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Characterization of a multifunctional PEG-based gene delivery system containing nuclear localization signals and endosomal escape peptides ☆

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Abstract

Endosomal escape and nuclear localization are two barriers to gene delivery that need to be addressed in the design of new nonviral gene delivery vehicles. We have previously synthesized low-toxicity polyethylene glycol (PEG)-based vehicles with endosomal escape functionalities, but it was determined that the transfection efficiency of PEG-based vehicles that escaped the endosome was still limited by poor nuclear localization. Two different nuclear localization signal (NLS) peptides, SV40 and TAT, were coupled to PEG-based vehicles with DNA-binding peptides (DBPs) to determine the effect of NLS peptides on the transfection efficiency of PEG-based gene delivery vehicles. Coupling one SV40 peptide, a classical NLS, or two TAT peptides, a nonclassical NLS, to PEG–DBP vehicles increased the transfection efficiency of PEG–DBP/DNA particles 15-fold and resulted in similar efficiency to that of a common cationic polymer vehicle, polyethylenimine (PEI). The transfection efficiency of both types of PEG–DBP–NLS particles was further increased 7-fold in the presence of chloroquine, suggesting that the transfection efficiency of PEG–DBP–NLS particles is limited by their ability to escape the endosome. To develop particles that could escape the endosome and target the nucleus, a mixture of PEG–DBP–NLS vehicles and PEG-based vehicles with DBPs and endosomal escape peptides were complexed with plasmid DNA to form multifunctional particles that had a transfection efficiency 2–3 times higher than that of PEI. Additionally, the PEG-based vehicles were less toxic and more resistant to nonspecific protein adsorption than PEI, making them an attractive alternative for nonviral gene delivery.

Keywords: Nuclear localization; Endosomal escape; Nonviral; Gene delivery; PEG

1. Introduction

The design of effective multifunctional gene delivery vehicles with low toxicity for systemic gene transfer could be useful for many medical applications. Common synthetic vehicles are based on cationic polymers and lipids. Cationic polymers, such as polyethylenimine (PEI), are able to condense DNA through electrostatic interactions to form positively charged particles that can bind to the negatively charged cell surface proteoglycans [1]. The transfection efficiency of PEI vehicles has been shown to increase with the addition of functional groups, such as targeting ligands or nuclear localization signal (NLS) peptides [2,3]. However, several groups have reported that PEI is cytotoxic to many different cell lines. At PEI concentrations typically used in transfection protocols, cell membrane damage can occur due to the electrostatic interaction of PEI and the plasma membrane [4]. Additionally,

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cationic polymers and lipids interact strongly with plasma proteins, leading to decreased circulation times and low transfection efficiency in vivo [5–7].

Conjugating a hydrophilic polymer, such as polyethylene glycol (PEG), to the surface of cationic DNA particles reduces the charge density on the surface of the polymer–DNA particles. PEG-conjugated PEI particles have longer blood clearance times than unmodified PEI particles. However, conjugation of PEG to the surface of cationic polymer particles decreases their transfection efficiency. Surface-conjugated PEG hinders the ability of the cationic polymers and functional groups to interact with cell surface receptors [8,9]. We previously described a synthetic vehicle that uses PEG as the backbone of the vehicle, rather than conjugated to the surface of the polymer–DNA particle [10]. This vehicle is nontoxic, resistant to nonspecific protein adsorption and can be modified with functional groups to target key steps in the gene delivery pathway [10,11].

In previous work, PEG-based vehicles were coupled with endosomal escape peptides (EEPs), INF7, to design a vehicle capable of destabilizing the endosome. INF7 peptides contain glutamate residues, which protonate under acidic conditions (pH 5–5.5) in the endosome, triggering a conformational change from a random coil to α-helix leading to endosomal disruption [12–16]. We found that PEG-based vehicles have transfection efficiencies similar to PEI when EEPs and DNA-binding peptides (DBPs) were coupled to the PEG backbone [17]. However, time-dependent studies measuring the release of PEG–DNA particles from the endosome demonstrated that an increased rate of endosomal escape is not directly related to an increase in transfection efficiency. Other barriers such as nuclease degradation in the cytoplasm and nuclear localization were hypothesized to potentially limit the efficiency of PEG-based vehicles.

This result was similar to previous studies of PEI/DNA particles, which demonstrated that localization and transport of particles to the nucleus is a key barrier in gene delivery [18]. Particles larger than 25 nm in diameter cannot freely diffuse across the nuclear membrane and require active transport through the nuclear pore complex or cell mitosis for nuclear translocation [19–25]. Incorporation of NLS peptides, which assist in nuclear translocation through nuclear pore complexes, onto PEI particles or naked DNA has been shown to increase the transfection efficiency up to 300-fold [26–29]. Therefore, it was hypothesized that the addition of NLS peptides to PEG-based vehicles would increase their transfection efficiency.

This study aimed to examine the effects of classical and nonclassical NLS peptides on transfection efficiency of PEG-based vehicles. SV40 peptide, a classical NLS derived from the SV40 virus, or Tat peptide, a nonclassical NLS derived from the protein transduction domain of the human immunodeficiency virus (HIV), were coupled to a PEG-based vehicle in combination with DBPs via a Michael-type addition [30–33]. The classical NLS, SV40 peptide, binds to importin α to form a complex with importin β to achieve nuclear transport [30,31], whereas Tat peptide translocates into the nucleus by passive diffusion through nuclear pore complexes or by conjugation with importin β [32,33]. Therefore, we can analyze how different NLS peptides affect transfection efficiency of PEG-based vehicles.

In this study, the transfection efficiencies were measured with one or two NLS peptides per PEG molecule at multiple charge ratios (ratio of positive charges on PEG–DBP–NLS to negative charges on DNA) to determine the most efficient PEG–DBP–NLS vehicle combinations. These PEG–DBP–NLS vehicles were then mixed with previously described PEG-based vehicles with EEPs [17] to develop a multifunctional vehicle that is more efficient and less toxic than PEI.

2. Materials and methods

2.1. Materials

Branched 50 kDa PEI was purchased from Sigma (St. Louis, MO). PEG tetraol (10,000 MW) was purchased from Shearwater Polymers (Huntsville, AL) and acrylated as previously described [34]. Plasmid DNA encoding for β-Galactosidase (pSV-β-Galactosidase, Promega, Madison, WI) and green fluorescent protein (pSv-GFP, Packard Biosciences, Wellesley, MA) were purified from overnight bacterial culture using a Qiagen (Valencia, CA) Megaprep kit.

2.2. Peptide synthesis

DBP (Ac-GCGKRKEFLERNRVAASKFRKRK-NH_2), SV40 peptide (PKKKRKVEPYC-NH_2), Tat peptide (Ac-GCGYGRKKRRQRRRG-NH_2) and INF7 peptide (GLFE AIEGFIENGWEGMIEGWYGCG) were synthesized with an ABI 433A peptide synthesizer (Applied Biosystems, Foster City, CA) using standard solid phase Fmoc chemistry as described previously [10]. DBP and INF7 peptide were purified as previously described [11,17]. NLS peptides, SV40 and Tat, were purified with reverse-phase high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) using 0.1% TFA in water and acetonitrile (Fisher, Hampton, NH, HPLC grade) as the mobile phase. Peptides were eluted from a C18 Xterra Prep RP15 column (19 × 150 mm) (Waters, Milford, MA). Matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) (Applied Biosystems) mass spectrometry was used to verify peptide masses. Purified peptides were lyophilized and stored at −20 °C.

2.3. Vehicle synthesis

NLS peptides and DBPs were coupled to PEG–tetraacrylate (TA) using a Michael-type addition [34] (Fig. 1). PEG–TA and Tat peptide or SV40 peptide were dissolved in 0.1 M phosphate buffer at pH 8 and then coupled for

ID	Title	Pages
2222	Characterization of a multifunctional PEG-based gene delivery system containing nuclear localization signals and endosomal escape peptides ☆	11