Self-assembling peptide dimers with multifunctionalities for gene delivery systems

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ABSTRACT

Peptides have been used in various biomedical fields including gene and drug delivery systems. In this study, multifunctional peptide dimer (MPD) was utilized for gene delivery systems. The peptide monomer consists of cellular penetrating peptide moiety (R_8), MMP-2 specific sequence (GPLGV), pH-responsive moiety (H_5) and hydrophobic moiety (palmitic acid) ($CR_8GPLGVH_5$-Pal). MPD was formed by DMSO oxidation of thiols in terminal cysteine residues. MPD could condense pDNA successfully and self-assemble into sub-micron particles via hydrophobic interaction. MPD showed low cytotoxicity in comparison with PEI25k. MPD polyplex showed low transfection efficiency in HEK293 cells expressing no MMP-2 but high transfection efficiency in A549 cells expressing MMP-2, meaning the enhanced transfection efficiency by MMP-induced structural change. This result shows the potential of MPD for targeted gene delivery systems.

Keywords: self-assemble, peptide-based gene delivery, DMSO oxidation, MMP-2, multifunctionality

1 INTRODUCTION

Peptide-based gene delivery systems have many advantages for using gene delivery carriers. [1]. There are many peptide sequences which can interact with cellular molecules or components. These biological interactions of peptide represented many advantages for using gene delivery carriers targeted for cancer treatment. Representatively RGD peptide can enhance cellular recognition and attachment by integrin receptors. Some peptides also represent cell penetrating ability, pH-responsiveness, and breakage by specific enzymes. Especially, cellular penetrating peptide such as penetratin, Tat sequence, or oligoarginine have been extensively examined in gene delivery field because they can form nanosized complexes with nucleic acids due to their cationic property and possess high cellular uptake efficiency [2, 3]. Furthermore, some oligopeptides which are linked via disulfide bonds and represented bioreducibility also have been developed for gene delivery systems. They can be degraded in reductive environment such as cytosol, showing controlled release of nucleic acids and low cytotoxicity [4].

In this work, self-assembling multifunctional peptide was designed for gene delivery systems. The multifunctional peptide (MP) consists of cellular penetrating peptide moiety (R_8), matrix metalloproteinase-2 (MMP-2) specific sequence (GPLGV), pH-responsive moiety (H_5), and hydrophobic moiety (palmitic acid) ($CR_8GPLGVH_5$-Pal). It is expected that R8 moiety would condense pDNA into polyplex particles and facilitate the cellular uptake of the polyplexes. GPLGV is a substrate sequence of matrix metalloproteinase-2 (MMP-2), which is one of the collagenases overexpressed from several tumor cells and plays important roles in tumor progression and metastasis [5]. H_5 moiety was employed for endosome buffering ability of imidazole groups to escape from endosomes after cellular uptake. Palmitic acid at C-terminal would induce micelle formation via self-assembly of the peptide by hydrophobic interaction.

MP was oxidized to form multifunctional peptide dimer (MPD) by DMSO oxidation of thiols in terminal cysteine residues [6]. MPD could condense pDNA successfully at a weight ratio of 5. MPD itself could self-assemble into submicron micelle particles via hydrophobic interaction, of which critical micelle concentration is about 0.01mM. MPD showed concentration-dependent but low cytotoxicity in comparison with PEI25k. MPD polyplexes showed low transfection efficiency in HEK293 cells expressing low level of MMP-2 but high transfection efficiency in A549 and C2C12 cells expressing high level of MMP-2, meaning the enhanced transfection efficiency probably due to MMP-induced structural change of polyplexes. Bafilomycin A1-treated transfection results suggest that the transfection of MPD is mediated via endosomal escape by endosome buffering ability. These results show the potential of MPD for MMP-2 targeted gene delivery systems due to its multifunctionality.

2 MATERIALS & METHODS

2.1 Materials

Multifunctional peptide (CR8GPLGVH5-Pal) was purchased by GL Biochem (Shanghai, China). Thiazolyl Blue Tetrazolium Bromide (MTT reagent), agarose, and 5,5′-Dithiobis(2-nitrobenzoic acid) (Ellman′s reagent) were purchased by Sigma-Aldrich (St. Louis, MO). Dimethyl sulfoxide (DMSO) was purchased by Merck (Germany).

Luciferase assay system was purchased by Promega (Madison, WI). BCA™ protein assay kit was purchased from Pierce (Rockford, Il). Fetal bovine serum (FBS), 0.25% trypsin-EDTA, Dulbecco's phosphate buffered saline (DPBS), and Dulbecco's modified Eagle's medium (DMEM) were purchased from Invitrogen (Carlsbad, CA). The plasmid, pCN-Luci, was amplified in Escherichia coli DH5α and isolated by Nucleobond® Xtra Midi kit (Macherey-Nagel, Germany).

2.2 Synthesis of peptide dimers

Multifunctional peptide dimer (MPD) was synthesized by using DMSO auto oxidation reaction. Multifunctional peptide (MP) was dissolved into DMSO and the solution was stirred at room temperature for 24 hr. After oxidation reaction, peptide dimer was purified by ether precipitation.

2.3 Agarose gel electrophoresis

Agarose gel (0.7 wt %) containing ethidium bromide was prepared in Tris-Acetate-EDTA (TAE buffer). The polyplexes having various concentrations were prepared. The electrophoresis was carried out for 15 min at 100 V (Mupid-2plus, Takara Bio Inc., Japan). DNA retardation ability was confirmed by gel documentation system. (ChemiDoc XRS+ gel documentation system, Bio-Rad, Hercules, CA)

2.4 Average size and zeta-potential measurements

Average size and Zeta-potential values of MP and MPD were measured by Zetasizer Nano ZS (Malvern Instruments, UK).

2.5 Cytotoxicity

Cytotoxicity of MPD was characterized by MTT assay. All cells were seeded into a 96-well cell culture plate, the cells were treated to 100 µL of peptide solutions for 4 h. After peptide treatment, the fresh media was exchanged and incubated for 24 h. After incubation, MTT stock solution was added and 2 h later, the formazan was dissolved into 150 µL of DMSO. The absorbance was measured at 570 nm using microplate reader. (Synergy H1, BioTek, USA).

2.6 Transfection experiments

The transfection efficiencies of MPD were confirmed by measuring luciferase expression. All cells were seeded into a 24-well cell culture plate, after 24 h, the confluencies of each wells reached 70-80 %, the polyplex solutions were added to cells. The polyplexes having various weight ratios were prepared by mixing peptide, peptide dimer solution and pDNA (pCN-Luci). After 4 h incubation, the polyplex solutions were removed, and fresh DMEM with 10% FBS was added into each wells. The cells were incubated for 2 days and after incubation, the media was removed and 120 µL of lysis buffer was added and shaken for 30 min. The cell lysates were centrifugated and the supernatants were used to luciferase assay. The proteins in cell lysates were examined by BCA™ protein assay kit. The transfection efficiency results were calculated in terms of RLU/mg protein.

3 RESULTS & DISCUSSION

MPD was dimerized to synthesize MPD by DMSO oxidation via disulfide bond formation between cysteine thiols at N-terminal. pDNA condensing ability of MPD was investigated by agarose gel electrophoresis.

MPD showed pDNA retardation weight ratio of 2 completely. Because of octaarginine (R_8) moiety, pDNA retardation ability was found.

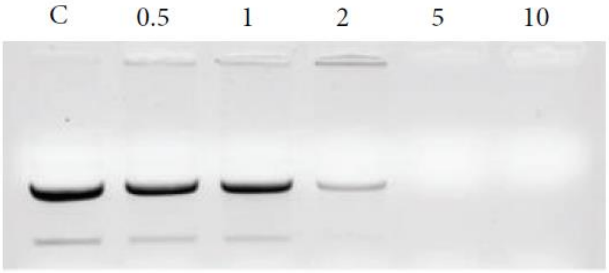

Figure 1 : Agarose gel electrophoresis result of MPD polyplexes. C: pDNA only. Numbers mean weight ratios of the polyplexes.

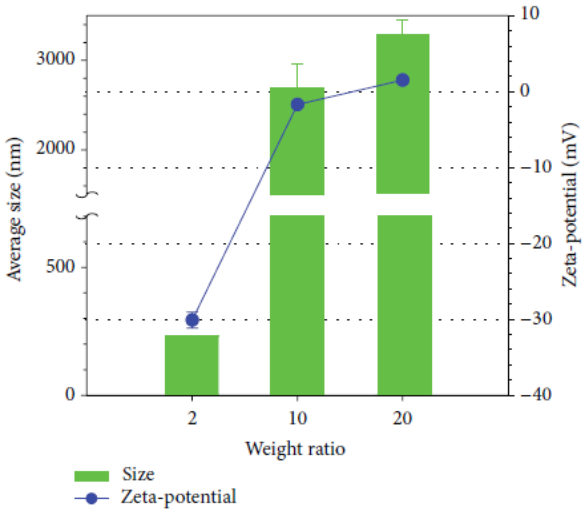

Figure 2 : Average sizes and zeta-potential values measurement results of MPD polyplexes.

Average sizes and Zeta-potential values of MPD polyplexes were measured by Zetasizer (Figure 2). The sizes of MPD polyplexes was 230 nm at a weight ratio of 2

and increased to about 2-3 μm over weight ratios of 20. However, Zeta-potential values of MPD polyplexes were found to be negative (−30.1mV) at a weight ratio of 2 and to be increased to almost zero over weight ratios of 20. Considering Zeta-potential values, it is thought that MPD polyplexes could form large aggregates via hydrophobic interaction at high weight ratios due to the neutralization of surface charges by interaction of cationic MPD micelles with anionic pDNA.

Cellular toxicity was examined by MTT assay. The viability of MPD-treated cells was more than 80% at the same concentration in all two cell lines (Figure 3, 4). It means that cytotoxicity of MPD was low at this condition, although it was found to be concentration-dependent.

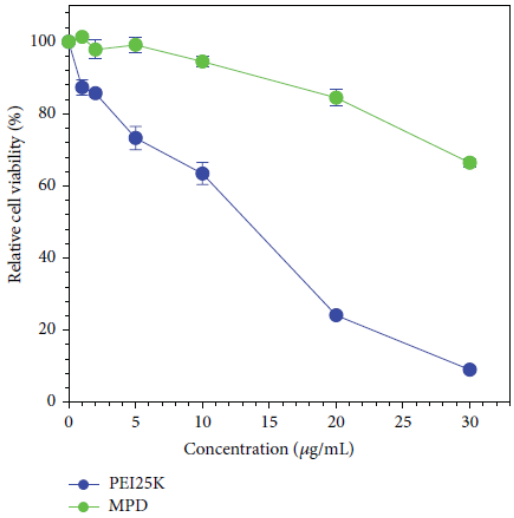

Figure 3 : MTT assay results of MPD in A549

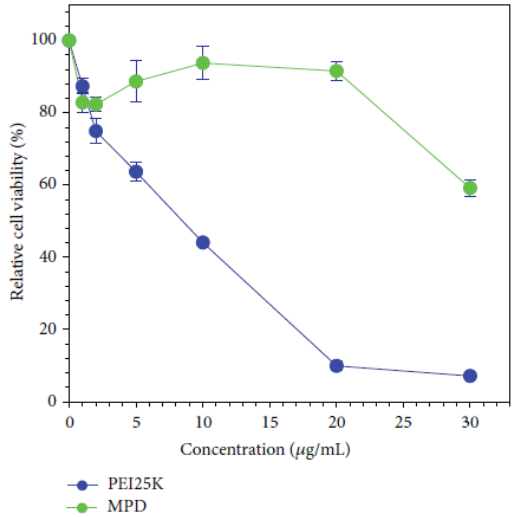

Figure 4 : MTT assay results of MPD in HEK293

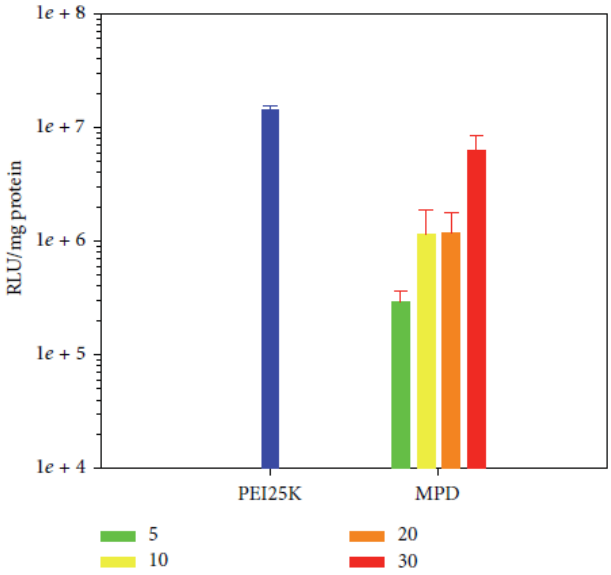

Figure 5 :Transfection experiment results of MPD polyplexes in A549.

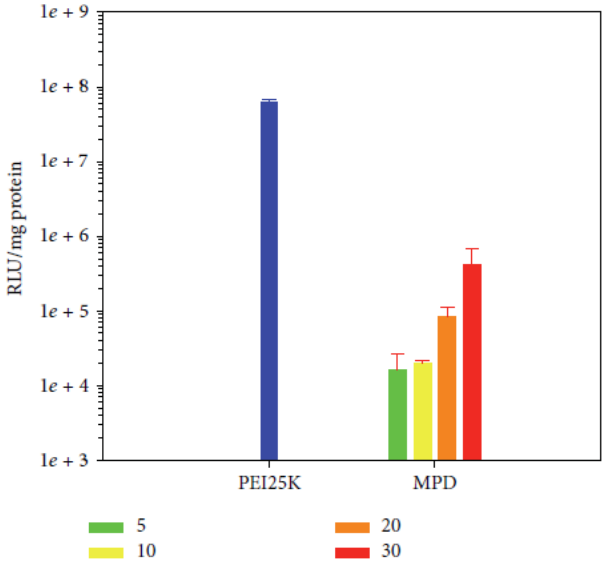

Figure 6 : Transfection experiment results of MPD polyplexes in HEK293.

A549 cells express high levels of MMP-2 but HEK293 does not. MPD, therefore, showed high transfection efficiency comparable to PEI25k in A549 cells expressing high level of MMP-2. (Figure 5, 6) In HEK293 cells, however, represented low level of MMP-2, MPD showed lower transfection efficiency than A549 cells. This result means that MPD possess the potential for gene delivery systems, especially targeting MMP-2 expressing cancer cells.

4 CONCLUSION

In this study, we designed multifunctional peptide dimers for gene therapy targeted to cancers. MP had many

structural moieties which have biological functionalities. MPD was oxidized successfully by DMSO auto oxidation reaction and captured pDNA at a weight ratio of 2. MPD showed positive zeta-potential, however the zeta-potential of MPD polyplexes was decreased. MPD represented better cell viability than PEI25K. MPD represented high transfection efficiency in MMP-2 rich cell line, A549, in comparison with no MMP-2 expression cell line, HEK293. We need additional researches about the reason of enhanced transfection efficiency related to secondary structure, morphology, and so on. As a result, we suggested that MPD has a potential for peptide-based gene delivery systems.

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