Available online at www.sciencedirect.com
ScienceDirect
Acta Biomaterialia 5 (2009) 903–912
Acta BIOMATERIALIA
www.elsevier.com/locate/actabiomat

Self-assembling peptide–lipoplexes for substrate-mediated gene delivery ☆

Jennifer C. Rea [a], Romie F. Gibly [b], Annelise E. Barron [c], Lonnie D. Shea [a,d,*]

[a] Department of Chemical and Biological Engineering, Northwestern University, 2145 Sheridan Road, Tech E156, Evanston, IL 60208-3120, USA
[b] Department of Interdepartmental Biological Sciences, Northwestern University, 2145 Sheridan Road, Tech E156, Evanston, IL 60208-3120, USA
[c] Department of Bioengineering, Stanford University, W300B James H. Clark Center, 318 Campus Drive, Stanford, CA 94305-5440, USA
[d] Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, IL 60611, USA

Received 1 July 2008; received in revised form 27 August 2008; accepted 8 October 2008
Available online 21 October 2008

Abstract

The efficiency of biomaterial-based gene delivery is determined by the interaction of the material and the vector. For lipoplexes, surface immobilization has been used to transfect cells for applications such as cell arrays and model tissue formation through patterned transfection. Further increases in the delivery efficiency are limited by cellular internalization, which may be overcome by altering the material/vector interactions. In this report, we investigated the modification of the lipoplex physical properties through self-assembly with cationic peptides, and subsequently quantified cellular association, internalization and nuclear accumulation of DNA and transfection. Relative to lipid alone, peptide–lipoplexes enhanced transfection by up to 4.6-fold. The presence of the peptide in the lipoplex increased internalization efficiency by up to 4.5-fold, decreased the percentage of lysosomal DNA by 2.1-fold and increased the efficiency of nuclear accumulation by 3.0-fold. In addition, an analysis of internalization pathways for peptide–lipoplexes indicated a greater role of clathrin and caveolae-mediated endocytosis relative to macropinocytois, which was not observed for peptide-free lipoplexes. These results demonstrate peptide-induced enhancement of gene transfer by surface immobilization due to increased cellular internalization and nuclear accumulation, which has numerous applications ranging from cell-based assays to regenerative medicine.

Keywords: Gene delivery; Peptide; Lipoplex; Substrate-mediated; Self-assembly

1. Introduction

Biomaterials have been used for many therapeutic and research applications such as tissue engineering and gene delivery. Gene transfer from the surface of a biomaterial, termed reverse transfection [1], solid-phase delivery [2] or substrate-mediated delivery [3], immobilizes DNA vectors onto a surface as opposed to more typical bolus delivery from the media. Several approaches have been employed for DNA immobilization, including DNA entrapment in gelatin [1], polyelectrolyte layering of DNA [4,5] and immobilization of preformed complexes followed by specific tethering [3,6,7] or nonspecific adsorption [2,8–12]. Immobilization by non-specific adsorption can reduce the amount of DNA required for expression and increase transgene expression and the number of cells expressing the transgene relative to similar quantities delivered as a bolus [8,9]. However, the delivery efficiency is a function of not only the properties of the surface but also the properties of the DNA complexation vectors, such as cationic polymers or lipids [3,8,10].

Although immobilizing complexes places the vector directly in the cell microenvironment, the immobilization must still allow for efficient cell internalization and intracellular trafficking of the DNA. While transfection following

☆ Part of the Self-Assembling Biomaterials Special Issue, edited by William L. Murphy and Joel H. Collier.

* Corresponding author. Address: Department of Chemical and Biological Engineering, Northwestern University, 2145 Sheridan Road, Tech E156, Evanston, IL 60208-3120, USA. Tel.: +1 847 491 7043; fax: +1 847 491 3728.
E-mail address: l-shea@northwestern.edu (L.D. Shea).

1742-7061/$ - see front matter © 2008 Acta Materialia Inc. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.actbio.2008.10.003

surface immobilization with cationic polymers is limited by intracellular transport, transfection with lipoplexes delivered from the surface is limited by internalization [13]. Increased cellular association of lipoplexes has been achieved for bolus delivery by altering lipoplex physical properties, such as size and zeta potential, and adding functional groups to the lipoplex surface to mediate cellular interactions [14]. However, while DNA complexes formed with cationic polymers, termed polyplexes, have been modified for surface delivery, lipoplexes are more difficult to alter due to the chemical structure of the lipids, such as the lack of covalent binding sites [3].

The physiochemical properties of lipoplexes that influence gene transfer can be manipulated through peptide addition during complex formation [15,16]. We have previously reported that transfection efficiency of lipoplexes delivered as a bolus is significantly enhanced by the addition of short, cationic peptides to form self-assembled particles, termed peptide–lipoplexes [14]. Specifically, these peptides contained the SV40 T-antigen nuclear localization sequence (NLS) or a scrambled NLS sequence, and have amines that facilitate binding between the peptide and plasmid. Peptide–lipoplexes are assembled by first incubating cationic peptides with DNA prior to incubation with lipids. The peptides interact with the DNA without fully shielding its negative charges, as shown by gel electrophoresis, thus allowing the cationic lipids to condense the DNA [14]. Peptide–lipoplexes with a size of less than 500 nm, a positive zeta potential and a relatively high amount of surface-displayed amines had the highest transfection efficiency and highest amount of cell-associated DNA, regardless of peptide sequence [14]. These results suggest that increasing the peptide quantity added to DNA increases the incorporation of peptides into the lipoplexes, which can facilitate cellular interactions through modified lipoplex surface properties [17].

In this report, we investigate the incorporation of peptides as a means of overcoming the limiting steps to substrate-mediated lipofection. We hypothesize that the physical properties of the lipoplexes, which are determined by the amount of peptide added, affect the lipoplex interaction with the substrate, thus affecting cell association, internalization, nuclear accumulation and transfection efficiency. Peptide–lipoplexes were formed at peptide/DNA weight ratios ranging from 0 to 100 by adding cationic peptides to DNA prior to complexation with lipids. Protein expression was quantified as a function of peptide incorporation in the lipoplexes. Lipoplexes were visualized on the surface using fluorescence microscopy to determine lipoplex morphology. Cell association of DNA, DNA internalization efficiency and nuclear accumulation efficiency were measured as a function of peptide amount. Finally, the pathway for internalization and the subsequent lysosomal distribution of DNA were investigated by using endocytic inhibitors and confocal microscopy, respectively. These studies identify mechanisms by which lipoplex delivery from biomaterials can be enhanced and may contribute to the design of gene delivery vectors for use in a range of cell-based assays or regenerative medicine.

2. Materials and methods

2.1. Materials

Plasmids encoding for β-galactosidase (pβGal) and luciferase/enhanced green fluorescent fusion protein (pEGFP-Luc) with a CMV promoter were purified from bacteria culture using Qiagen (Valencia, CA) reagents and stored in Tris–EDTA buffer (10 mM Tris, 1 mM EDTA, pH 7.4). Lipofectamine, SYTO 61 nucleic acid stain and LysoTracker Yellow HCK-123 reagent were purchased from Invitrogen (Carlsbad, CA). Label IT Cy3 Labeling Kit was purchased for Mirus Bio Corporation (Madison, WI). [α-^{32}P]dATP was purchased from PerkinElmer (Waltham, MA). Amino acids and resin for peptide synthesis were purchased from Novabiochem (San Diego, CA). The remaining peptide synthesis reagents were purchased from Applied Biosystems (Foster City, CA). Peptides were also purchased from Celtek Bioscience LLC (Nashville, TN). Amiloride hydrochloride, filipin and chlorpromazine hydrochloride were purchased from Sigma-Aldrich (St. Louis, MO). All other reagents were obtained from Fisher Scientific (Waltham, MA) unless otherwise noted.

2.2. Peptide synthesis and purification

An Applied Biosystems (Foster City, CA) 433A peptide synthesizer was used to synthesize the SV40 T-antigen nuclear localization sequence (NLS) and a scrambled peptide control. The SV40 peptide, EGPKKKRKVG, containing the minimal SV40 T-antigen NLS, and the sSV40 peptide, EKRGKVKPKG, a scrambled version of SV40, were synthesized using standard solid phase methods and FastMocTM chemistry. The peptides were cleaved with a mixture of 90% trifluoroacetic acid, 2.5% triisopropylsilane (TIS), 2.5% thioanosole and 5% water for 1-2 h at room temperature, then lyophilized. The crude peptides were analyzed for purity by reversed-phase high-pressure liquid chromatography (RP-HPLC). The peptides were purified by preparative RP-HPLC with a gradient of 0–20% acetonitrile with 0.1% trifluoroacetic acid (TFA) in water with 0.1% TFA. Peptide molecular weights were confirmed using electrospray ionization (ESI) mass spectroscopy and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry at Northwestern University's Analytical Services Laboratory. Peptide purities were analyzed using analytical RP-HPLC and were determined to be >95%.

2.3. Peptide–lipoplex formation and immobilization

Lipofectamine (Invitrogen, Carlsbad, CA), a 3:1 w/w formulation of the polycationic lipid 2,3-dioleyloxy-*N*-[2(sperminecarboxamido)ethyl]-*N*,*N*-dimethyl-1-propanaminium trifluoroacetate (DOSPA) and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE), was used at a 10:1 w/w ratio with DNA, within the manufacturer's