Relationship between the gene transfer efficacy and the facilitated disassembly of polyplexes composed of self-assembling amphiphilic polycations

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Statement of Purpose: Each step in the non-viral gene transfection, such as cellular uptake of polyplexes, their release from the endosome, and the localization into the nucleus6 have been studied. In contrast, the transcription of the polyplexes, which is the last step of the gene transfer, was not well understood. We previously reported that the introduction of nonionic hydrophilic groups to conventional poly(L-lysine) carrier was found to effectively suppress the polyplex compaction and its transcription efficiency was greatly improved.[1] In the present study, we have tried to improve the intra-nucleus transcription efficiency by changing the molecular topology of the polycation carriers from linear-type to micelle-like for the first time.

Methods: In order to prepare both of liner-type and micelle-like carriers, slight amount of hydrophobic moieties was introduced to the conventional linear polycations. A series of amphiphilic polycations containing 2-methacryloyloxy ethylphosphoryl choline (MPC) were synthesized, and the micelle forming property and the gene transfection ability were investigated. Random copolymerization of N-[3-(dimethylamino) propyl] acrylamide (DMAPAA), MPC, and stearylacrylate (SA) were carried out in methanol using AIBN as an initiator (M/I=200) at 60^{o}C for 4h, dialyzed against methanol, and lyophilized. The compositions were determined by ^{1}H-NMR. The copolymers were expressed as p(DMAPAAx-*co*-MPCy-*co*-SAz), where x,y, and z represent the molar composition of each monomers. The molecular weight determined by gel permeation chromatography (GPC) of p(DMAPAA46-*co*-MPC53-*co*-SA1), p(DMAPAA62-*co*-MPC37-*co*-SA1), p(DMAPAA40-*co*-MPC60), and p(DMAPAA62-*co*-MPC38) were, 52,000, 42,000, 102,000, and 65,000, respectively. The critical micelle concentration (CMC) of the obtained polymers was measured using pyrene as hydrophobic probe molecules at various polymer concentrations from 0.01 to 1.0g/L. The CMC of p(DMAPAA46-*co*-MPC53-*co*-SA1) and p(DMAPAA62-*co*-MPC37-*co*-SA1) was 3.1 and 6.6 mg/L, respectively. pCMV-Luc endcoding luciferase gene and the copolymers were incubated in TE buffer for 30min at room temperature resulting in the polyplex formation. The charge ratio of mixed plasmid DNA and polymers (C/A ratio) was ranged from 0.5 to 10.0. The agarose gel electrophoresis showed that the DNA formed polyplex at the C/A ratio higher than 1.0 completly. COS-1 cells were transfected with the polyplexes by the chloroquine method.[2] The amount of plasmid DNA ingested by the cell was assessed using 32P-labeled pDNA.

Results / Discussion: Each polymer showed no cytotoxicity. When copolymers with similar DMAPAA contents were compared, p(DMAPAA-*co*-MPC-*co*-SA)s showed about three times higher expression than the p(DMAPAA-*co*-MPC)s. On the other hand, cellular uptake of polyplexes and their intracellular distribution were not affected by the SA unit. These results indicate that the higher efficiency of p(DMAPAA46-*co*-MPC53-*co*-SA1) was resulted from the efficient transcription of the transgene complexing with the micelle-like carriers after their internalization. We then examined the dissociation tendency of the polyplexes. The exchange reaction of DNA with potassium polyvinyl sulfate (PVSK) was evaluated on the agarose gel. Complete release of DNA from the polyplexes with p(DMAPAA46-*co*-MPC53-*co*-SA1) was observed by adding PVSK solution. In comparison, dissociation of the p(DMAPAA40-*co*-MPC60)/pDNA polyplexes has not occurred at all. The micelle-like structure was found to facilitate the release of plasmid DNA from the polyplexes even in the intracellular environment.

In vitro transcription/translation experiment using TNT® T7 coupled Reticulocyte Lysate System (Promega Corporation, USA) was carried out for these polyplexes. The suppressed transcription in the case of p(DMAPAA40-*co*-MPC60)/pDNA polyplexes was recovered by introducing only 1 mole percent of SA unit, which is in a good agreement with the above agarose gel electrophoresis.

Conclusions: In conclusion, it was found that DNA complexing with the copolymers having stearyl residues is much more easily replaced with added PVSK than the case of the linear-type copolymers. The amphiphilic copolymers were found to form micelle-like structure. These carriers promote the transcription caused by easiness of DNA release from the polyplexes and by less entanglement. These results suggest that the factor of carrier topology is important to detemine the expression efficiency.

References:

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