PREPARATION OF EFFICIENT GENE CARRIERS USING POLYAMIDOAMINE DENDRON-BEARING CATIONIC LIPIDS WITH DIFFERENT ALKYL CHAINS

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Introduction

Preparation of non-viral vectors which deliver therapeutic gene to target cells has been desired for efficient and safe gene therapy. Among these non-viral vectors, cationic lipids and cationic polymers have been intensively studied, but their activity still requires improvement. These vectors can associate with plasmid DNA and form complexes, which are termed lipoplexes for the complexes with cationic lipids and polyplexes for the complexes with cationic polymers (1, 2). These complexes bind to the cell surface through electrostatic interactions and are taken up by cells mainly via endocytosis. Subsequently, some parts of plasmid DNA contained in the complexes reach the nucleus, where gene transcription occurs. However, most of the complexes are likely to be trapped in the endosome and eventually degraded in the lysosome. Therefore, avoidance of plasmid DNA degradation in the lysosome and its transfer into cytosol is considered to be a key process for efficient transfection (3).

Cationic lipid-based systems are considered to promote this process by fusing with and destabilizing the endosomal membrane with the help of fusogenic lipids, such as dioleoyl-phosphatidylethanolamine (DOPE) (4). Some synthetic cationic polymers such as polyethylenimine (5) and polyamidoamine dendrimers (6) also induce efficient transfection of cells by enhancing the transfer of plasmid DNA into cytosol through the so-called proton sponge effect (7).

In a previous study (8), we developed a novel type of cationic lipids that consist of two dodecyl groups and a polyamidoamine (PAMAM) dendron as a head group. Since PAMAM dendrons have many tertiary amino groups in their interior, they can show the proton sponge effect. In addition, these dendron-bearing lipids can form lipoplexes with a fusogenic lipid DOPE and plasmid DNA, and achieve efficient transfection of cells by the synergetic action of membrane fusion and the proton sponge effect. In fact, in combination with DOPE, the lipid with the third generation dendron, DL-G3-2C_{12} (Fig. 1), achieved more efficient transfection of CV1 cells, a green monkey kidney cell line, than widely used nonviral vectors in the presence of serum as well as in the absence of serum (9).

Molecular structure of dendron-bearing lipids, such as generation of the dendron moiety and length of alkyl groups, will influence formation, stability and transfection activity of complexes. In a previous study (8), we found that transfection activity of the dendron-bearing lipids increases as the generation of their dendron moiety. However, the effect of the alkyl chain length of the dendron-bearing lipids on their transfection activity was still to be clarified.

In this study, PAMAM dendron-bearing lipid with two octadecyl chains DL-G3-2C_{18} was additionally synthesized (Fig 1). We compared abilities of the dendron-bearing lipids, DL-G3-2C_{18} and DL-G3-2C_{12}, to form complexes with plasmid DNA and to transfect cells. The influence of the alkyl chain length of these dendron-bearing lipids on their functions as non-viral gene vectors was investigated.

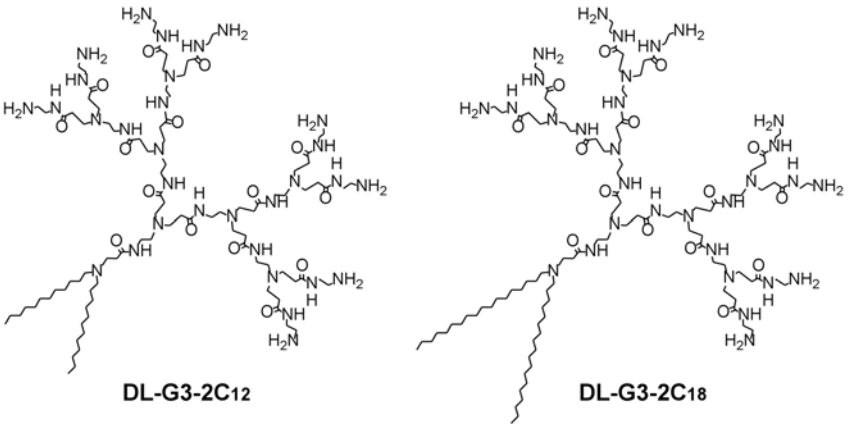

Figure 1. Structures of DL-G3-2C_{12} and DL-G3-2C_{18}.

Experimental Part

DL-G3-2C_{18} and DL-G3-2C_{12} were synthesized by repetition of exhaustive Michael addition with methyl acrylate using dioctadecylamine or didodecylamine as the core material and subsequent exhaustive amidation with ethylenediamine, according to the method previously reported (8)

Lipoplexes were prepared via the common technique. Briefly, to a dry thin membrane of the dendron-bearing lipid, phosphate-buffered saline (PBS) was added and sonicated for 2 min using a bath-type sonicator to afford a lipid suspension. Plasmid DNA was dissolved in 20 mM Tris-HCl buffer (pH7.4), was mixed with the lipid suspension, and incubated for 30 min at room temperature to afford a lipoplex with a given ratio of primary amino group of DL-G3 to plasmid DNA phosphate (N/P ratios).

Particle size of lipoplexes was measured by dynamic light scattering (DLS).

The stability of the lipoplexes consisting of DL-G3-2C_{18} or DL-G3-2C_{12} was investigated by addition of heparin, which is a polysaccharide with negatively charged groups. The effect of addition of heparin on dissociation of plasmid DNA from the DL-G3-2C_{18} and DL-G3-2C_{12} lipoplexes was evaluated by agarose gel retardation assay. Lipoplexes were incubated with varying amounts of heparin for 30 min, and then electrophoresed on an agarose gel.

Transfection of CV1 cells was done according to the following procedures. The cells were seeded in DMEM supplemented with 10 % FCS in 24-well culture plates at 5.0×10^4 cells per well the day before transfection. The cells were washed with PBS and then covered with DMEM in the presence or absence of 10% FCS. The lipoplex containing plasmid DNA was added gently to the cells and incubated for 4 h at 37ºC. Then, the cells were rinsed with PBS, covered with DMEM containing 10% FCS, and incubated at 37ºC. After 40 h, the cells were lysed, and luciferase activity of the lysate was analyzed using a luminometer. The protein content of the lysate was measured by Coomassie Protein Assay Reagent using bovine serum albumin as the standard.

Results and Discussion

We prepared two kinds of PAMAM dendron-bearing lipids with different alkyl chain lengths, DL-G3-2C_{12} and DL-G3-2C_{18}, and compared their ability to form lipoplexes and their transfection activity.

Influence of the alkyl chain length of dendron-bearing lipids on lipoplex size was investigated by DLS. In the case of DL-G3-2C_{12} lipoplexes, the particle size was not altered significantly and maintained at about 2 μm between the N/P ratios of 1 to 8. In the case of DL-G3-2C_{18} lipoplexes, large particles (2 μm) were observed at N/P ratio of 1.0, but the lipoplex particle size became smaller with increasing the N/P ratio. The lipoplexes of DL-G3-2C_{18} might be stabilized by their long alkyl chains, whereas DL-G3-2C_{12} lipoplexes could not be well stabilized because of their short alkyl chains. Therefore, the latter with low stability might tend to form aggregates with larger diameters. These results indicate that the hydrophobicity of alkyl groups of the dendron-bearing lipids strongly affects the particle size of their lipoplexes.

As mentioned above, DL-G3-2C_{18} was indicated to form a stable complex with plasmid DNA compared with DL-G3-2C_{12}. Therefore, we investigated the stability of the lipoplexes consisting of the dendron-bearing lipids with different alkyl chain length. Some polyanions, such as heparin and dextran sulfate, are known to induce dissociation of DNA from lipoplexes by binding to cationic lipids of lipoplexes. Therefore, these polyanions have been used to estimate the stability of the lipoplexes. We examined the effect of addition of heparin on dissociation of plasmid DNA from the DL-G3-2C_{18} and DL-G3-2C_{12} lipoplexes to evaluate their stability. In this experiment, the lipoplexes with the N/P ratio of 2 were incubated with varying amounts of heparin for 30 min, and then electrophoresed on an agarose gel. Liberation of plasmid DNA was caused mainly by their electrostatic interaction. Therefore, we investigated the correlation between the amount of released plasmid DNA and the -/+ charge ratio of plasmid DNA and heparin to the dendron lipids. In the case of DL-G3-2C_{12}, the release of plasmid DNA

was observed above the -/+ charge ratio of 1, whereas in the DL-G3-2C_{18}, the release of plasmid DNA was observed above the -/+ charge ratio of 1.4. This result indicates that the DL-G3-2C_{18} lipoplex required more heparin than the DL-G3-2C_{12} lipoplex to cause release of plasmid DNA from the lipoplexes. Probably, hydrophobic interaction between the longer alkyl chains of DL-G3-2C_{18} might increase stability of the complex formed with plasmid DNA.

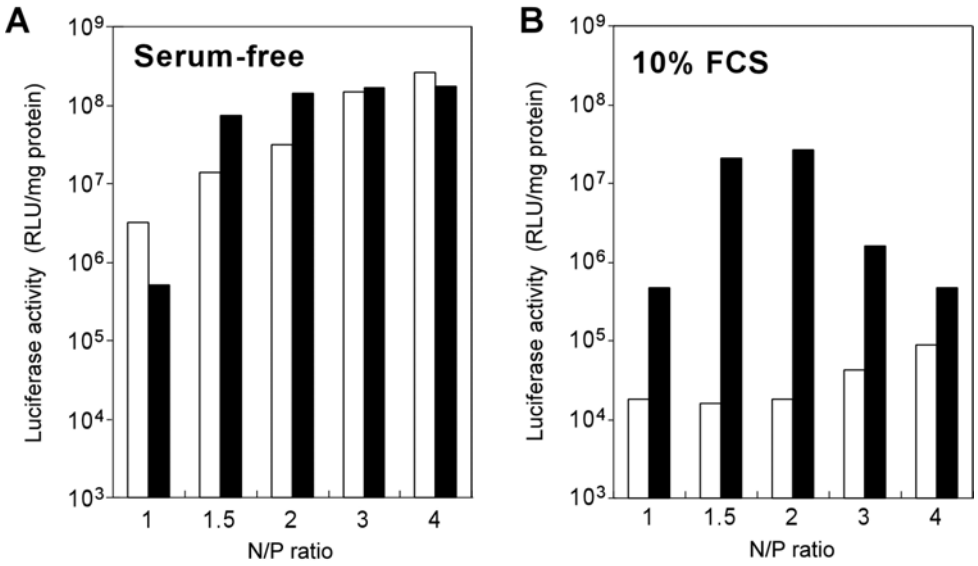

Figure 2. Luciferase activities of CV1 cells treated with DL-G3-2C_{12} lipoplexes (open bars) and DL-G3-2C_{18} lipoplexes (closed bars). The cells ($5x10^4$) were treated with the lipoplex containing 1μg plasmid DNA in the absence (A) and presence (B) of 10% FCS.

We also investigated the influence of the alkyl chain length of dendron-bearing lipids on their transfection activity. It is well known that transfection activity of lipoplexes varies, depending on their cationic lipid/DNA ratios. Thus, we prepared the lipoplexes consisting of the dendron-bearing lipid, DL-G3-2C_{12} or DL-G3-2C_{18}, and plasmid DNA at various N/P ratios and examined transfection of CV1 cells using these lipoplexes in the absence of serum or in the presence of 10% FCS (Fig. 2). Figure 2A represents the expression of luciferase in the cells treated with these lipoplexes in the absence of serum. Transfection activity of these lipoplexes depended on their N/P ratios. These lipoplexes exhibited approximately the same activity above the N/P ratio of 3, although DL-G3-2C_{18} lipoplexes with the N/P ratios of 1.5 and 2 seem to have a slightly higher activity than DL-G3-2C_{12} lipoplexes with the same N/P ratios. Figure 2B represents the expression of luciferase in the cells treated with these lipoplexes in the presence of 10% FCS. DL-G3-2C_{12} lipoplexes with the N/P ratios between 1 and 4 did not induce expression of luciferase in CV1 cells in the presence of FCS. However, DL-G3-2C_{18} lipoplexes caused transfection of CV1 cells efficiently. Especially, DL-G3-2C_{18} lipoplexes with the N/P ratios of 1.5 and 2 exhibited a much higher activity than DL-G3-2C_{12} lipoplexes.

Many studies have revealed that the transfection activity of lipoplexes is reduced dramatically in the presence of serum because of their interaction with serum proteins. As mentioned above, the DL-G3-2C_{12} lipoplexes were highly serum-sensitive as other types of cationic lipid-based vectors. However, DL-G3-2C_{18} lipoplexes were serum-resistant. This result indicates that the hydrophobic interaction by the long alkyl chains of DL-G3-2C_{18} might improve stability of the lipoplexes and provide a high serum-resistant property to them.

In addition, it is apparent that the DL-G3-C_{18} lipoplexes with the N/P ratios of 1.5-2 possess relatively strong transfection activity among the lipoplexes with the N/P rations of 1-4. These lipoplexes with high transfection activities have relatively low charge density. Since electrostatic force is likely to play an important role in the interaction between the lipoplexes and serum proteins, the lipoplexes with low charge densities might weakly interact with serum proteins. Their weak interaction might prevent the loss of transfection activity induced by serum proteins.

These results indicate that hydrophobic interaction of alkyl chain moieties of the dendron-bearing lipids greatly contribute to increments of stability and transfection activity of their lipoplexes.

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