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Synergistic effect of amino acids modified on dendrimer surface in gene delivery

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ABSTRACT

Design of an efficient gene vector based on dendrimer remains a great challenge due to the presence of multiple barriers in gene delivery. Single-functionalization on dendrimer cannot overcome all the barriers. In this study, we synthesized a list of single-, dual- and triple-functionalized dendrimers with arginine, phenylalanine and histidine for gene delivery using a one-pot approach. The three amino acids play different roles in gene delivery: arginine is essential in formation of stable complexes, phenylalanine improves cellular uptake efficacy, and histidine increases pH-buffering capacity and minimizes cytotoxicity of the cationic dendrimer. A combination of these amino acids on dendrimer generates a synergistic effect in gene delivery. The dual- and triple-functionalized dendrimers show minimal cytotoxicity on the transfected NIH 3T3 cells. Using this combination strategy, we can obtain triple-functionalized dendrimers with comparable transfection efficacy to several commercial transfection reagents. Such a combination strategy should be applicable to the design of efficient and biocompatible gene vectors for gene delivery.

1. Introduction

Gene therapy remains a promising strategy in the treatment of hereditary and acquired diseases in recent years. However, the largest obstacle in gene therapy is to develop high efficient and nontoxic gene carriers [1,2]. An ideal gene carrier should possess multiple functions to overcome the barriers at different stages in gene transfection process [3]. First, the vector should condense genes into nanoparticles and the formed nanoparticles should be stable in serum. Then, the nanoparticles can be internalized into cells via specific endocytosis pathways and escaped from acidic vesicles such as endosomes and lysosomes. Finally, the vector should release the bound genes in the cytoplasm or nucleus [1]. Cationic polymers such as polyethylenimine (PEI) [4], chitosan [5], poly-L-lysine [6], diethylaminoethyl-dextran (DEAE-dextran) [7] and poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) [8] are widely used as nonviral gene carriers due to their versatile structures and unique properties, but inherent cytotoxicity and relatively low transfection efficiency are associated with these polymers, which limit their applications in gene therapy [9].

Dendrimers are a class of synthetic polymers with unique properties such as well-defined structure, spherical shape, low polydispersity, excellent solubility, and large number of surface functionalities and interior cavities [10–12]. Cationic dendrimers can effectively condense DNA into stable complexes due to the multivalency effect of the positive charges on dendrimer surface [13]. In addition, there is a high density of protonable tertiary amine groups in dendrimer interior, providing the "proton sponge effect" during endosomal escape process [14]. As a result, dendrimers and their conjugates were widely used as gene vectors during the past decade [15]. Dendrimer-based transfection reagents such as SuperFect and PolyFect have already entered the market. To further improve their transfection efficiency, dendrimers were modified with cyclodextrins [16], lipids, sugars, peptides, fluorous compounds [17,18], amino acids [19–24], mitochondrial targeting ligands [25] and nanoparticles [26]. Among these functionalized dendrimers, amino acid-dendrimer conjugates are of great interest to the researchers [20–24,27]. Amino acids have the same fundamental structure, differing only in their residues. They can be sorted into cationic, anionic and neutral amino acids, or hydrophilic and hydrophobic amino acids. Dendrimers can be modified with amino acids via facile condensation reactions. Conjugation of cationic amino acids such as arginine (Arg) and lysine (Lys) to dendrimer directly tailors the charge density on dendrimer surface

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[19–23,28,29]. Besides, the residues of these amino acids such as guanidinium and imidazole groups play essential roles in the gene delivery processes. The guanidine group has specific affinity with cell membranes, while the imidazole group provides additional pH-buffering capacity during endosomal escape [24,30]. Arginine-rich or histidine (His)-rich peptides can be directly used as gene vectors [31]. Conjugation of hydrophobic amino acids such as phenylalanine (Phe) and leucine to dendrimer surface tailors the hydrophobicity of dendrimer surface, which is essential in the endocytosis process [32,33]. Conjugation of these amino acids can increase the transfection efficacy of dendrimers through different mechanisms, however, there are multiple barriers in gene transfection and single-functionalization cannot overcome all the barriers.

A solution to this problem is multiple-functionalization of dendrimers with different amino acids such as Arg, His and Phe. Combination of these amino acids on dendrimer may simultaneously improve the membrane affinity, endocytosis and endosomal escape of the complexes. A recent study found that a combination of His and hydrophobic amino acids such as Phe and tyrosine can significantly improve the siRNA interference efficacy of a reduction-sensitive polymer [34]. Also, dual-functionalization of Arg and His on dendrimer surface allows high transfection efficacy [35]. However, these dual-functionalized polymers were synthesized in multi-steps and the synergistic effect of amino acids on these polymers still needs in-depth investigations.

Here, we systematically investigate the synergistic effect of Arg, Phe and His with distinct functions on dendrimer surface in gene delivery. A one-pot approach was adopted to construct single-, dual- and triple-functionalized dendrimers (Fig. 1). Generation 5 (G5) polyamidoamine (PAMAM) dendrimer with a molecular weight of 28826 Da was used as the scaffold material. A total number of 15 dendrimer-amino acid conjugates including 5 single-, 5 dual- and 5 triple-functionalized dendrimers, respectively were synthesized. The physicochemical properties, complex formation, transfection efficacy, transfection mechanisms and cytotoxicity of these amino acid-modified dendrimers were investigated. The aims of this study are to reveal the synergistic effect of amino acids on the transfection efficacy of amino acid-modified dendrimers and to prepare multiple-functionalized dendrimers as efficient gene vectors using a facile one-pot strategy.

2. Materials and methods

2.1. Materials

G5 PAMAM dendrimer with an ethylenediamine core and surface primary amine groups was purchased from Dendritech (Midland, MI). Boc-Arg(Pbf)-OH, Boc-His(Trt)-OH and Boc-Phe-OH were purchased from GL Biochem (Shanghai, China) Ltd. YOYO-1 and Lipofectamine 2000 were obtained from Invitrogen (Carlsbad, California). PolyFect was purchased from Qiagen (German). JetPEI was purchased from Polyplus-Transfection (France). Fetal bovine serum (FBS) and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO (Gaithersburg, MD). G5 dendrimer was received in methanol solution and the solvent was distilled before use. The dendrimer was characterized by ^{13}C NMR and polyacrylamide gel electrophoresis. All the other chemicals were used as received without further purification.

2.2. Synthesis and characterization of the single-, dual- and triple-functionalized dendrimers

Amino acid-modified G5 PAMAM dendrimers were synthesized by a facile condensation reaction as described elsewhere [28]. Briefly, different amounts of Boc-Arg(pbf)-OH, Boc-Phe-OH and Boc-His(Trt)-OH were dissolved in 1.5 mL dehydrated N,N-dimethyl formamide (DMF), followed by addition of dicyclohexylcarbodiimide (DCC, 1.3 molar equivalents of carboxyl group in the protected amino acids) and N-hydroxysuccinimide (NHS, 1.2 molar equivalents of carboxyl group in the protected amino acids) to activate the carboxyl groups of amino acids for 6 h. 50 mg G5 PAMAM dendrimer was dissolved in 2 mL anhydrous dimethyl sulfoxide (DMSO) and added dropwise into the activated amino acid solution. After that, the reaction mixture was stirred at room temperature for 7 d. The molar ratios of fed amino acids to each G5 dendrimer are listed in Table 1. The reaction mixture was dialyzed against DMSO (500 mL) for two times and freeze-dried, the obtained solid was dissolved in 2 mL trifluoroacetic acid (TFA) and stirred at room temperature for 6 h to de-protect the protected groups such as Boc, Pbf and Trt. Then, TFA was removed by rotary evaporation and the crude materials were dialyzed against DMSO (500 mL, three times), PBS buffer (500 mL, three times) and distilled water (500 mL, ten times). The purified product was freeze-dried as white powders. The yielding products were characterized by ^{1}H NMR in D_2O (Varian 699.804 MHz).

2.3. Preparation of polymer/DNA complexes

All the polymer/DNA complexes were freshly prepared before characterization or gene transfection experiments. Generally, amino acid-dendrimer conjugates were added into 0.8 μg plasmid DNA (EGFP or luciferase plasmid) at different N/P ratios and the sample was incubated for 30 min at room temperature. The N/P ratio was calculated according to the cationic groups (N number) on the dendrimer surface to anionic phosphate groups (P number) in plasmid DNA. The corresponding polymer/DNA weight ratios for the complexes at different N/P ratios are also shown in Table S1. For His- and Phe-functionalized dendrimers, the N number for each conjugate was a constant of 128. For Arg-functionalized dendrimers, the N number was a sum of 128 and the number of conjugated Arg moieties since Arg has two cationic groups. Since the imidazole group in His and the tertiary amine group in dendrimer are not protonated at pH 7.4, these groups were not considered when calculating the N numbers. For the commercial transfection reagents such as Lipofectamine 2000,

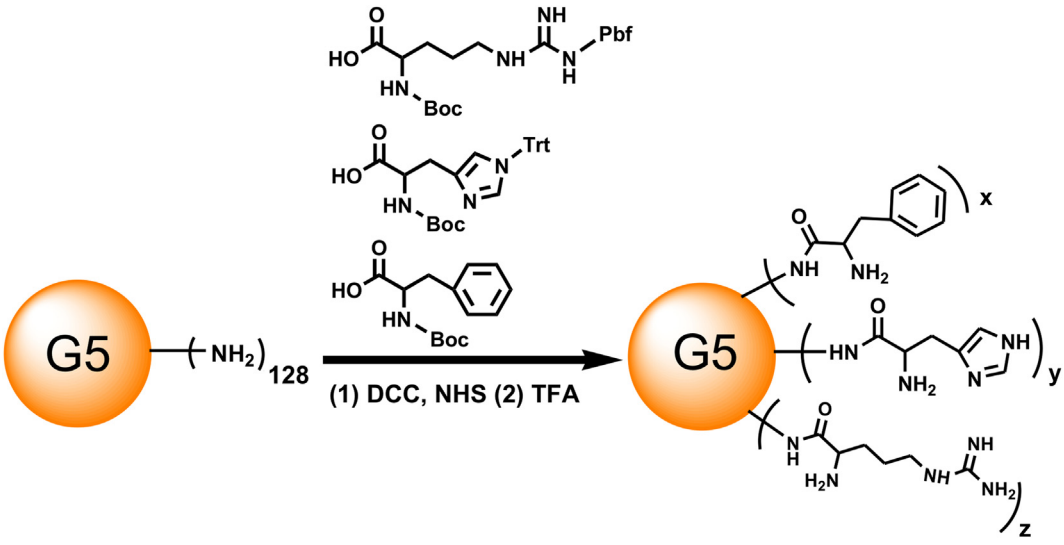

Fig. 1. Synthesis of multi-functionalized dendrimers using a one-pot approach.