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Decaarginine-PEG-liposome enhanced transfection efficiency and function of arginine length and PEG

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ABSTRACT

Oligoarginine-conjugated lipids ((Arg)n-PEG-lipid) (n = 4, 6, 8, and 10: number of arginine residues) are novel gene delivery vectors. We prepared two oligoarginine-modified liposomes using (Arg)n-lipid without and with poly(ethylene glycol) (PEG) spacer ((Arg)n-L and (Arg)n-PEG-L), and investigated the effect of PEG spacer and oligoarginine length of liposomes on cellular uptake, gene transfection, and its mechanism in HeLa cells, using complexes with plasmid DNA (DNA) or oligodeoxynucleotide. Transfection efficiency increased as the number of arginine residues increased and Arg10-PEG-L/DNA complexes (lipoplexes) showed the highest gene transfection efficiency among (Arg)n- and (Arg)n-PEG-lipoplexes. Arg4- and Arg4-PEG-lipoplexes were taken up greatly into cells, but showed lower transfection efficiency than Arg10- and Arg10-PEG-lipoplexes, respectively. The different gene expression by Arg4-L to Arg10-L with or without PEG spacer may be explained by the different intracellular uptake mechanism. The main cellular uptake mechanism of Arg10-L and Arg10-PEG-L was the macropinocytosis pathway, whereas that of Arg4-L and Arg4-PEG-L was not. PEG spacer was more effective for intracellular trafficking than Arg length and surface charge of lipoplex which depends on Arg length at the almost same size of lipoplexes. The findings suggested that Arg10-PEG-L was a superior vector since Arg10 induced the macropinocytosis uptake pathway.

1. Introduction

Several cell-penetrating peptides (CPPs), less than 30 amino acid residues in length, such as HIV-1 Tat fragments, have attracted much attention as cellular delivery vectors. CPPs contain a number of basic amino acid residues, and can deliver their associated molecules into cells (Derossi et al., 1994; Vives et al., 1997; Oehlke et al., 1998; Pooga et al., 1998; Futaki et al., 2001b; Morris et al., 2001). CPP-modified nanoparticles (Lewin et al., 2000) and liposomes (Torchilin et al., 2001) can also be taken up into cells. Oligoarginine has similar characteristic to CPPs (Mitchell et al., 2000; Wender et al., 2000; Futaki et al., 2001a,b). Accordingly, oligoarginine-modified liposomes that have the ability to cross a plasma membrane are anticipated for the efficient delivery of plasmid DNA (DNA). The nature of the spacer between the CPP and the liposome might be an important issue. The use of a longer poly(ethylene glycol) (PEG) spacer in the attached molecule increased the level of binding to the biological targets (Gabizon et al., 1999). Torchilin et al. (2001, 2003) were the first to report the cytosolic delivery of DNA complexed with liposomes modified with a Tat peptide attached to the terminal ends of PEG chains.

Investigations delineating the influence of oligoarginine length on the uptake of oligoarginine peptides alone have been reported (Mitchell et al., 2000; Wender et al., 2000; Futaki et al., 2001b). Concerning gene delivery, it was reported that stearylation of Arg8 (stearyl-Arg8), giving the highest transfection efficiency among stearyl-Arg4 to stearyl-Arg16 (Futaki et al., 2001a), and stearyl-Arg8-modified liposome-encapsulated DNA, improved transfection efficiency (Kogure et al., 2004).

We previously synthesized two oligoarginine lipids, conjugating oligoarginine and 3,5-bis (dodecyloxy)benzamide (BDB) as the lipid component ((Arg)n-BDB) (Fig. 1A; Furuhata et al., 2006a), and introducing a PEG spacer into (Arg)n-BDB ((Arg)n-PEG-BDB) (Fig. 1B; Furuhata et al., 2006b). We used (Arg)n-PEG-BDB as a novel micelle vector of DNA (Furuhata et al., 2006b); however, there is no report about the influence of the PEG spacer and the arginine length of oligoarginine-modified liposomes complexed with DNA on transfection efficiency.

In this study, liposomes containing (Arg)n-BDB or (Arg)n-PEG-BDB of various oligoarginine lengths (n = 4, 6, 8, and 10) were prepared ((Arg)n-L, (Arg)n-PEG-L). The effect of the PEG spacer and oligoarginine length of (Arg)n-L or (Arg)n-PEG-L complexed

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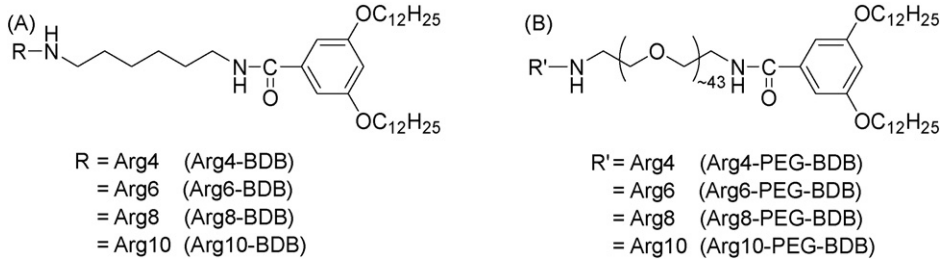

Fig. 1. Chemical structures of (Arg)n-BDBs (A) and (Arg)n-PEG-BDBs (B).

with DNA ((Arg)n- or (Arg)n-PEG-L/D, lipoplex) on cellular uptake, gene expression and its mechanism were investigated in human cervical carcinoma HeLa cells. We demonstrate that the different gene expressions between Arg4-L and Arg10-L with or without the PEG spacer may be explained by the different intracellular uptake mechanism by oligoarginine length.

2. Materials and methods

2.1. Materials

Egg phosphatidylcholine (EPC) was purchased from Q.P. Co., Ltd. (Tokyo, Japan). Cholesterol (Chol) and sucrose were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The Pica gene luciferase assay kit was purchased from Toyo Ink Mfg. Co., Ltd. (Tokyo, Japan). Bicinchoninic acid (BCA) protein assay reagent was obtained from Pierce (Rockford, IL, USA). DMEM, FBS and FITC-labeled transferrin were purchased from Invitrogen Corp. (Carlsbad, CA, USA). 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was obtained from Lambda Probes & Diagnostics (Graz, Austria). 5-(*N*-Ethyl-*N*-isopropyl)amiloride (EIPA) and filipin were from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals were of reagent grade. (Arg)n-BDB and (Arg)n-PEG-BDB (Fig. 1A and B) were synthesized as previously reported (Furuhata et al., 2006a,b).

2.2. DNA, rhodamine-labeled DNA and FITC-labeled oligodeoxynucleotide

DNA encoding the luciferase gene under the control of the CMV promoter (pCMV-luc) was constructed as previously described (Igarashi et al., 2006). Protein-free preparation of pCMV-luc was purified following alkaline lysis using maxiprep columns (Qiagen, Hilden, Germany). Labeling of pCMV-luc was performed using the protocol of the Label IT TM-rhodamine labeling kit (Mirus, Madison, WI, USA). The FITC-labeled 20-mer randomized oligodeoxynucleotide (FITC-ODN) was synthesized with a phosphodiester backbone (Sigma Genosys Japan, Hokkaido, Japan).

2.3. Cell culture

HeLa cells were kindly provided by Toyobo Co., Ltd. (Osaka, Japan) and grown in DMEM supplemented with 10% FBS at 37 °C in a humidified 5% CO_2 atmosphere. Cells cultures were prepared by plating cells in a 35-mm culture dish 24 h prior to each experiment.

2.4. Preparation of liposomes

Four liposomal formulae were prepared by the dry film method with water (Furuhata et al., 2006a): EPC, Chol and (Arg)n-BDB or (Arg)n-PEG-BDB in a 7:3:0.5 molar ratio for (Arg)n-L or (Arg)n-PEG-L, respectively. Particle size distributions and zeta-potentials were measured by the dynamic and electrophoresis light scattering method, respectively (ELS-800, Otsuka Electronics Co., Ltd., Osaka, Japan), at 25 °C after the dispersion was diluted to an appropriate volume with water. DiI-labeled liposomes were prepared as described above but with post-addition of DiI at 0.04 mol% of total lipids.

2.5. Gene transfection

Each lipoplex was left at room temperature for 10–15 min. For transfection, (Arg)n-L or (Arg)n-PEG-L/D at a charge ratio (+/−) of (Arg)n-BDB to 2 μg of DNA or FITC-ODN of 1 or 3 was diluted with serum-free DMEM to 1 mL, and then gently applied to the cells. Here, the charge of (Arg)n-BDB or (Arg)n-PEG-BDB was considered the same (+1), not depending on the number of arginine residues.

2.6. Luciferase assay

All samples were incubated with cells for 3 h at 37 °C in serum-free DMEM, DMEM (1 mL) containing 10% FBS was added, and the cells were further incubated for 21 h. Luciferase expression was measured according to the instructions accompanying the Pica gene luciferase assay kit, as described previously (Furuhata et al., 2006b). The protein concentration of the supernatants was determined with BCA reagent using bovine serum albumin (BSA) as the standard, and cps/μg protein was calculated.

2.7. Cellular uptake

(Arg)n-L or (Arg)n-PEG-L/FITC-ODN, (Arg)n-L/FITC-DNA, and DiI-labeled (Arg)n-L/D or (Arg)n-PEG-L/D were diluted with serum-free DMEM to 1 mL, and then gently applied to the cells. In this study, all samples were incubated with cells for 3 h at 37 °C in serum-free DMEM. At the end of the incubation of lipoplexes with cells, the cells were washed three times with 1 mL of PBS, and detached from the plate by incubating with 0.05% trypsin and EDTA solution at 37 °C for 3 min. Flow cytometric analysis was described previously (Furuhata et al., 2006a,b).

To investigate the cellular uptake mechanism, cells were washed with serum-free medium and preincubated for 30 min at 4 °C, or with medium containing sucrose (0.4 M), EIPA (50 μM) or filipin (5 μg/mL) at 37 °C. After the medium was replaced with fresh medium containing complexes, cells were incubated for 1 h at 4 °C, or at 37 °C in the presence of sucrose, EIPA or filipin, and then treated with trypsin before flow cytometry.

2.8. Confocal laser scanning microscopy

To investigate intracellular fate of (Arg)n- and (Arg)n-PEG-lipoplexes in cytoplasm, cells were incubated for 2 and 3 h with DiI-labeled liposomes/FITC-labeled DNA and were observed by confocal microscopy. To investigate cellular uptake mechanism, cells were co-incubated for 1 h with (Arg)n-L or (Arg)n-PEG-L/rhodamine-labeled DNA (+/− = 3/1) with 50 μg/mL FITC-labeled

transferrin. After the medium was removed, the cells were washed five times with PBS. Live cells were observed with a Radiance 2100 confocal laser scanning microscope (BioRad, CA, USA). For DiI-labeled liposome and rhodamine-labeled DNA, maximum excitation was performed by a 543-nm line of internal He-Neon laser, and fluorescence emission was observed with long-pass barrier filter 560DCLP. FITC-labeled DNA and transferrin were imaged using the 488-nm excitation beam of an argon laser, and fluorescence emission was observed with a filter HQ515/30.

2.9. Data analysis

Significant differences in the mean values were evaluated by Student's unpaired *t*-test. A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Luciferase expression of lipoplexes

We used 5 mol% oligoarginine-modified liposomes because the zeta potential of the prepared liposomes was not increased >5 mol% Arg10 (Furuhata et al., 2006a). Each particle size of liposomes was adjusted to about 200 nm by sonication. Zeta-potentials of (Arg)n-L were increased from 39 to 54 mV as Arg length increased (Furuhata et al., 2006a) while those of (Arg)n-PEG-L did not. The size- and zeta-potentials of lipoplexes at a charge ratio (+/−) of 1/1 and 3/1 were not greatly different (Supplementary Table 1). We evaluated the transfection efficiency of (Arg)n-L or (Arg)n-PEG-L complexed with pCMV-luc ((Arg)n- or (Arg)n-PEG-lipoplexes) at a charge ratio (+/−) of 1/1 in HeLa cells by assaying luciferase activity. The results shown in Fig. 2 demonstrate that longer oligoarginine shows stronger luciferase activity. Decaarginine had the highest level of activity among the series of liposomes, indicating that the optimal number of arginine residues for transfection was 10 or more. Arg10-PEG-L showed about 50-fold higher transfection efficiency than Arg10-L.

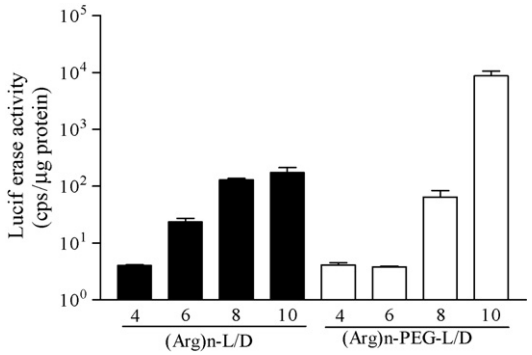

Fig. 2. Luciferase activity after transfection of HeLa cells using (Arg)n- or (Arg)n-PEG-lipoplexes ((Arg)n-BDB or (Arg)n-PEG-BDB: 2 μg of DNA = 1:1, (+/−) charge ratio). All samples were incubated with cells for 3 h at 37 °C in serum-free DMEM, and further incubated for 21 h in DMEM containing 10% FBS. Each value is the mean ± S.D. of three separate determinations.

3.2. Cellular uptake of lipoplexes

To confirm the ability of oligoarginine-modified liposomes to carry genes into cells, we assayed the cell internalization of their lipoplexes at a charge ratio (+/−) of 1/1 by flow cytometry (Fig. 3). Cells were exposed for 3 h to the lipoplexes in the absence of serum, and then trypsinized. Flow cytometric analysis confirmed cell internalization. Surprisingly, the cellular uptake of FITC-ODN in (Arg)n- and (Arg)n-PEG-lipoplexes decreased as the number of arginine

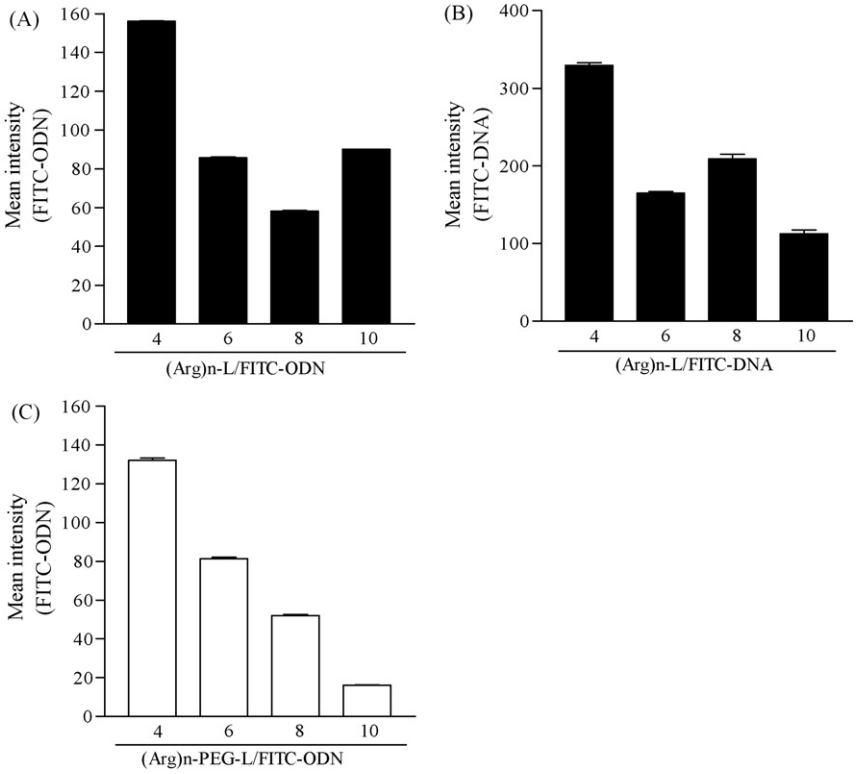

Fig. 3. Cellular uptake of (Arg)n-L/FITC-ODN (A), (Arg)n-L/FITC-DNA (B) and (Arg)n-PEG-L/FITC-ODN (C) lipoplexes at a charge ratio (+/−) of 1/1. Cells were incubated for 3 h in serum-free DMEM and treated with trypsin before FACS analysis. Each bar represents the mean ± S.D. of three experiments.

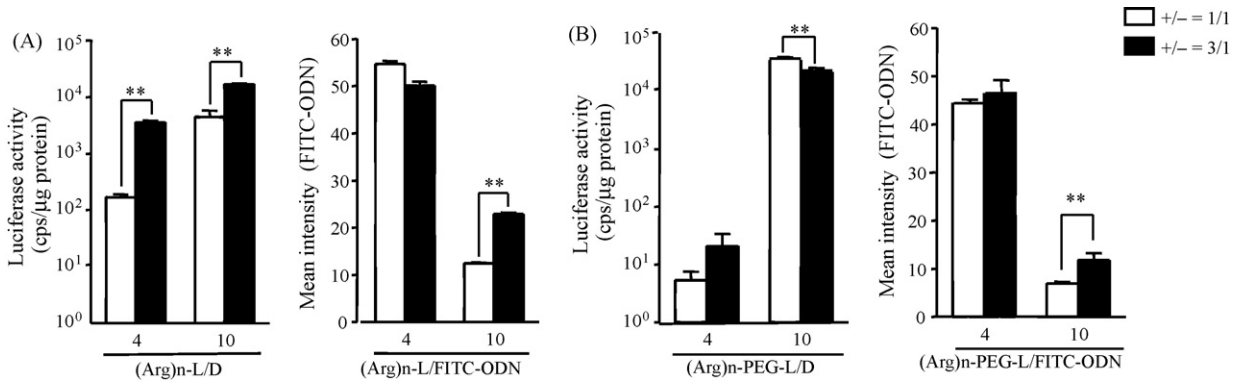

Fig. 4. Influence of charge ratio of Arg4- and Arg10-lipoplexes (A), Arg4-PEG- and Arg10-PEG-lipoplexes (B) on transfection efficiency and cellular uptake (+/− = 1/1 or 3/1). Cellular uptake was examined using FITC-ODN complexes. Each value is the mean ± S.D. of three separate determinations (**$p < 0.01$). The experimental conditions were the same as Fig. 3.

residues increased, except Arg10-L, where Arg4-L and Arg4-PEG-L showed the highest level of FITC-ODN uptake among the series of lipoplexes (Fig. 3A and C). A similar result was obtained when FITC-DNA was used instead of FITC-ODN at a charge ratio (+/−) of 1/1 (Fig. 3B). The difference in transfection efficiency was not correlated with the difference in cellular uptake (Figs. 2 and 3). Hence, we focused on Arg4- or Arg10-modified liposomes in subsequent experiments.

3.3. *Effect of the charge ratio (+/−) on luciferase expression and cellular uptake*

To examine effect of the charge ratio (+/−) of the delivery of DNA, we increased the charge ratio (+/−) from 1/1 to 3/1 (Fig. 4). When the charge ratio (+/−) was increased at 3/1, the transfection efficiency of Arg4-L and Arg10-L vectors significantly increased, but that of Arg10-PEG-L significantly decreased (Fig. 4A and B) ($p < 0.01$). From

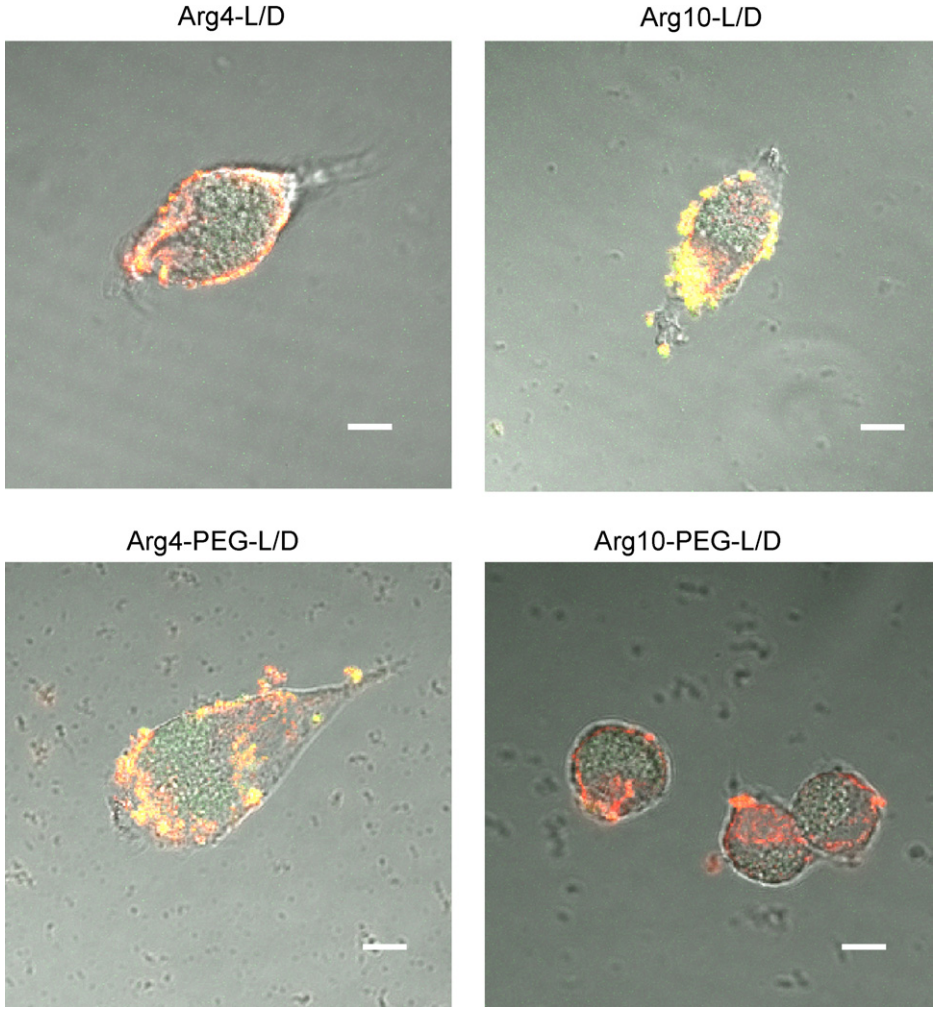

Fig. 5. Release of DNA from (Arg)n- and (Arg)n-PEG-lipoplexes 3 h after transfection by confocal microscopy. DiI-labeled Arg4- Arg10-, Arg4-PEG- and Arg10-PEG-L (red)/FITC-labeled DNA (green) were incubated for 3 h. Yellow color shows liposomes merged with DNA. Scale bar = 10 μm.

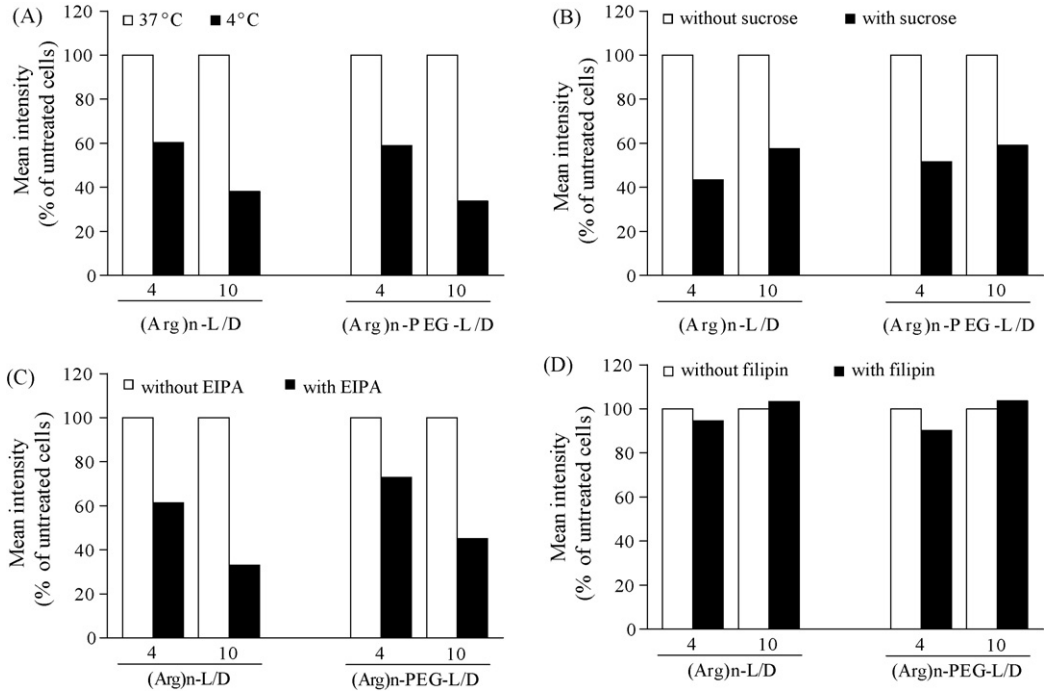

Fig. 6. Mechanism of cellular uptake of (Arg)n- and (Arg)n-PEG-lipoplexes. Effect of low temperature (A), sucrose (B), EIPA (C), and filipin (D) on the cellular uptake of DiI-labeled (Arg)n- and (Arg)n-PEG-lipoplexes at a charge ratio (+/−) = 3/1. HeLa cells were pretreated at 4 °C (A), with sucrose (0.4 M) (B), EIPA (50 μM) (C) or filipin (5 μg/mL) (D) at 37 °C for 30 min. After medium was replaced with fresh medium containing complexes, cells were incubated for 1 h at 4 °C, in the presence of sucrose, EIPA or filipin, and then treated with trypsin before flow cytometry. Each value is the mean of three separate determinations. All values of treated group in (A)–(C) were significantly different from their controls ($p < 0.05$).

the increase of the charge ratio (+/−), cellular uptake of FITC-ODN with Arg10-L and Arg10-PEG-L significantly increased, but that with Arg4-L and Arg4-PEG-L did not (Fig. 4A and B). These findings suggested that Arg4-L and Arg4-PEG-L may be taken up enough, and transfection efficiency may be affected by other factors such as release DNA in the endosome.

3.4. Intracellular fate of lipoplexes in cytoplasm

Because cellular uptake of lipoplexes was not reflected in the transfection efficiency, intracellular fate of (Arg)n- and (Arg)n-PEG-lipoplexes in cytoplasm was examined by colocalization study between DiI-labeled liposomes (red) and FITC-labeled DNA (green) using confocal microscopy. After 2 h-incubation, intracellular (Arg)n- and (Arg)n-PEG-lipoplexes were highly colocalized with DNA (data not shown). After 3 h-incubation, Arg10-PEG-lipoplexes did not show any colocalization whereas other lipoplexes were still colocalized as shown in Fig. 5. This finding suggested that release of DNA from Arg10-PEG-lipoplexes was faster than that from other lipoplexes.

3.5. Mechanism of the cellular uptake of lipoplexes

The difference in the cellular uptake and gene transfection efficiency by oligoarginine-modified liposome may be due to different uptake mechanisms. To investigate the internalization mechanism of (Arg)n- and (Arg)n-PEG-lipoplexes further, we examined the effect of several endocytosis inhibitors on the cellular uptake of DiI-labeled liposomes. Endocytosis is inhibited by low temperature (4 °C) through energy depletion. Cells were exposed to lipoplexes at either 4 °C or 37 °C, then trypsinized, and analyzed by flow cytometry. Low temperature inhibited the uptake of all lipoplexes (Fig. 6A), indicating that transport occurred through energy-dependent endocytosis and supporting the contribution of endocytosis as a major uptake pathway of (Arg)n- and (Arg)n-PEG-lipoplexes. Endocytosis, which occurs in most cells as pinocytosis, represents at least four basic mechanisms: clathrin-mediated endocytosis, macropinocytosis, caveolae-mediated endocytosis, and clathrin- and caveolae-independent endocytosis (Conner and Schmid, 2003). Clathrin-mediated endocytosis is the major endocytotic pathway, and is inhibited by hypertonic medium (0.4 M sucrose), which induces the dissociation of a clathrin lattice (Heuser and Anderson, 1989). In the presence of sucrose, the cellular uptake of Arg4-, Arg10-, Arg4-PEG- and Arg10-PEG-lipoplexes decreased about 57%, 42%, 48% and 41%, respectively, compared with that in the absence of sucrose (Fig. 6B). Macropinocytosis is inhibited by EIPA through the interaction with Na^+/H^+ exchange protein (West et al., 1989). In the presence of EIPA, the cellular uptake of Arg4-, Arg10-, Arg4-PEG- and Arg10-PEG-lipoplexes decreased about 39%, 67%, 27% and 55%, respectively, compared with that in the absence of EIPA (Fig. 6C). Caveolae-mediated endocytosis is inhibited by filipin through cholesterol depletion (Lamaze and Schmid, 1995). Filipin hardly influenced the cellular uptake of all lipoplexes (Fig. 6D), indicating the minor contribution of caveolae in the uptake process. This indicated that Arg10-L and Arg10-PEG-L use macropinocytosis as the major uptake route, whereas Arg4-L and Arg4-PEG-L mainly use a different pathway, probably clathrin-mediated endocytosis.

Furthermore, we examined this by observation of the colocalization of Arg4-, Arg10-, Arg4-PEG- and Arg10-PEG-L/rhodamine-labeled DNA (red) in live cells to intracellular endocytotic vesicles with FITC-labeled transferrin (green), a marker of clathrin-mediated endocytosis (Fig. 7). Arg4- and Arg4-PEG-L/rhodamine-labeled DNA (red) showed colocalization with transferrin, but Arg10- and Arg10-PEG-L/D showed few colocalization with transferrin under the same condition, suggesting that the cellular uptake mechanism was different between Arg4- and Arg4-PEG-L, and Arg10- and Arg10-PEG-L.

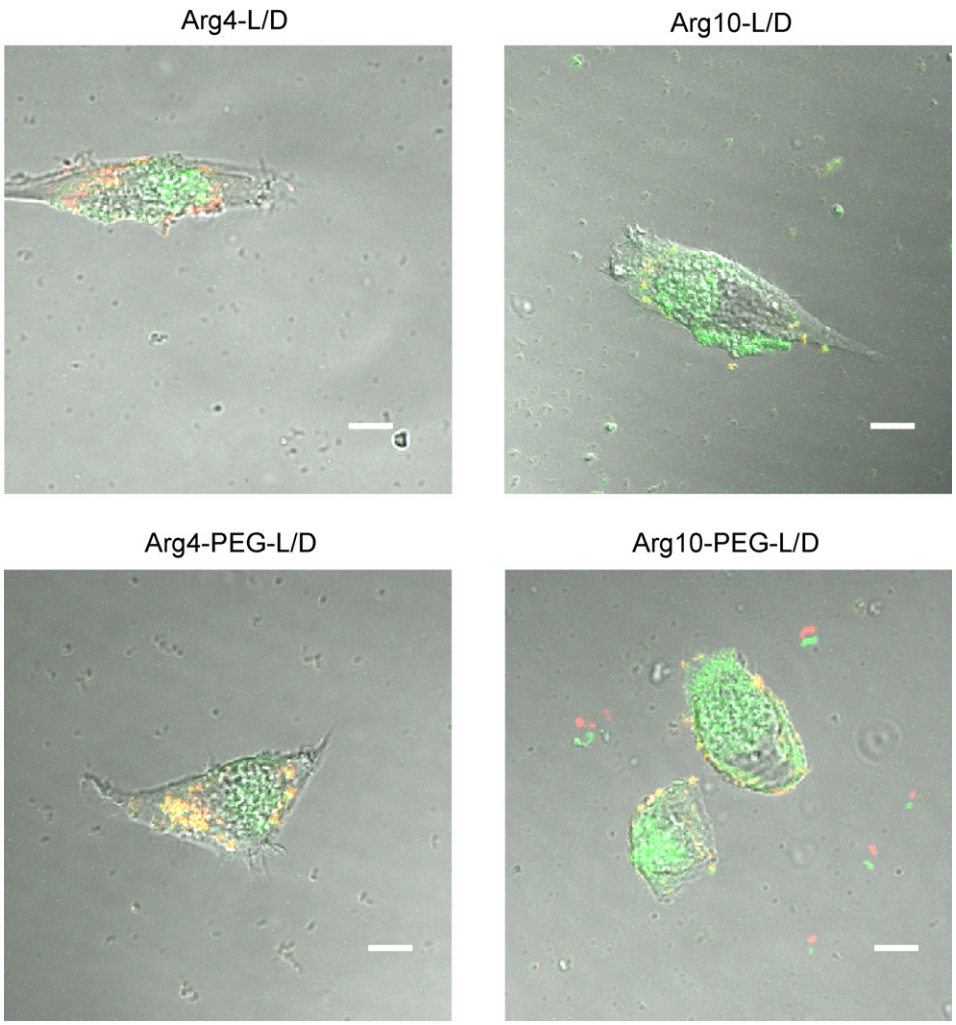

Fig. 7. Cellular uptake mechanism of Arg4- Arg10-, Arg4-PEG- and Arg10-PEG-lipoplexes. Arg4- Arg10-, Arg4-PEG- and Arg10-PEG-L/rhodamine-labeled DNA (red) and FITC-labeled transferrin (green) were incubated for 1 h and visualized using confocal laser scanning microscopy. Yellow color shows DNA merged with transferrin. Scale bar = 10 μm.

4. Discussion

The cellular uptake of ODN with (Arg)n-L decreased with the increase of oligoarginine length. Moreover, in calcein-entrapped (Arg)n-L as a marker of the aqueous phase, a similar result was obtained by measuring calcein uptake in the cells (Furuhata et al., 2006a). However, the uptake of oligoarginine peptides alone (Wender et al., 2000; Futaki et al., 2001b; Mitchell et al., 2000), and ODN with (Arg)n-PEG-BDB micelle vector (Furuhata et al., 2006b) increased with the increase of oligoarginine length (Supplementary Fig. S1); therefore, this discrepancy may be caused by the uniqueness of liposomes.

Surface functionalization through the PEG spacer would allow oligoarginine flexibility for more efficient interactions with the cell membrane. Torchilin et al. (2001) reported that cellular uptake and the transfection efficiency of Tat peptide attached to the liposome via the PEG spacer were higher than those attached directly to the liposome. In our case, cellular uptake of (Arg)n-PEG-L did not increase depending on oligoarginine length. Furthermore, different from micelle vectors (Furuhata et al., 2006b), the effect of oligoarginine length on the transfection efficiency of oligoarginine-modified liposome was in inverse relation to cellular uptake. Longer arginine length exhibited lower uptake and higher transfection efficiency.

Cellular uptake changes by oligoarginine length in both ODN and DNA in lipoplexes were similar (Fig. 3A and B). To the best of our knowledge, cellular uptake of both DNA and ODN by CPP-modified liposomes has not been reported. We showed that ODN and DNA as lipoplexes were taken up similarly into cells.

Considering that Arg10-L/D and Arg10-PEG-L/D contained more charges than Arg4-L/D and Arg4-PEG-L/D, the increase of charge ratio (+/−) could increase effectively cellular uptake of Arg4-L/D and Arg4-PEG-L/D. In our study, the increase of the charge ratio (+/−) increased cellular uptake of Arg10-L/D and Arg10-PEG-L/D, but not that of Arg4-L/D and Arg4-PEG-L/D. This difference may be explained that Arg4-L and Arg4-PEG-L interacted with DNA stronger than Arg10-L and Arg10-PEG-L, and therefore, could not release DNA. It was supported by the report that Arg4-BDB interacted with DNA stronger than Arg10-BDB (Fujita et al., in press). Another explanation is that the cellular uptake mechanism might be different between them. The increase of the charge ratio (+/−) significantly decreased the transfection efficiency of Arg10-PEG-L vectors despite increased cellular uptake (Fig. 4B). Arg10-PEG-L/D might tightly bind to heparan sulfate proteoglycans (HSP) via PEG spacer. HSP could delay and/or limit the release of lipoplexes in cytosol. These results suggested that intracellular trafficking was one of the rate-limiting stages for transfection efficiency in not only Arg4-L, but also Arg10-L.

The internalization of CPPs was originally described as being unaffected by low-temperature incubation or by treatment with typical endocytosis inhibitors. The pathway was proposed to be

independent of endocytosis (Derossi et al., 1994; Vives et al., 1997); however, later it was reported that this was due to artifacts (Richard et al., 2003). The internalization mechanism of CPP itself or its cargo remains a controversial issue (Nakase et al., 2008). The cellular translocation of Arg4-L and Arg4-PEG-L in lipoplexes decreased by sucrose, but did not decrease much by EIPA compared with Arg10-L and Arg10-PEG-L. Their internalization might involve a different mechanism from macropinocytosis. Furthermore the colocalization image of Arg4- and Arg4-PEG-lipoplexes with transferrin was observed, but that of Arg10- and Arg10-PEG-L was not (Fig. 7). These results suggested that the internalization of Arg10- and Arg10-PEG-lipoplexes (modification with 5 mol% of Arg10) occurs mainly through macropinocytosis, and that of Arg4- and Arg4-PEG-lipoplexes mainly through clathrin-mediated endocytosis. Similar to our result, Khalil et al. (2006) reported that 5 mol% Arg8-modified liposomes containing condensed DNA showed higher gene expression through macropinocytosis.

Decaarginine might induce macropinocytosis because the cellular uptake mechanism of decaarginine-containing vectors, such as Arg10-PEG-BDB micelles (Furuhata et al., 2008), Arg10-L and Arg10-PEG-L, was macropinocytosis, regardless of particle type and the PEG spacer, which exhibited the highest transfection efficiency in a series of oligoarginine vectors. The data suggest the superiority of macropinocytosis as a cellular uptake pathway in gene transfection. It is reported that macropinosomes leak (Meier et al., 2002) and DNA may be released easily into the cytoplasm. Macropinosomes decrease their pH, but do not merge with lysosomes, thus avoiding DNA degradation (Conner and Schmid, 2003).

PEG spacer was more effective for intracellular trafficking than Arg length and surface charge of lipoplex which depends on Arg length at the almost same size of lipoplex. PEG might promote the escape from macropinosomes of DNA because the transfection efficiency of Arg10-PEG-L was significantly higher than that of Arg10-L (Fig. 2) and faster release of DNA from Arg10-PEG-lipoplexes in cytoplasm was observed than Arg10-lipoplexes by confocal microscopy images (Fig. 5).

Liposomes are versatile vectors because they can easily modify their functions by changing components such as oligoarginine lipid and the PEG spacer, maintaining their shape as liposomes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijpharm.2008.12.011.

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