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New amphiphilic *N*-phosphoryl oligopeptides designed for gene delivery

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ABSTRACT

Gene therapy is a potent tool for the treatment of cancer and other gene defect diseases, which involves using DNA that encodes a functional, therapeutic gene to replace a mutated gene. However, the DNA transfection efficiency is restricted by its negative charges and low susceptibility to endonucleases which prevent them penetrating tissue and cellular membranes. Both viral and non-viral vectors have been used for gene delivery, but the former are limited by their immunogenicity, while the latter are less efficient than their viral counterpart. Cationic amphiphilic lipopeptides whose structures can be easily modified and transformed have been used as non-viral vectors in gene delivery system due to their low cytotoxicity and high transfection efficiency. In this study, a series of cationic amphiphilic *N*-phosphoryl oligopeptides with varied lengths of hydrophobic tails and oligopeptide headgroups (C12-K6, C14-K6, C16-K6, Chol-K6 and C12-H6) were synthesized and used as gene delivery vectors. The affinities, abilities to condense pDNA and transfection efficiencies of the K6-lipopeptides were better than those of the H6-lipopeptides. In addition, the hydrophobic chains of the lipopeptides also affected their transfection efficiencies. The K6-lipopeptide with a hydrophobic chain of twelve carbons (C12-K6) showed the highest transfection efficiency in all these synthetic lipopeptides. At an optimal P/N ratio of 20, C12-K6 showed comparable pDNA transfection efficiency to PEI-25k, a well-defined gene delivery vector, but the cytotoxicity of C12-K6 was much lower. With acceptable gene transfection efficiency and low cytotoxicity, this cationic amphiphilic lipopeptide will have promising applications in gene therapy.

1. Introduction

Many diseases, such as cancer and hemophilia, are associated with the acquired and inherited defects of related genes. For example, about 30% of tumors, including lung, colon, thyroid, and pancreatic carcinomas, have a mutation in RAS gene. Another example is hemophilia, which is associated with defects of clotting factors IIX, IX and XI. Gene therapy has attracted much attention in the treatment of this kind of diseases as it is accomplished by transporting a therapeutic gene (pDNA, siRNA, ODNA, etc.) into target cells to replace a deficient gene or lead to a modulation of the expression of genes, thereby restoring production of a functional protein (Friend et al., 1996).

However, the DNA transfection efficiency is restricted by its negative charges and low susceptibility to endonucleases which prevent them penetrating tissue and cellular membranes. Thus, gene delivery vectors are used to facilitate genes transfection and protect them from being degraded. Depending on the vectors used for nucleic acid transfer, gene delivery is usually divided into two main systems: viral and non-viral gene delivery. Although viral vectors are widely studied and have some advantages, their immunogenicity and potential oncogenicity may cause serious problems in terms of safety and limited their application *in vivo* (Luo and Saltzman, 2000). A broad range of non-viral delivery systems including mechanical, electrical and chemical methods have been established. For example, naked DNA can be directly injected into a cell's nucleus by microinjection (Harland and Weintraub, 1985). Cellular uptake of exogenous DNA can be also realized by electrical pulses which are able to transiently permeabilize cell membranes (Heiser, 2000). The first chemical method for DNA delivery was introduced in the late 1950s, and high salt concentration and polycationic proteins were used to improve the ability of nucleic acids to enter cells (Felgner, 1990). DEAE-dextran (Pagano, 1970) and calcium phosphate (Schenborn and Goiffon, 2000) were also used for interaction with DNA to form DEAE-dextran-DNA and calcium phosphate-DNA complexes, respectively, which were internalized via endocytosis after being deposited onto cells. So far, synthetic gene delivery

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chemicals, including cationic lipids (Martin et al., 2005), cationic polymers (Boussif et al., 1995; Furgeson et al., 2003; Suh et al., 1994; Xu and Yang, 2011), cell-penetrating peptides (CPPs) (Furgeson et al., 2003; Parente et al., 1990; Suh et al., 1994; Trabulo et al., 2010) and some dendrimers (Furgeson et al., 2003; Kukowska-Latallo et al., 1996; Suh et al., 1994; Zhou et al., 2006) have become the most widely used vectors in biological studies and pre-clinical gene therapies.

The basic principle of non-viral vectors mediated transfection is as follows: (i) Non-viral vectors compact large DNA molecules into small particles by electrostatic interactions between the positively charged vectors and negatively charged DNA molecules (Furgeson et al., 2003; Gershon et al., 1993; Ma et al., 2007; Suh et al., 1994). The formation of vector/DNA complex can also protect DNA from degradation by enzymes in the external environment. (ii) An excess addition of cationic vectors equip the complex surfaces with positive charges, which is presumed to facilitate subsequent cellular uptake via interaction with the negative cell surface structures such as heparin sulphates and other proteoglycans (Mislick and Baldeschwieler, 1996). These complexes are taken up by cells through clathrin-mediated endocytosis, caveolae-mediated endocytosis and macropinocytosis (Conner and Schmid, 2003; Kirkham and Parton, 2005; Nichols, 2003; Suh et al., 1994). (iii) Once in the endocytic pathway, the plasmids may be degraded when reaching the lysosomes. Accordingly, for effective transfections, the plasmids need to acquire cytosolic access at an earlier stage by escaping from the endosomes (Mukherjee et al., 1997). Different mechanisms such as pore formation in the endosomal membranes (Huang et al., 2004), pH-buffering effect of protonable groups (Moreira et al., 2009; Pack et al., 2000; Suh et al., 1994; Varkouhi et al., 2011) and fusion into the lipid bilayer of endosomes (Xu and Szoka, 1996) have been proposed to facilitate the endosomal escape.

Felgner et al. (Felgner et al., 1987) reported the first cationic lipid named DOTMA (*N*-(1-(2,3-dioleyloxy) propyl)-*N*,*N*,*N*-trimethyl-ammonium chloride), which consists of a quaternary amine connected to two unsaturated aliphatic hydrocarbon chains via ether groups. DOTMA was 5 to over 100 folds more effective than either the DEAE-dextran or the calcium phosphate transfection technique. Cationic lipids are amphiphilic molecules that consist of the following three structural segments: (i) a hydrophilic headgroup which is positively charged, usually via the protonation of monovalent or multivalent amine groups; (ii) a hydrophobic region composed of a steroid or alkyl chains (saturated or unsaturated); (iii) a linker connecting the cationic headgroup with the hydrophobic anchor, whose nature and length may impact on the stability and biodegradability of the vector.

Cell-penetrating peptides (CPPs) are short peptides with potential abilities to translocate across the plasma membrane of living cells. Many CPPs are derived from the transduction domains of viral proteins involved in interaction with cell membranes (Vivès et al., 1997). A typical example of CPPs is HIV Tat (Ignatovich et al., 2003) consisting of 6 arginines and 2 lysines in its 13 amino acid residues, which is effective in binding to plasmid DNA through charge interaction and condensing it. CPPs have been studied during the last decades as materials for drug delivery vehicles due to their biodegradability, biocompatibility, low toxicity and ease of synthesis (Torchilin et al., 2003).

In this study, a series of cationic amphiphilic *N*-phosphoryl oligopeptides (C12-K6, C14-K6, C16-K6, Chol-K6 and C12-H6) were designed and synthesized using the phosphite method (Ma and Zhao, 1992; Moreira et al., 2009; Musiol et al., 1994; Suh et al., 1994) and Fmoc solid-phase synthesis. Results showed that the length and structures of the *N*-terminal phosphoryl ester chains could dramatically affect the transfection efficiency of lipopeptide/pDNA complexes. At an optimal P/N ratio of 20, C12-K6 showed best pDNA transfection efficiency which is comparable to the commercial gene delivery vector PEI-25k, but its cytotoxicity was much lower. With acceptable gene transfection efficiency and low cytotoxicity, this cationic amphiphilic lipopeptide could be potentially applied in gene therapy.

2. Materials and methods

2.1. Materials

Dodecanol, tetradecanol, hexadecanol and cholesteryl chloroformate were purchased from Alfa Aesar Co. (Ward Hill, MA). Fmoc-Lys(Boc)-Wang resin, H-His(Trt)-2-Chlorotrityl resin, Fmoc-Lys(Boc)-OH, Fmoc-His(Trt)-OH, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetra-methyluroniumhexafluorophosphate (HBTU), *N*-hydroxybenzotriazole (HOBt), piperdine, diisopropylethylamine (DIEA), thioanisole, dithioglycol and trifluoroacetic acid (TFA) were obtained from GL Biochem (Shanghai) Ltd. (Shanghai, China). Dimethylformamide (DMF) and dichloromethane (DCM) were provided by DiKMA Technologies Inc. (Beijing, China). Branched poly(ethylene imine) (PEI-25k, Mw = 25,000) was supplied by Sigma–Aldrich Co. (St. Louis, MO). Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin were purchased from Gibco (Grand Island, NY). Dimethylsulfoxide (DMSO) and thiazoyl blue tetrazolium bromide (MTT) were purchased from BioDee (Beijing, China). pEGFP-N2 and 293T cell line were generously provided by the laboratory of Prof. Yanmei Li (School of Life Science, Tsinghua University).

2.2. Synthesis of the lipopeptides

The *N*-phosphoryl oligopeptides were synthesized from phosphites and oligopeptides, which could be divided into two major steps: synthesis of phosphites and its incorporation to the oligopeptides (Ma and Zhao, 1992; Moreira et al., 2009; Musiol et al., 1994; Suh et al., 1994). The synthesis of phosphites with long dialkoxyl chains was obtained through direct esterification of phosphorus trichloride by 3 equivalents of alcohol in benzene (Fig. 1). Separation of the products was accomplished by reduced pressure distillation.

Peptides were synthesized on resin utilizing standard Fmoc solid phase procedure. Phosphites (Fig. 1A) and cholesteryl chloroformate (Fig. 1B) were incorporated as the last amino acid. The resulting dry resin bound lipopeptides were cleaved and side-chains were deprotected using a cocktail of TFA:H_2O:thioanisole:phenol:ethanedithiol (82.5:5:5:5:2.5). The resin was then removed by filtration, and TFA was evaporated with a slow stream of N_2. The lipopeptides were precipitated from the filtrate with cold diethyl ether. The resulting crude lipopeptides were then purified by RP-HPLC (Cosmosil C18 & C4 peptide/protein column). Purified lipopeptide solutions were evaporated to remove organic phase and then lyophilized, obtaining a pure lipopeptide powder utilized for all subsequent experiments. The purity (>90%) of each lipopeptide was assessed by analytical HPLC and ESI-MS.

2.3. Agarose gel retardation assay

To examine the ability of each cationic lipopeptide to bind pDNA, an agarose gel retardation assay was performed (Weyland et al., 2013). The complex solution was prepared under a predetermined N/P charge ratio (the molar ratio of the amine groups in the lipopeptides to the phosphates in pDNA) by mixing pDNA (0.8 μg pDNA in 8 μl TE buffer) with an equal volume of the lipopeptides in PBS buffer solution (pH 7.4). Each mixture was vortexed for 30 s and incubated for 30 min at room temperature. Then, the mixture was analyzed on a 0.8% agarose gel containing

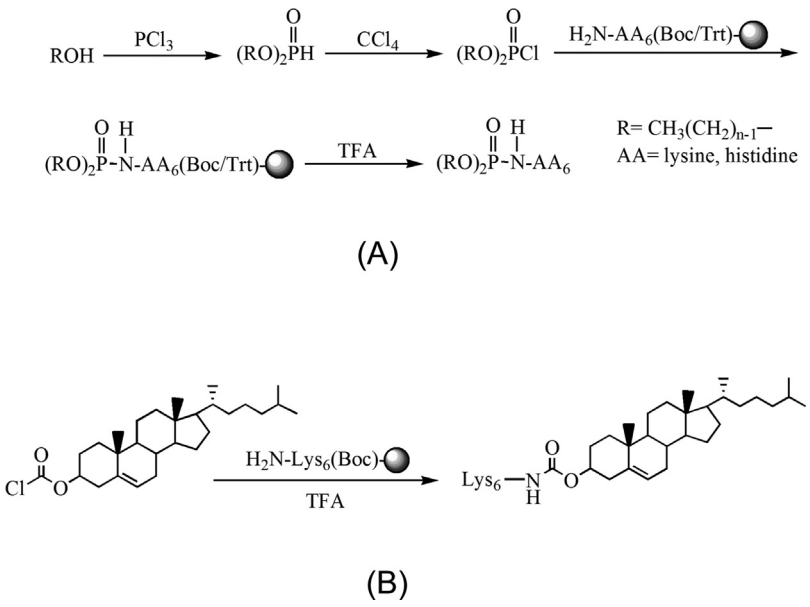

Fig. 1. (A) Synthetic pathways of cationic *N*-phosphoryl oligopeptides. (B) Synthetic pathways of Chol-K6 cationic lipopeptides.

0.45 mg/ml ethidium bromide (EtBr). Gel electrophoresis was carried out in TAE running buffer (40 mM Tris–acetate, 1 mM EDTA) at a voltage of 110 V for 30 min. DNA bands were visualized and photographed by a UV transilluminator.

2.4. Particle size and zeta potential analysis

Sizes and zeta potentials of the lipopeptide/pDNA complexes were analyzed at room temperature on a Malvern Zetasizer Nano ZS90 (Malvern Instruments Ltd., UK). The complex solutions with various N/P ratios were prepared by adding lipopeptide solutions to 50 μl PBS solutions (pH 7.4) containing 3.0 μg pDNA. After incubation at room temperature for 30 min, the complex solutions were diluted to 1 ml with PBS and subjected to the zetasizer for particle size and zeta potential analysis.

2.5. Atomic force microscope (AFM) imaging

C12-K6/pDNA complex formed at N/P ratio of 3 was prepared as described above and characterized at room temperature on a Nanoscope Innova atomic force microscopy (AFM) (Veeco Instruments Inc., USA) in tapping mode. The samples for AFM analysis were preliminarily prepared by dropping the complex solution onto a piece of fresh mica, and air-dried at room temperature prior to AFM analysis.

2.6. Cell culture

The 293T cells are isolated from human embryonic kidneys and transformed with large T antigen, and commonly used for transient transfection. The cells used in this study were cultured at 37 °C under an atmosphere with 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) containing 4.5 g/l glucose, 10% FBS, 100 units/ml of penicillin and 100 μg/ml of streptomycin.

2.7. MTT cytotoxicity assay

MTT assays were conducted with 293T cells to evaluate the cytotoxicities of the synthesized lipopeptides and their pDNA complexes. Briefly, the cells were seeded in a 96-well plate at a density of 7000 cells/well, and incubated for 24 h to form a monolayer. Subsequently, the medium was replaced with fresh DMEM containing lipopeptides or lipopeptide/pDNA complexes of different N/P ratios and incubated for another 24 h. Then, 10 μl sterile-filtered MTT (5.0 mg/ml) was added into each well, reaching a final MTT concentration of 0.5 mg/ml. After incubation for 4 h, the medium was removed and DMSO (100 μl/well) was added to dissolve the formed crystals. Finally, after gently shaking the plate for 10 min, the absorbance was measured on a microplate reader (BioTek, Synergy TM 4, USA) at 570 nm. In this study, a branched PEI-25k was applied as a positive reference.

2.8. EGFP transfection assay

For the EGFP assay, 293T cells were seeded into a 24-well plate at a density of 50,000 cells/well and incubated for 24 h. Complex solutions were preliminarily prepared at various N/P ratios by mixing the lipopeptides and pEGFP-N2 (1.0 μg/well). After 30 min standing, the prepared complex solutions were added into the wells, and continued to incubate for 4 h in FBS-free DMEM. Then, the medium was replaced with fresh DMEM containing 10% FBS, and incubated for another 24 h. Consequently, the 293T cells were harvested and washed with PBS. To compare the transfection efficiency, cells were analyzed using a FACSCalibur flow cytometer (BD Biosciences, USA).

3. Results and discussion

3.1. Synthesis and identification of the lipopeptides

During the separation of phosphite ester from the reaction mixture, the temperature should be carefully controlled, because

Table 1
Molecular weights of the synthetic lipopeptide vectors.

Vector	Molecular weight	
	Expected	Found
K6-C12	1201.92	M + H = 1203.84, (M + 2H)/2 = 602.75
K6-C14	1258.98	M + H = 1259.92, (M + 2H)/2 = 630.73
K6-C16	1315.05	M + H = 1315.94, (M + 2H)/2 = 658.75
K6-Chol	1198.91	M + H = 1199.83, (M + 2H)/2 = 600.71
H6-C12	1256.71	M + H = 1257.41, (M + 2H)/2 = 629.45

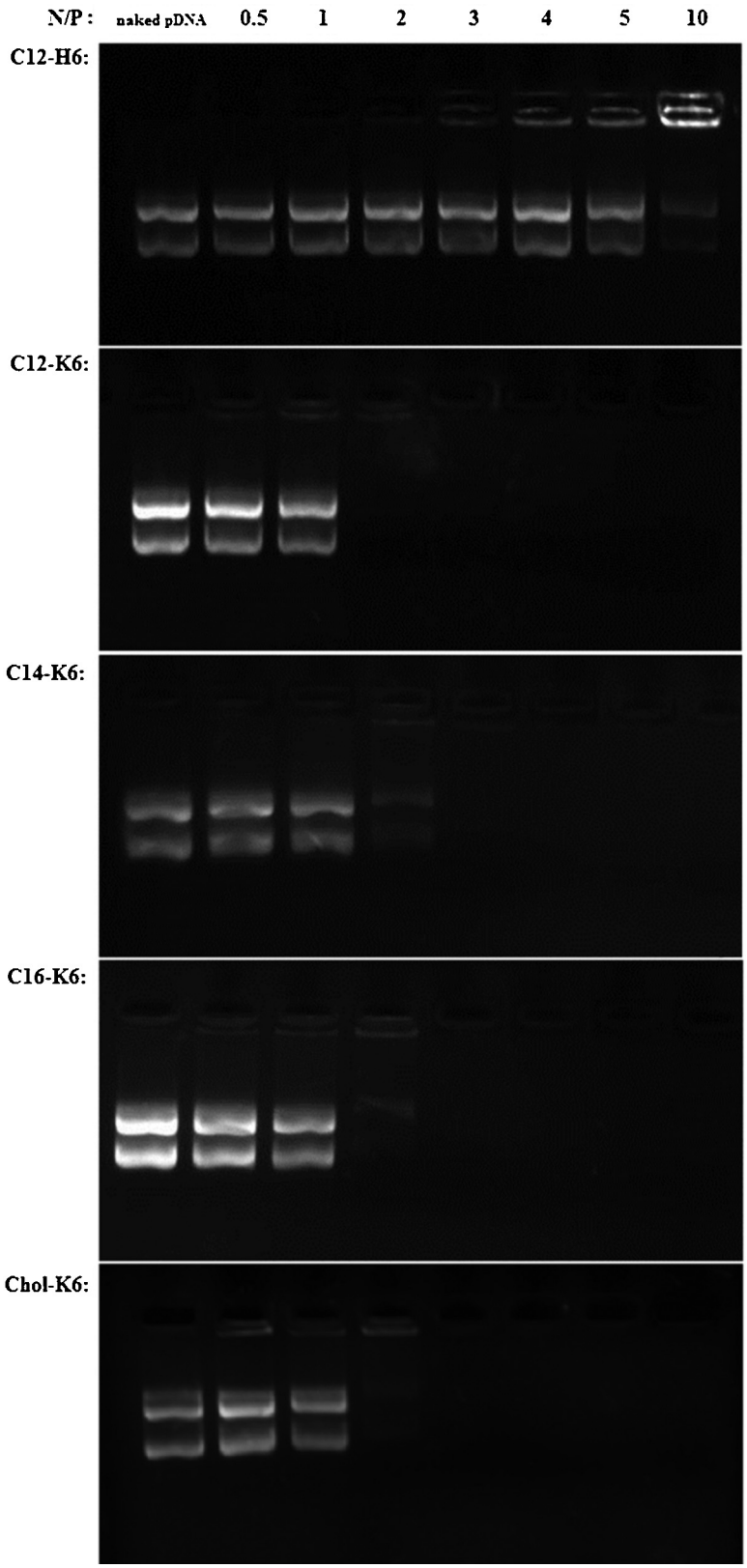

Fig. 2. Agarose gel retardant electrophoresis assay of lipopeptides/pDNA complexes. Lipopeptide/pDNA complexes with various N/P charge ratios were analyzed on a 0.8% agarose gel containing 0.45 mg/ml ethidium bromide (EtBr). Gel electrophoresis was carried out in TAE running buffer (40 mM Tris–acetate, 1 mM EDTA) with a voltage of 110 V for 30 min. DNA bands were visualized and photographed by a UV transilluminator.

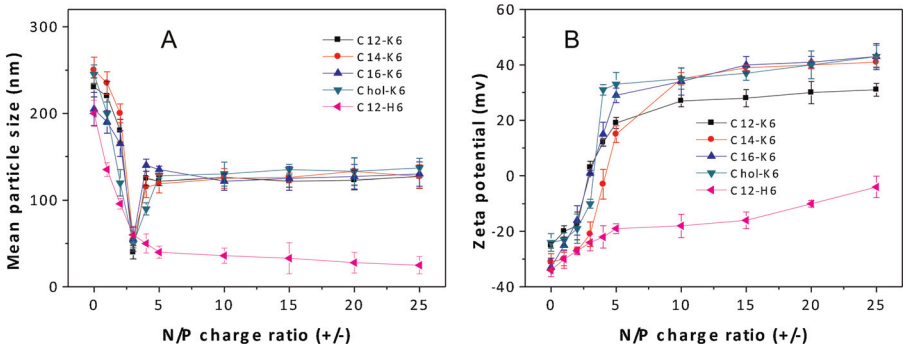

Fig. 3. Particle sizes and zeta potentials of the lipopeptide/pDNA complexes under various N/P ratios. Lipopeptides were mixed with pDNA at various N/P ratios and incubated at room temperature for 30 min, and then diluted to 1 ml with PBS. The particles sizes (A) and zeta potentials (B) were measured using a zetasizer. Data represents the mean and SD of three independent tests.

too high a temperature would cause the formation of a large amount of by-products. Oligopeptides were synthesized employing a standard Fmoc solid-phase peptide synthesis (SPPS) technique. Lipopeptides containing K6 were synthesized manually on the Fmoc-Lys(Boc)-Wang Resin, while C12-H6 was synthesized on the 2-chlorotrityl chloride resin in case of racemization of histidines. The molecular weights determined by an electrospray ionization mass spectrometry (ESI/MS) were listed in Table 1.

3.2. Compaction of pDNA by the lipopeptides

The binding capability of materials to condense pDNA is a prerequisite for gene delivery vectors. The electrostatic interactions between negatively charged nucleic acids and cationic lipopeptides will influence the efficiency of packing gene cargoes and their subsequent release into cytoplasm. In order to evaluate the abilities of these synthesized lipopeptides to bind pDNA, an agarose gel electrophoresis analysis of the lipopeptide/pDNA complexes with various N/P ratios was performed. For the lipopeptides containing K6, the pDNA bands were attenuated as the N/P ratio increased and disappeared at N/P ratios higher than 2, suggesting an efficient compaction of the pDNA by these lipopeptides. However, there were still pDNA bands for the samples of C12-H6 at N/P ratios up to 10, which meant pDNA was hardly bound by C12-H6. As the isoelectric points of lysine and histidine side chains were ~9.60 and ~7.60, respectively, lipopeptides containing K6 were much more positively charged than that containing H6 at pH 7.4, which led to a higher binding affinity of those lipopeptides containing K6 to the negatively charged pDNA. The length and structures of the hydrophobic chains in the lipopeptides seemed to have little effects on their condensing abilities to pDNA, since all K6-containing lipopeptides with different hydrophobic chains started to retard pDNA at the same N/P ratio of 2, but C12-K6 might have a slightly higher efficiency due to its short alkane chain (Fig. 2).

To further investigate the complexation of pDNA with the lipopeptides, the physicochemical properties of the lipopeptide/pDNA complexes were analyzed. According to the agarose gel retardation assays, pDNA was condensed by the cationic lipopeptides, and as a result, the particle sizes of K6-lipopeptide/pDNA complexes were dramatically decreased along with the N/P ratios increasing from 0 to 3. In addition, the particle sizes of K6-lipopeptide/pDNA complexes reached to a minimum of about 50 nm at the N/P ratio of 3, and then increased and maintained at a plateau of 125 nm as the N/P ratios increased (Fig. 3A). These suggested that pDNA was completely condensed by K6-lipopeptides at the N/P ratio of 3, which is in consistent with the agarose gel eletrophoresis, and a shell of cationic lipopeptides were formed on the surface of lipopeptide/pDNA complexes when extra lipopeptides were added into the solutions. Stable cationic lipopeptide/pDNA complexes were formed when N/P ratios were over 5, as no more cationic lipopeptides were able to bind to the complexes due to the electrostatic repulsion. For the case of C12-H6, the particle sizes of C12-H6/pDNA complexes were continuously declined as the N/P ratios increased until it was over 5. The relatively stable particle sizes of C12-H6/pDNA complex at the N/P

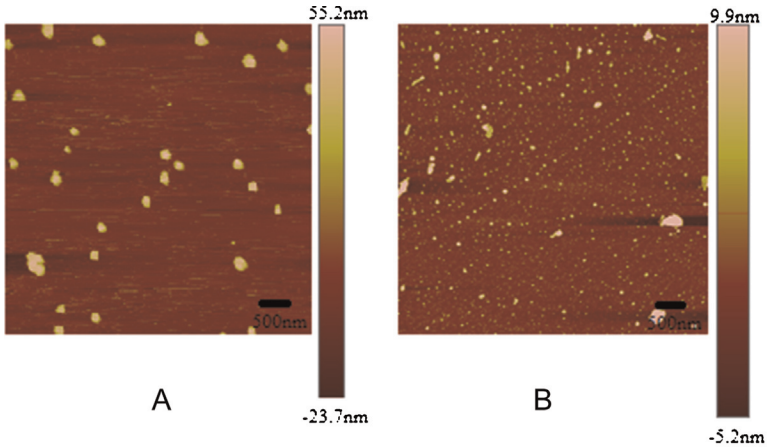

Fig. 4. Morphologies of naked pDNA (A) and C12-K6/pDNA complex (N/P = 3) (B) under AFM. The samples were prepared by depositing a drop of complex solution onto a piece of fresh mica for 10 min, and then air-dried at room temperature prior to AFM measurements.

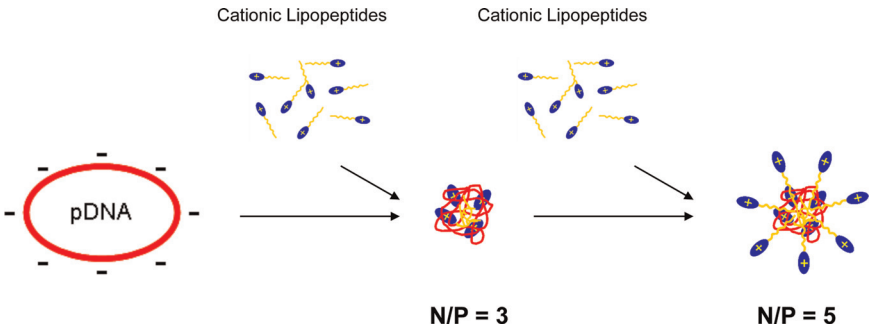

Fig. 5. A schematic graph of the pDNA compaction by the lipopeptides under varied N/P ratios.

ratios over 5 were comparable to the minimum particle sizes of the K6-lipopeptide/pDNA complexes at the N/P ratio of 3, suggesting no lipopeptide shell was formed on its surface (Fig. 3A).

The morphology of cationic lipopeptide/pDNA complexes were observed under AFM. A typical image of C12-K6/pDNA complex was provided as a representative, showing an average size of less than 50 nm, which was significantly lower than that of the naked pDNA whose average size was about 200 nm (Fig. 4). This along with the agarose gel eletrophoresis and particle size measurements confirmed the condensation of pDNA by these cationic lipopeptides.

As complexes were formed by the lipopeptides and pDNA, zeta potentials were measured to indicate the electrostatic properties of these particles. A sharp increase of the potentials were observed in the N/P ratio range of 0 to 5, and then reached to a plateau. The transition points of the K6-lipopeptide/pDNA complexes from negative to positive were at the N/P ratio of around 3, which were in accordance with their minimum points of the particle size measurement. The potentials of K6-lipopeptide/pDNA complexes became more positive until reached the N/P ratio of 5, which also suggested the formation of cationic shells on the particle surfaces. However, although the potential of C12-H6/pDNA complex was also increased along with N/P ratios, it never became positive, implying no cationic shell formation for this complex (Fig. 3B). According to the above results, a schematic picture was drawn to illustrate the process of pDNA compaction by these cationic lipopeptides (Fig. 5).

3.3. In vitro *cytotoxicity*

The cytotoxicities of the lipopeptides used in the present study was assessed on 293T cells by MTT assay, and the branched PEI-25k, a defined non-viral gene delivery vector, was used as a positive control. Both synthetic lipopeptides and PEI-25k showed dose-dependent cytotoxicities (Fig. 6A). The cell viability decreased up to 60% when the branched PEI-25k concentration was increased to 200 mg/l. This high cytotoxicity was originated from the cytolysis due to its high cationic charge density as reported by Moein Moghimi et al. (Moghimi et al., 2005). With regard to C12-K6, C14-K6 and C16-K6, cell viabilities were still maintained over 50% at 200 mg/l. C12-H6 showed the lowest cytotoxicity in all the tested vectors due to its low charge density. Chol-K6 showed the highest cytotoxicity which may be related to the structure of cholesterol, but detailed mechanism was unclear.

Meanwhile, cell viabilities of the lipopeptide/pDNA complexes were also evaluated (Fig. 6B). In general, the complexes showed lower cytotoxicity than the lipopeptides under the same concentrations, which was probably because of the reduced positive charges when binding to pDNA.

3.4. Gene transfection efficiency of the lipopeptides

According to the agarose gel retardation assay, C12-K6 seemed to be more efficient than the other lipopeptides, and its cytotoxicity was acceptable, which was much lower than that of the commercial gene delivery vector PEI-25k, so C12-K6 was chosen to determine the optimal N/P ratio for gene transfection. The transfection percentage increased dramatically until the N/P ratio reached 20, and then decreased a little. As indicated by the particle size and zeta potential measurement, cationic lipopeptides were not able to assemble onto the formed lipopeptide/pDNA complexes if the N/P ratio was over 5, thus there might be a large number of free lipopeptides at N/P ratio of 20. The pDNA transfection efficiency was continuously enhanced at the N/P ratios from 3 to 20, suggesting the free lipopeptides might facilitate the complexes translocation and unpacking of the pDNA (Moghimi et al., 2005). However, over-dosed free lipopeptides was toxic to the cells, so the transfection efficiency dropped when

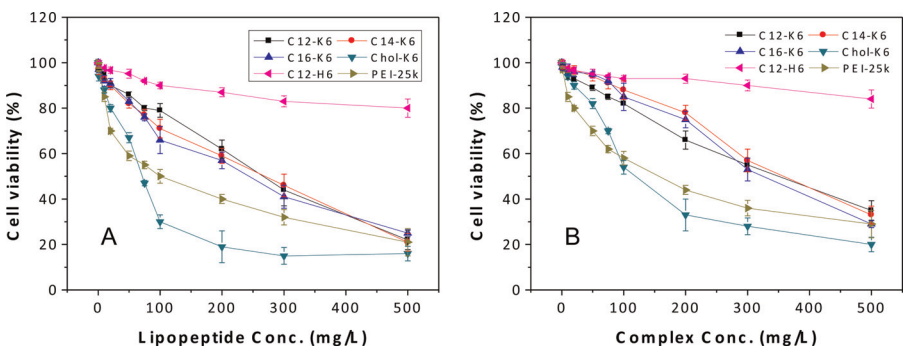

Fig. 6. (A) Dose-dependent cytotoxicities of lipopeptides and PEI-25k on 293T cells. (B) Dose-dependent cytotoxicities of lipopeptides/pDNA complexes and PEI-25k/pDNA complex prepared at N/P = 20 on 293T cells. MTT assays were performed after these vectors or complexes were incubated with 293T cells for 24 h. Data represents the mean and SD of three independent tests.

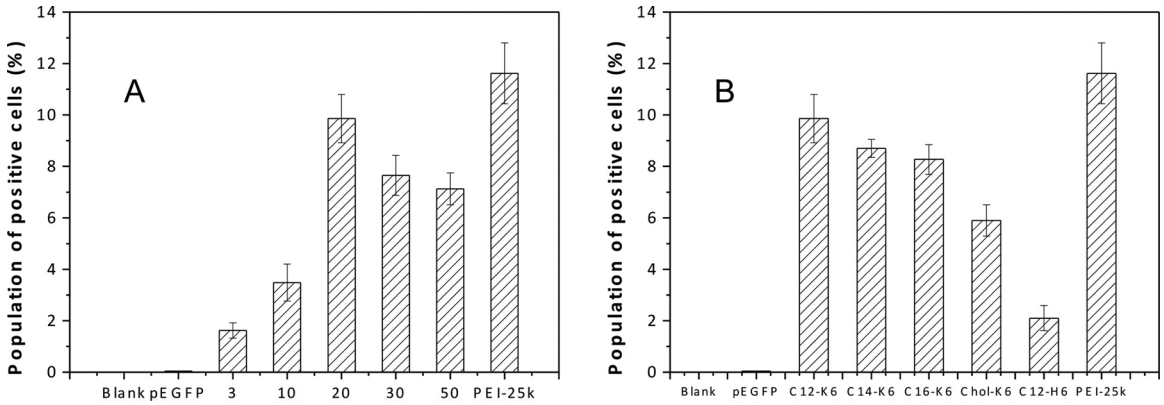

Fig. 7. (A) Transfection efficiency of C12-K6 at various N/P ratios determined by flow cytometry. (B) Transfection efficiency of different vectors at the N/P ratio of 20. Data represents the mean and SD of three independent tests.

the N/P ratio was over 20. The transfection efficiency of C12-K6 at N/P ratio of 20 was comparable to that of PEI-25k (Fig. 7A). This suggested that C12-K6 was an efficient gene delivery vector.

A further investigation of the transfection efficiencies of these vectors at N/P ratio of 20 was tested. For the K6-lipopeptides, hydrophobic chains did affect their transfection efficiency, as the positive cell percentages were decreased as the lengths of phosphite esters chains were prolonged (C12-K6 > C14-K6 > C16-K6), and that of chol-K6 was in further decreased (Fig. 7B). Because the particle sizes and potentials of these K6-lipopeptide/pDNA complexes were similar at the P/N ratio of 20, packing and translocation of them should be varied little. Therefore, the hydrophobic chains might affect the unpacking of the condensed pDNA, as the more complicated was the hydrophobic chain, the more difficult for the pDNA to dissociate from the complexes.

C12-H6 showed much lower transfection efficiency due to its less positive charges and weak pDNA binding affinity. Although the pDNA might be completely compacted by C12-H6 at the N/P ratio of 20, but it seemed no cationic shell was formed on the complex surface, as the zeta potential of this complex was still negative even at the N/P ratio of 20. The cationic shell was believed to be critical for cell membrane binding and translocation, thus the C12-H6/pDNA complex was difficult to enter the cell and exhibited low transfection efficiency.

Non-viral gene delivery vectors are widely studied for gene delivery due to their low immunogenicity, but their transfection efficiencies are usually lower compared with viral vectors. The positive charges of non-viral vectors like PEIs, play an important role in their interaction with cell membranes, translocation and escape from the endosomes. However, high positive charges are also associated with high cytotoxicity (Moghimi et al., 2005). Therefore, a balance between the transfection efficiency and cytotoxicity becomes an issue. On the one hand, the designed cationic lipopeptides used in this study were composed of a short cationic peptide chain of K6, which is from a cell penetrating peptide and facilitates their interaction with pDNA and membrane translocation. The peptide component was believed to endow these cationic lipopeptides with good biocompatibility and low cytotoxicity. On the other hand, the hydrophobic ester chain components were incorporated to help the assembly of the complexes, but their length and complexity might affect the escape of the complexes from endosomes. Among all the designed lipopeptide vectors, C12-K6 showed a much lowered cytotoxicity and comparable gene transferring ability in comparison with the commercial gene delivery vector, PEI-25k, which could have a promising application prospect in gene delivery.

4. Conclusion

In conclusion, a series of lipopeptides with varied phosphoryl ester chains were designed and synthesized as gene delivery vectors. The K6-lipopeptides were much more efficient than H6-lipopeptides in facilitating pDNA transfection. Moreover, the types of hydrophobic phosphoryl ester chains were also related to their transfection efficiency. At an optimal N/P ratio of 20, C12-K6 showed comparable pDNA transfection efficiency to PEI-25k, a defined gene delivery vector. However, the cytotoxicity of C12-K6 was much lower than PEI-25k (IC_{50} of C12-K6 *vs.* PEI-25k: 307 mg/l *vs.* 100 mg/l). With a comparable transfection efficiency but low cytotoxicity, the cationic amphiphilic lipopeptide C12-K6 will have a promising application prospect in gene therapy as a delivery vector.

Acknowledgements

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