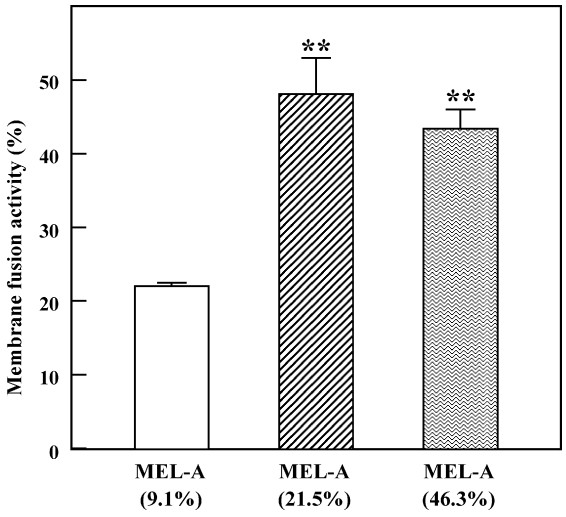

Fig. 2. Effect of different MEL-A unsaturated fatty acid ratios on cationic liposome membrane fusion. The efficiency of membrane fusion between liposomes containing MEL-A (9.1%), MEL-A (21.5%), and MEL-A (46.3%) and anionic liposomes was measured by FRET assay. The membrane fusion activity was calculated from the NBD fluorescence intensity relative to the maximum NBD fluorescence intensity when the anionic liposomes were completely disrupted by 0.1% Triton X-100. All data represent the mean ± S.D. ($n = 4$). $^{**}P < 0.01$ compared with MEL-A (9.1%) by t-test.

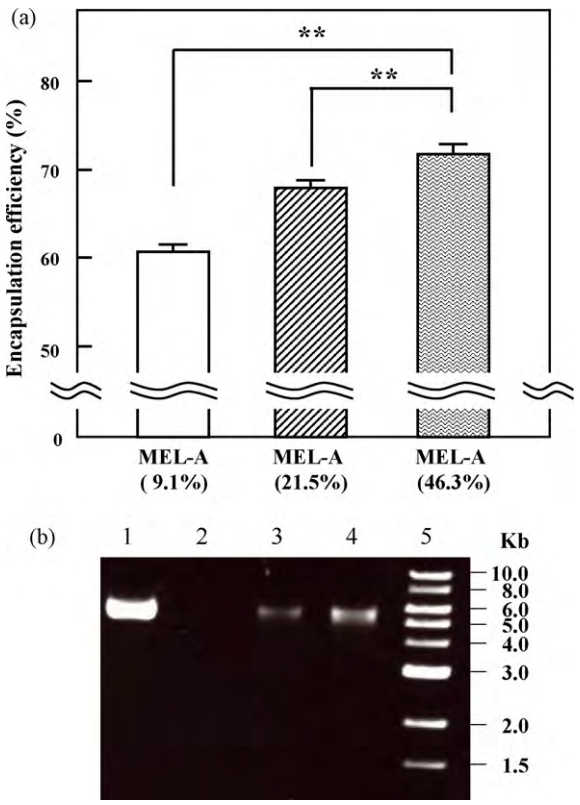

Fig. 3. Efficiency of cationic liposome encapsulation of plasmid DNA. (a) Ethidium bromide was added to the MEL-A-containing cationic liposome–DNA complexes and the encapsulation efficiency was calculated from the fluorescence intensity of ethidium at 595 nm (excited at 520 nm). All data represent the mean ± S.D. ($n = 4$). $^{**}P < 0.01$ compared with MEL-A (9.1%) by t-test. (b) MEL-A-containing cationic liposome–DNA complexes were formed and DNaseI-mediated digestion of the plasmid DNA was measured by 1% agarose gel electrophoresis. Lane 1: naked plasmid DNA without DNaseI, lane 2: naked plasmid DNA incubated with DNaseI for 1 h, lane 3: MEL-A (21.5%) cationic liposome–DNA complex incubated with DNaseI for 1 h, lane 4: MEL-A (46.3%) cationic liposome–DNA complex incubated with DNaseI for 1 h, lane 5: DNA ladder marker.

3.3. Cationic liposome plasmid DNA encapsulation efficiency

To investigate the effect of different ratios of unsaturated fatty acids in MEL-A on cationic liposome encapsulation of plasmid DNA, we used an ethidium intercalation assay. MEL-A (9.1%)-containing liposomes, which had low transfection efficiency, also had the weakest encapsulation efficiency of the three types of MEL-A-containing liposomes (Fig. 3a). MEL-A (46.3%)-containing liposomes had higher encapsulation efficiency than MEL-A (21.5%)-containing liposomes, although the transfection efficiency of MEL-A (21.5%)-containing liposomes was much higher than that of MEL-A (46.3%)-containing liposomes. When we examined the DNA encapsulation by MEL-A (21.5%)- and MEL-A (46.3%)-containing liposomes using agarose gel electrophoresis, the amount of DNA encapsulated by MEL-A (46.3%)-containing liposomes was higher than that by MEL-A (21.5%)-containing liposomes (Fig. 3b). This result correlated well with the encapsulation efficiency determined by ethidium intercalation assay.

3.4. DNA release from the MEL-A liposomes

To understand the difference in transfection efficiency between the MEL-A (21.5%)- and MEL-A (46.3%)-containing liposomes, we investigated the release of plasmid DNA from the cationic liposomes by measuring ethidium bromide intercalation into the plasmid DNA before and after the addition of anionic liposomes. In

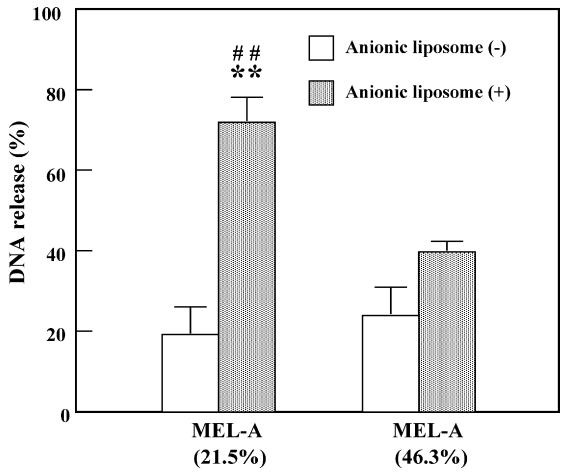

Fig. 4. Efficiency of plasmid DNA release from the cationic liposome–DNA complex. Ethidium bromide was added to the MEL-A-containing cationic liposome–DNA complexes and the proportion of plasmid DNA present was calculated from the fluorescence intensity of ethidium at 595 nm (excited at 520 nm) after the addition of anionic liposomes to the complexes. All data represent the mean ± S.D. ($n = 3$). $^{**}P < 0.01$ compared with the value in the absence of anionic liposomes by t test. $^{\#\#}P < 0.01$ compared with the value of MEL-A (46.3%) by t-test.

case of MEL-A (21.5%)-containing cationic liposomes, only 20% of the DNA was released from the cationic liposomes when no anionic liposomes were added, whereas approximately 70% was released after the addition of anionic liposomes (Fig. 4). On the other hand, in case of MEL-A (46.3%)-containing liposomes, the only 40% DNA was released after the addition of anionic liposomes. This was probably why gene transfection by MEL-A (21.5%)-containing liposomes was higher than that by MEL-A (46.3%)-containing liposomes.

3.5. Localization of the MEL-A-containing liposomes and the DNA oligonucleotide

Finally, we compared the intracellular distribution of the DNA oligonucleotide and the liposomes containing 3 types of MEL-A using CLSM after the oligonucleotide was transfected into NIH-3T3 cells by the liposomes. In case of MEL-A (9.1%)-containing liposomes, neither the fluorescence-labeled lipid from the cationic liposomes nor the DNA oligonucleotide was detected inside the NIH-3T3 cells 1 h after the addition of the liposome–DNA complex (Fig. 5a). On the other hand, in case of MEL-A (21.5%)- and MEL-A (46.3%)-containing cationic liposomes, fluorescence-labeled lipids composed of liposomes were observed on the plasma membrane of the target NIH-3T3 cells (Fig. 5b and c), suggesting that these cationic liposomes were fused with the plasma membrane. In contrast, the DNA oligonucleotide was delivered into the nucleus by MEL-A (21.5%)-containing liposomes, but MEL-A (46.3%)-containing liposomes delivered very little DNA into the nucleus. The intracellular distribution of the lipids and oligonucleotide was consistent with the physicochemical properties of each MEL-A-containing cationic liposome. The differences in the efficiency of DNA release from the liposomes and that of DNA transfer into the nucleus appear to be the primary reason why the gene transfection efficiency of MEL-A (21.5%)-containing liposome was much higher than that of MEL-A (46.3%)-containing liposome despite their similar membrane fusion activities.

4. Discussion

It is well known that in non-viral vectors for gene delivery, lipid physicochemical properties, such as lipid packing density, shape, and zeta-potential, have a significant effect on transfec-

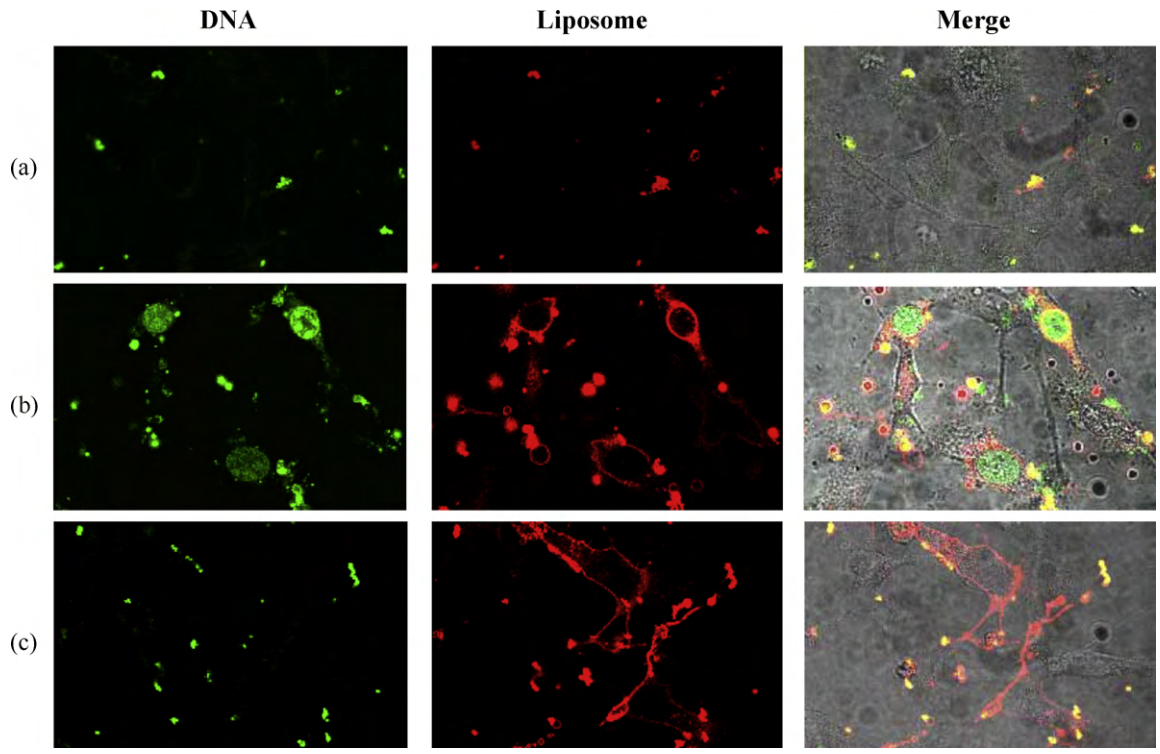

Fig. 5. Intracellular distribution of MEL-A-containing liposomes and oligonucleotide DNA. NIH-3T3 cells were incubated with the FITC-conjugated oligonucleotide DNA complex and the liposomes containing the rhodamine-conjugated lipids with (a) MEL-A (9.1%), (b) MEL-A (21.5%), and (c) MEL-A (46.3%) for 1 h. Fluorescence images of oligonucleotide DNA (left) and lipids in the liposomes (middle) are shown. The image to the right shows the merged fluorescence and transmittance images (green for DNA, red for the liposome, and yellow for the liposome–DNA complex). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

tion efficiency (Takeuchi et al., 1996; Xu et al., 1999; Lin et al., 2003; Wittenberg et al., 2008). MEL-A-containing liposomes transfer efficiently foreign genes into target cells by fusing with the plasma membrane as well as by endocytosis. This suggests that the fatty acids in MELs significantly affect gene transfection efficiency by altering the physicochemical properties of the associated liposomes (Inoh et al., 2001; Ueno et al., 2007b; Nakanishi et al., 2009). Further, in case of MEL-A monolayers, we have shown that the presence of unsaturated fatty acids in a MEL-A monolayer changes the surface pressure and packing density (Imura et al., 2008). Because changes in the lipid packing density in the lipid bilayers are known to cause shape changes in the lipid bilayers (commonly observed in cellular events such as cell division, endocytosis, and exocytosis) during membrane fusion, we speculated that the MEL-A unsaturated fatty acid ratio of the liposomes would also affect the properties of the liposome–DNA complex, and thus, improve the efficiency of gene transfection into target cells (Wittenberg et al., 2008).

Given this background, to develop a more efficient system for gene transfer using MEL-A-containing cationic liposomes, examination of the effect of the MEL-A unsaturated fatty acid ratio on gene transfection by cationic liposomes and on the physicochemical properties of the cationic liposomes could prove valuable. In this context, we prepared three types of cationic liposomes containing MEL-A with different ratios of unsaturated fatty acids (9.1%, 21.5%, and 46.3%) and measured their transfection efficiency.

As shown in Fig. 1, the gene transfection efficiency of MEL-A (21.5%)-containing liposomes was much higher than that of MEL-A (9.1%)- and MEL-A (46.3%)-containing liposomes. In our previous studies, we found that membrane fusion between the target cells and cationic liposomes was the most important factor for achieving high transfection efficiency using MEL-A cationic liposomes (Inoh et al., 2004; Ueno et al., 2007a). Therefore, we investigated the effect of the MEL-A unsaturated fatty acid ratio on membrane fusion. MEL-A (21.5%)- and MEL-A (46.3%)-containing liposomes showed high membrane fusion compared with MEL-A (9.1%)-containing liposomes (Fig. 2). Because DNA encapsulation by cationic liposomes is also essential for high transfection efficiency (Sorgi et al., 1997; Noguchi et al., 2002), we examined the DNA encapsulated by the MEL-A-containing liposomes. MEL-A (46.3%)-containing liposomes encapsulated DNA more efficiently than MEL-A (21.5%)-containing liposomes (Fig. 3), whereas the efficiency of DNA release from MEL-A (21.5%)-containing cationic liposomes was much better than that from MEL-A (46.3%)-containing liposomes following the addition of anionic liposomes (Fig. 4). This indicates that although MEL-A (21.5%)- and MEL-A (46.3%)-containing cationic liposomes had the same level of fusion with the plasma membrane, the MEL-A (21.5%)-containing cationic liposomes released DNA more efficiently than the MEL-A (46.3%)-containing liposomes. Actually, in MEL-A (21.5%)- and MEL-A (46.3%)-containing liposomes, although both cationic liposomes were observed to fuse with the plasma membrane of the target NIH-3T3 cells, the DNA oligonucleotide was transferred to the nucleus by MEL-A (21.5%)-containing liposomes, but MEL-A (46.3%)-containing liposomes transferred very little DNA to the nucleus (Fig. 5a). These data demonstrate why MEL-A (21.5%)-containing liposome complexes showed higher transfection efficiency even though membrane fusion between the MEL-A (46.3%)-containing cationic liposome complexes and the plasma membrane of the target cells was more efficient. These results suggest that DNA release from cationic liposomes is essential or is even more essential than DNA encapsulation by them for high transfection efficiency.

Recently, it has been reported that unsaturated fatty acids in bilayers facilitate membrane fluidity within the bilayer, leading to high membrane fusion activity (Papahadjopoulos et al., 1990), and that an increase in chain unsaturation in the hydrophobic moiety

of cationic lipids promotes lipoplex-membrane fusion, and thus, high transfection efficiency (Koynova et al., 2009). In case of MEL-A-containing cationic liposomes, a high MEL-A unsaturated fatty acid ratio (21.5% and 46.5%) promoted membrane fusion between the liposome–DNA complexes and the plasma membrane. However, the efficiency of DNA release from MEL-A (46.3%)-containing liposomes was much lower than that of MEL-A (21.5%)-containing liposomes following fusion with the plasma membrane. This suggests that the MEL-A unsaturated fatty acid ratio might influence DNA encapsulation, membrane fusion, and DNA release, and that an optimum unsaturated fatty acid ratio may be necessary for highly efficient gene transfection into target cells.

In this study, we demonstrated that the MEL-A unsaturated fatty acid ratio significantly affects transfection efficiency due to changes in membrane fusion activity and the efficiency of DNA release from the liposomes. Although more detailed studies are needed, this study has shown that the ratio of unsaturated fatty acids in MEL-A cationic liposome is important for high gene transfection efficiency. These results may aid the development of not only cationic liposomes but also general non-viral vectors that have gene transfection efficiencies comparable with that of viral vectors.

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