Structure/function relationship in the polyplexes containing cationic polypeptides for gene delivery

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ABSTRACT
Various cationic polypeptides of linear or highly branched structures were synthesized by introducing tertiary or quaternary ammonium groups and hydroxyl groups to poly(L-lysine) (PL) or polyamideamine (PAMAM) dendrimers. These polycations were mixed with plasmid DNA to form polyplexes and subjected to in vitro gene introduction experiment. The transient gene expression was greatly affected by the side groups of PL derivatives or the surface cation charge density of PAMAM dendrimers. This difference in gene expression was found to result from two independent factors as follows: one is the cellular uptake of the polyplexes and the other is the compaction of the polyplexes. Lower charge density of PAMAM dendrimers suppressed the polyplex formation and cellular uptake, resulting the lower gene expression. Only the polycations that form polyplexes compacted at an adequate extent lead an effective gene expression, suggesting that the physicochemical properties of the polyplexes defined by the chemical structures of the polycations play an important role in the effective gene transfer.

INTRODUCTION
Many cationic polymers have been widely studied as gene carriers for transfecting foreign genes into mammalian cells in vitro. Recently, branched polyethyleneimine and polyamideamine (PAMAM) dendrimers, is reported to be effective gene carriers for genetic materials compared with linear cationic polymer such as DEAE-dextran, poly(2-dimethylaminoethyl methacrylamide), poly-L-lysine (PL) and various derivatives. Although these results indicate that the structure of gene carriers closely relates with transfection efficiency, little is known about its mechanism. It is then important to examine the factors influencing the transfection capability of the gene carriers.

To date, we evaluated various water-soluble polycations in transfecting plasmid DNA to mammalian cells and found that the important features of gene-carriers are their tertiary or quaternary ammonium groups and non-ionic hydrophilic groups such as hydroxyl groups or amide groups. In the present study, polypeptides having different chemical structures were synthesized and used for the gene transfection in vitro to evaluate the physicochemical properties of polypeptide/DNA polyplexes influencing the transfection efficacy.

Branched polypeptides

Linear polypeptides

MATERIALS and METHODS
Plasmid DNAs encoding enhanced green fluorescence protein (EGFP) genes or luciferase (Luc) genes were used. PL and poly(lysine-co-serine) (PLS; a random copolymer of L-lysine and L-serine with the composition of 3:1) were used. Trimethyl polylysine (PtmL) and Trimethyl poly(lysine-co-serine) (PtmLS) were prepared by methylation of the ε amino groups in lysine residues of PL and PLS with dimethylsulfate. PAMAM dendrimers of 4th generation with various surface charge density were synthesized by reacting 3.5th generation dendrimers with diethylethylenediamine (DE-EDA), ethylenediamine (EDA), and ethanolamine (EA) at a given feed ratio in methanol at 40°C for 3hours.

The amount of polyplexes phagocytized by the cells by the transfection processes was measured using 32P-labeled plasmid DNA. The properties of the polyplexes of the polypeptides and plasmid DNA were investigated by agarose gel electrophoresis and the
extent of the compaction of the polyplexes were evaluated by measuring the decrement of the fluorescent intensity of the intercalating ethidium bromide (EtBr) molecules in forming polyplexes with the polycations. The transfection into COS-1 cells was carried out by the hypertonic or chloroquine treatment method using these polypeptides at the different C/A ratio (ratio of cationic groups of cationic polypeptide to anionic groups of DNA).

RESULTS AND DISCUSSION

The C/A ratio dependence of the transgene expression using the PAMAM dendrimers and PL derivatives were shown in Figure 2 and Table 1. As shown in Figure 2, PAMAM dendrimers having the high charge density lead effective gene expression but that with low charge density did not. The type of cationic groups of the dendrimers surfaces, primary or tertiary ammonium groups, had no effect on the gene expression. In the case of linear polypeptides, the transfection activity was greatly affected by the side groups, and only PtmLS having both of quaternary ammonium groups and hydroxyl groups showed high transfection efficiency (Table 1).

The amount of ingested plasmid DNA by cells was almost equal for each PL derivatives but those for 4G(A30/OH) and 4G(DE24/OH) were about 10 times as few as the case for 4G(A100) and 4G(DE100). This low uptake results from the low ability of 4G(A30/OH) and 4G(DE24/OH) dendrimers in forming polyplexes with plasmid DNA, which is also confirmed by the agarose gel electrophoresis (data not shown).

Figure 3 shows the decrement of the fluorescence intensity of the intercalating EtBr molecules by polyplex formation. The conformation of DNA molecules are changed by forming compacted polyplexes with polycations, resulting in the decrease in the fluorescent intensity of intercalating EtBr. Therefore, the extent of the polyplex compaction can be evaluated by monitoring the change in the fluorescence. In the case of dendrimers having lower charge density at the surface, a constant EtBr fluorescence was observed irrespective of C/A ratios. It is in good agreement with their low capacity of the polyplex formation observed under the agarose gel electrophoresis and cellular uptake experiment. For PL and PLS, the EtBr fluorescence decreased with increased C/A ratio, indicating the formation of highly compacted polyplexes.

![Figure 2](https://example.com/figure2.png)

Figure 2. Gene expression of pCMV-Luc complexed with (O) 4G(A100), (●) 4G(A30/OH), (●) 4G(DE100) and (▪) 4G(DE24) at various C/A ratios.

![Figure 3](https://example.com/figure3.png)

Figure 3. Fluorescence intensity of ethidium bromide added to solution mixture of DNA and (A) (O) 4G(A100), (●) 4G(A30/OH), (●) 4G(DE100) (▪) 4G(DE24/OH), (△) PL, (▲)PLS, (△)PtmL and (◆) PtmLS at various C/A ratios. Ex: 519nm. Em: 590nm.

In the case of the PtmL, PtmLS and dendrimers with higher surface charge density, which lead high gene expression (Figure 2 and Table 1), the decrease of EtBr fluorescence is significantly inhibited in comparison with PL and PLS. This result indicates that these polyplexes seem to be formed by looser interaction between DNA and polycations than the case of PL and PLS, and only the polyplexes at the moderate compaction (about 20 to 60% decrease in the EtBr fluorescence) can lead high gene expression in our gene delivery system. Although its mechanism is unclear, the moderately compacted polyplexes may allow the dissociation of the polyplexes or the recognition by the transcription factors. The improved amount of plasmid release from the polyplexes by exchanging reaction in the presence of another polyanion and the enhanced luciferase expression from the polyplexes in in vitro transcription/translation system using rabbit reticulo lysate were observed only for the moderately compacted polyplexes.

### Table 1. Transfection efficiency of pEGFP-N1 complexes with various polypeptides at various C/A ratio.

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<thead>
<tr>
<th>Polypeptide</th>
<th>Fluorescence intensity of EGFP expressed</th>
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<tbody>
<tr>
<td></td>
<td>C/A=1</td>
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<tr>
<td>PL</td>
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<tr>
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<tr>
<td>PtmLS</td>
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### REFERENCES